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An unusual glucoside from *Cleistanthus gracilis*

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Dedicated to Professor Rodney Croteau in honour of his 60th birthday.

Abstract

Extraction of roots and stems of *Cleistanthus gracilis* furnished common triterpenes, plant sterols and the unusual glucoside (+) gracicleistanthoside, the glucoside of 2-β-hydroxy-8-azabicyclo-(5,2,0)-4β,9β-epoxynona-5,7-diene.

Keywords: *Cleistanthus gracilis*; Phyllanthaceae; Gracicleistanthoside; Glucoside of 2-hydroxy-8-azabicyclo-(5,2,0)-4,9-epoxynona-5,7-diene

1. Introduction

Chemical examinations of the mainly Southeast Asian genus *Cleistanthus* (Phyllanthaceae), in particular all parts of the toxic *Cleistantus collinus* Roxb. from India, have resulted in isolation of a number of aryl lignans and their glycosides (Ananjeyulu et al., 1975a,b, 1977, 1981; Chimmani et al., 2003; Govindachari et al., 1967a,b, 1969; Lakshmi et al., 1970; Latha et al., 1985; Ramesh et al., 2003; Sastry and Rao, 1983; Sastry et al., 1987; Satyanarayana et al., 1984, 1998) whose biological effects have been the subject of numerous studies. A few other *Cleistanthanus* species from Southeast Asia have been examined and appear to be chemically somewhat similar (Paris and Nothis, 1970; Sastry and Rao, 1983; Sastry et al., 1987), while *C. schlechteri* from South Africa was the first reported source of members of the diterpene class of cleistanthanes (Candy et al., 1970; McGarry et al., 1969; Pegel et al., 1970, 1971). We now report the results of our study of the roots and stems of *Cleistanthus gracilis* Hook. f. from Southwest Thailand whose roots are said to be used locally as a diuretic and for the relief of fever.

2. Results and discussion

After extraction of the roots of *C. gracilis* with hexane which removed lupeol, β-sitosterol and stigmasterol, further extraction with ethanol and extensive chromatography afforded a non-crystalline substance $C_{14}H_{20}NO_4$ (HRMS) whose ^1H and ^{13}C NMR spectra (Table 1) showed that it was β-D-glucoside of a secondary alcohol $C_8H_9O_2N$ containing one disubstituted and one tetrasubstituted double bond. The second oxygen atom appeared to be that of an unusual ether because of the presence in the ^1H NMR spectrum (Table 1) of two H–C–O signals not associated with alcohols. One of these, at δ 5.60, was long range coupled to the proton on the carbon atom involved in the ether linkage to

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Table 1
^{1}H, ^{13}C, HMBC and NOESY spectroscopic data for compound **1**[a]

Position	δH	δC	HMBC	NOESY
1	–	117.1 *s*		
2	4.76 *ddd*(9.7, 3.7, 1.2)	70.4 *d*	C-4*,7*,9*,1′*	H-3a,b,4,1′
3a	2.44 *ddd* (12.5,4.5,4.5)	35.7 *t*	C-2,4,5*,7	H-2,4,1′
3b	1.74 *ddd*(12.5,9.7.8)			H-2,3a
4	4.28 *m*	64.2 *d*		H-2,3a,5
5	6.15 *dd*(10,2.5)	141.3 *d*	C-3*,7	H-4,6
6	6.24 *dd* (10,1)	126.1 *d*	C-2,4,7*,9	H-4,5,9
7	–	155.8 *s*		
8	8.47 *brs*			
9	5.60 *brs*	95.1 *d*	C-1,2*,6*,7*	H-2,6
1′	4.43 *d*(8)	99.4 *d*	C-1*,3′*	H-2,3a,b,3′
2′	3.17 *t*(9)	72.8 *d*	C-3′, C-4′	
3′	3.19 *t*(10)	76.9 *d*		
4′	3.06 *t*(9)	70.3 *d*	C-3′*	
5′	3.18 *m*	76.7 *d*		
6′a	3.71 *dd* (12,2)	61.6 *t*		
6′b	3.45 *m*			

[a] Assignments based on COSY, HSSQ and DEPT experiments. Chemical shifts in ppm relative to TMS; coupling constants in parentheses are in Hz. Asterisks indicate strong cross peaks.

Table 2
^{1}H, ^{13}C and HMBC spectroscopic data for compound **2** [a,b]

Position	δH	δC	HMBC
1	–	116.4 *s*	
2	4.76 *t*(4)	73.5 *d*	C-3,9(W),1′
3a	2.1–2.3 *m*	32.8 *t*	
3b	2.1–2.3 *m*		
4	5.35 *dd*(8.4,4.5)	64.0 *d*	C-6,7[c]
5	6.18 *dd*(10,4.5)	133.0 *d*	C-3
6	6.36 *d*(10)	128.9 *d*	C-2,4,7(W),9
7	–	152.9 *s*	
9	5.36 *s*	99.4 *d*	C-6,7[c]
1′	4.85 *d*(8)	102.2 *d*	C-2
2′	5.08 *dd*(10,8)	71.1 *d*	C-3′
3′	5.21 *t*(9.5)	72.8 *d*	C-2′,4′
4′	5.19 *t*(10)	69.0 *d*	C-5′
5′	3.78 *m*	72.2 *d*	
6′a	4.29 *dd*(12.4,2.3)	61.6 *t*	
6′b	4.12 *dd*(12.4,4.4)		
Ac-Me[b]	2.10 *s*,2.09 *s*,2.04 *s*, 2.03 *s*,2.00 *s*	21.0, 20.8, 20.6 20.6, 20.6	
Ac-CO		171.0,170.6,170.2 169.4,169.0	

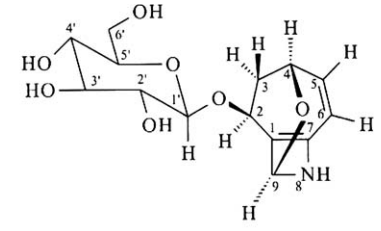
[a] Assignments based on COSY, HMSQ and DEPT experiments. Chemical shifts in ppm relative to TMS; coupling constants in parentheses are in Hz.
[b] Intensity three protons.
[c] As a result of near superposition of H-4 and H-9.

C-1′ of the glucose moiety, the other, at δ 4.28, was vicinally coupled to one of the vinylic protons, that at δ 6.15, of the disubstituted *cis* double bond and also vicinally coupled to two protons on a carbon next to the glycosidic linkage.

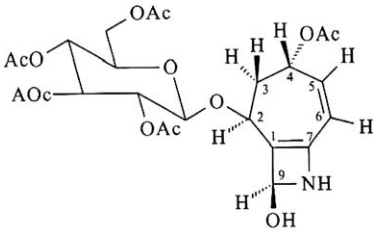
A structure which incorporates these features as well as a second tetrasubstituted double bond required by the presence of two additional carbon singlets at δ 117.1 and 155.8 and the nitrogen atom required by the mass spectrum is represented by the unusual and highly strained formula **1** for the glycoside, i.e. where the nitrogen atom is of the aminal type and incorporated in an unsaturated four-membered ring the double bond of which is part of a 1,3-diene system. Vicinal and long range coupling constants as well as the information gleaned from the NOESY data (Table 1) and the existence of the ether bridge then required that H-2, H-4 and H-9 be *cis* to each other. Noteworthy also are the NOE's (Table 1) between H-1 of the glucose half and H-2 and H3a,b of the heterocyclic half which indicates that in solution the relative orientation between the sugar and the aglycone part depicted in the planar formula is not necessarily preferred.

We have named the new substance gracicleistanthoside **1**. In an attempt to characterize it further an attempt was made to acetylate the glucose portion and perhaps the NH group as well. However, acetylation of the glucose moiety was accompanied by attack of acetate, with inversion, on C-4 concomitant with cleavage of the oxygen bridge, thus relieving the strain and leading to **2** as demonstrated by the ^{1}H and ^{13}C NMR spectra (Table 2). Aside from the expected downfield shifts in the glucose half, the ^{1}H NMR spectrum of **2** differs from that of **1** significantly only in the chemical shift changes of H-4 and H-9 expected from opening the oxide ring and acetylation at C-4. On the other hand, relief of strain has resulted in chemical shift changes of all carbon atoms within the new seven-membered ring. Inversion at C-4 during the acylation process is further demonstrated by the NOESY spectrum of **2** which did not exhibit the strong cross peak between H-2 and H-4 characteristic of **1**. The high resolution mass spectrum of **2** did not exhibit the molecular ion as a result of the facile loss of water to form a fused azete.

Extraction of the stems of *C. gracilis* followed by extensive chromatography in the manner described for the roots yielded only friedelin in addition to lupeol, β-sitosterol and stigmasterol.



1



2

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded at ambient temperature in $CDCl_3$ on a Bruker AMC instrument operating at 300.13 resp. 75.47 MHz or a Bruker DRX instrument operating at 500 resp. 125 MHz. The usual Bruker HMBC pulse sequence was used in which the long range C–H coupling constant was optimized for 7 Hz and the $^{1}J_{CH}$ was optimized for 147 Hz. The coupling constants are optimal for observing $^{3}J_{CH}$ in aromatic compounds but permit observation of $^{2}J_{CH}$ and $^{4}J_{CH}$ correlations in saturated systems, particularly those which have ''zig-zag'' orientation. EI mass spectra were measured on a Hitachi Perkin–Elmer RMV-6M instrument. HRMS mass spectra were measured on a Kratos Concept II 2 sector/mass spectrometer. Rotations were determined on a Polax-2 L instrument. Si gel 60(0.2–0.5 mm Merck) was used for analytical chromatography and Si 60 GF 254 Merck for preparative TLC.

3.2. Plant material

Roots and stems of *C. gracilis* Hook. f. (Phyllanthaceae) were collected in Prachuab Kirikan Province, Southwest Thailand, in September 2001. The plant material was identified by Mr. C. Phengklai; a voucher specimen (BKF 128193) was deposited in the Royal Forest Department, Paholyothin Road, Bangkok, Thailand.

3.3. Extraction and isolation

3.3.1. Roots

Air dried and powdered roots (2.8 kg) of *C. gracilis* were percolated by hexane (3 × 15 L) at rt. Filtration and evaporation of the filtrate at reduced pressure gave a residue (crude hexane extract, 12 g). The hexane-insoluble material was air-dried and percolated with EtOH (3 × 15 L) at rt. Evaporation of the EtOH solution at reduced pressure gave a residue (39 g) which was extracted with $CHCl_3$ (3 × 5 L). Combination of the $CHCl_3$ extracts and evaporation at reduced pressure gave crude $CHCl_3$ extract (8 g); the material remaining after the extraction with $CHCl_3$ was air-dried to constitute the crude EtOH extract (30 g). The crude hexane extract (11 g) was applied to a Si gel column (150 g) and eluted with hexane and hexane–EtOAc, 200 ml fractions being collected as follows: Frs. 1–10 (hexane), 11–19 (hexane–EtOAc, 9:1) 20–24 (hexane–EtOAc, 4:1), 25–30 (hexane–EtOAc, 7:3), 31–34 (hexane–EtOAc, 3:2), 35–43 (hexane–EtOAc, 1:1), 44–50 (hexane–EtOAc, 2:3), 51–65 (hexane–EtOAc, 3:7), 66–71 (hexane–EtOAc, 1:4), 72–78 (hexane–EtOAc, 1:9). Frs 25–30 (2 g) were combined and subjected to Si gel (50 g) CC, five 100 ml subfractions being collected by elution with hexane–EtOAc (19:1). Subfr. 2 (530 mg) on recrystallization from MeOH afforded lupeol (110 mg) whose structure was established by HRMS, ^{1}H NMR and ^{13}C NMR spectrometry. Frs 34–43 (4.2 g) were combined and similarly applied to a Si gel column (150 g) with hexane-EtOAc, 9:1, ten 100 ml subfractions being collected. Subfrs. 2–5 (800 mg) were combined; recrystallization from MeOH gave 540 mg of a mixture of β-sitosterol and stigmasterol.

A portion of the crude EtOH extract (5 g) was subjected to Si gel (50 g) CC and eluted with $CHCl_3$–MeOH, 150 ml frs. being collected as follows: Frs. 1–5 ($CHCl_3$–MeOH, 19:1), 6–26 ($CHCl_3$–MeOH, 9:1), 27–30 (MeOH). Frs. 10–16 (173 mg) were combined and purified by TLC (Si gel, $CHCl_3$–MeOH, HCO_2H, 85:15:0.1) to give **1** as yellow viscous material (143 mg).

3.3.2. Stems

Dried and powdered stem wood (2.4 kg) was percolated by hexane (3 × 15 l) at rt. Filtration and evaporation of the filtrate at reduced pressure gave a residue (crude hexane extract, 14 g). The hexane-insoluble material was dried and then percolated with EtOH (3 × 15 L) at rt. Evaporation of the EtOH solution at reduced pressure gave a crude EtOH extract (38 g).

Chromatography of the crude hexane extract (14 g) over Si gel (200 g) and elution with hexane and hexane–EtOAc, gave 200 ml fractions being collected as follows. Frs. 1–10 (hexane), 11–19 (hexane–EtOAc, 1:9), 20–24 (hexane–EtOAc, 4:1), 25–30 (hexane–EtOAc, 7:3), 31–34 (hexane–EtOAc, 3:2), 35–43 (hexane–EtOAc, 1:1), 44–50 (hexane–EtOAc, 2:3), 51–65 (hexane–EtOAc, 3:7). Frs. 4–7 (1.3 g) were combined and applied to a Si gel (30 g) column. Elution with hexane–EtOAc (19:1), using thirteen 50 ml subfractions gave in subfr. 5 (90 mg) of friedelin (54 mg) after crystallization from MeOH, identified by EI-HRMS, ^{1}H and ^{13}C NMR spectroscopy. Subfr. 6 (83 mg) gave lupeol (41 mg), identified by HRMS, ^{1}H and ^{13}C NMR spectroscopy. Elution with hexane gave six 50 ml subfractions. Subfr. 4 (253 mg) after crystallization from methanol gave lupeol (35 mg). Subfr. 6 (50 mg) after crystallization from methanol gave 25 mg of a mixture of β-sitosterol and stigmasterol.

3.3.3. (β-D-glucopyranosyl)-8-azabicyclo(5,2,0)-4,9-epoxynona-5,7-diene (1)

Viscous brown gum, $[\alpha]_D^{25}$ − 35.7 (c 0.7, MeOH); For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2; HREMS *m/z* 314.12380; calcd. for $C_{14}H_{20}NO_4 + H^+$ *m/z* 314.12398.

Acetylation of **1** (16 mg) with acetic anhydride and work-up in the usual fashion followed by chromatography furnished 12 mg of 2-(2′,3′,4′,6′-tetraacetoxy-β-D-glucopyranosyl)-4-acetoxy-9-hydroxy-8-azabicyclo(5,2,0)-5,7-diene(**2**) as a brown gum, $[\alpha]_D^{25}$166.6 (c 0.3 g/100 ml, $CHCl_3$), ^{1}H and ^{13}C NMR spectra, see Tables 1 and 2. FAB HRMS in NBA *m/z* 524.17665; calcd. for $C_{24}H_{31}$-$NO_{13} + H^+$-H_2O *m/z* 524.17680.

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