Part I

Chemical Constituents from the Seeds of *Cerbera manghas*

CHAPTER 1.1

INTRODUCTION

1.1.1 Introduction

Cerbera manghas Linn., a mangrove plant belonging to the Apocynaceae family, is distributed widely in the coastal areas of Southeast Asia and countries surrounding the Indian Ocean. The Apocynaceae family contains about 155 genus and 1700 species. In Thailand only 42 genus and 125 species are found, from *Cerbera* genera only 2 species are found, *C. manghas* and *C. odollam* (The Forest Herbarium, Royal Forest Department, 1999). *C. manghas* was found in Prachuap Khiri Khan, Chonburi, Rayong, Phuket, Songkhla, Satun and Narathiwat while *C. odollam* was found in Bangkok, Ranong, Surat Thani, Phangga, Krabi, Satun and Narathiwat

Cerbera manghas is a small tree, 4 - 6 m tall, stem soft, glabrous with milky juice, leaves alternate, closely set or whorled at the apices of branchlets, 10 -15 x 3 - 5 cm, ovate-oblong or oblaceolate, acuminate at apex, rounded at base, flowers large, bracteate, 3 - 4 cm long, arranged in terminal paniculate cyme, funnel shaped, white with yellow throat, turning purple or red on ageing, fruit large, 7 - 9 x 4 - 6 cm, globose, ovoid or ellipsoid, drupaceous with fibrous pericarp, seeds 1 -2 , each 2 - 2.5 cm across, broad, compressed, fibrous.

Figure 1 *Cerbera manghas* (Apocynaceae)

1.1.2 Review of literatures

Plants in the *Cerbera* genus (Apocynaceae) are well known to be rich in a variety of compounds: cardenolide glycosides (Abe, *et.al.,* 1977; Yamauchi, 1987); lignan (Abe, *et.al.,* 1988; 1989); iridoid monoterpenes (Abe, *et.al.,* 1977; Yamauchi, *et.al.,*1990) normonoterpene glycosides (Abe, *et.al.,* 1988; 1996) and dinormonoterpeniod glycosides (Abe, *et.al.,* 1996) etc.

Chemical constituents isolated from 6 species of the genus *Cerbera* were summarized by Surat Laphookhieo in 2002 (Laphookhieo, 2002). Additional constituents from *Cerbera manghas* obtained from NAPRALERT database developed by University of Illinois at Chicago and Chemical Abstracts of the year 2003 will be presented and classified into groups, such as carbohydrate, cardenolides and lignans (see **Table 1**).

1.1.3 Biological activities of Cerbera species

The importance of *Cerbera* in traditional medicine throughout the tropical world is apparent from NAPRALERT database. The significant biological activities of the extract of *Cerbera* species are summarized in **Table 2** and the importance ethnomedical applications are summarized in **Table 3**.

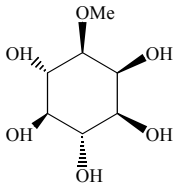
Table 1 Compounds from *Cerbera manghas*

a : Carbohydrates **b** : Cardenolides **c** : Lignans

Scientific name	Compound	Bibliography
C. manghas		
-leaves	Bornesitol, **1a**	Nishibe, *et. al*., 2001
-roots	Cycloolivil, **5c**	Chang, *et. al.,* 2000
	14β-Hydroxy-3β-(3-*O*-methyl-6-deoxy-α-L-glucopyranosyl)-11β,12β-epoxy-(5β,17βH)-card-20(22)-enolide,**2b**	
	14β-Hydroxy-3β-(3-*O*-methyl-6-deoxy-α-L-rhamnosyl)-11β,12β-epoxy-(5β,17βH)-card-20(22)-enolide, **3b**	
	Neriifolin, **4b**	
	Olivil, **6c**	

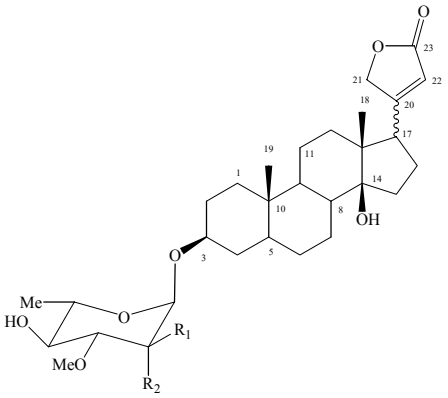
Structures

a : Carbohydrate



1a: (+)-Bornesitol

b : Cardenolide glycosides

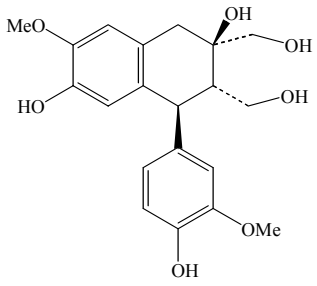


2b: 14β-Hydroxy-3β-(3-*O*-methyl-6-deoxy-α-L-glucopyranosyl)-11β,12β-epoxy-(5β,17βH)-card-20(22)-enolide,

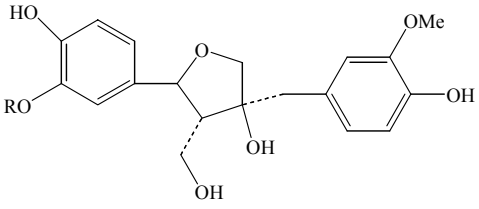
3b: 14β-Hydroxy-3β-(3-*O*-methyl-6-deoxy-α-L-rhamnosyl)-11β,12β-epoxy-(5β,17βH)-card-20(22)-enolide,

4b: R_1=OH, R_2=H; 17β-Neriifolin

c: Lignans



5c: (-)-Cycloolivil



6c: R = H; (-)-Olivil

Table 2 Biological activities of *Cerbera* species

Scientific name	Type of Biological activity	Bibliography
C. dilatata		
- kernels	Cardiotonic activity	Thorp, *et.al.*, 1953
C. floribunda		
- kernels	Cardiotonic activity	Thorp, *et.al.*, 1953
C. manghas		
- entire plants	Toxic effect (general)	Wee, *et.al.*, 1988
- dried entire plants	Prostaglandin synthesis inhibition	Dunstan, *et.al.*, 1997
-flesh entire plants	Spasmolytic activity	Cox, *et.al.*, 1989
-flowers	Toxicity assessment (quantitative)	Mahran, *et.al.*, 1972
-fruits	Antitumor activity	Norton, *et.al.*, 1973
	Antiviral activity	
	Hypotensive activity	
-kernels	Cardiotonic activity	Thorp, *et.al.*, 1953 and
		Chen, *et.al.*, 1942
	Anticonvulsant activity	Bansinath, *et.al.*, 1982
	Barbiturate potentiation	
- leaves	Toxicity assessment (quantitative)	Mahran, *et.al.*, 1972
	Antitumor activity	Norton, *et.al.*, 1973
	Antiviral activity	
	Hypotensive activity	
- dried leaves	Antioxidant activity	Masuda, *et.al.*, 1999
	Cytoxic activity	Masuda, *et.al.*, 2002
	Radical scavenging effect	Masuda, *et.al.*, 1999
	Analgesic activity	Tran, *et.al.*, 1991
	Anticonvulsant activity	
	Barbiturate potentiation	

Table 2 (Continued)

Scientific name	Type of Biological activity	Bibliography
C. manghas	Locomotor activity	
- dried leaves	Toxicity assessment (quantitative)	
	Tranquilizing effect	
-leaves and stems	Antibacterial activity	Nakanishi, *et.al.*, 1965
	Antitumor activity	
	Toxicity assessment (quantitative)	
- roots	Toxicity assessment (quantitative)	Mahran, *et.al.*, 1972
	Antitumor activity	Norton, *et.al.*,1973
	Antiviral activity	
	Hypotensive activity	
-dried roots	Antioxidant activity	Lee, *et. al*., 1998
	Radical scavenging effect	
	Antiestrogenic effect	Chang, *et.al.*, 2000
	Antiproliferation activity	
- seeds	Cardiotonic activity	Chopra, *et.al.*, 1942
	Toxic effect (general)	Iyer and Narendranath, 1975
- stems	Antitumor activity	Nakanishi, *et.al.*, 1965 and
	Toxicity assessment (quantitative)	Norton, *et.al.*,1973
	Antiviral activity	Norton, *et.al.*,1973
	Hypotensive activity	
-stem barks	Toxicity assessment (quantitative)	Mahran, *et.al.*, 1972
	Antitumor activity	Mahmoud, *et.al.*, 1979
	Cytoxic activity	
- dried stem barks	Cytoxic activity	Pezzuto, *et.al.*, 1991
	DNA binding effect	
- stem woods	Toxicity assessment (quantitative)	Mahran, *et.al.*, 1972

Table 2 (Continued)

Scientific name	Type of Biological activity	Bibliography
C. odollam		
- fresh seeds	Anticrustacean activity	MacKeen, *et.al.*, 2000
C. peruviana		
- fruits	Toxic effect (general)	Samal, *et.al.*, 1989
- seeds	Toxic effect (general)	
C. thevetia		
- fresh flowers	Toxic effect (general)	Samal, *et.al.*, 1990

Table 3 Ethnomedical applications of *Cerbera* species

Scientific name	Ethnomedical application	Bibliography
C. floribunda		
- dried barks	Malaria	Holdsworth, 1983
	Tropical ulcers	
	Childbirth	
	Gonorrhea	
	Jaundice	
- part not specified	Contraceptive	Holdsworth, 1989
C. manghas		
-fresh flowers	Inflammation	Mc clatchey, 1996
	Ichtheotoxin/Sting	
	Rheumatism	Duke, *et.al*., 1985
-fruits	Criminal abortion	Quisumbing, 1951
	Abortifacient	Saha, *et.al*., 1980
- dried fruits	Anesthetic	Sakushima, *et.al*., 1980
	Fish poison	Pickard, 1986
- kernel	Abortifacient	Bansinath, *et.al*., 1982
- part not specified	Abortifacient	Hefez, 1982 and Casey, 1960
	Antifertility agent	Casey, 1960
- fresh plant juice	Abortifacient	Kambo, 1988

This research involved isolation, purification and structure elucidation of chemical constituents isolated from the seeds of *C. manghas.* It is a part of the basic research on the utilization of Thai plant for pharmaceutical purposes.

CHAPTER 1.2

EXPERIMENTAL

1.2.1 Instruments and Chemicals

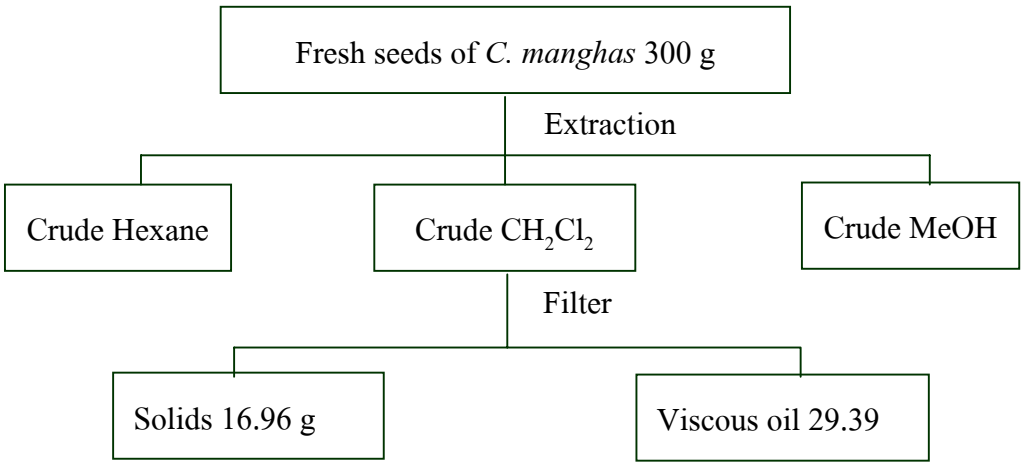
Melting point was recorded in °C and was measured on an Electrothermal Melting Point Apparatus. Infrared spectra were recorded using FTS FT-IR spectrophotometer and major bands (ν) were recorded in wave number (cm^{-1}). Ultraviolet (UV) absorption spectra were recorded using UV-160A spectrophotometer (SHIMADZU) and principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in chloroform and methanol solution. Nuclear magnetic resonance spectra were recorded on 500 MHz Varian UNITY INOVA spectrometer. Spectra were recorded in deuterochloroform and deuteromethanol solution and were recorded as δ value in ppm downfield from TMS (internal standard δ 0.00). Single-crystal X-ray diffraction measurements were collected using SMART 1-K CCD diffractometer with monochromated Mo-Kα radiation (λ = 0.71073 A°) using ω-scan mode and SHELXTL for structure solution and refinement. Optical rotation was measured in chloroform solution with sodium D line (590 nm) on an AUTOPOL^{R} II automatic polarimeter. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except diethyl ether, which was analytical grade reagent. Quick column chromatography was performed on silica gel 60 GF_{254} (Merck). Column chromatography was performed on silica gel (Merck) type 100 (0.063 – 0.200). Precoated plates of silica gel 60 GF_{254} or reversed-phase C_{18} were used for analytical purposes.

1.2.2 Plant material

The seeds of *Cerbera manghas* (Apocynaceae) were collected from Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand, in March 2002. The plant was identified by Professor Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and the voucher specimen was deposited in the herbarium (No. 0012281).

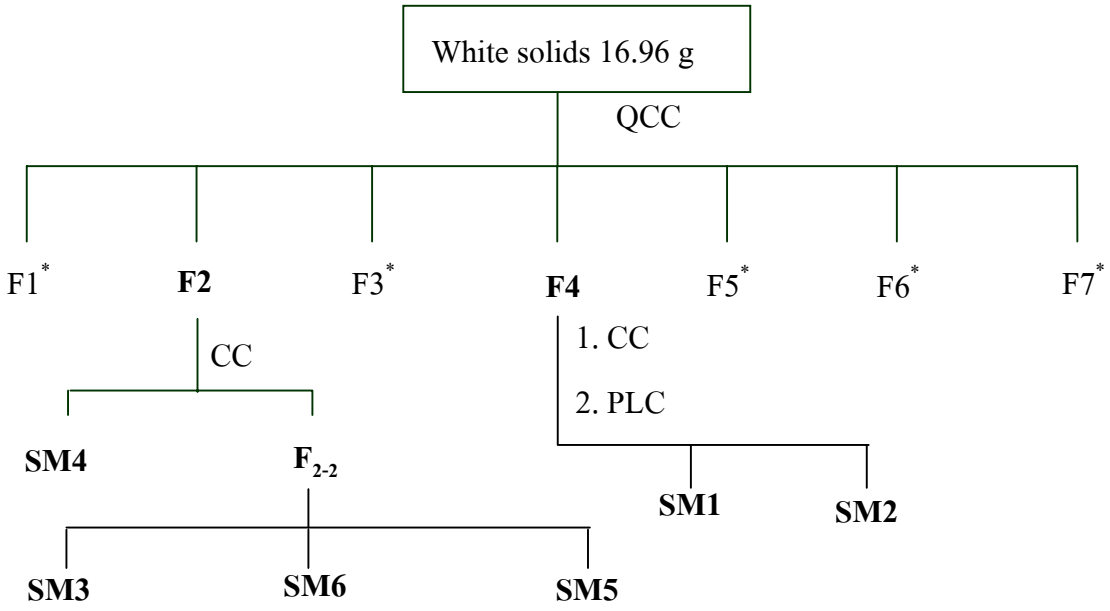
1.2.3 Extraction

Seeds from the fresh fruits of *C. manghas* (300 g) were extracted twice with methylene chloride (2.5 L), over the period of 5 days each at room temperature. The mixture was filtered and concentrated under reduced pressure to give white solid (16.96 g) and crude methylene chloride extract as a yellow oil (29.39 g) **Scheme 1**.



Scheme 1 Extraction of the seeds of *C. manghas*

1.2.4 Investigation of the crude methylene chloride extract from the seeds of C. manghas



* Not further investigated

Scheme 2 Isolation of compounds **SM1, SM2, SM3, SM4, SM5** and **SM6** from the seeds of *C. manghas*

The white solid (16.96 g) was purified by column chromatography on silica gel using hexane as eluent and increasing polarity with diethyl ether and methanol, respectively, to give seven fractions (**Scheme 2**).

Fraction F_2 (0.640 g) was purified by column chromatography using 5% methanol in methylene chloride as eluent to give two subfractions.

Subfraction $F_{2\text{-}1}$ (0.420 g) was purified by column chromatography using 10% acetone in methylene chloride as eluent to give **SM4** (0.003 g) as a white solid (R_f = 0.28, 10% acetone in methylene chloride).

Subfraction F_{2-2} (0.122 g) was purified by PLC using 70% diethyl ether in hexane as eluent to give **SM3** (0.055 g) as white solid (R_f = 0.21, 3% methanol in methylene chloride), **SM6** (0.008 g) as white solid (R_f = 0.23, 70% diethyl ether in hexane), **SM5** (0.010 g) as white solid (R_f = 0.21, 70% diethyl ether in hexane).

Fraction F_4 (1.900 g) was purified by column chromatography using diethyl ether in methanol as eluent and increasing polarity with methanol and recrystalized in methanol-chloroform to give **SM1** (0.055 g) as white solid (R_f = 0.28, 2% methanol in diethyl ether) and **SM2** (0.017 g) as white solid (R_f = 0.19, 2% methanol in diethyl ether).

Compound SM1 : White solid; mp: 216-218 °C; $[\alpha]_D^{27}$: -62.50° (*c* = 0.016, $CHCl_3$); UV (CH_3OH) λ_{max} (nm) (log ε): 218 (4.18); IR (KBr) ν (cm^{-1}): 3424 (O-H stretching), 2937 (C-H stretching), 1734 (C=O stretching), 1612 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 5**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 5**; DEPT-135° ($CDCl_3$): see **Table 5**.

Compound SM2 : White solid; mp: 226-227 °C; $[\alpha]_D^{27}$: -100.00° (*c* = 0.010, $CHCl_3$); UV (CH_3OH) λ_{max} (nm) (log ε): 242 (3.32); IR (KBr) ν (cm^{-1}): 3473 (O-H stretching), 2943 (C-H stretching), 1746 (C=O stretching), 1717 (C=O stretching), 1632 (C=C stretching), 1203 (C-O stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 9**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 9**; DEPT-135° ($CDCl_3$): see **Table 9**.

Compound SM3 : White solid; mp: 230-232.5 °C; $[\alpha]_D^{27}$: -66.67° (*c* = 0.03, $CHCl_3$); UV (CH_3OH) λ_{max} (nm) (log ε): 217 (4.24), 270 (3.82); IR (KBr) ν (cm^{-1}): 3483 (O-H stretching), 2922 (C-H stretching), 1782, 1745 (C=O stretching),

1627 (C=C stretching), 1034 (C-O stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 12** ; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 12**; DEPT -135° ($CDCl_3$): see **Table 12**.

Compound SM4 : White solid; mp: 209-211 °C; $[\alpha]_D^{27}$: -62.00° (*c* = 0.048, $CHCl_3$); UV (CH_3OH) λ_{max} (nm) (log ε): 210 (4.02); IR (KBr) ν (cm^{-1}): 3446 (O-H stretching), 2929 (C-H stretching), 1741, 1740, 1697 (C=O stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 16**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 16**; DEPT -135°($CDCl_3$): see **Table 16**.

Compound SM5 : White solid; mp: 211-213 °C; $[\alpha]_D^{27}$: -90.50° (*c* = 0.022, $CHCl_3$); UV (CH_3OH) λ_{max} (nm) (log ε): 218 (4.23); IR (KBr) ν (cm^{-1}): 3453 (O-H stretching), 2929 (C-H stretching), 1741 (C=O stretching), 1627 (C=C stretching); ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 20**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 20**; DEPT -135°($CDCl_3$): see **Table 20**.

Compound SM6 : White solid; mp 103-105°C; $[\alpha]_D^{26}$: -166.10° (c 0.024, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε): 217 (4.04); IR ν_{max} cm^{-1}; 3461 (OH), 1745, 1716 (C=O) and 1645 (C=C); HRFABMS ($[M+1]^+$) *m/z* 575.3205, calcd. 575.3220; ^{1}H NMR ($CDCl_3$) (δ ppm) (500 MHz): see **Table 24**; ^{13}C NMR ($CDCl_3$) (δ ppm) (125 MHz): see **Table 24**; DEPT -135°($CDCl_3$): see **Table 24**.

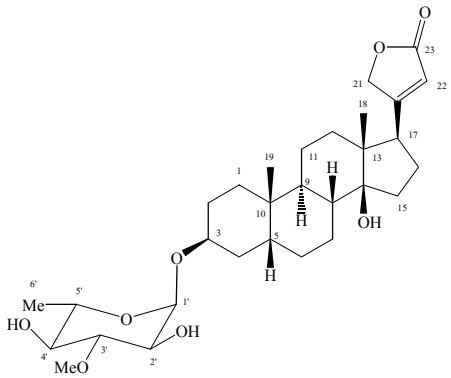
CHAPTER 1.3

RESULTS AND DISCUSSION

1.3.1 Structural elucidation of compounds from the seeds of C. manghas

The methylene chloride extract of seeds of *C. manghas* was subjected to chromatography and/or crystallization and/or PLC to give six cardenolide glycosides, **SM1, SM2, SM3, SM4, SM5** and **SM6**. Only **SM6** is a new compound. Structure elucidations of all compounds were determined from 1D and 2D NMR spectroscopic data. All carbons of aglycone unit were assigned by ^{13}C NMR, HMQC and HMBC data. The chemical shift of sugar moiety was determined using 1D ^{1}H NMR and ^{1}H-^{1}H COSY spectroscopic data.

1.3.1.1 Compound SM1

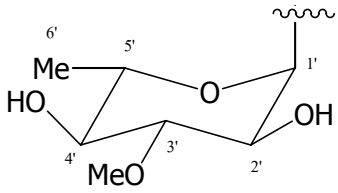


Compound **SM1** was obtained as a white solid, mp = 216-218 °C, $[\alpha]_D^{27}$= -62.50° (*c* = 0.016, $CHCl_3$). The IR spectrum showed absorption bands at 3424 cm^{-1} and 1734 cm^{-1} corresponding to a hydroxy group and carbonyl group, respectively. The presence of carbonyl carbon at δ 175.06 from ^{13}C NMR spectrum supported the above conclusion. The UV spectrum showed maximum at 218 nm.

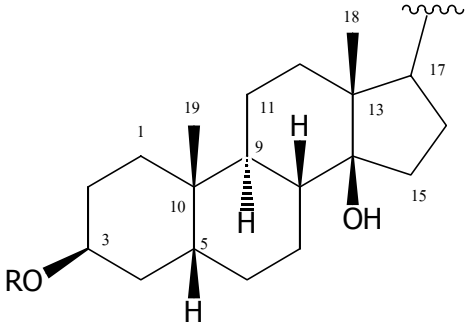
The ^{13}C NMR spectral data (see **Table 5**) showed 30 signals for 30 carbon atoms. Analysis of the DEPT-90° and DEPT-135° spectra of this compound suggested the presence of four methyl carbon atoms (δ 60.54, 23.78, 17.40 and 15.71), ten methylene carbon atoms (δ 73.60, 39.95, 32.91, 30.55, 29.91, 26.55, 26.44, 26.28, 21.29 and 21.14), eleven methine carbon atoms (δ 117.48, 97.15, 85.24, 74.68, 73.39, 72.66, 67.60, 50.90, 41.64, 36.52 and 35.66) and five signals for quaternary carbon atoms (δ 175.06, 175.04, 84.55, 49.63 and 35.19).

The ^{1}H NMR spectral data (see **Table 5**) recorded in $CDCl_3$ was a typical of cardenolide glycoside. The *singlet* signal at δ 5.87 (1H, H-22) accompanied by AB system at δ 4.79 (1H, *dd, J* = 18.5 and 1.5 Hz, H-21b) and 4.97 (1H, *dd, J* = 18.5 and 1.5 Hz, H-21a), were characteristic peaks of a butenolide ring, together with the sugar protons at δ 4.83 (1H, *d, J* = 3.5 Hz), 3.71 (1H, *dq, J* = 9 and 6 Hz), 3.66 (3H, *s*), 3.53 (1H, *dd, J* = 9 and 3.5 Hz), 3.24 (1H, *t, J* = 9 Hz), 3.10 (1H, *t, J* = 9 Hz), and 1.22 (3H,

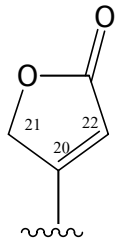
d, J = 6 Hz) which were assigned to H-1′, H-5′, 3′-OMe, H-2′, H-3′, H-4′ and 3H-6′, respectively. The sugar unit was identified as L-thevetose by comparison with the previously reported data (Yamauchi, *et.al.,* 1987). The two methyl protons, one oxymethine and one methine proton of the steroidal ring were shown at δ 0.86 (3H, *s*), 0.94 (3H, *s*), 3.95 (1H, *br s*) and 2.77 (1H, *dd, J* = 5 and 9 Hz), which were assigned to CH_3-18, CH_3-19, H-3 and H-17, respectively. The remaining methylene protons appeared between δ 1.21 and 2.21. Thus, this compound exhibited tetracyclic of steroidal skeleton, butenolide ring and the sugar moiety as indicated by ^{1}H NMR.



Sugar unit



Steroid unit



Butenolide unit

The complete assignment of ^{13}C and ^{1}H NMR (see **Table 5**) signals were made with the information from ^{1}H-^{1}H COSY (see **Table 4**), HMQC and HMBC spectrum (see **Table 5**). In the HMBC spectrum the carbon signals at δ 67.60 (C-5′), 73.39 (C-3) and 85.24 (C-3′) showed the correlation peaks with the H-1′(4.83), indicating that the glycosidic linkage was formed between sugar moiety and the steroid at C-3 (73.39). The carbon signals at δ 39.95 (C-12), 49.63 (C-13), 73.60 (C-21), 117.48 (C-22) and 175.04 (C-20) showed the correlation peaks with the H-17 (2.77), confirming that the butenolide ring was attached to C-17 (50.90) of the steroid ring D.

In NOE experiment, irradiation of methine proton at δ2.77 (H-17) resulted in the enhancement of the signals at 5.87 (H-22), 4.97 (H-21a) and 4.79 (H-21b) while the signal at δ0.86 (CH_3-18) has not changed. These observations suggested that CH_3-18 and H-17 are opposite. Thus, this compound should be β-butenolide at C-17.

Table 4 500 MHz COSY Correlation of some protons of compound **SM1**

δ_H (ppm)	**Proton Correlation with δ_H (ppm)**
H-21a (4.97)	H-21b
H-21b (4.79)	H-21a
H-1′ (4.83)	H-2′
H-2′ (3.53)	H-1′ and H-3′
H-3′ (3.24)	H-2′ and H-4′
H-4′ (3.10)	H-3′ and H-5′
H-5′ (3.71)	H-4′ and H-6′
H-6′ (1.22)	H-5′

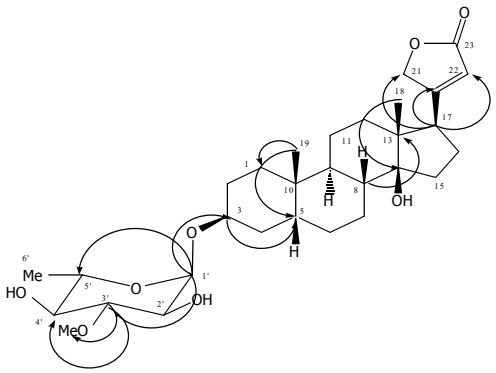
Table 5 ^{1}H, ^{13}C and HMBC spectral data of compound **SM1**

Position	$\delta_C^{\#}$ *(ppm)*		δ_H **(ppm)**	**HMBC**
1	29.91	CH_2	1.45 (1H, *m*), 1.76 (1H, *m*)	-
2	26.55[a]	CH_2	1.54 (1H, *m*), 1.62 (1H, *m*)[*]	-
3	73.39	CH	3.95 (1H, *br s*)	C-5
4	30.55	CH_2	1.37 (1H, *m*), 1.54 (1H, *m*)	-
5	36.52	CH	1.62 (1H, *m*)	-
6	26.28[a]	CH_2	1.24 (1H, *m*), 1.62 (1H, *m*)[*]	-
7	21.14[b]	CH_2	1.70 (2H, *m*)[**]	-
8	41.64	CH	1.55 (1H, *m*)	C-6, C-13 and C-14
9	35.66	CH	1.60 (1H, *m*)	-
10	35.19	C	-	-
11	21.29[b]	CH_2	1.21 (1H, *m*), 1.42 (1H, *m*)[**]	-
12	39.95	CH_2	1.37 (1H, *m*), 1.50 (1H, *m*)	-
13	49.63	C	-	-
14	84.55	C	-	-
15	32.91	CH_2	1.68 (1H, *m*), 2.08 (1H, *m*)	-
16	26.44[a]	CH_2	1.86 (1H, *m*), 2.15 (1H, *m*)	-

Table 5 (Continued)

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
17	50.90	CH	2.77 (1H, *dd, J* = 9, 5 Hz)	C-12 , C-13 , C-20 , C-21 and C-22
18	15.71	CH_3	0.86 (3H, *s*)	C-12 , C-13, C-14 and C-17
19	23.78	CH_3	0.94 (3H, *s*)	C-1, C-5 and C-9
20	175.04	C	-	-
21a	73.60	CH_2	4.97 (1H, *dd, J* = 18.5, 1.5 Hz)	C-20, C-22 and C-23
21b			4.79 (1H, *dd, J* = 18.5, 1.5 Hz)	
22	117.48	= CH	5.87 (1H, *s*)	C-17, C-20, C-21 and C-23
23	175.06	C	-	-
1′	97.15	CH	4.83 (1H, *d, J* = 3.5 Hz)	C-3, C-3′ and C-5′
2′	72.66	CH	3.53 (1H, *dd, J* = 9, 3.5 Hz)	-
3′	85.24	CH	3.24 (1H, *t, J* = 9 Hz)	C-2′, C-4′ and C-3′OMe
4′	74.68	CH	3.10 (1H, *t, J* = 9 Hz)	C-3′, C-5′ and C-6′
5′	67.60	CH	3.71 (1H, *dq, J* = 9, 6 Hz)	C-3′
6′	17.40	CH_3	1.22 (3H, *d, J* = 6 Hz)	C-4′ and C-5′
3′-OMe	60.54	CH_3	3.66 (3H, *s*)	C-3′

[a, b,] Assignment with the same superscripts may be interchanged, [#] Carbon type deduced from DEPT experiment, [*, **] Assignment with the same superscripts may be interchanged.



Selected HMBC correlation of **SM1**

Comparison of ^{1}H and ^{13}C NMR spectral data (**Table 6-7**) between compound **SM1** and 17β-neriifolin showed similarity. Thus compound **SM1** was identified as 3β-*O*-(l-thevetosyl)-14β-hydroxy-5β-card-20(22)-enolide, (17β-neriifolin) which was previously isolated from the leaves of *C. odollam* and *C. manghas* (Yamauchi, *et.al.,* 1987).

Table 6 Comparison of ^{1}H NMR spectral data between 17β-neriifolin and compound **SM1**

Position	**17β-neriifolin δ_H (ppm) (recorded in pyridine)**	**Compound SM1 δ_H (ppm) (recorded in $CDCl_3$)**
3	4.18 (1H, *br s*)	3.95 (1H, *br s*)
17	2.80 (1H, *dd, J* = 9, 6 Hz)	2.77 (1H, *dd, J* = 9, 5 Hz)
18	0.83 (3H, *s*)	0.86 (3H, *s*)
19	1.02 (3H, *s*)	0.94 (3H, *s*)
21	5.03 (1H, *dd, J* = 18, 1 Hz)	4.97 (1H, *dd, J* = 18.5, 1.5 Hz)
	5.31 (1H, *dd*, *J* = 18, 1 Hz)	4.79 (1H, *dd, J* = 18.5, 1.5 Hz)
22	6.13 (1H, *br s*)	5.87 (1H, *s*)
1′	5.24 (1H, *d, J* = 4 Hz)	4.83 (1H, *d, J* = 3.5 Hz)
2′	4.08 (1H, *dd, J* = 9, 4 Hz)	3.53 (1H, *dd, J* = 9, 3.5 Hz)
3′	4.00 (1H, *t, J* = 9 Hz)	3.24 (1H, *t, J* = 9 Hz)
4′	3.66 (1H, *t, J* = 9 Hz)	3.10 (1H, *t, J* = 9 Hz)
5′	4.31 (1H, *m*)	3.71 (1H, *dq, J* = 9, 6 Hz)

6′	1.41 (1H, *d, J* = 6 Hz)	1.22 (1H, *d, J* = 6 Hz)

Table 7 Comparison of ^{13}C NMR spectral data between 17β-neriifolin and compound **SM1**

Position	**17β-neriifolin, δ_C (ppm) (recorded in pyridine)**	**Compound SM1, δ_C (ppm) (recorded in $CDCl_3$)**
1	30.3	29.9
2	26.9[a]	26.6[a]
3	73.7	73.4
4	31.0	30.6
5	36.8	36.5
6	27.1[a]	26.3[a]
7	21.5[b]	21.1[b]
8	41.9	41.6
9	35.8	35.7
10	35.5	35.2
11	21.9[b]	21.3[b]
12	39.8	40.0
13	50.1	49.6
17	51.5	50.9
18	16.2	15.7
19	23.8	23.8
20	175.9	175.0
21	73.6	73.6
22	117.6	117.5
23	174.4	175.1
1′	98.8	97.2
2′	73.4	72.7
3′	85.4	85.2

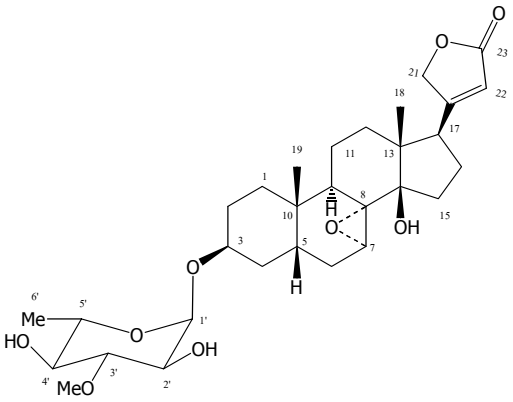
4′	76.6	74.7

Table 7 (Continued)

Position	**17β-neriifolin, δ_C (ppm) (recorded in pyridine)**	**Compound SM1, δ_C (ppm) (recorded in $CDCl_3$)**
5′	68.9	67.6
6′	18.5	17.4
3′-OMe	60.5	60.5

[a, b, c] Assignment with the same superscripts may be interchanged.

1.3.1.2 Compound SM2



Compound **SM2** was isolated as a white solid, mp = 226-227.5 °C, $[\alpha]_D^{27}$= -100.00° (*c* = 0.010, $CHCl_3$). The IR spectrum showed absorption bands which were ascribed to OH stretching (3473 cm^{-1}), C=O stretching (1746 cm^{-1}) and C-O stretching (1203 cm^{-1}). The UV spectrum showed maximum at 242 nm.

The complete analysis of ^{13}C and ^{1}H NMR spectral data of compound **SM2** (**Table 9**) were assigned with informations provided from ^{1}H-^{1}H COSY (**Table 8**), ^{1}H-^{13}C correlation (HMQC) and ^{1}H-^{13}C correlation by long-range coupling (HMBC) (**Table 9**), along with comparison of ^{1}H NMR spectral data to deacetyltanghinin

(**Table 10**). The ^{13}C NMR spectral data (**Table 9**) of compound **SM2** recorded in $CDCl_3$ showed 30 signals for 30 carbon atoms. Analysis of the DEPT-90° and DEPT-135° spectra of this compound suggested the presence of four methyl carbon atoms (δ 60.62, 24.23, 17.47 and 17.00), nine methylene carbon atoms (δ 73.31, 40.89, 34.35, 32.60, 31.49, 28.29, 27.71, 26.93 and 20.27), eleven methine carbon atoms (δ 117.79, 97.23, 84.46, 74.66, 72.82, 72.65, 67.54, 51.07, 50.56, 34.13 and 31.44) and six quaternary carbon atoms (δ 174.26, 173.54, 80.94, 63.84, 52.16 and 33.59).

Compound **SM2**, a derivative of compound **SM1**, showed similar characteristic bands in IR and UV spectrum to those of **SM1**. Comparison of the ^{1}H NMR spectral data of the two compounds revealed close structural similarity. Difference in the spectrum of compound **SM2** was shown as a signal of oxymethine proton at δ 3.23 (1H, *d, J* = 6.5 Hz) which was not observed in compound **SM1**. The ^{1}H and ^{13}C-NMR spectral data indicated the presence of the epoxy group connected to the oxymethine carbon at δ 51.07 (C-7) and 63.84 (C-8) from the HMQC experiment. The H-7 in compound **SM2** (δ 3.23, 1H, *d, J* = 6.5 Hz) appeared at the lower field than H-7 in compound **SM1** (δ 1.70, 2H, *m*). The characteristic bands of butenolide ring were shown as a *singlet* at δ 5.88 (1H) and two *doublets* AB system at δ 4.95 (1H, *J* = 18.5 Hz) and 4.79 (1H, *J* = 18.5 Hz) which were assigned to H-22, H-21a and H-21b, respectively. The moiety of sugar appeared at δ 4.84 (1H, *d, J* = 4 Hz), 3.73 (1H, *m*), 3.68 (-O$\underline{CH_3}$), 3.58 (1H, *br t*), 3.23 (1H, *t, J* = 9.5 Hz), 3.15 (1H, *t, J* = 9.5 Hz), 1.25 (3H, *d, J* = 6 Hz), which could be assigned to H-1′, H-5′, 3′-O$\underline{CH_3}$, H-2′, H-3′, H-4′ and 3H-6′, respectively.

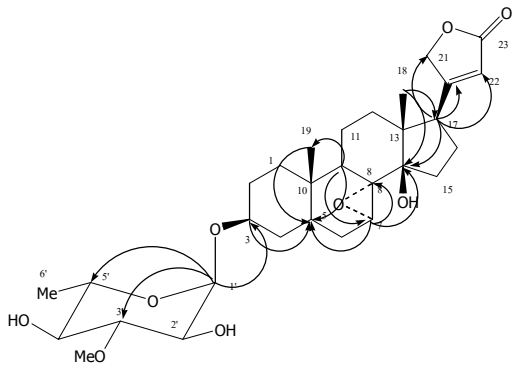
The HMBC correlation of compound **SM2** (**Table 9**) showed the same correlation with compound **SM1** (**Table 5**). The carbon signals at δ 34.13 (C-5), 28.29 (C-6), 63.84 (C-8) and 80.94 (C-14) showed the correlation peaks with H-7 (3.23). The position 7 of compound **SM2** should be connected to the epoxy group. In NOE

experiment, irradiation of methine proton at δ2.82 (H-17) resulted in the enhancement of the signals at δ 5.88 (H-22), 4.95 (H-21a) and 4.79 (H-21b) while the signal at δ 0.92 (CH_3-18) has not changed. These observations suggested that CH_3-18 and H-17 are opposite. Thus, this compound should be β-butenolide at C-17.

Compound **SM2** was identified as 3β-*O*-(l-thevetosyl)-7β, 8β-epoxy-14β-hydroxy-5β-card-20(22)-enolide, (deacetyltanghinin) which was the compound previously isolated from the leaves, seeds and barks of *C. manghas.* (Abe, *et.al.,* 1977). The structure of **SM2** was also confirmed by X-ray diffraction (see **Figure 2**).

Table 8 500 MHz COSY Correlation of some protons of compound **SM2**

δ_H (ppm)	**Proton Correlation with δ_H (ppm)**
H-21a (4.95)	H-21b
H-21b (4.79)	H-21a
H-1′ (4.84)	H-2′
H-2′ (3.58)	H-1′ and H-3′
H-3′ (3.23)	H-2′ and H-4′
H-4′ (3.15)	H-3′ and H-5′
H-5′ (3.73)	H-4′ and H-6′
H-6′ (1.25)	H-5′



Selected HMBC correlation of compound **SM2**

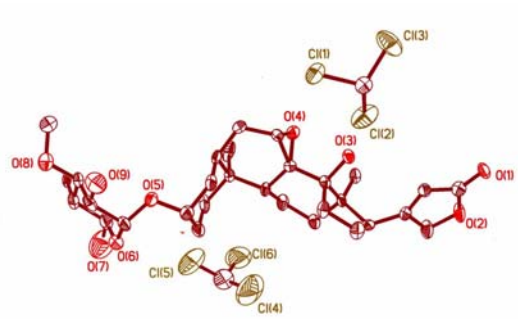


Figure 2 X-ray ORTEP diagram of compound **SM2**

Table 9 ^{1}H, ^{13}C and HMBC spectral data of compound **SM2**

Position	δ_C [#] *(ppm)*		δ_H **(ppm)**	**HMBC**
1	31.49	CH_2	1.32 (1H, *m*), 1.54 (1H, *m*) [**]	C-3 and C-9
2	26.93	CH_2	1.66 (2H, *m*) [**]	-
3	72.82	CH	3.93 (1H, *s*)	C-1 and C-5
4	32.60	CH_2	1.42 (1H, *m*), 1.53 (1H, *m*) [**]	-
5	34.13	CH	2.28 (1H, *m*)	C-7 and C-19
6	28.29	CH_2	1.98 (1H, *m*), 2.24 (1H, *m*) [**]	C-8 and C-10
7	51.07	CH	3.23 (1H, *d*, *J* = 6.5 Hz) [*]	C-5, C-6, C-8 and C-14
8	63.84	C	-	-
9	31.44	CH	2.25 (1H, *m*) [**]	C-5, C-7 , C-8 and C-19
10	33.59	C	-	-
11	20.27	CH_2	1.57 (2H, *m*) [**]	C-8, C-9, C-12 and C-13
12	40.89	CH_2	1.51 (1H, *m*), 1.72 (1H, *m*) [**]	-
13	52.16	C	-	-
14	80.94	C	-	-
15	34.35	CH_2	1.59 (1H, *m*), 1.78 (1H, *m*) [**]	C-13, C-14 and C-17
16	27.71	CH_2	1.51 (1H, *m*), 2.31 (1H, *m*) [**]	C-14 and C-20

Table 9 (Continued)

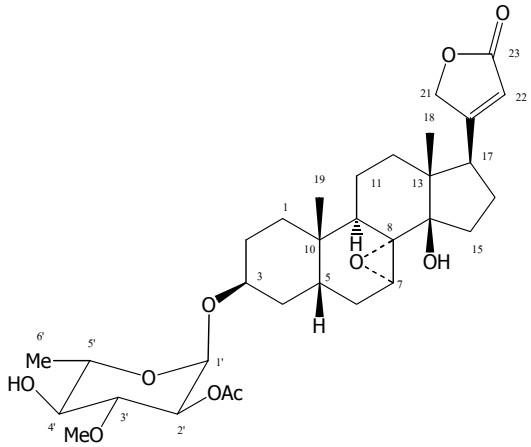
Position	δ_C[#] (ppm)		δ_H (ppm)	HMBC
17	50.56	CH	2.82 (1H, *t, J* = 6 Hz)	C-12, C-14, C-20, C-21 and C-22
18	17.00	CH_3	0.92 (3H, *s*)	C-12, C-13, C-14 and C-17
19	24.23	CH_3	0.98 (3H, *s*)	C-1 and C-5
20	173.54	C	-	-
21	73.31	CH_2	4.95 (1H, *d, J* = 18.5 Hz)	C-22 and C-23
			4.79 (1H, *d, J* = 18.5 Hz)	
22	117.79	= CH	5.88 (1H, *s*)	C-17, C-21 and C-23
23	174.26	C	-	-
1′	97.23	CH	4.84 (1H, *d*, *J* = 4 Hz)	C-3, C-3′ and C-5′
2′	72.65	CH	3.58 (1H, *br t*)	C-3′
3′	84.46	CH	3.23 (1H, *t, J* = 9.5 Hz)[*]	C-4′, C-5′ and 3′- OMe
4′	74.66	CH	3.15 (1H, *t, J* = 9.5 Hz)	C-3′, C-5′ and C-6′
5′	67.54	CH	3.73 (1H, *m*)	C-4′
6′	17.47	CH_3	1.25 (3H, *d, J* = 6 Hz)	C-4′ and C-5′
3′-OMe	60.62	CH_3	3.68 (3H, *s*)	C-3′

[#] Type of carbon deduced by DEPT, [*] Superimposed, [**] Position of proton deduced by HMQC and HMBC.

Table 10 Comparison of ^{1}H NMR spectral data between compound **SM2** and deacetyltanghinin

Position	Compound SM2, δ_H (ppm)	Deacetyltanghinin, δ_H (ppm) (recorded in pyridine)
7	3.23 (1H, *d, J* = 6.5 Hz)	3.41 (1H, *d, J* = 6 Hz)
18	0.92 (3H, *s*)	1.00 (3H, *s*)
19	0.98 (3H, *s*)	1.04 (3H, *s*)
21	4.95 (1H, *d, J* = 18.5 Hz)	5.11 (2H, *d, J* = 8 Hz)
	4.79 (1H, *d, J* = 18.5 Hz)	
22	5.88 (1H, *s*)	6.14 (1H, *br s*)
1′	4.84 (1H, *d, J* = 4 Hz)	5.24 (1H, *d, J* = 3 Hz)
6′	1.25 (3H, *d, J* = 6 Hz)	1.67 (3H, *d, J* = 6 Hz)
3′-OMe	3.68 (3H, *s*)	3.86 (3H, *s*)

1.3.1.3 Compound SM3



Compound **SM3** was isolated as a white solid, mp = 230-232.5 °C, $[\alpha]_D^{27}$ = -66.67° (*c* = 0.03, $CHCl_3$). The IR spectrum of this compound showed absorption of OH group (3483 cm^{-1}), C=O group (1782 and 1745 cm^{-1}) and C-O stretching (1034 cm^{-1}). The UV spectrum showed maxima at 270 and 217 nm.

The ^{13}C NMR spectral data (see **Table 12**) of compound **SM3** recorded in $CDCl_3$ showed 32 signals for 32 carbon atoms. Analysis of the DEPT-90° and DEPT-135° spectra of this compound suggested the presence of five methyl carbon atoms (δ 60.54, 24.21, 20.85, 17.46 and 16.94), nine methylene carbon atoms (δ 73.29, 40.82, 34.26, 32.20, 31.22, 28.22, 27.65, 26.97 and 20.21), eleven methine carbon atoms (δ 117.65, 93.71, 80.75, 75.14, 74.19, 71.56, 67.02, 51.05, 50.47, 33.66 and 31.35) and seven signals for quaternary carbon atoms (δ 174.29, 173.66, 170.16, 80.93, 63.90, 52.12 and 33.48).

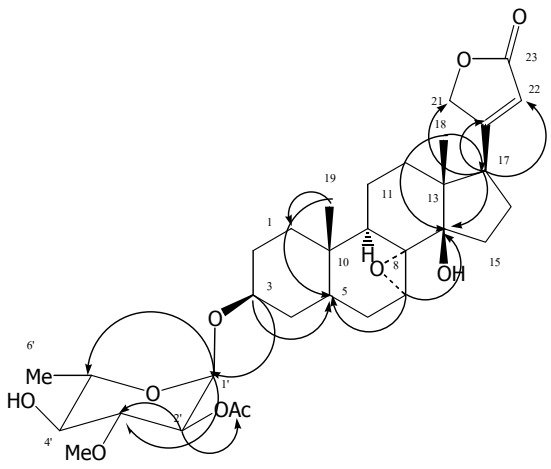
Compound **SM3**, a derivative of compound **SM2**, showed similar characteristic bands in IR and UV spectrum with **SM2**. Comparison of the ^{1}H NMR spectral data (see **Table 13**) of the two compounds revealed close structural similarity. Difference in the spectrum of compound **SM3** was shown as a signal of acetoxy proton at δ 2.07 (3H, *s*) which was not observed in compound **SM2**. The characteristic bands of butenolide ring were shown as a *broad triplet* at δ 5.89 (1H, J = 1.5 Hz) and *doublet of doublet* AB system at δ 4.96 (1H, J = 18.5, 2.0 Hz) and 4.80 (1H, J = 18.5, 2.0 Hz) which were assigned to H-22 H-21a and H21-b, respectively. The moiety of sugar appeared at δ 5.05 (1H, *d,* J = 4 Hz), 4.63 (1H, *dd,* J = 10, 4 Hz), 3.78 (1H, *dq,* J = 10, 6.5 Hz), 3.56 (1H, *t,* J = 10 Hz), 3.58 (-OCH_3), 3.20 (1H, *t,* J = 10 Hz), 1.26 (3H, *d,* J = 6.5 Hz), which could be assigned to H-1′, H-2′, H-5′, H-3′, 3′-O$\underline{CH_3}$, H-4′and 3H-6′, respectively. The H-2′ in compound **SM3** (4.63, 1H, *dd,* J = 10, 4 Hz) appeared at a lower field than H-2′ in compound **SM2** (3.58, 1H, *br t*). These observations indicated that position 2′ of compound **SM3** should be connected with the acetyl group.

The HMBC correlation of compound **SM3** (see **Table 12**) showed the same correlation with compound **SM2** (see **Table 9**) except the proton signal at C-2′ (δ 4.63, 1H, *dd*, J = 10, 4 Hz). This signal gave correlation peaks with carbonyl carbon (170.16) and C-3′ (80.75), thus confirming the position of the acetoxy group at C-2′ in

the sugar moiety. Compound **SM3** was identified as 3*β*-*O*-(2′-*O*-acetyl-l-thevetosyl)-7*β*, 8*β*-epoxy-14*β*-hydroxy-5*β*-card-20(22)-enolide, (tanghinin) which was the compound previously isolated from the leaves, seeds and barks of *C. manghas.* (Abe, *et.al.,* 1977).

Table 11 500 MHz COSY Correlation of some protons of compound **SM3**

δ_H (ppm)	**Proton Correlation with δ_H (ppm)**
H-21a (4.96)	H-21b
H-21b (4.80)	H-21a
H-1′ (5.05)	H-2′
H-2′ (4.63)	H-1′ and H-3′
H-3′ (3.56)	H-2′ and H-4′
H-4′ (3.20)	H-3′ and H-5′
H-5′ (3.78)	H-4′ and H-6′
H-6′ (1.26)	H-5′



Selected HMBC correlation of compound **SM3**

Table 12 ^{1}H, ^{13}C and HMBC spectral data of compound **SM3**

Position	$\delta_C^{\#}$ **(ppm)**		δ_H **(ppm)**	**HMBC**
1	31.22	CH_2	1.48 (1H, *m*), 2.19 (1H, *m*)	C-3 , C-5 and C-19
2	26.97	CH_2	1.54 (1H, *m*), 1.63 (1H, *m*)	C-3 and C-10
3	71.56	CH	3.84 (1H, *br t*, J = 2.5 Hz)	C-1 , C-5 and C-1′
4	32.20	CH_2	1.29 (1H, *m*), 1.40 (1H, *m*)	C-2 , C-6 and C-10
5	33.66	CH	1.56 (1H, *m*)	C-1, C-7 and C-19
6	27.65	CH_2	1.44 (1H, *m*), 2.26 (1H, *m*)	C-4, C-7, C-8 and C-10
7	51.05	CH	3.22 (1H, *d*, J = 6 Hz)	C-5, C-6, C-8 and C-14
8	63.90	C	-	-
9	31.35	CH	1.31 (1H, *m*)	C-1, C-5 and C-19
10	33.48	C	-	-
11	20.21	CH_2	1.54 (2H, *m*)	C-1, C-7, C-8 and C-13
12	40.82	CH_2	1.54 (1H, *m*), 1.71 (1H, *m*)	C-9, C-14, C-17 and C-18
13	52.12	C	-	-
14	80.93	C	-	-
15	34.26	CH_2	1.73 (1H, *m*), 2.22 (1H, *m*)	C-8, C-13 and C-17
16	28.22	CH_2	1.94 (1H, *m*), 2.22 (1H, *m*)	C-13, C-14 and C-20
17	50.47	CH	2.82 (1H, *dd*, J = 9, 6 Hz)	C-12, C-13, C-14, C-16, C-20, C-21 and C-22

Table 12 (Continued)

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
18	16.94	CH_3	0.91 (3H, *s*)	C-12, C-14 and C-17
19	24.21	CH_3	0.98 (3H, *s*)	C-1 and C-5
20	173.66	C	-	-
21a	73.29	CH_2	4.96 (1H, *dd, J* = 18.5, 2.0 Hz)	C-22 and C-23
21b			4.80 (1H, *dd*, *J* = 18.5, 2.0 Hz)	
22	117.65	= CH	5.89 (1H, *br t, J* = 1.5 Hz)	C-17, C-21 and C-23
23	174.29	C	-	-
1′	93.71	CH	5.05 (1H, *d*, *J* = 4 Hz)	C-3, C-3′ and C-5′
2′	74.19	CH	4.63 (1H, *dd*, *J* = 10, 4 Hz)	2′-C=O, C-3′ and C-4′
3′	80.75	CH	3.56 (1H, *t*, *J* = 10 Hz)	C-2′, 3′-OMe and C-5′
4′	75.14	CH	3.20 (1H, *t, J* = 10 Hz)	C-3′ and C-5′
5′	67.02	CH	3.78 (1H, *dq*, *J* = 10, 6.5 Hz)	C-3′
6′	17.46	CH_3	1.26 (1H, *d*, *J* = 6.5 Hz)	C-4′ and C-5′
2′-OAc	20.85	CH_3	2.07 (3H, *s*)	C-2′
2′-C=O	170.16	C	-	-
3′-OMe	60.54	CH_3	3.58 (3H, *s*)	C-3′

[#] Carbon type deduced from DEPT experiment.

Table 13 Comparison of ^{1}H NMR spectral data between compound **SM3** and **SM2**

Position	**Compound SM3, δ_H (ppm)**	**Compound SM2, δ_H (ppm)**
1	1.48 (1H, *m*), 2.19 (1H, *m*)	1.32 (1H, *m*), 1.54 (1H, *m*)
3	3.84 (1H, *br t*, *J* = 2.5 Hz)	3.93 (1H, *s*)
4	1.29 (1H, *m*), 1.40 (1H, *m*)	1.42 (1H, *m*), 1.53 (1H, *m*)
5	1.56 (1H, *m*)	2.28 (1H, *m*)
7	3.22 (1H, *d*, *J* = 6 Hz)	3.23 (1H, *d*, *J* = 6.5 Hz)
9	1.31 (1H, *m*)	2.25 (1H, *m*)
12	1.54 (1H, *m*), 1.71 (1H, *m*)	1.51 (1H, *m*), 1.72 (1H, *m*)
15	1.73 (1H, *m*), 2.22 (1H, *m*)	1.59 (1H, *m*), 1.78 (1H, *m*)
17	2.82 (1H, *dd*, *J* = 9, 6 Hz)	2.82 (1H, *t, J* = 6 Hz)
18	0.91 (3H, *s*)	0.92 (3H, *s*)
19	0.98 (3H, *s*)	0.98 (3H, *s*)
21	4.96 (1H, *dd*, *J* = 18.5, 2.0 Hz)	4.95 (1H, *d*, *J* = 18.5 Hz)
	4.80 (1H,dd, *J* = 18.5, 2.0 Hz)	4.79 (1H, *d*, *J* = 18.5 Hz)
22	5.89 (1H, *br t, J* = 1.5 Hz)	5.88 (1H, *s*)
1′	5.05 (1H, *d*, *J* = 4 Hz)	4.84 (1H, *d, J* = 4 Hz)
2′	4.63 (1H, *dd, J* = 10, 4 Hz)	3.58 (1H, *br t*)
3′	3.56 (1H, *t, J* = 10 Hz)	3.23 (1H, *t, J* = 9.5 Hz)
4′	3.20 (1H, *t, J* = 10 Hz)	3.15 (1H, *t, J* = 9.5 Hz)
5′	3.78 (1H, *dq*, *J* = 10, 6.5 Hz)	3.73 (1H, *m*)
6′	1.26 (3H, *d, J* = 6.5 Hz)	1.25 (3H, *d, J* = 6 Hz)
2′-OAc	2.07 (3H, *s*)	-
3′-OMe	3.58 (3H, *s*)	3.68 (3H, *s*)

Table 14 Comparison of ^{13}C NMR spectral data between compound **SM3** and **SM2**

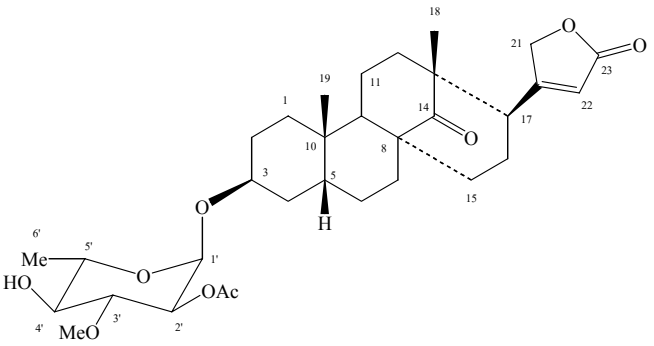
Position	Compound SM3, δ_C (ppm)	Compound SM2, δ_C (ppm)
1	31.22	31.49
2	26.97	26.93
3	71.56	72.82
4	32.20	32.60
5	33.66	34.13
6	27.65	28.29
7	51.05	51.07
8	63.90	63.84
9	31.35	31.44
10	33.48	33.59
11	20.21	20.27
12	40.82	40.89
13	52.12	52.16
14	80.93	80.94
15	34.26	34.35
16	28.22	27.71
17	50.47	50.56
18	16.94	17.00
19	24.21	24.23
20	173.66	173.54
21	73.29	73.31
22	117.65	117.79
23	174.29	174.26
1′	93.71	97.23
2′	74.19	72.65
3′	80.75	84.46
4′	75.14	74.66

Table 14 (Continued)

Position	Compound SM3, δ_C (ppm)	Compound SM2, δ_C (ppm)
5′	67.02	67.54
6′	17.46	17.47
2′-OAc	20.85	-
2′-C=O	170.16	-
3′-OMe	60.54	60.62

[a, b, c] Assignment with the same superscripts may be interchanged.

1.3.1.4 Compound SM4



Compound **SM4** was isolated as a white solid, mp = 209-211 °C, $[\alpha]_D^{27}$ = - 62.0° (*c* = 0.048, $CHCl_3$). Its UV absorption spectrum showed maximum at 210 nm. The IR spectrum of compound **SM4** showed absorption bands at 3446 cm^{-1} (hydroxy group) and 1741, 1740 and 1697 cm^{-1} (carbonyl groups).

The complete analysis of ^{13}C and ^{1}H NMR spectral data of compound **SM4** (**Table 16**) were assigned with the informations provided from ^{1}H-^{1}H COSY (**Table 15**), HMQC, and HMBC (**Table 16**). The ^{13}C NMR spectrum of compound **SM4** recorded in $CDCl_3$ (**Table 16**) showed 32 signals for 32 carbon atoms, however, no C-14 carbinol

carbon peak was observed between δ 83 and 85 when compared with compound **SM1** (**Table 5**). Analysis of the DEPT-90° and DEPT-135° spectrum of this compound indicated the existence of five methyl carbon atoms (δ 60.55, 26.52, 23.33, 20.89 and 17.58), ten methylene carbon atoms (δ 72.77, 44.04, 42.57, 31.65, 29.80, 28.96, 28.96, 26.92, 24.02 and 21.30), ten methine carbon atoms (δ 116.63, 93.85, 80.83, 75.28, 74.26, 72.15, 66.98, 53.10, 45.80 and 36.87) and seven quaternary carbon atoms (δ 220.93, 173.56, 170.45, 170.24, 48.75, 47.35 and 37.26).

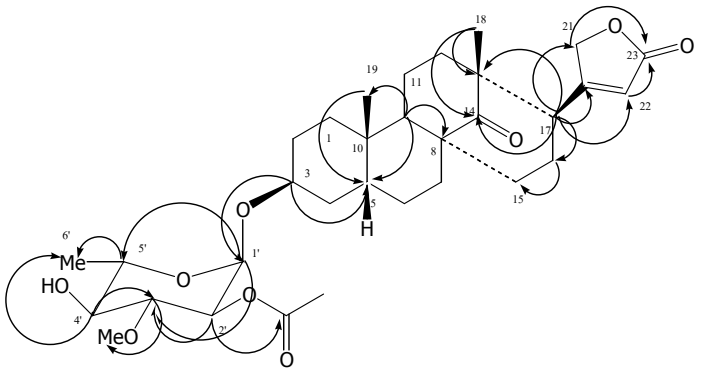
The ^{1}H NMR spectrum of compound **SM4** (**Table 16**) was a typical of cardenolide glycoside (similar to compound **SM1**, **Table 5**). The normal typical characteristic peaks were shown at δ 5.71 (1H, *t*, *J* = 1.5 Hz) accompanied by AB system at δ 4.69 (1H, *dd, J* = 18.0, 1.5 Hz) and 4.57 (1H, *dd*, *J* = 18.0, 1.5 Hz) which were attributed to a butenolide ring and these protons were assigned to H-22, H-21a and H-21b, respectively. The sugar protons appeared at δ 5.07 (1H, *d, J* = 4 Hz), 4.65 (1H, *dd, J* = 9, 4 Hz), 3.81 (1H, *dq, J* = 9, 6 Hz), 3.59 (1H, *t*, *J* = 9 Hz), 3.59 (3H, *s*), 3.23 (1H, *t, J* = 9 Hz), 2.08 (3H, *s*) and 1.27 (3H, *d*, *J* = 6 Hz) which were assigned to H-1′, H-2′, H-5′, H-3′, 3′-O<u>Me</u>, H-4′, 2′-O<u>Ac</u> and 3H-6′, respectively. The two methyl protons, one oxymethine and one methine proton of steroidal ring appeared at δ 0.97 (3H-19, *s*), 0.89 (3H-18, *s*), 3.88 (1H-3, *br s*) and 3.10 (1H-17, *d*, *J* = 7 Hz), respectively.

The correlation peaks in the HMBC spectra (**Table 16**) of H-3 (δ 3.88) with the carbons at δ 31.65 (C-1), 36.87 (C-5) and 93.85 (C-1′); and H-1′ (δ 5.07) with the carbons at δ 72.15 (C-3) and 80.83 (C-3′), indicating that the glycosidic linkage was formed between sugar moiety and the steroid at C-3. The proton signal at δ 3.10 (H-17) showed the correlation by long-range coupling with the carbon signals at 42.57 (C-12), 44.04 (C-15), 47.35 (C-13), 72.77 (C-21), 116.63 (C-22) and 170.24 (C-20) and proton at δ 2.87 (1H, *m*) showed correlation peaks with 48.75 (C-8), 53.10 (C-17) and 170.24 (C-20), thus the position of the butenolide ring at C-17 was confirmed. The proton at δ 2.87 should be

assigned to H-16a. Thus compound **SM4** was identified as 3β-*O*-(2′-*O*-acetyl-l-thevetosyl)-15(8→14)-*abeo*-5β-(8R)-14-oxo-card-20(22)-enolide, (2′-acetoxy-cerleaside A) which was the compound previously isolated from the seeds of *C. odollam* (Laphookhieo, *et.al*., 2004)

Table 15 500 MHz COSY Correlation of some protons of compound **SM4**

δ_H (ppm)	**Proton Correlation with δ_H (ppm)**
H-17 (3.10)	H-16a (2.88) and 16b (1.58)
H-21a (4.69)	H-21b
H-21b (4.57)	H-21a
H-1′(5.07)	H-2′
H-2′ (4.65)	H-1′ and H-3′
H-3′ (3.59)	H-2′ and H-4′
H-4′ (3.23)	H-3′ and H-5′
H-5′ (3.81)	H-4′ and H-6′
H-6′ (1.27)	H-5′



Selected HMBC correlation of compound **SM4**

Table 16 ^{1}H, ^{13}C and HMBC spectral data of compound **SM4**

Position	δ_C [#] (ppm)		δ_H **(ppm)**	**HMBC**
1	31.65	CH_2	1.45 (1H, *m*), 1.59 (1H, *m*)	-
2	29.80	CH_2	1.33 (1H, *m*), 1.61 (1H, *m*)	-
3	72.15	CH	3.88 (1H, *br s*)	C-1, C-5 and C-1′
4	28.96	CH_2	1.09 (1H, *m*), 1.96 (1H, *m*)	-
5	36.87	CH	1.60 (1H, *m*)	-
6	28.96	CH_2	1.06 (1H, *m*), 1.94 (1H, *m*)	-
7	24.02	CH_2	1.11 (1H, *m*), 2.18 (1H, *m*)	-
8	48.75	C	-	-
9	45.80	CH	2.50 (1H, *d*, *J* = 8.5 Hz)	C-1, C-5 , C-8 , C-12 , C-14 and C-19
10	37.26	C	-	-
11	21.30	CH_2	1.84 (1H, *m*), 2.47 (1H, *m*)	-
12	42.57	CH_2	2.10 (2H, *m*)	-
13	47.35	C	-	-
14	220.93	C	-	-
15	44.04	CH_2	2.17 (2H, *m*)	-
16	26.92	CH_2	1.57 (1H, *m*), 2.87 (1H, *m*)	C-15 , C-17 , C-8 and C-20
17	53.10	CH	3.10 (1H, *d*, *J* = 7 Hz)	C-12 , C-13 , C-15 , C-20 , C-21 and C-22

Table 16 (Continued)

Position	δ_C[#] (ppm)		δ_H (ppm)	HMBC
18	23.33	CH_3	0.89 (3H, *s*)	C-15, C-13, C-16 and C-17
19	26.52	CH_3	0.97 (3H, *s*)	C-1, C-5 and C-9
20	170.24	C	-	-
21a	72.77	CH_2	4.69 (1H, *dd*, *J* = 18.0, 1.5 Hz)	C-20, C-22 and C-23
21b			4.57 (1H, *dd*, *J* = 18.0, 1.5 Hz)	
22	116.63	= CH	5.71 (1H, *t*, *J* = 1.5 Hz)	C-20, C-21 and C-23
23	173.56	C	-	-
1′	93.85	CH	5.07 (1H, *d*, *J* = 4 Hz)	C-3, C-3′and C-5′
2′	74.26	CH	4.65 (1H, *dd*, *J* = 9, 4 Hz)	C-3′and C-2′-C=O
3′	80.83	CH	3.59 (1H, *t*, *J* = 9 Hz)	C-2′ and C-3′-OMe
4′	75.28	CH	3.23 (1H, *t*, *J* = 9 Hz)	C-3′ , C-5′ and 6′
5′	66.98	CH	3.81 (1H, *dq*, *J* = 9, 6 Hz)	C-3′, C-4′ and 6′
6′	17.58	CH_3	1.27 (3H, *d*, *J* = 6 Hz)	C-4′ and C-5′
2′-O<u>Ac</u>	20.89	CH_3	2.08 (3H, *s*)	C-2′
2′-C=O	170.45	C	-	-
3′-O<u>Me</u>	60.55	CH_3	3.59 (3H, *s*)	C-3′

[#] Carbon type deduced from DEPT experiment.

Table 17 Comparison of ^{1}H NMR spectral data between compound **SM4** and **SM1**

Position	δ_H (ppm) compound SM4	δ_H (ppm) compound SM1
1	1.45 (1H, *m*), 1.59 (1H, *m*)	1.45 (1H, m), 1.76 (1H, *m*)
2	1.33 (1H, *m*), 1.61 (1H, *m*)	1.54 (1H, m), 1.62 (1H, *m*)
3	3.88 (1H, *br s*)	3.95 (1H, *br s*)
4	1.09 (1H, *m*), 1.96 (1H, *m*)	1.37 (1H, m), 1.54 (1H, *m*)
5	1.60 (1H, *m*)	1.62 (1H, *m*)
6	1.06 (1H, *m*), 1.94 (1H, *m*)	1.24 (1H, *m*), 1.62 (1H, *m*)
7	1.11 (1H, *m*), 2.18 (1H, *m*)	1.70 (2H, *m*)
9	2.50 (1H, *d*, *J* = 8.5 Hz)	1.60 (1H, *m*)
11	1.84 (1H, *m*), 2.47 (1H, *m*)	1.21 (1H, *m*), 1.42 (1H, *m*)
12	2.10 (2H, *m*)	1.37 (1H, *m*), 1.50 (1H, *m*)
15	2.17 (2H, *m*)	1.68 (1H, *m*), 2.08 (1H, *m*)
16	1.57 (1H, m), 2.87 (1H, *m*)	1.86 (1H, *m*), 2.15 (1H, *m*)
17	3.10 (1H, *d*, *J* = 7 Hz)	2.77 (1H, *dd*, *J* = 9, 5 Hz)
18	0.97 (3H, *s*)	0.86 (3H, *s*)
19	0.89 (3H, *s*)	0.94 (3H, *s*)
21	4.69 (1H, *dd, J* = 18.0, 1.5 Hz)	4.79 (1H, *dd, J* = 18.5, 1.5 Hz)
	4.57 (1H, *dd, J* = 18.0, 1.5 Hz)	4.97 (1H, *dd*, *J* = 18.5, 1.5 Hz)
22	5.71 (1H, *t*, *J* = 1.5 Hz)	5.87 (1H, *s*)
1′	5.07 (1H, *d*, *J* = 4 Hz)	4.83 (1H, *d*, *J* = 3.5 Hz)
2′	4.65 (1H, *dd, J* = 9, 4 Hz)	3.53 (1H, *dd, J* = 9, 3.5 Hz)
3′	3.59 (1H, *t*, *J* = 9 Hz)	3.24 (1H, *t, J* = 9 Hz)
4′	3.23 (1H, *t*, *J* = 9 Hz)	3.10 (1H, *t*, *J* = 9 Hz)
5′	3.81 (1H, *dq*, *J* = 9, 6 Hz)	3.71 (1H, *dq*, *J* = 9, 6 Hz)
6′	1.27 (3H, *d*, *J* = 6 Hz)	1.22 (3H, *d*, *J* = 6 Hz)
2′-OAc	2.08 (3H, *s*)	-
3′-OMe	3.59 (3H, *s*)	3.66 (3H, *s*)

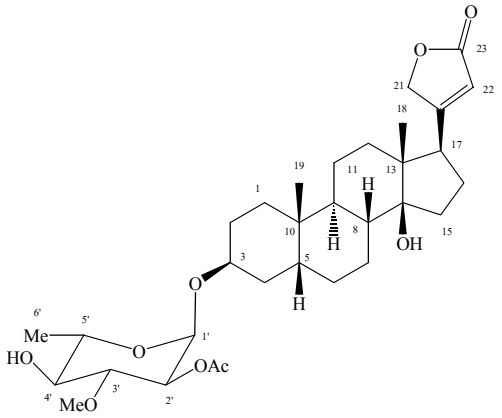
Table 18 Comparison of ^{13}C NMR spectral data between compound **SM4** and **SM1**

Position	δ_C **(ppm) compound SM4**	δ_C **(ppm) compound SM1**
1	31.65	29.91
2	29.80	26.55
3	72.15	73.39
4	28.96	30.55
5	36.87	36.52
6	28.96	26.28
7	24.02	21.14
8	48.75	41.64
9	45.80	35.66
10	37.26	35.19
11	21.30	21.29
12	42.57	39.95
13	47.35	49.63
14	220.93	84.55
15	44.04	32.91
16	26.92	26.44
17	53.10	50.90
18	23.33	15.71
19	26.52	23.78
20	170.24	175.04
21	72.77	73.60
22	116.63	117.48
23	173.56	175.06
$1'$	93.85	97.15
$2'$	74.26	72.66
$3'$	80.83	85.24

Table 18 (Continued)

Position	δ_C (ppm) compound SM4	δ_C (ppm) compound SM1
4′	75.28	74.68
5′	66.98	67.60
6′	17.58	17.40
2′-OAc	20.89	-
2′-C=O	170.45	-
3′-OMe	60.55	60.54

1.3.1.5 Compound SM5



Compound **SM5** was isolated as a white solid, mp = 211-213 °C, $[\alpha]_D^{27}$= -90.50° (*c* = 0.022, $CHCl_3$). The IR spectrum showed absorption bands which were ascribed to OH stretching of hydroxy group (3453 cm^{-1}) and C=O stretching of carbonyl (1741 and 1624 cm^{-1}). The UV spectrum showed maximum at 218 nm.

The complete analysis of ^{13}C and ^{1}H NMR spectral data of compound **SM5** (**Table 20**) were assigned with informations provided from ^{1}H-^{1}H COSY (**Table 19**), ^{1}H-^{13}C correlation (HMQC) and ^{1}H-^{13}C correlation by long-range coupling (HMBC)

(**Table 20**), along with comparison of ^{1}H NMR spectral data to compound **SM1** (**Table 5**). The ^{13}C NMR (**Table 20**) spectrum of compound **SM5** recorded in $CDCl_3$ showed 32 signals for 32 carbon atoms. Analysis of the DEPT-90° and DEPT-135° spectra of this compound suggested the presence of five methyl carbon atoms (δ 60.49, 23.89, 20.89, 17.54 and 15.76), ten methylene carbon atoms (δ 73.48, 40.00, 33.12, 30.36, 29.90, 26.88, 26.62, 26.59, 21.34 and 21.19), eleven methine carbon atoms (δ 117.61, 93.73, 80.93, 75.33, 74.30, 72.25, 67.08, 50.92, 41.82, 36.47 and 35.65) and six quaternary carbon atoms (δ174.69, 174.68, 170.27, 85.49, 49.62 and 35.19).

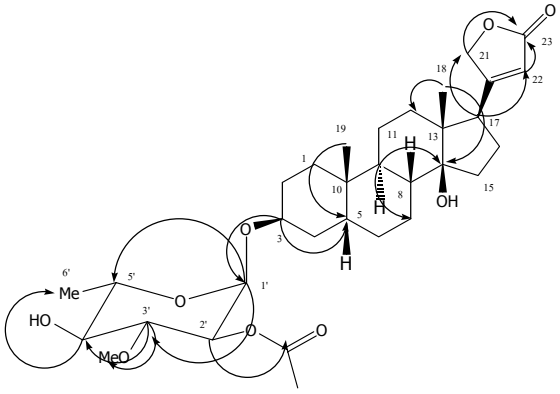
Compound **SM5**, a derivative of compound **SM1**, showed similar characteristic bands in IR and UV spectrum with those of **SM1**. Comparison of the ^{1}H NMR spectral data (**Table 21**) of the two compounds revealed close structural similarity. Difference in the spectrum of compound **SM5** was shown as a signal of acetoxy proton at δ 2.07 (3H, *s*) which was not observed in compound **SM1**. The characteristic bands of butenolide ring were shown as a *singlet* at δ 5.88 (1H) and a *doublet of doublet* AB system at δ 5.00 (1H, J = 18.5, 2.0 Hz) and 4.82 (1H, J = 18.5, 2.0 Hz) which were assigned to H-22, 21a and H21-b, respectively. The moiety of sugar appeared at δ 5.05 (1H, *d, J* = 3.5 Hz), 4.63 (1H, *dd, J* = 9, 3.5 Hz), 3.79 (1H, *dq, J* = 9, 6 Hz), 3.66 (1H, *t, J* = 9 Hz), 3.66 (-OCH_3), 3.21 (1H, *t, J* = 9 Hz), 1.27 (3H, *d, J* = 6 Hz), which could be assigned to H-1′, H-2′, H-5′, H-3′, 3′-OCH_3, H-4′ and 3H-6′, respectively. The H-2′ in compound **SM5** (4.63, 1H, *dd, J* = 9, 3.5 Hz) appeared at the lower field than H-2′ in compound **SM1** (3.53, 1H, *dd, J* = 9, 3.5 Hz). These observations indicated that the position 2′ of compound **SM5** should be connected to the acetoxy group.

The HMBC correlation of compound **SM5** (**Table 20**) showed the same correlation with compound **SM1** (**Table 5**) except the proton signal at C-2′ (δ 4.63, 1H, *dd*, J = 9, 4 Hz). This signal gave correlation peaks with carbonyl carbon (170.27)

and C-3′ (80.93), thus confirming the position of the acetoxy group at C-2′ in the sugar moiety. Compound **SM5** was identified as 3β-*O*-(2′-*O*-acetyl-l-thevetosyl) - 14β-hydroxy-5β-card-20(22)-enolide, (cerberin) which was the compound previously isolated from the leaves of *C. odollam* and *C. manghas* (Yamauchi, *et.al.,* 1987).

Table 19 500 MHz COSY Correlation of some protons of compound **SM5**

δ_H (ppm)	**Proton Correlation with δ_H (ppm)**
H-21a (5.00)	H-21b
H-21b (4.82)	H-21a
H-1′ (5.05)	H-2′
H-2′ (4.63)	H-1′ and H-3′
H-3′ (3.66)	H-2′ and H-4′
H-4′ (3.21)	H-3′ and H-5′
H-5′ (3.79)	H-4′ and H-6′
H-6′ (1.27)	H-5′



Selected HMBC correlation of compound **SM5**

Table 20 ^{1}H, ^{13}C and HMBC spectral data of compound **SM5**

Position	δ_C (ppm)		δ_H (ppm)	HMBC
1	29.90	CH_2	1.31 (1H, *m*), 1.68 (1H, *m*)	-
2	26.88[a]	CH_2	1.50 (2H, *m*)*	-
3	72.25	CH	3.87 (1H, *br s*)	C-1, C-5 and C-1′
4	30.36	CH_2	1.48 (2H, *m*)	-
5	36.47	CH	1.64 (1H, *m*)	-
6	26.62[a]	CH_2	1.22 (2H, *m*)*	-
7	21.19[b]	CH_2	1.70 (2H, *m*)**	-
8	41.82	CH	1.55 (1H, *m*)	-
9	35.65	CH	1.60 (1H, *m*)	-
10	35.19	C	-	-
11	21.34[b]	CH_2	1.21 (1H, *m*), 1.42 (1H, *m*)**	-
12	40.00	CH_2	1.38 (1H, *m*), 1.51 (1H, *m*)	-
13	49.62	C	-	-
14	85.49	C	-	-
15	33.12	CH_2	1.68 (1H, *m*), 2.10 (1H, *m*)	-
16	26.59[a]	CH_2	1.84 (1H, *m*), 2.15 (1H, *m*)	-
17	50.92	CH	2.78 (1H, *dd, J* = 9, 5 Hz)	C-12 , C-13 , C-14 , C-16 , C-20 , C-21 and C-22

Table 20 (Continued)

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
18	15.76	CH_3	0.87 (3H, *s*)	C-12 , C-13 , C-14 and C-17
19	23.89	CH_3	0.95 (3H, *s*)	C-1 and C-5
20	174.69	C	-	-
21a	73.48	CH_2	5.00 (1H, *dd, J* = 18.5, 2.0 Hz)	C-17, C-20 and C-23
21b			4.82 (1H, *dd, J* = 18.5, 2.0 Hz)	
22	117.61	= CH	5.88 (1H, *s*)	C-17, C-20, C-21, C-20 and C-23
23	174.68	C	-	-
1′	93.73	CH	5.05 (1H, *d*, *J* = 3.5 Hz)	C-3, C-3′and C-5′
2′	74.30	CH	4.63 (1H, *dd, J* = 9, 3.5 Hz)	C-3′ and 2′-C=O
3′	80.93	CH	3.66 (1H, *t, J* = 9 Hz)	C-2′, C-4′ and 3′- O<u>Me</u>
4′	75.33	CH	3.21 (1H, *t, J* = 9 Hz)	C-3′, C-5′ and C-6′
5′	67.08	CH	3.79 (1H, *dq, J* = 9, 6 Hz)	C-4′
6′	17.54	CH_3	1.27 (3H, *d, J* = 6 Hz)	C-4′ and C-5′
2′-O<u>Ac</u>	20.89	CH_3	2.07 (3H, *s*)	C-2′
2′-C=O	170.27	C	-	-
3′-O<u>Me</u>	60.49	CH_3	3.66 (3H, *s*)	C-3′

[a, b, c] Assignment with the same superscripts may be interchanged, [#] Type of carbon deduced by DEPT, [*, **] Assignment with the same superscripts may be interchanged.

Table 21 Comparison of ^{1}H NMR spectral data between compound **SM5** and **SM1**

Position	Compound SM5, δ_H (ppm)	Compound SM1, δ_H (ppm)
1	1.31 (1H, *m*), 1.68 (1H, *m*)	1.45 (1H, *m*), 1.76 (1H, *m*)
3	3.87 (1H, *br s*)	3.95 (1H, *br s*)
4	1.48 (2H, *m*)	1.37 (1H, *m*), 1.54 (1H, *m*)
5	1.64 (1H, *m*)	1.62 (1H, *m*)
8	1.55 (1H, *m*)	1.55 (1H, *m*)
9	1.60 (1H, *m*)	1.60 (1H, *m*)
12	1.38 (1H, *m*), 1.51 (1H, *m*)	1.37 (1H, *m*), 1.50 (1H, *m*)
15	1.68 (1H, *m*), 2.10 (1H, *m*)	1.68 (1H, *m*), 2.08 (1H, *m*)
17	2.78 (1H, *dd, J* = 9, 5 Hz)	2.77 (1H, *dd, J* = 9, 5 Hz)
18	0.87 (3H, *s*)	0.86 (3H, *s*)
19	0.95 (3H, *s*)	0.94 (3H, *s*)
21	5.00 (1H, *dd, J* = 18.5, 2.0 Hz)	4.97 (1H, *dd, J* = 18.5, 1.5 Hz)
	4.82 (1H, *dd, J* = 18.5, 2.0 Hz)	4.79 (1H, *dd, J* = 18.5, 1.5 Hz)
22	5.88 (1H, *s*)	5.87 (1H, *s*)
1′	5.05 (1H, *d, J* = 3.5 Hz)	4.83 (1H, *d, J* = 3.5 Hz)
2′	4.63 (1H, *dd*, *J* = 9, 3.5 Hz)	3.53 (1H, dd, *J* = 9, 3.5 Hz)
3′	3.66 (1H, *t, J* = 9 Hz)	3.24 (1H, *t, J* = 9 Hz)
4′	3.21 (1H, *t, J* = 9 Hz)	3.10 (1H, *t*, *J* = 9 Hz)
5′	3.79 (1H, *dq*, *J* = 9, 6 Hz)	3.71 (1H, *dq*, *J* = 9, 6 Hz)
6′	1.27 (3H, *d, J* = 6 Hz)	1.22 (3H, *d, J* = 6 Hz)
2′-OAc	2.07 (3H, *s*)	-
3′-OMe	3.66 (3H, *s*)	3.66 (3H, *s*)

Table 22 Comparison of ^{13}C NMR spectral data between compound **SM5** and **SM1**

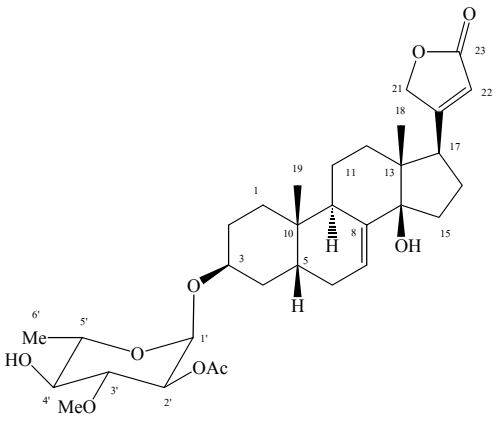
Position	Compound SM5, δ_C (ppm)	Compound SM1, δ_C (ppm)
1	29.90	29.91
2	26.88[a]	26.55[a]
3	72.25	73.39
4	30.36	30.55
5	36.47	36.52
6	26.62[a]	26.28[a]
7	21.19[b]	21.14[b]
8	41.82	41.64
9	35.65	35.66
10	35.19	35.19
11	21.34[b]	21.29[b]
12	40.00	39.95
13	49.62	49.63
14	85.49	84.55
15	33.12	32.91
16	26.59	26.44[a]
17	50.92	50.90
18	15.76	15.71
19	23.89	23.78
20	174.69[c]	175.04[c]
21	73.48	73.60
22	117.61	117.48
23	174.68[c]	175.06[c]
1′	93.73	97.15
2′	74.30	72.66
3′	80.93	85.24
4′	75.33	74.68

Table 22 (Continued)

Position	Compound SM5, δ_C (ppm)	Compound SM1, δ_C (ppm)
5′	67.08	67.60
6′	17.54	17.40
2′-C=O	170.27	-
2′-OAc	20.89	-
3′-OMe	60.49	60.54

[a, b, c] Assignment with the same superscripts may be interchanged.

1.3.1.6 Compound SM6



Compound **SM6** was obtained as a white solid, and its molecular formula was determined as $C_{32}H_{46}O_9$ by HR FABMS ($[M+1]^+$ *m/z* 575.3205, calcd. 575.3220). The IR spectrum showed the presence of hydroxyl (3461 cm^{-1}) and carbonyl (1745, 1716 cm^{-1}), the UV spectrum (λ_{max} 217 nm) suggested the presence of an α, β-unsaturated γ-lactones (Siddiqui, et.al., 1997).

The ^{1}H-NMR spectrum (**Table 24**) showed signals characteristic of cardenolide framework such as methylene protons at C-21 (δ4.82 and 4.99, each *dd*, *J* = 18.0 and 1.5 Hz), an olefinic proton at C-22 (δ 5.92, *br t*, *J* = 1 Hz) and a methine

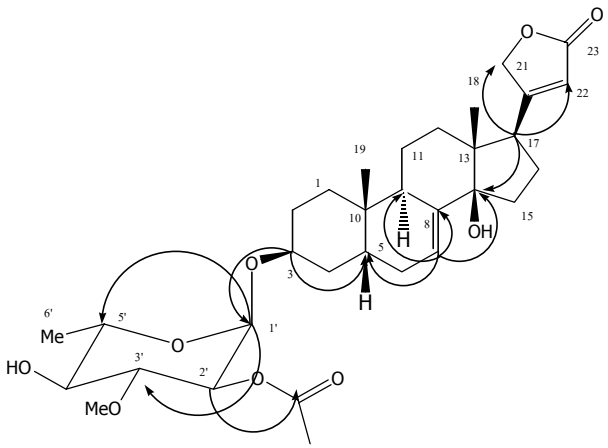
proton at C-17 (δ2.84, *dd*, *J* = 9.5 and 6 Hz) (Abe, et.al., 1977). The ^{1}H- and ^{13}C-NMR spectral data indicated the presence of one sugar molecule according to an anomeric proton at δ5.06 (*d*, *J* = 3.5 Hz) for H-1′ connected to an anomeric carbon at δ 93.84 from the HMQC experiment. The connectivity of all protons of the sugar moiety was assigned by means of the COSY spectrum and comparison with 2′-*O*-acetyl-l-thevetose, the sugar moiety of cerberin (Yamauchi, *et.al*., 1987). Clear separations of the proton signals were shown at δ4.65 (1H, *dd*, *J* = 10 and 3.5 Hz, H-2′), 3.59 (1H, *t*, *J* = 10 Hz, H-3′), 3.22 (1H, *t*, *J* = 10 Hz, H-4′), 3.80 (1H, *m*, H-5′), 1.27 (3H, *d*, *J* = 6.5 Hz, H-6′) and 3.59 (3H, *s*, 3′-OMe). Two methyl *singlets* at δ 0.80 and 0.86 were assigned to 3H-18 and 3H-19, respectively. One oxymethine proton at δ 3.83 (*m*) was assigned to H-3. The ^{13}C NMR (**Table 24**) spectrum of compound **SM6** recorded in $CDCl_3$ showed 32 signals for 32 carbon atoms. Analysis of the DEPT-90° and DEPT-135° spectra of this compound suggested the presence of five methyl carbon atoms (δ 60.54, 24.81, 20.93, 17.55 and 15.98), nine methylene carbon atoms (δ 73.43, 39.77, 39.18, 31.12, 30.27, 29.87, 28.48, 27.68 and 27.40), eleven methine carbon atoms (δ 117.89, 117.71, 93.84, 80.84, 75.27, 74.27, 72.24, 66.95, 50.26, 34.72 and 33.89) and six quaternary carbon atoms (δ 174.45, 174.30, 170.22, 138.72, 85.19 and 50.80).Comparison of its ^{1}H and ^{13}C-NMR spectral data with cerberin (Yamauchi *et. al*., 1987) revealed their close structural similarity except **SM6** showed an olefinic proton at δ 5.80 (*br d*, *J* = 5.5 Hz) which was not observed in cerberin (**Table 25**). This olefinic proton was connected to an olefinic carbon at δ117.89 from the HMQC experiment and attributed to the tri-substituted alkene of H-7, thus suggesting **SM6** to be a Δ^{7}-cardenolide. The assignment was confirmed by HMBC, in which H-7 (δ5.80) showed correlations with C-5 (34.72), C-6 (29.87) and C-14 (85.19); H-11 (δ 1.58) with C-8 (138.72) and C-13 (50.80). Additional correlations were observed between H-17 (δ2.84) with C-12 (39.18), C-14 (85.19), C-20 (174.45), C-21 (73.43) and C-22

(117.71); and between H-3 (δ 3.83) with C-5 (34.72) and C-1′ (93.84), indicating that the α, β-unsaturated γ-lactone was located at C-17 (50.26) and the sugar moiety was located at C-3 (72.24), respectively. The H-2′ (δ 4.65) showed correlation with carbonyl carbon of acetate group (170.22), indicating that the acetate group was located at C-2′.

The relative stereochemistry of **SM6** was supported from NOE correlations observed between interactions of H-17 (δ 2.84) with H-21 and H-22, and interactions of 3H-18 (δ 0.80) with H-21 and H-22, indicating that H-17 must be α-oriented whereas irradiation of H-2′ (δ 4.65) resulted in strong NOE enhancements of H-1′ and H-4′. Irradiation of H-3 (δ 3.83) showed interaction with only H-1′. Thus, the protons position at C-3, C-1′, C-2′ and C-4′ were α-oriented. These observations together with a small coupling constant of H-1′ (J = 3.5 Hz) revealed that the sugar was β-2′-O-acetyl-l-thevetose. The carbon chemical shifts were conclusively assigned on the basis of ^{13}C-NMR, DEPT and HMQC experiment (**Table 24**) and by comparison with cerberin. Therefore compound **SM6** was determined as 3β-*O*-(2′-*O*-acetyl-l-thevetosyl)-7-en-card-20(22)-enolide, (7,8-dehydrocerberin). This compound has not been reported before.

Table 23 500 MHz COSY Correlation of some protons of compound **SM6**

δ_H (ppm)	Proton Correlation with δ_H (ppm)
H-21a (4.99)	H-21b
H-21b (4.82)	H-21a
H-1′ (5.06)	H-2′
H-2′ (4.65)	H-1′ and H-3′
H-3′ (3.59)	H-2′ and H-4′
H-4′ (3.22)	H-3′ and H-5′
H-5′ (3.80)	H-4′ and H-6′
H-6′ (1.27)	H-5′



Selected HMBC correlation of **SM6**

Table 24 ^{1}H, ^{13}C and HMBC spectral data of compound **SM6**

Position	**δ_C[#] (ppm)**		**δ_H (ppm)**	**HMBC**
1	30.27	CH_2	1.51 (2H, *m*)	-
2	27.40[a]	CH_2	**	-
3	72.24	CH	3.83 (1H, *m*)	C-5 , C-6 and C-1′
4	31.12	CH_2	1.29 (1H, *m*), 1.31 (1H, *m*)	-
5	34.72	CH	1.71 (1H, *m*)	-
6	29.87	CH_2	2.43 (1H, *m*), 1.60 (1H, *m*)	-
7	117.89	CH	5.80 (1H, *br d, J* = 5.5 Hz)	C-5 , C-6 and C-14
8	138.72	C	-	-
9	33.89	CH	2.27 (1H, *m*)	-
10	-	C	-	-
11	28.48	CH_2	1.58 (2H, *m*)[§]	C-8 and C-13
12	39.18	CH_2	1.54 (2H, *m*)[§]	C-14 and C-18
13	50.80	C	-	-
14	85.19	C	-	-
15	39.77	CH_2	**	-
16	27.68[a]	CH_2	**	-
17	50.26	CH	2.84 (1H, *dd, J* = 9.5, 6 Hz)	C-12 , C-14 , C-20, C-21 and C-22

Table 24 (Continued)

Position	δ_C [#] (ppm)		δ_H (ppm)	HMBC
18	15.98	CH_3	0.80 (3H, *s*)	C-12 , C-14 and C-17
19	24.81	CH_3	0.86 (3H, *s*)	C-1 and C-5
20	174.45 [b]	C	-	-
21	73.43	CH_2	4.82 (1H, *dd, J* = 18, 1.5 Hz)	C-17, C-20 and C-22
			4.99 (1H, *dd, J* = 18, 1.5 Hz)	
22	117.71	= CH	5.92 (1H, *br t*, *J* = 1.5 Hz)	C-17, C-20 and C-21
23	174.30 [b]	C	-	-
1′	93.84	CH	5.06 (1H, *d*, *J* = 3.5 Hz)	C-3, C-3′ and C-5′
2′	74.27	CH	4.65 (1H, *dd, J* = 10, 3.5 Hz)	C-3′ , 2′-C=O and C-4′
3′	80.84	CH	3.59 (1H, *t, J* = 10 Hz)	C-4′ , C-5′ and 3′- O<u>Me</u>
4′	75.27	CH	3.22 (1H, *t, J* = 10 Hz)	C-3′ , C-5′ and C-6′
5′	66.95	CH	3.80 (1H, *m*)	-
6′	17.55	CH_3	1.27 (3H, *d, J* = 6.5 Hz)	C-4′ and C-5′
2′-O<u>Ac</u>	20.93	CH_3	2.09 (3H, *s*)	C-2′
2′-C=O	170.22	C	-	-
3′-O<u>Me</u>	60.54	CH_3	3.59 (3H, *s*)	C-3′

[a, b] Assignment with the same superscripts may be interchanged, [#] Type of carbon deduced by DEPT, [§] Type of proton deduced by HMQC. [**] The chemical shifts of proton resonated at δ 1.25-2.15.

Table 25 Comparison of ^{1}H NMR spectral data between compound **SM6** and **SM3**

Position	Compound SM6, δ_H (ppm)	Compound SM3, δ_H (ppm)
1	1.51 (2H, *m*)	1.48 (1H, *m*), 2.19 (1H, *m*)
3	3.83 (1H, *m*)	3.84 (1H, *t*, *J* = 2.5 Hz)
4	1.29 (1H, *m*), 1.31 (1H, *m*)	1.29 (1H, *m*), 1.40 (1H, *m*)
5	1.71 (1H, *m*)	1.56 (1H, *m*)
7	5.80 (1H, *br d*, *J* = 5.5 Hz)	3.22 (1H, *d, J* = 6 Hz
9	2.27 (1H, *m*)	1.31 (1H, *m*)
12	1.54 (2H, *m*)	1.54 (1H, *m*), 1.71 (1H, *m*)
17	2.84 (1H, *dd, J* = 9.5, 6 Hz)	2.82 (1H, *dd, J* = 9, 6 Hz)
18	0.80 (3H, *s*)	0.91 (3H, *s*)
19	0.86 (3H, *s*)	0.98 (3H, *s*)
21	4.82 (1H, *dd, J* = 18, 1.5 Hz)	4.80 (1H, *dd, J* = 18.5, 2.0 Hz)
	4.99 (1H, *dd, J* = 18, 1.5 Hz)	4.96 (1H, *dd, J* = 18.5, 2.0 Hz)
22	5.92 (1H, *br t*, *J*= 1Hz)	5.89 (1H, *t, J* = 1.5 Hz)
1′	5.06 (1H, *d, J* = 3.5 Hz)	5.05 (1H, *d, J* = 4 Hz)
2′	4.65 (1H, *dd*, *J* = 10, 3.5 Hz)	4.63 (1H, *dd*, *J* = 10, 4 Hz)
3′	3.59 (1H, *t, J* = 10 Hz)	3.56 (1H, *t, J* = 10 Hz)
4′	3.22 (1H, *t, J* = 10 Hz)	3.20 (1H, *t*, *J* = 10 Hz)
5′	3.80 (1H, *m*)	3.78 (1H, *dq*, *J* = 10, 6.5 Hz)
6′	1.27 (3H, *d, J* = 6.5 Hz)	1.26 (3H, *d, J* = 6.5 Hz)
2′-OAc	2.09 (3H, *s*)	2.07 (3H, *s*)
3′-OMe	3.59 (3H, *s*)	3.58 (3H, *s*)

Table 26 Comparison of ^{13}C NMR spectral data between compound **SM6** and **SM3**

Position	Compound SM6, δ_C (ppm)	Compound SM3, δ_C (ppm)
1	30.27	31.22
2	27.40[a]	26.97
3	72.24	71.56
4	31.12	32.20
5	34.72	33.66
6	29.87	27.65
7	117.89	51.05
8	138.72	63.90
9	33.89	31.35
10	-	33.48
11	28.48	20.21
12	39.18	40.82
13	50.80	52.12
14	85.19	80.93
15	39.77	34.26
16	27.68[a]	28.22
17	50.26	50.47
18	15.98	16.94
19	24.81	24.21
20	174.45	173.66
21	73.43	73.29
22	117.71	117.65
23	174.30	174.29
1′	93.84	93.71
2′	74.27	74.19
3′	80.84	80.75
4′	75.27	75.14

Table 26 (Continued)

Position	Compound SM6, δ_C (ppm)	Compound SM3, δ_C (ppm)
5′	66.95	67.02
6′	17.55	17.46
2′-C=O	170.22	170.16
2′-OAc	20.93	20.85
3′-OMe	60.54	60.54

[a] Assignment with the same superscripts may be interchanged.