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(24*R*)- AND (24*S*)-14α-METHYL-5α-ERGOST-9(11)-EN-3β-OLS FROM *GYNOSTEMMA PENTAPHYLLUM*

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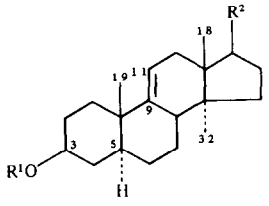
Key Word Index—*Gynostemma pentaphyllum*, Cucurbitaceae, sterol, (24*R*)- and (24*S*)-14α-methyl-5α-ergost-9(11)-en-3β-ol

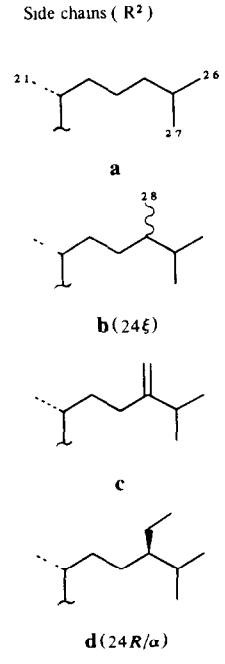
Abstract—Two new sterols were isolated as a mixture from the aerial parts of *Gynostemma pentaphyllum* and shown to be the (24*R*)- and (24*S*)-epimers of 14α-methyl-5α-ergost-9(11)-en-3β-ol.

INTRODUCTION

Gynostemma pentaphyllum Makino (Japanese name, Amachazuru) has been shown by our recent studies to contain several uncommon sterols including a 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] (**1c**) [1], and four 24,24-dimethylsterols: 24,24-dimethyl-5α-cholest-7-en-3β-ol (24,24-dimethyllathosterol), (22*E*)-24,24-dimethyl-22-dehydrolathosterol and 24,24-dimethyl-25-dehydrolathosterol [2], and 24,24-dimethyl-5α-cholestan-3β-ol [3], in addition to major (22*E*,24*R*/β)-24-ethyl-22-dehydrolathosterol (chondrillasterol) and other sterols [4–6]. Our continuing study on the sterol constituents of *G. pentaphyllum* has led to the isolation as a mixture and identification of two further 14α-methylsterols, (24*R*/α)- and (24*S*/β)-epimers of 14α-methyl-5α-ergost-9(11)-en-3β-ol [or 14α,24-dimethyl-5α-cholest-9(11)-en-3β-ol] (**1b**).



1 R^1 = H
2 R^1 = Ac



RESULTS AND DISCUSSION

Steryl acetate **2b** was isolated from the acetylated sterol fraction of *G. pentaphyllum* by virtue of the procedure described in the Experimental section. The mass spectrum of **2b** showed $[M]^+$ at *m/z* 456, corresponding to

$C_{31}H_{52}O_2$, accompanied with fragmentation ions at *m/z* 441 $[M-Me]^+$, 381 $[M-HOAc-Me]^+$, 329 $[M-C_9H_{19}$ (side chain)$]^+$ and 269 (329 − HOAc) indicating that it was an acetate of a C_{29}-sterol with a saturated C_9 side chain, and a monounsaturated skeleton with an additional methyl group. The presence of further ions at *m/z* 287 $[M-$side chain$-C_3H_6$ (ring D$)]^+$ and 273 (287 $-CH_2$) suggested that the additional methyl group in the skeleton was located at C-14 [7, 8] The ^{1}H NMR spectrum of the steryl acetate **2b** showed the skeletal olefinic signal at δ 5 286 (*dt*, $J = 5.9$ and 1 9 Hz, 11-H) and three angular methyl singlets at δ 0 657 (18-H_3), 0 754 (32-H_3) and 0 980 (19-H_3) in addition to the signals due to the 3β-acetoxyl and 3α-methine protons [δ 2 024 (3H, *s*, 3β-OAc) and 4.676 (1H, *tt*, $J = 5$ 1 and 11.2 Hz, 3α-H)] The spectrum showed four methyl doublets attributed to the side chain methyl protons at δ 0 780 (28-H_3), 0 807 (27-H_3), 0 855 (26-H_3) and 0.872 (21-H_3) The overall ^{1}H NMR spectral pattern was indistinguishable from that of the 24*R*/α-epimer of authentic 14α-methyl-5α-ergost-9(11)-en-3β-yl acetate (**2b**) (Table 1) [1], and hence, the steryl acetate was identified as (24*R*)-14α-methyl-5α-ergost-9(11)-en-3β-yl acetate The mass spectral data mentioned above were fully consistent with those of authentic **2b** [1] and supported the identification However, the steryl acetate **2b** from *G. pentaphyllum* displayed, further weak but distinctive three methyl doublets in the ^{1}H NMR spectrum at δ 0.785, 0 860 and 0.881 which were consistent with those arising from 27-H_3, 26-H_3 and 21-H_3, respectively, of the 24*S*/β-epimer of authentic **2b** (Table 1) [1] This suggests that the steryl acetate was not a pure 24*R*-epimer but contained a small proportion of its 24*S*-epimer The ratio of the 24*R*- and 24*S*-epimers was estimated to be 89:11 based on the averaged intensity of the ^{1}H signals under consideration

This study has, thus, demonstrated the occurrence of two 14α-methylsterols, (24*R*)- and (24*S*)-epimers of **1b**, in the aerial parts of *G pentaphyllum* as the minor sterol constituents Although the mixture of C-24 epimers of **1b** has been synthesized recently by hydrogenation from the corresponding 24-methylene derivative **1c**, which also was isolated from the same plant [1], this seems to be the first report of the occurrence of (24*R*)- and (24*S*)-**1b** in a plant. *G. pentaphyllum* is now shown to contain three 14α-methyl-$\Delta^{9(11)}$-sterols, **1c** [1] and both C-24 epimers of **1b**. A species of Cucurbitaceae, *Cucumis sativus* (cucumber), has recently been shown to contain the 24-ethyl homologue of **1b**, (24*R*)-14α-methyl-5α-stigmast-9(11)-en-3β-ol (**1d**) [9]. The lower homologue of **1b**, 14α-methyl-5α-cholest-9(11)-en-3β-ol (**1a**), has recently been detected in the seeds of *Solanum melongena* (eggplant, Solanaceae) [10]

Table 1 ^{1}H NMR spectral data of 14α-methyl-5α-ergost-9(11)-en-3β-yl acetate (**2b**) (400 MHz, $CDCl_3$, TMS as int standard)

H	**2b**† (24*R*/α)	**2b**† (24*S*/β)	**2b**‡ (24*R*/α)	**2b**‡ (24*S*/β)
18-H_3 (*s*)	0 657		0 656	
19-H_3 (*s*)	0 980		0 979	
32-H_3 (*s*)	0 754		0 754	
21-H_3 (*d*)	0 872(6 8)*	0 881(6 2)	0 874(7 0)	0 882(6 4)
26-H_3 (*d*)	0 855(6 8)	0 860(6 4)	0 854(6 8)	0 859(6 8)
27-H_3 (*d*)	0.807(6 8)	0 785(6 4)	0 807(6 8)	0 785(6 8)
28-H_3 (*d*)	0 780(6 4)		0 781(6 8)	
3β-OAc (*s*)	2 024		2 024	
3α-H (*tt*)	4 677(4 9, 11 2)		4 676(5 1, 11 2)	
11-H (*dt*)	5 286(5 9, 1 9)		5 285(6 1, 1 5)	

*Figures in parentheses denote *J* values (Hz)

†Isolated from *G pentaphyllum* in this study Mixture of C-24 epimers (24*R* 24*S* = 89 11)

‡Authentic sterol prepared from **2c** by hydrogenation (cf ref [1]) Mixture of C-24 epimers (24*R* 24*S* = 1 1)

EXPERIMENTAL

Argentation TLC silica gel–$AgNO_3$ (4 1) developed × 3 with CCl_4–CH_2Cl_2 (5 1); HPLC Altex Ultrasphere ODS column (Beckman Altex, 5 μm, 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. 255° *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 A mixture of the C-24 epimers of **2b** which was prepared from **2c** by hydrogenation [1] was used as the ref specimen The dried aerial parts of *G pentaphyllum* were purchased from Kinokuniya Kan-Yaku Kyoku Co (Tokyo) For the ^{1}H NMR data of the natural and synthetic **2b**, see Table 1

Extraction and isolation Air-dried aerial parts (20 kg) 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 for 3 hr and then unsaponifiable lipid was subjected to CC on silica gel (700 g) which was successively eluted with hexane (2 5 l), hexane–Et_2O (9 1, 3 0 l), hexane–Et_2O (4 1 2 5 l), hexane–EtOAc (6 1, 9 0 l), hexane–EtOAc (3 1, 2 5 l), and then with MeOH (2 0 l) The fractions eluted with hexane–EtOAc (6·1) 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 from Me_2CO–MeOH which gave crystalline (18 3 g) and brown pasty filtrate (8 9 g) fractions CC of the filtrate on silica gel (300 g) using hexane–Et_2O (4 1) as an eluant afforded purified steryl acetate mixture (2 0 g) This was subjected to argentation TLC to give seven bands The fraction (135 mg) recovered from the least polar band (R_f 0 53–0 62) was then subjected to HPLC which yielded the fraction containing **2b** from which was isolated **2b** (2 mg, mixture of C-24 epimers) by repetitive HPLC

14α-Methyl-5α-ergost-9(11)-en-3β-yl acetate (**2b**, C-24 *epimeric mixture*) *isolated from* G pentaphyllum *RR*$_t$ 0 97 and 1 49 on HPLC and GC, respectively MS *m/z* (rel int) 456 3955 $[M]^+$ (11, $C_{31}H_{52}O_2$, requires 456 3963), 441 3750 (39, $C_{30}H_{49}O_2$), 396 3745 (2, $C_{29}H_{48}$), 381 3500 (12, $C_{28}H_{45}$), 329 2483 (2, $C_{22}H_{33}O_2$), 287 1981 (3, $C_{19}H_{27}O_2$), 273 1866 (3, $C_{18}H_{25}O_2$) 269 2307 (3, $C_{20}H_{29}$), 261 1835 (3, $C_{17}H_{25}O_2$), 255 2091 (3, $C_{19}H_{27}$), 227 1812 (5, $C_{17}H_{23}$), 213 1692 (4, $C_{16}H_{21}$), 201 1655 (5, $C_{15}H_{21}$), 43 0533 (C_3H_7) and 43 0167 ($C_2H_3O_1$) (100)

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A PREGNANE TRIGLYCOSIDE ESTER FROM *DREGEA SINENSIS* VAR *CORRUGATA*

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Key Word Index—*Dregea sinensis* var *corrugata*, Asclepiadaceae, dregeoside, steroid, pregnane glycoside ester

Abstract—A new pregnane glycoside ester, dregeoside, was isolated from the dried rhizome of *Dregea sinensis* var *corrugata* On the basis of chemical reactions and spectroscopic evidence, the structure was established as 12-*O*-benzoyl-drevogenin-3-*O*-*β*-D-oleandropyranosyl(1→4)-*O*-*β*-D-cymaropyranosyl(1→4)-*O*-*β*-D-cymaropyranoside

INTRODUCTION

In a previous paper [1], we reported the isolation and structure elucidation of a drevogenin from the rhizomes of *Dregea sinensis*. As a continuation of the studies on this plant, we present the spectral and chemical evidence for the structure of a new triglycoside (**1**)

RESULTS AND DISCUSSION

Dregeoside (**1**) was isolated by gel column chromatography and reverse-phase chromatography. The mass spectrum of compound **1** indicated that molecular formula was $C_{49}H_{76}O_{16}$ (FAB-MS $[M]^+$ at *m/z* 920). The UV spectrum showed absorption at 230 nm Its IR spectrum showed the presence of methoxy group (2925, 1440 cm^{-1}) and –C–O–C–O– (1195, 1160, 1080, 1050 cm^{-1}) Confirmation of the triglycoside structure of compound **1** and the position of its monobenzoate ester group on the genin moiety was provided by the ^1H NMR (400 MHz) spectrum of **1** which indicated the presence of three methoxy group at 3.38 (3H, *s*, OMe), 3.45 (6H, *s*, 2 × OMe) and three methyl group at 1 23 (6H, *d*, $J=6$ Hz, scc. 2 × Me), 1.34 (3H, *d*, $J=6$ Hz, sec. Me) A methyl group and a methoxy group could be assigned to C-3 and C-6, respectively, of a deoxy sugar

As cymaropyranose possesses only two hydroxy groups at C-1 and C-4, the sugar sequence in **1** was linear We assigned the ^{13}C NMR signals of the sugar chain in **1** as shown in Table 1 in comparison with the data on the ^{13}C NMR chemical shifts of methyl *β*-D-cymaroside and *α*,*β*-D-oleandroside [2] [3] From the ^{13}C NMR chemical shifts of the anomeric carbons the D-cymarose and D-oleandrose moieties in **1** are suggested to have a *β*-configuration at C-1. The ^{13}C NMR data of **1** is presented in Table 1. Mass spectral fragment ion peaks at *m/z* 145 ($C_7H_{13}O_3$), 144 ($C_7H_{12}O_3$), and 499 ($C_7H_{13}O_3$–$C_7H_{12}O_3$–$C_7H_{12}O_4$) suggested that there were three deoxy sugars in the molecule.

Mild acid hydrolysis of the acetate of **1** with dilute sulphuric acid yielded 4-*O*-acetyl-oleandrose, cymarose and the monoacetate of **2** which was identical with an authentic sample as determined by TLC and GLC Oleandrose was indicated to be the terminal sugar

Mild acid hydrolysis of **1** using the earlier reported method of Mannich and Siewert [4] yielded drevogenin (**2**) and cymarose (**4**), oleandrose **5** ($[\alpha]_D$ mp, TLC) [5]. Alkaline hydrolysis of **2** yielded deacyldrevogenin (**3**), which was identical with authentic dihydrosarcostin (mp, mmp, and IR) [6]. The monobenzoate nature of the ester function in **1** was supported by its IR, UV (λ_{max}^{EtOH} 282 nm, log *ε* 3.05) and ^1H and ^{13}C NMR spectral data. The difference of $C_{21}H_{36}O_9$ between the formula of glycoside **1** and its aglycone **2** indicated that **1** was a triglycoside The same conclusion could be drawn from the mass spectrum of **1** which recorded fragment ions for a trisaccharide unit (*m/z* 450) and the genin moiety (*m/z* 470) besides the prominent fragment ions of drevogenin monobenzoate giving ions for benzoic acid at *m/z* 122 and the other expected ions of the drevogenin moiety including the fragment ions due to the sequential losses of its four molecules of water at *m/z* 452, 434, 416, and 398. In