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4α,14α-DIMETHYL-5α-ERGOSTA-7,9(11),24(28)-TRIEN-3β-OL FROM *PHASEOLUS VULGARIS* AND *GYNOSTEMMA PENTAPHYLLUM*

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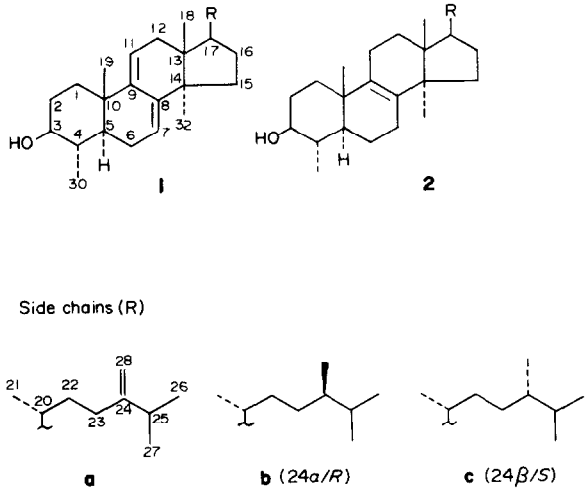
Key Word Index—*Phaseolus vulgaris*; Leguminosae; seeds; *Gynostemma pentaphyllum*; Cucurbitaceae; plant sterols; 4α-methylsterol; 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol.

Abstract—The structure of a new 4α-methylsterol isolated from the immature seeds of *Phaseolus vulgaris* and the aerial parts of *Gynostemma pentaphyllum* has been shown to be 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol by spectroscopic methods and by chemical correlation with obtusifoliol. This appears to be the first report of the isolation of a 4α-methylsterol with a $\Delta^{7,9(11)}$-diene system from nature. Abundances and chromatographic data of all identified 4α-methylsterols isolated from *G. pentaphyllum* are also given.

INTRODUCTION

In the course of our study on the isolation and characterization of higher plant sterols, we found that several plants belonging to the Cucurbitaceae contain sterols with side-chains which, hitherto, were supposed to be typical of marine sponge sterols. Examples are side-chains with 24,24-dimethyl groups [1] and acetylenic sterols [2] which were obtained from *Gynostemma pentaphyllum*. The isolation of the acetylenic sterols completed our work on the 4-demethylsterols from this source.

We now report the results of an investigation of the 4α-methylsterols, intermediates in the biosynthesis of 4-demethylsterols [3, 4], from the aerial parts of *G. pentaphyllum*. One novel sterol, viz. 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol (**1a**) and five known 4α-methylsterols were identified. The same novel sterol was also isolated from the immature seeds of *Phaseolus vulgaris*. Compound **1a** accounts for 1.9 and 1.8% of the 4α-methylsterol fraction of *G. pentaphyllum* and *P. vulgaris*, respectively. Other 4α-methylsterols from the immature seeds of *P. vulgaris* (including several new ones) have been reported recently [5]. A list of all identified 4α-methylsterols of *G. pentaphyllum*, their abundances and chromatographic data, is given in the Experimental.



RESULTS AND DISCUSSION

The acetyl derivative of a 4α-methylsterol **1a** was isolated from the 4α-methylsterol fraction of *P. vulgaris* seed extract as described in the Experimental. The mass spectrum of **1a**-acetate included peaks for $[M]^+$ at *m/z* 466 ($C_{32}H_{50}O_2$), and fragmentation ions at *m/z* 451 $[M-Me]^+$ and 391 $[M-Me-HOAc]^+$. This indicated that it was an acetate of a C_{30} 4α-methylsterol with three degrees of unsaturation. The base peak had at *m/z* 339 as a result of the loss of the side chain plus two hydrogens, a process typical of a sterol with one degree of unsaturation in the side chain and two in the nucleus [6, 7] possessing one additional methyl group in the nucleus and one in the side chain. The diagnostic peak at *m/z* 382 (loss of end of the side chain by McLafferty rearrangement [6–8]) had a low intensity because of competing skeletal fragmentation. Its occurrence limited the possibilities for the position of the double bond in the side chain to $\Delta^{24(28)}$ or $\Delta^{24(25)}$. The presence of fragment ions at *m/z* 299 $[M-\text{side chain}-C_3H_6$ (ring D)$]^+$ and 285 $[299-CH_2]$ suggested that the additional methyl group in the nucleus was located at C-14 [9, 10]. The ^{1}H NMR spectrum showed two olefinic protons at δ4.669 and 4.722 ($CDCl_3$) which were also present in the spectrum of obtusifoliol [4α,14α-dimethyl-5α-ergosta-8,24(28)-dien-3β-ol, **2a**] acetate (Table 1). This, together with the mass spectral evidence, proved the presence of the 24-methylene group.

Table 1. ^{1}H NMR data of the acetates of some 4α-methylsterols (400 MHz, TMS as int. standard)*

	Acetate									
				1b, c§		**1b**, c‖		**1b**, c (pyridine-d_5)‖		**2b**, c
H	**1a**†	**1a**‡	**1a** (pyridine-d_5)‡	(**1b**)	(**1c**)	(**1b**)	(**1c**)	(**1b**)	(**1c**)	**2a**	(**2b**)	(**2c**)
3α-H (*dt*)	4.397	4.397	4.598	4.396		4.396		4.593		4.379	4.383	
	(5.0, 11.0)	(4.4, 9.5)	(4.7, 11.5)	(4.4, 11.2)		(4.7, 11.1)		(4.7, 11.0)		(4.9, 11.0)	(5.0, 10.6)	
3β-OAc (*s*)	2.059	2.057	2.093	2.056		2.057		2.084		2.050	2.046	
7-H (*d*)	5.411	5.415	5.486	5.407		5.407		5.486		—	—	
	(8.2)	(7.2)	(6.3)	(8.2)		(8.5)		(6.7)				
11-H (*d*)	5.390	5.391	5.435	5.385		5.386		5.431		—	—	
	(9.0)	(8.8)	(5.8)	(8.7)		(7.8)		(6.3)				
18-H_3 (*s*)	0.581	0.583	0.696	0.576		0.574		0.699[a]	0.704[a]	0.710	0.708	
19-H_3 (*s*)	0.952	0.954	1.036	0.951		0.950		1.037		0.983	0.983	
21-H_3 (*d*)	0.922	0.923	0.985	0.882	0.890	0.881	0.891	0.963	0.966	0.929	0.893	0.902
	(6.6)	(6.6)	(5.0)	(7.1)	(6.6)	(7.2)	(6.4)	(6.4)	(6.5)	(6.6)	(6.0)	(6.0)
25-H (*sept*)	2.238	2.240	2.301	—		—		—		2.236	—	
	(7.0)	(7.1)	(7.2)							(6.6)		
26-H_3 (*d*)¶	1.029	1.029	1.084	0.856	0.860	0.855	0.860	0.890	0.896	1.025	0.856	0.859
	(7.2)	(6.6)	(6.8)	(6.6)	(6.6)	(6.8)	(6.9)	(6.4)	(6.4)	(6.6)	(7.0)	(6.4)
27-H_3 (*d*)¶	1.033	1.034	1.090	0.808	0.787	0.807	0.786	0.845	0.834	1.031	0.809	0.787
	(6.6)	(7.1)	(7.1)	(6.6)	(6.6)	(6.8)	(6.8)	(6.8)	(6.8)	(7.1)	(6.4)	(6.8)
28-H_2	4.668 (1H)	4.669 (1H)	4.883 (1H)	—		—				4.665 (1H)	—	
	(*d*, 1.1)	(*d*, 1.6)	(*s*)							(*d*, 1.6)		
	4.722 (1H)	4.722 (1H)	4.893 (1H)							4.716 (1H)		
	(*s*)	(*s*)	(*s*)							(*s*)		
28-H_3 (*d*)	—			0.785		0.784		0.845		—	0.784	0.782
				(6.6)		(6.8)		(6.8)			(6.7)	(6.4)
30-H_3 (*d*)	0.866	0.866	0.896	0.865		0.864		0.890		0.859	0.859	
	(6.6)	(6.0)	(7.0)	(6.0)		(6.4)		(6.6)		(6.6)	(6.4)	
31-H_3 (*s*)	0.896	0.899	0.991	0.891		0.890		0.988		0.891	0.889	

*Figures in parentheses denote *J* values (Hz). Unless stated otherwise, the spectra were recorded in $CDCl_3$. **2b**, **c**-Acetate was run at 600 MHz.

†Isolated from *Gynostemma pentaphyllum*.

‡Isolated from *Phaseolus vulgaris*.

§Prepared from **1a** isolated from *P. vulgaris*.

‖Prepared from obtusifoliol.

¶The higher-field signal is arbitrarily assigned to the 26-H_3, and the lower-field signal to the 27-H_3 as for the compound possessing side chain **a**.

[a]Assignment may be interchanged.

The UV spectrum (in EtOH) revealed maxima at 237, 243 and 251 nm typical of the presence of a $\Delta^{7,9(11)}$-diene system in the sterol nucleus [11, 12] which was associated with two olefinic methine signals at δ5.386 and 5.412 observed in the ^{1}H NMR spectrum ($CDCl_3$). The ^{1}H NMR spectrum ($CDCl_3$) further showed that **1a**-acetate possessed the common 3β-acetoxy-4α-methyl-5α-steroid nucleus by exhibiting the signals at δ0.866 (3H, *d*, 30-H_3, J = 6.0 Hz), 2.057 (3H, *s*, 3β-OAc), and 4.397 (1H, *dt*, 3α-H, J = 4.4, 9.5 Hz) which were almost identical with those of **2a**-acetate (Table 1). Thus, the structure of **1a** was assigned as 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol. This assignment was confirmed by chemical correlation with obtusifoliol (**2a**) acetate.

Δ^8-Mono-unsaturated 4,4,14-trimethylsteroids and triterpenoids yield the corresponding $\Delta^{7,9(11)}$-diene compounds upon dehydrogenation with selenium dioxide [12–14]. This reaction can be applied to the synthesis of $\Delta^{7,9(11)}$-di-unsaturated 4α-methylsterols from the corresponding Δ^8-monoenes. Thus, upon hydrogenation, **1a**-acetate afforded a mixture of the 24(28)-dihydro derivative, **1b**, **c**-acetate, which was identical (NMR) with a mixture of the epimers at C-24 prepared from obtusifoliol (**2a**) acetate by hydrogenation of the 24-methylene group followed by the selenium dioxide dehydrogenation of the resulting 24(28)-dihydro derivative, **2b**, **c**-acetate. The $\Delta^{7,9(11)}$-di-unsaturated 4α-methylsterol, **1a**, was also isolated from the acetylated 4α-methylsterol fraction of *Gynostemma pentaphyllum* extract. See Table 1 for a NMR comparison.

The ^{1}H NMR spectra of **1b**, **c** and **2b**, **c** were partially assigned using triterpene data of 24-methylene-5α-lanosta-7,9(11)-diene-3β-ol [15]. The assignment was supported by ^{1}H–^{1}H 2D COSY NMR data of synthetic **1b**, **c**-acetate in both chloroform-*d* and pyridine-d_5. The COSY experiments made it also possible to assign several ^{1}H signals, in addition to those listed in Table 1 (see Experimental section). The ^{13}C NMR spectral data for synthetic **1b**, **c**-acetate are listed in Table 2. These assignments were based on the DEPT experiments and by the comparison of the ^{13}C NMR data with those of relevant compounds reported in the literature [15–18].

This appears to be the first report of the occurrence of a 4α-methylsterol possessing a $\Delta^{7,9(11)}$-diene system from natural sources. The presence of **1a** in plants of the taxonomically not closely correlated Leguminosae and Cucurbitaceae families may suggest its wider distribution in higher plants.

Several $\Delta^{7,9(11)}$-di-unsaturated 4,4,14-trimethylsterols and 4,14-demethylsterols are known to occur in nature. The known 4,4,14-trimethylsterols (lanostane type triterpene alcohols) are agnosterol [5α-lanosta-7,9(11),24-trien-3β-ol] and its 24-dihydro derivative, 24-dihydroagnosterol from wool fat [3, 12], and 24-methylene-5α-lanosta-7,9(11)-dien-3β-ol from *Artabotrys odoritissimus* (Annonaceae) [15] and *Neolitsea sericea* (Lauraceae) (K. Yano, T. Akihisa, T. Tamura and T. Matsumoto, unpublished results). The known 4,14-demethylsterols are 5α-stigmasta-7,9(11),*Z*-24(28)-trien-3β-ol from sunflower oil [19] and 5α-ergosta-7,9(11),24(28)-trien-3β-ol from a Black Sea sponge, *Haliclona flavescens* [20].

After about 20 years of intense isolation work marine organisms might perhaps be almost exhausted as sources of new sterols. The isolation of **1a** from two different sources shows again that it is not too hard to find new sterols in higher plants. The number of known plant sterols [21, 22] is still far behind the number of known marine sterols [23–25] (≈140 vs >200).

Table 2. ^{13}C NMR spectral data (100.62 MHz, $CDCl_3$) of the epimeric acetates of compounds **1b**, **c***

C	**1b**-acetate	**1c**-acetate
1	34.34 (CH_2)	
2	27.36 (CH_2)	
3	78.33 (CH)	
4	36.72 (CH)	
5	45.58 (CH)	
6	26.42 (CH_2)	
7	118.97 (CH)	
8	142.97 (C)	
9	143.13 (C)	
10	36.31 (C)	
11	117.40 (CH)	
12	37.88 (CH_2)	
13	43.73 (C)	
14	50.38 (C)	
15	31.48 (CH_2)	
16	27.86 (CH_2)	27.90 (CH_2)
17	51.05 (CH)	50.91 (CH)
18	15.70 (Me)	
19	20.53 (Me)	
20	36.41 (CH)	36.60 (CH)
21	18.49 (Me)	18.67 (Me)
22	33.93 (CH_2)	
23	30.63 (CH_2)	30.94 (CH_2)
24	38.86 (CH)	39.09 (CH)
25	32.43 (CH)	31.42 (CH)
26	20.20 (Me)	17.56 (Me)
27	18.29 (Me)	20.60 (Me)
28	15.41 (Me)	15.45 (Me)
30	15.11 (Me)	
32	25.60 (Me)	
COMe	21.34 (Me)	
COMe	170.91 (C)	

*Carbon types were determined by DEPT experiments.

EXPERIMENTAL

Crystallizations were performed from Me_2CO–MeOH. Mps: uncorr. Prep. TLC: silica gel plates were developed ×3 with hexane–EtOAc (6:1); argentation prep. TLC: silica gel–$AgNO_3$ (4:1) plates were developed ×3 with CCl_4–CH_2Cl_2 (5:1). HPLC: Altex Ultrasphere ODS 5 μ column (Beckman; 25 cm × 10 mm i.d.), MeOH–H_2O (49:1) as mobile phase (flow rate, 4 ml/min). GC: SCOT OV-17 glass capillary column (30 m × 0.3 mm i.d.), column temp. 255°. RR_t on HPLC and GC listed are relative to cholesteryl acetate. All EIMS were run at 70 eV with a probe. The MS data do not include peaks with m/z <200. ^{1}H NMR (400 or 600 MHz) and ^{13}C NMR (100.62 MHz) spectra were determined in $CDCl_3$ or in pyridine-d_5 with TMS as int. standard. UV spectra were recorded in EtOH. Acetylation: Ac_2O–pyridine at room temp. overnight. Alkaline hydrolysis of the steryl acetate was performed in 5% KOH–MeOH at room temp. overnight. Hydrogenation was carried out in EtOH using pre-reduced PtO_2 at atm. pres. and room temp. overnight.

Obtusifoliol (**2a**) was isolated from the latex of *Euphorbia regis-jubae* [26].

Isolation of 4α,14α-*dimethyl*-5α-*ergosta*-7,9(11),24(28)-*trien*-3β-*ol* (**1a**) *from* Phaseolus vulgaris *seeds.* The 4α-methylsterol fraction separated from the MeOH extract of the immature seeds of *P. vulgaris* was acetylated, and the acetate fraction (167 mg) subjected to argentation TLC which afforded 4 bands [5]. The fraction (27 mg) from the most polar band (R_f 0.07–0.21), contained **1a**-acetate, was further fractionated by HPLC yielding **1a**-acetate (RR_t: HPLC, 0.66; GC, 1.45) (0.6 mg) isolated. See ref. [5] for a chart of other 4α-methylsterols from the same source. Abundance of **1a**-acetate in the total acetylated 4α-methylsterols was found to be 1.8% as determined by GC. Mp 123–124° MS *m/z* (rel. int.): 466.3806 $[M]^+$ (97, $C_{32}H_{50}O_2$, requires 466.3807), 451.3621 (19, $C_{31}H_{47}O_2$), 423.3231 (5, $C_{29}H_{43}O_2$), 406.3641 (8, $C_{30}H_{46}$), 391.3340 (24, $C_{29}H_{43}$), 382.2871 (5, $C_{26}H_{38}O_2$), 367.2638 (14, $C_{25}H_{35}O_2$), 339.2303 (100, $C_{23}H_{31}O_2$), 325.2218 (5, $C_{22}H_{29}O_2$), 314.2198 (5, $C_{21}H_{30}O_2$), 309.2619 (5, $C_{23}H_{33}$), 307.2423 (5, $C_{23}H_{31}$), 300.2057 (22, $C_{20}H_{28}O_2$), 299.1993 (32, $C_{20}H_{27}O_2$), 298.1899 (22, $C_{20}H_{26}O_2$), 287.2013 (5, $C_{19}H_{27}O_2$), 285.2262 (8, $C_{19}H_{27}O_2$), 281.2262 (11, $C_{21}H_{29}$), 274.1927 (14, $C_{18}H_{26}O_2$), 265.1940 (5, $C_{20}H_{25}$), 259.1657 (8, $C_{17}H_{23}O_2$), 239.1769 (65, $C_{18}H_{23}$), 227.1779 (22, $C_{17}H_{23}$), 226.1723 (35, $C_{17}H_{22}$), 225.1646 (30, $C_{17}H_{21}$), 213.1635 (22, $C_{16}H_{21}$), 211.1487 (22, $C_{16}H_{19}$). UV λ_{max} nm: 237, 243, 251. For the ^{1}H NMR data, see Table 1. Hydrogenation of **1a**-acetate afforded 4α,14α-dimethyl-5α-ergosta-7,9(11)-dien-3β-ol (**1b, c**) acetate (C-24 epimeric mixture).

4α,14α-*Dimethyl*-5α-*ergosta*-7,9(11)-*dien*-3β-*ol* (**1b, c**) *acetate prepared from* **1a**-*acetate by hydrogenation.* RR_t: HPLC, 0.91; GC, 1.43. MS *m/z* (rel. int.): 468.3973 $[M]^+$ (100, $C_{32}H_{52}O_2$, requires 468.4965), 453.3769 (17, $C_{31}H_{49}O_2$), 408.3733 (6, $C_{30}H_{48}$), 393.3480 (15, $C_{29}H_{45}$), 341.2428 (6, $C_{23}H_{33}O_2$), 299.2025 (15, $C_{20}H_{27}O_2$), 287.2032 (11, $C_{19}H_{27}O_2$), 281.2264 (9, $C_{21}H_{29}$), 274.1918 (9, $C_{18}H_{26}O_2$), 239.1789 (30, $C_{18}H_{23}$), 226.1736 (23, $C_{17}H_{22}$), 225.1668 (15, $C_{17}H_{21}$), 213.1676 (9, $C_{16}H_{21}$), 211.1488 (11, $C_{16}H_{19}$). UV λ_{max} nm: 233, 243, 250. For the ^{1}H NMR data, see Table 1.

Isolation of 4α-*methylsterols including* 4α,14α-*dimethyl*-5α-*ergosta*-7,9(11),24(28)-*trien*-3β-*ol* (**1a**) *from* Gynostemma pentaphyllum. Air-dried aerial parts (20 kg) of *G. pentaphyllum* were extracted with CH_2Cl_2 under reflux for 7 hr to give 580 g lipid which was saponified (5% KOH in MeOH) under reflux for 3 hr and then unsaponifiable lipid (107 g) were subjected to CC over silica gel (700 g). Elution in order of increasing polarity: 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) [2, 27]. The fractions (8.68 g) eluted with hexane–Et_2O (4:1) were found to contain 4α-methylsterols (the elution was monitored by TLC on precoated silica gel). They yielded a clean 4α-methylsterol mixture (30 mg) by successive CC and prep. TLC purification. The 4α-methylsterol mixture was acetylated, and the acetates (25 mg) were subjected to HPLC fractionation to give six isolated 4α-methylsteryl acetates: **1a**-acetate (RR_t: HPLC, 0.70; GC, 1.46) (0.3 mg; abundance in the 4α-methylsterol mixture as determined by GC, 1.9%); **2a**-acetate (RR_t: HPLC, 0.83; GC, 1.49) (1.1 mg; 1.7%); cycloeucalenol [4α-methyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol] acetate (RR_t: HPLC, 0.90; GC, 1.77) (1.0 mg; 13.4%); gramisterol [4α-methyl-5α-ergosta-7,24(28)-dien-3β-ol] acetate (RR_t, HPLC, 0.99; GC, 1.79) (4.5 mg; 60.0%); 24β-ethyl-25-dehydrolophenol [4α-methyl-24β-ethyl-5α-cholesta-7,25-dien-3β-ol] acetate (RR_t: HPLC, 1.13; GC, 2.17) (1.7 mg; 7.4%); and citrostadienol [4α-methyl-5α-stigmasta-7*Z*,24(28)-dien-3β-ol] acetate (RR_t: HPLC, 1.21; GC, 2.38) (1.1 mg; 4.9%). Identification of the latter five 4α-methylsteryl acetates was based on their HPLC and GC data [5]. MS of **1a**-acetate: *m/z* (rel. int.): 466 $[M]^+$ (100), 451 (23), 423 (7), 406 (10), 391 (30), 382 (7), 367 (17), 339 (92), 300 (27), 299 (40), 298 (27), 287 (17), 281 (13), 274 (17), 239 (80), 227 (27), 226 (43), 225 (37), 213 (25), 211 (27). For ^{1}H NMR data of **1a**-acetate, see Table 1. Identification of 24β-ethyl-25-dehydrolophenyl acetate was supported by the following spectroscopic data [28]: Mp 166–168°. MS *m/z* (rel. int.): 468 $[M]^+$ (49), 453 (23), 408 (4), 393 (5), 357 (6), 356 (5), 327 (100), 313 (5), 302 (9), 287 (7), 269 (34), 243 (11), 241 (14), 227 (23), 215 (7), 213 (5). ^{1}H NMR data ($CDCl_3$, 400 MHz). Assignment, δ (multiplicity, *J* in Hz): 3α-H, 4.401 (*dt*, 3.8, 11.0); 3β-OAc, 2.052 (*s*); 18-H_3, 0.525 (*s*); 19-H_3, 0.836 (*s*); 21-H_3, 0.909 (*d*, 6.6); 26-H_3, 1.565 (*d*, 1.0); 27-H_2, 4.642 (1H, *d*, 2.8), 4.729 (1H, *dt*, 3.8, 1.4); 29-H_3, 0.803 (*t*, 7.4); 30-H_3, 0.850 (*d*, 6.6).

Synthesis of 4α,14α-*dimethyl*-5α-*ergosta*-7,9(11)-*dien*-3β-*ol* (**1b, c**) *acetate from obtusifoliol* (**2a**) *acetate.*

4α,14α-*Dimethyl*-5α-*ergost*-8-*en*-3β-ol (**2b, c**) *acetate.* Catalytic hydrogenation of **2a**-acetate (mp. 113–115°; MS *m/z*: 468 $[M]^+$; see Table 1 for the ^{1}H NMR data) (100 mg) yielded the C-24 (28) dihydro derivative, **2b, c**-acetate (RR_t: GC, 1.46) (97 mg). Mp 126–128°. MS *m/z*: 470 $[M]^+$. For ^{1}H NMR data, see Table 1.

Synthetic 4α,14α-*dimethyl*-5α-*ergosta*-7,9(11)-*dien*-3β-*ol* (**1b, c**) *acetate.* A soln of SeO_2 (100 mg) in 96% HOAc (2 ml) was added to a soln of **2b, c**-acetate (50 mg) in glacial HOAc (14 ml) and the mixture gently refluxed for 20 hr. After the usual work-up and argentation TLC, 4α,14α-dimethyl-5α-ergosta-7,9(11)-dien-3β-ol (**1b, c**) acetate was obtained (RR_t: HPLC, 0.91; GC, 1.42) (13 mg). Mp 129–131°. MS *m/z* (rel. int.): 468 $[M]^+$ (100), 453 (17), 408 (5), 393 (14), 341 (10), 299 (17), 287 (10), 281 (6), 274 (9), 239 (23), 226 (18), 225 (12), 213 (7), 211 (7). UV λ_{max}: 233, 242, 250 nm. The ^{1}H and ^{13}C NMR spectral data are listed in Tables 1 and 2, respectively. ^{1}H NMR data not included in Table 1 (400 MHz) are given below. Assignment ($CDCl_3$), δ(multiplicity, *J* in Hz): 1α-H, *ca* 1.33 (*m*); 1β-H, *ca* 1.58 (*m*); 2α-H, 1.941 (*ddt*, 12.4, 12.6, 4.1); 2β-H, 1.414 (*ddt*, 4.1, 13.0, 13.7); 4β-H, 1.568 (*ddq*, 12.5, 14.0, 5.9); 5α-H, 1.091 (*dt*, 4.1, 12.2); 6α-H, 2.212 (*ddd*, 4.1, 8.0, 17.2); 6β-H, 1.759 (*dd*, 11.1, 17.5); 12α-H, 2.103 (*ddd*, 1.6, 6.7, 17.5); 12β-H, 2.230 (*br d*, 15.8). ^{1}H NMR data (pyridine-d_5). Assignment, δ (multiplicity, *J* in Hz): 1α-H, *ca* 1.37 (*m*); 1β-H, *ca.* 1.47 (*m*); 2α-H, 2.007 (*ddt*, 7.6, 8.5, 4.3); 2β-H, *ca* 1.72 (*m*); 4β-H, 1.588 (*ddq*, 13.4, 15.8, 6.7); 5α-H, 1.115 (*dt*, 3.6, 11.1); 6α-H, 2.180 (*ddd*, 6.2, 6.3, 17.8); 6β-H, 1.739 (*dd*, 12.1, 17.4); 12α-H, 2.137 (*ddd*, 4.7, 4.8, 17.7); 12β-H, 2.271 (*br d*, 17.7).

Synthetic 4α,14α-*dimethyl*-5α-*ergosta*-7,9(11)-*dien*-3β-*ol* (**1b, c**). Alkaline hydrolysis of **1b, c**-acetate gave free sterol **1b, c**. Mp 169–170°. MS *m/z* (rel. int.): 426.3879 $[M]^+$ (100, $C_{30}H_{50}O_1$, requires 426.3859), 411.3638 (22, $C_{29}H_{47}O_1$), 393.3545 (6, $C_{29}H_{45}$), 299.2342 (11, $C_{21}H_{31}O_1$), 257.1901 (37, $C_{18}H_{25}O_1$), 244.1812 (15, $C_{17}H_{24}O_1$), 239.1768 (12, $C_{18}H_{23}$), 232.1825 (9, $C_{16}H_{24}O_1$), 226.1713 (12, $C_{17}H_{22}$), 217.1598 (6, $C_{15}H_{21}O_1$), 211.1492 (6, $C_{16}H_{19}$). ^{1}H NMR data ($CDCl_3$, 600 MHz). Assignment, δ (multiplicity, *J* in Hz): 3α-H, 3.118 (*dt*, 4.7, 11.0); 7-H, 5.421 (*d*, 6.7); 11-H, 5.386 (*d*, 6.1); 18-H_3, 0.581 (either for 24α- or 24β-epimer) and 0.583 (either for 24β- or 24α-epimer) (*s*); 19-H_3, 0.942 (*s*); 21-H_3: 0.887 (24α, *d*, 6.5), 0.895 (24β, *d*, 6.3); 26-H_3: 0.858 (24α, *d*, 6.9), 0.863 (24β, *d*, 7.1); 27-H_3: 0.811 (24α, *d*, 7.0), 0.791 (24β, *d*, 7.1); 28-H_3: 0.788 (24α, *d*, 6.6), 0.787 (24β, *d*, 6.9); 30-H_3, 1.001 (*d*, 6.5); 32-H_3, 0.895 (*s*).

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