Pergamon

0031-9422(95)00557-9

Phytochemistry, Vol. 41, No. 1, pp. 279–281, 1996

0031-9422/96 $15.00 + 0.00

A STEROL GLYCOSIDE FROM LEAVES OF *CLERODENDRON COLEBROOKIANUM*

PRATUL GOSWAMI, JIBON KOTOKY,* ZE-NAI CHEN*† and YANG LU†

Division of Life Science, Institute of Advanced Study in Science and Technology, Khanapara, Guwahati-781022, India; †Department of Chemistry, Shanghai Second Medical University, Shanghai 200025, China

(*Received* 8 *March* 1995)

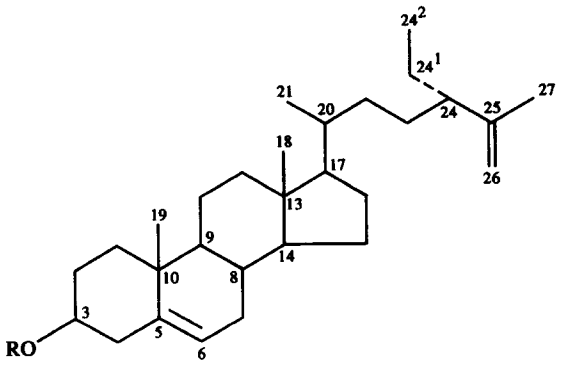
Key Word Index—*Clerodendron colebrookianum*; Verbenaceae; Plant chemistry; Sterol; Sterol Glycoside; Clerosterol 3β-O-[β-D-glucoside].

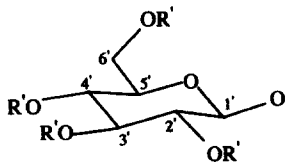
Abstract—A new sterol glycoside clerosterol 3β-O-[β-D-glucoside] along with clerosterol, sitosterol, octacosanol and fatty acids has been isolated from the leaves of *Clerodendron colebrookianum*. Their structures have been characterised by spectral analysis and the C-24 (*S*/β) configuration in clerosterol was also confirmed.

INTRODUCTION

The genus *Clerodendron*, which belongs to the family Verbenaceae, is a widely distributed genera. Some species of it have been used as medicine in clinics for treatment of different diseases, such as asthma, pyreticosis, diseases of the blood and catarrhal affections of the lungs [1]. In China the leaves of *Clerodendron trichotomum* Thunb. are used as a traditional drug for hyertension; the constituents of this have been studied [2].

Clerodendron colebrookianum Walp. is a shrub generally growing in the moist and waste places of north-eastern region of India up to an altitude of 1700 meters. In traditional folk medicine, *C. colebrookianum* has been also used as a remedy for hypertension, and chemical investigation of its root has been reported [3]. Because of this medicinal importance, we were prompted to investigate the leaves of this species. The present paper deals with the isolation and strutural elucidation of a new sterol glycoside (**1**) along with clerosterol (**2**) isolated from this plant for the first time and with some other compounds (i.e. sitosterol, octacosanol, stearic acid and palmitic acid). All these constituents were isolated from different fractions eluted from silica gel columns with solvents of increasing polarity. Their structures were elcidated by spectroscopic methods, in particular by mass spectrometry, ^{1}H NMR, NOEDS, and 2D NMR (^{1}H–^{1}H COSY, NOESY).



	R	R'
1		H
1a		Ac
2	H	—
2a	Ac	—

RESULTS AND DISCUSSION

To new sterol glycoside (**1**), mp 258–262° (dec.) was obtained as white powder and had the molecular formula $C_{35}H_{58}O_6$ which was deduced from the FAB mass spectrum and NMR data. Two quasimolecular ions at *m/z* 597 $[M + Na]^+$ and 613 $[M + K]^+$ in the FAB-mass spectrum indicated its molecular weight to be 574. The fragment ions in EI-mass spectrum at *m/z* 412 $[M - C_6H_{10}O_5]^+$, 394 $[M - C_6H_{10}O_5 - H_2O]^+$, 255 $[M - C_6H_{10}O_5 - H_2O - C_{10}H_{19}$ (side chain)$]^+$ and *m/z* 163, 127, 73, 57 obtained from the sugar moiety suggested that **1** may be monoglycoside with a C_{29}-sterol

*Author to whom correspondence should be addressed.

(*m/z* 412) aglycone moiety. The IR spectrum displayed the presence of hydroxyl (3500–3300 cm^{-1}) and olefinic (1620 cm^{-1}) groups.

The sterol aglycone moiety in **1** was identified by its 400 MHz ^{1}H NMR spectrum. The olefinic signals at δ4.620 (H-26) and δ4.714 (H-26) indicated the presence of a terminal methylene group in the side chain. The other signals of the side chain appeared at δ0.868 (H-21), 0.744 (H-24^2) and 1.516 (H-27). The angular methyl groups resonated at δ0.633 (H-18) and 0.945 (H-19). The signal at δ5.320 showed an olefinic proton (1H, *br*, *d*, J = 5.0 Hz, H-6) and the multiplet at δ3.459 (1H, *m*, $W_{1/2}$ = 17 Hz, H-3) is a characteristic of Δ^5-3β-hydroxyl sterols. These spectral data were identical with those of **2**. Since all δ_H values of **2** were consistent with the published data [4] of clerosterol, the structure of the sterol aglycon in **1** was elucidated as poriferasta-5,25(26)-dien-3β-ol (i.e. clerosterol).

In order to confirm the glycosyl moiety in **1**, its tetracetyl derivative **1a** was prepared in Ac_2O-pyridine. The signals at δ1.986, 2.003, 2.030 and 2.059 in the ^{1}H NMR spectrum indicated the presence of four acetyl groups in **1a**. The anomeric proton H-1′ (δ4.571, *d*, $J_{1',2'}$ = 8.1 Hz) of **1a** indicated the β-glycosidic linkage. The non-equivalent protons of H-6′ appeared as two *dd* signals at δ4.237 and 4.091 with J values of 12.1 Hz ($J_{6'A,6'B}$), 4.8 Hz ($J_{6'A,5}$) and 2.3 Hz ($J_{6'B,5'}$) and an H-5′ signal as a multiplet appeared at δ3.653, while the H-2′ H-3′ and H-4′ gave rise to triplet signals at δ4.938, 5.185 and 5.060, respectively. All the signals of the sugar moiety (H-1′ to H-6′) were consistent with the known data in the acetyl derivative of β-glucoside reported [5, 6] and their assigments could be confirmed from their ^{1}H–^{1}H COSY spectrum (Table 1).

From the NOESY spectrum (Table 1), we found the NOE correlations among H-1′, H-3′ and H-5′. It is a characteristic of β-glucopyranosyl. From NOEDS spectrum (Table 2), a significant NOE correlation between H-1′ and H-3 (δ3.465, *m*, $W_{1/2}$ = 17 Hz) could be clearly observed. Thus, it is confirmed that the glucosyl unit of **1** is attached at C-3 position of clerosterol moiety.

In most previous cases the configuration at C-24 of Δ^{25}-unsaturated 24-alkyl sterols in plants was found to be 24 *S*/β. Recently it was reported that both epimers at C-24 (24*S*/β and 24*R*/α) occur together in the same plant. The discrimination between the two epimers is possible on the basis of the shift of H-21 and H-29 signals of their acetates in the 400 MHz ^{1}H NMR data. The 24(*R*)-epimer has its H-21 doublet at lower field (ca. 3 Hz, 0.008 ppm) and its H-29 triplet at higher field (2–3 Hz, 0.005–0.007 ppm) than those of the 24(*S*)-epimer [7].

In order to confirm the C-24 configuration of clerosterol **2**, its acetyl derivative **2a** was prepared in Ac_2O/Py since the NMR data of both epimers were reported in their acetate forms [7, 8]. The 24 *S*/β configurtion of **2** was confirmed by comparison of its ^{1}H NMR values of **2a** with the reference data in the literature (Table 3).

Table 1. 2D-NMR Correlations of **1a**

Proton	COSY (^{1}H)	NOESY (^{1}H)
1′	2′	3′, 5′
2′	1′, 3′	4′, 6′$_A$, 6′$_B$
3′	2′, 4′	1′
4′	3′, 5′	2′
5′	4′, 6′$_A$, 6′$_B$	1′
6′$_A$	5′, 6′$_B$	6′$_B$, 2′
6′$_B$	5′, 6′$_A$	6′$_A$, 2′
3	4$_{A,B}$	

Table 2. NOEDS spectrum of **1a**. NOE correlation between H-1′ and H-3

Proton irradiated	NOE observed (enhancement ratio)
H-1′	H-3 (+ 4.9), H-5′ (+ 8.5)
H-3	H-1′ (+ 5.07)

Table 3. Comparing some $\Delta\delta$ values from ^{1}H NMR ($CDCl_3$) of **2a** with those of the reference compounds (the signal of H-19 as reference standard)

Compound	H-19	H-21	$\Delta\delta$	H-19	H-24^2	$\Delta\delta$
2a	0.996	0.884	**0.112**	0.996	0.780	**0.216**
24*S*/β [7]	1.017	0.904	**0.113**	1.017	0.801	**0.216**
24*S*/β [8]	1.015	0.904	**0.111**	1.015	0.800	**0.215**
24*R*/β [7]	1.016	0.912	0.104	1.016	0.796	0.220

The configuration of **2a** could be deduced from the $\Delta\delta$ values in bold.

EXPERIMENTAL

Plant Material. The plant materials were collected from the hilly areas of the northeastern region of India and a vouher specimen has been deposited at the herbarium of the Institute of Advanced Study in Science and Technology.

Extraction and Isolation. Air-dried and finely powdered leaves were exhaustively extracted with petrol at ambient temperature for 5 weeks and the petrol solution was concentrated *in vacuo* to give a dark residue. It was subjected to CC on silica gel (60–120 mesh). The petrol–benzene (3:1) eluated **2**, in the further elutions of the column with petrol–benzene (1:9) **1** was obtained and recrystalized from MeOH-$CHCl_3$. Other fractions afforded sitosterol, octacosanol, stearic acid, and palmitic acid. These compounds were identified by spectral data or by direct comparison with authentic samples.

Clerosterol 3β-*O*-[β-D-*glucoside*] (**1**). mp 258–262° (dec.); FAB-MS *m/z*: 597 $[M + Na]^+$, 631 $[M + K]^+$; EI-MS *m/z* (rel. int.): 412 $[M - C_6H_{10}O_5]^+$ ($C_{29}H_{48}O$) (5), 394 $[M - C_6H_{10}O_5 - H_2O]^+$ (100), 255 $[M - C_6H_{10}O_5 - H_2O - C_{10}H_{19}$ (side chain, SC)$]^+$ (18), 253 $[M - C_6H_{10}O_5 - H_2O - SC - 2H]^+$ (19), 163

(7), 127 (8), 73 (38), 57 (54). ^{1}H NMR (400 MHz, DMSO-d_6): δ0.633 (3H, *s*, H-18), 0.744 (3H, *t*, J = 7.2 Hz, H-24^2), 0.868 (3H, *d*, J = 6.2 Hz, H-21), 0.945 (3H, *s*, H-19), 1.516 (3H, *s*, H-27), 2.89–3.14 (4H, *br m*, H-2′, H-3′, H-4′, and H-5′), 3.401 (1H, *dd*, J = 11.6/5.6 Hz, H-6′), 3.459 (1H, *m*, $W_{1/2}$ = 17 *Hz*, *H*-3), 3.638 (1*H*, *br d*, J = 11.4 Hz, H-6′), 4.217 (1H, *d*, J = 7.8 Hz, H-1′), 4.620 (1H, *br s*, H-26), 4.714 (1H, *br d*, H-26), 5.320 (1H, *br d*, J = 5.0 Hz, H-6).

Clerosterol tetracetyl-3β-*O*-[β-D-*glucoside*] (**1a**). ^{1}H NMR (400 MHz, $CDCl_3$): δ0.650 (3H, *s*, H-18), 0.781 (3H, *t*, J = 7.2 Hz, H-24^2), 0.883 (3H, *d*, J = 6.3 Hz, H-21), 0.964 (3H, *s*, H-19), 1.545 (3H, *s*, H-27), 1.986, 2.003, 2.030, 2.059 (12H, *s*, 4 × MeC=O), 3.465 (1H, *m*, $W_{1/2}$ = 17 Hz, H-3), 3.653 (1H, *m*, $J_{5',6'A}$ = 4.8 Hz, $J_{5',6'B}$ = 2.3 Hz, $J_{5',4'}$ = 9.6 Hz, H-5′), 4.091 (1H, *dd*, $J_{6'B,6'A}$ = 12.1 Hz, $J_{6'B,5'}$ = 2.3 Hz, H-6′$_B$), 4.237 (1H, *dd*, $J_{6'A,6'B}$ = 12.1 Hz, $J_{6'A,5'}$ = 4.8 Hz, H-6′$_A$), 4.571 (1H, *d*, J = 8.1 Hz, H-1′), 4.620 (1H, *d*, J = 2.1 Hz, H-26), 4.704 (1H, *d*, J = 1.7 Hz, H-26), 4.938 (1H, *dd*, J = 8.1 Hz, 9.6 Hz, H-2′), 5.060 (1H, *t*, J = 9.6 Hz, H-4′), 5.185 (1H, *t*, J = 9.6 Hz, H-3′), 5.336 (1H, *d*, J = 4.5 Hz, H-6).

Clerosterol, (24*S*/β) (*poriferasta*-5,25(26)-*dien*-3β-*ol* (**2**). $C_{29}H_{48}O$, mp 120–121° (lit. [5] 119.8–121°); IR ν_{max}^{KBr} cm^{-1} 3450; HR-MS: 412.3701 (calc. 412.3705, $C_{29}H_{48}O$); EI-MS *m/z* (rel. int.): 412 $[M]^+$ (100), 397 $[M - Me]^+$ (22), 394 $[M - H_2O]^+$ (16), 379 $[M - Me - H_2O]^+$ (34), 314 $[M - C_7H_{14}]^+$ (39), 300 $[M - C_7H_{12}O]^+$ (27), 299 $[M - C_7H_{14} - Me]^+$ (58), 273 $[M - C_{10}H_{19}(SC)]^+$ (17), 271 $[M - SC - 2H]^+$ (72), 255 $[M - SC - H_2O]^+$ (24), 253 $[M - SC - H_2O - 2H]^+$ (21), 231 $[M - SC - C_3H_6]^+$ (16), 229 $[M - SC - C_3H_8]^+$ (21), 213 $[M - SC - C_3H_6 - H_2O]^+$ (43), 211 $[M - SC - C_3H_8 - H_2O]^+$ (18). ^{1}H NMR (400 MHz, $CDCl_3$): δ0.644 (3H, *s*, H-18), 0.773 (3H, *t*, J = 7.4 Hz, H-24^2), 0.878 (3H, *d*, J = 6.4 Hz, H-21), 0.979 (3H, *s*, H-19), 1.538 (3H, *s*, H-27), 3.505 (1H, *m*, $W_{1/2}$ = 22 Hz, H-3), 4.618 (1H, *s*, H-26), 4.702 (1H, *s*, H-26), 5.324 (1H, *d*, J = 4.8 Hz, H-6).

Clerosteryl acetate (**2a**): ^{1}H NMR (400 MHz, $CDCl_3$): δ0.649 (3H, *s*, H-18), 0.780 (3H, *t*, J = 7.4 Hz, H-24^2), 0.884 (3H, *d*, J = 6.4 Hz, H-21), 0.996 (3H, *s*, H-19), 1.545 (3H, *s*, H-27), 2.012 (3H, *s*, CH_3-C=O), 2.287, 2.305 (1H, each, *m*, H-4) 4.581 (1H, *m*, $W_{1/2}$ = 22 Hz, H-3), 4.620 (1H, *d*, J = 2 Hz, H-26), 4.704 (1H, *d*, J = 1.4 Hz, H-26), 5.352 (1H, *d*, J = 4.7 Hz, H-6).

Acknowledgements—The authors are thankful to Dr K. Barua, Director, FSL, Shillong and Dr Guoqiang Song, Director of NMR lab., Shanghai Institute of Materia Medica, Academia Sinica for spectral analysis and Dr M. K. Kalita and Mr M. C. Barua for their encouragement. Also thanks are due to the ASTEC, Govt of Assam as well as the State Educational Committee and the Ministry of Health of PR China for financial assistance.

REFERENCES

1. Prakash, L. and Garg, G. (1980) *Pharmaz.* **35**, H. 8, 500.
2. Chen, Z. N., Xu, P. J. and Yao, T. R. (1988) *Yaoxue Xuebao* **23**, 789.
3. Joshi, K. C., Singh, P. and Mehra, A. (1979) *Planta Med.* **37**, 64.
4. Garg, V. K. and Nes, W. R. (1984) *Phytochemistry* **23**, 2925.
5. Guevara, A. P., Lim-Sylianco, C. Y., Dayrit, F. M. and Finch, P. (1989) *Phytochemistry* **28**, 1721.
6. Abraham, W., Wertz, P. W., Burken, R. R. and Downing, D. T. (1987) *J. Lipid Res.* **28**, 446.
7. Akihisa, T., Kokke, W. C. M. C., Tamura, T. and Matsumoto, T. (1991) *Lipids* **26**, 660.
8. Thakur, S., Ghosh, P., Akihisa, T., Shimizu, N., Tamura, T. and Matsumoto, T. (1988) *Indian J. Chem.* **27B**, 17.