Phytochemistry, Vol. 20, No. 8, pp. 1929–1933, 1981.
Printed in Great Britain.

0031-9422/81/081929-05 $02.00/0

24β-ETHYL-31-NORLANOSTA-8,25(27)-DIEN-3β-OL AND 24β-ETHYL-25(27)-DEHYDROLOPHENOL IN SEEDS OF THREE CUCURBITACEAE SPECIES

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(*Received* 21 *November* 1980)

Key Word Index—*Cucumis sativus*; *Lagenaria leucantha* var. *Gourda*; *Citrullus battich*; Cucurbitaceae; seeds; 4α-methylsterols; 24β-ethyl-31-norlanosta-8,25(27)-dien-3β-ol; 24β-ethyl-25(27)-dehydrolophenol.

Abstract—The structures of two 4α-methylsterols isolated from *Cucumis sativus* (Cucurbitaceae) seeds were determined based mainly on their ^{13}C NMR spectra as 24β-ethyl-31-norlanosta-8,25(27)-dien-3β-ol and 24β-ethyl-25(27)-dehydrolophenol, respectively, of which the former is a new sterol from natural sources. These two 4α-methylsterols were identified in the seeds of two other Cucurbitaceae species, *Lagenaria leucantha* var. *Gourda* and *Citrullus battich*. The probable biogenetic significance of the two 4α-methylsterols is discussed. Other 4α-methylsterols identified in the seeds of the three Cucurbitaceae species were obtusifoliol, cycloeucalenol and gramisterol.

INTRODUCTION

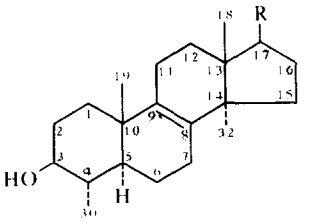
Seeds of the family Cucurbitaceae contain the 24β-ethylsterols, 24β-ethylcholesta-7,22,25(27)-trien-3β-ol* [1–4] and 24β-ethylcholesta-7,25(27)-dien-3β-ol [3, 4], together with chondrillasterol [4–6] and/or spinasterol, a 24α-ethylsterol [3, 4]. In view of the importance of the biosynthetic intermediates of 4-desmethylsterols and the fact that almost no detailed work has been done so far with Cucurbitaceae species, a study was undertaken of the 4α-methylsterol constituents of the seeds of cucumber (*Cucumis sativus*), bottle gourd (*Lagenaria leucantha* var. *Gourda*) and water melon (*Citrullus battich*). Previously known 4α-methylsterols in some Cucurbitaceae seeds were obtusifoliol (**1d**), gramisterol (**2d**), cycloeucalenol (**3d**), citrostadienol [7], and 24ξ-ethyl-25(27)-dehydrolophenol [8].

RESULTS

The 4α-methylsterol fraction that separated from the unsaponifiable lipid of cucumber seed oil was acetylated, and the resulting acetate fraction was separated into four bands (referred to as bands 1–4 in order of polarity, beginning with the least polar) by argentation TLC. The mass spectrum of the steryl acetate recovered from band 1 showed that it was an acetate (M^+, *m/z* 482) of a sterol with the formula $C_{31}H_{52}O$, which possessed two double bonds in the molecule. The ion at *m/z* 341 ($M - C_{10}H_{19}$ [side chain, SC] − 2 H) indicated that one of the double bonds was located in the C_{10} side chain [9]. The other fragments at *m/z* 287 ($M - SC - C_3H_6$ [part of ring D] $- CH_2$) and 227 (*m/z* 287 − HOAc) indicated the presence of an additional C-32 methyl group in the ring system [10]. The ^{1}H NMR spectrum showed a methyl singlet at δ 2.05 due to an acetoxy methyl probably at the usual 3β-position; a broad multiplet at 4.38 (1 H, $W_{1/2}$ = 25 Hz) attributable to a C-3 axial proton was also observed. The spectrum exhibited three further methyl singlets at 0.70, 0.87 and 0.98 and one methyl doublet at 0.84 (J = 7 Hz). Since these four methyl signals agreed with the C-18, C-32, C-19 and C-30 methyl signals, respectively, of obtusifoliol (**1d**) acetate [11], the sterol was shown to possess the ring system **1**. A singlet at 1.57 indicated the presence of a methyl group connected with a double bond; the double bond was shown to be an exo methylene group by the apperance of two broad singlets (each 1 H) at 4.65 and 4.71. A methyl triplet at 0.82 (J = 7 Hz) suggested the presence of an ethyl group at the C-24 position. Since these three signals agreed with those of the C-26, C-27 and C-29 protons, respectively, of 24-ethyl-25(27)-dehydrosterols [12–17], the sterol was shown to have a 24-ethyl-$\Delta^{25(27)}$ side chain. Though experimentally unproved in this study, the 25(27) designation rather than 25(26) of the Δ^{25}-bond was made here for natural 25-dehydrosterols according to the considerations given by Nes *et al.* [18]. Thus the structure of the 4α-methylsterol was 24ξ-ethyl-31-norlanosta-8,25(27)-dien-3β-ol (**1a**).

The structure was finally confirmed and the β-orientation of the 24-ethyl group was revealed by

*Nomenclature: 24β-Ethyl-31-norlanosta-8,25(27)-dien-3β-ol = 4α, 14α-dimethyl-24β-ethyl-5α-cholesta-8,25(27)-dien-3β-ol; 24β-ethyl-25(27)-dehydrolophenol = 4α-methyl-24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol; obtusifoliol = 4α,14α,24-trimethyl-5α-cholesta-8,24(28)-dien-3β-ol; gramisterol = 4α,24-dimethyl - 5α - cholesta - 7,24(28) - dien - 3β - ol; cycloeucalenol = 4α,14α,24-trimethyl-9β,19-cyclo-5α-cholest-24(28)-en-3β-ol; citrostadienol = 4α-methyl-24-ethyl-5α-cholesta-7,*trans*-24(28)-dien-3β-ol; cyclotrichosantol = 4α,14α-dimethyl-24ξ-ethyl-9β,19-cyclo-5α-cholest-25(27)-en-3β-ol; 24β-ethylcholesta-7,22,25(27)-trien-3β-ol = 24β-ethyl-5α-cholesta-7,*trans*-22,25(27)-trien-3β-ol; 24β-ethylcholesta-7,25(27)-dien-3β-ol = 24β-ethyl-5α-cholesta-7,25(27)-dien-3β-ol; chondrillasterol = 24β-ethyl-5α-cholesta-7,*trans*-22-dien-3β-ol; spinasterol = 24α-ethyl-5α-cholesta-7,*trans*-22-dien-3β-ol; cholesterol = cholest-5-en-3β-ol; 24-dihydrolanosterol = 5α-lanost-8-en-3β-ol.



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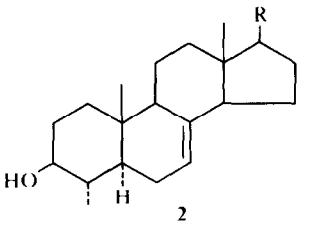

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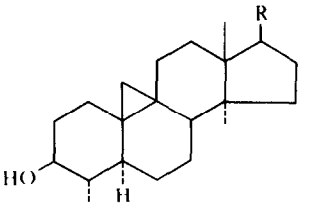

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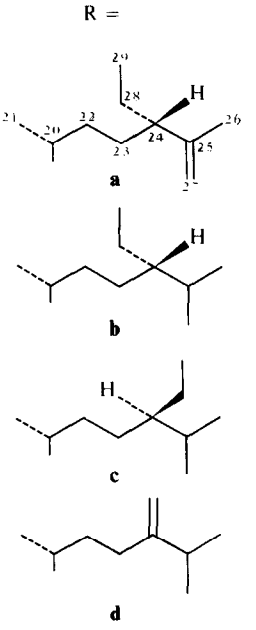

^{13}CNMR spectroscopy. As shown in Table 1, the chemical shifts of the signals due to the ring system carbons (C-1 through C-19, C-30 and C-32) of the steryl (**1a**) acetate agreed well with those of obtusifoliol (**1d**) acetate, and thus confirmed that the sterol had the ring system **1**. The assignment of the signals due to ring system **1** carbons was applied to **1d** and its acetate. The C-1 through C-5 signal assignments were based on the acetylation shift values [19–22], calculated from the chemical shifts in Table 1, which were almost identical with those of 24α-ethyllophenol (**2c**) [23]. Assignment of the signals due to C-6 through C-19 and C-32 carbons was facilitated by comparison with those reported for 24-dihydrolanosterol and its acetate [24], while the C-30 signal was consistent with that of **2c** and this enabled the signal to be assigned. Side chain carbon signals (C-20 through C-28) of **1d** were assigned by comparison with the published data on cycloeucalenol (**3d**) [21]. On the other hand, **1a**-acetate showed side chain carbon signals (C-20 through C-29) almost identical with those of 24β-ethylcholesta-7,25(27)-dien-3β-ol acetate [4], and hence the 4α-methylsterol (**1a**) to have a 24-ethyl-$\Delta^{25(27)}$ side chain, probably with the 24β-ethyl configuration. In order to verify the β-orientation of the ethyl group, 24-ethyl-31-norlanost-8-en-3β-ol (**1b**) acetate was prepared following hydrogenation from **1a**-acetate. The dihydro derivative, **1b**-acetate, provided side chain carbon signals consistent with those of 24β-ethyllophenyl (**2b**) acetate (described later) as shown in Table 1. The assignment of the signals was facilitated by comparison with those of the published spectral data of 24β-ethylsterol [25]. Some differences were observed in the chemical shifts of the C-23 and C-29 carbon signals of the epimeric pair of 24β- and 24α-ethylsterols, i.e. **2b**- and **2c**-acetates, respectively (Table 1). Hence, the 24β-ethyl orientation of the dihydro derivative **1b** was confirmed and, the structure of the 4α-methylsterol was 24β-ethyl-31-norlanosta-8,25(27)-dien-3β-ol (**1a**).

Band 2 from the argentation TLC afforded a 4α-methylsteryl acetate which showed a molecular ion in the mass spectrum at *m/z* 468 indicating that it was an acetate of a C_{30} sterol (molecular formula $C_{30}H_{50}O$) with two double bonds of which one was located in the C_{10} side chain (*m/z* 327, M − $C_{10}H_{19}$[SC] − 2H, base peak) [9]. That the fragment ion at *m/z* 327 was the base peak indicated moreover the possible location of the ring system double bond at C-7 [9, 26]. The ^{1}HNMR spectrum of the steryl acetate exhibited a methyl singlet at δ 2.05 arising from an acetoxy methyl and associated with a broad multiplet at 4.40 (1H, $W_{1/2}$ = 25 Hz), this indicated that the acetoxy group was situated probably at the usual 3β-position. Two methyl singlets at 0.52 and 0.83, one methyl doublet at 0.85 (J = 6.1 Hz) and one methine broad doublet at 5.16 (J = 4.4 Hz) were observed. Since these signals agreed well with those of the C-18, C-19 and C-30 methyls and C-7 methine, respectively, of 24α-ethyllophenol (**2c**) acetate [11], the sterol was recognized to have a lophenol ring system, i.e. **2**. It also showed further signals due to side chain protons at δ 0.91 (3H, *d*, J = 5.6 Hz), 0.83 (3H, *t*, J = 7 Hz), 1.55 (3H, *s*), and 4.65 and 4.71 (each 1H and *br s*). These signals agreed well with those of the C-21, C-29, C-26 and C-27 protons, respectively, reported for 24-ethyl-25(27)-dehydrosterols [12–17], and hence the sterol was indicated to carry a 24-ethyl-$\Delta^{25(27)}$ side chain. Moreover, the spectrum was consistent with that reported for 24-ethyl-25(27)-dehydrolophenyl acetate [14], and therefore the structure of the 4α-methylsterol was 24ξ-ethyl-25(27)-dehydrolophenol (**2a**). This structure was confirmed and the β-orientation of the 24-ethyl group was established by ^{13}CNMR spectroscopy. The chemical shifts of signals arising from ring system carbons (C-1 through C-19, and C-30) of **2a**-acetate were almost identical with those of **2c**-

Table 1. ^{13}C NMR chemical shifts (δ) of 4α-methylsterols

	Acetates							
Carbon	**1a***	**1b†**	**1d***		**2a†**	**2b***	**2c*‡**	
C-1	34.6	34.6	34.6	(34.9)§	36.6	36.6	36.6	(37.0)§
C-2	27.2	27.2	27.1	(31.1)	27.1	27.1	27.1	(31.0)
C-3	78.8	78.8	78.7	(76.4)	78.3	78.4	78.4	(76.2)
C-4	36.0	36.1	36.0	(39.2)	37.0	37.0	37.0	(40.2)
C-5	47.1	47.1	47.0	(47.0)	46.7	46.8	46.7	(46.7)
C-6	20.7	20.7	20.7	(20.7)	26.7	26.6	26.6	(26.7)
C-7	28.1	28.2	28.2	(28.2)	117.2	117.2	117.2	(117.4)
C-8	133.3‖	133.3‖	133.2‖	(133.5‖)	139.0	139.1	139.1	(139.1)
C-9	134.8‖	134.8‖	134.6‖	(134.5‖)	49.4	49.5	49.4	(49.7)
C-10	36.2	36.1	36.2	(36.3)	34.6	34.8	34.7	(34.8)
C-11	21.8	21.8	21.7	(21.7)	21.4	21.4	21.3	(21.4)
C-12	25.4	25.5	25.4	(25.5)	39.5	39.5	39.5	(39.6)
C-13	44.5	44.5	44.4	(44.5)	43.3	43.3	43.3	(43.4)
C-14	49.8	49.9	49.8	(49.8)	55.0	55.0	54.9	(55.0)
C-15	31.0	31.0	31.2	(31.2)	22.9	22.9	22.9	(23.0)
C-16	30.7	30.7	31.0	(31.0)	27.9	27.9	28.0	(28.0)
C-17	50.4	50.4	50.3	(50.3)	56.0	56.0	56.0	(56.0)
C-18	15.7	15.7	15.7	(15.7)	12.2	11.8	11.8	(11.8)
C-19	18.6	18.8	18.7	(18.7)	14.1	14.1	14.0	(14.2)
C-30	15.0	15.0	15.0	(15.0)	15.2	15.1	15.2	(15.2)
C-32	24.4	24.4	24.4	(24.4)	—	—	—	—
$\underline{C}$OMe	170.9	170.9	170.8	—	170.7	170.7	170.8	—
CO$\underline{C}$H$_3$	21.3	21.3	21.3	—	21.4	21.4	21.3	—
C-20	36.2	37.0	36.4	(36.4)	35.9	36.6	36.6	(36.6)
C-21	18.6	18.8	18.1	(18.2)	18.8	18.9	18.9	(18.9)
C-22	33.9	34.2	34.9	(34.9)	33.6	33.9	33.9	(33.9)
C-23	29.7	26.7	30.7	(30.7)	29.5	26.6	26.2	(26.2)
C-24	49.5	46.0	156.8	(156.8)	49.4	46.1	45.9	(45.9)
C-25	147.6	29.0	33.8	(33.8)	147.3	29.0	29.1	(29.1)
C-26	17.8	18.8	21.8¶	(21.8¶)	17.7	18.9	19.8	(19.8)
C-27	111.4	19.6	22.0¶	(22.0¶)	111.4	19.6	19.0	(19.0)
C-28	26.6	23.0	105.9	(105.9)	26.7	22.9	23.1	(23.1)
C-29	12.0	12.3	—	—	11.9	12.3	12.0	(12.0)

*Recorded at 25.05 MHz.
†Recorded at 22.50 MHz.
‡Reported in ref. 23.
§Figures in parentheses refer to chemical shifts for the free alcohol.
‖,¶Assignment in any vertical column may be reversed although those given are preferable.

acetate (Table 1) [23], while those due to side chain carbons (C-20 through C-29) agreed with the corresponding signals of **1a**-acetate and the other 24β-ethyl-25(27)-dehydrosterols [4]. Moreover, 24-ethyllophenol (**2b**) acetate prepared from **2a**-acetate by hydrogenation showed that the side chain carbon signals were consistent with those reported for 24β-ethyllophenol (**2b**) acetate [23] but differed enough for differentiation from 24α-ethyllophenol (**2c**) acetate in the chemical shifts of the C-23 and C-29 signals (Table 1). Hence, the structure of the 4α-methylsterol was 24β-ethyl-25(27)-dehydrolophenol (**2a**). A small discrepancy was observed in the ^{13}C chemical shifts of some of the side chain signals between the compounds with the ring systems **1** and **2** attached to the side chains **a** and **b**, but this is most probably due to the difference in the ring system structures.

The most bulky band 3 from the argentation TLC contained the acetates of obtusifoliol (**1d**) and cycloeucalenol (**3d**) as the major constituents, and the most polar band 4 consisted of gramisterol (**2d**) acetate accompanied by some minor unidentified components.

The seeds of bottle gourd and water melon were also investigated for their 4α-methylsterol constituents and **2a** was isolated from both of the seeds and identified in the same way as described above. The other major constituents in the 4α-methylsterol fractions of both of the seed materials were **1a**, **1d**, **2d** and **3d**, which were identified by argentation TLC, GLC and MS.

DISCUSSION

The occurrence of 24β-ethyl-31-norlanosta-8,25(27)-dien-3β-ol (**1a**), 24β-ethyl-25(27)-dehydrolophenol (**2a**), obtusifoliol (**1d**), gramisterol (**2d**) and cycloeucalenol (**3d**) as the major components in the 4α-methylsterol fractions from the unsaponifiable lipids of the seed oils of three Cucurbitaceae species, cucumber, bottle gourd and water melon, has now been demonstrated. Compound **1a** is a

new 4α-methylsterol from natural sources. Although the configuration at C-24 of **1a** from bottle gourd and water melon seeds was not determined by ^{13}C NMR spectroscopy, there can be little or no doubt that the sterol was the 24β-ethyl configuration because evidence has been given of the 24β-alkyl configuration for the 24-alkylsterols bearing a $\Delta^{25(27)}$-bond in tracheophytes [18, 27]. For the same reason, though the configuration at C-24 remained undetermined, 24ξ-ethyl-25(27)-dehydrolophenol (**2a**) isolated from the leaves of *Clerodendrum campbellii* [14] and from the seeds of *Echinocystis lobata* [8], and cyclotrichosantol (**3a**) isolated from the leaves of *Trichosantes palmata* [15], are considered to have the 24β-ethyl configuration.

4-Desmethylsterols are biosynthesized from 4,4-dimethylsterols (lanostane triterpene alcohols) via 4α-methylsterols. The 4α-methylsterols with the ring systems of **1**, **2** and **3**, such as **1d**, **2d** and **3d** which were identified also in this study in the seeds of the three Cucurbitaceae species, are the representative biosynthetic intermediates in photosynthetic plants. The following sequence is known to operate in plants: 4,4-dimethylsterols→→**3**→**1**→**2**→→4-desmethylsterols [28, 29]. Taking this into account, it is highly probable that **1a** and **2a** participate as the intermediates for the biosynthesis of 24β-ethylcholesta-7,25(27)-dien-3β-ol and other 24β-ethyl-4-desmethylsterols [1–6] in the seeds of the Cucurbitaceae species. Moreover, **1a** is a compound which was proposed as a possible intermediate in the conversion of **1d** to 24β-ethyl-31-norlanost-8-en-3β-ol (**1b**) in *Chlorella emersonii*, a green alga, though the occurrence of **1a** in this organism has remained unproved [30]. In the seeds of the Cucurbitaceae species, **1d** [29] and **3a**, though the latter was unidentified in this study, are the possible biosynthetic precursors of **1a**.

EXPERIMENTAL

Recrystallizations were performed in MeOH or in MeOH–Me_2CO. Mps were taken on a heated block and are uncorr. ^{13}C FT NMR spectra were recorded at 22.50 or 25.05 MHz using 0.10–0.15 M soln in $CDCl_3$. The chemical shifts (δ) are expressed in ppm relative to TMS and are estimated to be accurate ±0.05 ppm. The probe temp. was *ca* 30°. FT NMR measurement conditions were as follows: spectral width: 4.5–6.0 kHz, pulse width: 5–9 μsec, acquisition time: 1.0 or 1.5 sec, and number of data points: 4096 or 8192. ^1H FT NMR spectra were obtained with a 90 or 100 MHz FT instrument in $CDCl_3$ with TMS as internal reference. MS (70 eV, $m/z > 100$) were taken with a GC/MS (2% OV-17, 2 m × 3 mm glass column). GLC was carried out on an OV-17 SCOT glass capillary column (30 m × 0.3 mm, 260°, split ratio 100:1) and RR_t is given relative to cholesteryl acetate. $AgNO_3$-Si gel (1:4) TLC (0.5 mm) was developed 4× with CH_2Cl_2–CCl_4 (1:5). Hydrogenation of 24β-ethyl-31-norlanosta-8,25(27)-dien-3β-ol (**1a**) acetate was performed in EtOH–C_6H_6 (1:1) over PtO_2 at room temp. Reduction of 24β-ethyl-25(27)-dehydrolophenol (**2a**) acetate was carried out with the addition of a trace amount of pyridine to the reaction mixture to inhibit migration of the Δ^7-bond to the $\Delta^{8(14)}$-position [31]. Other techniques used in this work have been described previously [7, 32].

The origin of the seeds of bottle gourd (*L. leucantha* var. *Gourda*) and water melon (*C. battich*) has been described previously [4], and the seeds of cucumber (*C. sativus*) were obtained from mature pepos which were harvested at a local farm in 1978. Obtusifoliol (**1d**), gramisterol (**2d**) and cycloeucalenol (**3d**) were used as the reference specimens [11]. Identification of the isolated compounds was performed by mp, argentation TLC, GLC, MS, and ^1H and ^{13}C NMR spectra, while that of unisolated ones was by argentation TLC, GLC and MS. The ^{13}C NMR chemical shifts recorded for the 4α-methylsterols described below are summarized in Table 1. In every spectrum, off-resonance decoupling was used to aid assignment.

4α-Methylsterols of cucumber seeds. The 4α-methylsterol fraction (64 mg) separated by Si gel TLC from the unsaponifiable lipid (5.8 g) of cucumber seed oil (700 g), which was obtained by Soxhlet extraction using CH_2Cl_2 as solvent from dried and ground seeds (2.3 kg), was acetylated. The acetate fraction (56 mg) was separated into 4 bands by argentation TLC. The fraction from band 1 (R_f 0.53) gave **1a**-acetate (RR_t 1.80, 8 mg) after purification by repeated argentation TLC, mp 129–130°. MS *m/z* (rel. int.): 482 $[M]^+$ (43), 467 (100), 407 (45), 369 (5), 287 (10), 269 (20), 227 (9), 215 (11), 201 (10), 175 (10), 159 (10), 137 (10), 119 (10), 109 (15), 107 (10), 105 (10). ^1H NMR (100 MHz): δ 0.70 (3 H, *s*, H-18), 0.87 (3 H, *s*, H-32), 0.98 (3 H, *s*, H-19), 1.57 (3 H, *s*, H-26), 2.05 (3 H, *s*, H-3β-OAc), 4.65 and 4.71 (each 1 H, *br s*, H-27), 0.84 (3 H, *d*, J = 7 Hz, H-30), 0.82 (3 H, *t*, J = 7 Hz, H-29), 4.38 (1 H, *m*, $W_{1/2}$ = 25 Hz, H-3α). Hydrogenation for 3 hr of **1a**-acetate gave **1b**-acetate (RR_t 1.78), mp 130–132°. MS *m/z* (rel. int.): 484 $[M]^+$ (23), 469 (100), 424 (3), 409 (43), 301 (9), 287 (5), 283 (4), 241 (6), 227 (6), 215 (9), 201 (6), 175 (7), 161 (10), 159 (7), 121 (7), 119 (10), 109 (7), 107 (9), 105 (8). ^1H NMR (90 MHz): δ 0.71 (3 H, *s*, H-18), 0.89 (3 H, *s*, H-32), 0.98 (3 H, *s*, H-19), 2.05 (3 H, *s*, H-3β-OAc), 0.80 (3 H, *d*, J = 6 Hz, H-27), 0.82 (3 H, *d*, J = 6 Hz, H-26), 0.86 (3 H, *d*, J = 7 Hz, H-30), 0.85 (3 H, *t*, J = 7 Hz, H-29), 4.40 (1 H, *m*, $W_{1/2}$ = 25 Hz, H-3α).

The fraction from band 2 (R_f 0.38) afforded **2a**-acetate (RR_t 2.20, 10 mg) after repeated argentation TLC, mp 170–172°. MS *m/z* (rel. int.): 468 $[M^+]$ (34), 453 (37), 408 (10), 393 (18), 356 (8), 327 (100), 287 (9), 269 (42), 267 (11), 243 (12), 241 (24), 227 (39), 173 (11), 161 (19), 159 (22), 147 (25), 145 (17), 135 (15), 133 (22), 131 (14), 121 (30), 120 (12), 119 (29), 109 (26), 107 (27). ^1H NMR (100 MHz): δ 0.52 (3 H, *s*, H-18), 0.83 (3 H, *s*, H-19), 1.55 (3 H, *s*, H-26), 2.05 (3 H, *s*, H-3β-OAc), 4.65 and 4.71 (each 1 H, *br s*, H-27), 0.85 (3 H, *d*, J = 6.1 Hz, H-30), 0.91 (3 H, *d*, J = 5.6 Hz, H-21), 5.16 (1 H, *br d*, J = 4.4 Hz, H-7), 0.83 (3 H, *t*, J = 7 Hz, H-29), 4.40 (1 H, *m*, $W_{1/2}$ = 25 Hz, H-3α). Hydrogenation for 12 hr of **2a**-acetate yielded **2b**-acetate (RR_t 2.16), mp 154–156°. MS *m/z* (rel. int.): 470 $[M]^+$ (100), 455 (15), 410 (6), 395 (10), 329 (6), 327 (10), 269 (44), 243 (12), 227 (16), 147 (13), 135 (11), 121 (11), 119 (13), 105 (10). ^1H NMR (90 MHz): δ 0.53 (3 H, *s*, H-18), 0.84 (3 H, *s*, H-19), 2.05 (3 H, *s*, H-3β-OAc), 0.81 (3 H, *d*, J = 7 Hz, H-27), 0.83 (6 H, *d*, J = 7 Hz, H-26, H-30), 0.91 (3 H, *d*, J = 7 Hz, H-21), 5.17 (1 H, *br d*, J = 6 Hz, H-7), 0.84 (3 H, *t*, J = 7 Hz, H-29), 4.46 (1 H, *m*, $W_{1/2}$ = 25 Hz, H-3α).

The fraction (24 mg) from band 3 (R_f 0.22) was a mixture of the acetates of **1d** (RR_t 1.49) and **3d** (RR_t 1.77) accompanied by an unidentified minor component (RR_t 2.07). The fraction (6 mg) from band 4 (R_f 0.15) gave **2d**-acetate (RR_t 1.79) accompanied by unidentified minor components with RR_t 1.58 and 1.71.

4α-Methylsterols of bottle gourd and water melon seeds. Soxhlet extraction of dried and ground seeds (1.9 kg) of bottle gourd gave an oil (380 g) which upon saponification gave an unsaponifiable lipid (4.8 g). The 4α-methylsterol fraction (26 mg), separated from the unsaponifiable lipid by Si gel TLC, afforded the acetate fraction (19 mg) upon acetylation, which separated into 4 bands by argentation TLC. Band 1 gave **1a**-acetate (2 mg) accompanied by unidentified minor components with RR_t 1.54 and 1.86. The fraction from band 2 gave **2a**-acetate (4 mg, mp 169–173°) after purification by further argentation TLC, which upon hydrogenation yielded **2b**-acetate (mp 152–155°). The fraction (7 mg) from band 3 was a mixture of the acetates of **1d**, **3d** and an

unidentified minor component (RR_t 2.07), and band 4 yielded **2d**-acetate (2 mg) accompanied by unidentified components with RR_t 1.58 and 1.71.

Dried and ground seeds (1.8 kg) of water melon gave an oil (553 g) by Soxhlet extraction, which upon saponification yielded an unsaponifiable lipid (4.5 g). Si gel TLC of the unsaponifiable lipid gave 4α-methylsterol fraction (37 mg) which after acetylation afforded an acetate fraction (34 mg). The acetate fraction separated into 4 bands by argentation TLC. The fraction (2 mg) from band 1 was a mixture of **1a**-acetate and unidentified components with RR_t 1.54 and 1.86. The fraction from band 2, upon further argentation TLC, gave **2a**-acetate (6 mg, mp 170–173°). Hydrogenation of **2a**-acetate yielded **2b**-acetate (mp 153–156°). Band 3 afforded the mixture (13 mg) of the acetates of **1d**, **3d** and unidentified components with RR_t 2.07 and 2.40. The fraction (3 mg) from band 4 contained **2d**-acetate and an unidentified minor component (RR_t 1.71).

Acknowledgements—We are grateful to Y. Kawasaki for technical assistance and to Japan Electron Optics Laboratory Co., T. Ishikawa, and Dr. T. Takido for the measurement of NMR spectra. Our thanks are also due to Professor Y. Ichinohe for his valuable comments.

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