Phytochemistry, Vol 28, No 4, pp 1219–1224, 1989
Printed in Great Britain

0031-9422/89 $3.00+0.00

25-METHYLGRAMISTEROL AND OTHER 4α-METHYLSTEROLS FROM *PHASEOLUS VULGARIS* SEEDS

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(*Received* 18 *July* 1988)

Key Word Index—*Phaseolus vulgaris*; Leguminosae, seeds; 4α-methyl sterol, 25-methylgramisterol; 24-methylene-25-methyllophenol, 24*Z*-ethylidene-4α-methyl-5α-cholest-8(14)-en-3β-ol, 24*E*- and 24*Z*-ethylidene-4α, 14α-dimethyl-5α-cholest-8-en-3β-ol, 24*Z*-ethylidene-4α, 14α-dimethyl-5α-cholest-9(11)-en-3β-ol

Abstract—The structures of five new 4α-methylsterols isolated from the immature seeds of *Phaseolus vulgaris* have been shown to be 25-methylgramisterol, 24*Z*-ethylidene-4α-methyl-5α-cholest-8(14)-en-3β-ol, 24*E*- and 24*Z*-ethylidene-4α, 14α-dimethyl-5α-cholest-8-en-3β-ol and 24*Z*-ethylidene-4α, 14α-dimethyl-5α-cholest-9(11)-en-3β-ol. In addition, four known but uncommon 4α-methylsterols, 28-isocitrostadienol, 4α-methylfecosterol, 24*Z*-ethylidene-4α-methylcholest-8-en-3β-ol, and 24-methylene-4α, 14α-dimethyl-5α-cholest-9(11)-en-3β-ol, together with several common 4α-methylsterols were isolated from the seeds and identified

INTRODUCTION

Our recent study has shown that the immature seeds of *Phaseolus vulgaris* cv Kentucky Wonder contained 24-methylene-25-methylcholest-5-en-3β-ol (24-methylene-25-methylcholesterol, **7f**) as one of the sterol constituents [1]. The structure of **7f** is unusual because it possesses a tertiary butyl moiety in the side chain. Moreover, detection of **7f** in the seeds of *P. vulgaris* was of interest from the standpoint of structural correlation between sterols and brassinosteroids since the seeds have been demonstrated to contain 25-methyldolichosterone [(22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-25-methyl-5α-ergost-24(28)-en-6-one], a brassinosteroid possessing a 24-methylene-25-methyl moiety in the side chain, as one of the major brassinosteroids [2]. This paper describes an investigation on the 4α-methylsterols, intermediates for (4-demethyl)sterol biosynthesis [3,4], which led to the isolation and characterization of a new 4α-methylsterol possessing a 24-methylene-25-methyl-cholestane side chain, 25-methylgramisterol (**1f**), in addition to several other new and known 4α-methyl sterols

RESULTS

4α-Methylsterols were isolated as the acetyl derivatives from *P. vulgaris* seeds by the procedure described in the Experimental section. The composition of the 4α-methylsterol fraction, and molecular ion peaks, mp, and *RRt* in GC and HPLC of the 15 4α-methylsterols isolated and identified or newly characterized are given in Table 1. Composition of the 4α-methylsterol fraction was determined based on the argentation TLC and GC data. Identification of the following eight 4α-methylsterols (see Table 1 for the name of compounds) was performed by comparison of the mp, chromatographic (GC [5], HPLC and argenation TLC), MS and ^{1}H NMR data with those of authentic compounds as the acetates: **1a**, **1b**, **1c**, **1d**, **1e**, **4b**, **5b**, and **6b**. The ^{1}H NMR signal assignments were performed by comparison with the literature data [6–9]. Identification of two further known compounds, **2b** and **2d**, for which reference specimens were unavailable, was performed by comparison of ^{1}H NMR [6, 10, 11] and mass spectral [11] data. The characterization of the five 4α-methylsterols, **1f**, **3d**, **5d**, **5e**, and **6d**, the natural occurrence of which has so far been unreported, is described below.

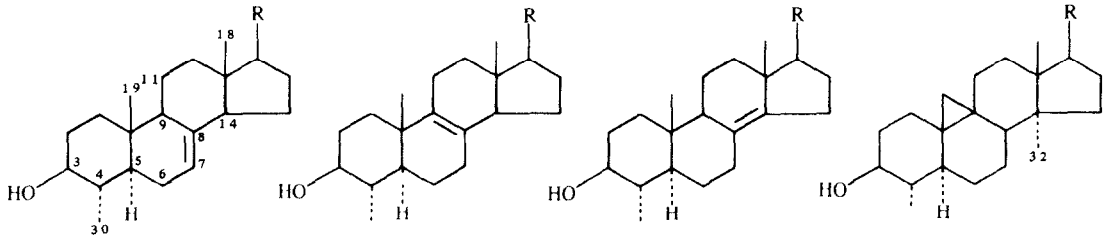
The mass spectrum of **1f**-acetate showed $[M]^+$ at *m/z* 468 ($C_{32}H_{52}O_2$) accompanied with fragmentation ions at *m/z* 453 $[M-Me]^+$, 393 $[M-Me-HOAc]^+$, and 327 $[M-C_{10}H_{19}$ (side chain) $-2H]^+$ indicating that it was an acetate of a C_{30} 4α-methylsterol with two double bonds, one of which was in the C_{10} side chain and the other in the ring system [12, 13]. The presence of a further ion at *m/z* 370 $[M-C_7H_{13}$ (part of side chain)$-1H]^+$, 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 C-24 [12–14]. The ^{1}H NMR spectrum of **1f**-acetate showed signals due to the side chain protons at δ 0.969 (3H, *d*, *J* = 6.4 Hz, 21-H_3), 1.059 (9H, *s*, 26-H_3, 27-H_3, 29-H_3), and 4.661 (1H, *d*, *J* = 1.0 Hz) and 4.837 (1H, *s*) (28-H_2), in addition to those arising from the 3β-acetoxy-4α-methyl-Δ^7 5α-steroid skeleton [6] (Table 2). The ^{1}H NMR spectral signals for the side chain protons were almost consistent with those of **7f**-acetate [1, 15] (Table 2) and its Δ^7-isomer, 24-methylene-25-methyl-5α-cholest-7-en-3β-ol acetate [16], and hence, **1f** was considered to have the structure 24-methylene-4α,25-dimethyl-5α-cholest-7-en-3β-ol (25-methylgramisterol or 24-methylene-25-methyllophenol)

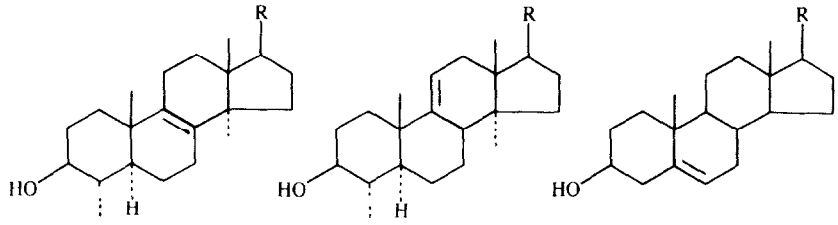
Table 1 Composition, the molecular ion $[M]^+$, mp and chromtographic data of the 4α-methylsterols from *Phaseolus vulgaris* seeds

4α-Methylsterol*	Composition	Acetate $[M]^+$ (*m/z*)	Acetate Mp (°)	Acetate RR_t (GC)†	Acetate *RRt* (HPLC)†
1a 24α-Methyllophenol (4α, 24α-dimethylcholest-7-enol)	0.9	456	140–141	1 72	1 34
1b Gramisterol (24-methylenelophenol)	5 6	454	137–138	1 79	0 97
1c 24α-Ethyllophenol	6 3	470	156–157	2 10	1 54
1d Citrostadienol (24*Z*-ethylidenelophenol)	22 2	468	146–148	2 40	1 21
1e 28-Isocitrostadienol (24*E*-ethylidenelophenol)	0 9	468	138–141	2 29	1.21
1f 25-Methylgramisterol (24-methylene-25-methyllophenol)	0 3	468	137–140	2 20	1 14
2b 4α-Methylfecosterol (24-methylene-4α-methylcolest-8-enol)	4 0	454	111–113	1 58	0 91
2d 24*Z*-Ethylidene-4α-methylcholest-8-enol	3 3	468	130–132	2 13	1 13
3d 24*Z*-Ethylidene-4α-methylcholest-8(14)-enol	3 1	468	116–117	2 03	1 10
4b Cycloeucalenol (24-methylene-9β, 19-cyclo-4α, 14α-dimethylcholestanol)	6 2	468	110–112	1 77	0 96
5b Obtusifoliol (24-methylene-4α, 14α-dimethylcholest-8-enol)	27 6	468	111–113	1 49	0 84
5d 24*Z*-Ethylidene-4α, 14α-dimethylcholest-8-enol	6 1	482		1 97	1 08
5e 24*E*-Ethylidene-4α, 14α-dimethylcholest-8-enol	2 3	482	—	1 88	1 08
6b 24-Methylene-4α, 14α-dimethylcholest-9(11)-enol	0 8	468	105–107	1 69	0.84
6d 24*Z*-Ethylidene-4α, 14α-dimethylcholest-9(11)-en-3β-ol	3 0	482	125–127	2 25	1 07
Others, unidentified	7 4	—	—	—	—

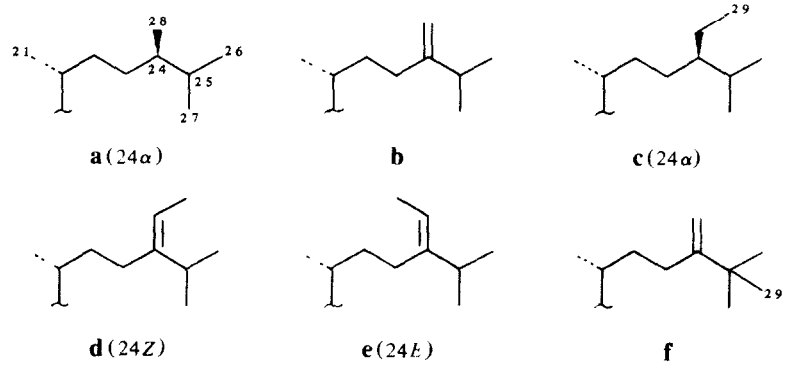
*All 4α-methylsterols possess the 3β-hydroxy-5α-steroid structure

†RR_t were expressed relative to cholesterol acetate





Side chains (R)



The mass spectrum of **3d**-acetate showed $[M]^+$ at *m/z* 468 ($C_{32}H_{52}O_2$) accompanied with the fragmentation ions at *m/z* 453 $[M-Me]^+$, 393 $[M-Me-HOAc]^+$, 370 $[M-C_7H_{13}$ (part of side chain) $-1H]^+$, and 327 $[M-C_{10}H_{19}$ (side chain) $-2H]^+$ suggesting that it was an acetate of a C_{30} 4α-methylsterol with two double bonds, one of which was in the ring system and the other was at C-24 in the C_{10} side chain [12–14] The ^{1}H NMR spectrum of **3d**-acetate showed the side chain ^{1}H signals almost consistent with those of **1d**-acetate (Table 2) indicating that it possesses 24*Z*-ethylidene-cholestane side chain. The ^{1}H NMR spectral signals for the skeletal protons at δ 0 728 (*s*, 19-H_3), 0.841 (*s*, 18-H_3), 0.849 (3H, *d*, *J* = 6.6 Hz, 30-H_3), 2.050 (*s*, 3β-OAc), and 4.415 (1H, *dt*, *J* = 4 4, 10.4 Hz, 3α-H) were almost indistinguishable from those of authentic $\Delta^{8(14)}$-4α-methylsterol acetate, **3a**-acetate (Table 2), and thus, **3d**-was regarded to have the structure 24*Z*-ethylidene-4α-methyl-5α-cholest-8(14)-en-3β-ol.

The mass spectrum of **5d**-acetate showed $[M]^+$ at *m/z* 482 ($C_{33}H_{54}O_2$) accompanied with the fragmentation ions at *m/z* 467 $[M-Me]^+$, 407 $[M-Me-HOAc]^+$, 384 $[M-C_7H_{13}$ (part of side chain) $-1H]^+$, 369 (*m/z* 384 $-$ Me), and 341$[M-C_{10}H_{19}$ (side chain) $-2H]^+$ suggesting that it was an acetate of C_{31} 4α-methylsterol with two double bonds, one of which was in the skeleton and the other was at C-24 in the C_{10} side chain [12 – 14] Further ions at *m/z* 301 $[M-$ ring D$]^+$ and 287 (*m/z* 301 $-CH_2$) suggested the presence of a 14α-methyl group [17]. The ^{1}H NMR spectrum of **5d**-acetate displayed signals due to the side chain protons at δ 0.926 (3H, *d*, *J* = 6.6 Hz, 21-H_3), 0.980 (6H, *d*, *J* = 7 1 Hz, 26-H_3, 27-H_3), 1.593 (3H, *t*, *J* = 6.6 Hz, 29-H_3), 2.834 (1H, *sept*, *J* = 6.9 Hz, 25-H), and 5.124 (1H, *q*, *J* = 6.6 Hz, 28-H), in addition to the signals arising from the 3β-acetoxy-4α, 14α-dimethyl-Δ^8 5α-steroid skeleton which were almost consistent with those of **5b**-acetate (Table 2). The ^{1}H NMR signals for the side chain protons of **5d**-acetate were indistinguishable from those of 24*Z*-ethyliene-5α-lanost-8-en-3β-ol acetate [18] and very similar to those of **1d**-acetate (Table 2), and hence, **5d** was considered to have the structure 24*Z*-ethylidene-4α, 14α-dimethyl-5α-cholest-8-en-3β-ol. The mass spectral data of **5e**-acetate were indistinguishable from those of **5d**-acetate. While the ^{1}H NMR spectrum of **5e**-acetate showed the signals arising from the skeletal protons consistent with those of **5d**-acetate (Table 2), it showed the signals due to the side chain protons at δ 0.969 (3H, *d*, *J* = 6.6 Hz, 29-H_3), 2.202 (1H, *sept*, *J* = 6.8 Hz, 25-H), and 5 124 (1H, *q*, *J* = 6 6 Hz, 28-H), which were indistinguishable from those of 24*E*-ethylidene-5α-lanost-8-en-3β-ol acetate [18] and very similar to those of **1e**-acetate (Table 2). Thus, **5e** was considered to be the 24*E*-isomer of **5d**, i e. 24*E*-ethylidene-4α, 14α-dimethyl-5α-cholest-8-en-3β-ol.

6d-Acetate showed $[M]^+$ at *m/z* 482 ($C_{33}H_{54}O_2$) accompanied by the prominent fragmentation ions at *m/z* 467, 407, 384, 369, 341, 301, and 287 in the mass spectrum, analogous to those of **5d**- and **5e**-acetates described above, suggesting that **6d** also was a C_{31} 4α-methylsterol with a methyl group at C-14 and two double bonds, one of which was in the skeleton and the other was at C-24 in the C_{10} side chain [12–14, 17]. The ^{1}H NMR spectrum of **6d**-acetate showed signals arising from the skeletal protons at δ 0.658 (*s*, 18-H_3), 0 748 (*s*, 32-H_3), 0.845 (3H, *d*, *J* = 6.3 Hz, 30-H_3), 1.006 (*s*, 19-H_3), 2.053 (*s*, 3β-OAc), 4.366 (1H, *dt*, *J* = 5 0, 10.8 Hz, 3α-H), and 5 303 (1H, *m*, 11-H), in addition to those due to a 24*Z*-ethylidene side chain. The skeletal proton signals were consistent with those of **6b**-acetate possessing a 3β-acetoxy-4α, 14α-dimethyl-$\Delta^{9(11)}$ 5α-steroid skeleton, and hence, **6d** was regarded to be 24*Z*-ethylidene-4α, 14α-dimethyl-5α-cholest-9(11)-en-3β-ol

DISCUSSION

We have identified or newly characterized the fifteen 4α-methylsterols isolated from the immature seeds of *Phaseolus vulgaris* in this study (Table 1). Among them, five 4α-methylsterols, **1f**, **3d**, **5d**, **5e**, and **6d**, were new 4α-methylsterols from natural sources. On the other hand, the other four 4α-methylsterols, **1e**, **2b**, **2d**, and **6b**, have so far been reported to occur only in a few restricted higher plants, for example, **1e** in ricebran oil, some species of Solanaceae, and olive oil [19], **2b** and **2d** in suspension cultures of bramble cells [11], and **6b** in banana peel and olive oil [19]. Although two 24-alkyl-Δ^7-4α-methylsterols, **1a** (24ξ) and **1c** (24ξ), are known to present as the minor 4α-methylsterols in several higher plants [19, 20], this study seems to be one of the rare cases [19, 21] where the configuration at C-24 of these compounds was unambiguously determined. The remaining four 4α-methylsterols, **1d**, **5b**, **1b**, and **4b**, of which the first two were the most abundant 4α-methylsterols of *P. vulgaris* seeds (Table 1), are known to be the most common and dominant 4α-methylsterols in the great majority of higher plants [3, 19, 21, 22].

The occurrence of **1f** in *P. vulgaris* seeds is of interest from the structural as well as the biogenetic point of view concerning sterols and brassinosteroids since it has recently been demonstrated that the seeds contain 24-methylene-25-methylcholesterol (**7f**) [1] and 25-methyldolicohosterone [2], both of which possess a 24-methylene-25-methyl moiety in the side chain. 4-Demethyl sterols are biosynthesized via 4α-methylsterols as intermediates [3, 4], and taking this into account, the 4α-methylsterol **1f** might be expected to be an intermediate of the biosynthesis of **7f** in *P. vulgaris* seeds. Moreover, **1f** and **7f** are considered to be closely correlated to the biosynthesis of 25-methyldolichosterone.

Although $\Delta^{8(14)}$-4α-methylsterols are reported to constitute the dominant 4α-methylsterols of an oyster [23], the occurrence of this type of 4α-methyl sterol in higher plants is rare. 4α-Methyl-5α-cholest-8(14)-en-3β-ol, detected in several species of Solanaceae [19], might be the only $\Delta^{8(14)}$-4α-methylsterol so far been detected in higher plants, and this study seems to be the second instance for the detection of this type of compound, i. e **3d**, in a higher plant. $\Delta^{8(14)}$-Sterols have been suggested as intermediates arising during sterol biosynthesis as a consequence of the C-14 demethylation [21, 24], and hence, the co-occurrence of **3d** with **1d**, **2d**, and **5d** in *P. vulgaris* seeds may imply that the biosynthetic pathway of sterols in this study proceeds in the following way: **5d**→**3d**→**2d**→**1d**.

Co-occurrence of two 24*E*-ethylidene-4α-methylsterols, **1e** and **5e**, in the 4α-methylsterol fraction, and fucosterol (24*E*-ethylidenecholesterol, **7e**) in the 4-demethyl sterol fraction [1], in *P. vulgaris* seeds might suggest the presence of the 24*E*-ethylidene route of sterol biosynthesis via **5e** and **1e** to afford **7e** in the seeds. Two $\Delta^{9(11)}$-4α, 14α-dimethylsterols, **6b** and **6d**, have been detected in *P. vulgaris* seeds in this study, and one possible origin of these 4α-methylsterols is the $\Delta^{9(11)}$-

Table 2 ^{1}H NMR data of the acetates of the 4α-methylsterols from *Phaseolus*

Acetate	18-H_3 (*s*)	19-H_3 (*s*) or 19-H_2	30-H_3 (*d*)	32-H_3 (*s*)	21-H_3 (*d*)	26-H_3 (*d*)	27-H_3 (*d*)	28-H_3 or 28-H_2	29-H_3 (*d* or *t*)
1a	0.533	0.840	0.852(6.6)	--	0.915(6.6)	0.852(6.6)	0.805(7.1)	0.776(*d*, 6.6)	--
1b	0.537	0.840	0.853(6.4)	—	0.955(6.4)	1.024(6.8)		4.659(1.5)†	—
						1.030(6.8)		4.716(1H,*s*)	
1c	0.535	0.829	0.853(5.4)	--	0.926(6.4)	0.838(6.8)	0.817(6.4)	-	0.846(8.3)
1d	0.537	0.841	0.853(6.1)	—	0.951(6.6)	0.978(6H,7.1)			1.591(7.1)
1e	0.542	0.843	0.853(6.4)	-	0.995(5.9)	0.980(6.8)		-	1.574(7.2)
						0.983(6.8)			
1f	0.544	0.842	0.853(6.4)	—	0.969(6.4)	1.059(6H,*s*)		4.661(1.0)‡	1.059(*s*)
								4.837(1H,*s*)	
2b	0.619	0.987	0.856(6.5)	—	0.959(6.5)	1.024(6.8)		4.659(1.5)‡	-
						1.031(6.8)		4.714(1H,*s*)	
2d	0.609	0.987	0.856(6.6)	—	0.955(6.6)	0.978(6H,7.1)			1.591(7.1)
3a†	0.835	0.727	0.847(6.4)	—	0.928(6.3)	0.863(6.3)			--
						0.867(6.3)			
3d	0.841	0.728	0.849(6.6)	--	0.959(6.6)	0.976(6.6)		--	1.593(6.4)
						0.977(7.7)			
4b	0.972	0.151(3.9)‡	0.845(6.6)	0.902	0.899(6.6)	1.027(6.6)		4.665(1.7)‡	-
		0.402(3.8)‡				1.032(6.6)		4.718(1H,*s*)	
5b	0.710	0.983	0.859(6.6)	0.891	0.929(6.6)	1.025(6.6)		4.665(1.6)‡	—
						1.031(7.1)		4.716(1H,*s*)	
5d	0.710	0.984	0.859(6.6)	0.888	0.926(6.6)	0.980(6H,7.1)			1.593(6.6)
5e	0.715	0.984	0.859(6.6)	0.888	0.969(6.0)	0.980(6H,7.1)			1.579(6.6)
6b	0.660	1.003	0.842(6.3)	0.750	0.912(6.4)	1.026(6.8)		4.664(1.5)‡	—
						1.031(6.8)		4.717(1H,*s*)	
6d	0.658	1.006	0.845(6.0)	0.748	0.909(6.5)	0.980(6H,7.0)			1.594(6.8)
7f	0.688	1.021	—	—	0.964(6.6)	1.057(6H,*s*)		4.661(1.1)‡	1.057(*s*)
								4.833(0.8)	

*Figures in parentheses denote coupling constants (*J* values) as for *d*, *t*, *q*, and *sept* signals, whereas half-width
†Authentic compounds
‡1H and *d*

lanostene compound, 24-methylene-5α-lanost-9(11)-en-3β-ol, the occurrence of which was shown in the 4, 4-dimethylsterol fraction of the seeds (unpublished results). The $\Delta^{9(11)}$-4α, 14α-dimethylsterols can lead to $\Delta^{9(11)}$-14α-methylsterols through enzymatic demethylation at C-4 and the presence of this type of sterol is known in some higher plants [19, 25] It is worth noting that whereas the 4α, 24-dimethylsterol **1a** isolated from *P vulgaris* seeds in this study was shown to possess a 24α-methyl configuration, 24-methylcholesterol isolated from the seeds has been demonstrated to be a mixture of C-24 epimers, campesterol (24α) and 22-dihydrobrassicasterol (24β) [1] This suggests that the formation of the 24β-methylsterol occurs during the later stage of sterol biosynthesis, i e at the 4-demethylsterol level

EXPERIMENTAL

Crystallizations were performed in MeOH Mp' uncorr Argentation TLC. silica gel–$AgNO_3$ (4.1) developed x3 with CCl_4–CH_2Cl_2 (5 1). HPLC Altex Ultrasphere ODS column (Beckman Altex, 5 μm, 25 cm × 10 mm i d), MeOH–H_2O (98 2) as mobile phase (flow rate, 4 ml/min) GC OV-17 SCOT glass capillary column (30 m × 0 3 mm i d), column temp 255° *RR*, on HPLC and GC expressed relative to cholesterol acetate EIMS (70 eV). probe; ^{1}H NMR 400 MHz, $CDCl_3$, TMS as int standard, acetylation Ac_2O–pyridine at room temp overnight The acetates of the following 4α-methylsterols were used as the reference specimens **1a**, **1b**, **4b**, and **5b** [26], **1c** [20], **1d** and **1e** [27], **3a** [28], **6b** [29], and **7f** [1, 15] The 24*E*- and 24*Z*-isomers of 24-ethylidene-4α-methylsterols showed the same mobility in the HPLC under the conditions used, and hence, the separation of these isomers from each other was performed by the argentation TLC (cf [27]) (The separation factor for 24*E*-/24*Z*-isomers was *ca* 1 14)

Isolation of 4α-methylsterols Immature seeds of *P vulgaris* (136 kg) were homogenized and extracted × 3 with excess MeOH The combined extract was reduced to the aq phase *in vacuo*, and then subjected to solvent partitioning between $CHCl_3$ and H_2O The $CHCl_3$ fraction was concd and partitioned further between hexane and 85% MeOH, and the hexane fraction afforded an oil (800 g) after evapn A portion (87 g) of the oil was saponified by reflux with 5% KOH in 80% EtOH The unsaponifiable lipid (10 2 g) extracted with hexane was chromatographed on silica gel (300 g), eluted with a mixture of hexane and CH_2Cl_2 (1 1) in which the eluate was collected in 20 ml each fraction The fractions 145–165 corresponding to 4α-methylsterol (the elution was monitored by TLC on precoated silica gel) was acetylated, and the acetate fraction (167 mg) was subjected to argentation TLC which afforded four bands (referred to as bands 1–4 in the order of polarity, beginning with the least polar) The least polar fraction (11 mg) from band 1 (R_f 0 74–0.82) was a mixture mainly constituted of the acetates of **1a** and **1c** Band 2 (R_f 0 31–0.41) gave a mixture (13 mg) contained the acetates of

vulgaris seeds (400 MHz, $CDCl_3$, TMS as int. standard)*

3β-OAc (*s*)	3α-H (*dt*)	7-H or 6-H(*m*)	11-H (*m*)	25-H (*sept*)	28-H (*q*)
2.050	4.404(3.8,11.0)	5.174(10)	—	—	—
2.051	4.404(3.9,10.7)	5.177(9.4)	—	2.381(6.8)	—
2.052	4.404(3.9,10.7)	5.176(9.4)	—	—	—
2.053	4.404(4.4,11.0)	5.176(10)	—	2.831(6.9)	5.109(7.0)
2.054	4.406(3.9,10.8)	5.177(10)	—	2.201(6.8)	5.185(6.8)
2.053	4.405(3.9,10.7)	5.181(10)	—	—	—
2.055	4.375(5.0,11.2)	—	—	2.233(6.8)	—
2.053	4.375(4.9,11.0)	—	—	—	5.109(6.6)
2.048	4.413(4.4,10.8)	—	—	—	—
2.050	4.415(4.4,10.4)	—	—	—	5.1141(6.6)
2.054	4.510(4.4,10.4)	—	—	2.237(6.6)	—
2.050	4.379(4.9,11.0)	—	—	2.236(6.6)	—
2.050	4.380(4.9,10.7)	—	—	2.834(6.9)	5.124(6.6)
2.050	4.380(4.9,10.7)	—	—	2.202(6.8)	5.186(6.6)
2.052	4.368(4.9,10.7)	—	5.302(11)	2.236(6.8)	—
2.053	4.366(5.0,10.8)	—	5.303(11)	2.833(6.5)	5.115(6.8)
2.032	4.602(*m*,2.5)	5.376(10)	—	—	—

(W_{max}) values as for multiplet signals.

3d, **5d**, and **5e** Band 3 (R_f 0.21–0.31) yielded a mixture (52 mg) of the acetates of **1d**, **1e**, **2d**, **4b**, and **5b**. Band 4 (R_f 0.07–0.21) afforded a mixture (27 mg) of the acetates of **1b**, **1f**, **2b**, **5d**, and **6b**. Isolation of the acetates of individual 4α-methylsterols from each fraction was performed by the combination of argentation TLC and HPLC. The MS data of the acetates of **1e**, **1f**, **2b**, **2d**, **3d**, **5d**, **6b**, and **6d** isolated from *P. vulgaris* seeds in this study are as follows. (The MS data **5e**-acetate were very similar to those of **5d**-acetate and, hence, these were omitted from below.)

28-*Isocitrostadienol* (**1e**) *acetate*. MS *m/z* (rel. int.): 468.4012 $[M]^+$ (12, $C_{32}H_{52}O_2$, requires 468.3964), 453 (10), 393 (9), 370 (66), 355 (5), 327 (100), 310 (8), 302 (5), 295 (5), 287 (4), 269 (19), 267 (9), 243 (9), 242 (5), 241 (9), 227 (22), 215 (8), 213 (6).

25-*Methylgramisterol* (**1f**) *acetate*. MS *m/z* (rel. int.): 468.3945 $[M]^+$ (9, $C_{32}H_{52}O_2$), 453.3666 (13, $C_{31}H_{49}O_2$), 408.3708 (2, $C_{30}H_{48}$), 393.3568 (4, $C_{29}H_{45}$), 370.2821 (49, $C_{25}H_{38}O_2$), 356.2664 (9, $C_{24}H_{36}O_2$), 341.2513 (4, $C_{23}H_{33}O_2$), 327.2285 (97, $C_{22}H_{31}O_2$), 310.2617 (4, $C_{23}H_{34}$), 302.2198 (6, $C_{20}H_{30}O_2$), 295.2486 (4, $C_{22}H_{31}$), 287.2039 (5, $C_{19}H_{27}O_2$), 269.2219 (12, $C_{20}H_{29}$), 267.2115 (9, $C_{20}H_{27}$), 241.1945 (13, $C_{18}H_{25}$), 227.1829 (18, $C_{17}H_{23}$), 215.1839 (6, $C_{16}H_{23}$), 213.1690 (6, $C_{16}H_{21}$), 43.0545 ($C_2H_3O_1$) and 43.0175 (C_3H_7) (100).

4α-*Methylfecosterol* (**2b**) *acetate*. MS *m/z* (rel. int.) 454.3802 $[M]^+$ (80, $C_{31}H_{50}O_2$, requires 454.3808), 439 (28), 394 (10), 379 (14), 353 (7), 342 (3), 327 (19), 313 (5), 302 (4), 295 (4), 287 (4), 269 (8), 253 (6), 243 (14), 241 (26), 227 (30), 225 (12), 215 (7), 213 (11), 55 (100).

24*Z*-*Ethylidene*-4α-*methyl*-5α-*cholest*-8-*en*-3β-*ol* (**2d**) *acetate*. MS *m/z* (rel. int.): 468.3971 $[M]^+$ (38, $C_{32}H_{52}O_2$), 453 (13), 422 (2), 408 (3), 393 (8), 370 (9), 341 (1), 327 (9), 302 (2), 295 (3), 287 (2), 269 (5), 255 (2), 253 (2), 243 (9), 241 (6), 227 (16), 213 (4), 55 (100).

24*Z*-*Ethylidene*-4α-*methyl*-5α-*cholest*-8(14)-*en*-3β-*ol* (**3d**) *acetate*. MS *m/z* (rel. int.): 468.4003 $[M]^+$ (80, $C_{32}H_{52}O_2$), 453.3754 (24, $C_{31}H_{49}O_2$), 408.3348 (8, $C_{30}H_{48}$), 393.3552 (9, $C_{29}H_{45}$), 370.2841 (18, $C_{25}H_{38}O_2$), 356.2719 (4, $C_{24}H_{36}O_2$), 327.2319 (13, $C_{22}H_{31}O_2$), 302.2212 (5, $C_{20}H_{30}O_2$), 295.2418 (2, $C_{22}H_{31}$), 287.1989 (4, $C_{19}H_{27}O_2$), 269.2258 (8, $C_{20}H_{29}$), 267.2123 (3, $C_{20}H_{27}$), 255.2109 (2, $C_{19}H_{27}$), 243.2115 (15, $C_{18}H_{27}$), 241.1969 (12, $C_{18}H_{25}$), 227.1806 (20, $C_{17}H_{23}$), 215.1846 (4, $C_{16}H_{23}$), 213.1668 (3, $C_{167}H_{21}$), 55.0541 (100, C_4H_7).

24*Z*-*Ethylidene*-4α,14α-*dimethyl*-5α-*cholest*-8-*en*-3β-*ol* (**5d**) *acetate*. MS *m/z* (rel. int.): 482.4137 $[M]^+$ (27, $C_{33}H_{54}O_2$, requires 482.4121), 467.3871 (57, $C_{32}H_{51}O_2$), 439.3550 (3, $C_{30}H_{47}O_2$), 422.3911 (1, $C_{31}H_{50}$), 407.3616 (17, $C_{30}H_{47}$), 384.2990 (5, $C_{26}H_{40}O_2$), 369.2778 (11, $C_{25}H_{37}O_2$), 341.2470 (1, $C_{23}H_{33}O_2$), 309.2616 (10, $C_{23}H_{33}$), 301.2217 (6, $C_{20}H_{29}O_2$), 287.1962 (9, $C_{19}H_{27}O_2$), 283.2384 (3, $C_{21}H_{31}$), 281.2272 (1, $C_{21}H_{29}$), 275.1981 (8, $C_{18}H_{27}O_2$), 269.2287 (9, $C_{20}H_{29}$), 241.1999 (9, $C_{18}H_{25}$), 227.1803 (9, $C_{17}H_{23}$), 55.0542 (100, C_4H_7).

24-*Methylene*-4α,14α-*dimethyl*-5α-*cholest*-9(11)-*en*-3β-*ol* (**6b**) *acetate*. MS *m/z* (rel. int.): 468.3943 $[M]^+$ (14, $C_{32}H_{51}O_2$), 453 (36), 425 (19), 393 (18), 383 (7), 369 (7), 341 (70), 309 (8), 301 (10), 299 (5), 287 (13), 275 (9), 241 (13), 227 (11), 215 (12), 43 (100).

24*Z*-*Ethylidene*-4α,14α-*dimethyl*-5α-*lanost*-9(11)-*en*-3β-*ol* (**6d**) *acetate*. MS *m/z* (rel. int.): 482.4129 $[M]^+$ (9, $C_{33}H_{54}O_2$), 467.3919 (16, $C_{32}H_{51}O_2$), 439.3536 (4, $C_{30}H_{47}O_2$), 407.3644 (11, $C_{30}H_{47}$), 384.3008 (53, $C_{26}H_{40}O_2$), 369.2799 (14, $C_{25}H_{37}O_2$),

341 2454 (34, $C_{23}H_{33}O_2$), 328 2396 (4, $C_{22}H_{32}O_2$), 325 2981 (3, $C_{24}H_{37}$), 316.2445 (4, $C_{21}H_{32}O_2$), 309 2590 (13, $C_{23}H_{33}$), 302 2202 (27, $C_{20}H_{30}O_2$), 301 2155 (29, $C_{20}H_{29}O_2$), 300 2100 (10, $C_{20}H_{28}O_2$), 299 1997 (19, $C_{20}H_{27}O_2$), 287 1988 (6, $C_{19}H_{27}O_2$), 275 1987 (7, $C_{18}H_{27}O_2$), 274 1926 (7, $C_{18}H_{26}O_2$), 269 2250 (4, $C_{20}H_{29}$), 256 2184 (3, $C_{19}H_{28}$), 253 1949 (2, $C_{19}H_{25}$), 241 1959 (27, $C_{18}H_{25}$), 227 1845 (8, $C_{17}H_{23}$), 215 1846 (9, $C_{16}H_{23}$), 55 0543 (100, C_4H_7)

Acknowledgements—We thank Drs T Takido and M Aimi for NMR and mass spectra

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