4α-Methyl-5α,14β-ergosta-8,24(24[1])-dien-3β-ol ("triticusterol"): the first naturally occurring 14β(H)-steroid

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The structure of triticusterol isolated from the germ oil of wheat (*Triticum aestivum* L.; Gramineae) was established to be 4α-methyl-5α,14β-ergosta-8,24(24[1])-dien-3β-ol on the basis of spectroscopic data. This is the first example of a naturally occurring compound with a 14β(H)-steroid skeleton.

Introduction

In photosynthetic organisms, sterols are biosynthesized from cycloartenol (5α-cycloart-24-en-3β-ol), the first cyclization product of squalene 2,3-oxide, by sequential oxidative C-4 and C-14 demethylation, double-bond isomerization, and C-24 alkylation through a pathway such as cycloartenol ⟶⟶ cycloeucalenol [4α,14α-dimethyl-9β,19-cyclo-5α-ergost-24(24[1])-en-3β-ol] ⟶ obtusifoliol [4α,14α-dimethyl-5α-ergosta-8,24(24[1])-dien-3β-ol] ⟶ 4α-methyl-5α-ergosta-8,14,24(24[1])-trien-3β-ol **2** ⟶ 4α-methylfecosterol [4α-methyl-5α-ergosta-8,24(24[1])-dien-3β-ol; **3**] ⟶ gramisterol [4α-methyl-5α-ergosta-7,24(24[1])-dien-3β-ol] ⟶ citrostadienol {[24(24[1])*Z*]-4α-methyl-5α-stigmasta-7,24(24[1])-dien-3β-ol} ⟶⟶ 4-non-methylated sterols (Scheme 1).[1–3] Even though this route creates ample opportunity for the formation of structural isomers during double-bond migration and reduction, so far none of these isomers have been found in nature.[4,5] We report here the isolation and characterization of 4α-methyl-5α,14β-ergosta-8,24(24[1])-dien-3β-ol (**1**, "triticusterol"), along with another novel sterol, 4α-methyl-5α-ergosta-8,14,24(24[1])-trien-3β-ol **2**, and 4α-methylfecosterol **3**, from the germ oil of wheat (*Triticum aestivum* L.; Gramineae).

Results and discussion

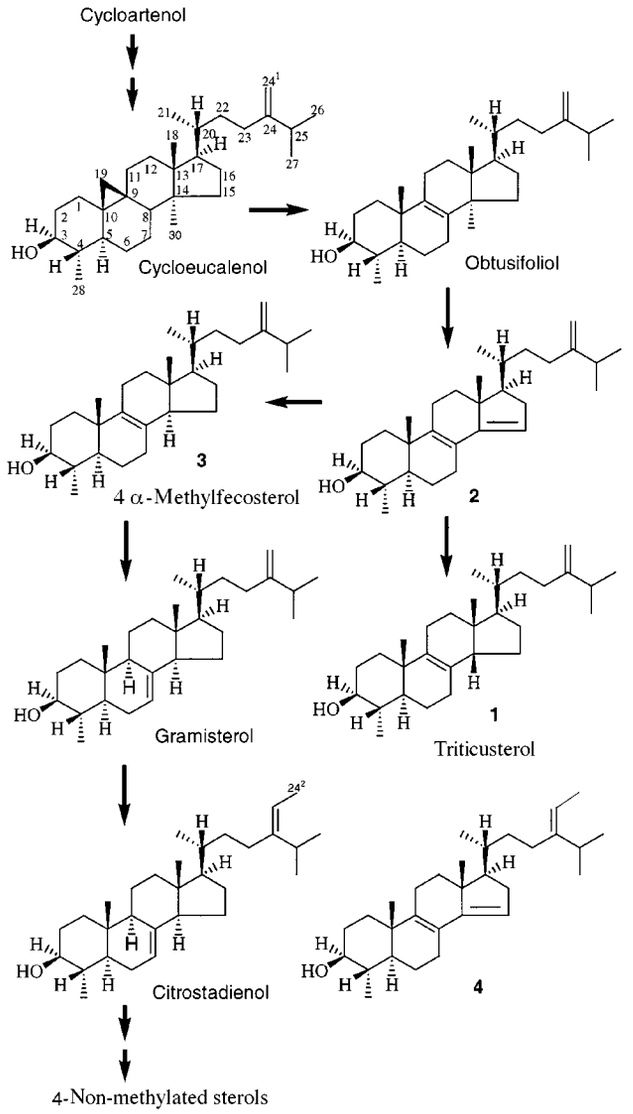
The isolation procedure involved saponification of the oil, fractionation of the neutral nonsaponifiable lipid over silica gel, and separation of the components of the 4α-methylsterol fraction by reversed-phase chromatography and preparative reversed-phase HPLC.

Compounds **1** and **3** have virtually identical mass spectra (MW 412, $C_{29}H_{48}O$). 500 MHz ^{1}H NMR spectra (see Table 1) of compounds **1**, **2** (MW 410, $C_{29}H_{46}O$) and **3** showed that they were 4α-methylsterols with the same side chain [3-H, ddd, δ 3.1; three methyl doublets (21-H_3, 26-H_3 and 27-H_3); two exo-methylene protons]. Compound **3** was readily identified by comparison with literature data.[1,7] Both compounds **1** and **3** have a tetrasubstituted double bond in the skeleton (125 MHz ^{13}C NMR). Because the ^{1}H NMR spectrum of compound **1** did not fit that of the $\Delta^{8(14)}$-isomer[8] of compound **3** (a known compound[9–11]), the possibility of an isomeric steroid skeleton had to be considered. Compound **1** has angular-methyl signals at δ 0.86 and 0.95 ppm (Table 1). The Zürcher[12] values for 18-H_3 and 19-H_3 in 5α,14β-cholest-8-en-3β-ol are 0.86 and 0.90, respectively. This agreement suggested compound **1** to be 4α-methyl-5α,14β-ergosta-8,24(24[1])-dien-3β-ol. Analysis of ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, HMQC, and HMBC spectra supported the proposed structure. The stereochemistry of triticusterol **1** was established by comparison of the phase-sensitive NOESY and NOE difference spectra with those of stereoisomer **3**. Compound **1** showed NOE correlations between [5α-H ~ 1α-H, 3α-H, 28-H_3 (4α-Me), and 7α-H] and [19-H_3 (10β-Me) ~ 2β-H, 4β-H, and 6β-H] (Fig. 1), which were observed also for compound **3**. This demonstrated that triticusterol **1** possessed the same stereochemistry as compound **3** as far as rings A and B were concerned. Compound **3** showed NOE correlation between [18-H_3 (13β-Me) ~ 19-H_3 (10β-Me)]. This was not observed for triticusterol **1**, but compound **1** did exhibit NOE correlation between [18-H_3 (13-Me) ~ 14-H]. This suggested that triticusterol **1** possessed a *cis*-fused C/D ring junction (13β,14β) which caused ring D to be deflected down from the

Table 1 Diagnostic ^{1}H NMR spectral data (δ-values; 500 MHz; $CDCl_3$) of two novel sterols (**1**, **2**) and two reference compounds (**3**, **4**)[a]

	1	**2**	**3**	**4**[b]
3-H (ddd)	3.10	3.11	3.10	3.12
15-H (br s)		5.36		5.36
18-H_3 (s)	0.86	0.82	0.61	0.82
19-H_3 (s)	0.95	1.02	0.98	1.01
21-H_3 (d)	0.96 (6.5)	0.97 (6.4)	0.96 (6.6)	0.97 (5.9)
25-H (septet)	2.23 (6.8)	2.24 (6.7)	2.23 (6.8)	2.84 (6.2)
26-H_3 (d)	1.02 (6.5)	1.03 (7.0)	1.02 (6.8)	0.98 (6.2)
27-H_3 (d)	1.03 (7.0)	1.04 (6.7)	1.03 (6.8)	0.98 (6.2)
28-H_3 (d)	1.00 (6.0)	1.02 (6.4)	0.99 (6.4)	1.01 (6.2)
24[1]-H_2	4.66 4.72	4.68 4.73	4.66 4.71	5.12 (1H, q, 6.2)
24[2]-H_3 (d)				1.60 (6.6)

[a] *J*-Values (Hz) are in parentheses. [b] Data taken from ref. 15 (determined at 400 MHz).



Scheme 1 Structures of 4α-methylsterols described in this paper, and the possible biosynthetic sequence leading to 4-non-methylated sterols in wheatgerm.

plane of the steroid ring system, and also spatially oriented 18-H_3 away from 19-H_3. Further NOE correlations [7α-H ~ 15α-H ~ 16α-H ~ 17α-H] supported the above proposition and demonstrated that compound **1** had a 14β(H),17α(H)-cholestane ring system. Other significant NOE correlations were observed between [18-H_3 ~ 20-H] (which was observed also for isomer **3**) and between [12β-H ~ 18-H_3] and [12α-H ~ 21-H_3]. Based on the spectral evidence we concluded that triticusterol **1** is 4α-methyl-5α,14β-ergosta-8,24(24^1)-dien-3β-ol (20*R*), a 14β-isomer of 4α-methylfecosterol **3**.

The most stable conformation of stereoisomers **1** and **3** was simulated using MacroModel. The results of the calculations [13,14] are shown in Fig. 1 together with the significant NOEs (⟷). The conformer of simulated **1** deflected ring D down from the plane of the ring system and C-22 of the side chain at C-17 oriented into a "right-handed" conformation (C-22 *trans*-oriented to C-13). The "right-handed" conformation of the side chain was in accord with that of compound **3** (Fig. 1). This was consistent with the NOE experiment done in solution, thus confirming the proposed structure of triticusterol **1**.

Elucidation of the structure of compound **2** was more straightforward. It has three degrees of unsaturation (MW 410), two in the skeleton and one in the side chain. The UV spectrum supported a *cis*-diene chromophore [λ_{max} 248 nm (EtOH)].[1,15] Comparison of our spectral data with published data for 4α-

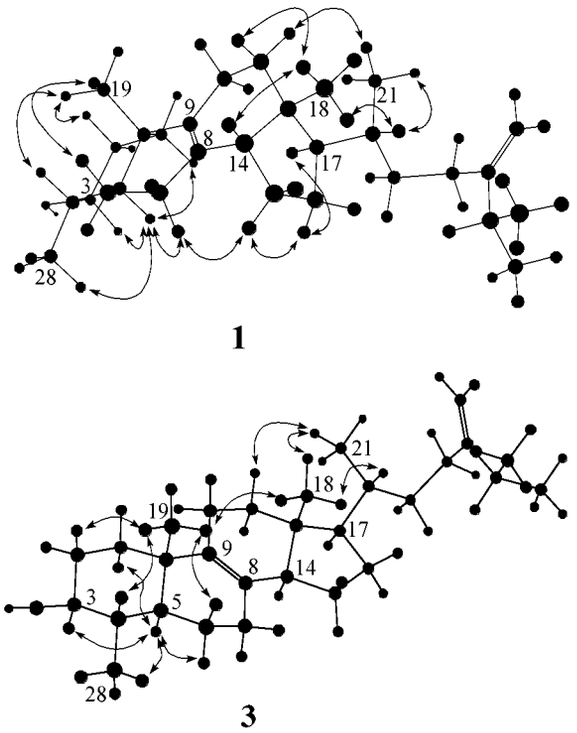


Fig. 1 Energy-minimized conformations and major NOE correlations (↔) (determined in $CDCl_3$) for triticusterol **1** and 4α-methylfecosterol **3**.

methylvernosterol {[24(24^1)*Z*]-4α-methyl-5α-stigmasta-8,14,24(24^1)-trien-3β-ol; **4**} [15] (Table 1) left no doubt that compound **2** was 4α-methyl-5α-ergosta-8,14,24(24^1)-trien-3β-ol.

14β(H)-Steroids, including 5α,14β-cholest-8-en-3β-ol,[16] are known synthetic compounds [17–21] but triticusterol **1** is the first example of a naturally occurring steroid with a 14β(H) configuration. It was isolated, together with another novel sterol, 4α-methyl-5α-ergosta-8,14,24(24^1)-trien-3β-ol **2**, and 4α-methylfecosterol **3**, from the 4α-methylsterol fraction of the oil.[22,25] Compound **2** is the precursor of 4α-methylfecosterol **3** and also the most likely precursor of triticusterol **1**. Apparently, in wheatgerm, the conversion of triene **2** into diene **3** is not completely stereoselective.[26]

Experimental

General

Crystallizations were performed from methanol. Mps were measured on a Yanagimoto micro mp apparatus and are uncorrected. Reversed-phase chromatography was performed on an ODS (Chromatorex-ODS DM1020T, 100–200 mesh, Fuji Silysia Chemical Ltd., Aichi, Japan) column with MeOH as the mobile phase, and HPLC on an ODS column (25 cm × 10 mm i.d.; Superiorex ODS S-5 μm column, Shiseido Co., Tokyo, Japan) with MeOH (4 ml min^{-1}) as the mobile phase, at 25 °C. GLC was performed using a DB-17 fused silica capillary column (30 m × 0.3 mm i.d., column temperature 275 °C). In both HPLC and GLC, cholesterol (cholest-5-en-3β-ol) was the standard for the determination of relative retention time (Rt_R). EI-MS were recorded on a Hitachi M-80B double-focussing GC-MS instrument (70 eV) using a direct inlet system. NMR Spectra were recorded with JEOL α-500 and Varian INNOVA 500 spectrometers at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$ with Me_4Si (^{1}H NMR) and $CDCl_3$ at δ_C 77.0 (^{13}C NMR) as internal standard. *J*-Values are given in Hz. UV spectra on a Shimadzu UV-300 spectrometer and IR spectra on a JASCO IR300 IR spectrometer were obtained in EtOH and KBr pellets, respectively. Optical rotations were measured on a JASCO DIP-370 polarimeter; $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. Acetylation was performed in Ac_2O–pyridine

at room temperature overnight. Crude wheatgerm oil was donated by Summit Oil Mills Co. (Chiba, Japan). Fully assigned ^{1}H and ^{13}C NMR data of compounds **1** and **2** and their acetyl derivatives, and of reference compounds **3** and **3**-acetate, which were unavailable in the literature, are described below accompanied with some NMR data obtained in C_5D_5N.

Isolation procedure

Alkaline hydrolysis (5% KOH in MeOH; reflux; 3 h) of crude wheatgerm oil (3.65 kg) yielded neutral nonsaponifiable lipid (175 g), which was crystallized from acetone–MeOH to yield a crystallized portion (62 g), consisting of a mixture of 4-non-methylated sterols, and a filtrate portion. The filtrate portion was chromatographed over silica gel (900 g) with hexane and hexanes–EtOAc (9 : 1, 4 : 1, v/v) as eluent. The hexanes–EtOAc (9 : 1) eluate, after re-chromatography over silica gel, yielded the 4α-methylsterol fraction (5.9 g). Chromatography of this fraction over an ODS column followed by HPLC afforded compounds **1** (25 mg), **2** (6 mg), and **3** (20 mg).

Triticusterol 1. Fine needles; mp 107–109 °C; Rt_R 0.62 (HPLC), 1.03 (GLC); $[\alpha]_D^{25}$ + 118.6 (*c* 0.60, $CHCl_3$); ν_{max}(KBr)/ cm^{-1} 3429, 1642 and 887; δ_C- and δ_H($CDCl_3$) C-1 [34.5; 1.17 (α), 1.80 (β)], C-2 [31.1; 1.86 (α), 1.53 (β)], C-3 [76.7; 3.10, ddd, *J* 5.1, 10.0 and 11.0], C-4 [38.9; 1.35], C-5 [47.8; 0.95, dt, *J* 2.2 and 11.8], C-6 [20.8; 1.74 (α), 1.27 (β)], C-7 [30.7; 2.10 (α), 1.72 (β)], C-8 [130.5], C-9 [135.4], C-10 [36.9], C-11 [20.7; 1.88 (2H)], C-12 [35.4; 1.64 (α; dt, *J* 13.2 and 5.1), 1.26 (β)], C-13 [41.5], C-14 [54.0; 1.77], C-15 [30.0; 1.95 (α), 1.11 (β)], C-16 [28.3; 1.28 (α), 1.75 (β)], C-17 [50.3; 1.42], C-18 [23.7; 0.86, s], C-19 [18.4; 0.95, s], C-20 [33.6; 1.48], C-21 [19.8; 0.96, d, *J* 6.5], C-22 [34.4; 1.15, 1.59], C-23 [31.7; 1.91, 2.13], C-24 [156.9], C-25 [33.8; 2.23, sept, *J* 6.8], C-26 [22.0; 1.02, d, *J* 6.5], C-27 [21.9; 1.03, d, *J* 7.0], C-28 [15.1; 1.00, d, *J* 6.0], C-24^1 [106.0; 4.66, d, *J* 1.5 and 4.72, s]; δ_C- and δ_H(C_5D_5N) C-1 [35.1; 1.20 (α), 1.82 (β)], C-2 [32.2; 2.06 (α), 1.83 (β)], C-3 [76.0; 3.32, ddd, *J* 5.1, 10.5 and 10.5], C-4 [39.7; 1.59], C-5 [48.1; 1.05], C-6 [21.4; 1.79 (α), 1.30 (β)], C-7 [31.1; 2.15 (α), 1.76 (β)], C-8 [130.7], C-9 [136.2], C-10 [37.3], C-11 [21.1; 1.99 (2H)], C-12 [35.8; 1.72 (α), 1.35 (β)], C-13 [41.9], C-14 [54.4; 1.84], C-15 [30.4; 1.22 (α), 2.00 (β)], C-16 [28.7; 1.81 (α), 1.32 (β)], C-17 [50.8; 1.54], C-18 [24.0; 0.94, s], C-19 [18.6; 1.05, s], C-20 [34.0; 1.53], C-21 [20.0; 1.04, d, *J* 6.4], C-22 [34.8; 1.26, 1.70], C-23 [32.1; 2.02, 2.22], C-24 [156.8], C-25 [34.1; 2.28, sept, *J* 7.0], C-26 [22.2; 1.06, d, *J* 7.2], C-27 [22.0; 1.07, d, *J* 6.8], C-28 [15.8; 1.28, d, *J* 6.2], C-24^1 [106.7; 4.86 and 4.87, each 1H and s]. The NOE correlations determined in C_5D_5N were consistent with those determined in $CDCl_3$; *m/z* 412.3686 ($C_{29}H_{48}O$, M^+, 57% requires *M*, 412.3702), 397.3458 ($C_{28}H_{45}O$, 43), 394.3558 ($C_{29}H_{46}$, 1), 379.3355 ($C_{28}H_{43}$, 9), 369.3150 ($C_{26}H_{41}O$, 1), 328.2722 ($C_{23}H_{36}O$, 2), 313.2517 ($C_{22}H_{33}$, 7), 295.2430 ($C_{22}H_{31}$, 5), 287.2422 ($C_{20}H_{31}O$, 6), 285.2296 ($C_{20}H_{29}O$, 11), 245.1917 ($C_{17}H_{25}O$, 10), 227.1829 ($C_{17}H_{23}$, 8) and 55.0557 (C_4H_7, 100). On acetylation, triticusterol **1** yielded the acetyl derivative.

Triticusterol 1 acetate. Amorphous gum; $[\alpha]_D^{25}$ + 148.4 (*c* 0.20, $CHCl_3$); ν_{max}(KBr)/ cm^{-1} 1738, 1641, 1244 and 887; δ_C- and δ_H($CDCl_3$) C-1 [34.1; 1.21 (α), 1.80 (β)], C-2 [27.1; 1.90 (α), 1.54 (β)], C-3 [78.9; 4.38, ddd, *J* 4.8, 11.0 and 11.0], C-4 [35.8; 1.57], C-5 [47.8; 1.04], C-6 [20.8; 1.74 (α), 1.29 (β)], C-7 [30.5; 2.09 (α), 1.71 (β)], C-8 [130.6], C-9 [135.1], C-10 [36.7], C-11 [20.7; 1.87 (2H)], C-12 [35.4; 1.65 (α; dt, *J* 13.2 and 5.1), 1.26 (β)], C-13 [41.5], C-14 [54.0; 1.78], C-15 [30.1; 1.94 (α), 1.11 (β)], C-16 [28.3; 1.30 (α), 1.76 (β)], C-17 [50.4; 1.41], C-18 [23.7; 0.86, s], C-19 [18.2; 0.96, s], C-20 [33.6; 1.48], C-21 [19.8; 0.96, d, *J* 6.6], C-22 [34.3; 1.16, 1.58], C-23 [31.7; 1.91, 2.10], C-24 [156.9], C-25 [33.8; 2.23, sept, *J* 6.6], C-26 [22.0; 1.02, d, *J* 7.0], C-27 [21.9; 1.03, d, *J* 7.0], C-28 [15.1; 0.86, d, *J* 6.6], C-24^1 [106.0; 4.67, d, *J* 1.5 and 4.72, s], O*C*OMe [170.9] and OCO*Me* [21.4; 2.05, s]; δ_C- and δ_H(C_5D_5N) C-1 [34.3; 1.11 (α), 1.74 (β)], C-2 [27.5; 1.97 (α), 1.58 (β)], C-3 [78.7; 4.57, ddd, *J* 5.2, 11.0 and 11.0], C-4 [36.1; 1.61], C-5 [48.0; 1.00], C-6 [21.1; 1.70 (α), 1.25 (β)], C-7 [30.7; 2.11 (α), 1.75 (β)], C-8 [130.8], C-9 [135.6], C-10 [37.0], C-11 [21.0; 1.90 (2H)], C-12 [35.6; 1.73 (α), 1.32 (β)], C-13 [41.8], C-14 [54.3; 1.83], C-15 [30.3; 1.23 (α), 2.00 (β)], C-16 [28.7; 1.84 (α), 1.33 (β)], C-17 [50.6; 1.53], C-18 [23.9; 0.93, s], C-19 [18.3; 0.98, s], C-20 [33.9; 1.55], C-21 [20.0; 1.04, d, *J* 6.1], C-22 [34.8; 1.29, 1.72], C-23 [32.0; 2.03, 2.22], C-24 [156.8], C-25 [34.1; 2.29, sept, *J* 6.7], C-26 [22.2; 1.07, d, *J* 7.0], C-27 [22.0; 1.07, d, *J* 6.7], C-28 [15.3; 0.92, d, *J* 6.7], C-24^1 [106.7; 4.87 and 4.88, each 1H and s], O*C*OMe [170.6] and OCO*Me* [21.2; 2.08, s]; *m/z* 454.3789 ($C_{31}H_{50}O_2$; M^+, 31% requires *M*, 454.3807), 439 (7), 411 (1), 394 (3), 379 (35), 370 (2), 355 (4), 327 (7), 295 (8), 287 (6), 269 (3), 255 (3), 227 (10) and 43 (100).

4α-Methyl-5α-ergosta-8,14,24(24^1)-trien-3β-ol 2. Fine needles; mp 124–126 °C; Rt_R 0.69 (HPLC), 1.03 (GLC); λ_{max}(EtOH)/nm 248; ν_{max}(KBr)/cm^{-1} 3417, 1639, 887 and 800; δ_H($CDCl_3$) 3α-H (3.11, ddd, *J* 4.6, 9.8 and 11.3), 15-H (5.36, br s), 18-H_3 (0.82, s), 19-H_3 (1.02, s), 21-H_3 (0.97, d, *J* 6.4), 26-H_3 (1.03, d, *J* 7.0), 27-H_3 (1.04, d, *J* 6.7), 28-H_3 (1.02, d, *J* 6.4) and 24^1-H_2 (4.68, d, *J* 1.2 and 4.73, s); *m/z* 410.3516 ($C_{29}H_{46}O$; M^+, 61%; requires *M*, 410.3545), 395.3280 ($C_{28}H_{43}O$, 35), 377.3207 ($C_{28}H_{41}$, 6), 367.2971 ($C_{26}H_{39}O$, 7), 326.2627 ($C_{23}H_{34}O$, 2), 311.2377 ($C_{23}H_{31}O$, 37), 293.2282 ($C_{22}H_{29}$, 3), 285.2266 ($C_{20}H_{29}O$, 9), 269.1898 ($C_{19}H_{25}O$, 20), 253.1950 ($C_{19}H_{25}$, 6), 227.1807 ($C_{17}H_{23}$, 12), 69.0714 (C_5H_9, 78) and 55.0549 (C_4H_7, 100).

4α-Methyl-5α-ergosta-8,14,24(24^1)-trien-3β-ol 2 acetate. λ_{max}(EtOH)/nm 248; δ_C- and δ_H($CDCl_3$) C-1 [34.9; 1.34 (α), 1.88 (β)], C-2 [27.3; 1.94 (α), 1.53 (β)], C-3 [78.6; 4.38, ddd, *J* 5.2, 11.0 and 11.0], C-4 [36.1; 1.60], C-5 [47.2; 1.18], C-6 [20.7; 1.86 (α), 1.36 (β)], C-7 [26.7; 2.14 (2H)], C-8 [123.0], C-9 [140.6], C-10 [37.0], C-11 [21.8; 2.22 (α), 2.13 (β)], C-12 [37.0; 1.43 (α), 2.03 (β)], C-13 [45.0], C-14 [151.0], C-15 [117.5; 5.38, br s], C-16 [35.9; 2.39 (α), 2.11 (β)], C-17 [57.1; 1.58], C-18 [15.7; 0.82, s], C-19 [19.3; 1.03, s], C-20 [34.0; 1.67], C-21 [18.9; 0.97, d, *J* 6.4], C-22 [34.6; 1.23, 1.57], C-23 [30.8; 1.90, 2.15], C-24 [156.9], C-25 [33.8; 2.24, sept, *J* 6.7], C-26 [22.0; 1.03, d, *J* 6.7], C-27 [21.9; 1.04, d, *J* 6.7], C-28 [15.1; 0.88, d, *J* 6.1], C-24^1 [106.0; 4.68, d, *J* 1.2, and 4.73, s], O*C*OMe [170.9] and OCO*Me* [21.4; 2.06, s]; *m/z* 452.3631 ($C_{31}H_{48}O_2$, M^+, 62%; requires *M*, 452.3651), 437 (25), 409 (19), 392 (1), 377 (15), 368 (2), 353 (32), 349 (11), 327 (8), 326 (17), 311 (13), 293 (6), 265 (9), 251 (14), 227 (13), 69 (65) and 43 (100).

4α-Methylfecosterol 3. δ_C- and δ_H($CDCl_3$) C-1 [35.1; 1.26 (α), 1.77 (β)], C-2 [31.2; 1.83 (α), 1.48 (β)], C-3 [76.5; 3.10, ddd, *J* 4.9, 9.8 and 11.0], C-4 [39.3; 1.31], C-5 [46.9; 1.02], C-6 [20.9; 1.73 (α), 1.25 (β)], C-7 [27.5; 1.88 (α), 2.00 (β)], C-8 [128.1], C-9 [135.1], C-10 [36.3], C-11 [22.8; 2.12 (α), 2.02 (β)], C-12 [37.1; 1.37 (α), 1.97 (β)], C-13 [42.2], C-14 [51.9; 2.04], C-15 [23.7; 1.56 (α), 1.28 (β)], C-16 [28.8; 1.92 (α), 1.32 (β)], C-17 [54.8; 1.17], C-18 [11.3; 0.61, s], C-19 [18.9; 0.98, s], C-20 [36.3; 1.43], C-21 [18.8; 0.96, d, *J* 6.6], C-22 [34.7; 1.13, 1.53], C-23 [31.1; 1.87, 2.08], C-24 [156.9], C-25 [33.8; 2.23, sept, *J* 6.8], C-26 [22.0; 1.02, d, *J* 6.8], C-27 [21.9; 1.03, d, *J* 6.8], C-28 [15.1; 0.99, d, *J* 6.4], C-24^1 [105.9; 4.66, d, *J* 1.2, and 4.71, s]; δ_C- and δ_H(C_5D_5N) C-1 [35.2; 1.23 (α), 1.62 (β)], C-2 [32.3; 2.07 (α), 1.82 (β)], C-3 [75.9; 3.31, ddd, *J* 4.9, 10.7 and 10.7], C-4 [40.0; 1.55], C-5 [47.6; 1.08], C-6 [21.5; 1.81 (α), 1.30 (β)], C-7 [27.9; 2.02 (2H)], C-8 [128.2], C-9 [135.7], C-10 [36.7], C-11 [23.1; 2.20 (α), 2.06 (β)], C-12 [37.5; 1.45 (α), 2.00 (β)], C-13 [42.6], C-14 [52.3; 2.14], C-15 [24.1; 1.60 (α), 1.33 (β)], C-16 [29.1; 1.32 (α), 1.90 (β)], C-17 [55.2; 1.20], C-18 [11.6; 0.70, s], C-19 [19.2; 1.05, s], C-20 [36.5; 1.46], C-21 [19.0; 1.03, d, *J* 6.7], C-22 [35.8; 1.28, 1.78], C-23 [31.5; 1.95, 2.19], C-24 [156.8], C-25 [34.2; 2.29, sept, *J* 6.7], C-26 [22.2; 1.07, d, *J* 7.0], C-27 [22.1; 1.08, d, *J* 6.7],

C-28 [15.8; 1.26, d, *J* 6.1] and C-24[1] [105.7; 4.85, d, *J* 1.2, and 4.87, s]. The NOE correlations determined in C_5D_5N were consistent with those determined in $CDCl_3$; *m/z* 412.3683 ($C_{29}H_{48}O$, M^+, 60%; requires *M*, 412.3702), 397 (23), 394 (3), 379 (6), 369 (1), 328 (2), 313 (4), 295 (2), 287 (6), 285 (16), 267 (3), 245 (10), 227 (13) and 55 (100). On acetylation, compound **3** yielded the acetyl derivative.

4α-Methylfecosterol 3 acetate. δ_C- and $\delta_H(CDCl_3)$ C-1 [34.8; 1.29 (α), 1.79 (β)], C-2 [27.2; 1.88 (α), 1.51 (β)], C-3 [78.8; 4.38, ddd, *J* 4.9, 11.0 and 11.0], C-4 [36.1; 1.55], C-5 [46.9; 1.09], C-6 [20.9; 1.76 (α), 1.26 (β)], C-7 [27.3; 1.88 (α), 2.02 (β)], C-8 [128.2], C-9 [134.8], C-10 [36.2], C-11 [22.8; 2.11 (α), 2.00 (β)], C-12 [37.0; 1.40 (α), 1.97 (β)], C-13 [42.1], C-14 [51.8; 2.07], C-15 [23.7; 1.58 (α), 1.30 (β)], C-16 [28.8; 1.93 (α), 1.35 (β)], C-17 [54.8; 1.19], C-18 [11.3; 0.61, s], C-19 [18.8; 0.99, s], C-20 [36.3; 1.43], C-21 [18.7; 0.96, d, *J* 6.7], C-22 [34.7; 1.15, 1.54], C-23 [31.1; 1.89, 2.10], C-24 [156.9], C-25 [33.8; 2.23, sept, *J* 7.0], C-26 [22.0; 1.02, d, *J* 6.7], C-27 [21.9; 1.03, d, *J* 7.0], C-28 [15.1; 0.86, d, *J* 6.4], C-24[1] [105.9; 4.66, d, *J* 1.5 and 4.71, s], O*C*OMe [170.9] and OCO*Me* [21.4; 2.05, s]; *m/z* 454.3793 ($C_{31}H_{50}O_2$, M^+, 70%; requires *M*, 454.3807), 439 (20), 394 (8), 379 (11), 370 (2), 355 (3), 327 (15), 295 (3), 287 (4), 269 (6), 241 (23), 227 (26) and 43 (100).

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