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24,24-DIMETHYL-5α-CHOLESTAN-3β-OL, A STEROL FROM *GYNOSTEMMA PENTAPHYLLUM*

TOSHIHIRO AKIHISA, HIDEKI MIHARA, TOMOYUKI FUJIKAWA, TOSHITAKE TAMURA and TARO MATSUMOTO

College of Science and Technology, Nihon University, 1–8, Kanda Surugadai, Chiyoda-ku, Tokyo, 101 Japan

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Key Word Index—*Gynostemma pentaphyllum*; Cucurbitaceae; sterol; 24, 24-dimethyl-5α-cholestan-3β-ol; 24α-ethyl-5α-cholestan-3β-ol.

Abstract—A new sterol isolated from the aerial parts of *Gynostemma pentaphyllum* has been shown to be 24,24-dimethyl-5α-cholestan-3β-ol based on the spectroscopic and chemical correlation with 24,24-dimethyl-5α-cholest-7-en-3β-ol. The other sterol isolated was identified as 24α-ethyl-5α-cholestan-3β-ol.

INTRODUCTION

Gynostemma pentaphyllum (Japanese name, Amachazuru) has been shown by our recent studies to contain several uncommon sterols including three 24,24-dimethylsterols: 24,24-dimethyl-5α-cholest-7-en-3β-ol (**2a**), 24,24-dimethyl-5α-cholesta-7,22*E*-dien-3β-ol and 24,24-dimethyl-5α-cholesta-7,25-dien-3β-ol [1]; and 24-methylene-14α-methyl-5α-cholest-9(11)-en-3β-ol [2], in addition to major 24β/*R*-ethyl-5α-cholesta-7,22*E*-dien-3β-ol (chondrillasterol) and other compounds [3–5] as the sterol components. Our continuing study on the sterol constituents of *G. pentaphyllum* has led to the isolation and characterization of a further 24, 24-dimethylsterol, 24,24-dimethyl-5α-cholestan-3β-ol (**1a**), besides 24α/*R*-ethyl-5α-cholestan-3β-ol (**1b**).

RESULTS AND DISCUSSION

The two sterols **1a** and **1b** were isolated as the acetyl derivatives from *G. pentaphyllum* by virtue of the procedure described in the Experimental section. The mass spectrum of **1a**-acetate showed $[M]^+$ at *m/z* 458, corresponding to $C_{31}H_{54}O_2$, accompanied by fragmentation ions at *m/z* 443 $[M-Me]^+$, 398 $[M-HOAc]^+$, 383 $[M-Me-HOAc]^+$ and 257 $[M-C_{10}H_{21}$ (side chain) $-HOAc]^+$ indicating that it was an acetate of a saturated C_{29}-sterol. Other prominent ions at *m/z* 415 $[M-C_3H_7]^+$ and 355 $[M-C_3H_7-HOAc]^+$, which might be correlated with a loss of the terminal isopropyl group (C_{25}–C_{27}), suggested the presence of a 24,24-dimethyl group in the side chain [1]. The ^1H NMR spectrum of **1a**-acetate showed signals due to the side chain methyl protons at δ 0.757 (6H, *s*, 28-H_3, 29-H_3), 0.799 and 0.803 (each 3H, *d* and *J* = 7.1 Hz, 26-H_3, 27-H_3), and 0.896 (3H, *d*, *J* = 6.6 Hz, 21-H_3), in addition to those arising from the conventional 3β-acetoxy 5α-saturated sterol skeleton [6] (Table 1). The ^1H NMR spectral signals for the side chain protons were almost consistent with those of 24, 24-dimethyl-5α-cholest-7-en-3β-ol (**2a**) acetate [1] (Table 1), and hence, **1a** was considered to have the structure 24, 24-dimethyl-5α-cholestan-3β-ol. This was confirmed by comparison with authentic **1a**-acetate synthesized from **2a**-acetate. **2a**-Acetate afforded its $\Delta^{8(14)}$-isomer, 24,24-dimethyl-5α-cholest-8(14)-en-3β-ol (**3a**) acetate, under the hydrogenation condition over pre-reduced PtO_2 in acetic acid at atmospheric pressure and temp. for 16 hr [7], which was then isomerized to the Δ^{14}-isomer, 24,24-dimethyl-5α-cholest-14-en-3β-ol (**4a**) acetate, by treating with dried hydrogen chloride gas in chloroform solution at 0° for two hr. Hydrogenation of **4a**-acetate over pre-reduced PtO_2 in ethanol at atmospheric pressure and temp. for 24 hr yielded **1a**-acetate. The mp, chromatographic and spectroscopic data of the synthetic **1a**-acetate were consistent with those of the naturally occurring one (see Tables 1 and 2). The other sterol, **1b**-acetate, was identified as 24α-ethyl-5α-cholestan-3β-yl acetate by comparison with the authentic compound.

This study has thus demonstrated the occurrence of the fourth 24, 24-dimethylsterol, **1a**, which is considered to be a new sterol, in *G. pentaphyllum*. It might be worthy to note here that all of the 24, 24-dimethylsterols described in this, and in our previous paper [1], afforded a characteristic fragmentation ion in the mass spectrum of $[M-C_3H_7]^+$, which was considered to be formed by a loss of the terminal isopropyl group (C-25 to C-27) of the side chain from $[M]^+$. This was accompanied by the ion of $[M-C_3H_7-HOAc]^+$. This fragmentation ion is known also to occur by a loss of the side chain isopropyl

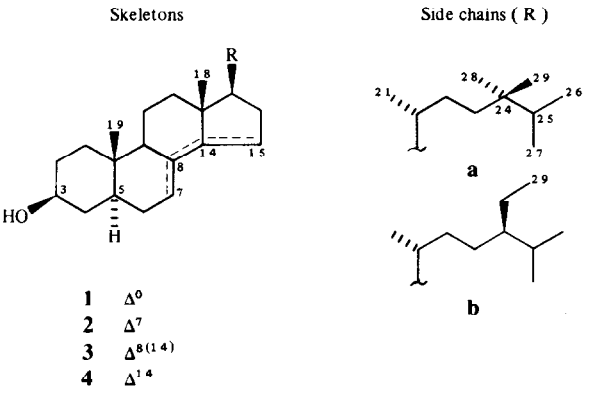

Table 1. ^{1}H NMR spectral data of the acetyl derivatives of some sterols (400 MHz, $CDCl_3$, TMS as int. standard)*

Protons	Acetate					
	1a†	**1a**	**2a**†‡	**3a**	**4a**	**1b**†
18-H_3 (*s*)	0.645	0.643	0.529	0.837	0.896	0.647
19-H_3 (*s*)	0.816	0.815	0.808	0.704	0.837	0.816
21-H_3 (*d*)	0.896 (6.6)	0.897 (6.6)	0.917 (5.8)	0.928 (6.6)	0.910 (6.6)	0.904 (6.6)
26-H_3 (*d*)	0.799 (7.1)	0.799 (7.3)	0.803 (6.7)	0.804 (6.6)	0.805 (6.6)	0.831 (6.6)
27-H_3 (*d*)	0.803 (7.1)	0.802 (6.7)			0.809 (6.6)	0.809 (6.6)
28-H_3 (*s*)	0.757	0.752	0.764	0.764	0.770	
29-H_3 (*s*)						0.842 (*t*, 7.4)
3β-OAc (*s*)	2.019	2.018	2.026	2.021	2.021	2.017
3α-H (*tt*)	4.685 (11.5, 6.6)	4.688 (11.5, 6.6)	4.694 (*m*, 25)	4.716 (*m*, 25)	4.686 (11.5, 6.6)	4.683 (11.3, 4.9)
7-H (*m*)	—	—	5.142 (13)	—	—	—
15-H (*s*)	—	—	—	—	5.150	—

*Chemical shifts given in δ values. Figures in parentheses denote J values (Hz) as for doublet and triplet signals, and $W_{1/2}$ values (Hz) for multiplet signals.

†Natural sterols isolated from *Gynosotemma pentaphyllum*. Others were synthetic sterols.

‡Determined at 250 MHz (cf. ref. [1]).

Table 2. Mp and chromatographic data of some sterols

Sterol	Acetate			
	Mp (°)	RR_t* GC	RR_t* HPLC	R_c*
24,24-Dimethyl-5α-cholestan-3β-ol (**1a**)†	148.5–149.0	1.74	1.61	1.20
24,24-Dimethyl-5α-cholestan-3β-ol (**1a**)	146.5–148.0	1.74	1.61	1.20
24,24-Dimethyl-5α-cholest-7-en-3β-ol (**2a**)†‡	180.0–183.0	2.02	1.30	0.94
24, 24-Dimethyl-5α-cholest-8(14)-en-3β-ol (**3a**)	122.5–123.0	1.77	1.17	1.18
24, 24-Dimethyl-5α-cholest-14-en-3β-ol (**4a**)	146.0–146.5	1.72	1.13	0.81
24α-Ethyl-5α-cholestan-3β-ol (**1b**)†	130.8–131.2	1.64	1.50	1.20

*RR_t on GC and HPLC, and Rc-values on argentation TLC were expressed relative to cholesterol acetate.

†Natural sterols isolated from *Gynostemma pentaphyllum*. Others were synthetic sterols.

‡Cf. ref [1].

group (C-25 to C-27) due to allylic cleavage in the mass spectrum of 24-methyl- and 24-ethyl-Δ^{22}-sterols [8,9]. The other noteworthy finding in the mass spectra of 24, 24-dimethylsterols was that **1a**—acetate gave a prominent ion at m/z 315 ($C_{21}H_{31}O_2$) which might correspond to $[M - \text{side chain} - 2H]^+$ and this has so far been regarded as a characteristic ion for the sterols possessing an unsaturated side chain [10].

EXPERIMENTAL

Mp: uncorr. Argentation TLC: silica gel–$AgNO_3$ (4:1) developed three times with CCl_4–CH_2Cl_2 (5:1); HPLC: Ultrasphere ODS 5μ column (Altex; 25 cm x 10 mm i.d.), MeOH as mobile phase (flow rate, 4 ml/min), RI detector; GC: OV-17 SCOT glass capillary column (30 m × 0.3 mm i.d.), column temp. 255°. R_c on argentation TLC, and RR_t on HPLC and GC expressed relative to cholesteryl acetate. EIMS (70 eV): probe. ^{1}H NMR: 400 MHz, $CDCl_3$, TMS as int. standard. Acetylation: Ac_2O–pyridine at room temp. overnight. Sterols **1b** [5] and **2a** [1] were used as the reference compounds, **2a** was used as the starting material for the synthesis of **1a**. The dried aerial parts of *G. pentaphyllum* were purchased from Kinokuniya Kan-Yaku Kyoku Co. (Tokyo). For the mp and chromatographic data of the sterols described in this paper, see Table 2, and the ^{1}H NMR data, see Table 1.

Isolation of new sterol. Dried aerial parts (20 kg; leaves and stems) of *G. Pentaphyllum* were extracted with CH_2Cl_2 under reflux for 7 hr to give 580 g of lipid which was saponified (5% KOH in MeOH) under reflux and then the unsaponifiable lipid (107 g) was extracted with isopropyl ether. Column chromatography on silica gel (700 g) (hexane, hexane–Et_2O,

hexane–EtOAc and then MeOH as eluants) gave the sterol mixture (31 g). (The elution was monitored by TLC on precoated silica gel). The sterol mixture was acetylated, and the resulting acetate fraction (30 g) was crystallized three times from Me_2CO–MeOH which gave the 1st (13.8 g), 2nd (2.9 g) and 3rd (1.6 g) crystallized portions and a brown pasty filtrate portion (8.9 g). The 3rd crystallized portion was subjected to argentation TLC to give eight bands. The fraction (26 mg) recovered from the least polar band was then subjected to repetitive reverse-phase HPLC yielding the acetates of **1a** (1 mg) and **1b** (1 mg).

24, 24-*Dimethyl-5α-cholestan-3β-ol* (**1a**) *acetate*. MS: *m/z* 458.4137 (M^+, $C_{31}H_{54}O_2$, rel. int. 56% requires 458.4121), 443.3867 ($C_{30}H_{51}O_2$, 5%), 415.3608 ($C_{28}H_{47}O_2$, 6%), 398.3883 ($C_{29}H_{50}$, 47%), 383.3627 ($C_{28}H_{47}$, 23%), 355.3402 ($C_{26}H_{43}$, 40%), 315.2324 ($C_{21}H_{31}O_2$, 18%), 290.2957 ($C_{21}H_{38}$) and 290.2225 ($C_{19}H_{30}O_2$, 19%), 276.2047 ($C_{18}H_{28}O_2$, 45%), 275.1988 ($C_{18}H_{27}O_2$, 33%), 257.2290 ($C_{19}H_{29}$, 15%), 243.2110 ($C_{18}H_{27}$, 13%), 230.2042 ($C_{17}H_{26}$, 31%), 215.1819 ($C_{16}H_{23}$, 100%).

1a-*Acetate synthesized from* **2a**-*acetate*. MS *m/z* (rel. int.): 458 $[M]^+$ (44), 443 (6), 415 (15), 398 (45), 383 (24), 355 (37), 315 (32), 290 (11), 276 (33), 275 (28), 257 (20), 243 (16), 230 (28), 215 (100).

24, 24-*Dimethyl-5α-cholest-8(14)-en-3β-ol* (**3a**) *acetate*. MS *m/z* (rel. int.): 456 $[M]^+$ (100), 441 (18), 413 (5), 398 (4), 396 (2), 381 (4), 355 (3), 315 (12), 288 (4), 276 (4), 274 (2), 255 (14), 229 (21), 213 (16).

24, 24-*Dimethyl-5α-cholest-14-en-3β-ol* (**4a**) *acetate*. MS *m/z* (rel. int.): 456 $[M]^+$ (6), 441 (5), 413 (9), 381 (2), 353 (3), 315 (100), 255 (64), 241 (2), 228 (1), 213 (2).

24α-*Ethyl-5α-cholestan-3β-ol* (**1b**) *acetate*. MS *m/z* (rel. int.): 458 $[M]^+$ (56), 443 (4), 398 (47), 383 (21), 344 (3), 290 (13), 276 (37), 257 (8), 230 (31), 215 (100).

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