CHAPTER 1.2

EXPERIMENTAL

1.2.1 Chemicals and Instruments

Melting points were recorded in °C and were measured on a digital Electrothermal 9100 Melting Point Apparatus. Infrared spectra (IR) were recorded using FTS165 FT-IR spectrometer. Major bands (ν) were recorded in wave number (cm^{-1}). Nuclear magnetic resonance spectra were recorded on 500 MHz Varian UNITY INOVA spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in deuterochloroform or pentadeuteropyridine solution and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). Optical rotation was measured in methanol, chloroform, ethanol or pyridine solution with sodium D line (590 nm) on either an AUTOPOL^{R} II automatic polarimeter or a JASCO DIP-370 polarimeter. The solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except for petroleum ether and diethyl ether which were analytical grade reagent. Quick column chromatography, thin-layer chromatography (TLC) and precoated thin-layer chromatography were performed on silica gel 60 GF_{254} (Merck) or reverse-phase C-18. Column chromatography was performed on silica gel (Merck) type 100 (70-223 mesh ASTM) or reverse-phase C_{18}.

1.2.2 Plant Material

Twigs and stems of *C. serratum* were collected from Satoon Province in Thailand. The plant was identified by Ajarn Charan Leeratiwong, Department of Biology, Faculty of Science, Prince of Songkla University.

1.2.3 Extraction

Twigs and stems of *C. serratum* (6,200 g) were extracted with methanol (23.5 L) over period of 7 days at room temperature for 4 times. The filtered solution was then evaporated to dryness under reduced pressure to afford crude methanol extracts as a brown viscous-gum in 379.43 g.

1.2.4 Isolation and Chemical Investigation of the Crude Methanol Extract from the Twigs and Stems of C. serratum

The crude methanol extract of *C. serratum* was tested for solubility in various solvents as shown in **Table 2**.

Table 2 Solubility of the crude methanol extract of *C. serratum*

Solvent	Solubility at room temperature
Petroleum ether	+ yellow solution
$CHCl_3$	+ yellow solution
EtOAc	+ yellow solution
MeOH	++ yellow solution mixed with yellow solid

Table 2 (continued)

Solvent	Solubility at room temperature
H_2O	++ brown solution
10% HCl	++ brown solution
10% NaOH	+++ yellow solution
10% $NaHCO_3$	+++ brown solution

+ partially soluble ++ moderately soluble +++ well soluble

It was concluded, based on solubility results, that the major component in the crude methanol extract was polar and acidic.

The crude methanol extract (104.51 g) was purified by quick column chromatography over silica gel. Elution was conducted initially with hexane, followed by increasing amount of ethyl acetate in hexane and finally with methanol. Fractions with the similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford thirteen fractions, as shown in **Table 3**.

Table 3 Fractions obtained from the crude methanol extract by quick column chromatography over silica gel

Fraction	Weight (g)	Physical appearance
A	0.024	colourless solid
B	0.008	colourless solid
C	0.004	yellow solid
D	0.594	yellow semi-solid
E	0.129	yellow semi-solid
F	1.119	yellow semi-solid

Table 3 (continued)

Fraction	Weight (g)	Physical appearance
G	0.725	green semi-solid
H	0.423	green semi-solid
I	4.765	green viscous liquid
J	4.560	green semi-solid
K	24.873	red-brown viscous liquid
L	41.496	red-brown viscous liquid
M	9.243	red-brown viscous liquid

Fraction A contained two spots on normal phase TLC with hexane as a mobile phase which showed one UV- active spot with the R_f value of 0.77. After spraying the TLC plate with vanilin sulfuric acid reagent and subsequently heating, one additional spot was observed with the R_f value of 0.75. It was not further purification because it was obtained in low quantity.

Fractions B-C showed no definite spot on normal phase TLC and they were not investigated further.

Fraction D was separated by column chromatography over silica gel. Elution was conducted initially with hexane, followed by increasing amount of ethyl acetate in hexane and finally with ethyl acetate. Fractions with the similar TLC chromatogram were combined and evaporated to dryness *in vacuo* to afford **subfractions D1-D7**, as shown in **Table 4**.

Table 4 Subfractions obtained from **fraction D** by column chromatography over sililca gel

Subfraction	Weight (g)	Physical appearance
D1	0.001	yellow viscous liquid
D2	0.371	yellow liquid
D3	0.034	yellow liquid
D4	0.067	yellow-brown solid
D5	0.056	yellow-brown solid
D6	0.014	yellow-brown solid
D7	0.015	yellow semi-solid

Subfraction D1 showed no definite spot on normal phase TLC. Thus, it was not investigated.

Subfraction D2 was further rechromatography over silica gel. Elution was conducted initially with hexane, followed by increasing amount of ethyl acetate in hexane and finally with ethyl acetate. Fractions with the similar TLC chromatogram were combined and evaporated to dryness *in vacuo* to afford four subfractions, as shown in **Table 5**.

Table 5 Subfractions obtained from **subfraction D2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D2.1	0.193	yellow liquid
D2.2	0.147	yellow liquid

Table 5 (continued)

Subfraction	Weight (g)	Physical appearance
D2.3	0.003	yellow viscous-liquid
D2.4	0.001	yellow viscous-liquid

Subfraction D2.1 was separated by column chromatography over silica gel, eluting with 30% dichloromethane in hexane. All fractions were examined by TLC and combined on the basis of their TLC chromatograms. The solvents were evaporated to dryness under reduced pressure to afford three subfractions, as shown in **Table 6**.

Table 6 Subfractions obtained from **subfraction D2.1** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
D2.1A	0.046	yellow viscous- liquid
D2.1B	0.098	yellow viscous- liquid
D2.1C	0.006	yellow viscous- liquid

Subfraction D2.1A showed no definite spot on normal phase TLC. No further separation was then carried out.

Subfractions D2.1B and **D2.2** were combined because of their similar TLC chromatograms and then chromatographed by column chromatography over silica gel, eluting with hexane and finally with 1% acetone in hexane. Fractions with the similar TLC chromatogram were combined and evaporated to dryness *in vacuo* to afford three subfractions, as shown in **Table 7**.

Table 7 Subfractions obtained from **subfractions D2.1B** and **D2.2** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
DA	0.030	Colourless liquid
DB	0.069	yellow viscous- liquid
DC	0.090	yellow viscous- liquid

Subfractions DA-DB showed no definite spot on normal phase TLC. Thus, they were not investigated.

Subfraction DC was recrystallized from the dichloromethane to afford **CS-S3** as a white solid (2.0 mg). Its chromatogram on normal phase TLC using 3% ethyl acetate in hexane (4 runs) as a mobile phase showed a single spot with the R_f value of 0.42 which visualized as a purple spot in vanillin sulfuric acid reagent and subsequently heating.

Compound CS-S3 (White solid)

$[\alpha]_D^{30}$ **:** +34.00° (c = 0.10, $CHCl_3$)

mp **:** 199.0 - 202.0 °C

IR (KBr) ν (cm^{-1}) **:** 2937, 2863 (C-H stretching), 1709 (C=O stretching)

^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz) **:** 5.29 (*d*, *J* = 6.5 Hz, 1H), 2.72 (*ddd*, *J* = 15.5, 13.5 and 6.5 Hz, 1H), 2.40 (*ddd*, *J* = 15.5, 5.5 and 3.5 Hz, 1H), 2.09 (*ddd*, *J* = 13.5, 6.5 and 3.5

			Hz, 1H), 2.07 (*m*, 1H), 1.86 (*m*, 1H), 1.78 (*m*, 1H), 1.70 (*m*, 2H), 1.60 (*m*, 2H), 1.46 (*m*, 1H), 1.44 (*m*, 1H), 1.38 (*m*, 1H), 1.35 (*m*, 1H), 1.34 (*m*, 1H), 1.30 (*m*, 1H), 1.26 (*m*, 1H), 1.24 (*m*, 1H), 1.23 (*m*, 2H), 1.21 (*s*, 3H), 1.07 (*s*, 6H), 0.98 (*q*, J = 9.5 Hz, 1H), 0.89 (*d*, *J* = 6.5 Hz, 3H), 0.83 (*d*, *J* = 6.0 Hz, 3H), 0.81 (*s*, 3H), 0.79 (*s*, 3H), 0.77 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		**:**	217.30, 147.41, 115.61, 59.59, 53.26, 51.99, 47.64, 42.80, 41.04, 39.30, 38.18, 36.75, 36.64, 36.07, 35.86, 34.89, 30.77, 29.62, 28.18, 26.27, 25.53, 22.99, 22.57, 22.10, 22.04, 21.64, 20.15, 16.95, 15.29, 13.98
DEPT 135°	**CH3**	**:**	25.53, 22.99, 22.10, 22.04, 21.64, 16.95, 15.29, 13.98
	CH2	**:**	36.64, 36.07, 35.86, 34.89, 29.62, 28.18, 26.27, 22.57, 20.15
	CH	**:**	115.61, 59.59, 53.26, 51.99, 41.04, 30.77

Subfraction D2.1C showed no definite spot on normal phase TLC. Therefore, it was not investigated.

Subfractions D2.3-D2.4 showed no definite spot on normal phase TLC. Further investigations were not carried out.

Subfractions D3-D5 contained many spots on normal phase TLC. Therefore, they were not further investigated.

Subfractions D6-D7 showed no definite spot on normal phase TLC. Further purifications were not performed.

Fracton E showed no definite spot on normal phase TLC. Thus, it was not investigated.

Fraction F was fractionated by column chromatography over silica gel. Elution was conducted initially with hexane, followed by increasing amount of ethyl acetate in hexane and finally with ethyl acetate. All fractions were examined by TLC and combined on the basis of TLC chromatogram. The solvents were evaporated to dryness *in vacuo* to afford eleven subfractions, as shown **Table 8**.

Table 8 Subfractions obtained from **fraction F** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
FA	0.008	white solid
FB	0.026	yellow solid
FC	0.039	yellow solid
FD	0.183	yellow semi-solid
FE	0.031	yellow solid
FF	0.014	yellow viscous-liquid
FG	0.139	yellow solid
FH	0.061	yellow solid
FI	0.188	yellow viscous-liquid
FJ	0.220	yellow viscous-liquid
FK	0.008	yellow viscous-liquid

Subfraction FA showed no definite spot on normal phase TLC. Thus, investigation was not carried out.

Subfractions FB-FC contained many spots on normal phase TLC without major component. Therefore, further separations were not performed.

Subfraction FD was chromatographed by column chromatography over silica gel. Elution was conducted initially with 50% dichloromethane in hexane, followed by increasing amount of dichloromethane in hexane and finally with dichloromethane. Fractions with the similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford five subfractions, as shown in **Table 9**.

Table 9 Subfractions obtained from **subfraction FD** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
FD.1	0.004	yellow viscous-liquid
FD.2	0.023	colourless viscous-liquid
FD.3	0.032	yellow viscous-liquid
FD.4	0.001	colourless viscous-liquid
FD.5	0.037	colourless viscous-liquid

Subfraction FD.1 showed no definite spot on normal phase TLC. Thus, it was not investigated.

Subfraction FD.2 was subjected to preparative TLC over silica gel plates using 30% dichloromethane in hexane as an eluent (4 runs) to afford **subfractions A-D**.

Subfraction A (2.0 mg) contained two spots on normal phase TLC. Because of low quantity, it was not further separated.

Subfraction B (1.0 mg) showed no definite spot on normal phase TLC. Therefore, it was not further investigated.

Subfraction C (2.0 mg) contained two spots on normal phase TLC. Because of low quantity, it was not carried out.

Subfraction D (2.0 mg) contained three spots on normal phase TLC. Because of low quantity, it was not further separated.

Subfraction FD.3 contained many spots on normal phase TLC. Thus, it was not investigated.

Subfractions FD.4-FD.5 showed no definite spot on normal phase TLC. Therefore, they were not investigation further.

Subfractions FE-FF showed no definite spot on normal phase TLC. Therefore, further separations were not performed.

Subfraction FG was fractionated by column chromatography over silica gel. Elution was conducted initially with 20% dichloromethane in hexane, followed by increasing amount of dichloromethane in hexane and finally with 50% dichloromethane in hexane. All fractions were examined by TLC and combined on the basis of TLC chromatogram. The solvents were evaporated to dryness under reduced pressure to afford seven subfractions, as shown in **Table 10**.

Table 10 Subfractions obtained from **subfraction FG** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
FG.1	0.009	yellow viscous-liquid
FG.2	0.003	yellow solid
FG.3	0.009	yellow viscous-liquid
FG.4	0.005	yellow viscous-liquid
FG.5	0.004	yellow solid
FG.6	0.019	yellow solid
FG.7	0.013	white solid

Subfractions FG.1-FG.2 showed no definite spot on normal phase TLC. They were not further purification because they were obtained in low quantity.

Subfraction FG.3 was therefore rechromatographed by preparative TLC on silica gel plates, with 2% ethyl acetate in petroleum ether (15 runs) to afford **subfractions FG.3A-FG.3C**.

Subfraction FG.3A was a yellow solid (2.0 mg). It contained two spots on normal phase TLC. Because of low quantity, it was not further separated.

Subfraction FG.3B was a yellow solid (1.0 mg). It contained three spots on normal phase TLC. Because of low quantity, it was not further separated.

Subfraction FG.3C was a yellow solid (2.0 mg). It contained two spots on normal phase TLC. Because of low quantity, it was not further separated.

Subfractions FG.4-FG.6 contained many spots without major component. Further separations were not carried out.

Subfraction FG.7 showed no definite spot on normal phase TLC. Thus, it was not investigated.

Subfraction FH showed no definite spot on normal phase TLC. Thus, further separation was not performed.

Subfraction FI was crystallized upon standing at room temperature to afford **CS-S1** (43.0 mg) as a major component separated from fraction C.

Fraction G was recrystallized from the chloroform in methanol in hexane to give **CS-S1** as a colourless needle (33.0 mg). It showed a single spot on normal phase TLC with 30% ethyl acetate in hexane with the R_f value of 0.34 which was visualized as a purple spot in vanillin sulfuric acid reagent and subsequently heating.

Compound CS-S1 (Colourless needles)

$[\alpha]_D^{30}$	**:**	+10.91° (c = 0.11, $CHCl_3$)
mp	**:**	156.5 - 159.5 °C
IR (KBr) ν (cm^{-1})	**:**	3454 (O-H stretching), 2945, 2871 (C-H stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz)	**:**	5.16 (*dd* , *J* = 15.0 and 8.5 Hz, 1H), 5.16 (*brm*, 1H), 5.03 (*dd*, *J* = 15.0 and 8.5 Hz, 1H), 3.61 (*tt*, *J* = 11.0 and 4.5 Hz, 1H), 2.03 (*m*, 1H) 2.00 (*m*, 1H), 1.80 (*m*, 2H), 1.79 (*m*, 1H), 1.74 (*m*, 2H), 1.70 (*m*, 1H), 1.64 (*m*, 1H), 1.59 (*m*, 1H), 1.52 (*m*, 2H), 1.44 (*m*, 1H), 1.38 (*m*, 1H), 1.36 (*m*, 1H), 1.34 (*m*, 2H), 1.30 (*m*, 1H), 1.26 (*m*, 1H), 1.25 (*m*, 1H), 1.24 (*m*, 1H), 1.22 (*m*, 1H), 1.06 (*m*, 1H), 1.03 (*d*, *J* = 6.5 Hz, 3H), 0.85 (*d*, *J* = 6.5

			Hz, 3H), 0.80 (*d*, *J* = 6.0 Hz, 3H), 0.81 (*t*, *J* = 7.5 Hz, 3H), 0.80 (*s*, 3H), 0.55 (*s*, 3H)
^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz)		**:**	139.55, 138.17, 129.39, 117.44, 71.05, 55.84, 55.10, 51.23, 49.40, 43.26, 40.84, 40.22, 39.42, 37.96, 37.11, 34.19, 31.86, 31.45, 29.61, 28.51, 25.39, 23.00, 21.52, 21.36, 21.10, 18.97, 13.04, 12.25, 12.03
DEPT 135°	**CH_3**	**:**	21.36, 21.10, 18.97, 13.04, 12.25, 12.03
	CH2	**:**	39.42, 37.96, 37.11, 31.45, 29.61, 28.51, 25.39, 23.00, 21.52
	CH	**:**	138.17, 129.39, 117.44, 71.05, 55.84, 55.10, 51.23, 49.40, 40.84, 40.22, 31.86

The mother liquor of **fraction G** was separated by column chromatography over silica gel. Elution was conducted initially with hexane, followed by increasing amount of ethyl acetate in hexane and finally with methanol. Fractions with the similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford fifteen subfractions, as shown in **Table 11**.

Table 11 Subfractions obtained from the mother liquor of **fraction G** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
MG.1	0.019	yellow viscous- liquid
MG.2	0.029	brown viscous -liquid
MG.3	0.022	green semi - liquid
MG.4	0.041	green semi - liquid
MG.5	0.022	green semi - liquid
MG.6	0.036	green semi - liquid
MG.7	0.012	green solid
MG.8	0.014	green semi- solid
MG.9	0.021	green semi- solid
MG.10	0.005	green viscous - liquid
MG.11	0.015	green viscous - liquid
MG.12	0.013	green viscous - liquid
MG.13	0.032	green viscous - liquid
MG.14	0.048	green viscous - liquid
MG.15	0.059	green viscous - liquid

Subfractions MG.1-MG.2 contained many spot on normal phase TLC, overlapping with other. Therefore, investigations were not further carried out.

Subfractions MG.3-MG.5 showed no definite spot on normal phase TLC. Therefore, further separations were not performed.

Subfraction MG.6 contained three spots on normal phase TLC, overlapping with other. Therefore, investigation was not further carried out.

Subfraction MG.7 contained three spots on normal phase TLC. Because of low quantity, it was not further separated.

Subfractions MG.8-MG.15 showed no definite spot on normal phase TLC. Because of low quantity, they were not carried out.

Fractions H-I showed no definite spot on normal phase TLC. Thus, they were not investigated.

Fraction J was fractionated by column chromatography over silica gel with various proportions of acetone in chloroform and finally with methanol. Fractions with the similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford seven subfractions, as shown in **Table 12**.

Table 12 Subfractions obtained from **subfraction J** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
J1	0.002	brown viscous -liquid
J2	0.005	white solid
J3	0.004	yellow solid
J4	0.019	white semi-solid
J5	0.003	white solid
J6	0.002	white solid
J7	0.019	white solid

Subfraction J1 showed no definite spot on normal phase TLC. Therefore, investigation was not carried out.

Subfraction J2 contained one UV-active spot and two purple spots with vanilin sulfuric acid reagent and subsequently heating. Because of low quantity, purification was not performed further.

Subfraction J3 contained one UV-active spot and one purple spot with vanilin sulfuric acid reagent and subsequently heating. Because of low quantity, purification was then not performed further.

Subfraction J4 was crystallized from 30% acetone in chloroform to give **CS-S4** as a white solid (13.0 mg). It showed a single spot on normal phase TLC with 40% acetone in chloroform (4 runs) with the R_f value of 0.12 which was visualized as a purple spot in vanillin sulfuric acid reagent and subsequently heating.

Compound CS-S4 (White solid)

$[\alpha]_D^{29}$	**:**	-90.00° (c = 0.10, $CHCl_3$)
mp	**:**	262.4 - 262.7 °C
IR (KBr) ν (cm^{-1})	**:**	3410 (O-H stretching), 2945, 2871 (C-H stretching)
^{1}H NMR (Pyridine-d_5+$CDCl_3$) (δ ppm) (500 MHz)	**:**	5.21 (*dd* , *J* = 15.5 and 9.0 Hz, 1H), 5.17 (*brm*, 1H), 5.07 (*dd*, *J* = 15.5 and 9.0 Hz, 1H), 4.93 (*d*, *J* = 8.0 Hz, 1H), 4.48 (*brd*, *J* = 12.0 Hz, 1H), 4.31 (*brdd*, *J* = 12.0 and 5.5 Hz, 1H), 4.18 (*t*, *J* = 8.5 Hz, 1H), 4.13 (*t*, *J* = 8.5 Hz, 1H), 3.93 (*m*, 1H), 3.91 (*m*, 1H), 3.89 (*m*, 1H), 2.06 (*m*, 1H), 2.00 (*m*, 1H), 1.97 (*m*, 2H), 1.94 (*m*, 1H), 1.82 (*m*, 1H),1.79 (*m*, 1H), 1.70 (*m*, 1H), 1.69 (*m*,

			1H), 1.60 (*m*, 1H), 1.57 (*m*, 1H), 1.56 (*m*, 1H), 1.55 (*m*, 2H), 1.43 (*m*, 1H), 1.32 (*m*, 1H), 1.30 (*m*, 2H), 1.28 (*m*, 1H), 1.22 (*m*, 1H), 1.08 (*d*, *J* = 6.5 Hz, 3H),), 0.94 (*m*, 1H), 0.91 (*d*, *J* = 6.5 Hz, 3H), 0.88 (*t*, *J* = 7.5 Hz, 3H), 0.86 (*d*, *J* = 7.0 Hz, 3H), 0.72 (*s*, 3H), 0.58 (*s*, 3H)
^{13}C NMR (Pyridine-d_5+$CDCl_3$) (δ ppm) (125 MHz)		**:**	137.29, 136.42, 127.42, 115.62, 99.91, 76.05, 75.90, 75.03, 72.80, 69.43, 60.61, 53.84, 53.10, 49.27, 47.37, 41.26, 38.94, 37.97, 37.44, 35.13, 32.40, 32.33, 29.96, 27.77, 27.69, 26.68, 23.50, 21.12, 19.52, 19.46, 19.17, 17.05, 10.91, 10.36, 10.09
DEPT 135°	**CH_3**	**:**	19.46, 19.17, 17.05, 10.91, 10.36, 10.09
	CH2	**:**	60.61, 37.44, 35.13, 32.40, 27.77, 27.69, 26.68, 23.50, 21.12, 19.52
	CH	**:**	136.42, 127.42, 115.62, 99.91, 76.05, 75.90, 75.03, 72.80, 69.43, 53.84, 53.10, 49.27, 47.37, 38.94, 37.97, 29.96

Subfractions J5-J7 showed no definite spot on normal phase TLC. No further purifications were performed because of low quantity.

Fraction K was separated by column chromatography over silica gel using various proportions of methanol in ethyl acetate and finally with methanol. Fractions with the similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford ten subfractions, as shown in **Table 13**.

Table 13 Subfractions obtained from **fraction K** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
K1	0.072	green semi-solid
K2	0.062	green semi-solid
K3	0.027	green-brown viscous-liquid
K4	0.181	green-brown semi-solid
K5	0.287	brown semi-solid
K6	2.050	brown-yellow semi-solid
K7	1.360	brown-yellow semi-solid
K8	4.454	brown viscous-liquid
K9	4.979	brown viscous-liquid
K10	6.564	brown viscous-liquid

Subfraction K1 contained many spots on normal phase TLC. These spots were not well-separation. Thus, it was not investigated further.

Subfraction K2 was crystallized upon standing at room temperature to afford **CS-S1** as the major compound separated from **fraction H**.

Subfractions K3-K4 contained many spots on normal phase TLC. Therefore, they were not investigated.

Subfraction K5 was crystallized upon standing at room temperature to afford **CS-S5** as the major component separated from **subfraction J4**.

Subfractions K6-K8 and the mother liquor of **subfraction K5** were combined and then chromatographed by column chromatography over silica gel. Elution was conducted initially with dichloromethane, followed by increasing amount of methanol

in dichloromethane and finally with methanol. Fractions with the similar TLC chromatogram were combined and evaporated to dryness under reduced pressure to afford nine subfractions, as shown in **Table 14**.

Table 14 Subfractions obtained from **subfractions K6-K8** and the mother liquor of **subfraction K5** by column chromatography over silica gel

Subfraction	Weight (g)	Physical appearance
K58.1	0.004	yellow viscous- liquid
K58.2	0.010	green solid
K58.3	0.057	yellow-brown viscous-liquid
K58.4	0.137	yellow viscous-liquid
K58.5	0.334	yellow-brown semi-solid
K58.6	0.937	yellow-brown semi-solid
K58.7	0.535	yellow-brown semi-solid
K58.8	2.032	brown viscous-liquid
K58.9	1.862	brown viscous-liquid

Subfractions K58.1-K58.9 contained many spots on normal phase TLC. Therefore, further purifications were not performed.

Subfractions K9-K10 showed no definite spot on normal phase TLC. Therefore, they were not investigated.

Fraction L was crystallized from 30% methanol in ethyl acetate to afford **CS-S2** as a colourless crystal (1.817 g), melting at 175.2-176.9 °C. It showed a gray spot in vanillin sulfuric reagent and subsequently heating.

Compound CS-S2 (Colourless crystal)

$[\alpha]_D^{28}$		**:**	+78.37° (c = 0.25, Pyridine)
mp		**:**	175.2 - 176.9 °C
IR (KBr) ν (cm^{-1})		**:**	3373 (O-H stretching), 2930 (C-H stretching), 1130, 1071 (C-O stretching)
^{1}H NMR (Pyridine-d_5) (δ ppm) (500MHz)		**:**	5.94 (*d*, *J* = 4.0 Hz, 1H), 4.78 (*t*, *J* = 8.0 Hz, 1H), 4.67 (*d*, *J* = 8.0 Hz, 1H), 4.46 (*ddd*, *J* = 9.5, 4.5 and 2.5 Hz, 1H), 4.39 (*t*, *J* = 9.5 Hz, 1H), 4.28 (*dd*, *J* = 12.0 and 2.5 Hz, 1H), 4.24 (*td J* = 8.0 and 4.0 Hz, 1H), 4.14 (*dd*, *J* = 12.0 and 4.5 Hz, 1H), 4.13 (*d*, *J* = 12.0 Hz, 1H), 4.05 (*d*, *J* = 12.0 Hz, 1H), 3.98 (*dd*, *J* = 9.5 and 4.0 Hz, 1H)
^{13}C NMR (Pyridine-d_5) (δ ppm) (125 MHz)		**:**	103.35, 91.31, 82.03, 78.25, 73.09, 72.67, 72.63, 70.98, 69.40, 62.90, 60.25, 60.12
DEPT 135°	**CH_2**	**:**	62.90, 60.25, 60.12
	CH:	**:**	91.31, 82.03, 78.25, 73.09, 72.67, 72.63, 70.98, 69.40

CS-S2 (25.0 mg) was acetylated by dissolved in acetic anhydride (2.0 mL) in the present of catalytic amount of pyridine. The reaction mixture was stirred at the room temperature for one day. The reaction mixture was poured into ice-water and the aqueous solution was extracted with dichloromethane (5X10 mL). The combined dichloromethane extracts were washed with 10% HCl, 10% $NaHCO_3$ and water, respectively, and then dried over anhydrous sodium sulphate. After removal the

dichloromethane solvent, a colourless viscous-liquid was obtained in 55.8 mg (**AcCS-S2**).

Octaacetate of CS-S2 (AcCS-S2) (colourless viscous-liquid)

$[\alpha]_D^{28}$	**:**	+21.00° (c = 0.34, $CHCl_3$)
IR (KBr) ν (cm^{-1})	**:**	3018, 2967 (C-H stretching), 1749 (C=O stretching), 1229, 1034 (C-O stretching)
^{1}H NMR ($CDCl_3$) (δ ppm) (300MHz)	**:**	5.65 (*d*, *J* = 3.6 Hz, 1H), 5.42 (*d*, *J* = 5.7 Hz, 1H), 5.40 (*d*, *J* = 10.2 Hz, 1H) 5.33 (*t*, *J* = 5.7 Hz, 1H), 5.04 (*t*, *J* = 10.2 Hz, 1H), 4.83 (*dd*, *J* = 10.2 and 3.6 Hz, 1H), 4.31 (*dd*, *J* = 12.0 and 4.8 Hz, 1H), 4.28 (*m,* 1*H*), 4.26 (*m,* 1H), 4.18 (*m,* 1H), 4.15 (*s,* 2H), 4.14 (*m,* 1H)
^{13}C NMR ($CDCl_3$) (δ ppm) (75 MHz)	**:**	170.68, 170.47, 170.08 (3xC), 169.90, 169.65, 169.50, 103.96, 89.89, 79.07, 75.65, 74.94, 70.23, 69.59, 68.46, 68.18, 63.61, 62.84, 61.71, 20.60 (8xC)
DEPT 135° CH2	**:**	63.61, 62.84, 61.71
CH	**:**	89.89, 79.07, 75.65, 74.94, 70.23, 69.59, 68.46, 68.18

Fraction M showed no definite spot on normal phase TLC. No further investigation was conducted.