

## 2. EXPERIMENTAL

### 2.1 Chemicals and instruments

Melting points were measured in degree Celcius ( $^{\circ}\text{C}$ ) on an Electrothermal 9100 digital melting point apparatus and uncorrected. Infrared spectra (IR) were obtained on FTS165 FT-IR spectrometer. Major bands ( $\nu$ ) were recorded in wavenumber ( $\text{cm}^{-1}$ ). Ultraviolet (UV) absorption spectra were measured with Specord S100 spectrophotometer (Analytik Jena Ag) or Shimadzu UV-1601 spectrophotometer. Principle bands ( $\lambda_{\text{max}}$ ) were recorded as wavelengths (nm) and  $\log \varepsilon$  in methanol solution.  $^1\text{H}$  and  $^{13}\text{C}$ -Nuclear magnetic resonance spectra were recorded on 300 MHz Bruker AVANCE, 400 MHz Bruker AMX 400 or 500 MHz Varian UNITY INOVA spectrometer. Spectra were recorded in deuteriochloroform solution, unless otherwise stated, and  $\delta$  value in ppm downfield from TMS (internal standard  $\delta$  0.00). Optical rotations were measured in methanol or chloroform solution with sodium D line (589 nm) on an AUTOPOL<sup>R</sup> II or a JASCO P-1020 automatic polarimeters. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except for petroleum ether (bp. 40-60  $^{\circ}\text{C}$ ), diethyl ether and ethyl acetate which were analytical grade reagent. Quick column chromatography was performed on silica gel 60 GF<sub>254</sub> (Merck). Column chromatography was performed on silica gel (Merck) type 100 (70-230 mesh ASTM), Sephadex LH-20, or reverse-phase C<sub>18</sub> silica gel. Precoated TLC plates of silica gel 60 F<sub>254</sub> or reverse-phase C<sub>18</sub> silica gel were used for analytical purposes.

## 2.2 Plant material

The fruits of *G. scortechinii* were collected at the Ton Nga Chang Wildlife Sanctuary, Hat Yai, Songkhla, Thailand in June 2000 while those of *G. hanburyi* were collected at the Sri Pang Nga National Park, Kura Buri and Tagua Pa, Pang Nga, Thailand in May 2004. The plants were identified by Dr. Prakart Sawangchote and Miss Katesarin Maneenoon, Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, where voucher specimens have been deposited.

## 2.3 Chemical investigation from the fruits of *G. scortechinii*

### 2.3.1 Extraction

The fruits (1,120 g) of *G. scortechinii*, cut into small segments, were extracted with MeOH (2.5 L) over the period of 7 days at room temperature for three times. After filtration, the filtrate was evaporated to dryness under reduced pressure to give a crude methanol extract as a brown-yellow gum in 94.39 g.

### 2.3.2 Chemical investigation of the crude methanol extract of the fruits

The crude methanol extract was primarily tested for its solubility in various solvents at room temperature. The results were shown in **Table 2**.

**Table 2** Solubility of the crude methanol extract in various solvents at room temperature

Solvent	Solubility at room temperature
Petroleum ether	-
CH <sub>2</sub> Cl <sub>2</sub>	+ yellow solution
CHCl <sub>3</sub>	+ yellow solution

**Table 2** (continued)

Solvent	Solubility at room temperature
EtOAc	+ pale yellow solution
Acetone	++ yellow solution
MeOH	+++ yellow solution mixed with white solid
H <sub>2</sub> O	+ pale yellow solution
10%HCl	+ pale yellow solution
10%NaOH	++ brown-yellow solution
10%NaHCO <sub>3</sub>	++ yellow solution

Symbol meaning: - insoluble, + partially soluble, ++ moderately soluble, +++ well soluble

It was shown that the crude methanol extract dissolved slightly in dichloromethane, chloroform, ethyl acetate, water and 10% aqueous HCl but it was soluble well in acetone, methanol, 10% aqueous NaOH and 10% aqueous NaHCO<sub>3</sub>. These indicated that major components were moderately polar and acidic compounds.

Chromatogram characteristics of the crude methanol extract dissolved in CH<sub>2</sub>Cl<sub>2</sub>, CHCl<sub>3</sub>, EtOAc and acetone on normal phase TLC with 40% ethyl acetate-petroleum ether showed two major yellow spots under UV-S with the R<sub>f</sub> values of 0.31 and 0.65 but the one dissolved in methanol showed no major spots. Therefore, the crude methanol extract was separated into two parts by dissolving in chloroform.

### 2.3.2.1 Investigation of the chloroform-soluble part

Chloroform-soluble part (**GFA**) was obtained as a brown-yellow gum in 24.82 g. Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform showed three major spots under UV-S with the R<sub>f</sub> values of 0.06, 0.34 and 0.71. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with chloroform and gradually enriched with pure methanol to give thirty one fractions. All fractions were examined by TLC, combined

on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford thirteen fractions, as shown in **Table 3**.

**Table 3** Fractions obtained from **GFA** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
GFA1	0.713	Yellow liquid
GFA2	0.606	Orange-yellow liquid
GFA3	2.240	Orange-yellow gum
GFA4	0.758	Yellow gum
GFA5	1.370	Green gum
GFA6	12.458	Dark green gum
GFA7	1.378	Green gum
GFA8	1.122	Green gum
GFA9	2.620	Yellow-green gum mixed with solid
GFA10	0.798	Orange gum
GFA11	0.329	Orange-yellow gum
GFA12	1.234	Yellow-brown solid
GFA13	0.544	Brown solid

**Fraction GFA1** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed one major spot under UV-S with the  $R_f$  value of 0.79. After dipping the TLC plate in ASA reagent and subsequent heating, it showed many violet spots. Therefore, it was not further investigated.

**Fraction GFA2** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed two major spots under UV-S with the  $R_f$  values of 0.31 and 0.39 and two spots with the  $R_f$  values of 0.65 and 0.78 which appeared as a violet spot after dipping the TLC plate in ASA reagent and subsequent heating. It was purified by column chromatography on silica gel. Elution was conducted initially with 5% ethyl acetate-petroleum ether and gradually increased the polarity until pure methanol to give fifty one fractions. All fractions were examined

by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 4**.

**Table 4** Subfractions obtained from fraction **GFA2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A2-1	0.025	White solid and pale yellow gum
A2-2	0.005	Colorless gum
A2-3	0.024	Pale yellow gum
A2-4	0.238	Yellow gum

**Subfraction A2-1** Chromatogram characteristics on normal phase TLC with 2% ethyl acetate-petroleum ether showed many pale spots under UV-S. Therefore, it was not further investigated.

**Subfraction A2-2** Chromatogram characteristics on normal phase TLC with 2% ethyl acetate-petroleum ether showed two pale UV-active spots with the  $R_f$  values of 0.44 and 0.52. Because it was obtained in low quantity, it was not further investigated.

**Subfraction A2-3** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (3 runs) showed three UV-active spots with the  $R_f$  values of 0.38, 0.40 and 0.48 and two spots with the  $R_f$  values of 0.35 and 0.48 which appeared as a violet spot after dipping the TLC plate in ASA reagent and subsequent heating. Further purification was performed on precoated TLC, using 10% ethyl acetate-petroleum ether as a mobile phase (5 runs), to afford two bands.

**Band 1 (GF1)** was obtained as a colorless gum in 2.3 mg. Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether (3 runs) showed only one UV-active spot with the  $R_f$  value of 0.30.

$[\alpha]_D^{29}$  +266° (c = 0.08, CHCl<sub>3</sub>)  
 IR (neat)  $\nu_{\max}$  (cm<sup>-1</sup>) 3468 (OH stretching), 2930 (CH stretching),

	1732, 1673 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	240 (3.88)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (300 MHz)	6.95 ( <i>dq</i> , $J = 6.3$ and $1.5$ Hz, 1H), 3.17 ( <i>s</i> , 3H), 2.60 ( <i>ddd</i> , $J = 10.2$ , $6.3$ and $5.1$ Hz, 1H), 2.37 ( <i>m</i> , 2H), 2.25 ( <i>m</i> , 1H), 1.85 ( <i>sept d</i> , $J = 7.0$ and $2.5$ Hz, 1H), 1.79 ( <i>t</i> , $J = 1.5$ Hz, 3H), 1.78 ( <i>m</i> , 1H), 1.47 ( <i>m</i> , 1H), 1.36 ( <i>m</i> , 2H), 1.29 ( <i>m</i> , 1H), 1.12 ( <i>s</i> , 3H), 0.92 ( <i>d</i> , $J = 7.0$ Hz, 3H), 0.89 ( <i>d</i> , $J = 7.0$ Hz, 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (75 MHz)	199.55, 150.98, 134.69, 75.01, 48.90, 42.99, 42.63, 36.90, 35.42, 30.26, 27.80, 21.52, 21.35, 19.19, 16.00, 15.74
DEPT (135°) (CDCl <sub>3</sub> )	CH 150.98, 42.99, 42.63, 35.42, 27.80 CH <sub>2</sub> 36.90, 30.26, 19.19 CH <sub>3</sub> 48.10, 21.52, 21.35, 16.00, 15.74
EIMS ( $m/z$ ) (% rel. int.)	250 (19), 218 (16), 207 (24), 175 (51), 162 (17), 135 (19), 85 (100), 72 (23), 69 (13)

**Band 2 (GF2)** was obtained as a colorless gum in 3.9 mg. Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether (3 runs) showed only one UV-active spot with the  $R_f$  value of 0.24.

$[\alpha]_D^{29}$	+72° (c = 0.17, CHCl <sub>3</sub> )
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3468 (OH stretching), 2935 (CH stretching), 1738, 1681 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	238 (3.85)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (300 MHz)	6.80 ( <i>brs</i> , 1H), 3.20 ( <i>s</i> , 3H), 2.71 ( <i>dd</i> , $J = 15.0$ and $1.8$ Hz, 1H), 2.24 ( <i>sept d</i> , $J = 6.9$ and $2.1$ Hz, 1H), 2.14 ( <i>m</i> , 1H), 2.07 ( <i>dd</i> , $J = 15.0$ and $13.5$ Hz, 1H), 1.98 ( <i>m</i> , 1H), 1.87 ( <i>m</i> , 1H), 1.78 ( <i>dd</i> , $J = 2.1$ and $1.5$ Hz, 3H), 1.69 ( <i>m</i> , 1H), 1.48 ( <i>m</i> , 1H), 1.24

	( <i>m</i> , 1H), 1.18 ( <i>m</i> , 1H), 1.12 ( <i>s</i> , 3H), 0.98 ( <i>d</i> , <i>J</i> = 6.9 Hz, 3H), 0.83 ( <i>d</i> , <i>J</i> = 6.9 Hz, 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) (δ ppm) (75 MHz)	200.38, 146.15, 135.34, 74.78, 48.20, 47.78, 45.04, 40.48, 38.28, 34.86, 26.16, 21.46, 21.01, 17.86, 15.94, 15.18
DEPT (135°) (CDCl <sub>3</sub> )	CH 146.15, 47.78, 45.04, 40.48, 26.16 CH <sub>2</sub> 38.28, 34.86, 21.01 CH <sub>3</sub> 48.20, 21.46, 17.86, 15.94, 15.18
EIMS ( <i>m/z</i> ) (% rel. int.)	250 (13), 218 (16), 207 (20), 175 (65), 165 (21), 147 (10), 135 (19), 91 (16), 85 (100), 72 (29), 69 (12)

**Subfraction A2-4** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether showed none of well-separated spots. Therefore, it was not further investigated.

**Fraction GFA3** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed three major spots; two yellow spots with the *R<sub>f</sub>* values of 0.49 and 0.60 and one UV-active spot with the *R<sub>f</sub>* value of 0.38. It was further separated by column chromatography on silica gel. Elution was conducted initially with chloroform and gradually increased the polarity until 20% methanol-chloroform to give fifty nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions, as shown in **Table 5**.

**Table 5** Subfractions obtained from fraction **GFA3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A3-1	0.079	Green-brown liquid
A3-2	0.043	Yellow-brown gum
A3-3	0.084	Green gum

**Table 5** (continued)

Subfraction	Weight (g)	Physical appearance
A3-4	1.377	Yellow gum
A3-5	0.533	Yellow gum
A3-6	0.590	Yellow gum

**Subfraction A3-1** Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether showed none of well-separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction A3-2** Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether showed one yellow spot with the  $R_f$  value of 0.55. Attempted purification by repeated chromatography was unsuccessful.

**Subfraction A3-3** Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether showed many UV-active spots without any major spots. Therefore, it was not further investigated.

**Subfraction A3-4** Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether showed one major yellow spot with the same  $R_f$  value as **scortechinone A**, obtained from its twigs.

**Subfraction A3-5** Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether showed two yellow spots with the  $R_f$  values of 0.25 and 0.39. It was further separated by flash column chromatography. Elution was conducted with chloroform to give eleven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 6**.



**Table 6** Subfractions obtained from subfraction **A3-5** by flash column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A3-5-1	0.038	Yellow gum
A3-5-2	0.428	Yellow gum
A3-5-3	0.003	Pale yellow gum

**Subfraction A3-5-1** Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether showed one major yellow spot with the same  $R_f$  value as **scortechinone A**, obtained from its twigs.

**Subfraction A3-5-2** Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether showed four spots; two yellow spots with the  $R_f$  values of 0.18 and 0.28, and two UV-active spots with the  $R_f$  values of 0.13 and 0.36. Further purification (60.0 mg) was performed on precoated TLC, using 8% ethyl acetate-petroleum ether as a mobile phase (13 runs), to afford five bands.

**Band 1 (GF3)** was obtained as a yellow gum in 10.1 mg. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether showed one yellow spot with the same  $R_f$  value as **scortechinone A**, obtained from its twig.

$[\alpha]_D^{29}$	+18° (c = 0.03, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3468 (OH stretching), 2966, 2928 (CH stretching), 1746, 1635 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	361 (3.88)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	13.16 (s, 1H), 7.49 (d, $J$ = 1.0 Hz, 1H), 5.21 (tm, $J$ = 7.0 Hz, 1H), 4.37 (m, 1H), 4.36 (q, $J$ = 6.5 Hz, 1H), 3.62 (s, 3H), 3.21 (d, $J$ = 7.0 Hz, 2H), 2.68 (dm, $J$ = 14.0 Hz, 1H), 2.55 (dd, $J$ = 14.0 and 10.0 Hz, 1H), 2.55 (d, $J$ = 9.5 Hz, 1H), 2.33 (d, $J$ =

13.5 Hz, 1H), 1.75 (*s*, 3H), 1.71 (*s*, 3H), 1.67 (*s*, 3H), 1.65 (*dd*,  $J = 13.5$  and  $9.5$  Hz, 1H), 1.58 (*s*, 3H), 1.41 (*d*,  $J = 6.5$  Hz, 3H), 1.36 (*s*, 3H), 1.28 (*s*, 3H), 1.16 (*s*, 3H), 1.06 (*s*, 3H)

**Band 2 (GF4)** was obtained as a yellow gum in 5.1 mg. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether showed only one yellow spot with the  $R_f$  value of 0.55 (**scortechinone L**).

$[\alpha]_D^{29}$	-176° ( $c = 0.02$ , CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3440 (OH stretching), 2927, 2855 (CH stretching), 1745, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	355 (4.25)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	13.24 ( <i>s</i> , 1H), 7.51 ( <i>d</i> , $J = 1.5$ Hz, 1H), 5.23 ( <i>tm</i> , $J = 7.0$ Hz, 1H), 4.55 ( <i>q</i> , $J = 6.5$ Hz, 1H), 4.36 ( <i>dm</i> , $J = 10.0$ Hz, 1H), 3.64 ( <i>s</i> , 3H), 3.22 ( <i>d</i> , $J = 7.0$ Hz, 2H), 2.67 ( <i>dm</i> , $J = 14.5$ Hz, 1H), 2.58 ( <i>d</i> , $J = 9.5$ Hz, 1H), 2.54 ( <i>dd</i> , $J = 14.5$ and $10.0$ Hz, 1H), 2.34 ( <i>d</i> , $J = 13.5$ Hz, 1H), 1.76 ( <i>s</i> , 3H), 1.72 ( <i>s</i> , 3H), 1.68 ( <i>s</i> , 3H), 1.67 ( <i>dd</i> , $J = 13.5$ and $9.5$ Hz, 1H), 1.49 ( <i>s</i> , 3H), 1.42 ( <i>s</i> , 3H), 1.36 ( <i>s</i> , 3H), 1.30 ( <i>d</i> , $J = 6.5$ Hz, 3H), 1.29 ( <i>s</i> , 3H), 1.02 ( <i>s</i> , 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (125 MHz)	202.28, 178.11, 166.54, 163.27, 154.32, 135.49, 133.91, 132.42, 132.01, 121.62, 117.08, 111.91, 105.79, 101.36, 91.24, 89.30, 84.88, 84.36, 83.18, 53.91, 49.95, 43.70, 30.94, 30.69, 28.98, 28.92, 28.17, 25.69, 25.44, 21.34, 20.11, 17.79, 16.66, 16.35
DEPT (135°) (CDCl <sub>3</sub> )	CH 133.91, 121.62, 117.08, 91.24, 49.95 CH <sub>2</sub> 30.69, 28.92, 21.34

	CH <sub>3</sub> 53.91, 30.94, 28.98, 28.17, 25.69, 25.44, 20.11, 17.79, 16.66, 16.35
EIMS ( <i>m/z</i> ) (% rel. int.)	562 (4), 535 (34), 534 (100), 519 (22), 478 (19), 465 (44), 451 (20), 437 (28), 381 (70), 289 (32), 247 (66), 245 (60), 229 (35), 209 (31), 149 (27)

**Band 3 (GF5)** was obtained as a yellow solid in 4.7 mg. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether showed only one yellow spot with the same  $R_f$  value as **scortechinone D**, obtained from its latex. It melted at 172-174 °C.

$[\alpha]_D^{29}$	+222° ( <i>c</i> = 0.02, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	2930, 2856 (CH stretching), 1746, 1641 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	358 (3.65)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	13.03 ( <i>s</i> , 1H), 7.52 ( <i>d</i> , <i>J</i> = 1.5 Hz, 1H), 6.04 ( <i>s</i> , 1H), 4.39 ( <i>q</i> , <i>J</i> = 6.5 Hz, 1H), 4.38 ( <i>dm</i> , <i>J</i> = 10.5 Hz, 1H), 3.64 ( <i>s</i> , 3H), 2.71 ( <i>dm</i> , <i>J</i> = 14.5 Hz, 1H), 2.59 ( <i>d</i> , <i>J</i> = 9.5 Hz, 1H), 2.58 ( <i>dd</i> , <i>J</i> = 14.5 and 10.5 Hz, 1H), 2.36 ( <i>d</i> , <i>J</i> = 13.0 Hz, 1H), 1.72 ( <i>s</i> , 3H), 1.66 ( <i>dd</i> , <i>J</i> = 13.0 and 9.5 Hz, 1H), 1.59 ( <i>s</i> , 3H), 1.41 ( <i>d</i> , <i>J</i> = 6.5 Hz, 3H), 1.37 ( <i>t</i> , <i>J</i> = 1.5 Hz, 3H), 1.30 ( <i>s</i> , 3H), 1.17 ( <i>s</i> , 3H), 1.09 ( <i>s</i> , 3H)

**Band 4 (GF6)** was obtained as a yellow gum in 2.3 mg. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether showed one yellow spot with the same  $R_f$  value as **scortechinone E**, obtained from its latex.

$[\alpha]_D^{29}$	-240° ( <i>c</i> = 0.03, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	2974, 2928 (CH stretching),

	1745, 1640 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	359 (3.76)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	13.09 ( <i>s</i> , 1H), 7.52 ( <i>d</i> , <i>J</i> = 1.0 Hz, 1H), 6.04 ( <i>s</i> , 1H), 4.55 ( <i>q</i> , <i>J</i> = 6.5 Hz, 1H), 4.36 ( <i>dm</i> , <i>J</i> = 10.5 Hz, 1H), 3.64 ( <i>s</i> , 3H), 2.69 ( <i>dm</i> , <i>J</i> = 14.0 Hz, 1H), 2.61 ( <i>d</i> , <i>J</i> = 9.5 Hz, 1H), 2.55 ( <i>dd</i> , <i>J</i> = 14.0 and 10.5 Hz, 1H), 2.36 ( <i>dd</i> , <i>J</i> = 13.0 and 1.5 Hz, 1H), 1.72 ( <i>s</i> , 3H), 1.67 ( <i>dd</i> , <i>J</i> = 13.0 and 9.5 Hz, 1H), 1.49 ( <i>s</i> , 3H), 1.42 ( <i>s</i> , 3H), 1.38 ( <i>t</i> , <i>J</i> = 1.5 Hz, 3H), 1.30 ( <i>d</i> , <i>J</i> = 6.5 Hz, 3H), 1.30 ( <i>s</i> , 3H), 1.07 ( <i>brs</i> , 3H)

**Band 5** was obtained as a yellow gum in 1.1 mg. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether showed one UV-active spot with the  $R_f$  value of 0.30. The <sup>1</sup>H NMR spectrum indicated that it contained two compounds. Because it was obtained in low quantity, it was not further investigated.

**Subfraction A3-5-3** Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether showed many pale spots under UV-S. Therefore, it was not further investigated.

**Subfraction A3-6** Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether showed none of well-separated spots under UV-S. Therefore, it was not further investigated.

**Fraction GFA4** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed five major UV-active spots with the  $R_f$  values of 0.19, 0.30, 0.48, 0.56 and 0.65. It was further separated by column chromatography on silica gel. Elution was conducted initially with chloroform and gradually increased the polarity until pure methanol to give seventy four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford eight subfractions, as shown in **Table 7**.

**Table 7** Subfractions obtained from fraction **GFA4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A4-1	0.043	Yellow solid
A4-2	0.060	Yellow gum mixed with solid
A4-3	0.070	Yellow gum mixed with solid
A4-4	0.145	Yellow gum
A4-5	0.129	Orange-yellow gum
A4-6	0.162	Green-yellow gum
A4-7	0.119	Green-yellow gum
A4-8	0.061	Yellow gum

**Subfraction A4-1** Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether (6 runs) showed one UV-active spot with the  $R_f$  value of 0.32 and two spots with the  $R_f$  values of 0.24 and 0.41 which appeared as violet spots after dipping the TLC plate in ASA reagent and subsequent heating. It was purified by column chromatography on silica gel. Elution was conducted with 5% ethyl acetate-hexane to give forty four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nine subfractions. **Stigmasterol** (0.004 g) was obtained from the sixth subfraction as a colorless gum. Other fractions were obtained in low quantity. Their chromatogram characteristics on normal phase TLC with 5% ethyl acetate-petroleum ether (3 runs) showed no major spots. Most of spots appeared as purple spots after dipping the TLC plate in ASA reagent and subsequent heating.

**Subfraction A4-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed one major UV-active spot with the  $R_f$  value of 0.46. It was separated by column chromatography on silica gel. Elution was conducted initially with 10% ethyl acetate-petroleum ether and gradually enriched with pure ethyl acetate to give thirty nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 8**.

**Table 8** Subfractions obtained from subfraction **A4-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A4-2-1	0.007	Yellow gum
A4-2-2	0.027	Yellow gum
A4-2-3	0.012	Yellow gum
A4-2-4	0.007	Yellow gum

**Subfraction A4-2-1** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed three UV-active spot with the  $R_f$  values of 0.45, 0.55 and 0.73. Because it was obtained in low quantity, it was not further investigated.

**Subfraction A4-2-2** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed three UV-active spots with the  $R_f$  values of 0.38, 0.42, and 0.46. Further purification by precoated TLC on silica gel plates with 10% ethyl acetate-petroleum ether (22 runs) as a mobile phase afforded two bands.

**Band 1** was obtained as a yellow gum in 0.036 g. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (4 runs) showed two UV-active spots with the  $R_f$  values of 0.35 and 0.39. It was combined with subfraction **A4-2-3** and further purified by precoated TLC on silica gel plates with EtOAc:CH<sub>2</sub>Cl<sub>2</sub>:Petrol (0.1:8:2) (3 runs) as a mobile phase to afford two bands.

**Band 1.1** was obtained as a yellow gum in 0.024 g. Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether showed one major yellow spot with the  $R_f$  value of 0.52 which was identical to **scortechinone A**. It was separated by column chromatography on silica gel. Elution was conducted with 8% ethyl acetate-petroleum ether to give ninety two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions.

**Subfraction 1.1A** was obtained as a yellow gum (0.009 g). Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (10 runs) showed one major yellow spot with the  $R_f$  value of 0.59 which was **scortechinone A**.

**Subfraction 1.1B** was obtained as a yellow gum (0.017 g). Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (10 runs) showed two major yellow spots with the  $R_f$  values of 0.54 and 0.59. This fraction was investigated together with subfraction **A4A-2** later.

**Band 1.2 (GF7)** was obtained as a pale yellow gum in 0.002 g. Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether showed only one UV-active spot with the  $R_f$  value of 0.45.

$[\alpha]_D^{29}$	-200° (c = 0.01, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3394 (OH stretching), 2952, 2925, 2848 (CH stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	280 (3.17), 236 (3.87)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	6.00 ( <i>d</i> , $J$ = 15.5 Hz, 1H), 5.43 ( <i>dd</i> , $J$ = 15.5 and 10.0 Hz, 1H), 5.28 ( <i>brs</i> , 1H), 5.00 ( <i>brs</i> , 1H), 4.93 ( <i>brs</i> , 1H), 4.85 ( <i>brs</i> , 1H), 3.77 ( <i>dd</i> , $J$ = 11.5 and 4.0 Hz, 1H), 2.63 ( <i>m</i> , 1H), 2.44 ( <i>td</i> , $J$ = 13.0 and 5.0 Hz, 1H), 2.20 ( <i>ddd</i> , $J$ = 13.0, 5.0 and 2.5 Hz, 1H), 2.06 ( <i>m</i> , 1H), 2.02 ( <i>m</i> , 1H), 1.80 ( <i>m</i> , 1H), 1.66 ( <i>m</i> , 1H), 1.64 ( <i>m</i> , 1H), 1.49 ( <i>sept</i> , $J$ = 6.5 Hz, 1H), 0.90 ( <i>d</i> , $J$ = 6.5 Hz, 3H), 0.82 ( <i>d</i> , $J$ = 6.5 Hz, 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (125 MHz)	153.49, 146.72, 137.97, 129.61, 112.91, 110.57, 76.03, 52.50, 36.27, 36.17, 34.52, 31.82, 29.93, 20.75, 20.49
DEPT (135°) (CDCl <sub>3</sub> )	CH 137.97, 129.61, 76.03, 52.50, 31.82 CH <sub>2</sub> 112.91, 110.57, 36.27, 36.17, 34.52, 29.93 CH <sub>3</sub> 20.75, 20.49

**Band 2** was obtained as a yellow gum in 0.001 g. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (4 runs) showed one UV-active spot with the  $R_f$  value of 0.29. Because it was obtained in low quantity, it was not further investigated.

**Subfraction A4-2-3** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed two UV-active spots with the  $R_f$  values of 0.35 and 0.39. It was further purified along with **band 1 of subfraction A4-2-2**.

**Subfraction A4-2-4** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed many UV-active spots. Because it was obtained in low quantity, it was not further investigated.

**Subfraction A4-3** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed two major UV-active spots with the  $R_f$  values of 0.36 and 0.46. Further separation with column chromatography on silica gel was performed. Elution was conducted initially with 10% ethyl acetate-petroleum ether and gradually enriched with pure ethyl acetate to give fifty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 9**.

**Table 9** Subfractions obtained from subfraction **A4-3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A4-3-1	0.005	Yellow gum
A4-3-2	0.029	Yellow gum
A4-3-3	0.024	Yellow gum mixed with solid
A4-3-4	0.005	Yellow gum

**Subfraction A4-3-1** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed two pale UV-active



spots with the  $R_f$  values of 0.51 and 0.72. Because it was obtained in low quantity, it was not further investigated.

**Subfraction A4-3-2** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed three major UV-active spots with the  $R_f$  values of 0.28, 0.40 and 0.44. It was further separated by column chromatography on silica gel. Elution was conducted with EtOAc:CH<sub>2</sub>Cl<sub>2</sub>:Petrol (0.1:8:2) to give seventy two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 10**.

**Table 10** Subfractions obtained from subfraction **A4-3-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A4A-1	0.003	Yellow gum
A4A-2	0.016	Yellow gum
A4A-3	0.011	Yellow gum

**Subfraction A4A-1** Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (11 runs) showed one yellow spot with the same  $R_f$  value as **scortechinone A**, obtained from its twigs.

**Subfraction A4A-2** Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (11 runs) showed one major yellow spot with the same  $R_f$  value as **scortechinone A** and one minor yellow spot with the same  $R_f$  value as **scortechinone L**.

**Subfraction A4A-3** Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (11 runs) showed two pale UV-active spots with the  $R_f$  values of 0.49 and 0.55. Because it was obtained in low quantity, it was not further investigated.

**Subfraction A4-3-3** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed three major UV-active spots with the  $R_f$  values of 0.34, 0.40 and 0.44. Further purification was performed by

precoated TLC, using 8% ethyl acetate-petroleum ether as a mobile phase (14 runs), to afford five bands.

**Band 1** was obtained as a yellow gum in 2.4 mg. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (3 runs) showed one yellow spot with the same  $R_f$  value as **scortechinone A**, obtained from its twig.

**Band 2** was obtained as a yellow gum in 2.2 mg. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (3 runs) showed one yellow spot with the  $R_f$  value of 0.22. Its was shown by TLC comparison that the major spot was **GF4**, obtained from subfraction **A3-5-2**.

**Band 3** was obtained as a yellow gum in 8.5 mg. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (3 runs) showed one major yellow spot with the  $R_f$  value of 0.19. Its was shown by TLC comparison that the major spot was **GF5**, obtained from subfraction **A3-5-2**.

**Band 4** was obtained as a yellow gum in 4.7 mg. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (3 runs) showed two major yellow spots with the  $R_f$  values of 0.17 and 0.19 which were **GF5** and **GF6**.

**Band 5** was obtained as a yellow gum in 3.1 mg. Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (3 runs) showed one yellow spot with the  $R_f$  value of 0.17 which was identical to that of **GF6**.

**Subfraction A4-3-4** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed none of well-separated spots. Because it was obtained in low quantity, it was not further investigated.

**Subfraction A4-4** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed two pale UV-active spots with the  $R_f$  values of 0.36 and 0.46. Further separation with column chromatography on silica gel was performed. Elution was conducted initially with 8% ethyl acetate-hexane and gradually enriched with pure ethyl acetate to give sixty nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions. Their

chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (4 runs) showed many pale UV-active spots with additional purple spots above UV-active spots after dipping the TLC plate in ASA reagent and subsequent heating. Thus, it was not further investigated.

**Subfraction A4-5** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed three major UV-active spots with the  $R_f$  values of 0.10, 0.39 and 0.50. Further separation with column chromatography on silica gel was performed. Elution was conducted initially with 8% ethyl acetate-hexane and gradually enriched with 40% ethyl acetate-hexane to give sixty nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 11**.

**Table 11** Subfractions obtained from subfraction **A4-5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A4-5-1	0.095	Yellow gum
A4-5-2	0.017	Yellow gum
A4-5-3	0.012	Yellow gum

**Subfraction A4-5-1** Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (4 runs) showed one major spot with the  $R_f$  value of 0.30. One additional violet spot with the  $R_f$  value of 0.26 was observed after dipping the TLC plate in ASA reagent and subsequent heating. Because it was obtained in low quantity, it was not further investigated.

**Subfraction A4-5-2** Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (4 runs) showed one major yellow spot with the  $R_f$  value of 0.07. Further purification was performed by precoated TLC, using 1% methanol-chloroform as a mobile phase (2 runs), to afford a yellow gum (**GF8**) in 11.8 mg. Chromatogram characteristics on normal phase TLC with 20%

ethyl acetate-petroleum ether (2 runs) showed only one yellow spot with the  $R_f$  value of 0.25.

$[\alpha]_D^{29}$	-36° (c = 0.10, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3432 (OH stretching), 2928 (CH stretching), 1749 (C=O stretching), 1614 (C=C stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	350 (4.02)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	12.87 (s, 1H), 7.08 (d, $J$ = 1.0 Hz, 1H), 5.25 (tm, $J$ = 7.0 Hz, 1H), 4.48 (m, 1H), 4.50 (q, $J$ = 6.5 Hz, 1H), 3.65 (d, $J$ = 11.5 Hz, 1H), 3.56 (d, $J$ = 11.5 Hz, 1H), 3.50 (s, 3H), 3.20 (d, $J$ = 7.0 Hz, 2H), 2.84 (d, $J$ = 12.5 Hz, 1H), 2.66 (dd, $J$ = 13.5 and 10.0 Hz, 1H), 2.62 (dm, $J$ = 13.5 Hz, 1H), 2.57 (d, $J$ = 10.0 Hz, 1H), 1.76 (dd, $J$ = 12.5 and 10.0 Hz, 1H), 1.75 (s, 3H), 1.69 (s, 3H), 1.61 (s, 3H), 1.59 (s, 3H), 1.55 (s, 3H), 1.43 (s, 3H), 1.41 (s, 3H), 1.38 (d, $J$ = 6.5 Hz, 3H), 1.36 (s, 3H), 1.19 (s, 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (125 MHz)	198.88, 176.47, 166.92, 162.96, 154.28, 136.45, 134.92, 132.03, 131.49, 121.71, 117.84, 113.71, 105.35, 101.91, 90.94, 87.96, 85.16, 84.86, 83.73, 67.88, 51.83, 43.74, 41.36, 33.80, 28.12, 25.80, 25.76, 25.24, 25.13, 21.40, 21.11, 17.86, 17.73, 14.53
DEPT (135°) (CDCl <sub>3</sub> )	CH 134.92, 121.71, 117.84, 90.94, 41.36 CH <sub>2</sub> 67.88, 33.80, 28.12, 21.40 CH <sub>3</sub> 51.83, 25.80, 25.76, 25.24, 25.13, 21.11, 17.86, 17.73, 14.53
EIMS ( $m/z$ ) (% rel. int.)	578 (4), 518 (74), 480 (100), 424 (28), 366 (27), 308 (19), 149 (44)

**Subfraction A4-5-3** Chromatogram characteristics on normal phase TLC with 8% ethyl acetate-petroleum ether (4 runs) showed no definite spot. Therefore, it was not further investigated.

**Subfraction A4-6** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed five major UV-active spots with the  $R_f$  values of 0.09, 0.18, 0.28, 0.36 and 0.48. Further separation with column chromatography on silica gel was performed. Elution was conducted initially with 15% ethyl acetate-hexane and gradually enriched with 20% methanol-acetone to give forty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions. Their chromatograms on normal phase TLC using 15% ethyl acetate-petroleum ether (4 runs) showed unseparable spots under UV-S. Therefore, they were not further purified.

**Subfraction A4-7** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed five major UV-active spots with the  $R_f$  values of 0.02, 0.09, 0.18, 0.23, and 0.28. This fraction was investigated together with subfraction **A5-B-5** later.

**Subfraction A4-8** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed three major UV-active spots with the  $R_f$  values of 0.02, 0.17 and 0.27. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 15% MeOH/H<sub>2</sub>O and gradually decreased the polarity until pure methanol to give twenty two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford seven subfractions. Their chromatograms on normal phase TLC using 20% ethyl acetate-petroleum ether showed many spots under UV-S. Therefore, they were not further purified.

**Fraction GFA5** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed five major UV-active spots with the  $R_f$  values of 0.07, 0.18, 0.30, 0.40 and 0.63. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with chloroform and gradually increased the polarity until pure methanol to give sixty two

fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 12**.

**Table 12** Subfractions obtained from fraction **GFA5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A5-1	0.991	Yellow-green gum
A5-2	0.258	Yellow-green gum
A5-3	0.121	Brown-yellow gum

**Subfraction A5-1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed five major UV-active spots with the  $R_f$  values of 0.04, 0.10, 0.18, 0.24 and 0.29. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 0.1% methanol-chloroform and gradually increased the polarity until pure methanol to give forty four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 13**.

**Table 13** Subfractions obtained from subfraction **A5-1** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A5-1-1	0.804	Yellow-green gum mixed with solid
A5-1-2	0.086	Yellow-brown gum

**Subfraction A5-1-1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed five major UV-active spots with the  $R_f$  values of 0.20, 0.26, 0.30, 0.36 and 0.45. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with

7% ethyl acetate-petroleum ether and gradually increased the polarity until 70% ethyl acetate-petroleum ether to give ninety fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 14**.

**Table 14** Subfractions obtained from subfraction **A5-1-1** by flash column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C1	0.425	Orange-yellow gum
C2	0.134	Orange-yellow gum mixed with solid
C3	0.252	Green-yellow gum

**Subfraction C1** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed three major UV-active spots with the  $R_f$  values of 0.42, 0.54 and 0.62. Further separation with flash column chromatography over silica gel was performed. Elution was conducted initially with 1% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of acetone in ethyl acetate and finally with pure acetone to give two hundred and nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford eight subfractions, as shown in **Table 15**.

**Table 15** Subfractions obtained from subfraction **C1** by flash column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C1-1	0.122	Yellow gum
C1-2	0.016	Yellow gum
C1-3	0.050	Yellow gum
C1-4	0.100	Yellow gum

**Table 15** (continued)

Subfraction	Weight (g)	Physical appearance
C1-5	0.043	Yellow gum
C1-6	0.040	Yellow-orange gum
C1-7	0.039	Yellow-orange gum
C1-8	0.022	Yellow-orange gum

**Subfraction C1-1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed no spots under UV-S. No further investigation was carried out.

**Subfraction C1-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the  $R_f$  value of 0.47. Further purification was performed by precoated TLC, using 10% ethyl acetate-petroleum ether as a mobile phase (15 runs), to afford a yellow solid in 4.2 mg. Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the  $R_f$  value of 0.26. Its  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Therefore, it was not further investigated.

**Subfraction C1-3** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed three major UV-active spots with the  $R_f$  values of 0.34, 0.47 and 0.53. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 10% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of acetone in ethyl acetate and finally with pure acetone. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford fifteen subfractions. Their chromatograms on normal phase TLC using 15% ethyl acetate-petroleum ether showed unseparable components under UV-S and all fractions were obtained in low quantity. Therefore, they were not further purified.

**Subfraction C1-4** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed four major UV-active



spots with the  $R_f$  values of 0.33, 0.39, 0.46 and 0.51. Further separation with flash column chromatography over silica gel was performed. Elution was conducted initially with 2% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with 50% methanol-ethyl acetate to give eighty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 16**.

**Table 16** Subfractions obtained from subfraction **C1-4** by flash column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C1-4-1	0.023	Yellow gum
C1-4-2	0.133	Yellow gum
C1-4-3	0.070	Brown-yellow gum

**Subfraction C1-4-1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (3 runs) showed no spots under UV-S. No further investigation was carried out.

**Subfraction C1-4-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (3 runs) showed four major UV-active spots with the  $R_f$  values of 0.27, 0.32, 0.38 and 0.42. It was further separated by column chromatography over silica gel. Elution was conducted initially with 11% ethyl acetate-petroleum ether and gradually enriched with pure ethyl acetate to give ninety one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 17**.

**Table 17** Subfractions obtained from subfraction **C1-4-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
CA1	0.013	Colorless gum
CA2	0.058	Yellow gum
CA3	0.067	Yellow gum

**Subfraction CA1** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (7 runs) showed two pale UV-active spots with the  $R_f$  values of 0.28 and 0.34. No further investigation was carried out.

**Subfraction CA2** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (7 runs) showed one major UV-active spot with the  $R_f$  value of 0.33. It was further separated by column chromatography over silica gel. Elution was conducted initially with 0.5% ethyl acetate-dichloromethane, gradually enriched with pure ethyl acetate and followed by increasing amount of acetone in ethyl acetate and finally with 50% acetone-ethyl acetate to give fifty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 18**.

**Table 18** Subfractions obtained from subfraction **CA2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
CA2-1	0.011	Yellow gum
CA2-2	0.029	Yellow gum
CA2-3	0.008	Yellow gum

**Subfraction CA2-1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed one major UV-active

spot with the  $R_f$  value of 0.50. Because it was obtained in low quantity, it was not investigated.

**Subfraction CA2-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the  $R_f$  value of 0.46. It was further separated by column chromatography over silica gel. Elution was conducted initially with 1% ethyl acetate-dichloromethane, gradually enriched with pure ethyl acetate and followed by increasing amount of acetone in ethyl acetate and finally with pure acetone to give a yellow gum (0.026 g). Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the  $R_f$  value of 0.54. It was further separated by column chromatography over silica gel. Elution was conducted with 8% ethyl acetate-petroleum ether to afford a yellow gum (**GF9**) in 16.0 mg. Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (7 runs) showed one major UV-active spot with the  $R_f$  value of 0.33. Further purification was performed by precoated TLC, using 20% ethyl acetate-petroleum ether as a mobile phase (5 runs), to afford a yellow gum (**GF9**) in 8.2 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed only one yellow spot with the  $R_f$  value of 0.49.

$[\alpha]_D^{29}$	-28° (c = 0.10, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3480 (OH stretching), 2968, 2929, 2856 (CH stretching), 1749, 1682, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	333 (3.62), 303 (4.16)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	12.08 (s, 1H), 9.48 (s, 1H), 7.01 (t, $J$ = 6.5 Hz, 1H), 5.24 (tm, $J$ = 7.0 Hz, 1H), 4.48 (s, 1H), 4.42 (q, $J$ = 6.5 Hz, 1H), 3.52 (s, 3H), 3.40 (s, 3H), 3.21 (m, 2H), 3.09 (s, 1H), 3.04 (dd, $J$ = 16.5 and 6.5 Hz, 1H), 2.96 (dd, $J$ = 16.5 and 6.5 Hz, 1H), 2.73 (d, $J$ = 8.5 Hz, 1H), 2.07 (d, $J$ = 14.5 Hz, 1H), 1.76 (s, 6H), 1.69 (s, 3H), 1.64 (dd, $J$ = 14.5

		and 8.5 Hz, 1H), 1.45 ( <i>s</i> , 3H), 1.42 ( <i>s</i> , 3H), 1.35 ( <i>d</i> , <i>J</i> = 6.5 Hz, 3H), 1.22 ( <i>s</i> , 3H), 1.09 ( <i>s</i> , 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) (δ ppm) (125 MHz)		205.41, 195.05, 191.68, 166.88, 161.72, 152.01, 148.64, 139.79, 132.96, 121.45, 113.60, 105.61, 102.37, 90.15, 87.07, 86.08, 82.21, 81.67, 75.32, 57.72, 52.32, 49.34, 45.26, 43.92, 30.54, 27.89, 27.26, 26.24, 25.79, 23.77, 22.35, 21.44, 17.74, 13.84, 9.38
DEPT (135°) (CDCl <sub>3</sub> )	CH	195.05, 148.64, 121.45, 90.15, 75.32, 49.34, 45.26
	CH <sub>2</sub>	27.89, 23.77, 21.44
	CH <sub>3</sub>	57.72, 52.32, 30.54, 27.26, 26.24, 25.79, 22.35, 17.74, 13.84, 9.38
EIMS ( <i>m/z</i> ) (% rel. int.)		608 (30), 553 (20), 438 (29), 381 (34), 291 (67), 289 (80), 259 (73), 233 (100)

**Subfraction CA2-3** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (3 runs) showed many spots under UV-S. No further investigation was carried out.

**Subfraction CA3** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (7 runs) showed two major UV-active spots with the *R<sub>f</sub>* values of 0.25 and 0.29. It was further separated by column chromatography over silica gel. Elution was conducted initially with 10% ethyl acetate-petroleum ether, gradually enriched with pure ethyl acetate and followed by increasing amount of acetone in ethyl acetate and finally with 50% acetone-ethyl acetate to give sixty nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 19**.

**Table 19** Subfractions obtained from subfraction **CA3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
CA3-1	0.037	Yellow gum
CA3-2	0.027	Brown-yellow gum

**Subfraction CA3-1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (8 runs) showed two major UV-active spots with the  $R_f$  values of 0.54 and 0.58. It was further separated by column chromatography over silica gel. Elution was conducted with 15% ethyl acetate-petroleum ether to give seventy eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 20**.

**Table 20** Subfractions obtained from subfraction **CA3-1** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
CA3-1-1	0.004	Yellow gum
CA3-1-2	0.020	Yellow gum

**Subfraction CA3-1-1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (7 runs) showed no spots under UV-S. It was therefore not investigated.

**Subfraction CA3-1-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (7 runs) showed three major spots: two yellow spots with the  $R_f$  values of 0.49 and 0.53 and one UV-active spot with the  $R_f$  value of 0.56. Further separation by precoated TLC on silica gel plates using 15% ethyl acetate-petroleum ether as a mobile phase (16 runs) afforded three bands of which their chromatograms on normal phase TLC using 15% ethyl acetate-petroleum

ether (8 runs) showed unseparable spots under UV-S. Thus, they were not further purified.

**Subfraction CA3-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (8 runs) showed two major UV-active spots with the  $R_f$  values of 0.54 and 0.58. Further separation by precoated TLC on silica gel plates using 15% ethyl acetate-petroleum ether as a mobile phase (23 runs) afforded two bands of which their chromatograms on normal phase TLC using 10% ethyl acetate-petroleum ether (2 runs) showed unseparable spots under UV-S. Its  $^1\text{H}$  NMR spectrum showed that they contained many compounds. Thus, they were not further purified.

**Subfraction C1-4-3** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (3 runs) showed no definite spot under UV-S. Thus, it was not investigated.

**Subfraction C1-5** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed three major UV-active spots with the  $R_f$  values of 0.06, 0.09 and 0.33. It was further separated by column chromatography over silica gel. Elution was conducted with 15% ethyl acetate-petroleum ether to give thirty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 21**.

**Table 21** Subfractions obtained from subfraction **C1-5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C1-5-1	0.012	Yellow gum
C1-5-2	0.023	Yellow gum

**Subfraction C1-5-1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (8 runs) showed one UV-active spot with the  $R_f$  value of 0.52 and one yellow spot with the  $R_f$  value of 0.48. Further purification was performed by precoated TLC, using 15% ethyl acetate-petroleum

ether as a mobile phase (21 runs), to afford a yellow gum (**GF10**) in 2.4 mg. Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed one yellow spot with the  $R_f$  value of 0.36 which was identical to that of **scortechinone H**, obtained from its latex.

$[\alpha]_D^{29}$	-242° (c = 0.11, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3446 (OH stretching), 2926 (CH stretching), 1744, 1682, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	339 (3.59)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	13.03 (s, 1H), 9.23 (s, 1H), 7.59 (s, 1H), 6.29 (ddm, J = 8.4 and 6.8 Hz, 1H), 5.21 (t, J = 6.4 Hz, 1H), 4.39 (q, J = 6.5 Hz, 1H), 3.63 (s, 3H), 3.20 (m, 2H), 2.90 (dd, J = 14.8 and 5.2 Hz, 1H), 2.64 (d, J = 9.2 Hz, 1H), 2.61 (dd, J = 14.8 and 8.4 Hz, 1H), 2.37 (d, J = 13.0 Hz, 1H), 1.75 (s, 3H), 1.74 (s, 3H), 1.69 (s, 3H), 1.68 (dd, J = 13.0 and 9.2 Hz, 1H), 1.42 (s, 3H), 1.41 (s, 3H), 1.40 (s, 3H), 1.31 (s, 3H), 1.30 (d, J = 6.5 Hz, 3H)

**Subfraction C1-5-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (8 runs) showed one major yellow spot with the  $R_f$  value of 0.48. Further purification was performed by precoated TLC, using 15% ethyl acetate-petroleum ether as a mobile phase (21 runs), to afford a yellow gum in 0.8 mg. Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed at least two components. Because it was obtained in low quantity, it was not further investigated.

**Subfraction C1-6** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed four major UV-active spots with the  $R_f$  values of 0.06, 0.09, 0.23 and 0.33. It was further separated by column chromatography over silica gel. Elution was conducted with 15% ethyl acetate-petroleum ether to give fifty eight fractions. All fractions were examined by TLC,

combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 22**.

**Table 22** Subfractions obtained from subfraction **C1-6** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C1-6-1	0.013	Yellow gum
C1-6-2	0.013	Yellow gum

**Subfraction C1-6-1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (8 runs) showed four major UV-active spots with the  $R_f$  values of 0.32, 0.38, 0.39 and 0.47. Further separation by precoated TLC on silica gel plates using 15% ethyl acetate-petroleum ether as a mobile phase (20 runs) afforded four bands of which their chromatograms on normal phase TLC using 10% ethyl acetate-petroleum ether (2 runs) showed unseparable components under UV-S. Thus, they were not further purified.

**Subfraction C1-6-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (8 runs) showed no definite spot under UV-S. Thus, it was not further investigated.

**Subfraction C1-7** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed three major UV-active spots with the  $R_f$  values of 0.07, 0.12 and 0.33. It was further separated by flash column chromatography over silica gel. Elution was conducted with 10% ethyl acetate-petroleum ether to give sixty one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 23**.



**Table 23** Subfractions obtained from subfraction **C1-7** by flash column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
C1-7-1	0.015	Yellow gum
C1-7-2	0.012	Yellow gum

**Subfraction C1-7-1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.30. Further separation by precoated TLC on silica gel plates using 15% ethyl acetate-petroleum ether as a mobile phase (17 runs) afforded four bands of which their chromatograms on normal phase TLC using 10% ethyl acetate-petroleum ether (2 runs) showed unseparable components under UV-S. Thus, they were not further purified.

**Subfraction C1-7-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (3 runs) showed no definite spot under UV-S. Therefore, it was not further investigated.

**Subfraction C1-8** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed many pale UV-active spots without any major spots. Therefore, it was not further investigated.

**Subfraction C2** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed three major UV-active spots with the  $R_f$  values of 0.46, 0.56 and 0.63. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 15% ethyl acetate-hexane, gradually enriched with pure ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with 20% methanol-ethyl acetate to give forty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 24**.

**Table 24** Subfractions obtained from subfraction **C2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
C2-1	0.051	Yellow gum
C2-2	0.075	Yellow gum

**Subfraction C2-1** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (12 runs) showed one major UV-active spot with the  $R_f$  value of 0.33. Further separation with column chromatography over silica gel was performed. Elution was conducted with 1% ethyl acetate-dichloromethane to give sixty two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford fifteen subfractions. Their chromatograms on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed many compounds without any major components. Thus, they were not further investigated.

**Subfraction C2-2** Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (12 runs) showed two major UV-active spots with the  $R_f$  values of 0.17 and 0.22. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with 1% ethyl acetate-dichloromethane and gradually increased the polarity until pure ethyl acetate to give one hundred and eleven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford twenty one subfractions. Their chromatograms on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed unseparable spots. Thus, they were not further investigated.

**Subfraction C3** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed three major UV-active spots with the  $R_f$  values of 0.23, 0.28 and 0.35. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 75% methanol-water and gradually decreased the polarity until pure methanol to give forty four fractions. All fractions were examined by TLC, combined on the basis of their

chromatogram characteristics and then evaporated to dryness *in vacuo* to afford twelve subfractions. They were not further investigated because their chromatograms on the normal phase TLC using 20% ethyl acetate-petroleum ether showed many UV-active spots without any major spots and they were obtained in low quantity.

**Subfraction A5-1-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed similar chromatogram to that of subfraction **A5-1-1**. Therefore, it was not further investigated.

**Subfraction A5-2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed four major UV-active spots with the  $R_f$  values of 0.04, 0.08, 0.12 and 0.18. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give forty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions. Their chromatogram characteristics on normal phase TLC with 0.5% methanol-chloroform (2 runs) showed many UV-active spots without any major spots. Therefore, it was not further investigated.

**Subfraction A5-3** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether showed none of well-separated spots under UV-S. Attempted purification by column chromatography over reverse-phase  $C_{18}$  silica gel was unsuccessful.

**Fraction GFA6** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) showed one yellow spot which was **scortechinone B** and the other one spot with the  $R_f$  value of 0.50. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with chloroform and gradually increased the polarity until pure methanol to give seventy nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nine subfractions, as shown in **Table 25**.

**Table 25** Subfractions obtained from fraction **GFA6** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A6-1	0.036	Yellow gum
A6-2	0.050	Yellow solid
A6-3	0.056	Yellow gum mixed with solid
A6-4	0.155	Yellow gum
A6-5	0.114	Yellow gum
A6-6	7.502	Yellow gum
A6-7	3.768	Yellow gum
A6-8	0.585	Green-yellow gum
A6-9	0.192	Brown-yellow gum

**Subfraction A6-1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed no spots under UV-S. It was further not investigated.

**Subfraction A6-2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed one yellow spot with the  $R_f$  value of 0.71. Further purification was performed on precoated TLC, using 15% ethyl acetate-petroleum ether (4 runs), to afford four bands. Chromatogram characteristics of each band on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed many UV-active spots and additional purple spots after dipping the TLC plate in ASA reagent and subsequent heating. Therefore, they were not further investigated.

**Subfraction A6-3** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (4 runs) showed two yellow spots with the  $R_f$  values of 0.60 and 0.69 and one UV-active spot with the  $R_f$  value of 0.78. It was further separated by column chromatography over silica gel. Elution was conducted initially with 8% ethyl acetate-petroleum ether and gradually enriched with pure ethyl acetate to give a yellow solid (0.052 g). Chromatogram characteristics on normal phase TLC with 12% ethyl acetate-petroleum ether (4 runs) showed two yellow spots with the  $R_f$  values of 0.46 and 0.54 and one UV-active spot with the  $R_f$  value of 0.21.

Further purification was performed on precoated TLC, using 15% ethyl acetate-petroleum ether (10 runs), to afford four bands. Chromatogram of each band on normal phase TLC using 15% ethyl acetate-petroleum ether showed at least two components. Because they were obtained in low quantity, they were not further investigated.

**Subfraction A6-4** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed six yellow spots with the  $R_f$  values of 0.28, 0.43, 0.51, 0.55, 0.60 and 0.69. It was further separated by column chromatography over silica gel. Elution was conducted initially with 8% ethyl acetate-petroleum ether and gradually enriched with pure ethyl acetate to give eighty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 26**.

**Table 26** Subfractions obtained from subfraction **A6-4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A6-4-1	0.020	Yellow gum
A6-4-2	0.032	Yellow gum
A6-4-3	0.026	Orange-yellow gum
A6-4-4	0.028	Orange-yellow gum
A6-4-5	0.047	Green gum

**Subfraction A6-4-1** Chromatogram characteristics on normal phase TLC with 12% ethyl acetate-petroleum ether showed four yellow spots with the  $R_f$  values of 0.47, 0.56, 0.61 and 0.67. Because it was obtained in low quantity, it was not further purified.

**Subfraction A6-4-2** Chromatogram characteristics on normal phase TLC with 12% ethyl acetate-petroleum ether showed three yellow spots with the  $R_f$  values of 0.27, 0.31 and 0.39. It was further separated by column chromatography over silica gel. Elution was conducted initially with 15% ethyl acetate-petroleum ether

and gradually enriched with pure acetone to give fifty five fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions. Their chromatograms on normal phase TLC using 15% ethyl acetate-petroleum ether showed unseparable components under UV-S. Therefore, they were not further purified.

**Subfraction A6-4-3** Chromatogram characteristics on normal phase TLC with 12% ethyl acetate-petroleum ether showed three yellow spots with the  $R_f$  values of 0.16, 0.23 and 0.31. Further purification was performed on precoated TLC, using 15% ethyl acetate-petroleum ether (16 runs), to afford five bands. Chromatogram of each band on normal phase TLC using 15% ethyl acetate-petroleum ether showed at least two components. Because they were obtained in low quantity, they were not further investigated.

**Subfraction A6-4-4** Chromatogram characteristics on normal phase TLC with 12% ethyl acetate-petroleum ether showed one yellow spot with the  $R_f$  value of 0.16. Further purification was performed on precoated TLC, using 15% ethyl acetate-petroleum ether (16 runs), to afford two bands. Further purification of these bands by precoated TLC using 50% chloroform-petroleum ether (14 runs) was unsuccessful.

**Subfraction A6-4-5** Chromatogram characteristics on normal phase TLC with 12% ethyl acetate-petroleum ether showed two major yellow spots with the  $R_f$  values of 0.33 and 0.52. Further purification was performed on precoated TLC, using 40% ethyl acetate-petroleum ether (8 runs), to afford two bands. Their  $^1\text{H}$  NMR spectra indicated that they contained many compounds. Thus, they were not further investigated.

**Subfraction A6-5** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed one major yellow spot which was **scortechinone B**. It was not further purified.

**Subfraction A6-6** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed two major spots which were **scortechinones B** and **I**, obtained from its latex. It was not further purified.

**Subfraction A6-7** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one major yellow spot with the  $R_f$  value of 0.28. It was further separated by column chromatography over silica gel. Elution was conducted initially with 1% methanol-chloroform and gradually increased the polarity until 50% methanol-chloroform to give eighty one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 27**.

**Table 27** Subfractions obtained from subfraction **A6-7** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A6-7-1	2.137	Yellow gum
A6-7-2	0.700	Green-yellow gum
A6-7-3	0.439	Green-yellow gum
A6-7-4	0.202	Green-yellow gum
A6-7-5	0.212	Brown-yellow gum

**Subfraction A6-7-1** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed two major spots which were **scortechinones B** and **I**. Thus, it was not further investigated.

**Subfraction A6-7-2** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one major spot which were **scortechinone I**. Thus, it was not further separated.

**Subfraction A6-7-3** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.51 and two minor yellow spots with the  $R_f$  values of 0.20 and 0.32. It was further separated by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol to give fifty five fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 28**.

**Table 28** Subfractions obtained from subfraction **A6-7-3** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
D1	0.110	Yellow gum
D2	0.056	Yellow gum
D3	0.092	Yellow gum
D4	0.062	Yellow gum
D5	0.020	Yellow gum

**Subfraction D1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed three major yellow spots with the  $R_f$  values of 0.18, 0.23 and 0.45. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give seventy one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 29**.

**Table 29** Subfractions obtained from subfraction **D1** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
D1-1	0.021	Yellow gum
D1-2	0.045	Yellow gum
D1-3	0.050	Yellow-green gum

**Subfraction D1-1** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed none of well-separated spots under UV-S. But the chromatogram on reverse-phase TLC using 60% methanol-water (2 runs) showed one yellow major spot with the  $R_f$  value of 0.25. Further separation by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted



initially with 60% methanol-water and gradually decreased the polarity until pure methanol to give four subfractions which were obtained in low quantity. Their chromatograms on normal phase TLC, using 2% methanol-chloroform (3 runs), showed many UV-active spots. Therefore, they were not further investigated.

**Subfraction D1-2** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.19 and 0.27. But its chromatogram on reverse-phase TLC with 60% methanol-water (2 runs) showed many spots under UV-S. Thus, it was not further purified.

**Subfraction D1-3** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed many UV-active spots without any major spots. Thus, it was not further purified.

**Subfraction D2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed two major UV-active spots with the  $R_f$  values of 0.23 and 0.49. It was further separated by column chromatography over silica gel. Elution was conducted initially with 30% ethyl acetate-petroleum ether, gradually enriched with pure ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with 10% methanol-ethyl acetate to give fifty five fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 30**.

**Table 30** Subfractions obtained from subfraction **D2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D2-1	0.012	Yellow gum
D2-2	0.005	Yellow gum
D2-3	0.026	Yellow gum

**Subfraction D2-1** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether showed two major UV-active spots with

the  $R_f$  values of 0.42 and 0.55. Further purification was performed on precoated TLC, using 30% ethyl acetate-petroleum ether (9 runs), to afford a yellow gum in 5.4 mg (**GF11**). Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (5 runs) showed only one UV-active spot with the  $R_f$  value of 0.20 which was identical to **scortechinone I**.

$[\alpha]_D^{29}$	+8° (c = 0.22, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3600-2500 (OH stretching), 2972, 2922 (CH stretching), 1751, 1687, 1634 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	303 (4.06)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (400 MHz)	12.07 (s, 1H), 6.59 (tm, $J = 7.0$ Hz, 1H), 5.23 (tm, $J = 7.5$ Hz, 1H), 4.47 (d, $J = 1.0$ Hz, 1H), 4.40 (q, $J = 6.5$ Hz, 1H), 3.50 (s, 3H), 3.37 (s, 3H), 3.21 (m, 2H), 3.20 (ddm, $J = 17.5$ and 7.0 Hz, 1H), 3.16 (brs, 1H), 3.11 (ddm, $J = 17.5$ and 7.0 Hz, 1H), 2.70 (d, $J = 9.0$ Hz, 1H), 2.01 (d, $J = 14.0$ Hz, 1H), 1.97 (s, 3H), 1.76 (s, 3H), 1.68 (s, 3H), 1.63 (dd, $J = 14.5$ and 9.0 Hz, 1H), 1.44 (s, 3H), 1.42 (s, 3H), 1.34 (d, $J = 6.5$ Hz, 3H), 1.21 (s, 3H), 1.11 (s, 3H)

**Subfraction D2-2** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether showed one major spot which was **scortechinone I**.

**Subfraction D2-3** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether showed five major UV-active spots with the  $R_f$  values of 0.13, 0.16, 0.24, 0.33 and 0.38. It was further separated by flash column chromatography. Elution was conducted initially with 0.5% methanol-chloroform and gradually increased the polarity until 10% methanol-chloroform to give sixty one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to

afford a yellow gum (0.014 g). Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed four UV-active spots with the  $R_f$  values of 0.37, 0.53, 0.65 and 0.70. Further purification was performed on precoated TLC, using 40% ethyl acetate-petroleum ether as a mobile phase (9 runs), to afford two bands. Their chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (9 runs) showed many unseparable UV-active spots. Because they were obtained in low quantity, they were not further investigated.

**Subfraction D3** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed three major UV-active spots with the  $R_f$  values of 0.23, 0.39 and 0.49. It was further separated by flash column chromatography over silica gel. Elution was conducted initially with 0.5% methanol-chloroform and gradually increased the polarity until 10% methanol-chloroform to give sixty one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 31**.

**Table 31** Subfractions obtained from subfraction **D3** by flash column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D3-1	0.063	Yellow gum
D3-2	0.015	Yellow gum

**Subfraction D3-1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) showed three major spots with the  $R_f$  values of 0.34, 0.44 and 0.55. It was further separated by column chromatography. Elution was conducted initially with 0.5% methanol-chloroform and gradually increased the polarity until 50% methanol-chloroform to give fourteen fractions. Their chromatograms on normal phase TLC using 30% ethyl acetate-petroleum ether showed many UV-active spots. Therefore, they were not further investigated.

**Subfraction D3-2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) showed four major spots with the  $R_f$  values of 0.14, 0.24, 0.33 and 0.43. Thus, it was not further investigated.

**Subfraction D4** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the  $R_f$  value of 0.49. It was further separated by column chromatography over silica gel. Elution was conducted initially with 1% methanol-chloroform and gradually increased the polarity until 50% methanol-chloroform to give forty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 32**.

**Table 32** Subfractions obtained from subfraction **D4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D4-1	0.033	Yellow gum
D4-2	0.017	Yellow gum

**Subfraction D4-1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed one major spot with the  $R_f$  value of 0.36. It was further separated by column chromatography. Elution was conducted initially with 0.5% methanol-chloroform and gradually increased the polarity until 50% methanol-chloroform to give eight three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford a yellow gum (0.022 g). Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed two major spots with the  $R_f$  values of 0.18 and 0.36. Further purification was performed on precoated TLC, using 30% ethyl acetate-petroleum ether as a mobile phase (10 runs), to afford two bands.

**Band 1** was obtained as a yellow gum in 4.5 mg. Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-

petroleum ether (3 runs) showed one yellow spot which was **scortechinone I**, obtained from its stem bark.

**Band 2 (GF12)** was obtained as a yellow gum in 3.4 mg. Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed one yellow spot which was **scortechinone M**, obtained from its stem bark.

$[\alpha]_D^{29}$	-376° (c = 0.15, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3600-2500 (OH stretching), 2974, 2928 (CH stretching), 1744, 1689, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	364 (3.83)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (400 MHz)	13.26 ( <i>s</i> , 1H), 7.51 ( <i>brs</i> , 1H), 5.37 ( <i>dm</i> , <i>J</i> = 9.0 Hz, 1H), 5.06 ( <i>brs</i> , 1H), 4.90 ( <i>brs</i> , 1H), 4.55 ( <i>q</i> , <i>J</i> = 6.4 Hz, 1H), 4.51 ( <i>d</i> , <i>J</i> = 10.8 Hz, 1H), 3.63 ( <i>s</i> , 3H), 3.57 ( <i>dd</i> , <i>J</i> = 15.0 and 11.5 Hz, 1H), 2.96 ( <i>dd</i> , <i>J</i> = 14.4 and 10.8 Hz, 1H), 2.73 ( <i>m</i> , 1H), 2.71 ( <i>m</i> , 1H), 2.63 ( <i>d</i> , <i>J</i> = 9.6 Hz, 1H), 2.31 ( <i>d</i> , <i>J</i> = 13.0 Hz, 1H), 1.86 ( <i>s</i> , 3H), 1.72 ( <i>m</i> , 1H), 1.72 ( <i>s</i> , 3H), 1.67 ( <i>s</i> , 3H), 1.49 ( <i>s</i> , 3H), 1.40 ( <i>s</i> , 3H), 1.39 ( <i>d</i> , <i>J</i> = 6.4 Hz, 3H), 1.28 ( <i>s</i> , 3H)

**Subfraction D4-2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed two major spots with the  $R_f$  values of 0.18 and 0.36. Further purification was performed on precoated TLC, using 25% ethyl acetate-petroleum ether as a mobile phase (14 runs), to afford two bands.

**Band 1** was obtained as a yellow gum in 1.2 mg. Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) showed at least two UV-active compounds. Because it was obtained in low quantity, it was not further investigated.

**Band 2 (GF13)** was obtained as a yellow gum in 1.6 mg. Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) showed one yellow spot with the same  $R_f$  value as **scortechinone F**, obtained from its latex.

$[\alpha]_D^{29}$	-102° (c = 0.11, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3500-2500 (OH stretching), 2974, 2928 (CH stretching), 1745, 1693, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	361 (3.84)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	13.09 ( <i>s</i> , 1H), 7.60 ( <i>brs</i> , 1H), 6.38 ( <i>ddq</i> , <i>J</i> = 10.0, 5.5 and 1.5 Hz, 1H), 5.21 ( <i>tm</i> , <i>J</i> = 7.0 Hz, 1H), 4.55 ( <i>q</i> , <i>J</i> = 6.5 Hz, 1H), 3.63 ( <i>s</i> , 3H), 3.21 ( <i>m</i> , 2H), 2.80 ( <i>ddm</i> , <i>J</i> = 15.0 and 5.5 Hz, 1H), 2.61 ( <i>d</i> , <i>J</i> = 9.5 Hz, 1H), 2.56 ( <i>dd</i> , <i>J</i> = 15.0 and 10.0 Hz, 1H), 2.34 ( <i>d</i> , <i>J</i> = 13.0 Hz, 1H), 1.74 ( <i>s</i> , 3H), 1.72 ( <i>s</i> , 3H), 1.69 ( <i>dd</i> , <i>J</i> = 13.0 and 9.5 Hz, 1H), 1.68 ( <i>s</i> , 3H), 1.47 ( <i>s</i> , 3H), 1.41 ( <i>s</i> , 3H), 1.38 ( <i>s</i> , 3H), 1.30 ( <i>d</i> , <i>J</i> = 6.5 Hz, 3H), 1.29 ( <i>s</i> , 3H)

**Subfraction D5** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed one pale UV-active spot with the  $R_f$  value of 0.42. Therefore, it was not further purified.

**Subfraction A6-7-4** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed four major UV-active spots with the  $R_f$  values of 0.10, 0.20, 0.32 and 0.51. It was further separated by column chromatography. Elution was conducted initially with 2% methanol-chloroform and gradually increased the polarity until 50% methanol-chloroform to give fifty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 33**.

**Table 33** Subfractions obtained from subfraction **A6-7-4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
E1	0.009	Yellow gum
E2	0.019	Yellow gum
E3	0.133	Brown-yellow gum

**Subfraction E1** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed one major yellow spot with the  $R_f$  value of 0.52. Further purification was performed on precoated TLC, using 40% ethyl acetate-petroleum ether as a mobile phase (10 runs), to afford a yellow gum in 3.5 mg (**GF14**). Its chromatogram on normal phase TLC with 40% ethyl acetate-petroleum ether showed only one spot with the same  $R_f$  value as **scortechinone C**, obtained from its twigs.

$[\alpha]_D^{29}$	-154° (c = 0.16, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3500-2500 (OH stretching), 2966, 2928 (CH stretching), 1744, 1693, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	364 (3.91)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	13.14 (s, 1H), 7.54 (d, $J$ = 1.0 Hz, 1H), 5.20 (dm, $J$ = 11.5 Hz, 1H), 5.08 (brs, 1H), 4.92 (brs, 1H), 4.57 (q, $J$ = 7.0 Hz, 1H), 4.32 (dm, $J$ = 11.0 Hz, 1H), 3.82 (dd, $J$ = 15.0 and 11.5 Hz, 1H), 3.65 (s, 3H), 2.98 (dd, $J$ = 14.0 and 3.5 Hz, 1H), 2.71 (dm, $J$ = 15.0 Hz, 1H), 2.65 (dd, $J$ = 14.0 and 11.0 Hz, 1H), 2.64 (d, $J$ = 9.5 Hz, 1H), 2.34 (d, $J$ = 13.0, 1H), 1.87 (s, 3H), 1.72 (dd, $J$ = 13.0 and 9.5 Hz, 1H), 1.71 (s, 3H), 1.63 (m, 3H), 1.56 (s, 3H), 1.44 (d, $J$ = 7.0 Hz, 3H), 1.38 (s, 3H), 1.29 (s, 3H)

**Subfraction E2** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed many spots without any major spots under UV-S. Therefore, it was not investigated.

**Subfraction E3** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed one major yellow spot with the  $R_f$  value of 0.27. Further separation on column chromatography was performed. Elution was conducted with 5% methanol-chloroform to give thirty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 34**.

**Table 34** Subfractions obtained from subfraction **E3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
E3-1	0.073	Yellow gum
E3-2	0.025	Yellow gum

**Subfraction E3-1** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed one major yellow spot with the  $R_f$  value of 0.28. Further separation on column chromatography was performed. Elution was conducted initially with 40% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with pure methanol to give seventy one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions. The chromatogram on normal phase TLC with 40% ethyl acetate-petroleum ether (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.26. Attempted purification by repeated chromatography was unsuccessful.

**Subfraction E3-2** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed one major yellow spot with the  $R_f$  value of 0.27. It was further separated by column chromatography. Elution



was conducted initially with 10% acetone-chloroform, gradually enriched with acetone and followed by increasing amount of methanol in acetone and finally with 10% methanol-acetone to give seventy four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nine subfractions. Their chromatograms on normal phase TLC with 10% acetone-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.34. Attempted purification by repeated chromatography was not successful.

**Subfraction A6-7-5** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.21 and 0.37. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give seventy eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions. Their chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed many spots without any major spots under UV-S. Thus, it was not further investigated.

**Subfraction A6-8** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one major yellow spot with the  $R_f$  value of 0.07. It was further separated by column chromatography over reversed-phase  $C_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give twenty seven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 35**.

**Table 35** Subfractions obtained from subfraction **A6-8** by column chromatography over reverse-phase C<sub>18</sub> silica gel

Subfraction	Weight (g)	Physical appearance
A6-8-1	0.192	Yellow gum
A6-8-2	0.108	Yellow gum
A6-8-3	0.035	Yellow solid
A6-8-4	0.148	Brown-yellow solid

**Subfraction A6-8-1**, upon standing at room temperature, afforded a yellow solid in 5.2 mg and a yellow gum in 187.0 mg (**SolA6-8-1**). Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one major yellow spot with the R<sub>f</sub> value of 0.07. It was further separated by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol to give fifty seven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford a yellow gum (0.181 g). Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed four major UV-active spots with the R<sub>f</sub> values of 0.07, 0.12, 0.24 and 0.30. It was further separated by column chromatography over reverse-phase C<sub>18</sub> silica gel. Elution was conducted initially with 60% methanol-water and gradually decreased the polarity until pure methanol to give fifty two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 36**.

**Table 36** Subfractions obtained from subfraction **G2** by column chromatography over reverse-phase C<sub>18</sub> silica gel

Subfraction	Weight (g)	Physical appearance
G2-1	0.026	Yellow gum
G2-2	0.035	Yellow gum
G2-3	0.120	Yellow gum

**Subfraction G2-1** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed none of well-separated spots under UV-S. But the chromatogram on reverse-phase TLC with 70% methanol-water showed three major yellow spots with the R<sub>f</sub> values of 0.51, 0.63 and 0.69. It was further separated by column chromatography over reverse-phase C<sub>18</sub> silica gel. Elution was conducted initially with 60% methanol-water and gradually decreased the polarity until pure methanol to give twenty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford seven subfractions of which chromatograms showed many spots without any major spots. No further investigation was performed.

**Subfraction G2-2** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed none of well-separated spots under UV-S. But the chromatogram on reverse-phase TLC with 70% methanol-water showed two major yellow spots with the R<sub>f</sub> values of 0.29 and 0.42. It was further separated by column chromatography over reverse-phase C<sub>18</sub> silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give forty four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions of which chromatograms showed many components. No further investigation was performed.

**Subfraction G2-3** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed four major yellow spots with the R<sub>f</sub> values of 0.12, 0.23, 0.41 and 0.50. It was further separated by column chromatography over silica gel. Elution was conducted initially with 5% methanol-

chloroform and gradually increased the polarity until 30% methanol-chloroform to give fifty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions of which chromatograms showed many spots without any major spots. Attempted purification by column chromatography over reverse-phase C<sub>18</sub> silica gel was unsuccessful.

**Subfraction A6-8-2** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed four major yellow spots with the R<sub>f</sub> values of 0.09, 0.16, 0.20 and 0.25. It was further separated by column chromatography over silica gel. Elution was conducted initially with 1% methanol-chloroform and gradually increased the polarity until 50% methanol-chloroform to give fifty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 37**.

**Table 37** Subfractions obtained from subfraction **A6-8-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
H1	0.017	Yellow gum
H2	0.060	Yellow gum

**Subfraction H1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed two major yellow spots with the R<sub>f</sub> values of 0.34 and 0.47. Further purification was performed by precoated TLC, using 25% ethyl acetate-petroleum ether as a mobile phase (13 runs), to afford two bands.

**Band 1** was obtained as a yellow gum in 4.4 mg. Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed one yellow spot with the same R<sub>f</sub> value as **scortechinone M**, obtained from its stem bark.

**Band 2 (GF15)** was obtained as a yellow gum in 2.3 mg. Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed only one yellow spot with the  $R_f$  value of 0.24.

$[\alpha]_D^{29}$	-58° (c = 0.04, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3500-2500 (OH stretching), 2922, 2848 (CH stretching), 1745, 1690, 1631 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	365 (4.01), 270 (4.52)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	13.26 ( <i>s</i> , 1H), 7.52 ( <i>d</i> , $J$ = 1.0 Hz, 1H), 5.40 ( <i>dm</i> , $J$ = 11.0 Hz, 1H), 5.06 ( <i>brs</i> , 1H), 4.89 ( <i>brs</i> , 1H), 4.53 ( <i>q</i> , $J$ = 6.5 Hz, 1H), 4.51 ( <i>dd</i> , $J$ = 10.5 and 3.5 Hz, 1H), 3.64 ( <i>s</i> , 3H), 3.57 ( <i>dd</i> , $J$ = 15.5 and 11.0 Hz, 1H), 2.93 ( <i>dd</i> , $J$ = 14.5 and 10.5 Hz, 1H), 2.78 ( <i>dm</i> , $J$ = 15.5 Hz, 1H), 2.71 ( <i>dd</i> , $J$ = 14.5 and 3.5 Hz, 1H), 2.62 ( <i>d</i> , $J$ = 9.5 Hz, 1H), 2.32 ( <i>d</i> , $J$ = 13.5 Hz, 1H), 1.85 ( <i>s</i> , 3H), 1.72 ( <i>dd</i> , $J$ = 13.5 and 9.5 Hz, 1H), 1.71 ( <i>s</i> , 3H), 1.67 ( <i>t</i> , $J$ = 1.5 Hz, 3H), 1.57 ( <i>s</i> , 3H), 1.39 ( <i>d</i> , $J$ = 6.5 Hz, 3H), 1.29 ( <i>s</i> , 3H), 1.15 ( <i>s</i> , 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (125 MHz)	203.15, 177.91, 168.29, 167.85, 164.06, 154.42, 147.12, 135.69, 134.75, 132.42, 129.43, 113.78, 110.62, 102.31, 101.33, 91.07, 89.08, 85.11, 84.03, 83.62, 74.82, 53.88, 49.62, 43.12, 30.72, 30.56, 28.91, 28.72, 28.31, 23.63, 21.23, 21.13, 18.33, 13.70
DEPT (135°) (CDCl <sub>3</sub> )	CH 135.69, 134.75, 91.07, 74.82, 49.62 CH <sub>2</sub> 110.62, 30.56, 28.91, 28.31 CH <sub>3</sub> 53.88, 30.72, 28.72, 23.63, 21.23, 21.13, 18.33, 13.70
EIMS ( $m/z$ ) (% rel. int.)	608 (4), 580 (30), 536 (27), 509 (100), 473 (32),

436 (33), 383 (43), 243 (38), 233 (52)

**Subfraction H2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the  $R_f$  value of 0.23. Attempted purification by column chromatography on silica gel and precoated TLC was unsuccessful.

**Subfraction A6-8-3** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed three major yellow spots with the  $R_f$  values of 0.09, 0.17 and 0.27. Further purification was performed by precoated TLC, using 25% ethyl acetate-petroleum ether as a mobile phase (8 runs), to afford three bands.

**Band 1 (GF16)** was obtained as a yellow gum in 3.3 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one yellow spot with the same  $R_f$  value as **scortechinone B**, obtained from its twigs.

$[\alpha]_D^{29}$	-226° (c = 1.05, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3500-2500 (OH stretching), 2977, 2929 (CH stretching), 1744, 1689, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	363 (4.03)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	13.14 (s, 1H), 7.57 (d, $J$ = 1.0 Hz, 1H), 5.65 (ddq, $J$ = 10.0, 5.0 and 1.5 Hz, 1H), 5.25 (tm, $J$ = 7.0 Hz, 1H), 4.52 (q, $J$ = 6.5 Hz, 1H), 3.63 (s, 3H), 3.26 (dd, $J$ = 16.0 and 10.0 Hz, 1H), 3.18 (d, $J$ = 7.0 Hz, 2H), 2.82 (dm, $J$ = 16.0 Hz, 1H), 2.61 (d, $J$ = 9.0, 1H), 2.33 (d, $J$ = 13.0 Hz, 1H), 1.75 (s, 3H), 1.72 (s, 6H), 1.69 (dd, $J$ = 13.0 and 9.0 Hz, 1H), 1.68 (s, 3H), 1.38 (s, 6H), 1.30 (d, $J$ = 6.5 Hz, 3H), 1.29 (s, 3H),

**Band 2** was obtained as a yellow gum in 5.7 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one major yellow spot with the  $R_f$  value of 0.24 which was **scortechinone F**, obtained from its stem bark.

**Band 3 (GF17)** was obtained as a yellow gum in 9.8 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed one yellow spot with the  $R_f$  value of 0.09. Its  $^1\text{H}$  NMR spectrum indicated that it was **scortechinone P**, obtained from its stem bark.

$[\alpha]_D^{29}$	+69° (c = 0.27, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3500-2500 (OH stretching), 2974, 2930 (CH stretching), 1749, 1690, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	304 (4.04)
$^1\text{H}$ NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	12.08 (s, 1H), 6.64 (tm, $J = 7.5$ Hz, 1H), 5.21 (tm, $J = 7.0$ Hz, 1H), 4.82 (s, 1H), 4.40 (q, $J = 6.6$ Hz, 1H), 3.48 (s, 3H), 3.20 (m, 2H), 3.19 (m, 2H), 3.19 (s, 1H), 2.71 (d, $J = 8.4$ Hz, 1H), 2.08 (d, $J = 14.4$ Hz, 1H), 1.97 (s, 3H), 1.76 (s, 3H), 1.69 (s, 3H), 1.57 (dd, $J = 14.4$ and 8.4 Hz, 1H), 1.43 (s, 6H), 1.42 (s, 3H), 1.34 (d, $J = 6.6$ Hz, 3H), 1.22 (s, 3H), 1.10 (s, 3H)

**Subfraction A6-8-4** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed many spots under UV-S. It was further separated by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol to give forty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions of which their chromatograms on normal phase TLC using 2% methanol-chloroform (3 runs) showed unseparable spots under UV-S. Thus, they were not further purified.

**Subfraction A6-9** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether showed two major yellow spots with the  $R_f$  values of 0.08 and 0.16. It was further separated by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol to give fifty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions. Their chromatograms on normal phase TLC with 5% methanol-chloroform (2 runs) showed many spots. Attempted purification by column chromatography over reverse-phase  $C_{18}$  silica gel or precoated TLC was not successful.

**Fraction GFA7** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (2 runs) showed four major spots; two yellow spots with the  $R_f$  values of 0.20 and 0.62 and two UV-active spots with the  $R_f$  values of 0.35 and 0.49. It was further separated by column chromatography on Sephadex LH-20. Elution was conducted with pure methanol to give seventy six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions, as shown in **Table 38**.

**Table 38** Subfractions obtained from fraction **GFA7** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
A7-1	0.170	Brown yellow gum
A7-2	0.899	Yellow gum
A7-3	0.250	Yellow gum
A7-4	0.033	Yellow gum
A7-5	0.026	Green yellow gum
A7-6	0.024	Green gum

**Subfraction A7-1** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (2 runs) showed none of well-separated spots under UV-S. Therefore, it was not further investigated.



**Subfraction A7-2** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (2 runs) showed three major yellow spots with the  $R_f$  values of 0.15, 0.36 and 0.44. It was further separated by column chromatography on Sephadex LH-20. Elution was conducted with pure methanol to give thirty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 39**.

**Table 39** Subfractions obtained from subfraction **A7-2** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
A7-2-1	0.154	Yellow gum
A7-2-2	0.480	Yellow gum
A7-2-3	0.256	Yellow gum

**Subfraction A7-2-1** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (2 runs) showed many UV-active spots without any major spots. Therefore, it was not further investigated.

**Subfraction A7-2-2** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (2 runs) showed three major yellow spots with the  $R_f$  values of 0.22, 0.39 and 0.46. Further separation on column chromatography over silica gel was performed. Elution was conducted initially with 0.5% methanol-chloroform and gradually increased the polarity until 50% methanol-chloroform to give fifty seven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions, as shown in **Table 40**.

**Table 40** Subfractions obtained from subfraction **A7-2-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
I1	0.140	Yellow gum
I2	0.071	Yellow gum
I3	0.052	Yellow gum
I4	0.166	Yellow gum
I5	0.052	Yellow gum
I6	0.007	Yellow gum

**Subfraction I1** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed none of well-separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction I2** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed two yellow spots with the  $R_f$  values of 0.41 and 0.65 and one pale UV-active spot with the  $R_f$  value of 0.72. It was further separated by column chromatography on silica gel. Elution was conducted initially with 30% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with 50% methanol-ethyl acetate to fifty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 41**.

**Table 41** Subfractions obtained from subfraction **I2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
I2-1	0.008	White solid mixed with yellow gum
I2-2	0.047	White solid mixed with yellow gum
I2-3	0.003	Yellow gum
I2-4	0.010	Yellow gum
I2-5	0.002	Yellow gum

**Subfraction I2-1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed one major spot with the  $R_f$  value of 0.25 which appeared as a violet spot after dipping the TLC plate in ASA reagent and subsequent heating. Because it was obtained in low quantity, it was not further investigated.

**Subfraction I2-2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed one major spot with the  $R_f$  value of 0.27 which appeared as a violet spot after dipping the TLC plate in ASA reagent and subsequent heating. The  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Therefore, it was not further investigated.

**Subfraction I2-3** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed one major spot with the  $R_f$  value of 0.21 which appeared as a violet spot after dipping the TLC plate in ASA reagent and subsequent heating. Because it was obtained in low quantity, it was not further investigated.

**Subfraction I2-4** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed one major yellow spot with the same  $R_f$  value as **scortechinone C**, obtained from its twigs.

**Subfraction I2-5** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed two major spots which were **scortechinones C** and **M**, obtained from its latex and stem bark.

**Subfraction I3** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed three major UV-active spots with the  $R_f$  values of 0.56, 0.65 and 0.72 which were similar to those found in subfraction **I2**. Therefore, it was not further investigated.

**Subfraction I4** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed four major UV-active spots with the  $R_f$  values of 0.37, 0.51, 0.59 and 0.65. It was further separated by column chromatography on silica gel. Elution was conducted initially with 40% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with 40% methanol-ethyl acetate to forty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford seven subfractions, as shown in **Table 42**.

**Table 42** Subfractions obtained from subfraction **I4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
I4-1	0.016	White solid mixed with yellow gum
I4-2	0.022	Yellow gum
I4-3	0.018	Yellow gum
I4-4	0.017	Yellow gum
I4-5	0.010	Yellow gum
I4-6	0.076	Yellow gum
I4-7	0.011	Yellow gum

**Subfraction I4-1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed two major spots with the  $R_f$  values of 0.26 and 0.35 which appeared as a violet spot after dipping the TLC plate in ASA reagent and subsequent heating. Because it was obtained in low quantity, it was not further investigated.

**Subfraction I4-2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed two pale UV-active spots with the  $R_f$  values of 0.52 and 0.63. One additional violet spot with the  $R_f$  value of 0.26 was observed after dipping the TLC plate in ASA reagent and subsequent heating. Because it was obtained in low quantity, it was not further investigated.

**Subfraction I4-3** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed two pale UV-active spots with the  $R_f$  values of 0.37 and 0.50. Because it was obtained in low quantity, it was not further investigated.

**Subfraction I4-4** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed two major UV-active spots with the  $R_f$  values of 0.26 and 0.37. Further purification was performed by precoated TLC, using 30% ethyl acetate-petroleum ether as a mobile phase (12 runs), to give a yellow gum (0.003 g) of which the chromatogram on normal phase TLC using 30% ethyl acetate-petroleum ether showed one UV-active spot with the  $R_f$  value of 0.22. Its  $^1\text{H}$  NMR spectrum showed that it contained many compounds. Thus, it was not purified because of low quantity.

**Subfraction I4-5** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed none of well-separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction I4-6** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed two major UV-active spots with the  $R_f$  values of 0.26 and 0.35. Further separation on column chromatography over silica gel was performed. Elution was conducted initially with 0.5% methanol-chloroform and gradually increased the polarity until 30% methanol-chloroform to give eighty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 43**.

**Table 43** Subfractions obtained from subfraction **I4-6** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
I4-6-1	0.013	White solid mixed with yellow gum
I4-6-2	0.014	Yellow gum
I4-6-3	0.006	Yellow gum
I4-6-4	0.028	Yellow gum
I4-6-5	0.004	Yellow gum

**Subfraction I4-6-1** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed many pale spots under UV-S. Therefore, it was not further investigated.

**Subfraction I4-6-2** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed three major UV-active spots with the  $R_f$  values of 0.38, 0.62 and 0.71. Further purification was performed by precoated TLC, using 10% acetone-chloroform as a mobile phase (6 runs), to afford three bands of which chromatograms showed one major spot. However, the  $^1\text{H}$  NMR spectra indicated that they contained many compounds. Since they were obtained in low quantity, no further investigation was performed.

**Subfraction I4-6-3** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.38 and 0.62. Further purification was performed by precoated TLC, using 10% acetone-chloroform as a mobile phase (6 runs), to afford a yellow gum in 2.5 mg (**GF18**) of which chromatogram showed one UV-active spot with the  $R_f$  value of 0.39.

$[\alpha]_D^{29}$	-39° (c = 0.08, CH <sub>3</sub> OH)
IR (neat) $\nu_{\text{max}}$ (cm <sup>-1</sup> )	3600-2500 (OH stretching), 2974, 2930 (CH stretching), 1749, 1693, 1633 (C=O stretching)

UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	338 (3.48), 304 (3.97)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	12.27 ( <i>s</i> , 1H), 6.59 ( <i>tm</i> , <i>J</i> = 7.0 Hz, 1H), 4.99 ( <i>s</i> , 1H), 4.84 ( <i>s</i> , 1H), 4.46 ( <i>s</i> , 1H), 4.41 ( <i>q</i> , <i>J</i> = 6.3 Hz, 1H), 4.27 ( <i>dd</i> , <i>J</i> = 9.0 and 4.0 Hz, 1H), 3.49 ( <i>s</i> , 3H), 3.39 ( <i>s</i> , 3H), 3.24 ( <i>ddm</i> , <i>J</i> = 16.0 and 7.0 Hz, 1H), 3.20 ( <i>s</i> , 1H), 3.12 ( <i>ddm</i> , <i>J</i> = 16.0 and 7.0 Hz, 1H), 2.90 ( <i>dd</i> , <i>J</i> = 14.0 and 4.0 Hz, 1H), 2.77 ( <i>dd</i> , <i>J</i> = 14.0 and 9.0 Hz, 1H), 2.72 ( <i>d</i> , <i>J</i> = 8.5 Hz, 1H), 2.04 ( <i>d</i> , <i>J</i> = 14.0 Hz, 1H), 1.97 ( <i>d</i> , <i>J</i> = 1.5 Hz, 3H), 1.84 ( <i>s</i> , 3H), 1.64 ( <i>dd</i> , <i>J</i> = 14.0 and 8.5 Hz, 1H), 1.44 ( <i>s</i> , 3H), 1.43 ( <i>s</i> , 3H), 1.34 ( <i>d</i> , <i>J</i> = 6.3 Hz, 3H), 1.22 ( <i>s</i> , 3H), 1.12 ( <i>s</i> , 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (125 MHz)	205.37, 192.19, 170.39, 167.37, 162.00, 152.71, 147.36, 137.29, 128.39, 113.90, 110.37, 102.62, 102.37, 90.56, 87.14, 86.46, 82.81, 81.43, 75.48, 75.12, 57.51, 52.42, 48.94, 45.28, 44.01, 30.46, 29.13, 28.39, 27.17, 26.10, 23.88, 22.07, 20.91, 18.15, 13.89
DEPT (135°) (CDCl <sub>3</sub> )	CH 137.29, 90.56, 75.48, 75.12, 48.94, 45.28 CH <sub>2</sub> 110.37, 29.13, 28.39, 23.88 CH <sub>3</sub> 57.51, 52.42, 30.46, 27.17, 26.10, 22.07, 20.91, 18.15, 13.89
EIMS ( <i>m/z</i> ) (% rel. int.)	608 (4), 579 (29), 536 (29), 508 (100), 382 (14), 276 (14), 233 (22)

**Subfraction I4-6-4** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed three major UV-active spots with the  $R_f$  values of 0.38 and 0.62. Further purification was performed by precoated TLC, using 10% acetone-chloroform as a mobile phase (6 runs), to afford a yellow gum in 7.1 mg of which chromatogram indicated the presence of **GF18**.

**Subfraction I4-6-5** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed none of well-separated spots. No further investigation was performed.

**Subfraction I4-7** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed none of well-separated spots. Therefore, it was not further investigated.

**Subfraction I5** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.37. It was further separated by column chromatography on silica gel. Elution was conducted initially with 30% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with 15% methanol-ethyl acetate to forty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 44**.

**Table 44** Subfractions obtained from subfraction **I5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
I5-1	0.009	Yellow gum
I5-2	0.006	Yellow gum
I5-3	0.024	Yellow gum
I5-4	0.011	Yellow gum
I5-5	0.002	Yellow gum

**Subfraction I5-1** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed none of well-separated spots. Therefore, it was not further investigated.

**Subfraction I5-2** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed one major UV-active spot with



the  $R_f$  value of 0.42. Therefore, it was not further investigated because of low quantity.

**Subfraction I5-3** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.42. Further purification was performed by precoated TLC, using 30% ethyl acetate-petroleum ether as a mobile phase (14 runs), to afford a yellow gum in 2.5 mg of which chromatogram on normal phase TLC with 40% ethyl acetate-petroleum ether showed two major UV-active spots with the  $R_f$  values of 0.42 and 0.52. Because it was obtained in low quantity, it was not investigated.

**Subfraction I5-4** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed two major UV-active spots with the  $R_f$  values of 0.34 and 0.42. Therefore, it was not further investigated.

**Subfraction I5-5** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed many spots without any major spots. Thus, no further purification was performed.

**Subfraction I6** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed none of well-separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction A7-2-3** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (2 runs) showed three major yellow spots which were **scortechinones B, C and M**, obtained its latex and stem bark .

**Subfraction A7-3** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (2 runs) showed five major UV-active spots with the  $R_f$  values of 0.11, 0.18, 0.28, 0.42 and 0.55. It was further separated by column chromatography on Sephadex LH-20. Elution was conducted with pure methanol to give twenty one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 45**.

**Table 45** Subfractions obtained from subfraction **A7-3** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
A7-3-1	0.008	Yellow gum
A7-3-2	0.062	Yellow gum
A7-3-3	0.181	Yellow gum

**Subfraction A7-3-1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed many spots without any major spots. Thus, no further purification was performed.

**Subfraction A7-3-2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed seven UV-active spots with the  $R_f$  values of 0.14, 0.20, 0.25, 0.33, 0.39, 0.46 and 0.56. Its chromatogram contained the same major spots as subfraction **A7-3-3**. Therefore, it was not investigated.

**Subfraction A7-3-3** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed six UV-active spots with the  $R_f$  values of 0.14, 0.20, 0.39, 0.46, 0.56 and 0.70. Further separation on column chromatography over silica gel was performed. Elution was conducted initially with 1% methanol-chloroform and gradually increased the polarity until 50% methanol-chloroform to give eighty seven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 46**.

**Table 46** Subfractions obtained from subfraction **A7-3-3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
J1	0.015	Yellow gum
J2	0.074	Yellow gum
J3	0.026	Yellow gum
J4	0.046	Yellow gum

**Subfraction J1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed many spots without any major spots. Thus, no further purification was performed.

**Subfraction J2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed four major UV-active spots with the  $R_f$  values of 0.40, 0.50, 0.55 and 0.65. Its  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Thus, it was not further investigated.

**Subfraction J3** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed three major UV-active spots with the  $R_f$  values of 0.14, 0.38, and 0.47. Further purification was performed on precoated TLC, using 2% methanol-chloroform (8 runs), to afford two bands. Their  $^1\text{H}$  NMR spectra indicated that they contained many compounds. Thus, they were not further investigated.

**Subfraction J4** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (3 runs) showed none of well-separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction A7-4** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (2 runs) showed two pale UV-active spots with the  $R_f$  values of 0.11 and 0.42, and one yellow spot with the  $R_f$  value of 0.55. Because it contained the same major spots as subfraction **A7-3**, it was not investigated.

**Subfraction A7-5** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (2 runs) showed one major UV-active

spot with the  $R_f$  value of 0.43. Further separation on column chromatography over silica gel was performed. Elution was conducted initially with 1% methanol-chloroform and gradually increased the polarity until 10% methanol-chloroform to give fifty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 47**.

**Table 47** Subfractions obtained from subfraction **A7-5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A7-5-1	0.004	Yellow gum
A7-5-2	0.001	Yellow gum
A7-5-3	0.008	Yellow gum
A7-5-4	0.017	Yellow gum

**Subfraction A7-5-1** Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform (2 runs) showed many spots without any major spots. Thus, no further purification was performed.

**Subfraction A7-5-2** Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.28 which was similar to that found in subfraction **A7-5-3**. Therefore, it was not investigated.

**Subfraction A7-5-3** Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.28. Further purification was performed by precoated TLC, using 30% ethyl acetate-petroleum ether as a mobile phase (5 runs), to afford a yellow solid (**GF19**) in 4.2 mg. It melted at 148.8-150.0 °C. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform showed only one yellow spot with the  $R_f$  value of 0.48.

$[\alpha]_D^{29}$	-273° (c = 0.06, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3427 (OH stretching), 2965, 2924 (CH stretching), 1640 (C=O stretching), 1609, 1574 (C=C stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	322 (4.14), 267 (4.48), 241 (4.46)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	13.90 ( <i>s</i> , 1H), 9.12 ( <i>brs</i> , 1H), 7.75 ( <i>d</i> , <i>J</i> = 3.5 Hz, 1H), 7.56 ( <i>d</i> , <i>J</i> = 3.5 Hz, 1H), 5.28 ( <i>tm</i> , <i>J</i> = 7.0 Hz, 1H), 4.63 ( <i>brs</i> , 1H), 4.55 ( <i>q</i> , <i>J</i> = 6.5 Hz, 1H), 3.30 ( <i>d</i> , <i>J</i> = 7.0 Hz, 2H), 1.87 ( <i>s</i> , 3H), 1.79 ( <i>s</i> , 3H), 1.78 ( <i>s</i> , 3H), 1.66 ( <i>s</i> , 6H), 1.44 ( <i>d</i> , <i>J</i> = 6.5 Hz, 3H), 1.33 ( <i>s</i> , 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (125 MHz)	181.37, 165.60, 161.81, 154.12, 151.64, 147.50, 140.62, 132.01, 122.58, 122.54, 122.33, 112.77, 108.00, 107.24, 103.72, 90.89, 71.52, 44.69, 30.55, 30.18, 25.95, 25.79, 22.19, 22.00, 17.79, 14.16
DEPT (135°) (CDCl <sub>3</sub> )	CH 122.58, 122.33, 108.00, 90.89 CH <sub>2</sub> 22.19 CH <sub>3</sub> 30.55, 30.18, 25.95, 25.79, 22.00, 17.79, 14.16
EIMS ( <i>m/z</i> ) (% rel. int.)	438 (21), 423 (15), 405 (16), 383 (100), 365 (23), 349 (30), 309 (23)

**Subfraction A7-5-4** Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform (2 runs) showed many spots without any major spots. Thus, no further purification was performed.

**Subfraction A7-6** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (2 runs) showed two major UV-active spots with the *R<sub>f</sub>* values of 0.20 and 0.35. Upon standing at room temperature, its chromatogram characteristics indicated the decomposition of this fraction. Therefore, it was not further investigated.

**Fraction GFA8** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed four major spots; two yellow spots with the  $R_f$  values of 0.07 and 0.30 and two UV-active spots with the  $R_f$  values of 0.10 and 0.16. It was further separated by column chromatography on Sephadex LH-20. Elution was conducted with pure methanol to give ninety one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford seven subfractions, as shown in **Table 48**.

**Table 48** Subfractions obtained from fraction **GFA8** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
A8-1	0.139	Green yellow gum
A8-2	0.299	Yellow gum
A8-3	0.414	Yellow gum
A8-4	0.206	Yellow gum
A8-5	0.038	Yellow solid
A8-6	0.014	Green yellow gum
A8-7	0.012	Green gum

**Subfraction A8-1** Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed none of well-separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction A8-2** Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed none of well-separated spots under UV-S. But the chromatogram on reverse-phase TLC with 70% methanol-water showed four major yellow spots with the  $R_f$  values of 0.14, 0.27, 0.38 and 0.46. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give one hundred fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and

then evaporated to dryness *in vacuo* to afford seven subfractions, as shown in **Table 49**.

**Table 49** Subfractions obtained from subfraction **A8-2** by column chromatography over reverse-phase C<sub>18</sub> silica gel

Subfraction	Weight (g)	Physical appearance
A8-2-1	0.024	Yellow gum
A8-2-2	0.120	Yellow gum
A8-2-3	0.056	Yellow gum
A8-2-4	0.020	Yellow gum
A8-2-5	0.019	Yellow solid
A8-2-6	0.014	Yellow gum
A8-2-7	0.063	Yellow gum

**Subfraction A8-2-1** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed none of well-separated spots under UV-S. No further investigation was performed.

**Subfraction A8-2-2** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed one major UV-active spot with the R<sub>f</sub> value of 0.24. Further separation on column chromatography over silica gel was performed. Elution was conducted initially with 2% methanol-chloroform and gradually increased the polarity until 30% methanol-chloroform to give forty five fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 50**.

**Table 50** Subfractions obtained from subfraction **A8-2-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
K1	0.013	Yellow gum
K2	0.057	Yellow gum
K3	0.033	Yellow gum
K4	0.024	Yellow gum

**Subfraction K1** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed many pale spots under UV-S. Because it was obtained in low quantity, it was not investigated.

**Subfraction K2** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.18 and one minor yellow spot with the  $R_f$  value of 0.25. Because its chromatogram is similar to that of subfraction **K3**, it was not further purified.

**Subfraction K3** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.18. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 40% methanol-water and gradually decreased the polarity until pure methanol to give eighty two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions. They were not further investigated because their chromatograms on the normal phase TLC using 4% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.37 and they were obtained in low quantity. Moreover, their  $^1\text{H}$  NMR spectra indicated that they contained many compounds.

**Subfraction K4** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (2 runs) showed none of well-separated spots under UV-S. No further investigation was performed.



**Subfraction A8-2-3** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed two pale UV-active spots with the  $R_f$  values of 0.20 and 0.29. Therefore, it was not further purified.

**Subfraction A8-2-4** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.20. It was further separated by column chromatography over silica gel. Elution was conducted initially with 1% methanol-chloroform and gradually increased the polarity until 10% methanol-chloroform to give fifty seven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions. They were not further investigated because their chromatograms on the normal phase TLC using 3% methanol-chloroform showed many UV-active spots without any major spots and they were obtained in low quantity.

**Subfraction A8-2-5** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.19 and 0.36. Further purification by column chromatography over silica gel was performed. Elution was conducted initially with 2% methanol-chloroform and gradually increased the polarity until 30% methanol-chloroform to give eighty four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions. They were not further investigated because their chromatograms on the normal phase TLC using 3% methanol-chloroform showed many UV-active spots without any major spots and they were obtained in low quantity.

**Subfraction A8-2-6** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed three major UV-active spots with the  $R_f$  values of 0.22, 0.27 and 0.37. It was further separated by column chromatography over silica gel. Elution was conducted initially with 1% methanol-chloroform and gradually increased the polarity until 10% methanol-chloroform to give sixty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford twelve subfractions. Their chromatograms on the normal phase TLC using 3%

methanol-chloroform showed many unseparable spots and they were obtained in low quantity. Therefore, they were not purified.

**Subfraction A8-2-7** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed many pale spots under UV-S. Thus, it was not investigated.

**Subfraction A8-3** Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed two major spots with the  $R_f$  values of 0.12 and 0.45. But the chromatogram on reverse-phase TLC with 70% methanol-water showed five major yellow spots with the  $R_f$  values of 0.21, 0.28, 0.35, 0.41 and 0.48. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 65% methanol-water and gradually decreased the polarity until pure methanol to give one hundred and eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford eight subfractions, as shown in **Table 51**.

**Table 51** Subfractions obtained from subfraction **A8-3** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
A8-3-1	0.034	Yellow gum
A8-3-2	0.103	Yellow gum
A8-3-3	0.070	Yellow gum
A8-3-4	0.063	Yellow gum
A8-3-5	0.052	Yellow gum
A8-3-6	0.048	Yellow gum
A8-3-7	0.010	Yellow gum
A8-3-8	0.027	Yellow gum

**Subfraction A8-3-1** Chromatogram characteristics on normal phase TLC with 4% methanol-chloroform (2 runs) showed none of well-separated spots under UV-S. No further investigation was performed.

**Subfraction A8-3-2** Chromatogram characteristics on normal phase TLC with 4% methanol-chloroform (2 runs) showed six major spots with the  $R_f$  values of 0.04, 0.09, 0.15, 0.21, 0.27 and 0.36. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 50% methanol-water and gradually decreased the polarity until pure methanol to give eighty one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford eleven subfractions. They were not further investigated because their chromatograms on the normal phase TLC using 3% methanol-chloroform (2 runs) showed many spots without any major spots and they were obtained in low quantity.

**Subfraction A8-3-3** Chromatogram characteristics on normal phase TLC with 4% methanol-chloroform (2 runs) showed four major spots with the  $R_f$  values of 0.13, 0.17, 0.25 and 0.31. It was further separated by column chromatography over silica gel. Elution was conducted initially with 0.8% methanol-chloroform and gradually increased the polarity until pure methanol to give eighty seven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nineteen subfractions. Their chromatograms on the normal phase TLC using 3% methanol-chloroform (2 runs) showed many unseparable spots and they were obtained in low quantity. Therefore, they were not purified.

**Subfraction A8-3-4** Chromatogram characteristics on normal phase TLC with 4% methanol-chloroform (2 runs) showed six major spots with the  $R_f$  values of 0.13, 0.17, 0.25, 0.29, 0.35 and 0.43. It was further separated by column chromatography over silica gel. Elution was conducted initially with 1% methanol-chloroform and gradually increased the polarity until 30% methanol-chloroform to give one hundred and two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 52**.

**Table 52** Subfractions obtained from subfraction **A8-3-4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
L1	0.002	Yellow gum
L2	0.019	Yellow gum
L3	0.031	Yellow gum

**Subfraction L1** Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform (2 runs) showed two pale UV-active spots with the  $R_f$  values of 0.64 and 0.78. Because it was obtained in low quantity, it was not further investigated.

**Subfraction L2** Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform (2 runs) showed three yellow spots with the  $R_f$  values of 0.33, 0.51 and 0.64. Further purification was performed on precoated TLC, using 3% methanol-chloroform as a mobile phase (5 runs), to afford three bands.

**Band 1** was obtained as a yellow gum in 1.2 mg. Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform showed one yellow spot with the same  $R_f$  value as **scortechinone C**, obtained from its latex.

**Band 2** was obtained as a yellow gum in 2.4 mg. Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform showed one yellow spot with the same  $R_f$  value as **scortechinone M**, obtained from its stem bark.

**Band 3** was obtained as a yellow gum in 4.9 mg. Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform showed one yellow spot with the  $R_f$  value of 0.23. Its  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Thus, it was not further investigated.

**Subfraction L3** Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform (2 runs) showed many spots without any major spots. Therefore, it was not further purified.

**Subfraction A8-3-5** Chromatogram characteristics on normal phase TLC with 4% methanol-chloroform (2 runs) showed three major spots under UV-S with the  $R_f$  values of 0.29, 0.42 and 0.71. Its  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Thus, it was not further investigated.

**Subfraction A8-3-6** Chromatogram characteristics on normal phase TLC with 4% methanol-chloroform (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.26 and 0.33. It was further separated by column chromatography over silica gel. Elution was conducted initially with 1% methanol-chloroform and gradually increased the polarity until 10% methanol-chloroform to give ninety five fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford fifteen subfractions. Their chromatograms on the normal phase TLC using 3% methanol-chloroform (2 runs) showed many unseparable spots and they were obtained in low quantity. Therefore, they were not investigated.

**Subfraction A8-3-7** Chromatogram characteristics on normal phase TLC with 4% methanol-chloroform (2 runs) showed five major UV-active spots with the  $R_f$  values of 0.32, 0.39, 0.48, 0.61 and 0.68. Attempted purification by precoated TLC was unsuccessful.

**Subfraction A8-3-8** Chromatogram characteristics on normal phase TLC with 4% methanol-chloroform (2 runs) showed no definite spot. Thus, it was not further investigated.

**Subfraction A8-4** Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed two major spots with the  $R_f$  values of 0.12 and 0.45. Further separation by column chromatography over silica gel was performed. Elution was conducted initially with 0.5 % methanol-chloroform and gradually increased the polarity until 80% methanol-chloroform to give eighty two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions, as shown in **Table 53**.

**Table 53** Subfractions obtained from subfraction **A8-4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A8-4-1	0.006	Yellow gum
A8-4-2	0.007	Yellow gum
A8-4-3	0.031	Yellow gum
A8-4-4	0.028	Yellow gum
A8-4-5	0.024	Yellow gum
A8-4-6	0.029	Yellow gum

**Subfraction A8-4-1** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed many pale spots under UV-S. Thus, it was not further investigated.

**Subfraction A8-4-2** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed two yellow spots with the same  $R_f$  values as **scortechinones B** and **C**, obtained from its twig.

**Subfraction A8-4-3** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed one major yellow spot with the same  $R_f$  value as **scortechinone B**, obtained from its twig.

**Subfraction A8-4-4** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed one yellow spot and one UV-active spot with the same  $R_f$  values as **scortechinones B** and **P**, obtained from its stem bark.

**Subfraction A8-4-5** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed one major UV-active spot with the same  $R_f$  value as **scortechinone P**, obtained from its stem bark.

**Subfraction A8-4-6** Chromatogram characteristics on normal phase TLC with 2% methanol-chloroform (3 runs) showed none of well-separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction A8-5** Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed many spots without any major spots. Thus, it was not further investigated.

**Subfraction A8-6** Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.68. Further purification was performed by precoated TLC, using 10% acetone-chloroform as a mobile phase (5 runs), to afford a yellow solid in 3.0 mg. Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (3 runs) showed one yellow spot with the  $R_f$  value of 0.52. Its  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Thus, it was not investigated.

**Subfraction A8-7** Chromatogram characteristics on normal phase TLC with 45% ethyl acetate-petroleum ether (2 runs) no definite spot. Therefore, it was not further investigated.

**Fraction GFA9** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed five major spots; two yellow spots with the  $R_f$  values of 0.07 and 0.35 and three UV-active spots with the  $R_f$  values of 0.10, 0.16 and 0.23. It was further separated by column chromatography on Sephadex LH-20. Elution was conducted with pure methanol to give one hundred fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford eight subfractions, as shown in **Table 54**.

**Table 54** Subfractions obtained from fraction **GFA9** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
A9-1	0.067	Brown yellow gum
A9-2	0.360	Brown yellow gum
A9-3	0.421	Brown yellow gum
A9-4	1.696	Brown yellow gum

**Table 54** (continued)

Subfraction	Weight (g)	Physical appearance
A9-5	0.018	Yellow gum
A9-6	0.031	Yellow gum
A9-7	0.028	Yellow gum
A9-8	0.009	Yellow green gum

**Subfraction A9-1** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) and chromatogram on reverse-phase TLC with 70% methanol-water showed no definite spot. Therefore, it was not further investigated.

**Subfraction A9-2** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed none of well-separated spots under UV-S. Its  $^1\text{H}$  NMR spectrum displayed proton signals in the high field region. Therefore, it was not further investigated.

**Subfraction A9-3** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one yellow spot with the  $R_f$  value of 0.19 and two UV-active spots with the  $R_f$  values of 0.35 and 0.50 which were similar to the chromatogram of subfraction **A9-4**. Thus, it was not investigated.

**Subfraction A9-4** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether (2 runs) showed one yellow spot with the  $R_f$  value of 0.19 and three UV-active spots with the  $R_f$  values of 0.35, 0.50 and 0.59. Further separation by column chromatography over silica gel was performed. Elution was conducted initially with 1% methanol-chloroform and gradually increased the polarity until pure methanol to give forty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford ten subfractions, as shown in **Table 55**.



**Table 55** Subfractions obtained from subfraction **A9-4** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A9-4-1	0.025	Yellow gum
A9-4-2	0.028	Yellow gum
A9-4-3	0.034	Yellow gum
A9-4-4	0.103	Yellow gum
A9-4-5	0.179	Yellow gum
A9-4-6	0.266	Yellow gum
A9-4-7	0.216	Yellow gum
A9-4-8	0.235	Yellow gum
A9-4-9	0.105	Yellow gum
A9-4-10	0.211	Brown yellow gum

**Subfraction A9-4-1** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether showed many pale spots under UV-S. Therefore, it was not further investigated.

**Subfraction A9-4-2** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether showed one major yellow spot with the  $R_f$  value of 0.40. It was further separated by column chromatography over silica gel. Elution was conducted initially with 20% ethyl acetate-petroleum ether, gradually enriched with pure ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with 20% methanol-ethyl acetate to give fifty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford ten subfractions. **Scortechinone B** (0.004 g) was obtained from the fifth subfraction as a yellow gum. Other subfractions were obtained in low quantity. Their chromatograms on normal phase TLC with 30% ethyl acetate-petroleum ether showed no major spots.

**Subfraction A9-4-3** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether showed many spots without any major spots. Thus, it was not further purified.

**Subfraction A9-4-4** Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether showed one UV-active spot with the  $R_f$  value of 0.10 and one yellow spot with the  $R_f$  value of 0.17. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give seventy eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford seven subfractions, as shown in **Table 56**.

**Table 56** Subfractions obtained from subfraction **A9-4-4** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
M1	0.004	Yellow gum
M2	0.008	Yellow gum
M3	0.017	Yellow gum
M4	0.012	Yellow gum
M5	0.013	Yellow gum
M6	0.019	Yellow gum
M7	0.018	Yellow gum

**Subfraction M1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-chloroform (2 runs) showed no definite spot. Therefore, it was not further investigated.

**Subfraction M2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.36. Its  $^1H$  NMR spectrum indicated that it was benzene derivative. Because it was obtained in low quantity, it was not purified.

**Subfraction M3** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-chloroform (2 runs) showed many spots without any major spots. No further investigation was performed.

**Subfraction M4** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-chloroform (2 runs) showed one yellow spot overlapping with one UV-active spot with the  $R_f$  value of 0.26. Further purification was performed on precoated TLC, using 25% ethyl acetate-petroleum ether as a mobile phase (43 runs), to afford two bands.

**Band 1 (GF20)** was obtained as a yellow solid in 3.3 mg, decomposed at 210 °C. Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed only one UV-active spot with the  $R_f$  value of 0.29

$[\alpha]_D^{29}$	+61° (c = 0.22, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3600-2500 (OH stretching), 2928 (CH stretching), 1704, 1656 (C=O stretching), 1609, 1585 (C=C stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	325 (4.07), 277 (4.20), 266 (4.24), 247 (4.25), 223 (4.41)
<sup>1</sup> H NMR (Acetone- <i>d</i> <sub>6</sub> ) ( $\delta$ ppm) (500 MHz)	12.87 ( <i>s</i> , 1H), 7.44 ( <i>s</i> , 1H), 6.58 ( <i>tm</i> , <i>J</i> = 7.5 Hz, 1H), 5.34 ( <i>tm</i> , <i>J</i> = 7.5 Hz, 1H), 4.58 ( <i>q</i> , <i>J</i> = 6.5 Hz, 1H), 4.09 ( <i>s</i> , 3H), 3.54 ( <i>ddm</i> , <i>J</i> = 15.0 and 7.5 Hz, 1H), 3.47 ( <i>d</i> , <i>J</i> = 7.5 Hz, 2H), 3.26 ( <i>ddm</i> , <i>J</i> = 15.0 and 7.5 Hz, 1H), 1.84 ( <i>d</i> , <i>J</i> = 1.0 Hz, 3H), 1.69 ( <i>d</i> , <i>J</i> = 1.0 Hz, 3H), 1.64 ( <i>d</i> , <i>J</i> = 1.0 Hz, 3H), 1.61 ( <i>s</i> , 3H), 1.53 ( <i>s</i> , 3H), 1.49 ( <i>s</i> , 3H), 1.41 ( <i>d</i> , <i>J</i> = 6.5 Hz, 3H), 1.24 ( <i>s</i> , 3H)
<sup>13</sup> C NMR (Acetone- <i>d</i> <sub>6</sub> ) ( $\delta$ ppm) (125 MHz)	182.03, 171.98, 168.92, 165.34, 157.03, 154.98, 150.75, 147.46, 145.10, 139.31, 132.48, 129.87, 127.95, 122.43, 117.43, 116.82, 109.26, 105.11, 103.49, 91.71, 91.60, 87.17, 57.02, 44.38, 35.98, 30.21, 28.85, 25.87, 25.44, 22.48, 20.81, 17.74, 14.61, 12.76

DEPT (135°) (Acetone- <i>d</i> <sub>6</sub> )	CH 139.31, 122.43, 109.26, 91.60
	CH <sub>2</sub> 35.98, 22.48
	CH <sub>3</sub> 57.02, 30.21, 28.85, 25.87, 25.44, 20.81, 17.74, 14.61, 12.76
EIMS ( <i>m/z</i> ) (% rel. int.)	562 (20), 489 (22), 463 (100), 435 (22), 375 (21), 349 (20), 323 (25)

**Band 2 (GF21)** was obtained as a yellow solid in 3.1 mg, melting at 213-215 °C. Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed only one yellow spot with the R<sub>f</sub> value of 0.29.

[α] <sub>D</sub> <sup>29</sup>	+28° (c = 0.22, CH <sub>3</sub> OH)
IR (neat) ν <sub>max</sub> (cm <sup>-1</sup> )	3600-2500 (OH stretching), 2927, 2848 (CH stretching), 1694, 1642 (C=O stretching), 1588 (C=C stretching)
UV (CH <sub>3</sub> OH) λ <sub>max</sub> (nm) (log ε)	328 (4.10), 277 (4.25), 267 (4.28), 243 (4.32), 222 (4.46)
<sup>1</sup> H NMR (Acetone- <i>d</i> <sub>6</sub> ) (δ ppm) (500 MHz)	13.15 ( <i>s</i> , 1H), 7.44 ( <i>s</i> , 1H), 6.59 ( <i>tm</i> , <i>J</i> = 7.5 Hz, 1H), 5.26 ( <i>tm</i> , <i>J</i> = 7.5 Hz, 1H), 4.64 ( <i>q</i> , <i>J</i> = 6.5 Hz, 1H), 4.10 ( <i>s</i> , 3H), 3.54 ( <i>dd</i> , <i>J</i> = 15.0 and 7.5 Hz, 1H), 3.28 ( <i>d</i> , <i>J</i> = 7.5 Hz, 2H), 3.28 ( <i>dd</i> , <i>J</i> = 15.0 and 7.5 Hz, 1H), 1.75 ( <i>s</i> , 3H), 1.70 ( <i>d</i> , <i>J</i> = 1.5 Hz, 3H), 1.64 ( <i>s</i> , 3H), 1.61 ( <i>s</i> , 6H), 1.53 ( <i>s</i> , 3H), 1.43 ( <i>d</i> , <i>J</i> = 6.5 Hz, 3H), 1.33 ( <i>s</i> , 3H)
<sup>13</sup> C NMR (Acetone- <i>d</i> <sub>6</sub> ) (δ ppm) (125 MHz)	181.43, 171.99, 168.94, 165.50, 161.40, 151.26, 150.76, 147.17, 145.07, 139.34, 132.23, 129.86, 128.19, 122.38, 117.01, 113.20, 109.38, 107.56, 104.47, 91.73, 91.62, 87.15, 57.29, 44.74, 36.03, 30.25, 28.84, 25.98, 25.79, 22.22, 21.44, 17.78,

		14.75, 12.77
DEPT (135°) (Acetone- <i>d</i> <sub>6</sub> )	CH	139.34, 122.38, 109.38, 91.73
	CH <sub>2</sub>	36.03, 22.22
	CH <sub>3</sub>	57.29, 30.25, 28.84, 25.98, 25.79, 21.44, 17.78, 14.75, 12.77
EIMS ( <i>m/z</i> ) (% rel. int.)		562 (80), 506 (46), 490 (77), 463 (100), 447 (34), 433 (54), 419 (50), 407 (76), 379 (80), 349 (36), 323 (43)

**Subfraction M5** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-chloroform (2 runs) showed one yellow spot overlap with one UV-active spot with the  $R_f$  value of 0.26. Further purification was performed on precoated TLC, using 25% ethyl acetate-petroleum ether as a mobile phase (38 runs), to afford four bands.

**Band 1** was obtained as a yellow gum in 3.6 mg. Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (4 runs) showed only one yellow spot with the same  $R_f$  value as **scortechinone P**, obtained from its stem bark.

**Band 2** was obtained as a yellow solid in 1.8 mg. Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (4 runs) showed only one UV-active spot with the same  $R_f$  value as **GF20**.

**Band 3** was obtained as a yellow solid in 1.2 mg. Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (4 runs) showed one UV-active spot with the  $R_f$  value of 0.29. Its <sup>1</sup>H NMR spectrum indicated that it contained many components. Thus, it was not further investigated.

**Band 4** was obtained as a yellow solid in 2.7 mg. Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (4 runs) showed only one UV-active spot with the same  $R_f$  value as **GF21**.

**Subfraction M6** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-chloroform (2 runs) showed one yellow spot overlap with one UV-active spot with the  $R_f$  value of 0.26. Further purification was performed on precoated TLC, using 25% ethyl acetate-petroleum ether as a mobile phase (36 runs), to afford two bands.

**Band 1** was obtained as a yellow solid in 0.6 mg. Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed one major yellow spot with the  $R_f$  value of 0.36 which was shown to be identical to that of **GF20**.

**Band 2** was obtained as a yellow solid in 3.0 mg. Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed one UV-active spot with the  $R_f$  value of 0.30. Its  $^1\text{H}$  NMR spectrum indicated that it was a mixture of **GF20** and **GF21**.

**Subfraction M7** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-chloroform (2 runs) showed many spots without any major spots. Therefore, it was not further investigated.

**Subfraction A9-4-5** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed one UV-active spot overlapping with one yellow spot with the  $R_f$  value of 0.41. It was further separated by column chromatography over reverse-phase  $\text{C}_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give ninety fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions, as shown in **Table 57**.

**Table 57** Subfractions obtained from subfraction **A9-4-5** by column chromatography over reverse-phase C<sub>18</sub> silica gel

Subfraction	Weight (g)	Physical appearance
N1	0.030	Yellow gum
N2	0.010	Yellow gum
N3	0.014	Yellow solid
N4	0.091	Yellow gum
N5	0.012	Yellow gum
N6	0.013	Yellow gum

**Subfraction N1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed no definite spot. Therefore, it was not further investigated.

**Subfraction N2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed two major UV-active spots with the R<sub>f</sub> values of 0.16 and 0.28. Attempted purification by precoated TLC was unsuccessful.

**Subfraction N3** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the R<sub>f</sub> value of 0.21. Further purification was performed by precoated TLC, using 30% ethyl acetate-petroleum ether as a mobile phase (30 runs), to afford a yellow solid in 4.8 mg. Chromatogram characteristics on normal phase TLC with 40% ethyl acetate-petroleum ether (2 runs) showed only one UV-active spot with the same R<sub>f</sub> value as **GF20**.

**Subfraction N4** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the R<sub>f</sub> value of 0.24 and one yellow spot with the R<sub>f</sub> value of 0.39. It was further separated by column chromatography over reverse-phase C<sub>18</sub> silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give one hundred and nine fractions. All fractions were

examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 58**.

**Table 58** Subfractions obtained from subfraction **N4** by column chromatography over reverse-phase C<sub>18</sub> silica gel

Subfraction	Weight (g)	Physical appearance
N4-1	0.008	Yellow gum
N4-2	0.016	Yellow gum
N4-3	0.037	Yellow solid
N4-4	0.026	Yellow gum
N4-5	0.011	Yellow gum

**Subfraction N4-1** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether showed no definite spot. Therefore, it was not further investigated.

**Subfraction N4-2** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether showed one UV-active spot with the same R<sub>f</sub> value as **GF21**.

**Subfraction N4-3** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether showed two UV-active spots with the same R<sub>f</sub> values as **GF20** and **GF21**.

**Subfraction N4-4** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether showed one UV-active spot with the same R<sub>f</sub> value as **GF20**.

**Subfraction N4-5** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether showed many pale UV-active spots. Thus, it was not further investigated.

**Subfraction N5** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed two major UV-active spots with the R<sub>f</sub> values of 0.24 and 0.32. Further purification was performed by precoated TLC, using 30% ethyl acetate-petroleum ether as a mobile phase (13 runs), to afford a



yellow solid in 5.6 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform showed only one UV-active spot. Its  $^1\text{H}$  NMR spectrum indicated that it was identical to that of **GF20**.

**Subfraction N6** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (4 runs) showed many spots without any major spots. Therefore, it was not further investigated.

**Subfraction A9-4-6** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed one UV-active spot overlapping with one yellow spot with the  $R_f$  value of 0.41. It was further separated by column chromatography over reverse-phase  $\text{C}_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give one hundred and twenty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 59**.

**Table 59** Subfractions obtained from subfraction **A9-4-6** by column chromatography over reverse-phase  $\text{C}_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
O1	0.060	Yellow gum
O2	0.042	Yellow gum
O3	0.152	Yellow solid

**Subfraction O1** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (3 runs) showed many pale spots without any major spots. Therefore, it was not further investigated.

**Subfraction O2** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (3 runs) showed two major UV-active spots with the  $R_f$  values of 0.27 and 0.45. Further purification by column chromatography over silica gel was performed. Elution was conducted initially with 30% ethyl acetate-petroleum ether, gradually enriched with pure ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with pure methanol to give

three hundred and forty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nine subfractions. They were not further investigated because their chromatograms on the normal phase TLC using 3% methanol-chloroform showed many UV-active spots without any major spots and they were obtained in low quantity.

**Subfraction O3** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate- petroleum ether (3 runs) showed two major UV-active spots with the  $R_f$  values of 0.31 and 0.46. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give fifty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 60**.

**Table 60** Subfractions obtained from subfraction **O3** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
O3-1	0.010	Yellow gum
O3-2	0.003	Yellow gum
O3-3	0.009	Yellow gum
O3-4	0.119	Yellow gum
O3-5	0.010	Yellow gum

**Subfraction O3-1** Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-petroleum ether (9 runs) showed many pale spots without any major spots. Therefore, it was not further investigated.

**Subfraction O3-2** Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-petroleum ether (9 runs) showed one major UV-active spot with the  $R_f$  value of 0.14. Its  $^1H$  NMR spectrum indicated that it was **GF22**.

**Subfraction O3-3** Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-petroleum ether (9 runs) showed one major UV-active spot with the  $R_f$  value of 0.12. Its  $^1\text{H}$  NMR spectrum indicated that it was a mixture of **GF20** and **GF21**.

**Subfraction O3-4** Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-petroleum ether (9 runs) showed two major UV-active spots with the  $R_f$  values of 0.14 and 0.21. It was further separated by column chromatography over reverse-phase  $\text{C}_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to give fifty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford ten subfractions. Their chromatograms on normal phase TLC with 35% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot. Its  $^1\text{H}$  NMR spectrum indicated that it was a mixture of **GF20** and **GF21**.

**Subfraction O3-5** Chromatogram characteristics on normal phase TLC with 25% ethyl acetate-petroleum ether (9 runs) showed no definite spot. Thus, it was not further investigated.

**Subfraction A9-4-7** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.35. It was further separated by column chromatography over reverse-phase  $\text{C}_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to eighty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 61**.

**Table 61** Subfractions obtained from subfraction **A9-4-7** by column chromatography over reverse-phase C<sub>18</sub> silica gel

Subfraction	Weight (g)	Physical appearance
P1	0.073	Yellow gum
P2	0.014	Yellow gum
P3	0.012	Yellow gum
P4	0.020	Yellow gum
P5	0.068	Yellow gum

**Subfraction P1** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (4 runs) showed many spots without any major spots. Thus, it was not further investigated.

**Subfraction P2** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the  $R_f$  value of 0.52. Further purification was performed by precoated TLC, using 3% methanol-dichloromethane as a mobile phase (25 runs), to afford a yellow gum in 4.2 mg. Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (5 runs) showed two UV-active spots with the  $R_f$  values of 0.31 and 0.47. Attempted purification by repeated precoated TLC, using 30% ethyl acetate-petroleum ether as a mobile phase (14 runs), gave a yellow gum in 0.6 mg. Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (7 runs) showed one UV-active spot with the same  $R_f$  value as **GF22**.

**Subfraction P3** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the  $R_f$  value of 0.68. Further purification was performed by precoated TLC, using 30% ethyl acetate-petroleum ether as a mobile phase (14 runs), to afford two bands.

**Band 1 (GF22)** was obtained as a yellow gum in 4.7 mg. Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (7 runs) showed only one UV-active spot with the  $R_f$  value of 0.38.

$[\alpha]_D^{29}$	+96° (c = 0.61, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3600-2500 (OH stretching), 2974, 2929 (CH stretching), 1697, 1651 (C=O stretching), 1609, 1583 (C=C stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	278 (3.94), 221 (4.09), 202 (4.06)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (500 MHz)	12.36 (s, 1H), 6.78 (tm, <i>J</i> = 7.5 Hz, 1H), 5.22 (tm, <i>J</i> = 7.0 Hz, 1H), 4.50 (t, <i>J</i> = 8.5 Hz, 1H), 4.47 (q, <i>J</i> = 6.5 Hz, 1H), 3.54 (s, 3H), 3.37 (m, 2H), 3.31 (dd, <i>J</i> = 16.0 and 8.0 Hz, 1H), 3.10 (dd, <i>J</i> = 16.0 and 8.0 Hz, 1H), 2.70 (dd, <i>J</i> = 18.0 and 8.0 Hz, 1H), 2.59 (dd, <i>J</i> = 18.0 and 8.0 Hz, 1H), 1.77 (s, 6H), 1.67 (s, 3H), 1.46 (s, 3H), 1.38 (s, 6H), 1.37 (d, <i>J</i> = 6.5 Hz, 3H), 1.21 (s, 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (125 MHz)	178.73, 174.99, 173.04, 163.29, 163.16, 155.44, 155.39, 154.20, 140.64, 140.43, 132.34, 129.05, 122.80, 121.56, 118.05, 111.55, 105.87, 102.78, 91.03, 90.63, 88.01, 74.76, 58.03, 43.76, 35.64, 27.11, 26.71, 26.58, 25.69, 25.09, 21.92, 20.46, 17.79, 14.36, 12.36
DEPT (135°) (CDCl <sub>3</sub> )	CH 140.64, 121.56, 90.63, 74.76 CH <sub>2</sub> 35.64, 27.11, 21.92 CH <sub>3</sub> 58.03, 26.71, 26.58, 25.69, 25.09, 20.46, 17.79, 14.36, 12.36
EIMS ( <i>m/z</i> ) (% rel. int.)	608 (8), 576 (19), 520 (42), 488 (73), 463 (70), 432 (100), 406 (48), 393 (71), 391 (36)

**Band 2** was obtained as a yellow gum in 1.5 mg. Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether (7 runs) showed one UV-active spot with the *R<sub>f</sub>* value of 0.23. Its <sup>1</sup>H NMR spectrum indicated that it contained many compounds. Therefore, it was not further purified.

**Subfraction P4** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the  $R_f$  value of 0.71 which was identical to that of **GF22**.

**Subfraction P5** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (4 runs) showed two major UV-active spots with the  $R_f$  values of 0.61 and 0.69 which were **GF20** and **GF21**.

**Subfraction A9-4-8** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.26 and 0.38. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 70% methanol-water and gradually decreased the polarity until pure methanol to eighty five fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 62**.

**Table 62** Subfractions obtained from subfraction **A9-4-8** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
Q1	0.064	Yellow gum
Q2	0.041	Yellow gum
Q3	0.046	Yellow gum
Q4	0.031	Yellow gum

**Subfraction Q1** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (3 runs) showed no definite spot. Thus, it was not further investigated.

**Subfraction Q2** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.17. Its  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Therefore, it was not further investigated.

**Subfraction Q3** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (3 runs) showed two major UV-active spots with the  $R_f$  values of 0.21 and 0.39. Further purification was performed by precoated TLC, using 30% ethyl acetate-petroleum ether as a mobile phase (14 runs), to afford two bands.

**Band 1** was obtained as a yellow gum in 5.5 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed only one UV-active spot with the  $R_f$  value of 0.38. Its  $^1\text{H}$  NMR spectrum indicated that it was identical to that of **GF22**.

**Band 2** was obtained as a yellow gum in 7.9 mg. Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed one UV-active spot with the  $R_f$  value of 0.39. Its  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Therefore, it was not further purified.

**Subfraction Q4** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.28. Its  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Therefore, it was not further purified.

**Subfraction A9-4-9** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed three major UV-active spots with the  $R_f$  values of 0.14, 0.26 and 0.37. Its chromatogram was similar to that of subfraction **A9-4-8**. Therefore, it was not further purified.

**Subfraction A9-4-10** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed no major spots. Thus, it was not further investigated.

**Subfraction A9-5** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform showed many spots without any major spots. Therefore, it was not further investigated.

**Subfraction A9-6** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform showed three major UV-active spots with the  $R_f$  values of 0.11, 0.26 and 0.37. Further purification was performed by precoated TLC, using 8% methanol-chloroform as a mobile phase (4 runs), to afford a yellow gum in 4.5 mg. Chromatogram characteristics on normal phase TLC with 8% methanol-

chloroform showed one UV-active spot with the  $R_f$  value of 0.33. Its  $^1\text{H}$  NMR spectrum indicated that it contained many components. Thus, it was not further investigated.

**Subfraction A9-7** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform showed many spots without any major spots. Therefore, it was not further investigated.

**Subfraction A9-8** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform showed three major UV-active spots with the  $R_f$  values of 0.28, 0.34 and 0.42. Therefore, it was not further investigated because of low quantity.

**Fraction GFA10** Chromatogram characteristics on normal phase TLC with 50% ethyl acetate-petroleum ether showed one major UV-active spot with the  $R_f$  value of 0.18. It was further separated by column chromatography on Sephadex LH-20. Elution was conducted with pure methanol to give eighty two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 63**.

**Table 63** Subfractions obtained from fraction **GFA10** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
A10-1	0.098	Yellow gum
A10-2	0.553	Yellow gum
A10-3	0.151	Yellow gum
A10-4	0.019	Yellow gum

**Subfraction A10-1** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (2 runs) showed no definite spot. Therefore, it was not further investigated.

**Subfraction A10-2** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (2 runs) showed five major UV-active spots with



the  $R_f$  values of 0.14, 0.36, 0.50, 0.60 and 0.66. Further separation by column chromatography over silica gel was performed. Elution was conducted initially with 0.5% methanol-chloroform and gradually increased the polarity until 70% methanol-chloroform to give one hundred and twenty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 64**.

**Table 64** Subfractions obtained from subfraction **A10-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
A10-2-1	0.017	Yellow gum
A10-2-2	0.022	Yellow gum
A10-2-3	0.051	Yellow gum
A10-2-4	0.100	Yellow gum
A10-2-5	0.296	Yellow gum

**Subfraction A10-2-1** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed many pale spots under UV-S. Therefore, it was not further investigated.

**Subfraction A10-2-2** Chromatogram characteristics on normal phase TLC with 30% ethyl acetate-petroleum ether showed none of well-separated spots under UV-S. No further investigation was performed.

**Subfraction A10-2-3** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform showed one major UV-active spot with the  $R_f$  value of 0.18. Its  $^1\text{H}$  NMR spectrum indicated that it contained many components. Thus, it was not further investigated.

**Subfraction A10-2-4** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether showed two major UV-active spots with the  $R_f$  values of 0.12 and 0.29. It was further separated by column chromatography over silica gel. Elution was conducted initially with 35% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of

methanol in ethyl acetate and finally with 60% methanol-ethyl acetate to give one hundred and thirty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford thirteen subfractions. Their chromatograms on the normal phase TLC using 40% ethyl acetate-petroleum ether showed many UV-active spots without any major spots. Attempted purification by column chromatography on silica gel and precoated TLC was unsuccessful.

**Subfraction A10-2-5** Chromatogram characteristics on normal phase TLC with 35% ethyl acetate-petroleum ether showed many pale UV-active spots. Therefore, it was not further investigated because its  $^1\text{H}$  NMR spectrum displayed proton signals in the high field region.

**Subfraction A10-3** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (2 runs) showed many pale spots. Therefore, it was not further investigated.

**Subfraction A10-4** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (2 runs) showed one major yellow spot with the  $R_f$  value of 0.20. Its  $^1\text{H}$  NMR spectrum indicated that it was a biflavone derivative. Therefore, it was not further investigated because of low quantity.

**Fraction GFA11** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.09 and 0.22. It was further separated by column chromatography on Sephadex LH-20. Elution was conducted with pure methanol to give forty two fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 65**.

**Table 65** Subfractions obtained from fraction **GFA11** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
A11-1	0.147	Yellow gum
A11-2	0.164	Yellow gum
A11-3	0.012	Yellow gum

**Subfraction A11-1** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (2 runs) showed many pale spots under UV-S. Therefore, it was not further investigated.

**Subfraction A11-2** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.17 and 0.39. It was partitioned between ethyl acetate (20 ml) and 0.1 M *di*-sodium tetraborate (60 ml) to give a yellow gum (0.080 g) from the organic phase. The chromatogram on normal phase TLC with 8% methanol-chloroform (3 runs) showed one UV-active spot with the  $R_f$  value of 0.17. Further separation by column chromatography was performed. Elution was conducted initially with 4% methanol-chloroform and gradually increased the polarity until 40% methanol-chloroform. Fractions with the similar chromatogram were combined and evaporated under reduced pressure to dryness to afford twelve subfractions. Their chromatograms on normal phase TLC with 50% acetone-hexane (2 runs) showed one major UV-active spot. The  $^1\text{H}$  NMR spectra indicated that they contained many compounds. The borate layer was acidified with 10% HCl and extracted with ethyl acetate (4x30 ml) to afford a yellow gum (0.063 g). The chromatogram on normal phase TLC with 8% methanol-chloroform (3 runs) showed many UV-active spots. Therefore, both parts were not further investigated.

**Subfraction A11-3** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.24. Its  $^1\text{H}$  NMR spectrum indicated that it was a biflavonoid derivative. Because it was obtained in low quantity, it was not further investigated.

**Fraction GFA12** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.37. It was further separated by column chromatography on Sephadex LH-20. Elution was conducted with pure methanol to give one hundred and three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 66**.

**Table 66** Subfractions obtained from fraction **GFA12** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
A12-1	0.608	Brown gum
A12-2	0.563	Brown gum
A12-3	0.024	Brown solid

**Subfraction A12-1** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.37. Attempted purification by column chromatography over silica gel was unsuccessful.

**Subfraction A12-2** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (2 runs) showed three pale UV-active spots with the  $R_f$  values of 0.09, 0.15 and 0.32. Its  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Thus, it was not further investigated.

**Subfraction A12-3** Chromatogram characteristics on normal phase TLC with 8% methanol-chloroform (2 runs) showed many pale UV-active spots. Thus, it was not further investigated.

**Fraction GFA13** Chromatogram characteristics on normal phase TLC with 5% methanol-chloroform (2 runs) showed no definite spot. Therefore, it was not further investigated.

### 2.3.2.2 Investigation of the chloroform-insoluble part

#### The first investigation

Chloroform-insoluble part (**GFB**) was obtained as a brown gum in 20.00 g. Chromatogram characteristics on normal phase TLC with 3% methanol-chloroform showed none of well-separated spots under UV-S. It was further separated by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol to give fifty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford seven fractions, as shown in **Table 67**.

**Table 67** Fractions obtained from **GFB** by column chromatography over Sephadex LH-20

Fraction	Weight (g)	Physical appearance
GFB-1	0.908	Black gum
GFB-2	13.00	Yellow brown gum
GFB-3	1.509	Brown gum mixed with solid
GFB-4	0.388	Brown gum mixed with solid
GFB-5	0.395	Brown gum mixed with solid
GFB-6	0.479	Yellow brown gum mixed with solid
GFB-7	0.719	Brown solid

**Fraction GFB-1** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed no definite spot. Thus, it was not further investigated.

**Fraction GFB-2** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed many pale UV-active spots. Attempted separation by column chromatography over Sephadex LH-20 was unsuccessful.

**Fraction GFB-3** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one major yellow spot with the  $R_f$  value of 0.09

and one UV-active spot with the  $R_f$  value of 0.17. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 30% methanol-water and gradually decreased the polarity until pure methanol to give seventy nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford nine subfractions, as shown in **Table 68**.

**Table 68** Subfractions obtained from fraction **GFB-3** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
B3-1	0.320	Brown gum
B3-2	0.159	Red brown gum
B3-3	0.236	Red brown gum
B3-4	0.192	Brown solid
B3-5	0.096	Brown solid
B3-6	0.040	Brown solid
B3-7	0.075	Brown solid
B3-8	0.349	Brown solid
B3-9	0.074	Brown solid

**Subfraction B3-1** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one pale UV-active spot with the  $R_f$  value of 0.33. The chromatogram on reverse-phase TLC with 50% methanol-water showed none of well separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction B3-2** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed no definite spot. No further purification was carried out.

**Subfraction B3-3** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.04. The chromatogram characteristics on reverse-phase TLC with 50%

methanol-water showed none of well separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction B3-4** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.08. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 60% methanol-water and gradually decreased the polarity until pure methanol to give nineteen fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 69**.

**Table 69** Subfractions obtained from subfraction **B3-4** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
B3-4-1	0.132	Red brown solid
B3-4-2	0.095	Red brown gum
B3-4-3	0.005	Yellow gum

**Subfraction B3-4-1** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (3 runs) showed no major spots under UV-S. Thus, it was not further investigated.

**Subfraction B3-4-2** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.08. Attempted purification by column chromatography over Sephadex LH-20 was not successful.

**Subfraction B3-4-3** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (3 runs) showed no definite spot. Therefore, it was not further investigated.

**Subfraction B3-5** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed two UV-active spots with the  $R_f$  values of 0.15 and 0.47. It was further separated by column chromatography over

reverse-phase C<sub>18</sub> silica gel. Elution was conducted initially with 50% methanol-water and gradually decreased the polarity until pure methanol to give thirty four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 70**.

**Table 70** Subfractions obtained from subfraction **B3-5** by column chromatography over reverse-phase C<sub>18</sub> silica gel

Subfraction	Weight (g)	Physical appearance
B3-5-1	0.011	Yellow brown solid
B3-5-2	0.072	Yellow brown solid
B3-5-3	0.007	Yellow solid
B3-5-4	0.005	Yellow solid

**Subfraction B3-5-1** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed no major spots under UV-S. Therefore, it was not further investigated.

**Subfraction B3-5-2** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed two major UV-active spots with the R<sub>f</sub> values of 0.05 and 0.21. Further separation was carried out by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol to give thirty one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions.

**Subfraction B3-5-2-1** It was obtained as brown solid (0.016 g). Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed one major UV-active spot near baseline. Because it was obtained in low quantity, it was not further investigated.

**Subfraction B3-5-2-2** It was obtained as brown solid (0.047 g). Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed one major UV-active spot with the R<sub>f</sub> value of 0.17. It was further



purified by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol to give thirty five fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to give a yellow gum in 0.039 g. The chromatogram on normal phase TLC with 10% methanol-dichloromethane (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.30. Further purification by column chromatography over reverse-phase  $C_{18}$  silica gel was carried out. Elution was conducted initially with 40% methanol-water and gradually decreased the polarity until 70% methanol-water. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford six subfractions of which chromatograms showed one UV-active spot with the same  $R_f$  value as the original fraction but their  $^1\text{H}$  NMR spectra indicated that they contained at least two components. Because they were obtained in low quantity, they were not further investigated.

**Subfraction B3-5-3** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed two major UV-active spots with the same  $R_f$  values as subfraction **B3-5-2**. Thus, it was not further investigated.

**Subfraction B3-5-4** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed two pale UV-active spots with the  $R_f$  values of 0.19 and 0.21. Therefore, it was not investigated because of low quantity.

**Subfraction B3-6** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one yellow spot and one UV-active spot with the  $R_f$  values of 0.40 and 0.47, respectively. Because its chromatogram was similar to that of subfraction **B3-7**, it was not further purified.

**Subfraction B3-7** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one yellow spot and two UV-active spots with the  $R_f$  values of 0.40, 0.12 and 0.47, respectively. It was further separated by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford five subfractions, as shown in **Table 71**.

**Table 71** Subfractions obtained from subfraction **B3-7** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
B3-7-1	0.016	Yellow brown solid
B3-7-2	0.029	Yellow solid
B3-7-3	0.008	Yellow solid
B3-7-4	0.020	Yellow solid
B3-7-5	0.015	Yellow solid

**Subfraction B3-7-1** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed no definite spot. Thus, it was not further investigated.

**Subfraction B3-7-2** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major yellow spot with the same  $R_f$  value as **GF23**.

**Subfraction B3-7-3 (GF23)** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed only one yellow spot with the  $R_f$  value of 0.43. It decomposed at 248 °C.

$[\alpha]_D^{29}$	+216° (c = 0.81, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3273 (OH stretching), 1643 (C=O stretching), 1605, 1515 (C=C stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	342 (4.46), 289 (4.63), 210 (4.95)
<sup>1</sup> H NMR (DMSO- <i>d</i> <sub>6</sub> ) ( $\delta$ ppm) (300 MHz)	13.50 ( <i>s</i> , 1H), 12.25 ( <i>s</i> , 1H), 7.40 ( <i>d</i> , <i>J</i> = 8.0 Hz, 1H), 7.38 ( <i>s</i> , 1H), 7.12 ( <i>d</i> , <i>J</i> = 8.4 Hz, 2H), 6.89 ( <i>d</i> , <i>J</i> = 8.0 Hz, 1H), 6.55 ( <i>s</i> , 1H), 6.36 ( <i>d</i> , <i>J</i> = 8.4 Hz, 2H), 6.21 ( <i>s</i> , 1H), 5.95 ( <i>s</i> , 2H), 5.68 ( <i>d</i> , <i>J</i> = 12.3 Hz, 1H), 4.86 ( <i>d</i> , <i>J</i> = 12.3 Hz, 1H)
<sup>13</sup> C NMR (DMSO- <i>d</i> <sub>6</sub> ) ( $\delta$ ppm)	196.50, 181.95, 166.88, 164.10, 163.82, 163.18,

(125 MHz)	162.16, 160.83, 157.63, 155.59, 150.02, 145.97, 128.77, 128.49, 121.39, 119.60, 116.48, 114.75, 113.54, 103.42, 102.53, 101.81, 100.89, 98.95, 96.54, 95.61, 81.24, 48.62
DEPT (90°) (DMSO- <i>d</i> <sub>6</sub> ) CH	128.77, 119.60, 116.48, 114.75, 113.54, 102.53, 98.95, 96.54, 95.61, 81.24, 48.62

**Subfraction B3-7-4** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed only one yellow spot with the same R<sub>f</sub> value as **GF23**.

**Subfraction B3-7-5** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed none of well separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction B3-8** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one yellow spot and one UV-active spot with the R<sub>f</sub> values of 0.40 and 0.58. It was further separated by column chromatography over silica gel. Elution was conducted initially with 5% methanol-chloroform and gradually increased the polarity until pure methanol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford six subfractions, as shown in **Table 72**.

**Table 72** Subfractions obtained from subfraction **B3-8** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
B3-8-1	0.005	Yellow brown solid
B3-8-2	0.003	Yellow solid
B3-8-3	0.093	Yellow solid
B3-8-4	0.028	Yellow solid
B3-8-5	0.019	Yellow solid
B3-8-6	0.030	Yellow solid

**Subfraction B3-8-1** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed many pale spots under UV-S. Thus, it was not further investigated.

**Subfraction B3-8-2** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed one major UV-active spot with the  $R_f$  value of 0.22 which was **GF24**.

**Subfraction B3-8-3** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed one major UV-active spot with the  $R_f$  value of 0.22. It was further purified by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford four subfractions, as shown in **Table 73**.

**Table 73** Subfractions obtained from subfraction **B3-8-3** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
B3A-1	0.001	Colorless gum
B3A-2	0.002	Yellow gum
B3A-3	0.028	Yellow solid
B3A-4	0.033	Yellow solid

**Subfraction B3A-1** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed one UV-active spot with the  $R_f$  value of 0.33. Because it was obtained in low quantity, it was not further investigated.

**Subfraction B3A-2** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed two UV-active spots with the  $R_f$  values of 0.30 and 0.33. No further purification was carried out because of low quantity.

**Subfraction B3A-3** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.30 which was **GF24**.

**Subfraction B3A-4 (GF24)** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform (2 runs) showed only one UV-active spot with the  $R_f$  value of 0.33. It decomposed at 258 °C.

$[\alpha]_D^{29}$	+133° (c = 0.80, CH <sub>3</sub> OH)
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3157 (OH stretching), 1638 (C=O stretching), 1611, 1515 (C=C stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	289 (4.53), 214 (4.75), 202 (4.77)
<sup>1</sup> H NMR (DMSO- <i>d</i> <sub>6</sub> ) ( $\delta$ ppm) (300 MHz)	13.20 ( <i>s</i> , 1H), 12.48 ( <i>s</i> , 1H), 8.10 ( <i>d</i> , <i>J</i> = 9.0 Hz, 2H), 7.25 ( <i>d</i> , <i>J</i> = 8.5 Hz, 2H), 7.09 ( <i>d</i> , <i>J</i> = 9.0 Hz, 2H), 6.80 ( <i>s</i> , 1H), 6.49 ( <i>d</i> , <i>J</i> = 8.5 Hz, 2H), 6.37 ( <i>s</i> , 1H), 6.15 ( <i>s</i> , 1H), 6.09 ( <i>s</i> , 1H), 5.82 ( <i>d</i> , <i>J</i> = 12.3 Hz, 1H), 5.16 ( <i>d</i> , <i>J</i> = 12.3 Hz, 1H)
<sup>13</sup> C NMR (DMSO- <i>d</i> <sub>6</sub> ) ( $\delta$ ppm) (75 MHz)	196.67, 182.16, 166.83, 164.26, 164.09, 163.23, 162.43, 161.48, 160.84, 157.80, 155.78, 129.12, 128.76, 127.80, 121.23, 116.47, 115.08, 104.00, 103.45, 102.15, 101.15, 99.14, 96.67, 95.69, 81.26, 48.67
DEPT (90°) (DMSO- <i>d</i> <sub>6</sub> ) CH	129.12, 128.76, 116.47, 115.08, 103.45, 99.14, 96.67, 95.69, 81.26, 48.67

**Subfraction B3-8-4** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed one major UV-active spot with the same  $R_f$  value as **GF24**.

**Subfraction B3-8-5** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed two pale UV-active spots with the  $R_f$  values of 0.16 and 0.20. Because it was obtained in low quantity, it was not further investigated.

**Subfraction B3-8-6** Chromatogram characteristics on normal phase TLC with 10% methanol-chloroform showed one major UV-active spot with the  $R_f$  value of 0.16 which was **GF23**.

**Subfraction B3-9** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed unseparable spots under UV-S. No further investigation was carried out.

**Fraction GFB-4** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one major yellow spot with the  $R_f$  value of 0.09. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 50% methanol-water and gradually decreased the polarity until 80% methanol-water to give fifty four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 74**.

**Table 74** Subfractions obtained from fraction **GFB-4** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
B4-1	0.297	Brown gum
B4-2	0.009	Yellow brown solid
B4-3	0.221	Yellow gum mixed with yellow solid
B4-4	0.019	Brown gum mixed with brown solid
B4-5	0.004	Brown gum

**Subfraction B4-1** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed unseparable spots under UV-S. No further investigation was carried out.

**Subfraction B4-2** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major yellow spot with the  $R_f$  value of 0.14 and one minor UV-active spot with the  $R_f$  value of 0.17. The major spot had the same  $R_f$  value as **GF23**.

**Subfraction B4-3** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one major yellow spot with the  $R_f$  value of 0.14 which was **GF23**.

**Subfraction B4-4** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed one yellow spot with the  $R_f$  value of 0.14 and one UV-active spot with the  $R_f$  value of 0.22 which were **GF23** and **GF24**, respectively.

**Subfraction B4-5** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform (2 runs) showed no definite spot. Thus, it was not further investigated.

**Fraction GFB-5** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed none of well separated spots under UV-S. Therefore, it was not further investigated.

**Fraction GFB-6** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed none of well separated spots under UV-S. Therefore, it was not further investigated.

**Fraction GFB-7** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform showed one pale UV-active spot with the  $R_f$  value of 0.07. No further purification was performed.

### **The second investigation**

The chloroform-insoluble part (**GFB**) was obtained as a brown gum in 9.0 g. It was further separated by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol. Fractions with similar chromatogram were combined and evaporated under reduced pressure to dryness to afford seven fractions, as shown in **Table 75**.

**Table 75** Fractions obtained from **2GFB** by column chromatography over Sephadex LH-20

Fraction	Weight (g)	Physical appearance
2GFB-1	7.521	Brown gum
2GFB-2	0.587	Brown gum
2GFB-3	0.648	Brown gum
2GFB-4	0.217	Brown gum
2GFB-5	0.555	Brown gum
2GFB-6	0.079	Brown solid
2GFB-7	0.412	Brown gum

**Fraction 2GFB-1** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed many pale UV-active spots. Thus, it was not further investigated.

**Fraction 2GFB-2** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed one major UV-active spot with the  $R_f$  value of 0.15 and many pale UV-active spots above the major spot. No further investigation was carried out.

**Fraction 2GFB-3** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed no definite spot. Therefore, it was not further investigated.

**Fraction 2GFB-4** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed one major UV-active spot with the same  $R_f$  value as **GF24**.

**Fraction 2GFB-5** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed two major spots; one yellow spot with the  $R_f$  values of 0.23 and one UV-active spot with the  $R_f$  value of 0.35. Its was shown by TLC comparison that the two major spots were **GF23** and **GF24**.

**Fraction 2GFB-6** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed two major spots; one yellow spot with the  $R_f$  values of 0.32 and one UV-active spot with the  $R_f$  value of 0.40. It was further



purified by Semi-preparative HPLC using a Nuc. 1.7 C<sub>18</sub> column (20 x 250 mm; Macherey-Nagel AG). Elution was conducted with 50% methanol-water to yield four subfractions, as shown in the **Table 76**.

**Table 76** Subfractions obtained from fraction **2GFB-6** by Semi-preparative HPLC

Subfraction	Weight (g)	Physical appearance
2GFB6-1	0.004	Brown gum
2GFB6-2	0.015	Brown solid
2GFB6-3	0.013	Brown gum
2GFB6-4	0.084	Brown gum

**Subfraction 2GFB6-1** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform (2 runs) showed many pale spots under UV-S. Thus, it was not further investigated because of low quantity.

**Subfraction 2GFB6-2** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform (2 runs) showed one only yellow spot with the R<sub>f</sub> value of 0.47 which was identical to **GF23**.

**Subfraction 2GFB6-3** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform (2 runs) showed one only UV-active spot with the R<sub>f</sub> value of 0.58 which was **GF24**.

**Subfraction 2GFB6-4** Chromatogram characteristics on normal phase TLC with 20% methanol-chloroform (2 runs) showed one only yellow spot with the same R<sub>f</sub> value as **GF23**.

**Fraction 2GFB-7** Chromatogram characteristics on normal phase TLC with 15% methanol-chloroform showed one pale UV-active spot with the R<sub>f</sub> value of 0.22. Therefore, it was not further investigated.

## 2.4 Chemical investigation from the fruits of *G. hanburyi*

### 2.4.1 Extraction

The fruits (16.13 g) of *G. hanburyi*, cut into small segments, were extracted with MeOH (0.6 L) over the period of 7 days at room temperature for three times. After filtration, the filtrate was evaporated to dryness under reduced pressure to give a crude methanol extract as a brown-yellow gum in 9.39 g. It was separated into two parts by dissolving in chloroform.

### 2.4.2 Investigation of the chloroform-soluble part

**Chloroform-soluble part (GSC)** was obtained as a brown-yellow gum in 1.15 g. Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether showed one major yellow spot with the  $R_f$  value of 0.34. Further separation with column chromatography over silica gel was performed. Elution was conducted initially with dichloromethane and gradually enriched with pure methanol to give thirty nine fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five fractions, as shown in the **Table 77**.

**Table 77** Fractions obtained from **GSC** by column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
GSC1	0.015	Yellow gum
GSC2	0.013	Yellow gum
GSC3	0.875	Brown-yellow gum
GSC4	0.078	Brown-yellow gum
GSC5	0.378	Brown gum

**Fraction GSC1** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed one pale UV-active spot with the

$R_f$  value of 0.27. After dipping the TLC plate in ASA reagent and subsequent heating, it showed many violet spots. Therefore, it was not further investigated.

**Fraction GSC2** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed one major yellow spot with the  $R_f$  value of 0.32 which has the same major spot as subfraction **GSC3-2-1**. Thus, it was combined with subfraction **GSC3-2-1**.

**Fraction GSC3** Chromatogram characteristics on normal phase TLC with 15% ethyl acetate-petroleum ether (2 runs) showed one pale yellow spot with the  $R_f$  value of 0.32 and two major UV-active spots with the  $R_f$  values of 0.08 and 0.21. Further separation with column chromatography on Sephadex LH-20 was performed. Elution was conducted with 50% methanol-chloroform. Fractions with the similar chromatogram were combined and evaporated under reduced pressure to afford four subfractions, as shown in the **Table 78**.

**Table 78** Subfractions obtained from fraction **GSC3** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
GSC3-1	0.012	Green gum
GSC3-2	0.284	Orange-yellow gum
GSC3-3	0.506	Orange-yellow gum
GSC3-4	0.003	Yellow gum

**Subfraction GSC3-1** Chromatogram characteristics on normal phase TLC with 70% chloroform-petroleum ether (3 runs) showed no definite spot. Therefore, it was not further investigated.

**Subfraction GSC3-2** Chromatogram characteristics on normal phase TLC with 70% chloroform-petroleum ether (3 runs) showed one major yellow spot with the  $R_f$  value of 0.19 and one pale UV-active spot with the  $R_f$  value of 0.67. It was further separated by column chromatography over silica gel. Elution was conducted initially with 70% chloroform-petroleum ether, gradually enriched with chloroform, followed by increasing amount of methanol up to 50% methanol-chloroform to give forty five

fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford seven subfractions, as shown in **Table 79**.

**Table 79** Subfractions obtained from subfraction **GSC3-2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
GSC3-2-1	0.009	Yellow gum
GSC3-2-2	0.032	Orange-yellow gum
GSC3-2-3	0.020	Orange-yellow gum
GSC3-2-4	0.012	Orange-yellow gum
GSC3-2-5	0.130	Orange-yellow gum
GSC3-2-6	0.026	Orange-yellow gum
GSC3-2-7	0.018	Orange-yellow gum

**Subfraction GSC3-2-1** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.64. Further separation was performed by column chromatography over silica gel. Elution was conducted with 15% ethyl acetate-petroleum ether. Fractions with the similar chromatogram were combined and evaporated under reduced pressure to afford three subfractions, as shown in the **Table 80**.

**Table 80** Subfractions obtained from subfraction **GSC3-2-1** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
GSCA-1	0.005	Yellow gum
GSCA-2	0.014	Orange-yellow gum
GSCA-3	0.004	Orange-yellow gum

**Subfraction GSCA-1** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed one pale UV-active spot with the  $R_f$  value of 0.49. Because it was obtained in low quantity, it was not further investigated.

**Subfraction GSCA-2** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.24 and 0.40. Further purification was performed by precoated TLC, using 15% ethyl acetate-petroleum ether as a mobile phase (9 runs), to afford a yellow gum (**GF25**) in 8.7 mg. Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed only one UV-active spot with the  $R_f$  value of 0.29.

$[\alpha]_D^{29}$	-44° (c = 0.11, CHCl <sub>3</sub> )
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3461 (OH stretching), 2976, 2928 (CH stretching), 1741, 1686, 1627 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	359 (3.73), 316 (4.19), 302 (4.16), 276 (4.66), 267 (4.63), 227 (4.60)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (300 MHz)	11.90 ( <i>s</i> , 1H), 9.43 ( <i>s</i> , 1H), 6.97 ( <i>tm</i> , <i>J</i> = 7.5 Hz, 1H), 6.62 ( <i>d</i> , <i>J</i> = 10.2 Hz, 1H), 5.53 ( <i>d</i> , <i>J</i> = 10.2 Hz, 1H), 4.97 ( <i>tm</i> , <i>J</i> = 7.5 Hz, 1H), 4.36 ( <i>dd</i> , <i>J</i> = 4.5 and 1.2 Hz, 1H), 3.34 ( <i>s</i> , 3H), 3.31 ( <i>dd</i> , <i>J</i> = 14.4 and 7.5 Hz, 1H), 3.19 ( <i>dd</i> , <i>J</i> = 14.4 and 6.0 Hz, 1H), 3.09 ( <i>d</i> , <i>J</i> = 1.2 Hz, 1H), 3.06 ( <i>dd</i> , <i>J</i> = 16.5 and 7.5 Hz, 1H), 2.94 ( <i>dd</i> , <i>J</i> = 16.5 and 6.3 Hz, 1H), 2.90 ( <i>dd</i> , <i>J</i> = 6.3 and 4.5 Hz, 1H), 2.54 ( <i>d</i> , <i>J</i> = 8.7 Hz, 1H), 2.00 ( <i>dd</i> , <i>J</i> = 14.7 and 6.3 Hz, 1H), 1.75 ( <i>s</i> , 6H), 1.63 ( <i>s</i> , 3H), 1.47 ( <i>s</i> , 3H), 1.44 ( <i>dd</i> , <i>J</i> = 14.7 and 8.7 Hz, 1H), 1.40 ( <i>s</i> , 3H), 1.37 ( <i>s</i> , 3H), 1.17 ( <i>s</i> , 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm)	208.10, 195.11, 193.25, 160.92, 156.41, 155.48, 148.79, 139.89, 131.47, 126.46, 122.36, 115.24,

(75 MHz)	109.10, 103.23, 101.81, 88.30, 85.97, 82.05, 78.57, 74.13, 55.89, 48.47, 43.69, 43.56, 29.77, 28.60, 28.19, 27.56, 27.31, 25.63, 21.55, 19.96, 18.06, 9.26
DEPT (135°) (CDCl <sub>3</sub> )	CH 195.11, 148.79, 126.46, 122.36, 115.24, 74.13, 48.47, 43.69, 43.56
	CH <sub>2</sub> 27.56, 21.55, 19.96
	CH <sub>3</sub> 55.89, 29.77, 28.60, 28.19, 27.31, 25.63, 18.06, 9.26

**Subfraction GSCA-3** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed many pale UV-active spots. Because it was obtained in low quantity, it was not further investigated.

**Subfraction GSC3-2-2** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed six spots under UV-S with the R<sub>f</sub> values of 0.20, 0.32, 0.40, 0.45, 0.54 and 0.64. Because it was obtained in low quantity, it was not further investigated.

**Subfraction GSC3-2-3** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed three major UV-active spots under UV-S with the R<sub>f</sub> values of 0.09, 0.16 and 0.20. Its <sup>1</sup>H NMR spectrum indicated that it contained many compounds. Therefore, it was not further investigated.

**Subfraction GSC3-2-4** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed two major UV-active spots with the R<sub>f</sub> values of 0.22 and 0.36. Further purification was performed on precoated TLC, using 20% ethyl acetate-petroleum ether as a mobile phase (9 runs), to afford two bands.

**Band 1** was obtained as a yellow gum in 0.8 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (3 runs) showed one UV-active spot with the R<sub>f</sub> value of 0.29. Because it was obtained in low quantity, it was not further investigated.

**Band 2** was obtained as a yellow gum in 4.1 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (3 runs) showed one UV-active spot with the  $R_f$  value of 0.18. It was combined with subfraction **3B-6-2**.

**Subfraction GSC3-2-5** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.12 and 0.27 which were similar to those found in subfraction **GSC3-3-6**. Therefore, it was not investigated.

**Subfraction GSC3-2-6** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.22. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted with 80% methanol-water to give forty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in **Table 81**.

**Table 81** Subfractions obtained from subfraction **GSC3-2-6** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
3A-8-1	0.012	Yellow gum
3A-8-2	0.002	Yellow gum
3A-8-3	0.007	Yellow solid
3A-8-4	0.001	Yellow gum
3A-8-5	0.015	Yellow gum

**Subfraction 3A-8-1** Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (3 runs) showed no definite spot. Thus, it was not further investigated.

**Subfraction 3A-8-2** Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (3 runs) showed one major UV-active spot

with the same  $R_f$  value as a major spot in subfraction **3A-8-3**. Therefore, it was not purified.

**Subfraction 3A-8-3** Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.16. Its  $^1\text{H}$  NMR spectrum indicated that it contained the same major spot as subfraction **GSCB-2**. Therefore, it was combined with subfraction **GSCB-2**.

**Subfraction 3A-8-4** Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.16. Its  $^1\text{H}$  NMR spectrum was similar to that of subfraction **GSCB1-1**. Thus, it was combined with subfraction **GSCB1-1**.

**Subfraction 3A-8-5** Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.16. Further purification was performed on precoated TLC, using 2% ethyl acetate-chloroform as a mobile phase (10 runs), to afford two bands.

**Band 1** was obtained as a yellow gum in 3.8 mg. Chromatogram characteristics on normal phase TLC with 2% ethyl acetate-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.30 which was similar to that found in subfraction **GSCB2-3**. Therefore, they were combined.

**Band 2** was obtained as a yellow gum in 0.3 mg. Chromatogram characteristics on normal phase TLC with 2% ethyl acetate-chloroform (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.19 and 0.23. Because it was obtained in low quantity, it was not further investigated.

**Subfraction GSC3-2-7** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed three pale UV-active spots with the  $R_f$  values of 0.05, 0.14 and 0.21. Its  $^1\text{H}$  NMR spectrum indicated that it has many compounds. Therefore, it was not investigated.

**Subfraction GSC3-3** Chromatogram characteristics on normal phase TLC with 70% chloroform-petroleum ether (3 runs) showed one major yellow spot with the  $R_f$  value of 0.19. It was further separated by column chromatography over silica gel. Elution was conducted initially with 80% chloroform-petroleum ether, gradually enriched with chloroform and followed by increasing amount of methanol in



chloroform and finally with 60% methanol-chloroform to give fifty one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford twelve subfractions, as shown in **Table 82**.

**Table 82** Subfractions obtained from subfraction **GSC3-3** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
GSC3-3-1	0.001	Yellow gum
GSC3-3-2	0.011	Yellow gum
GSC3-3-3	0.019	Yellow gum
GSC3-3-4	0.011	Yellow gum
GSC3-3-5	0.016	Yellow gum
GSC3-3-6	0.155	Yellow gum
GSC3-3-7	0.088	Orange-yellow gum
GSC3-3-8	0.050	Orange-yellow gum
GSC3-3-9	0.071	Orange-yellow gum
GSC3-3-10	0.009	Orange-yellow gum
GSC3-3-11	0.044	Orange-yellow gum
GSC3-3-12	0.016	Orange-yellow gum

**Subfraction GSC3-3-1** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the same  $R_f$  value as **GF25**.

**Subfraction GSC3-3-2** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.49 and 0.65. Further purification was performed on precoated TLC, using 15% ethyl acetate-petroleum ether as a mobile phase (5 runs), to afford two bands.

**Band 1** was obtained as a yellow gum in 3.1 mg. Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed one major UV-active spot with the  $R_f$  value of 0.29 which was **GF25**.

**Band 2 (GF26)** was obtained as a yellow gum in 3.1 mg. Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether (4 runs) showed only one UV-active spot with the  $R_f$  value of 0.18.

$[\alpha]_D^{29}$	-600° (c = 0.11, CHCl <sub>3</sub> )
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3461 (OH stretching), 2926, 2848 (CH stretching), 1738, 1689, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	357 (4.09), 287 (4.19), 278 (4.21), 227 (4.45), 203 (4.43)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (300 MHz)	12.75 (s, 1H), 9.25 (s, 1H), 7.56 (d, $J$ = 7.0 Hz, 1H), 6.61 (d, $J$ = 9.9 Hz, 1H), 6.40 (tm, $J$ = 7.5 Hz, 1H), 5.53 (d, $J$ = 9.9 Hz, 1H), 5.10 (tm, $J$ = 7.8 Hz, 1H), 3.53 (dd, $J$ = 7.0 and 4.5 Hz, 1H), 3.28 (m, 2H), 2.75 (dd, $J$ = 15.5 and 7.5 Hz, 1H), 2.64 (dd, $J$ = 15.5 and 7.5 Hz, 1H), 2.57 (d, $J$ = 9.0 Hz, 1H), 2.37 (dd, $J$ = 14.0 and 4.5 Hz, 1H), 1.75 (s, 3H), 1.74 (s, 3H), 1.65 (s, 3H), 1.49 (s, 3H), 1.47 (dd, $J$ = 14.0 and 9.0 Hz, 1H), 1.45 (s, 3H), 1.32 (s, 3H), 1.30 (s, 3H)
<sup>13</sup> C NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (75 MHz)	203.05, 194.49, 178.85, 161.35, 157.69, 157.15, 146.54, 140.11, 135.62, 133.65, 132.01, 126.41, 121.81, 115.29, 108.08, 103.28, 100.36, 90.80, 84.00, 83.40, 78.88, 48.99, 46.86, 29.97, 28.97, 28.95, 28.41, 25.78, 25.27, 21.68, 18.18, 8.60
DEPT (135°) (CDCl <sub>3</sub> )	CH 194.49, 146.54, 126.41, 121.81, 115.29, 48.99, 46.86 CH <sub>2</sub> 28.97, 25.27, 21.68 CH <sub>3</sub> 28.95, 28.41, 25.78, 18.18, 8.60

**Subfraction GSC3-3-3** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed four major UV-active spots with the  $R_f$  values of 0.22, 0.28, 0.36 and 0.49. It was further purified by column chromatography on silica gel. Elution was conducted with 20% ethyl acetate-petroleum ether. Fractions with the similar chromatogram were combined and evaporated under reduced pressure to afford four subfractions. Their chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (3 runs) showed one pale yellow spot. Attempted purification on precoated TLC using 80% dichloromethane-petroleum ether (12 runs) was unsuccessful.

**Subfraction GSC3-3-4** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed two pale UV-active spots with the  $R_f$  values of 0.11 and 0.19. Because it was obtained in low quantity, it was not further investigated.

**Subfraction GSC3-3-5** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed four major UV-active spots with the  $R_f$  values of 0.13, 0.23, 0.30 and 0.39. It was further separated by column chromatography over silica gel. Elution was conducted initially with 20% ethyl acetate-petroleum ether, gradually enriched with ethyl acetate and followed by increasing amount of methanol in ethyl acetate and finally with 10% methanol-ethyl acetate to give sixteen fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 83**.

**Table 83** Subfractions obtained from subfraction **GSC3-3-5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
3B-5-1	0.010	Yellow gum
3B-5-2	0.004	Yellow gum
3B-5-3	0.007	Yellow gum

**Subfraction 3B-5-1** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed no major spots under UV-S. Thus, it was not further investigated.

**Subfraction 3B-5-2** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.07 and 0.20. Further purification was performed by precoated TLC, using 20% ethyl acetate-petroleum ether as a mobile phase (11 runs), to afford a yellow gum in 2.9 mg. Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.29 which was identical to the major spot of **band 2 of subfraction GSC-3-2-4**. So, they were combined and purified by precoated TLC, using 1% methanol-dichloromethane as a mobile phase (7 runs), to give a yellow gum (**GF27**) in 4.0 mg. Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane showed only one UV-active spot with the  $R_f$  value of 0.33.

$[\alpha]_D^{29}$	-62° (c = 0.09, CHCl <sub>3</sub> )
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3600-3200 (OH stretching), 2974, 2930 (CH stretching), 1739, 1697, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	363 (3.45), 316 (4.02), 276 (4.51), 204 (4.35)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (300 MHz)	12.10 (s, 1H), 6.64 (d, $J$ = 10.0 Hz, 1H), 5.91 (ddq, $J$ = 10.5, 6.0 and 1.5 Hz, 1H), 5.53 (d, $J$ = 10.0 Hz, 1H), 4.30 (dd, $J$ = 4.5 and 1.2 Hz, 1H), 4.05 (dd, $J$ = 10.5 and 2.4 Hz, 1H), 3.54 (dd, $J$ = 13.8 and 10.5 Hz, 1H), 3.31 (s, 3H), 3.29 (d, $J$ = 1.2 Hz, 1H), 3.00 (dd, $J$ = 15.0 and 10.5 Hz, 1H), 2.99 (dd, $J$ = 13.8 and 6.0 Hz, 1H), 2.86 (dd, $J$ = 15.0 and 2.4 Hz, 1H), 2.83 (dd, $J$ = 6.0 and 4.5 Hz, 1H), 2.51 (d, $J$ = 8.7 Hz, 1H), 1.98 (dd, $J$ = 15.0 and 6.0 Hz, 1H), 1.88 (brs, 3H), 1.71 (s, 3H), 1.70 (s, 3H), 1.50 (s, 3H), 1.46 (s, 3H), 1.35 (dd,

		$J = 15.0$ and $8.7$ Hz, 1H), 1.29 ( <i>s</i> , 3H), 1.14 ( <i>s</i> , 3H)
$^{13}\text{C}$ NMR ( $\text{CDCl}_3$ ) ( $\delta$ ppm) (75 MHz)		207.98, 194.16, 170.73, 160.50, 157.17, 156.97, 132.49, 131.44, 125.99, 115.23, 105.32, 103.01, 102.07, 88.14, 86.12, 81.49, 79.01, 78.20, 74.78, 73.57, 55.89, 47.60, 44.48, 43.89, 29.53, 28.78, 28.71, 28.56, 28.54, 28.16, 27.49, 25.58, 20.46, 20.08
DEPT ( $135^\circ$ ) ( $\text{CDCl}_3$ )	CH	132.49, 125.99, 115.23, 78.20, 74.78, 47.60, 44.48, 43.89
	CH <sub>2</sub>	28.16, 25.58, 20.08
	CH <sub>3</sub>	55.89, 29.53, 28.78, 28.71, 28.56, 28.54, 27.49, 20.46
EIMS ( $m/z$ ) (% rel. int.)		608 (9), 592 (15), 575 (23), 537 (59), 504 (100), 476 (52), 420 (55), 303 (37), 231 (55)

**Subfraction 3B-5-3** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed many spots. Because it was obtained in low quantity, it was not further investigated.

**Subfraction GSC3-3-6** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the same  $R_f$  value as **GF28**.

**Subfraction GSC3-3-7** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed only one UV-active spot with the  $R_f$  value of 0.29 (**GF28**).

$[\alpha]_D^{29}$		$-39^\circ$ ( $c = 0.22$ , $\text{CHCl}_3$ )
IR (neat) $\nu_{\text{max}}$ ( $\text{cm}^{-1}$ )		3600-2500 (OH stretching), 2978, 2922 (CH stretching), 1742, 1682, 1633 (C=O stretching)
UV ( $\text{CH}_3\text{OH}$ ) $\lambda_{\text{max}}$ (nm) ( $\log \epsilon$ )		362 (3.57), 316 (4.12), 276 (4.59), 267 (4.55)

$^1\text{H}$ NMR ( $\text{CDCl}_3$ ) ( $\delta$ ppm) (300 MHz)	11.95 ( <i>s</i> , 1H), 6.69 ( <i>tm</i> , $J = 6.6$ and $1.5$ Hz, 1H), 6.61 ( <i>d</i> , $J = 9.9$ Hz, 1H), 5.51 ( <i>d</i> , $J = 9.9$ Hz, 1H), 5.01 ( <i>tm</i> , $J = 6.3$ and $1.2$ Hz, 1H), 4.34 ( <i>dd</i> , $J =$ 4.5 and $1.2$ Hz, 1H), 3.31 ( <i>m</i> , 1H), 3.30 ( <i>s</i> , 3H), 3.20 ( <i>m</i> , 2H), 3.19 ( <i>d</i> , $J = 1.2$ Hz, 1H), 3.13 ( <i>m</i> , 1H), 2.83 ( <i>t</i> , $J = 5.4$ Hz, 1H), 2.49 ( <i>d</i> , $J = 8.4$ Hz, 1H), 1.96 ( <i>dd</i> , $J = 14.5$ and $6.3$ Hz, 1H), 1.94 ( <i>d</i> , $J$ $= 1.5$ Hz, 3H), 1.73 ( <i>s</i> , 3H), 1.62 ( <i>s</i> , 3H), 1.46 ( <i>s</i> , 3H), 1.41 ( <i>dd</i> , $J = 14.5$ and $8.4$ Hz, 1H), 1.39 ( <i>s</i> , 3H), 1.35 ( <i>s</i> , 3H), 1.15 ( <i>s</i> , 3H)
$^{13}\text{C}$ NMR ( $\text{CDCl}_3$ ) ( $\delta$ ppm) (75 MHz)	208.59, 193.81, 172.76, 160.89, 156.35, 155.66, 140.15, 131.13, 126.81, 126.30, 122.55, 115.31, 109.08, 103.04, 101.87, 88.44, 86.30, 82.14, 78.14, 73.96, 55.78, 47.89, 43.89, 43.50, 29.73, 28.55, 28.19, 28.00, 27.20, 25.61, 21.51, 20.50, 19.96, 18.04
DEPT ( $135^\circ$ ) ( $\text{CDCl}_3$ )	CH 140.15, 126.30, 122.55, 115.31, 73.96, 47.89, 43.89, 43.50 CH <sub>2</sub> 28.00, 21.51, 19.96 CH <sub>3</sub> 55.78, 29.73, 28.55, 28.19, 27.20, 25.61, 20.50, 18.04

**Subfraction GSC3-3-8** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the same  $R_f$  value as **GF28**.

**Subfraction GSC3-3-9** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.24. Further separation was performed by column chromatography over silica gel. Elution was conducted with 1% methanol-chloroform. Fractions with the similar chromatogram were combined and evaporated under reduced pressure to afford four subfractions, as shown in the **Table 84**.

**Table 84** Subfractions obtained from subfraction **GSC3-3-9** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
GSCB-1	0.023	Yellow gum
GSCB-2	0.017	Yellow gum
GSCB-3	0.018	Yellow gum
GSCB-4	0.014	Yellow gum

**Subfraction GSCB-1** Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.20. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted with 80% methanol-water to give thirty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 85**.

**Table 85** Subfractions obtained from subfraction **GSCB-1** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
GSCB-1-1	0.004	Yellow gum
GSCB-1-2	0.008	Yellow gum
GSCB-1-3	0.016	Yellow gum

**Subfraction GSCB-1-1** Chromatogram characteristics on normal phase TLC with 10% acetone-hexane showed one pale UV-active spot with the  $R_f$  value of 0.07. Its  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Therefore, it was not further investigated.

**Subfraction GSCB-1-2** Chromatogram characteristics on normal phase TLC with 10% acetone-hexane showed one major UV-active spot with the  $R_f$

value of 0.10 which was the same major spot as subfraction **GSCB-1-3**. Thus, it was not further investigated.

**Subfraction GSCB-1-3** Chromatogram characteristics on normal phase TLC with 10% acetone-hexane showed one major UV-active spot with the  $R_f$  value of 0.10. Further purification was performed on precoated TLC, using 2% ethyl acetate-chloroform as a mobile phase (11 runs), to afford a yellow gum in 6.4 mg. Chromatogram characteristics on normal phase TLC with 5% ethyl acetate-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.21. It was combined and further purified with subfraction **GSCB2-3**.

**Subfraction GSCB-2** Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.15. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted with 80% methanol-water to give thirty fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford two subfractions, as shown in **Table 86**.

**Table 86** Subfractions obtained from subfraction **GSCB-2** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
GSCB-2-1	0.016	Yellow gum
GSCB-2-2	0.004	Yellow gum

**Subfraction GSCB-2-1** Chromatogram characteristics on normal phase TLC with 3% ethyl acetate-chloroform (3 runs) showed many spots. Therefore, it was not further investigated.

**Subfraction GSCB-2-2** Chromatogram characteristics on normal phase TLC with 3% ethyl acetate-chloroform (3 runs) showed one major UV-active spot with the  $R_f$  value of 0.43. Further purification was performed by precoated TLC, using 3% ethyl acetate-chloroform as a mobile phase (14 runs), to afford a yellow gum in 8.5 mg. Chromatogram characteristics on normal phase TLC with 3% ethyl



acetate-chloroform (2 runs) showed only one UV-active spot with the  $R_f$  value of 0.26. Its NMR data indicated this compound was **GF28**.

**Subfraction GSCB-3** Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.15. Further purification was performed by precoated TLC, using 2% ethyl acetate-chloroform as a mobile phase (10 runs), to afford two bands.

**Band 1** was obtained as a yellow gum in 6.0 mg. Chromatogram characteristics on normal phase TLC with 2% ethyl acetate-chloroform (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.25 which was **GF28**.

**Band 2** was obtained as a yellow gum in 7.1 mg. Chromatogram characteristics on normal phase TLC with 2% ethyl acetate-chloroform (2 runs) showed one major yellow spot with the  $R_f$  value of 0.24. Further purification was performed by precoated TLC, using 15% ethyl acetate-petroleum ether as a mobile phase (15 runs), to afford a yellow gum (**GF29**) in 4.0 mg. Chromatogram characteristics on normal phase TLC with 3% ethyl acetate-chloroform (2 runs) showed only one yellow spot with the  $R_f$  value of 0.23.

$[\alpha]_D^{29}$	-541° (c = 0.19, CHCl <sub>3</sub> )
IR (neat) $\nu_{\max}$ (cm <sup>-1</sup> )	3500-2500 (OH stretching), 2974, 2928 (CH stretching), 1738, 1692, 1633 (C=O stretching)
UV (CH <sub>3</sub> OH) $\lambda_{\max}$ (nm) (log $\epsilon$ )	359 (4.04), 288 (4.13), 219 (4.29), 203 (4.33)
<sup>1</sup> H NMR (CDCl <sub>3</sub> ) ( $\delta$ ppm) (300 MHz)	12.80 (s, 1H), 7.55 (d, $J$ = 6.9 Hz, 1H), 6.55 (d, $J$ = 9.9 Hz, 1H), 6.06 (tm, $J$ = 7.0 Hz, 1H), 5.45 (d, $J$ = 9.9 Hz, 1H), 5.03 (tm, $J$ = 6.6 Hz, 1H), 3.50 (dd, $J$ = 6.9 and 4.5 Hz, 1H), 3.31 (dd, $J$ = 14.7 and 8.4 Hz, 1H), 3.14 (dd, $J$ = 14.7 and 5.1 Hz, 1H), 2.95 (d, $J$ = 7.0 Hz, 2H), 2.53 (d, $J$ = 9.0 Hz, 1H), 2.32 (dd, $J$ = 13.5 and 4.5 Hz, 1H), 1.74 (s, 6H), 1.71 (s, 3H), 1.64 (s, 3H), 1.42 (s, 3H), 1.42 (dd, $J$ = 13.5 and 9.0 Hz, 1H), 1.38 (s, 3H), 1.29

	(s, 3H)
$^{13}\text{C}$ NMR ( $\text{CDCl}_3$ ) ( $\delta$ ppm) (75 MHz)	203.27, 178.95, 170.32, 161.17, 157.59, 157.23, 137.51, 135.34, 133.31, 131.51, 127.88, 126.04, 122.10, 115.37, 108.04, 103.14, 100.51, 90.84, 83.97, 83.77, 78.59, 48.98, 46.81, 29.86, 29.27, 28.84, 28.47, 28.23, 25.74, 25.16, 21.60, 20.75, 18.12
DEPT (135°) ( $\text{CDCl}_3$ )	CH 137.51, 135.34, 126.04, 122.10, 115.37, 48.98, 46.81 CH <sub>2</sub> 29.27, 25.16, 21.60 CH <sub>3</sub> 29.86, 29.27, 28.84, 28.47, 28.23, 25.74, 20.75, 18.12

**Subfraction GSCB-4** Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane (2 runs) showed one pale UV-active spot with the  $R_f$  value of 0.15 which was similar to that of subfraction **GSCB-3**. Therefore, it was not further investigated.

**Subfraction GSC3-3-10** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the same  $R_f$  value as that in subfraction **GSC3-3-9**. Therefore, it was not further investigated.

**Subfraction GSC3-3-11** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed two major UV-active spots with the  $R_f$  values of 0.11 and 0.22. Its  $^1\text{H}$  NMR spectrum indicated that it was a mixture of **GF28** and **GF29**. Thus, it was not further investigated.

**Subfraction GSC3-3-12** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed three pale UV-active spots with the  $R_f$  values of 0.04, 0.10 and 0.19. Thus, it was not further investigated.

**Subfraction GSC3-4** Chromatogram characteristics on normal phase TLC with 70% chloroform-petroleum ether (3 runs) showed no major spots. Therefore, it was not further investigated.

**Fraction GSC4** Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane showed none of well-separated spots under UV-S. Thus, it was not further investigated.

**Fraction GSC5** Chromatogram characteristics on normal phase TLC with 2% methanol-dichloromethane showed none of well-separated spots under UV-S. But the  $^1\text{H}$  NMR spectrum displayed signals of aromatic protons. So, it was further separated by column chromatography over Sephadex LH-20. Elution was conducted with 50% methanol-dichloromethane to give nineteen subfractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions. They were not further investigated because their chromatograms on the normal phase TLC using 3% methanol-dichloromethane showed none of well-separated spots under UV-S. In addition, their  $^1\text{H}$  NMR spectra indicated that they contained many compounds.

### 2.4.3 Investigation of the chloroform-insoluble part

**Chloroform-insoluble part (GSM)** was obtained as a brown gum in 8.23 g. Chromatogram characteristics on normal phase TLC with 10% ethyl acetate-petroleum ether showed one pale yellow spot with the  $R_f$  value of 0.34 and one brown spot at baseline. Further separation with column chromatography over Sephadex LH-20 was performed. Elution was conducted with pure methanol to give thirty seven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five fractions, as shown in the **Table 87**.

**Table 87** Fractions obtained from **GSM** by column chromatography over Sephadex LH-20

Fraction	Weight (g)	Physical appearance
GSM1	0.157	Brown gum
GSM2	6.533	Brown-yellow gum
GSM3	0.515	Brown-yellow gum
GSM4	0.527	Brown-yellow solid
GSM5	0.151	Brown solid

**Fraction GSM1** Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane showed no major spots under UV-S. Thus, it was not further investigated.

**Fraction GSM2** Chromatogram characteristics on normal phase TLC with 5% methanol-dichloromethane showed one pale UV-active spot with the  $R_f$  value of 0.54. It (3.79 g) was further separated by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol to afford twenty two fractions. Fractions with the similar chromatogram were combined and evaporated under reduced pressure to dryness to afford three subfractions. Further purification of the second subfraction (0.753 g) with column chromatography over silica gel was performed. Elution was initially conducted with 5% methanol-dichloromethane and gradually enriched with pure methanol to give twenty seven fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford three subfractions, as shown in the **Table 88**.

**Table 88** Subfractions obtained from **the second subfraction** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
GSMA-1	0.007	Yellow gum
GSMA-2	0.035	Yellow gum
GSMA-3	0.591	Yellow gum

**Subfraction GSMA-1** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed many pale UV-active spots. Because it was obtained on low quantity, it was not further investigated.

**Subfraction GSMA-2** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed one major UV-active spot with the same  $R_f$  value as **GF28**.

**Subfraction GSMA-3** Chromatogram characteristics on normal phase TLC with 20% ethyl acetate-petroleum ether (2 runs) showed one pale UV-active spot at baseline. Its  $^1\text{H}$  NMR spectrum indicated that it contained sugars as major constituents. Therefore, it was not further investigated.

**Fraction GSM3** Chromatogram characteristics on normal phase TLC with  $\text{CHCl}_3$  : MeOH :  $\text{H}_2\text{O}$  (65 : 35 : 3) showed two major UV-active spots with the  $R_f$  value of 0.40 and 0.47. It (239 mg) was further separated by column chromatography over Sephadex LH-20. Elution was conducted with pure methanol to give twenty three fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in the **Table 89**.

**Table 89** Subfractions obtained from fraction **GSM3** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
GSM3-1	0.157	Brown gum
GSM3-2	6.533	Brown-yellow gum
GSM3-3	0.276	Brown-yellow gum
GSM3-4	0.103	Brown-yellow solid
GSM3-5	0.048	Brown solid

**Subfraction GSM3-1** Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane showed no major spots under UV-S. Therefore, it was not further purified.

**Subfraction GSM3-2** Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane showed one major UV-active spot with the  $R_f$  value of 0.16. It was further acetylated with acetic anhydride (3.0 ml) and pyridine (0.8 ml). The mixture was stirred at room temperature for 24 hours. The reaction mixture was extracted with ethyl acetate (3x20 ml). The ethyl acetate layer was consecutively washed with 10% hydrochloric acid (2x20 ml), 10% sodium bicarbonate (3x20 ml) and water (2x20 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo* to yield a green-yellow gum in 40.0 mg. Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane showed three major UV-active spots with the  $R_f$  values of 0.07, 0.16 and 0.50. It was further purified by column chromatography on silica gel. Elution was conducted initially with pure dichloromethane and gradually enriched with pure methanol to afford twenty nine fractions. Fractions with the similar chromatogram were combined and evaporated under reduced pressure to afford nine subfractions. Their chromatogram characteristics on normal phase TLC with 0.5% methanol-dichloromethane (5 runs) showed many UV-active spots without any major spots. Therefore, they were not further investigated.

**Subfraction GSM3-3** Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane showed two major UV-active spots with

the  $R_f$  values of 0.11 and 0.16. It was further purified by column chromatography on silica gel. Elution was conducted with 10% methanol-dichloromethane to afford twenty four fractions. Fractions with the similar chromatogram were combined and evaporated under reduced pressure to afford five subfractions. Their chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane (2 runs) showed many UV-active spots without any major spots. In addition, their  $^1\text{H}$  NMR spectra showed that they contained many compounds. Therefore, they were not further investigated.

**Subfraction GSM3-4** Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane showed one major UV-active spot with the  $R_f$  value of 0.11 and one pale UV-active spot with the  $R_f$  value of 0.16. It was further subjected to acetylation reaction as described in subfraction **GSM3-2**. Chromatogram characteristics on normal phase TLC with 1% methanol-dichloromethane showed none of well-separated spots under UV-S. Therefore, it was not further investigated.

**Subfraction GSM3-5** Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane showed one major UV-active spot with the  $R_f$  value of 0.11 which was similar to that of subfraction **GSM3-4**. Thus, it was not purified.

**Fraction GSM4** Chromatogram characteristics on normal phase TLC with  $\text{CHCl}_3 : \text{MeOH} : \text{H}_2\text{O}$  (65 : 35 : 3) showed two major UV-active spots with the  $R_f$  values of 0.77 and 0.86. Further separation with column chromatography over Sephadex LH-20 was performed. Elution was conducted with pure methanol to give thirty four fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford five subfractions, as shown in the **Table 90**.

**Table 90** Subfractions obtained from fraction **GSM4** by column chromatography over Sephadex LH-20

Subfraction	Weight (g)	Physical appearance
GSM4-1	0.020	Brown gum
GSM4-2	0.022	Brown gum
GSM4-3	0.272	Brown gum
GSM4-4	0.192	Brown gum
GSM4-5	0.006	Brown solid

**Subfraction GSM4-1** Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane showed no major spots under UV-S. Therefore, it was not further investigated.

**Subfraction GSM4-2** Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane showed two major UV-active spots with the same  $R_f$  values as those found in subfraction **GSM4-3**. Thus, it was not further purified.

**Subfraction GSM4-3** Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane showed three major UV-active spots with the  $R_f$  values of 0.34, 0.44 and 0.54. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 40% MeOH/H<sub>2</sub>O and gradually decreased the polarity until pure methanol to give forty eight fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions, as shown in the **Table 91**.



**Table 91** Subfractions obtained from subfraction **GSM4-3** by column chromatography over reverse-phase C<sub>18</sub> silica gel

Subfraction	Weight (g)	Physical appearance
GSM4-3-1	0.018	Brown gum
GSM4-3-2	0.167	Yellow-brown gum
GSM4-3-3	0.065	Yellow gum
GSM4-3-4	0.006	Yellow gum
GSM4-3-5	0.008	Yellow gum
GSM4-3-6	0.006	Yellow solid mixed with white solid

**Subfraction GSM4-3-1** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane (2 runs) showed no major spots under UV-S. Therefore, it was not further investigated.

**Subfraction GSM4-3-2** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane (2 runs) showed one pale UV-active spot with the R<sub>f</sub> value of 0.27. It (56 mg) was further separated by column chromatography over reverse-phase C<sub>18</sub> silica gel. Elution was conducted with 50% MeOH/H<sub>2</sub>O to give thirty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions. Their chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane showed one major UV-active spot with the R<sub>f</sub> value of 0.18. But their chromatogram characteristics on reverse phase TLC with 50% methanol-water showed two major UV-active spots with the R<sub>f</sub> values of 0.15 and 0.31. Therefore, they were not further investigated.

**Subfraction GSM4-3-3** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane (2 runs) showed three major UV-active spots with the R<sub>f</sub> values of 0.21, 0.27 and 0.37. Its <sup>1</sup>H NMR spectrum displayed that it contained many compounds. Therefore, it was not further investigated.

**Subfraction GSM4-3-4** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane (2 runs) showed one major UV-active spot with the R<sub>f</sub> value of 0.37. However, the <sup>1</sup>H NMR spectrum indicated that it

contained many compounds. Therefore, it was not further investigated because of low quantity.

**Subfraction GSM4-3-5** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.34. It was combined with subfraction **GSM4-4-5**.

**Subfraction GSM4-3-6** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane (2 runs) showed no major spots under UV-S. Therefore, it was not further investigated.

**Subfraction GSM4-4** Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane showed four major UV-active spots with the  $R_f$  values of 0.03, 0.36, 0.44 and 0.52. It was further separated by column chromatography over reverse-phase  $C_{18}$  silica gel. Elution was conducted initially with 40% MeOH/H<sub>2</sub>O and gradually decreased the polarity until pure methanol to give forty six fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford six subfractions, as shown in the **Table 92**.

**Table 92** Subfractions obtained from subfraction **GSM4-4** by column chromatography over reverse-phase  $C_{18}$  silica gel

Subfraction	Weight (g)	Physical appearance
GSM4-4-1	0.036	Brown gum
GSM4-4-2	0.068	Yellow-brown gum
GSM4-4-3	0.034	Yellow-brown gum
GSM4-4-4	0.048	Yellow-brown gum
GSM4-4-5	0.006	Yellow-brown gum
GSM4-4-6	0.003	Yellow-brown gum

**Subfraction GSM4-4-1** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane showed no definite spot. Therefore, it was not further investigated.

**Subfraction GSM4-4-2** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane showed one major UV-active spot with the  $R_f$  value of 0.28. However, the  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Therefore, it was not further investigated.

**Subfraction GSM4-4-3** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane showed two major UV-active spots with the  $R_f$  values of 0.19 and 0.28. However, the  $^1\text{H}$  NMR spectrum indicated that it contained many compounds. Therefore, it was not further investigated.

**Subfraction GSM4-4-4** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane showed one major UV-active spot with the  $R_f$  value of 0.19. Its  $^1\text{H}$  NMR spectrum indicated that it might be a mixture of biflavone derivatives. Therefore, it was not further investigated.

**Subfraction GSM4-4-5** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane showed one major UV-active spot with the  $R_f$  value of 0.37. Further separation with column chromatography over reverse-phase  $\text{C}_{18}$  silica gel was performed. Elution was conducted with 50% methanol-water to give forty one fractions. All fractions were examined by TLC, combined on the basis of their chromatogram characteristics and then evaporated to dryness *in vacuo* to afford eight subfractions. Their chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane (2 runs) showed one major UV-active spot with the  $R_f$  value of 0.26. Their  $^1\text{H}$  NMR spectra indicated that they contained a mixture of biflavone derivatives. Therefore, they were not further investigated.

**Subfraction GSM4-4-6** Chromatogram characteristics on normal phase TLC with 8% methanol-dichloromethane showed one pale UV-active spot with the  $R_f$  value of 0.37. Therefore, it was not further investigated because of low quantity.

**Subfraction GSM4-5** Chromatogram characteristics on normal phase TLC with 10% methanol-dichloromethane showed no major spots under UV-S. Thus, it was not further investigated.

**Fraction GSM5** Chromatogram characteristics on normal phase TLC with  $\text{CHCl}_3 : \text{MeOH} : \text{H}_2\text{O}$  (65 : 35 : 3) showed no major spots under UV-S. Therefore, it was not further investigated.