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ACYL STERYL GLYCOSIDES FROM *PITHECELLOBIUM CAULIFLORUM*

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Key Word Index—*Pithecellobium cauliflorum*; Leguminosae; acyl steryl glycosides.

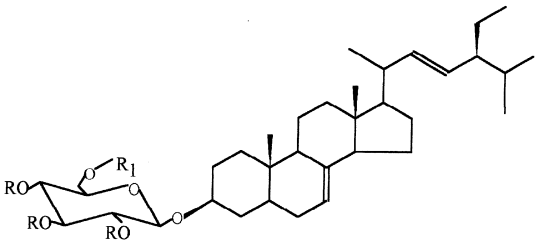
Abstract—One sterolglycoside and two sterolglycolipids, were found in the branches of *Pithecellobium cauliflorum* (Willd.) Mart., namely the β-D-glucoside of α-spinasterol (glucosylsterol, minor component), 3-*O*-[6′-*O*-palmitoyl-β-D-glucosyl]-spinasta-7, 22(23)-diene, and 3-*O*-[6′-*O*-stearoyl-β-D-glucosyl]-spinasta-7, 22(23)-diene (acylglucosylsterols, major components). Each was isolated and identified by a combination of different chromatographic, hydrolytic and spectroscopic methods, respectively.

INTRODUCTION

Various studies have been made on the chemical constituents of plants of the genus *Pithecellobium* (Leguminosae), most of which grow in tropical countries. In 1953, Weisner [1] reported the isolation of a pithecolobine alkaloid isolated from *P. saman* which exhibits local anaesthetic activity [2]. Subsequently, Varshney and Vyas [3–5] isolated and characterized a number of triterpene glycosides from the seeds of the fruits. Nigam and co-workers [6–8] studied the steroid, steroid glycoside, hydrocarbon and amino acid constituents of the seeds, bark and leaves of *P. dulce*, while Lee and co-workers studied the flavan-3-ol gallates from the leaves of *P. Lobatum* [9] Altman [10] reported an unidentified saponin and the present paper reports the isolation and characterization of two new glycosteroids from *P. cauliflorum*.

RESULTS

A mixture of glucosylsterol (**1**) and acyl steryl glycosides (**2, 3**) was isolated from the MeOH extract of the branches of *Pithecellobium cauliflorum* (Willd.) Mart. and purified by repeated and sequential column chromatography (cc) followed by centrifugal thin-layer chromatography (CTLC). TLC of glucosylsterol (**1**) showed a rose spot, and the TLC of acyl steryl glycosides (**2**, **3**) gave brown spots on spraying with 50% H_2SO_4. The ^1H NMR and ^{13}C NMR spectra of



	R	R_1
1	H	H
1a	Ac	Ac
2	H	Palmitate
3	H	Stearate

Fig. 1. Glucosylsterol (**1**), acetyled derivative of **1** (**1a**), acyl steryl glycosides (**2**, **3**).

the peracetylated derivative of **1** (**1a**) was identical to that of peracetyl 3β-*O*-β-D-glucopyranosylspinasterol first reported by Braz Filho and co-workers [11].

DISCUSSION

The ^1H NMR signals of **2** and **3**, which were attributed to the sterol moiety, were consistent with published data for 24ξ-ethyl-5α-colesta-7, 22-dien-3β-ol (spinasterol or chondrillasterol) [12]. The angular

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methyl groups signals appeared at δ 0.55 (3H, *s*, H-18) and δ 0.78 (3H, *s*, H-19); the side chain signals appeared at δ 1.03 (3H, *d*, $J = 6.6$ Hz, H-21), 0.86 (3H, *d*, $J = 7.0$ Hz), H-26), 0.84 (3H, *d*, $J = 6.2$ Hz, H-27) and 0.80 (3H, *t*, $J = 7.6$ Hz, H-29); and the olefinic signals at δ 5.01 (1H, *dd*, $J = 15.7$ and 8.0 Hz) and 5.18 (1H, *dd*, $J = 14.7$ and 7.9 Hz). These signals are consistent with a 24-ethyl, Δ [7,22(23)] sterol structure [12–14].

The identity of the sterol moiety was further confirmed by ^{13}C NMR DEPT spectra. Comparison with spectral data published, confirmed the presence of a Δ [7] nucleus, and shifts of the signals due to the side chain (C-20 to C-24″) were consistent with a 22(23) double bond [15, 16].

The good match of the sterol side chain ^{1}H and ^{13}C NMR chemical shifts and coupling constants with data published for C-24 alkyl sterols [17–19] with 24β-configuration suggested that the spinasta-5, 22(23)-diene moiety also possessed a 24β-ethyl configuration. Note that sterols with a 24β configuration at 24 *R* if a saturated side chain and 24 *S* if the Δ [22] derivatives, whereas sterols in most vascular plants have a 24α configuration [20].

The intense broad ^{1}H NMR singlet at δ 1.26 (ascribed to a long methylene chain) and the triplet at 2.34 (2H, *t*, $J = 7.7$ Hz, —CH_2—C═O), the ^{13}C NMR signal at 174.7 (ascribed to —C═O) and the intense signal at 29.7 (many —CH_2's) and the IR peak at 1730 cm^{-1} (ester linkage) were indicative of a long chain fatty acid. The CG-MS of the fatty acid methyl esters obtained from saponification followed by methylation of the acyl glucosyl sterols **2** and **3** confirmed the presence of palmitate and stearate.

The anomeric proton appears at δ 4.4 (1H, *d*, $J = 7.7$ Hz, C-1′). The glycosyl moiety of **2** and **3** indicated a β-D-glucosidic linkage, the non-equivalent protons at C-6′ appeared as doublets of doublets at δ 4,43 (1H, *dd*, $J_{6'a\,6'b} = 11$ Hz, $J_{6'a\,5'} = 5.2$ Hz, C-6′a) and 4.30 (1H, *dd*, $J_{6'b6'a} = 13$ Hz, $J_{6'b\,5'} = 2.5$ Hz, C-6′b); the C-5′ signal appeared at δ 3.60 (1H, *m*, C-5′), while C-2′, C-3′, and C-4′ appeared at δ 3.30 (3H, *m*). All the signals of the sugar moiety (C-1′ to C-6′) were confirmed from the ^{1}H–^{1}H COSY spectrum.

Six distinct signals are assigned to glucose in the ^{13}C NMR spectrum indicating the β-configuration and pyranose form [21]. The downfield shift of C-6′ (β-effect, +0.3 ppm) and upfield shift of C-5′ (γ-effect, −4.7 ppm) confirmed the attachment of the palmitoyl and stearoyl residues at C-6′.

Comparison of the ^{13}C NMR signals attributed to the glucosyl moieties of **2** and **3** with published data for model compounds [22, 23] confirmed the identity of β-D-glucose and the attachment of the palmitoyl (**2**) and stearoyl (**3**) residues at C-6′. Thus acyl steryl glycoside **2**, was identified as 3-*O*-[6′-*O*-palmitoyl-β-D-glucosyl]-spinasta 7, 22-diene and **3**, was identified as 3-*O*-[6′-*O*-stearoyl-β-D-glucosyl] spinasta 7, 22-diene.

EXPERIMENTAL

General

NMR spectra were recorded on a 200 MHz spectrometer using a Bruker AC-200 (^{1}H frequency 200 MHz, ^{13}C frequency 50.3), solvent $CDCl_3$, with TMS as an internal standard; FTIR spectra were recorded using a Perkin-Elmer model 1420 spectrometer; Gas Chromatography-Mass Spectrometry (GC-MS) used a Hewlett-Packard 5890 A chromatograph connected to a Hewlett-Packard 5988 A mass spectrometer, (25 m × 0.2 mm i.d., film thickness, 0.2 μm, fused-silica capillary columm coated with HP-FFAP; Helium 0.5 ml/min; temperature program from 160°, 15°/min to 200°; 70 eV; ion source at 300°; injection volume 0.5 μl, sample concentration 0.01 mg/ml and rel. amount 10%).

Plant material

Pithecellobium cauliflorum plant material was obtained from the Botanical Garden of Rio de Janeiro, Brazil (March 1995) by L. Rico and H.C. de Lima and a voucher specimen has been deposited at the Herbarium of the Botanical Garden of Rio de Janeiro with No. 4998 (RB).

Extraction and isolation

Air dried and finely powdered branches (800 g) were exhaustively extracted with hexane, CH_2Cl_2 and MeOH by reflux for 2 h, and these solutions were individually concentrated *in vacuo*. The dried MeOH extract (27.6 g) was dissolved in H_2O (500 ml) and extracted with *n*-BuOH (450 ml); the organic phase was then concentrated *in vacuo* to obtain a butanol extract (9.2 g). This crude extract was purified by repeated CC over Si gel 60 (Merck 0.063–0.200 mm) using $CHCl_3$ and $CHCl_3$-MeOH in a gradient of increasing polarity. Glycoside **1** (30 mg) was obtained from this extract after purification and the mixture of acyl steryl glycosides **2** (18 mg) and **3** (20 mg) were further purified by CTLC eluting with MeOH. The elution was monitored by analytical TLC on pre-coated silica gel, and detection was made by spraying with 50% H_2SO_4 and heating for a few minutes (105°).

Compound **1**. Mp 340°; IR ν^{Kbr} cm^{-1}: 3400 (OH), 2923 (C-H), 1161 (—C—O) and 973 (═C—H); ^{1}H NMR (200 MHz, pyridine): 5.30 (1H, *s*, C-7), 5.25 (2H, *m*, C-3′ and C-4′), 5.18 (1H, *dd*, $J = 9.9$ and 5.5 Hz, C-23), 5.01 (1H, *dd*, $J = 13.2$ and 6.6 Hz, C-22), 4.95 (2H, *t*, $J = 7.96$ Hz, C-2′ and C-3′), 4.60 (2H, *d*, $J = 7.9$ Hz, C-1′ and C-2′), 4.15 (3H, *m*, C-5′, C-6′a and C-6′b), 3.69 (2H, *ddd*, $J = 2.0$, 5.0 and 8.8 Hz, C-4′ and C-5′), 1.20 (3H, *d*, $J = 6.6$ Hz, C-21), 0.88 (1H, *d*, $J = 6.6$ Hz, C-24), 0.84 (3H, *d*, $J = 6,2$ Hz, C-27), 0.83 (3H, *d*, $J = 5.3$ Hz, C-26), 0.80 (3H, *t*, $J = 6.7$, C-29), 0.79 (3H, *s*, C-19), 0.66 (3H, *s*, C-18); ^{13}C NMR (pyridine, 50.3 MHz) δ 139.62 (C-8), 138.71 (C-22), 129.60 (C-23), 117.92 (C-7), 102.20 (C-1′), 78.62 (C-

5′), 78.60 (C-3), 77.13 (C-3′), 75.41 (C-2′), 71.80 (C-4′), 62.91 (C-6′), 56.01 (C-17), 55.32 (C-14), 51.53 (C-24), 49.60 (C-9), 43.51 (C-13), 41.21 (C-20), 40.21 (C-5), 39.62 (C-12), 37.10 (C-1), 34.70 (C-4), 34.50 (C-10), 32.22 (C-25), 29.41 (C-6), 29.03 (C-2), 29.00 (C-16), 25.32 (C-24′), 23.42 (C-11), 23.41 (C-15), 21.70 (C-21), 21.40 (C-26), 19.20 (C-27), 13.12 (C-19), 12.60 (C-18), 12.33 (C-24″).

Compound **2**. Mp 168–169°; IR ν^{KBr} cm^{-1}: 3400 (OH), 2923 and 2850 (aliphatic C-H), 1740 (—C═O of ester), 1180–1020 (—C—O), and 978 (═C—H); ^1H NMR (200 MHz, $CDCl_3$): 5.18 (1H, *dd*, $J = 14.5$ and 8.1, C-22 or C-23), 5.15 (1H, *sl*, C-7), 5.01 (1H, *dd*, $J = 15.5$ and 8.1 Hz, C-22 or C-23), 4.43 (1H, *dd*, $J = 12.0$ and 5.2 Hz, C-6′a), 4.40 (1H, *d*, $J = 8.0$ Hz, C-1′), 4.30 (1H, *dd*, $J = 12.0$ and 2.5 Hz, C-6′b), 3.70 (1H, *m*, C-5′), 3.31 (3H, *m*, C-2′, C-3′ and C-4′), 2.34 (2H, *t*, $J = 7.7$ Hz, C-2″), 1.26 [Very intense, brs, $(—CH_2)_n$], 0.90 (3H, *t*, $J = 6.0$ Hz, terminal Me), 1. 03 (3H, *d*, $J = 6.6$ Hz, C-21), 0.86 (3H, *d*, $J = 7$,0 Hz, C-26), 0.84 (3H, *d*, $J = 6.2$ Hz, C-27), 0.80 (3H, *t*, $J = 7.6$ Hz, C-24″), 0.78 (3H, *s*, C-19), 0.55 (3H, *s*, C-18); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 174.70 (C-1″), 138.50 (C-8), 138.10 (C-22), 129.40 (C-23), 117.30 (C-7), 101.10 (C-1′), 78.90 (C-3), 76.40 (C-3′), 73.90 (C-2′), 73.50 (C-5′), 70.10 (C-4′), 63.20 (C-6′), 55.90 (C-17), 55.10 (C-14), 51.20 (C-24), 49.30 (C-9), 43.20 (C-13), 40.90 (C-20), 40.20 (C-5), 39.40 (C-12), 37.10 (C-1), 34.23 (C-10), 34.21 (C-2″), 34.20 (C-4), 31.90 (C-25), 29.40 (C-6), 29.40 (C-2), 28.90 (C-16), 24.90/29.70 (C-3″/4″), 25.40 (C-24′), 23.00 (C-15), 22.70 (C-15″, C-11), 21.40 (C-26), 21.10 (C-21), 19.00 (C-27), 14.10 (C-16″), 13.00 (C-19), 12.30 (C-24″), 12.20 (C-18).

Methyl palmitate. GC-MS 70 eV, *m*/*z* (% rel. Int.): 270 $[M]^+$ ($C_{17}H_{34}O_2$) (6), 239 (2), 227 (5), 199 (3), 185 (3), 171 (4), 143 (18), 129(5), 87 (63), 74 (100), 43 (60), 41 (50). Identical to reference compound.

Compound **3**. Mp 168–169°; IR ν^{KBr} cm^{-1}: 3400 (OH), 2923 and 2850 (aliphatic C-H), 1740 (—C = O of ester), 1180–1020 (—C—O), and 978 (═C—H); ^1H NMR (200 MHz, $CDCl_3$): 5.18 (1H, *dd*, $J = 14.5$ and 8.1, C-22 or C-23), 5.15 (1H, *sl*, C-7), 5.01 (1H, *dd*, $J = 15.5$ and 8.1 Hz, C-22 or C-23), 4.43 (1H, *dd*, $J = 12.0$ and 5.2 Hz, C-6′a), 4.40 (1H, *d*, $J = 8.0$ Hz, C-1′), 4.30 (1H, *dd*, $J = 12.0$ and 2.5 Hz, C-6′b), 3.70 (1H, *m*, C-5′), 3.31 (3H, *m*, C-2′, C-3′ and C-4′), 2.34 (2H, *t*, $J = 7.7$ Hz, C-2″), 1.26 [Very intense, brs, $(—CH_2)_n$], 0.90 (3H, *t*, $J = 6.0$ Hz, terminal Me), 1. 03 (3H, *d*, $J = 6.6$ Hz, C-21), 0.86 (3H, *d*, $J = 7$,0 Hz, C-26), 0.84 (3H, *d*, $J = 6.2$ Hz, C-27), 0.80 (3H, *t*, $J = 7.6$ Hz, C-24″), 0.78 (3H, *s*, C-19), 0.55 (3H, *s*, C-18); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 174.70 (C-1″), 138.50 (C-8), 138.10 (C-22), 129.40 (C-23), 117.30 (C-7), 101.10 (C-1′), 78.90 (C-3), 76.40 (C-3′), 73.90 (C-2′), 73.50 (C-5′), 70.10 (C-4′), 63.20 (C-6′), 55.90 (C-17), 55.10 (C-14), 51.20 (C-24), 49.30 (C-9), 43.20 (C-13), 40.90 (C-20), 40.20 (C-5), 39.40 (C-12), 37.10 (C-1), 34.23 (C-10), 34.21 (C-2″), 34.20 (C-4), 31.90 (C-25), 29.40 (C-6), 29.40 (C-2), 28.90 (C-16), 24.90/29.70 (C-3″/4″), 25.40 (C-24′), 23.00 (C-15), 22.70 (C-15″, C-11), 21.40 (C-26), 21.10 (C-21), 19.00 (C-27), 14.10 (C-16″), 13.00 (C-19), 12.30 (C-24″), 12.20 (C-18).

Methyl stearate. GC-MS 70 eV, *m*/*z* (% rel. Int.): 298$[M]^+$ ($C_{19}H_{38}O_2$) (6), 255 (6), 199 (7), 185 (2), 143 (20), 129 (5), 87 (63), 74 (100), 43 (80), 41 (55). Identical to reference compound.

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REFERENCES

1. Weisner, K., MacDonald, D.M., Volante, Z. and Armstrong, R., *Can. J. Chem*., 1993, **30**, 761.
2. Leonard, B.E. and Sherratt, H. S. A., *Nature*, 1961, **15**, 287.
3. Varshney, I. P. and Vyas, P., *Indian J. Chem*., 1976, **14B**, 814.
4. Varshney, I.P. and Vyas, P., *Indian J. Chem*., 1977, **54**, 992.
5. Varshney, I.P. and Vyas, P., *Indian J. Chem*., 1978, **16B**, 166.
6. Nigam, S.K., Gupta, R.K. and Mitra, C.R., *J. Pharm. Sci*., 1963, **52** (6), 459.
7. Nigam, S.K., Gupta, R.K. and Mitra, C.R., *Indian J. Chem*., 1966, **5**(8), 395.
8. Nigam, S.K., Gupta, R.K. and Mitra, C.R., *Planta Med*., 1970, **18**, 44.
9. Lee M.,-W., Morimoto, S., Nonaka, G.I. and Nishioka,I., *Phytochemistry*, 1992, **31**(6), 2117.
10. Altman, R.I.A., *Bol. Tec. Inst. Agron. Norte* (Belém, Brazil), 1956, **31**(3), *Chem. Abstr*., 1958, **52**, 506.
11. Braz Filho, R., Gottlieb, H.E., Mourão, A.P. and Miranda, C.A.S., *An. Acad. Brasil. Cienc*., 1986, **58**(3), 363.
12. Garg, V.K. and Nes, W.R., *Phytochemistry*, 1984, **23**(12), 2919.
13. Garg, V.K. and Nes, W.R., *Phytochemistry*, 1984, **23**(12), 2925.
14. Itoh,, T., Sica, D. and Djerassi, C., *J. Chem. Soc., Perkin Trans*., 1983, 147.
15. Itoh, T., Kikuchi, Y., Tamura, T. and Masumoto, T., *Phytochemistry*, 1981, **20**(4), 761.
16. Itoh, T., Yoshida, K. Tamura, T. and Matsumoto, T., *Phytochemistry*, 1982, **21**(3), 727.
17. Adler, J. H. and Salt, T. A., *Lipids*, 1983, **18**(3), 229.
18. Akihisa, T., Kokke, W.C.M. C., Tamura, T. and Matsumoto, T., *Lipids*, 1991, **26**(8), 660.
19. Wright, J.L.C., McInnes, A.G., Shimizer, S., Smith, D.G., Walter, J.A., Idler, D. and Khalil, W., *Can. J. Chem*., 1978, **56**, 1898.
20. Goad, L.J., Lenton, J.R., Knapp, F.F. and Goodwin, T. W., *Lipids*, 1974, **9**, 582.
21. Tori, K., Seo, S., Yoshimura, Y., Arita, H. and Tomita, Y., *Tetrahedrom Lett*., 1977, **2,** 179.
22. Chaurasia, N. and Wichtt, M., *J. Nat. Prod*., 1987, **50**(5), 881.
23. Guevara, A.P., Lim-Sylianco, C.Y., Dayrit, F.M. and Frinch, P., *Phytochemistry*, 1989, **28**(6), 1721.