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SPATHODOL, A NEW POLYHYDROXYSTEROL FROM THE LEAVES OF *SPATHODEA CAMPANULATA*

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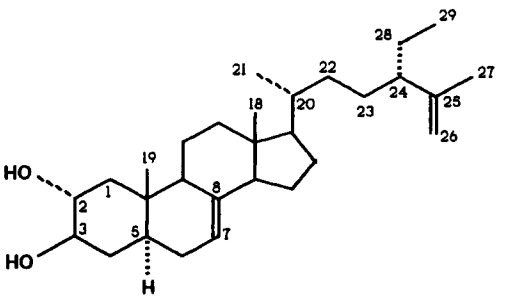
ABSTRACT.—Spathodol [**1**], a new dihydroxylated sterol, has been isolated from the leaves of *Spathodea campanulata*, along with the previously reported triterpenoids 3β-acetoxyoleanolic acid, siaresinolic acid, 3β-acetoxy-12-hydroxyoleanan-28,13-olide, oleanolic acid, and β-sitosterol-3-*O*-β-D-glucopyranoside. The structure of the new natural polyol was elucidated by spectroscopic methods.

Spathodea campanulata P. Beauvais (Bignoniaceae), called the African tulip tree, is widely distributed through Africa and found abundantly in Cameroon (1,2). It is used in traditional herbal medicine (2) for the treatment of ulcers, filaria, gonorrhea, diarrhea, and fever. More recently, the antimalarial properties of the bark and the leaves have been reported (3–5). We reported in our previous studies the isolation of sterols and triterpenoids from the bark (6,7). In this paper we report the results of an investigation of the dried leaves of *S. campanulata* and the isolation of a new dihydroxylated sterol, spathodol [**1**]. The structure of the new compound was determined to be (24*S*)-5α-stigmasta-7,25-diene-2α,3β-diol by spectroscopic methods.

RESULTS AND DISCUSSION

Si gel cc of the combined hexane/EtOAc extract of the dried leaves afforded several fractions. These fractions after repeated chromatography provided β-sitosterol, the new sterol **1**, 3β-acetoxyoleanolic acid, siaresinolic acid, 3β-acetoxy-12-hydroxyoleanan-28,13-olide, oleanolic acid, and β-sitosterol-3-*O*-β-D-glucopyranoside. The known compounds were identified by spectroscopic studies and comparison with authentic samples.

The new dihydroxylated sterol **1**, obtained as a powder, gave a positive Liebermann-Burchard reaction, indicative of a steroid skeleton. The elemental analysis and the eims, which contained the molecular ion peak at *m/z* 428, indicated a molecular formula of $C_{29}H_{48}O_2$ and two degrees of unsaturation in the nucleus and/or the side chain. The significant fragment ions at *m/z* 289 $[M-C_{10}H_{19}]^+$ (side chain), 287 $[M-(\text{side chain}+2H)]^+$ indicated the presence of a $C_{10}H_{19}$ side chain containing one double bond (8). The peak at *m/z*



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262 corresponding to loss of the side chain and part of ring D has been used to assign a Δ^7 unsaturation (9) in the sterol **1**. The large intensity of the peak at *m/z* 287 (73%), which indicated a facile loss of the side chain, is good evidence for a Δ^7 unsaturation (8) in **1**. Furthermore, the 344 $[M-C_6H_{12}]^+$ and 330 $[M-C_7H_{14}]^+$ peaks of sterol **1** are typical McLafferty rearrangements, diagnostic for Δ^{25} double bonds (8).

The ir spectrum of compound **1** showed bands for hydroxyl(s) (3600, 3400 cm^{-1}) and double bond(s) (1640 cm^{-1}) with a terminal methylene (880 cm^{-1}).

The ^{13}C-nmr data (Table 1), including DEPT experiments, disclosed the presence of five methyls, eleven methylenes, nine methines, and four quaternary carbons, thus accounting for all the 29 carbon atoms of **1**. The two methine signals at δ 72.6 and 76.0 indicated the presence of two sites of heteroatom functionality. The ^1H-nmr spectrum (Table 2) displayed a broad singlet at δ 5.16, characteristic of a Δ^7 vinyl proton (10,11). The signals at δ 0.52 (3H, s) and 0.83 (3H, s) assigned to the C-18 and C-19 methyl protons, respectively, confirmed the Δ^7 unsaturation (11). Resonances at δ 4.73 (1H, br

TABLE 1. ^{13}C nmr (50.3 MHz) of Compound **1** ($CDCl_3$/TMS).

Carbon	Compound		
	1	Reference Steroid[a]	Reference Steroid[b]
C-1	45.0		45.1
C-2	72.6		73.0
C-3	76.0		76.4
C-4	35.3		35.6
C-5	40.1	40.1	
C-6	29.0	29.5	
C-7	117.4	117.3	
C-8	139.5	139.5	
C-9	49.4	49.3	
C-10	36.3	34.2	
C-11	21.6	21.4	
C-12	39.4	39.5	
C-13	43.3	43.3	
C-14	54.4	55.0	
C-15	22.9	23.0	
C-16	27.9	27.9	
C-17	56.0	56.0	
C-18	12.1	12.1	
C-19	14.1	13.0	
C-20	35.9	36.0	
C-21	18.7	18.8	
C-22	33.6	33.6	
C-23	29.4	29.5	
C-24	49.5	49.5	
C-25	147.5	147.4	
C-26	111.4	111.4	
C-27	17.7	17.7	
C-28	26.5	26.5	
C-29	18.8	18.8	
MeCO	—	21.5	
Me*CO*	—	170.7	

[a](24*S*)-5α-Stigmasta-7,25-dien-3β-yl acetate (14).
[b]5α-Spirostane-2α,3β-diol (13).

TABLE 2. ^{1}H nmr (300 MHz) of Compound **1** ($CDCl_3$/TMS).

Proton	Compound		
	1	Reference steroid[a]	Reference steroid[b]
H-1α	1.10 m		
H-1β	2.06 dd (4.4, 12)		
H-2α	3.56 ddd (4.4, 9.1, 11.5)		
H-3β	3.40 ddd (4.1, 9.2, 10.8)		
H-4	1.80 m		
H-7	5.16 br s		5.15 m
H-18	0.52 s		0.54 s
H-19	0.83 s		0.82 s
H-21	0.91 d (6.5)	0.90 d (6.4)	
H-26	4.64 br s	4.64 br s	
H'-26	4.73 br s	4.72 br s	
H-27	1.57 s	1.57 s	
H-29	0.80 t (7.4)	0.80 t (7.4)	

[a]Clerosterol side chain (10).
[b]Steryl acetates (9).

s) and 4.64 (1H, br s) allowed the terminal methylene group to be placed at C-25 in the side chain. The doublet at δ 0.91 (3H, d, J = 6.5 Hz) was identified as the C-21 methyl group (10). The resonances at δ 1.57 (3H, s) and 0.80 (3H, t, J = 7.4 Hz), characteristic of a vinyl methyl group and a methyl group attached to a Csp^3 methylene, were assigned, respectively, to the C-27 protons and to the three protons at C-29 of the side chain 24-ethyl group. The good concordance in the ^{1}H-nmr shifts (Table 2) of the side chain in both clerosterol (12) and spathodol [**1**] allowed the assignment of the 24*S*/24-β configuration (10,13,14) at the C-24 carbon. The signals at δ 3.56 (1H, ddd, J = 4.4, 9.1, 11.5 Hz, H-2) and 3.40 (1H, ddd, J = 4.1, 9.2, 10.8 Hz, H-3) were assigned to two axial hydroxymethine protons of a 2,3 hydroxysterol on the basis of their multiplicity and their mutual coupling (J ca. 9 Hz).

The relative stereochemistry of the hydroxyl groups was further confirmed by spin decoupling experiments. Irradiation at δ 3.56 (H-2) simplified the H-3 signal at δ 3.40 (J = 4.1, 9.2, 10.8 Hz) to a doublet of doublets (J = 4 and 11 Hz) and the H-1β doublet of doublets at δ 2.06 (J = 4.2 and 12.0 Hz) to a doublet (J = 12.0 Hz). Likewise, irradiation of the H-1β signal at δ 2.06 (1H, dd, J = 4.4, 12.0 Hz) simplified the H-2 signal at δ 3.56 to a doublet of doublets (J = 9.0 and 11.0 Hz). Furthermore, nOe difference spectra indicated signal enhancements of H-2 by ca. 13% and 4% on irradiation of H-19 protons at δ 0.80 and H-1β at δ 2.06, respectively. The complete assignment of signals arising from the ring system and side-chain carbons was made by comparison with those of the literature data for 5α-spirostane-2α,3β-diol (C-1 to C-4) (15) and (24*S*)-5α-stigmasta-7,25-dien-3β-yl acetate (C-5 to C-29) (16).

Thus, the structure of spathodol [**1**] is (24*S*)-5α-stigmasta-7,25-diene-2α,3β-diol.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra were recorded at 300 MHz for the ^{1}H nmr and 50.3 MHz for the ^{13}C nmr. Chemical shifts are given as the δ (ppm) scale with TMS as internal standard. Mass spectra were obtained on a Nermag 10-10-C at 70 eV.

PLANT MATERIAL.—The leaves of *S. campanulata* were obtained in September 1987 from Dschang, in the West Province, Cameroon. A

voucher specimen (55489 HNC) is deposited in the National Herbarium, Yaoundé.

EXTRACTION.—Dried and finely powdered plant material (4 kg) was successively extracted with hexane and EtOAc at room temperature. After filtration and concentration of the solvent, the two extracts were combined on the basis of tlc to yield 60 g of crude extract.

ISOLATION OF TRITERPENOIDS AND STEROLS.—The crude extract was chromatographed on a column over Si gel using an *n*-hexane/EtOAc gradient elution system. Fractions of 100 ml were collected. The low polarity fractions 1–10 containing waxes and fats were discarded. β-Sitosterol, identified by standard sample comparison (mmp, ir, ^{1}H nmr, tlc) was the main component in fractions 11–43. Fractions 47–59 afforded a residue that was recrystallized in the hexane/EtOAc mixture to give compound **1** (80 mg).

(24S)-5α-Stigmasta-7,25-diene-2α,3β-diol [**1**].—Powder: mp 224–225°; ir ν max cm^{-1} (KBr) 3600, 3400, 1640, 880; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; eims *m/z* (rel. int. %) [M]$^+$ 428 (21), 413 (21), 344 (1), 330 (7), 289 (7), 288 (16), 287 (73), 271 (8), 262 (8), 253 (6), 248 (3), 159 (10), 145 (11), 133 (10), 119 (15), 107 (12), 105 (22), 97 (13), 91 (19), 84 (16), 83 (28), 81 (21), 79 (15), 69 (51), 67 (19), 54 (14), 56 (16), 55 (100), 43 (43), 41 (48). Calcd for $C_{29}H_{48}O_2$, C 81.10, H 10.92; found C 81.24, H 11.28.

KNOWN COMPOUNDS.—The more polar fractions 60–70, containing triterpenoids, were rechromatographed on Si gel. Known compounds, 3β-acetoxyoleanolic acid, siaresinolic acid, 3β-acetoxy-12-hydroxyoleanan-28,13-olide, oleanolic acid, and β-sitosterol-3-*O*-β-D-glucopyranoside, isolated and purified through repeated chromatographic separations, were identified by comparison of their physical and spectroscopic data with those of the literature (17–19) or authentic samples.

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