



SULFUR-CONTAINING GLUCOSIDES FROM *CLINACANTHUS NUTANS*

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Key Word Index—*Clinacanthus nutans*; Acanthaceae; sulfoxide; sulfone; amide; glucoside; clinacoside A.

Abstract—Five sulfur-containing glucosides were isolated from the butanol-soluble portion of the methanol extract of the stem and leaves of *Clinacanthus nutans*. Identification of compounds was achieved by chemical and spectroscopic means. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Clinacanthus nutans is an important herbal medicine in Thailand (Thai name: phayaa yo) and China (Chinese name: qing jian), being used as an anti-hepatitis and anti-herpes agent [1]. Until now, there were no reports on the constituents of this species.

RESULTS AND DISCUSSION

Six known C-glycosyl flavones, vitexin, isovitexin, shaftoside, isomollupentin 7-O- β -glucopyranoside, orientin and isoorientin have been isolated from the BuOH and water-soluble portion of the MeOH extract of the stems and leaves (1.0 kg) of *C. nutans* collected in Thailand, [2]. In continuation of the study on this extract, five new compounds (**1**–**5**) were isolated by repeated column chromatography followed by MPLC and HPLC, in yields of 0.013, 0.002, 0.003, 0.046 and 0.023%, respectively.

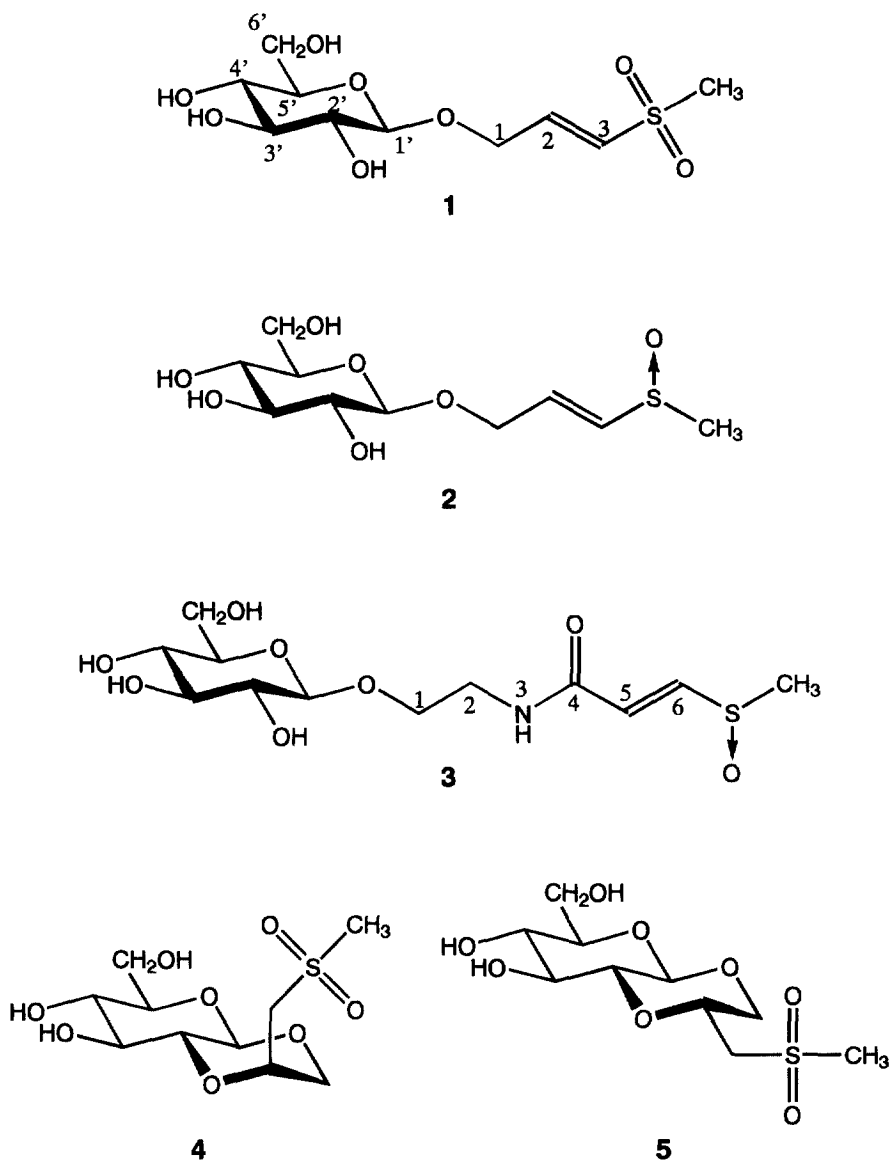
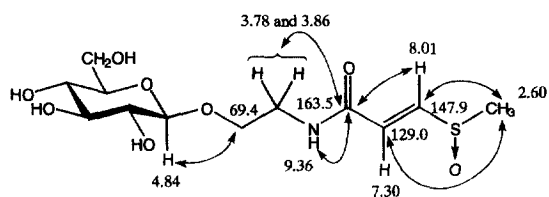
Compound **1** had a molecular formula of $C_{10}H_{18}O_8S$ and the IR spectrum showed strong absorption bands at 1296 and 1130 cm^{-1} characteristic of a sulfone group. The 1H and ^{13}C NMR spectra (Tables 1 and 2) showed the existence of a β -glucopyranosyl moiety at δ_H 4.75 (*d*, $J = 7.8$ Hz, H-1), δ_C 104.1, 75.0, 78.5, 71.4, 78.3 and 62.5 (C-1–C-6), a 1,2-*trans*-substituted double bond at δ_H 7.20 (*d*, $J = 15.1$ Hz) and 7.00 (*dt*, $J = 15.1$ and 4.8 Hz), with corresponding carbons at δ_C 143.8 and 130.5, respectively, an oxymethylene adjacent to the double bond at δ_C 66.8, δ_H 4.59 (1H,

dd, $J = 17.3$ and 4.8 Hz) and 4.38 (1H, *dd*, $J = 17.3$ and 4.8 Hz) and an *S*-methyl signal at δ_H 2.93 (3H, *s*), δ_C 42.6. The FG (field gradient) -HMBC results indicated correlation of the anomeric proton with the oxymethylene carbon, and the *S*-methyl proton with the double bond carbons. From these data, the structure of **1** was identified unambiguously as (*E*)-3-methylsulfonyl-2-propenyl β -D-glucoside, and was named clinacoside A.

Compound **2** had a molecular formula of $C_{10}H_{18}O_7S$ and the IR spectrum showed strong absorption bands at 1074 cm^{-1} characteristic of a sulfoxide group. The 1H and ^{13}C NMR spectra were closely related to those **1**, but with a significant difference of one of the double bond signals (Tables 1 and 2). The structure of **2** was accordingly decided as the corresponding sulfoxide of **1**, (*E*)-3-methylsulfinyl-2-propenyl β -D-glucoside, and named clinacoside B.

Compound **3** had a molecular formula of $C_{12}H_{21}NO_8S$ and the IR spectrum showed strong absorption bands at 1074 (sulfoxide) and 1658 and 1558 cm^{-1} , assignable to an amide group. The 1H and ^{13}C NMR spectra (Table 3) showed signals assignable to a β -glucopyranosyl group, a carbonyl group (δ_C 163.5), an amide proton [δ_H 9.36, (1H, *br t*)], a 1,2-substituted *trans*-double bond [δ_H 8.01 (1H, *d*, $J = 14.6$ Hz), 7.30 (1H, *d*, $J = 14.6$ Hz), δ_C 147.9 (*d*), 129.0 (*d*)], an oxymethylene [δ_H 4.17 (1H, *m*), 3.99 (1H, *m*), δ_C 69.4 (*t*)], a methylene group adjacent to the oxymethylene [δ_H 3.86 (1H, *m*), 3.78 (1H, *m*), δ_C 40.7 (*t*)], and an *S*-methyl group [δ_H 2.60 (3H, *s*), δ_C 39.9 (*q*)]. The HMBC data of **3** showed the multiple bond correlations shown in Fig. 1 and the structure of **3** was decided as shown and named clinacoside C.

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Formulae: Teshima *et al.*, Sulfur containing glucosides from *Clinacanthus nutans*Fig. 1. HMBC of compound 3 (in pyridine- d_5).

In the HMBC spectrum, unusual correlation through four bonds was observed between the *S*-methyl proton (δ_{H} 2.60) and C-5 (δ_{C} 129.0). This was justified by the measurement of non-decoupled ^{13}C NMR spectrum of **3**, where *S*-methyl signals appeared as *qdd* ($J = 138$, 1.5 and 1.2 Hz), indicating coupling with both H-

6 and H-5. This long-range (four bonds) coupling through a sulfur atom was also observed in the non-decoupled ^{13}C NMR spectrum of a model compound, ethyl methyl sulfone, where the C-methyl signal appeared at δ 6.9 as *qtq* ($J = 131$, 4.7 and 0.6 Hz). This observation also verified by HMBC of compounds **1** and **2**.

Compound **4** had the same molecular formula as **1**, $\text{C}_{10}\text{H}_{18}\text{O}_8\text{S}$ and the IR spectrum also showed sulfone absorptions at 1300 and 1133 cm^{-1} . The ^1H and ^{13}C NMR spectra were similar to those of **1**, but the existence of methylene and oxymethylene signals in place of the double bond signals in **1** were observed. Also, the chemical shifts of the carbon signals assignable to the glucosyl moiety were significantly different from those of **1**. On acetylation, **4** afforded a triacetate,

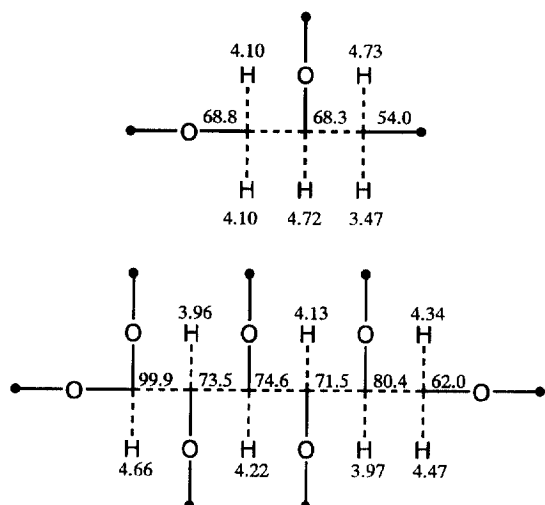


Fig. 2. Partial structure of compound **4** as deduced by H-H COSY and HMQC.

indicating the presence of a mono-substituted glucosyl moiety. Measurement of H-H COSY and FG-HMQC resulted in the deduction of the partial structures shown in Fig. 2. These two partial structures were combined as shown in Fig. 3 with the aid of FG-HMBC (shown by solid arrow) to give the planar structure and the stereochemistry was decided by NOEDS (shown by dotted arrow). Since the structure was considered as a cyclization product of **1**, it was named cycloclinacoside A1.

Compound **5** had the same molecular formula as **4**, and the IR and the NMR behaviour was very similar to those of **4**. H-H COSY and FG-HMQC resulted in the same planar structure as **4** and the stereochemistry was decided as shown (Fig. 4) based on NOE and named cycloclinacoside A2. It should be noted that since **1** was partially converted to **4** and **5** on standing at room temperature in MeOH, **4** and **5** might be artifacts.

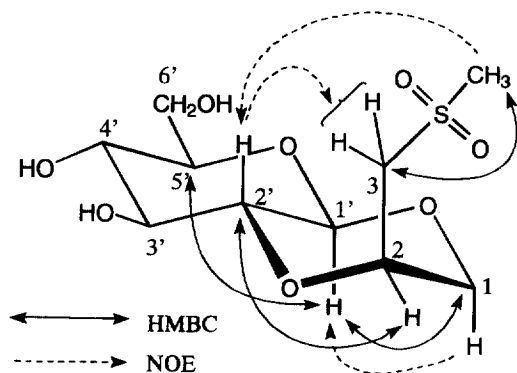


Fig. 3. HMBC and NOE of compound **4**.

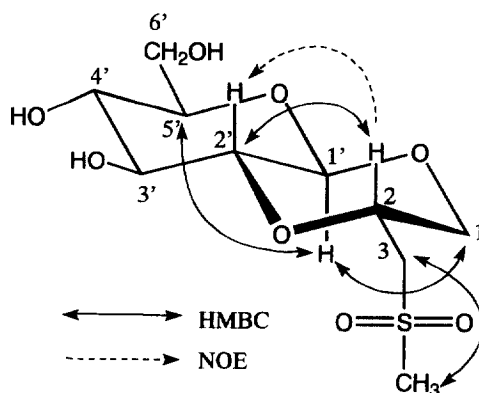


Fig. 4. HMBC and NOE of compound **5**.

EXPERIMENTAL

General

Mps: uncorr. ^1H NMR and ^{13}C NMR (TMS as int. standard): 500 and 125 MHz, respectively. FAB-MS: negative mode.

Plant material

Leaves and stems of *C. nutans* (Burm. f.) Lindau were collected in the botanical garden of Chiang Mai and Kong Kaen, Thailand. A voucher specimen is deposited in the Herbarium of Faculty of Pharmaceutical Sciences in Chiang Mai and Kong Kaen Universities.

Extraction and isolation

Dried leaves and stems (1 kg) were extracted with hot MeOH to give 119 g of extract, which was suspended in 70% MeOH and then extracted with hexane. The MeOH fr. was suspended in H_2O and extracted with *n*-BuOH. The BuOH fr. (17 g) was chromatographed on silica gel (CH_2Cl_2 -MeOH- H_2O , 100:10:1, 50:10:1, 30:10:1), MPLC (ODS; 5% MeOH) to give **3** (30 mg), and on silica gel again (CH_2Cl_2 -MeOH, 5:1), to give **1** (134 mg) and **2** (19 mg). From a part (20 g) of the aq. fr. (77 g), **4** (121 mg) and **5** (59 mg) were obtained by CC on silica gel (EtOAc-MeOH- H_2O , 8:2:1 and CH_2Cl_2 -MeOH- H_2O , 30:10:1, 15:6:1), MPLC (ODS; 5% MeOH) and HPLC (ODS; 1% MeCN).

Clinacoside A (1). Colourless oil. $[\alpha]_{\text{D}}^{21} -31^\circ$ ($\text{C}_3\text{H}_5\text{N}$, c 3.1). Negative ion HRFABMS: $[\text{M}-\text{H}]^-$ found m/z 297.0632. $\text{C}_{10}\text{H}_{17}\text{O}_8\text{S}$ requires 297.0644. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1296, 1130 (SO_2). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 257 (2.26). ^1H and ^{13}C NMR: Tables 1 and 2.

Enzymatic hydrolysis. **1** (1.6 mg) was dissolved in H_2O (1 ml) and incubated with almond β -glucosidase (Sigma) (1.6 mg) at 37° for 7 h to obtain β -D-glucose which was identified by comparison with an authentic sample by silica gel TLC (CH_2Cl_2 -MeOH- H_2O , 6:4:1).

Table 1. ^1H NMR spectral data of compounds **1**, **2**, **4** and **5** (δ in $\text{C}_5\text{D}_5\text{N}$)

	1	2	4	5
1	4.59 (1H, <i>dd</i> , $J = 17.3, 4.8$ Hz) 4.38 (1H, <i>dd</i> , $J = 17.3, 4.8$ Hz)	4.63 (1H, <i>dd</i> , $J = 14.4, 6.3$ Hz) 4.46 (1H, <i>dd</i> , $J = 14.4, 6.3$ Hz)	4.10 (2H, <i>m</i>)	4.15 (1H, <i>dd</i> , $J = 11.7, 2.8$ Hz) 3.72 (1H, <i>dd</i> , $J = 11.7, 10.7$ Hz)
2	7.00 (1H, <i>dt</i> , $J = 15.1, 4.8$ Hz)	6.68 (1H, <i>dt</i> , $J = 15.1, 6.3$ Hz)	4.72 (1H, <i>m</i>)	4.56 (1H, <i>dddd</i> , $J = 10.7, 9.2, 2.8, 2.8$ Hz)
3	7.20 (1H, <i>d</i> , $J = 15.1$ Hz)	7.01 (1H, <i>d</i> , $J = 15.1$ Hz)	4.73 (1H, <i>m</i>) 3.47 (1H, <i>m</i>)	3.65 (1H, <i>dd</i> , $J = 15.0, 9.2$ Hz) 3.65 (1H, <i>dd</i> , $J = 15.0, 2.8$ Hz)
S-Me	2.93 (3H, <i>s</i>)	2.46 (3H, <i>s</i>)	3.47 (3H, <i>s</i>)	3.33 (3H, <i>s</i>)
Glc-1	4.75 (1H, <i>d</i> , $J = 7.8$ Hz)	4.87 (1H, <i>d</i> , $J = 7.8$ Hz)	4.66 (1H, <i>d</i> , $J = 7.6$ Hz)	4.64 (1H, <i>d</i> , $J = 7.6$ Hz)
2	3.95 (1H, <i>dd</i> , $J = 7.8, 8.0$ Hz)	4.06 (1H, <i>d</i> , $J = 7.8, 8.5$ Hz)	3.96 (1H, <i>dd</i> , $J = 7.8, 9.5$ Hz)	3.62 (1H, <i>dd</i> , $J = 7.8, 9.5$ Hz)
3	4.12 (1H, <i>m</i>)	4.24 (1H, <i>m</i>)	4.22 (1H, <i>dd</i> , $J = 9.5, 8.5$ Hz)	4.13 (1H, <i>dd</i> , $J = 9.5, 9.5$ Hz)
4	4.10 (1H, <i>m</i>)	4.22 (1H, <i>m</i>)	4.13 (1H, <i>dd</i> , $J = 8.5, 8.5$ Hz)	4.18 (1H, <i>dd</i> , $J = 9.5, 9.5$ Hz)
5	3.81 (1H, <i>m</i>)	3.92 (1H, <i>m</i>)	3.97 (1H, <i>ddd</i> , $J = 8.5, 5.1, 2.2$ Hz)	3.99 (1H, <i>ddd</i> , $J = 9.5, 5.5, 2.1$ Hz)
6	4.41 (1H, <i>dd</i> , $J = 12.2, 2.2$ Hz) 4.23 (1H, <i>dd</i> , $J = 12.2, 5.6$ Hz)	4.53 (1H, <i>dd</i> , $J = 12.2, 2.2$ Hz) 4.37 (1H, <i>dd</i> , $J = 12.2, 5.2$ Hz)	4.47 (1H, <i>dd</i> , $J = 12.0, 2.2$ Hz) 4.34 (1H, <i>dd</i> , $J = 12.0, 5.1$ Hz)	4.50 (1H, <i>dd</i> , $J = 12.0, 2.1$ Hz) 4.33 (1H, <i>dd</i> , $J = 12.0, 5.5$ Hz)

Table 2. ^{13}C NMR spectral data of compounds **1**, **2**, **4** and **5** (δ in $\text{C}_5\text{D}_5\text{N}$)

	1	2	4	5
1	66.8 (<i>t</i>)	67.8 (<i>t</i>)	68.8 (<i>t</i>)	69.2 (<i>t</i>)
2	130.5 (<i>d</i>)	134.2 (<i>d</i>)	68.3 (<i>d</i>)	71.3 (<i>d</i>)
3	143.8 (<i>d</i>)	136.0 (<i>d</i>)	54.0 (<i>t</i>)	55.8 (<i>t</i>)
S-Me	42.6 (<i>q</i>)	40.5 (<i>q</i>)	43.0 (<i>q</i>)	43.4 (<i>q</i>)
Glc-1	104.1 (<i>d</i>)	104.2 (<i>d</i>)	99.9 (<i>d</i>)	99.1 (<i>d</i>)
2	75.0 (<i>d</i>)	75.2 (<i>d</i>)	73.5 (<i>d</i>)	80.6 (<i>d</i>)
3	78.5 (<i>d</i>)	78.6 (<i>d</i>)	74.6 (<i>d</i>)	74.8 (<i>d</i>)
4	71.4 (<i>d</i>)	71.5 (<i>d</i>)	71.5 (<i>d</i>)	71.9 (<i>d</i>)
5	78.3 (<i>d</i>)	78.5 (<i>d</i>)	80.4 (<i>d</i>)	80.4 (<i>d</i>)
6	62.5 (<i>t</i>)	62.7 (<i>t</i>)	62.0 (<i>t</i>)	62.3 (<i>t</i>)

Table 3. ^1H and ^{13}C NMR spectral data of compound **3** (δ in $\text{C}_5\text{D}_5\text{N}$)

	^{13}C	^1H
1	69.4 (<i>t</i>)	4.17 (1H, <i>m</i>) 3.99 (1H, <i>m</i>)
2	40.7 (<i>t</i>)	3.78 (1H, <i>m</i>) 3.86 (1H, <i>m</i>)
3		9.36 (1H, <i>t</i> , $J = 5.5$ Hz)
4	163.5 (<i>s</i>)	
5	129.0 (<i>d</i>)	7.30 (1H, <i>d</i> , $J = 14.6$ Hz)
6	147.9 (<i>d</i>)	8.01 (1H, <i>d</i> , $J = 14.6$ Hz)
S-Me	39.9 (<i>q</i>)	2.60 (3H, <i>s</i>)
Glc-1	105.1 (<i>d</i>)	4.84 (1H, <i>d</i> , $J = 7.9$ Hz)
2	75.1 (<i>d</i>)	4.00 (1H, <i>dd</i> , $J = 7.9, 7.9$ Hz)
3	78.5 (<i>d</i>)	4.20 (1H, <i>dd</i> , $J = 7.9, 7.9$ Hz)
4	71.6 (<i>d</i>)	4.15 (1H, <i>dd</i> , $J = 7.9, 7.9$ Hz)
5	78.5 (<i>d</i>)	3.92 (1H, <i>ddd</i> , $J = 7.9, 5.9, 2.4$ Hz)
6	62.7 (<i>t</i>)	4.53 (1H, <i>dd</i> , $J = 11.6, 2.4$ Hz) 4.32 (1H, <i>dd</i> , $J = 11.6, 5.9$ Hz)

Clinacoside B (**2**). Colourless oil. $[\alpha]_{\text{D}}^{21} +13^\circ$ ($\text{C}_5\text{H}_5\text{N}$, c 1.2). Negative ion HRFABMS: $[\text{M}-\text{H}]^-$ found m/z 281.0721, $\text{C}_{10}\text{H}_{17}\text{O}_7\text{S}$ requires 281.0695. IR $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 1074 (SO). ^1H and ^{13}C NMR: Tables 1 and 2.

Clinacoside C (**3**). Colourless oil. $[\alpha]_{\text{D}}^{21} +60^\circ$ ($\text{C}_5\text{H}_5\text{N}$, c 0.4). Negative ion HRFABMS: $[\text{M}-\text{H}]^-$ found m/z 338.0902, $\text{C}_{12}\text{H}_{20}\text{NO}_8\text{S}$ requires 338.0910. IR $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 1658, 1558 (CONH), 1074 (SO). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 252 (3.64). ^1H and ^{13}C NMR: Table 3.

Cycloclinacoside A1 (**4**). $[\alpha]_{\text{D}}^{21} +6^\circ$ ($\text{C}_5\text{H}_5\text{N}$, c 1.0). Negative ion HRFABMS: $[\text{M}-\text{H}]^-$ found m/z

297.0633, $\text{C}_{10}\text{H}_{17}\text{O}_8\text{S}$ requires 297.0644. IR $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 1294, 1130 (SO_2). ^1H and ^{13}C NMR: Tables 1 and 2.

Triacetylcycloclinacoside A1 (**4a**). Compound **4** (3 mg) in dry pyridine (1 ml) was reacted with Ac_2O at room temp. overnight to obtain the triacetate (**4a**, 6 mg). White powder. ^1H NMR (CDCl_3): δ 5.14 (1H, *dd*, $J = 9.3, 9.3$ Hz, Glc-3), 5.03 (1H, *dd*, $J = 9.3, 9.3$

Hz, Glc-4), 4.40 (1H, *dddd*, $J = 8.5, 4.2, 3.4, 1.1$, H-2), 4.39 (1H, *d*, $J = 7.8$ Hz, Glc-1), 4.19 (1H, *dd*, $J = 12.6, 4.7$ Hz, Glc-6), 4.10 (1H, *dd*, $J = 12.6, 2.2$ Hz, Glc-6), 4.04 (1H, *dd*, $J = 12.7, 3.4$ Hz, H-3), 3.96 (1H, *dd*, $J = 12.7, 1.1$ Hz, H-3), 3.78 (1H, *ddd*, $J = 9.3, 4.7, 2.2$ Hz, Glc-5), 3.71 (1H, *dd*, $J = 14.9, 8.5$ Hz, H-1), 3.44 (1H, *dd*, $J = 9.3, 7.8$ Hz, Glc-2), 3.15 (1H, *dd*, $J = 14.9, 4.2$ Hz, H-1), 2.92 (3H, *s*, S-Me), 2.03, 1.96, 1.86 (each 3H, *s*, CH₃CO). ¹³C NMR (CDCl₃): δ 170.5, 169.8, 169.5 (CH₃CO \times 3), 98.3 (Glc-1), 73.7 (Glc-5), 71.6 (Glc-3), 70.6 (Glc-4), 68.6 (Glc-2), 68.4 (C-2), 66.9 (C-3), 61.7 (Glc-6), 54.0 (C-1), 42.1 (-Me), 20.7, 20.6, 20.5 (CH₃CO \times 3).

Cycloclinacoside A2 (5). [α]_D²¹ + 63° (C₅H₅N, c 0.9). Negative ion HRFABMS: [M-H]⁻ found m/z 297.0658, C₁₀H₁₇O₈S requires 297.0644. IR ν_{\max}^{KBr} cm⁻¹: 1300, 1133 (SO₂). ¹H and ¹³C NMR: Tables 1 and 2.

Triacetylcycloclinacoside A2 (5a). Compound **5** (6 mg) in dry pyridine (1 ml) was reacted with Ac₂O (1 ml) at room temp. overnight to obtain the triacetate (**5a**, 9 mg). White powder. ¹H NMR (CDCl₃): δ 5.14 (1H, *dd*, $J = 9.4, 9.4$ Hz, Glc-3), 5.03 (1H, *dd*, $J = 9.4, 9.4$ Hz, Glc-4), 4.36 (1H, *d*, $J = 7.8$ Hz, Glc-1), 4.19

(1H, *m*, H-2), 4.17 (1H, *dd*, $J = 12.4, 4.9$ Hz, Glc-6), 4.10 (1H, *dd*, $J = 12.4, 2.2$ Hz, Glc-6), 3.97 (1H, *dd*, $J = 12.0, 2.7$ Hz, H-1), 3.78 (1H, *ddd*, $J = 9.4, 4.8, 2.2$ Hz, Glc-5), 3.59 (1H, *dd*, $J = 14.2, 14.2$ Hz, H-1), 3.37 (1H, *dd*, $J = 9.4, 7.8$ Hz, Glc-2), 3.11 (1H, *dd*, $J = 15.1, 9.0$ Hz, H-1), 2.86 (3H, *s*, S-Me), 2.85 (1H, *m*, H-3), 2.02, 1.98, 1.97 (each 3H, *s*, CH₃CO). ¹³C NMR (CDCl₃): δ 170.6, 170.2, 169.3 (CH₃CO \times 3), 97.5 (Glc-1), 76.9 (Glc-5), 73.6 (Glc-3), 71.5 (Glc-4), 70.5 (glc-2), 68.7 (C-2), 68.2 (C-3), 61.7 (Glc-6), 55.2 (C-1), 43.0 (S-Me), 20.7, 20.6, 20.5 (CH₃CO \times 3).

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