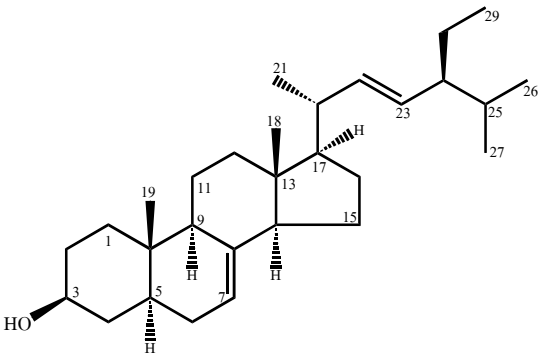
CHAPTER 1.3

RESULTS AND DISCUSSION

1.3.1 Structural Elucidation of Compounds from the Twigs and Stems of C. serratum

The crude methanol extract from the twigs and stems of *C. serratum* was purified by chromatographic methods to yield four compounds; one steroid (**CS-S1**), one disaccharide (**CS-S2**), one triterpene (**CS-S3**) and one steroid glucoside (**CS-S4**). All structures were determined by 1D and/or 2D NMR spectroscopic data and/or comparison of ^{1}H and/or ^{13}C spectral data with those reported in the literature. The ^{13}C NMR signals were assigned from ^{13}C, DEPT, HMQC and HMBC spectra.

1.3.1.1 CS-S1: Spinasterol



Compound **CS-S1** was obtained as colourless needles, melting at 156.5-159.5 °C; $[\alpha]_D^{30}$+10.91°, c = 0.11, $CHCl_3$. The IR spectrum **(Figure 3)** exhibited an absorption band for a hydroxyl group at 3454 cm^{-1}. The ^{1}H NMR spectrum (**Figure 4**)

(**Table 15**) of **CS-S1** revealed six methyl groups, two tertiary methyl groups as *singlet* at δ_H 0.55 and 0.80 which could be assigned to Me-18 and Me-19, respectively. The signals of three secondary methyl groups were observed as *doublets* at δ_H 1.03 (J = 6.5 Hz), δ_H 0.80 (J = 6.0 Hz) and δ_H 0.85 (J = 6.5 Hz), which were assigned to Me-21, Me-26 and Me-27, respectively. A *triplet* signal of Me-29 at δ_H 0.81 (*t*, J = 7.5 Hz) belonged to a primary methyl group. Two olefinic-proton resonanecs at δ_H 5.16 (*dd*, J = 15.5 and 8.5 Hz) and δ_H 5.03 (*dd*, J = 15.5 and 8.5 Hz) were assigned to the *trans*-olefinfc protons, H-22 and H-23, respectively. The remaining olefinic proton appeared at δ_H 5.16 (*brm*), which could be assigned to H-7. One oxymethine proton was resonated at δ_H 3.61 (*tt*, J = 11.0 and 4.5 Hz) with the large coupling constant (J = 11.0 Hz) could be assigned to α-H-3. The ^{13}C NMR spectrum (**Figure 5**) (**Table 15**) recorded in $CDCl_3$ showed 29 signals for 29 carbon atoms. Analysis of the DEPT spectrum (**Figure 6**) revealed six methyl carbon atoms (δ_C 21.36, 21.10, 18.97, 13.04, 12.25 and 12.03), nine methylene carbon atoms (δ_C 39.42, 37.96, 37.11, 31.45, 29.61, 28.51, 25.39, 23.00 and 21.52), eleven methine carbon atoms (δ_C 138.17, 129.39, 117.44, 71.05, 55.84, 55.10, 51.23, 49.40, 40.84, 40.22 and 31.86) and three quaternary carbon atoms (δ_C 139.55, 43.26 and 34.19). From these results, the remaining sixteen protons of methylene and six methine protons were resonated at δ_H 0.81-2.00.

Table 15 The NMR spectral data of compound **CS-S1** in $CDCl_3$

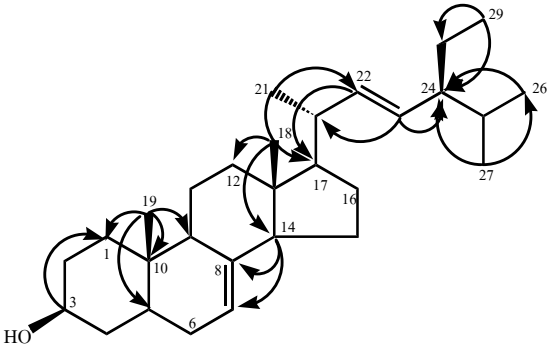
Position	δ_H, *mult*., J (Hz)	Type of C	δ_C	HMBC
1	1.80 (*m*, 1H); 1.06 (*m*, 1H)	CH_2	37.11	C-2, C-5, C-10
2	1.79 (*m*,1H); 1.36 (*m*, 1H)	CH_2	31.45	C-3
3	3.61 (*tt* , 11.0, 4.5, 1H)	CH	71.05	C-1
4	1.70 (*m*, 1H); 1.25 (*m*, 1H)	CH_2	37.96	C-2, C-3, C-10

Table 15 (continued)

Position	δ_H, *mult*., *J* (Hz)	Type of C	δ_C	HMBC
5	1.38 (*m*, 1H)	CH	40.22	
6	1.74 (*m*, 2H)	CH_2	29.61	
7	5.16 (*brm*, 1H)	CH	117.44	
8	-	C	139.55	
9	1.64 (*m*, 1H)	CH	49.40	
10	-	C	34.19	
11	1.59 (*m*, 1H); 1.44 (*m*, 1H)	CH_2	21.52	C-10, C-12, C-13
12	2.03 (*m*, 1H); 1.22 (*m*, 1H)	CH_2	39.42	C-11, C-13
13	-	C	43.26	
14	1.80 (*m*, 1H)	CH	55.10	C-7, C-8
15	1.34 (*m*, 2H)	CH_2	23.00	
16	1.30 (*m*, 1H); 1.26 (*m*, 1H)	CH_2	28.51	
17	1.24 (*m*, 1H)	CH	55.84	C-14
18	0.55 (*s*, 3H)	CH_3	12.03	C-12, C-13, C-14
19	0.80 (*s*, 3H)	CH_3	13.04[a]	C-1, C-5, C-9, C-10
20	2.00 (*m*, 1H)	CH	40.84	C-23
21	1.03 (*d*, 6.5, 3H)	CH_3	21.36	C-17, C-20, C-22
22	5.16 (*dd*, 15.5, 8.5, 1H)	CH	138.17	C-17, C-20, C-21, C-23, C-24
23	5.03 (*dd*, 15.5, 8.5, 1H)	CH	129.39	C-20, C-22, C-24, C-25, C-28
24	1.52 (*m*, 1H)	CH	51.23	
25	1.52 (*m*, 1H)	CH	31.86	C-22, C-24, C-26, C-27
26	0.80 (*d*, 6.0, 3H)[b]	CH_3	18.97	C-24, C-25
27	0.85 (*d*, 6.5, 3H)[b]	CH_3	21.10	C-24, C-25, C-26
28	*	CH_2	25.39	
29	0.81 (*t*, 7.5, 3H)	CH_3	12.25[a]	C-24, C-25

[a, b] Assignment with the same superscript maybe interchange, *not observed

The HMBC spectrum (**Figure 8**) (**Table 15**), Me-21 (δ_H 1.03) showed the correlation to C-17 (δ_C 55.84), C-22 (δ_C 138.17) and C-20 (δ_C 40.84) and H-22 (δ_H 5.16) showed correlation with C-17 (δ_C 55.84), indicating that the side chain in compound **CS-S1** was linked to the C-17 of the main skeleton. In addition, Me-19 (δ_H 0.80) showed correlation with C-1 (δ_C 37.11), C-9 (δ_C 49.40), C-10 (δ_C 34.19) and C-5 (δ_C 40.22), these results confirmed the location of Me-19. Finally, H-14 (δ_H 1.79) showed correlations with C-8 (δ_C 139.55) and C-7 (δ_C 117.44), indicating that the trisubstituted double bond should be located at C-7.

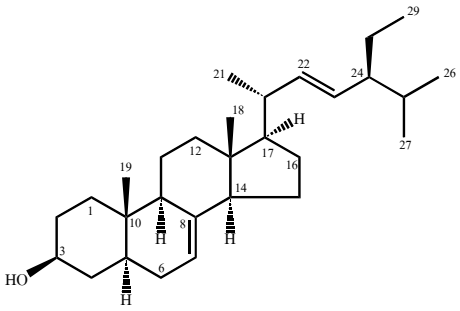


HMBC correlations

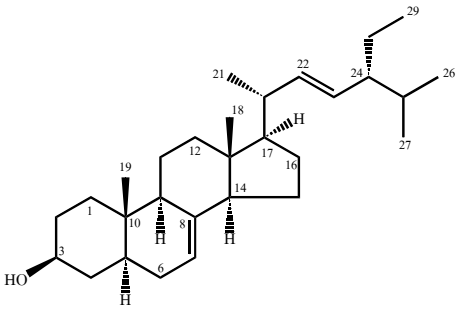
Only the relative stereochemistry between the H-3 and H-5 was found to be *cis* by the NOE difference results as no enhancement of H-5 was observed after irradiation at Me-19 (**Figure 9**). At this stage, **CS-S1** might possess the same relative sterochemistry as ether **spinasterol** or **chondrillasterol** which differed from **spinasterol** in the stereochemistry at C-24.

Comparison of ^{13}C NMR (**Table 16**) and ^{1}H NMR (**Table 17**) spectral data of compounds **CS-S1** with **spinasterol** (Kojima, *et al*., 1990) and **chondrillasterol** (Wandji, *et al*., 2002) showed similarity, but the differences were found in chemical shift of Me-26 and Me-27. **CS-S1** gave signals for both methyl groups almost identical to those of **spinasterol**. Furthermore, The ^{13}C NMR spectral data of **CS-S1** were in

agreement with those **spinasterol**. Thus, compound **CS-S1** was assigned as **spinasterol**.



spinasterol



chondrillasterol

Table 16 Comparison of ^{13}C NMR spectral data of compounds **CS-S1, spinasterol** and **chondrillasterol**

Position	**spinasterol**[a]	**chondrillasterol**[a]	**CS-S1**[b]
1	37.1	37.1	37.11
2	31.4	26.1	31.45
3	71.0	71.0	71.05
4	38.0	34.2	37.96
5	40.2	40.2	40.22
6	29.6	29.6	29.61
7	117.4	117.4	117.44
8	139.5	139.6	139.55
9	49.4	49.4	49.40
10	34.2	34.2	34.19
11	21.5	21.5	21.52
12	39.4	39.4	39.42
13	43.3	43.3	43.26
14	55.1	55.1	55.10
15	23.0	23.0	23.00

Table 16 (Continued)

Position	**spinasterol**[a]	**chondrillasterol**[a]	**CS-S1**[b]
16	28.5	28.5	28.51
17	55.8	55.9	55.84
18	12.0	12.0	12.03
19	13.0	13.0	13.04
20	40.8	40.8	40.84
21	21.4	21.1	21.36
22	138.7	138.2	138.17
23	129.4	129.4	129.39
24	51.2	51.2	51.23
25	31.9	31.9	31.86
26	21.2	19.0	18.97*
27	19.0	21.4	21.10*
28	25.4	25.4	25.39
29	12.3	12.2	12.25

[a]100 MHz in $CDCl_3$, [b]75 MHz in $CDCl_3$, * maybe interchange

Table 17 Comparison of ^{1}H NMR spectral data of compounds **CS-S1, spinasterol** and **chondrillasterol**

Position	δ_H, *mult.*, *J* (Hz)		
	spinasterol[a]	**chondrillasterol**[b]	**CS-S1**[c]
3	3.59 (*m*, 1H)	-	3.61 (*tt*, 11.0, 4.5, 1H)
7	5.15 (*m*, 1H)	-	5.16 (*brm*, 1H)
18	0.55 (*s*, 3H)	0.55 (*s*, 3H)	0.55 (*s*, 3H)
19	0.88 (*s*, 3H)	0.80 (*s*, 3H)	0.80 (*s*, 3H)
21	1.02 (*d*, 6.5, 3H)	1.03 (*d*, 6.5, 3H)	1.03 (*d*, 6.5, 3H)

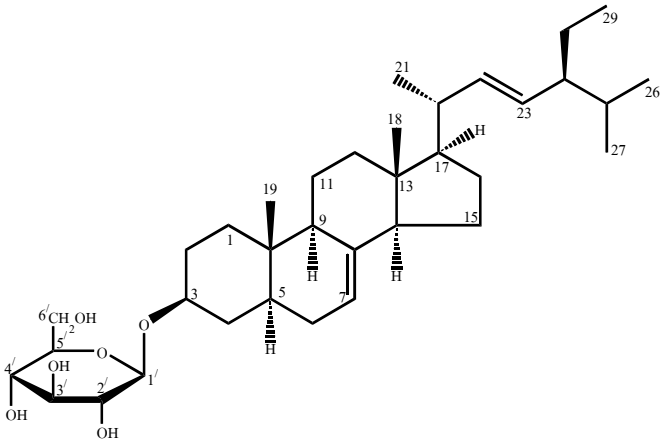
Table 17 (Continued)

Position	δ_H, *mult.*, *J* (Hz)		
	spinasterol[a]	**chondrillasterol**[b]	**CS-S1**[c]
22	5.15 (*dd*, 15.0, 9.0, 1H)	5.03 (*dd*, 7.5, 1H)	5.16 (*dd*, 15.5, 8.5, 1H)
23	5.02 (*dd*, 15.0, 9.0, 1H)	5.16 (*dd*, 7.5, 1H)	5.03 (*dd*, 15.5, 8.5, 1H)
26*	0.85 (*d*, 6.5, 3H)	0.83 (*d*, 6.5, 3H)	0.80 (*d*, 6.0*, 3H)
27*	0.80 (*d*, 6.5, 3H)	0.85 (*d*, 6.3, 3H)	0.85 (*d*, 6.5*, 3H)
29	0.80 (*t*, 7.5, 3H)	0.80 (*t*, 7.2, 3H)	0.81 (*t*, 7.5, 3H)

* maybe interchange, [a]400 MHz in $CDCl_3$, [b]360 MHz in $CDCl_3$ (Garg, 1984),

[c]300 MHz in $CDCl_3$

1.3.1.2 CS-S4: Spinasteryl-β-D-glucopyranoside



Compound CS-S4 was obtained as a white solid, melting at 262.4-262.7 °C; $[\alpha]_D^{29}$ –90.00, c = 0.10, $CHCl_3$. The IR spectrum (**Figure 10**) showed the absorption band for a hydroxyl group at 3410 cm^{-1}. Comparison of the ^1H NMR spectrum (**Figure 11**) (**Table 18**) of compound **CS-S4** with **CS-S1** revealed the same characteristic signals of the sterol unit.; [δ_H 5.21 (1H, *dd*, *J* = 15.5 and 9.0 Hz) and δ_H 5.07 (1H, *dd*, *J*

= 15.5 and 9.0 Hz) for *trans*-olefinic protons, H-22 and H-23, respectively, a *broad multiplet* signal of an olefinic proton at δ_H 5.17 , the signal of oxymethine proton, H-3, in ring A of sterol unit and six methyl groups]. The difference between **CS-S4** and **CS-S1** was the presence of characteristic signal of a sugar moiety. An anomeric proton at δ_H 4.93 (1H, *d*, *J* = 8.0 Hz), was infered to β-configuration of sugar moiety, based on the value of the coupling constant. Other proton signals of sugar moiety were resonated at δ_H 3.91 (1H, *m*), δ_H 4.18 (1H, *t*, *J* = 8.5 Hz), δ_H 4.13 (1H, *t*, *J* = 8.5 Hz), δ_H 3.89 (1H, *m*), δ_H 4.48 (1H, *brd*, *J* =12.0 Hz) and δ_H 4.31 (1H, *brdd*, *J* = 12.0 and 5.5 Hz), which were assigned to H-2′, H-3′, H-4′, H-5′, H_a-6′ and H_b-6′, respectively. The proton signals of sugar units were assigned by ^{1}H-^{1}H COSY spectrum (**Figure 16**) (**Table 18**) and HMBC correlation experiment (**Figure 15**) (**Table 18**). From these results, the sugar unit was identified as glucose. The ^{13}C NMR spectrum (**Figure 12**) (**Table 18**) showed 35 carbon atoms. Six carbon signals were in the glycosidic region corresponding to hexose moiety (δ_H 99.91, 76.05, 75.90, 72.80, 69.43 and 60.61). The remaining 29 carbon signals were due to the steroidal aglycone. Analysis of the DEPT 90° and DEPT 135° spectra of this compound suggested the presence of six methyl carbon atoms (δ_C 19.46, 19.17, 17.05, 10.91, 10.36 and 10.09), nine methylene carbon atoms (δ_C 37.44, 35.13, 32.40, 27.77, 27.69, 26.68, 23.50, 21.12 and 19.52), eleven methine carbon atoms (δ_C 136.42, 127.42, 115.62, 75.03, 53.84, 53.10, 49.27, 47.37, 38.94, 37.97 and 29.96) and three signals for quaternary carbon atom (δ_C 137.29, 41.26 and 32.33). The HMBC spectrum (**Figure 15**) (**Table 18**) showed the correlation between the anomeric proton with C-3 (δ_C 75.03), indicating that the glycoside linkage was formed between sugar moiety and steroid at C-3 (δ_C 75.03). On the basis of the evidence described above, the structure of **CS-S4** was assigned as **spinasteryl-β-D-glucopyranoside**.

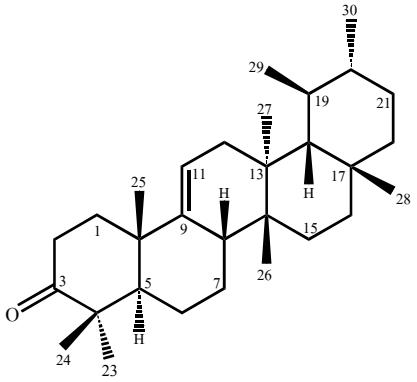
Table 18 The NMR spectral data of compound **CS-S4** in pyridine-d_5+$CDCl_3$

Position	δ_H , *mult*., *J* (Hz)	Type of C	δ_C	^{1}H-^{1}H COSY	HMBC
1	1.70 (*m*, 1H); 0.94 (*m*, 1H)	CH_2	35.13		
2	H_a: 1.94 (*m*, 1H)	CH_2	32.40		C-3, C-5
	H_b: 1.32 (*m*, 1H)				
3	3.93 (*m*, 1H)	CH	75.03	H-2_a, H-2_b, H-4_b	
4	H_a: 2.00 (*m*, 1H)	CH_2	27.69		C-6
	H_b: 1.55 (*m*, 1H)				
5	1.22 (*m*, 1H)	CH	37.97		C-19
6	1.69 (*m*, 2H)	CH_2	27.77		
7	5.17 (*brm*, 1H)	CH	115.62	H-6	
8	-	C	137.29		
9	1.60 (*m*, 1H)	CH	47.37		
10	-	C	32.33		
11	1.55 (*m*, 1H); 1.43 (*m*, 1H)	CH_2	19.52		
12	1.97 (*m*, 2H)	CH_2	37.44		C-11
13	-	C	41.26		
14	1.82 (*m*, 1H)	CH	53.10		
15	1.79 (*m*, 1H), 1.56 (*m*, 1H)	CH_2	21.12		
16	1.30 (*m*, 2H)	CH_2	26.68		
17	1.28 (*m*, 1H)	CH	53.84		C-16, C-20
18	0.58 (*s*, 3H)	CH_3	10.09		C-12, C-13, C-14
19	0.72 (*s*, 3H)	CH_3	10.91		C-1, C-5, C-9, C-10
20	2.06 (*m*, 1H)	CH	38.94	H-21, H-22	
21	1.08 (*d*, 6.5, 3H)	CH_3	19.46	H-20	C-17, C-20, C-22
22	5.21 (*dd*, 15.5, 9.0, 1H)	CH	136.42	H-20, H-23	C-20, C-23, C-24
23	5.07 (*dd*, 15.5, 9.0, 1H)	CH	127.42	H-22, H-24	C-20, C-22, C-24

Table 18 (continued)

Position	δ_H , *mult*., *J* (Hz)	Type of C	δ_C	^{1}H-^{1}H COSY	HMBC
24	1.57 (*m*, 1H)	CH	49.27		
25	1.56 (*m*, 1H)	CH	29.96		
26	0.86 (*d*, 7.0, 3H)	CH_3	17.05		C-28
27	0.91 (*d*, 6.5, 3H)	CH_3	19.17		C-24, C-25, C-26
28	1.42 (*m*, 2H)	CH_2	23.50		
29	0.88 (*t*, 7.5, 3H)	CH_3	10.36		C-24, C-25
1′	4.93(*d*, 8.0, 1H)	CH	99.91	H-2′	C-3
2′	3.91 (*m*, 1H)	CH	72.80	H-1′,H-3′	C-3′
3′	4.18 (*t*, 8.5, 1H)	CH	76.05	H-2′, H-4′	C-2′, C-4′
4′	4.13 (*t*, 8.5, 1H)	CH	69.43	H-3′, H-5′	C-5′
5′	3.89 (*m*, 1H)	CH	75.90	H-4′, H_a-6′, H_b-6′	
6′	H_a: 4.48 (*brd*, 12.0, 1H)	CH_2	60.61	H-5′	C-5′
	H_b: 4.31 (*brdd*, 12.0, 5.5, 1H)				

1.3.1.3 CS-S3: Bauer-9-en-3-one



Compound **CS-S3** was obtained as a white solid, melting at 199.0-202.0 °C; $[\alpha]_D^{30}$ +34.00, c = 0.10, $CHCl_3$. This compound showed the character of triterpene by giving a purple spot in vanillin sulfuric acid reagent. Its IR spectrum (**Figure 17**) showed the presence of a carbonyl group at 1709 cm^{-1}. The ^1H NMR spectrum (**Figure 18**) (**Table 19**) with the characteristic peaks of the triterpene, demonstrated the presence of six methyl *singlets* at δ_H 1.07 (2×Me), 1.21, 0.81, 0.79 and 0.77 and two methyl *doublets* at δ_H 0.89 (*J* = 6.5 Hz) and 0.83 (*J* = 6.0 Hz). The methylene protons at δ_H 2.72 (1H, *ddd*, *J* = 15.5, 13.5 and 6.5 Hz) and δ_H 2.40 (1H, *ddd*, *J* = 15.5, 5.5 and 3.5 Hz) which could be assigned to H_a-2 (βH) and H_b-2 (αH), respectively, were shifted to downfield according to the anisotropic effect of the carbonyl group at C-3. The olefinfic proton resonated at δ_H 5.29 (*d*, *J* = 6.5 Hz), which could be assigned the position C-11. The ^{13}C NMR spectrum (**Figure 19**) (**Table 19**) showed 30 signals for 30 carbon atoms. The DEPT 90° and DEPT 135° spectrua (**Figure 20**) indicated that the existence of eight methyl carbon atoms (δ_C 25.53, 22.99, 22.10, 22.04, 21.64, 16.95, 15.29 and 13.98), nine methylene carbon atoms (δ_C 36.64, 36.07, 35.86, 34.89, 29.62, 28.18, 26.27, 22.57 and 20.15), six methine carbon atoms (δ_C 115.61, 59.59, 53.26, 51.99, 41.04 and 30.77) and seven quaternary carbon atoms (δ_C 217.30, 147.41,

47.64, 42.80, 39.30, 38.18 and 36.75). The HMBC spectrum (**Figure 22**) (**Table 19**) showed the correlations of Me-25 (δ_H 1.21) with C-1 (δ_C 36.64), C-5 (δ_C 53.26), C-9 (δ_C 147.41) and C-10 (δ_C 39.30); olefinic proton H-11 (δ_H 5.29) with C-8 (δ_C 41.04), C-10 (δ_C 39.30) and C-13 (δ_C 36.75). These results confirmed the location of Me-25 and olefinic proton H-11. The correlations of H-1 (δ_H 2.09), H-2 (δ_H 2.72 and 2.40), Me-23 (δ_H 1.07) and Me-24 (δ_H1.07) with carbonyl carbon, indicated that the carbonyl carbon was located at C-3 (δ_C 217.30). In addition, the HMBC spectrum showed the correlations of Me-26 (δ_H 0.79) with C-18 (δ_C 51.99); Me-27 (δ_H 0.81) with C-12 (δ_C 29.62) and C-18 (δ_C 51.99); Me-28 (δ_H 0.77) with C-17 (42.80), C-18 (δ_C 51.99) and C-19 (δ_C 59.59); Me-29 (δ_H 0.83) with C-19 (δ_C 59.59), C-20 (δ_C 30.77) and C-30 (δ_C 22.10) and Me-30 (δ_H 0.89) with C-19 (δ_C 59.59), C-20 (δ_C 30.77) and C-29 (δ_C 22.99). These results confirmed the location of Me-26, Me-27, Me-28, Me-29 and Me-30, respectively. The relative stereochemistry of **CS-S3** was established by the NOE difference results. Firstly, the signals of Me-24 (δ_H 1.07) and Me-25 (δ_H 1.21) were enhanced when the H_a-2 (δ_H 2.72) was irradiated (**Figure 24**). Secondly, the enhancement of Me-25 (δ_H 1.21) and Me-26 (δ_H 0.79) signals by the irradiation of H-8 (δ_H 2.07) (**Figure 23**). These results indicated that H_a-2, Me-24, Me-25, H-8 and Me-26 located on the same side of the molecule, the β-side in **CS-S3**. Other relative stereochemistry could not be assigned by the NOE experiment. However, it is believed that **CS-S3** possessed the same relative stereochemistry as bauerene skeleton. On the basis of the evidence described above, the structure of **CS-S3** was assigned to **Bauer-9-en-3-one**.

Table 19 The NMR spectral data of compound **CS-S3** in $CDCl_3$

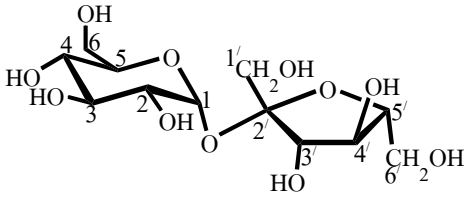
Position	δ_H, *mult*., *J* (Hz)	Type of C	δ_C	HMBC
1	2.09 (*ddd*, 13.5, 6.5, 3.5, 1H); 1.78 (*m*, 1H)	CH_2	36.64	C-3, C-5, C-9, C-10
2	H_a: 2.72 (*ddd*, 15.5, 13.5, 6.5, 1H); H_b: 2.40 (*ddd*, 15.5, 5.5, 3.5, 1H)	CH_2	34.89	C-1, C-3, C-10
3	-	C	217.30	
4	-	C	47.64	
5	1.35 (*m*, 1H)	CH	53.26	C-4, C-10, C-25
6	1.46 (*m*, 1H); 1.38 (*m*, 1H)	CH_2	35.86	
7	1.23 (*m*, 2H)	CH_2	28.18	
8	2.07 (*m*, 1H)	CH	41.04	C-5, C-9
9	-	C	147.41	
10	-	C	39.30	
11	5.29 (*d*, 6.5, 1H)	CH	115.61	C-8, C-10, C-13
12	1.44 (*m*, 1H); 1.26 (*m*, 1H)	CH_2	29.62	C-9, C-11, C-14
13	-	C	36.75	
14	-	C	38.18	
15	1.34 (*m*, 1H); 1.30 (*m*, 1H)	CH_2	20.15	
16	1.60 (*m*, 2H)	CH_2	22.57	C-13, C-17, C-19, C-27, C-28
17	-	C	42.80	
18	1.60 (*m*, 1H)	CH	51.99	
19	0.98 (*q,* 9.5, 1H)	CH	59.59	
20	*	CH	30.77	
21	1.86 (*m*, 1H); 1.24 (*m*, 1H)	CH_2	26.27	
22	1.70 (*m*, 2H)	CH_2	36.07	
23	1.07 (*s*, 3H)	CH_3	22.04	C-3, C-4, C-5, C-24
24	1.07 (*s*, 3H)	CH_3	25.53	C-3, C-4, C-5, C-23

Table 19 (continued)

Position	δ_H , *mult*., *J* (Hz)	Type of C	δ_C	HMBC
25	1.21 (*s*, 3H)	CH_3	21.64	C-1, C-5, C-9, C-10
26	0.79 (*s*, 3H)	CH_3	15.29	C-18
27	0.81 (*s*, 3H)	CH_3	16.95	C-12, C-18
28	0.77 (*s*, 3H)	CH_3	13.98	C-17, C-18, C-19
29	0.83 (*d*, 6.0, 3H)	CH_3	22.99	C-19, C-20, C-30
30	0.89 (*d*, 6.5, 3H)	CH_3	22.10	C-19, C-20, C-29

*not observed

1.3.1.4 CS-S2: Sucrose



Compound **CS-S2** was obtained as colourless crystals, melting at 175.2-176.9 °C; $[\alpha]_D^{28}$ +78.37, c = 0.25, pyridine. The IR spectrum (**Figure 25**) exhibited an absorption band for a hydroxyl group at 3373 cm^{-1}. The ^1H NMR spectrum (**Figure 26**) (**Table 20**) recorded in pyridine-d_5 showed typical signals of two sugar units. A *doublet* signal with a small coupling constant at δ_H 5.94 (*J* = 4.0 Hz) was assignable to the anomeric proton of the sugar unit. According to chemical shifts and splitting pattern of this proton as well as the presence of oxymethylene protons at δ_H 4.28 (*dd*, *J* = 12.0 and 2.5 Hz, 1H) and 4.14 (*dd*, *J* = 12.0 and 4.5 Hz, 1H), characteristic signals of H-6 of glucose unit. Four oxymethylene protons at δ_H 4.07 (*s*, 2H), δ_H 4.05 (1H, *d*, *J* = 12.0 Hz, 1H) and δ_H 4.13 (1H, *d*, *J* = 12.0 Hz, 1H) were attributed to H-1′ and H-6′ of

the fructose unit, respectively. Thus, two sugar units were determined as glucose and fructose. The proton signals of glucose and fructose units were assigned by ^{1}H-^{1}H COSY spectrum (**Figure 31**) (**Table 20**) and HMBC correlation experiment (**Figure 30**) (**Table 20**). The ^{13}C NMR spectrum (**Figure 27**) (**Table 20**) showed 12 carbon atoms. Analysis of the DEPT 90° and DEPT 135° spectra of this compound suggested the presence of three methylene carbon atoms (δ_C 62.90, 60.25 and 60.12), eight methine carbon atoms (δ_C 91.31, 82.03, 78.25, 73.09, 72.67, 72.63, 70.98 and 69.40) and one quaternary carbon atom (δ_C 103.35). These signals corresponded to signals of **sucrose.** This conclusion was confirmed by acetylation reaction of **CS-S2**. The ^{1}H NMR spectrum of its acetate showed the signals of sucrose unit together with methyl protons of eight acetyl groups [δ_H 2.18, 2.12 (2xMe), 2.11, 2.10 (2xMe), 2.05 and 2.02] and the ^{13}C NMR spectral data (**Figure 34**) (**Table 21**)exhibited eight carbonyl carbons (δ_C 170.68, 170.47, 170.08 (3xC), 169.90, 169.65 and 169.50) together with eight methine carbons [δ_C 89.89, 79.07, 75.65, 74.94, 70.23, 69.59, 68.46 and 68.18], three methylene carbons [δ_C 63.61, 62.84 and 61.71] and one sp^3 quaternary carbon atom (δ_C 103.96). This was further confirmed by comparing the NMR data of **AcCS-S2** with those of previousely reported for **sucrose octaacetate** (Nishida, *et al*., 1986) (**Table 21**). On the above accumulated evidence, the structure of **AcCS-S2** was established as **sucrose octaacetate**.

Table 20 The NMR spectral data of compound **CS-S2** in pyridine-d_5

Position	δ_H , *mult*., *J* (Hz)	Type of C	δ_C	^{1}H-^{1}H COSY	HMBC
1	5.94 (*d*, 4.0, 1H)	CH	91.31	H-2	C-3
2	3.98 (*dd*, 9.5, 4.0, 1H)	CH	70.98	H-1, H-3	
3	4.39 (*t*, 9.5, 1H)	CH	72.63	H-2, H-4	C-2, C-4
4	3.97 (*t*, 9.5, 1H)	CH	69.40	H-3, H-5	C-3

Table 20 (continued)

Position	δ_H, *mult*., *J* (Hz)	Type of C	δ_C	^{1}H-^{1}H COSY	HMBC
5	4.46 (*ddd*, 9.5, 4.5, 2.5, 1H)	CH	72.67	H-4, H_b-6	
6	H_a: 4.28 (*dd*, 12.0, 2.5, 1H);	CH_2	60.12 [a]	H_b-6	
	H_b: 4.14 (*dd*, 12.0, 4.5, 1H)			H-5, H_a-6	
1′	4.07 (*s,* 2H)	CH_2	60.25[a]		C-5′
2′	-	C	103.35		
3′	4.67 (*d*, 8.0, 1H)	CH	78.25	H-4′	
4′	4.78 (*t*, 8.0, 1H)	CH	73.09	H-3′, H-5′	C-4′, C-6′
5′	4.24 (*td*, 8.0, 4.0, 1H)	CH	82.03	H-4′, H_a-6′	C-3′, C-1′
6′	H_a: 4.05 (*d*, 12.0, 1H);	CH_2	62.90	H-5′	C-2′, C-3′
	H_b: 4.13 (*d*, 12.0, 1H)				

[a]maybe interchange

Table 21 The NMR spectral data of compounds **AcCS-S2** and **sucrose octaacetate**

Position	**AcCS-S2**		**sucrose octaacetate**	
	δ_H, *mult., J* (Hz)	δ_C (C-Type)	δ_H	δ_C (C-Type)
1	5.65 (*d*, 3.6, 1H)	89.89 (CH)	5.69 (1H)	89.93 (CH)
2	4.83 (*dd*, 10.2, 3.6, 1H)	70.23 (CH)	4.87 (1H)	70.26 (CH)
3	5.40 (*t*, 10.2, 1H)	69.59 (CH)	5.44 (1H)	69.61 (CH)
4	5.04 (*t*, 10.2, 1H)	68.18 (CH)	5.08 (1H)	68.17 (CH)
5	4.26 (*m*, 1H)	68.46 (CH)	4.28 (1H)	68.50 (CH)
6	4.14 (*m*, 1H), 4.28 (*m*, 1H)	61.71 (CH_2)	4.14 (1H), 4.28 (1H)	61.75 (CH_2)
1′	4.15 (*s,* 2H)	62.84 (CH_2)	4.17 (2H)	62.85 (CH_2)
2′	-	103.96 (C)	-	104.02 (C)
3′	5.42 (*d*, 5.7, 1H)	75.65 (CH)	5.47 (1H)	75.68 (CH)
4′	5.33 (*t*, 5.7, 1H)	74.94 (CH)	5.36 (1H)	74.98 (CH)

Table 21 (continued)

Position	**AcCS-S2**		**sucrose octaacetate**	
	δ_H, *mult., J* (Hz)	δ_C (C-Type)	δ_H	δ_C (C-Type)
5′	4.18 (*m*, 1H)	79.07 (CH)	4.21 (1H)	79.14 (CH)
6′	4.31 (*dd*, 12.0, 4.8, 2H)	63.61 (CH_2)	4.35 (1H), 4.29 (1H)	63.63 (CH_2)
$\underline{CO}CH_3$		170.68 (C)		170.07 (C)
$\underline{CO}CH_3$		170.47 (C)		170.01 (C)
$\underline{CO}CH_3$		170.08 (3xC)		169.50 (C)
$\underline{CO}CH_3$		169.90 (C)		170.66 (C)
$\underline{CO}CH_3$		190.65 (C)		170.09 (C)
$\underline{CO}CH_3$		169.50 (C)		169.65 (C)
$\underline{CO}CH_3$				169.88 (C)
$\underline{CO}CH_3$				170.46 (C)
$CO\underline{CH_3}$	2.10 (*s*, 3H)	20.60	2.10 (3H)	20.64 (CH_3)
$CO\underline{CH_3}$	2.02 (*s*, 3H)	(8xCH_3)	2.02 (3H)	20.66 (CH_3)
$CO\underline{CH_3}$	2.05 (*s*, 3H)		2.05 (3H)	20.61 (CH_3)
$CO\underline{CH_3}$	2.10 (*s*, 3H)		2.10 (3H)	20.58 (CH_3)
$CO\underline{CH_3}$	2.12 (*s*, 3H)		2.12 (3H)	20.69 (CH_3)
$CO\underline{CH_3}$	2.18 (*s*, 3H)		2.18 (3H)	20.56 (CH_3)
$CO\underline{CH_3}$	2.11 (*s*, 3H)		2.11 (3H)	20.72 (CH_3)
$CO\underline{CH_3}$	2.12 (*s*, 3H)		2.12 (3H)	20.63 (CH_3)