Phytochemistry, Vol. 26, No. 8, pp. 2412–2413, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

14α-METHYL-5α-ERGOSTA-9(11),24(28)-DIEN-3β-OL, A STEROL FROM *GYNOSTEMMA PENTAPHYLLUM*

TOSHIHIRO AKIHISA, TOSHITAKE TAMURA and TARO MATSUMOTO

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

(*Received* 14 *November* 1986)

Key Word Index—*Gynostemma pentaphyllum*; Cucurbitaceae; sterol; 14α-methyl-5α-ergosta-9(11),24(28)-dien-3β-ol.

Abstract—A new sterol isolated from the aerial parts of *Gynostemma pentaphyllum* has been shown to be 14α-methyl-5α-ergosta-9(11),24(28)-dien-3β-ol.

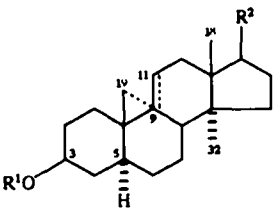
INTRODUCTION

Gynostemma pentaphyllum Makino (Japanese name, Amachazuru) is known to contain various kinds of dammarane saponins [1, and references cited therein]. We have recently shown that *G. pentaphyllum* contains (22*E*, 24*R*)-5α-stigmasta-7,22-dien-3β-ol (chondrillasterol), along with its (24*S*)-epimer (spinasterol), as the most predominant sterol component [2] accompanied with three new 24,24-dimethylsterols, 24,24-dimethyl-5α-cholest-7-en-3β-ol, (22*E*)-24,24-dimethyl-5α-cholesta-7,22-dien-3β-ol and 24,24-dimethyl-5α-cholesta-7,25-dien-3β-ol [3]. This paper describes a further study on the sterol constituents of the aerial parts of the plant, which led to the isolation and characterization of a new 14α-methylsterol, 14α-methyl-5α-ergosta-9(11),24(28)-dien-3β-ol [or 24-methylene-14α-methyl-5α-cholest-9(11)-en-3β-ol] (**1a**).

RESULTS AND DISCUSSION

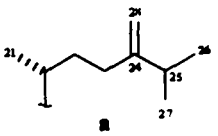
The new sterol (**1a**) by virtue of the purification procedure employed was isolated, and subsequently characterised as its monoacetate **2a**. The mass spectrum of **2a** showed $[M]^+$ at *m/z* 454, corresponding to $C_{31}H_{50}O_2$, accompanied with fragmentation ions at *m/z* 439 $[M - Me]^+$, 379 $[M - HOAc - Me]^+$ and 327 $[M - C_9H_{17}(\text{side chain}) - 2H]^+$ indicating that it was an acetate of a C_{29}-sterol with two double bonds, one of which was in the C_9 side chain and the other was in the skeleton [4, 5]. Other ions at *m/z* 287 [M – side chain – ring D]$^+$ and 273 (287 – CH_2) were characteristic of 14α-methylsterols [6, 7]. The presence of a further significant ion at *m/z* 370 $[M - C_6H_{12}]^+$ due to a McLafferty rearrangement involving cleavage of the C-22, C-23 bond with one H transfer from C-20, suggested that the side chain double bond was located at either the $\Delta^{24(25)}$- or the $\Delta^{24(28)}$-position [4, 5]. The side chain proton signals of **2a** in the ^{1}H NMR spectrum were observed at δ0.912 (3H, *d*, *J* = 6.4 Hz), 1.026 and 1.031 (each 3H and *d*, *J* = 6.4 Hz), 2.219 (1H, *sept.*, *J* = 7.0 Hz), 4.663 (1H, *d*, *J* = 1.5 Hz) and 4.715 (1H, *s*). The two olefinic signals indicated that the side chain double bond at C-24 might be oriented to C-24(28) as the terminal methylene group. 14α-Methyl-9β, 19-cyclo-5α-ergost-24(28)-en-3β-yl (24-methylenepollinastanyl) acetate (**3a**), a 24-methylene sterol, exhibited side chain signals almost identical with those of **2a** (Table 1) which supported the $\Delta^{24(28)}$ unsaturation. Steryl acetate **2a** showed the skeletal olefinic signal at δ5.29 (*m*, 11-H) and methyl signals at δ0.661 (3H, *s*, 18-H_3), 0.758 (3H, *s*, 32-H_3) and 0.981 (3H, *s*, 19-H_3), which were almost identical with the corresponding signals of authentic 14α-methyl-5α-ergost-9(11)-en-3β-yl acetate (**2b**, a mixture of C-24 epimers) (Table 1), and hence the 14α-methyl-$\Delta^{9(11)}$ skeletal structure was attributed for **2a**. Thus, the sterol was considered to have the structure 14α-methyl-5α-ergosta-9(11),24(28)-dien-3β-ol (**1a**). The proposed structure was supported from the hydrogenation of **2a** to give the dihydro derivative **2b** (*m/z* 456 $[M]^+$; *RRt* = 1.47 on GC) which was identified by comparison of its spectroscopic (MS and ^{1}H NMR) and GC data with those of authentic **2b**.

This study has, thus, demonstrated the occurrence of a 14α-methylsterol **1a**, which is considered to be a new sterol, in the aerial parts of *G. pentaphyllum* as the minor sterol constituent. It is noteworthy that another Cucurbitaceae, *Cucumis sativus* (cucumber), has recently

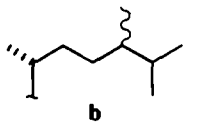


1 R^1 = H, $\Delta^{9(11)}$
2 R^1 = Ac, $\Delta^{9(11)}$
3 R^1 = Ac, 9β, 19-Cyclo

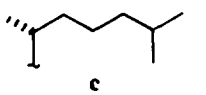
Side chain (R^2)



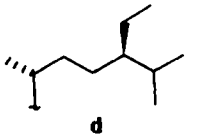
a



b



c



d

Table 1. ^{1}H NMR data of some 14α-methylsterols (400 MHz, $CDCl_3$, TMS as int. standard)

Sterol	18-H_3 (s)	19-H‡ (s)	32-H_3 (s)	21-H_3 (d)	26-H_3 (d)	27-H_3 (d)	28-H_2‡	3β-OAc (s)	3α-H (m)	11-H (m)	25-H (sept.)
2a	0.661	0.981	0.758	0.912 (6.4)*	1.026 (6.8) 1.031 (6.8)		4.663 (*d*, 1.5) 4.715 (*s*)	2.024	4.69 (23)	5.29 (7.8)	2.219 (7.0)
3a§	0.961	0.081 (*d*, 4.3) 0.442 (*d*, 4.0)	0.898	0.896 (6.4)	1.025 (6.7) 1.030 (6.7)		4.663 (*d*, 1.5) 4.714 (*s*)	2.026	4.80 (23)	—	2.237 (7.0)
(24*R*)				0.874 (7.0)	0.854 (6.8)	0.807 (6.8)					
2b¶	0.656	0.979	0.754				0.781 (*d*, 6.8)	2.024	4.68 (22)	5.29 (7.8)	—
(24*S*)				0.882 (6.4)	0.859 (6.8)	0.785 (6.8)					
(24*R*)				0.873 (6.4)	0.853 (7.0)	0.807 (6.7)					
2b§	0.656	0.978	0.753				0.780 (*d*, 6.4)	2.023	4.68 (22)	5.29 (7.8)	—
(24*S*)				0.881 (6.7)	0.858 (6.7)	0.785 (6.7)					

*Figures in parentheses denote *J* values (Hz) for doublet and septet signals, and $W_{\frac{1}{2}}$ values (Hz) for multiplet signals.
†Cyclo methylene signal as for **3a**.
‡Methyl doublet as for **2b**.
§Data for authentic sterols determined at 250 MHz (cf. ref. [8]).
¶Prepared from **2a** by hydrogenation.

been shown to contain the 24-ethyl homologue of **1a**, (24*R*)-14α-methyl-5α-stigmast-9(11)-en-3β-ol (**1d**) [8]. The other 14α-methyl-$\Delta^{9(11)}$-sterol so far known as the natural product is 14α-methyl-5α-cholest-9(11)-en-3β-ol (**1c**) detected in sea cucumbers [9, 10].

EXPERIMENTAL

Mp uncorr. Argentation TLC: silica gel–$AgNO_3$(4:1) developed three times with CCl_4–CH_2Cl_2 (5:1); HPLC: Partisil 5 ODS-2 column (Whatman; 25 cm × 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. 260°. *RR*, 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; Hydrogenation: EtOH over pre-reduced PtO_2 at atmos. pressure and temp. overnight. Two steryl acetates, **2b** and **3a**, were used as the reference specimens [8]. The dried aerial parts of *G. pentaphyllum* were purchased from Kinokuniya Kanyaku Kyoku Co. (Tokyo). For the ^{1}H NMR data of **2a**, **2b** and **3a**, see Table 1.

Isolation of new sterol. Dried aerial parts (10 kg; leaves and stems) of *G. pentaphyllum* were extracted with CH_2Cl_2 at room temp. for 1 week to give 242 g of lipid, which was saponified (5% KOH in MeOH) under reflux and then unsaponifiable lipids (50 g) extracted with isopropyl ether. CC of the unsaponiable lipid on silica gel (350 g) (hexane, hexene–Et_2O, hexane–EtOAc, and then MeOH as eluants) gave the sterol mixture (6.95 g). (The elution was monitored by TLC on precoated silica gel.) The sterol mixture was separated by further chromatography on silica gel (300 g) (hexane–EtOAc as eluant) into two fractions, one containing an appreciable amount of Δ^5-sterol in addition to Δ^7-sterol (fraction A, 3.5 g) and another consisting mainly of Δ^7-sterol (fraction B, 3.1 g) [2]. Fraction A was acetylated, and a portion of the acetate fraction A (1.1 g) subjected to argentation TLC to give eight bands. The fraction (61 mg) recovered from the fourth band from the solvent front (R_f 0.44) was subjected to reverse-phase HPLC yielding **2a** (2.6 mg; *RR*, 0.71 and 1.52 on HPLC and GC, respectively).

14α-Methyl-5α-ergosta-9(11),24(28)-dien-3β-yl acetate (**2a**). Mp. 95.0–96.5°. MS: *m/z* 454.3816 (M^+, $C_{31}H_{50}O_2$, rel. int. 9%, requires 454.3808), 439.3571 ($C_{30}H_{47}O_2$, 48%), 411.3289 ($C_{28}H_{43}O_2$, 17%), 379.3322 ($C_{28}H_{43}$, 14%), 370.2825 ($C_{25}H_{38}O_2$, 12%), 355.2584 ($C_{24}H_{35}O_2$, 8%), 327.2303 ($C_{22}H_{31}O_2$, 77%), 313.2133 ($C_{21}H_{29}O_2$, 15%), 287.2026 ($C_{19}H_{27}O_2$, 13%), 273.1846 ($C_{18}H_{25}O_2$, 11%), 261.1839 ($C_{17}H_{25}O_2$, 10%), 255.2095 ($C_{19}H_{27}$, 9%), 227.1819 ($C_{17}H_{23}$, 20%), 55.6567 (C_4H_7, 100%).

14α-Methyl-5α-ergost-9(11)-en-3β-yl acetate (**2b**; *C-24 epimeric mixture*) *prepared from* **2a** *by hydrogenation.* MS: *m/z* 456 (M^+, $C_{31}H_{52}O_2$, rel. int. 37%), 441 (86%), 396 (4%), 381 (23%), 329 (3%), 287 (6%), 273 (6%), 269 (7%), 261 (7%), 255 (5%), 227 (9%), 43 (100%).

Acknowledgements—We thank Drs. T. Takido and M. Aimi for NMR and mass spectra. Our thanks are also due to T. Fujikawa and T. Chikatsu for technical assistance.

REFERENCES

1. Takemoto, T., Arihara, S., Yoshikawa, K., Hino, K., Nakajima, Y. and Okuhira, M. (1984) *Yakugaku Zasshi* **104**, 1155.
2. Akihisa, T., Thakur, S., Rosenstein, F. U. and Matsumoto, T. (1986) *Lipids* **21**, 39.
3. Akihisa, T., Shimizu, N., Tamura, T. and Matsumoto, T. (1986) *Lipids* **21**, 515.
4. Knights, B. A. (1967) *J. Gas Chromatogr.* **5**, 273.
5. Massey, I. J. and Djerassi, C. (1979) *J. Org. Chem.* **44**, 2448.
6. Doyle, P. J., Patterson, G. W., Dutky, S. R. and Thompson, M. J. (1972) *Phytochemistry* **11**, 1951.
7. Knapp, F. F., Phillips, D. O., Goad, L. J. and Goodwin, T. W. (1972) *Phytochemistry* **11**, 3497.
8. Akihisa, T., Shimizu, N., Tamura, T. and Matsumoto, T. (1986) *Lipids* **21**, 491.
9. Kalinovskaya, N. I., Kalinovskii, A. I., Kuznetsova, T. A., Stonik, V. A. and Elyakov, G. B. (1984) *Dokl. Akad. Nauk. SSSR* **278**, 630.
10. Goad, L. J., Garneau, F.-X., Simard, J.-L., Ap Simon, J. W. and Girard, M. (1985) *Tetrahedron Letters* **26**, 3513.