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FOUR NEW AND OTHER 4α-METHYLSTEROLS IN THE SEEDS OF SOLANACEAE

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Abstract—Four new 4α-methylsterols in the seeds of Solanaceae were identified as 31-norlanost-9(11)-enol, 24-methyl-31-norlanost-9(11)-enol, 4α,24-dimethylcholesta-7,24-dienol and 4α-methyl-24-ethylcholesta-7,24-dienol. The other 4α-methylsterols identified in the seeds were 31-norcycloartanol, 31-norcycloartenol, cycloeucalenol, 31-norlanost-8-enol, 31-norlanosterol, obtusifoliol, 4α,14α,24-trimethylcholesta-8,24-dienol, 4α-methylcholest-8-enol, lophenol, 24-methyllophenol, 24-ethyllophenol, gramisterol and citrostadienol. The distribution of these seventeen 4α-methylsterols in the seeds of eight species of the Solanaceae was determined.

INTRODUCTION

In recent work, the unsaponifiable matters from the seed oils of Solanaceae plants were separated into 4,4-dimethylsterol, 4-desmethylsterol and 4-monomethylsterol fractions, and the approximate compositions of the 4,4-dimethylsterol [1] and 4-desmethylsterol [2] fractions were determined. This paper describes the identification of seventeen 4α-methylsterols, of which four are probably new sterols from plants, in the 4-monomethylsterol fractions from solanaceous seeds. The distribution of these sterols in the seeds of twelve Solanaceae also is determined. The 3β-monohydroxy 4α-methylsterols hitherto reported to occur in the materials from various solanaceous plants are cycloeucalenol (**3**) [3, 4], 31-norlanosterol (**5**) [3, 5], obtusifoliol (**6**) [3, 4], lophenol (**11**) [4, 6], (24*R*)-24-ethyllophenol (**13**) [7], gramisterol 24-methylenelophenol) (**14**) [3,4,8–10] and citrostadienol (**15**) [4, 6, 8, 10].

RESULTS AND DISCUSSION

Si gel PLC has heretofore been used in this laboratory to fractionate 4,4-dimethyl-, 4-monomethyl- and 4-desmethyl-sterols of the unsaponifiable matters from a number of vegetable seed oils [11–16]. An inspection of our results has revealed that the 32-methylated 4α-methylsterols with a Δ^8-or a $\Delta^{9(11)}$-bond, or a 9β,19-cyclopropyl group had a slightly larger R_f values on the PLC than the 32-desmethylated 4α-methylsterols with a Δ^7- or a Δ^8-bond. The following relative R_f-values (cholesterol, relative R_f 1.0) for the 4α-methylsterols determined by PLC on Si gel under the conditions used are illustrative: cycloeucalenol (**3**) (1.38), 31-norlanost-8-enol (**4**) (1.37), obtusifoliol (**6**) (1.35), 31-norlanost-9(11)-enol (**8**) (1.36), 4α-methylcholest-8-enol (**10**) (1.32), lophenol (**11**) (1.31), 24-ethyllophenol (**13**) (1.30), gramisterol (**14**) (1.26) and citrostadienol (**15**) (1.28). In this study, the 4-monomethylsterol fractions were subjected first to a further fractionation by Si gel PLC. The value of Si gel PLC for the separation of Δ^5 and Δ^7 4-desmethylsterols has been suggested previously [17, 18].

In the solanaceous seeds now examined, four new natural 4α-methylsterols were identified. MS of **8**-acetate, which was eventually isolated by argentation PLC from the acetylated 4-monomethylsterol fraction A (see Experimental) of *Lycopersicon esculentum* seeds, indicated that it is an acetate (IR, ν_{max} 1735 and 1240 cm^{-1}) of a C_{29} sterol with one double bond: *m/e* 456 (M^+, $C_{31}H_{52}O_2$), 441 (M^+-Me) and 381 (M^+-Me-AcOH). The presence of the ions at *m/e* 343 (M^+-C_8H_{17} [SC]), 301 (M^+-SC-C_3H_6 [part of ring D]) and 227 (M^+-SC-C_3H_6-CH_2-AcOH) showed the presence of a skeletal double bond, which was trisubstituted (IR, ν_{max} 3050 and 810 cm^{-1}), an additional C-32 methyl, and a saturated C_8 side chain. PMR spectrum showed a tertiary methyl singlet at δ 2.05 due to an acetoxy methyl probably at the usual 3β-position; a broad multiplet at δ 4.38 (1H, $W_{1/2}$ = 25 Hz) attributable to C-3 axial proton also was observed. It afforded further three tertiary methyl singlets at δ 0.65, 0.74 and 1.00. Since the first two agreed well with the chemical shifts of C-18 (δ 0.64) and C-32 (δ 0.74) methyl signals, for lanost-9(11)-enyl acetate, the skeletal double bond is likely to be located at the 9(11)-position. The singlet at δ 1.00 is attributable to C-19 methyl protons. Though lanost-9(11)-enyl acetate showed the corresponding signal at δ 1.07, a slightly larger chemical shift is explicable by the presence of C-31 methyl group [19]. That the skeletal double bond of **8**-acetate is located at the 9(11)-position and not at the 7-position was supported by the appearance of an olefinic methine multiplet at δ 5.30; lanost-9(11)-enyl acetate also showed it at an almost identical field, δ 5.28, whereas the Δ^7-compounds, lanost-7-enyl acetate and the acetates of 4α-methyl Δ^7-sterols, described in this paper showed it at a somewhat higher field, δ 5.17–5.19. The possibility of a Δ^7-bond instead of a $\Delta^{9(11)}$-bond was excluded since lanost-7-enyl acetate showed C-18 and C-32 methyl signals at δ 0.64 and 0.97 respectively. The spectrum showed further two doublets at δ 0.87 (6H) and 0.84 (3H) which are associated to the

C-26, C-27 *gem*-dimethyls [20–22] and C-30 methyl respectively. Hence **8** is recognized as 31-norlanost-9(11)-enol. The structure was further confirmed by argentation PLC, GLC, mp, IR, PMR and MS comparisons of **8**-acetate with authentic 31-norlanost-9(11)-enyl acetate.

The fraction of **8**-acetate just described above contained an additional minor component with RR_t 1.64 (**9**-acetate, ca 2%). GC–MS of **9**-acetate showed the ions at *m/e* 470 (M^+, $C_{32}H_{54}O_2$), 455 (M^+-Me), 410 (M^+-AcOH) and 395 (M^+-Me-AcOH) which indicated that it was an acetate of a C_{30} sterol with one double bond. That **9**-acetate possesses a saturated C_9 side chain, a skeletal double bond and an additional C-32 methyl group was indicated by the presence of the other fragments at *m/e* 343 (M^+-C_9H_{19} [SC]), 301 (M^+-SC-C_3H_6 [part of ring D]) and 287 (M^+-SC-C_3H_6-CH_2). The GLC and MS data agreed with those of authentic 24-methyl-31-norlanost-9(11)-enyl acetate. Thus **9** was regarded as the 24-methyl homologue of **8**, i.e. 24-methyl-31-norlanost-9(11)-enol.

4α-Methylsterol **17** was isolated from *Solanum melongena* seeds. The fraction, which was recovered from the more polar division of the 4-monomethylsterol band on Si gel PLC, after acetylation, was separated into four bands on argentation PLC. The band second from the solvent front afforded **17**-acetate accompanied with a trace amount of **16**-acetate. MS of **17**-acetate revealed that it was an acetate (IR, ν_{max} 1730 and 1238 cm^{-1}) of a C_{30} sterol with two double bonds, both or one of which was considered as trisubstituted (IR, ν_{max} 820 and 815 cm^{-1}), based on the ions at *m/e* 468 (M^+, $C_{32}H_{52}O_2$), 453 (M^+-Me) and 393 (M^+-Me-AcOH). The fragments at *m/e* 327(M^+-$C_{10}H_{19}$ [SC]-2H) and 227 (M^+-SC-C_3H_6 [part of ring D]-AcOH) indicated the presence of a C_{10} side chain with one double bond. Further, a prominent ion at *m/e* 370 (M^+-C_7H_{14}), which might be given as a result of McLafferty rearrangement, showed that the side chain double bond was located either at the 24(28)- or at the 24(25)-position [23]. PMR spectrum showed five tertiary methyl singlets at δ 0.54, 0.84, 1.63 (two overlapped singlets) and 2.05. The singlet at δ 2.05 arisen from an acetoxy methyl, associated with a broad multiplet at δ 4.41 (1H, $W_{1/2}$ = 26 Hz), indicated that the acetoxy group was situated probably at the usual 3β-position. The two singlets at δ 0.54 and 0.84, assignable to C-18 and C-19 methyls respectively, a doublet at δ 0.85 (3H, J = 6.7 Hz, C-30) and a multiplet at δ 5.17 (1H, $W_{1/2}$ = 11 Hz, C-7) suggested a 4α-methylated 5α-Δ^7-sten-3β-yl acetate skeleton. The absence of other olefinic proton signal showed that the double bond in the side chain was located at the 24(25)-position. This was supported by the two overlapped vinylic methyl singlets at δ 1.63 due to C-26 and C-27 methyls and a triplet at δ 0.93 (3H, J = 7.2 Hz) attributable to C-29 methyl. Thus **17** was 4α-methyl-24-ethylcholesta-7,24-dienol. The structure was finally confirmed by identification of the argentation PLC, GLC, mp, IR, PMR and MS data for the steryl acetate with those of authentic 4α-methyl-24-ethylcholesta-7,24-dienyl acetate.

The fraction from the band second from the solvent front on argentation PLC of the acetylated 4-monomethylsterol fraction B (see Experimental) of *L. esculentum* seeds was a mixture of the acetates of **16** and **17**. **16**- Acetate (RR_t 2.15) showed prominent ions in GC–MS at *m/e* 454 (M^+, $C_{31}H_{50}O_2$), 439 (M^+-Me) and 394 (M^+-AcOH) which indicated that it was an acetate of a C_{29} sterol with two double bonds. One of the double bonds was shown to be present in a C_9 side chain by the fragments at *m/e* 327 (M^+-C_9H_{17} [SC]-2H) and 277 (M^+-SC-C_3H_6[part of ring D]-AcOH). A prominent ion at *m/e* 370 (M^+-C_6H_{12}) as observed also for **17**-acetate indicated that the side chain double bond was located either at the 24(28)- or at the 24(25)-position [23]. Since the GLC and MS data were compatible with those of authentic 4α,24-dimethylcholesta-7,24-dienyl acetate, **16** was regarded as 4α,24-dimethylcholesta-7,24-dienol.

The sterols, **8** and **9**, identified in the Solanaceae appears to be the first examples of 4α-methylsterols with $\Delta^{9(11)}$-bond occurring in higher plants, though several $\Delta^{9(11)}$-tetracyclic triterpenes have been identified earlier in Sapotaceae [24–26], Lauraceae [27], Gramineae [28], Rutaceae [29] and Fagaceae [30]. Two other new sterols, **16** and **17**, and 4α,14α,24-trimethylcholesta-8,24-dienol (**7**) possess a 24-alkylated $\Delta^{24(25)}$-side chain. Such $\Delta^{24(25)}$-sterols have been demonstrated to be the biosynthetic intermediates of 24-alkylsterols with a saturated side chain in several plant genera [31–35]. Furthermore, recently, the $\Delta^{24(25)}$-sterols, preliminarily formed by an enzymatic isomerization of $\Delta^{24(28)}$-sterols, were shown to be converted to 24α-alkylsterols by reduction in the germinating seeds of *Pinus pinea* (Pinaceae) [36], and the phylogenetic distribution of sterols was extensively defined structurally and stereochemically in vascular plants [37]. Other than those identified in this study, there are five 24-alkylated $\Delta^{24(25)}$-sterols previously identified in higher plants: 4α,14α,24-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol [38]. cyclobranol (24-methyl-9β,19-cyclo-5α-lanost-24-en-3β-ol) [39], 24-ethyl-5α-cholesta-7,24-dien-3β-ol [17, 37], 24-methylcholesta-5,24-dien-3β-ol [2, 40] and 24-ethylcholesta-5,24-dien-3β-ol [2, 40].

The migration of a double bond at the 24(28)-position to the 24(25)-position of sterols has already been demonstrated to be achieved by I_2 in C_6H_6 [41–43] and by HCl in $CHCl_3$ [44] besides by H_2SO_4 employed in this study. Though it has been recognized during chromatography on Si gel that a $\Delta^{24(28)}$-bond isomerized to a $\Delta^{24(25)}$-bond of sterols [45], such migration of the double bond could not be observed on the Si gel PLC used in this study and also in a recent study [38].

In addition to the above four compounds, thirteen known 4α-methylsterols were identified in the solanaceous seeds: six 4α-methylsterols were isolated and identified as **4**, **6**, **11**, **13**, **14**, and **15**, and seven were identified by GLC and GC–MS as 31-norcycloartanol (**1**), 31-norcycloartenol (**2**), **3**, 31-norlanosterol (**5**), **7**, **10** and 24-methyllophenol (**12**).

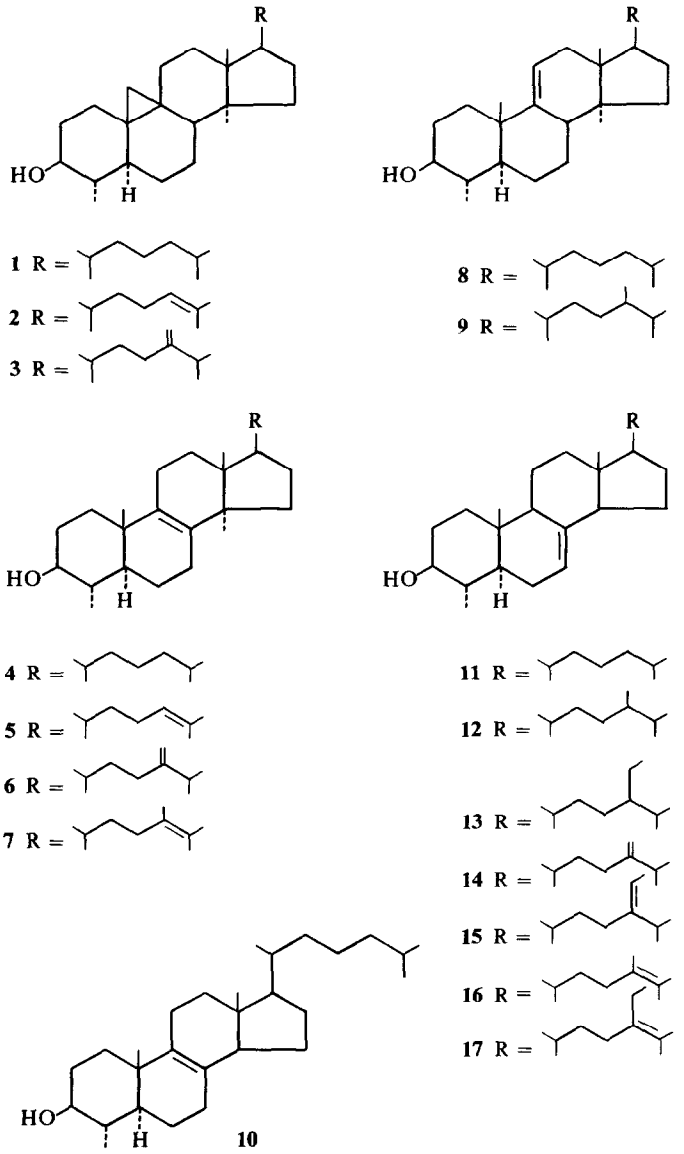
The C-24 ethyl group of sterol **13** isolated from *S. melongena* seeds was inferred to have a 24*R*-configuration because 24-ethyllophenol isolated from the same genus, *S. xanthocarpum*, was demonstrated to have a 24*R*-ethyl group [7]. That **13** possesses a 24*R*-ethyl group is admittable also by the co-occurrence of the said biosynthetic precursors [36], **15** and **17**, in the seeds. Though **4** was first identified in the pollens from *Taraxacum dens-leonis* (Compositae) by TLC, GLC and GC–MS [46], isolation of the sterol in this study from plants might be the first example.

Table 1 shows the distribution of 4α-methylsterols in the seeds of twelve plants among seven genera of Solanaceae. All of the seeds contained sixteen identified sterols. **1–6**, **8-17**, accompanied with several unidentified minor components. In addition to these sterols, **7** also

Table 1. Approximate composition (%) of 4α-methylsterols separated from the unsaponifiable matters of solanaceous seed oils

Seed materials	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**10**	**11**	**12**	**13**	**14**	**15**	**17**	Others*
Capsicum annuum L. (California chili)	4	3	3	13	9	6	5	7	23	tr	2	5	12	1	7
C. annuum L. (Takanotsume tougarashi)	3	9	1	7	9	3	15	4	30	tr	tr	5	5	1	8
C. annum L. var. *fasciculatum* Irish (Yatsubusa)	2	tr	1	4	tr	tr	19	tr	46	tr	tr	10	11	tr	7
C. annuum L. var. *cerasiforme* Irish (Goshiki tougarashi)	9	2	2	21	3	6	2	10	23	tr	tr	4	10	tr	8
C. annuum L. var. *angulosum* Mill.	5	1	3	14	2	5	3	8	28	tr	1	4	15	1	10
Lycopersicon esculentum Mill.	2	2	2	4	8	2	tr	1	44	tr	tr	8	23	tr	4
Physalis alkekengi L. var. *francheti* Hort.	1	4	8	7	2	14	tr	5	7	tr	3	36	8	tr	5
Lycium chinense Mill	tr	6	8	1	1	6	2	tr	9	tr	2	44	18	tr	3
Datura stramonium L.	tr	6	9	tr	2	13	1	1	6	tr	tr	43	13	tr	6
D. metel L.	tr	tr	4	1	2	73	2	2	3	tr	tr	tr	3	tr	10†
Nicotiana tabacum L. (MC-1)	tr	2	6	1	4	1	tr	1	14	1	1	5	60	1	3
Solanum melongena L (Shinkuro)	tr	tr	tr	2	1	tr	tr	1	13	4	65	3	8	1	2

*All of the seeds examined contained trace amounts of the sterols **9** and **16** in addition to an unidentified sterol with RR_t 1.15 and several other ones. † The sterol **7** was detected in a trace quantity.



was found in a trace quantity in *D. metel* seeds. The distribution pattern of the 4α-methylsterols in the solanaceous seeds was found to differ markedly from that in other seeds previously examined in this laboratory [12–14, 16], which have been shown to contain, in general, **3**, **6**, **14** and **15** as the principal 4α-methylsterol constituents. The peculiarity of Solanaceae seeds was observed also for the 4,4-dimethylsterol and 4-desmethylsterol fractions. The 4,4-dimethylsterol fractions contained significant amount of 5α-lanost-8-en-3β-ol, an unusual triterpene in higher plants, besides cycloartanol (9β,19-cyclo-5α-lanostan-3β-ol) and cycloartenol (9β,19-cyclo-5α-lanost-24-en-3β-ol) [1], and the 4-desmethylsterol fractions contained generally an unusually large proportion of cholesterol [2]. The Solanaceae examined can be classified into three groups based upon the compositions of the seed 4α-methylsterols. The first group, *Capsicum annuum* and *L. esculentum*, is characterized by the predominance of a sterol with a saturated C_8 side chain, **11**; the second group, *Physalis alkekengi*, *Lycium chinense*, *D. stramonium* and *D. metel*, contains the sterols with a C-24 methylenated side chain, **6** and **14**, as predominant components; and the third group, *Nicotiana tabacum* and *S. melongena*, contains those with a saturated or an unsaturated C_{10} side chain, **13** and **15**. These patterns are closely correlated with those of the other steroidal fractions; for instance, the 4-desmethylsterol fractions from the second group also contained a C-24 methylenated sterol, 24-methylenecholesterol (24-methylcholesta-5,24[28]-dien-3β-ol), besides 24-methylcholesta-5,24-dien-3β-ol and 28-isofucosterol (24-ethylcholesta-5,*Z*-24[28]-dien-3β-ol), as the major components [2].

EXPERIMENTAL

Recrystallizations were performed in Me_2CO–MeOH. Mps taken on a heat block are uncorr. IR spectra were recorded in KBr and PMR spectra were obtained with a 100 MHz FT-instrument, unless otherwise specified, in $CDCl_3$ with TMS as int. ref. MS (70 eV, > *m/e* 200) were taken with a GC–MS (2% OV-17 column) or with a probe injection. GLC were carried out on OV-17 SCOT glass capillary column (30 m × 0.3 mm, 260°, split ratio 50:1, sample vol. 1–2 μl) and $AgNO_3$–Si gel (1:4) PLC (0.5 mm) were developed 4 times with CH_2Cl_2–CCl_4 (1:5). The RR_t on GLC and *ca* relative R_f-value on argentation PLC for the acetates of authentic sterols and of the sterols isolated in this study were: cholesterol, RR_t 1.0 (relative R_f 1.0); 31-norcycloartanol (**1**), 1.33 (1.23); a mixture (0.82) of 31-norcycloartenol (**2**), 1.57, and 31-norlanosterol (**5**), 1.33; cycloeucalenol (**3**), 1.77 (0.42); 31-norlanost-8-enol (**4**), 1.11 (1.23); obtusifoliol (**6**), 1.48 (0.44); 4α,14α,24-trimethylcholesta-8,24-dienol (**7**), 1.79 (0.89); 31-norlanost-9(11)-enol (**8**), 1.26 (1.04); 24-methyl-31-norlanost-9(11)-enol (**9**), 1.64 (1.02); 4α-methylcholest-8-enol (**10**), 1.17 (1.13); lophenol (**11**), 1.34 (1.12); 24-methyllophenol (**12**), 1.75 (1.14); 24-ethyllophenol (**13**), 2.15 (1.13); gramisterol (**14**), 1.78 (0.30); citrostadienol (**15**), 2.40 (0.63); 4α,24-dimethylcholesta-7,24-dienol (**16**), 2.15 (0.84); 4α-methyl-24-ethylcholesta-7,24-dienol (**17**), 2.60 (0.84). The origin of the twelve seed samples now examined (Table 1) was reported in the previous paper [1] with one exception; the seeds of *D. metel* L. were courteously supplied by Prof. M. Takido of this university. Hydrogenation in Et_2O at room temp. was carried out over PtO_2. Extraction and saponification of seed oil and fractionation by PLC on Si gel of the unsaponifiable matter using hexane–Et_2O (7:3) as developer were performed as described previously [16]. The 4-monomethylsterol fraction obtained was further chromatographed on Si gel PLC to give two fractions. Each fraction was purified separately by rechromatography. The purified fraction was then acetylated by Ac_2O–Py and the resulting acetate fraction was further fractionated by argentation PLC. Approximate compositions of the 4-monomethylsterol fractions determined in Table 1 were based on the Si gel PLC, argentation PLC and GLC data. Identification of the compounds not described below was based upon the comparison of argentation PLC and GLC data and, if necessary, GC–MS data with those of authentic sterols and of the sterols authenticated in this study.

Separation of the 4-monomethylsterols of L. esculentum *seeds into two fractions by PLC on Si gel.* The 4-monomethylsterol fraction (850 mg) separated by PLC on Si gel from the unsaponifiable matter (10 g) of the seed oil, on further PLC, gave one band which was then divided into halves to give the fraction A from the less polar half and the fraction B from the more polar half. Both fractions were then rechromatographed separately on Si gel, yielding the refined fractions A (260 mg) and B (377 mg).

4α-Methylsterols of the fraction A of L. esculentum *seeds.* A portion of the acetylated fraction A (200 mg) was sepd into 5 bands (referred to bands 1–5 in the order of polarity, beginning with the least) by argentation PLC. The fraction (52 mg) from band 1 was a mixture of three components with RR_t 1.11 (34%), 1.15 (46%) and 1.33 (20%). The component with RR_t 1.33 showed the ions in GC–MS at *m/e* 456 (M^+), 441, 396, 381 (base peak), 341, 288, 283, 241 and 227. Since GLC and MS data were consistent with those of authentic 31-norcycloartanyl acetate (**1**-acetate), which was kindly supplied by Prof. H. J. Nicholas (St. Louis University, Missouri, USA), the component was regarded as the acetate of **1**. The fraction, on rechromatography by argentation PLC, afforded one band. The less polar section of the band on further purification by argentation PLC afforded **4**-acetate (RR_t 1.11, 3 mg), mp 101–103°. IR ν_{max} cm^{-1}: 1730, 1235 (OAc). PMR: δ 0.70 (3H, *s*, C-18), 0.89 (3H, *s*, C-32), 0.98 (3H, *s*, C-19), 2.05 (3H, *s*, C-3β-OAc), 0.86 (6H, *d*, *J* = 6 Hz, C-26, C-27), 4.38 (1H, *m*, $W_{1/2}$ = 26.4 Hz, C-3α). MS *m/e*: 456 (M^+), 441 (base peak), 381, 343, 301, 287, 283, 273, 269, 241, 227. A good agreement of the GLC and MS data with those of authentic 31-norlanost-8-enyl acetate (**4**-acetate) confirmed the structure **4**-acetate. Identification of the remaining one component with RR_t 1.15 is still underway. The fraction (15 mg) from band 2 afforded a fraction (10 mg) of **8**-acetate (RR_t 1.26) accompanied with a minor companion with RR_t 1.64 (**9**-acetate, ca 2%) after purification on argentation PLC, mp 113.5–115°. IR ν_{max} cm^{-1}: 3050, 1735, 1240, 810. PMR: δ 0.65 (3H, *s*), 0.74 (3H, *s*), 1.00 (3H, *s*), 2.05 (3H, *s*), 0.84 (3H, *d*, J = 5.4 Hz), 0.87 (6H, *d*, *J* = 6.1 Hz), 4.38 (1H, *m*, $W_{1/2}$ = 25 Hz), 5.30 (1H, *m*, $W_{1/2}$ = 9.9 Hz). MS *m/e* (rel. int.): 456 (M^+, 31), 441 (100), 396 (3), 381 (44), 343 (3), 301 (4), 283 (7), 275 (5), 273 (7), 241 (5), 227 (7), 215 (7), 201 (8). The chromatographic and spectral data as well as mp were compatible with those of authentic **8**-acetate. The minor component, **9**-acetate, showed the following ions in GC–MS at *m/e* (rel. int.): 470 (M^+, 19), 455 (100), 410 (9), 395 (76), 343 (5), 301 (6), 287 (17), 283 (10), 241 (13) and 227 (14). The GLC and MS data were identical with those of authentic 24-methyl-31-norlanost-9(11)-enyl acetate. The fraction (40 mg) from band 3 was a mixture of two components with RR_t 1.33 (70%) and 1.59 (30%). The faster eluted major component showed in GC–MS the molecular ion at *m/e* 454 ($C_{31}H_{50}O_2$, rel. int. 43) and other ions at *m/e* 439 (M^+ – Me, 100), 394 (M^+-AcOH, 12), 379 (M^+-Me-AcOH, 59), 301 (M^+-C_8H_{15} [SC]-C_3H_6 [part of ring D], 7), 287 (M^+-SC-C_3H_6-CH_2, 21) and 227 (*m/e* 287-AcOH, 33). The spectrum was almost indistinguishable from that reported for 31-norlanosteryl acetate (**5**-acetate) [5]. Thus the compound was considered to be **5**-acetate. This, on hydrogenation, afforded **4**-acetate (RR_t 1.11, M^+, *m/e* 456). The ratio (1.20) of the RR_t of the unhydrogenated compound (RR_t 1.33) to the RR_t of its dihydro derivative (**4**-acetate) is in good agreement with that observed for 4α-methylzymosteryl acetate (RR_t 1.40) and its dihydro derivative, **10**-acetate (RR_t 1.17). The GLC data support that the component with RR_t 1.33 possesses a Δ^{24}-bond in the side chain. GC–MS of the minor component (RR_t 1.59) showed ions at *m/e* 454 (M^+, $C_{31}H_{50}O_2$, rel. int. 13), 439 (M^+-Me, 12), 394 (M^+-AcOH, 100) and 379 (M^+-Me-AcOH, 99) indicating that it was an acetate of a C_{29} sterol with two double

bonds. The presence of the ions at *m/e* 287 (M^+-C_8H_{15} [SC]-C_3H_6 [part of ring D]-CH_2, 10) and 227 (*m/e* 287-AcOH, 15) showed the occurrence of one of the double bonds in the side chain and an additional C-32 methyl group in the ring system. Furthermore, the ion appeared at *m/e* 286 (M^+-$C_{10}H_{16}O_2$, 21) was probably due to the presence of a 9β,19-cyclopropyl group rather than the double bond in the ring system [47]. On hydrogenation, this compound gave **1**-acetate (RR_t 1.33; M^+, *m/e* 456). Based on the MS and the GLC correlation between this compound and its dihydro derivative, it was regarded as **2**-acetate. The fraction (34 mg) from band 4 was a mixture of four components in GLC with RR_t 1.05 (23%), 1.48 (26%), 1.77 (42%) and 2.33 (9%). GC–MS of the component eluted second (RR_t 1.48) showed ions at *m/e* 468 (M^+), 453 (base peak), 408. 393, 369, 341, 301, 287 and 227. The GLC and MS data were identical with those of authentic **6**-acetate isolated from the latex of *Euphorbia regis Jubae* W. B. (Euphorbiaceae) [38]. Thus the component is considered to be **6**-acetate. The component eluted third (RR_t 1.77) showed prominent ions in GC–MS at *m/e* 468 (M^+), 453, 408 (base peak), 393, 353, 300, 285 and 283. Since the GLC and MS data were in agreement with those of authentic **3**-acetate the component was regarded as **3**-acetate. The remaining two components (RR_t 1.05 and 2.33) in this fraction and several components in the fraction (19 mg) from band 5 remain as yet unidentified.

4α-Methylsterols of the fraction B of L. esculentum *seeds.* The aceylated fraction B (380 mg) was fractionated into 4 bands (referred to bands 1–4 in the order of polarity, beginning with the least) by argentation PLC. The fraction (178 mg) from the less polar major section of band 1 indicated a GLC peak with RR_t 1.34 accompanied with a minor component (RR_t 1.17, ca 5%), mp 113–114° IR $\nu_{max}cm^{-1}$: 1740, 1236 (OAc), 825, 815, 796 (>C=CH—). PMR: δ 0.53 (3H, *s*, C-18), 0.84 (3H, *s*, C-19), 2.05 (3H, *s*, C-3β-OAc), 0.87 (9H, *d*, *J* = 5.9 Hz, C-26, C-27, C-30), 0.92 (3H, *d*, *J* = 5.9 Hz, C-21), 4.40 (1H, *m*, $W_{1/2}$ = 25 Hz, C-3α), 5.18 (1H, *m*, $W_{1/2}$ = 10 Hz, C-7). MS *m/e* (rel. int.): 442 (M^+, $C_{30}H_{50}O_2$, 100), 427 (18), 382 (13), 367 (15), 329 (6), 287 (8), 269 (64), 243 (19), 227 (34). These data admit to interpret the structure of this compound as **11**-acetate (lit. [48] mp 112°, [49] mp 119–121°). GC–MS of the minor companion with RR_t 1.17 showed ions at *m/e* (rel. int.) 442 (M^+, 100), 427 (20), 382 (11), 367 (22), 329 (6), 287 (5), 269 (18), 243 (25) and 227 (31). The GLC and MS data were almost indistinguishable from those of authentic **10**-acetate. Hence the component was regarded as **10**-acetate. The fraction (6 mg) from the more polar minor section of band 1 comprized nearly equal proportion of three components with RR_t 1.75 and 2.15 and **11**-acetate accompanied with a trace amount of **10**-acetate. The component with RR_t 1.75 showed prominent ions in GC–MS at *m/e* 456 (M^+, base peak), 441, 396, 381, 329, 287, 269, 243 and 227. The GLC and MS data agreed with those of authentic **12**-acetate. Therefore, the component was regarded as **12**-acetate. Authentic **12**-acetate (24*RS* mixture) prepared from **14**-acetate by hydrogenation showed mp 138–139°. IR $\nu_{max}cm^{-1}$: 1735, 1238 (OAc), 824, 813 (>C=CH—). PMR (60 MHz): δ 0.54 (3H, *s*, C-18), 0.85 (3H, *s*, C-19). 2.05 (3H, *s*, C-3β-OAc), 0.80 (6H, *d*, *J* = 6 Hz, C-26, C-27) [20], 0.84 (3H, *d*, J = 6 Hz, C-30), 4.40 (1H, *m*, C-3α), 5.17 (1H, *m*, C-7). On the other hand, GLC and GC–MS (M^+, *m/e* 470) data of the component with RR_t 2,15 are identical with those of authentic **13**-acetate; therefore, the component was regarded as **13**-acetate. The fraction (4 mg) from the faint band 2 afforded a mixture of two components with RR_t 2.15 (60%) and 2.60 (40%). The faster eluted component with RR_t 2.15 indicated prominent ions in GC–MS at *m/e* (rel. int.) 454 (M^+, 13), 439 (13), 394 (6), 370 (36), 327 (100), 302 (6), 269 (19) 267 (9) and 227 (22). The GLC and MS data were close to those of authentic **16**-acetate. The component with RR_t 2.60 showed the GLC and GC–MS (M^+, *m/e* 468) data almost indistinguishable from those of authentic **17**-acetate. The fraction (112 mg) from band 3 afforded **15**-acetate (RR_t 2.40) after purification by argentation PLC, mp 149–151° (lit. [48] mp 143°, [50] mp 147–149°). IR ν_{max} cm^{-1}: 1720, 1250 (OAc) 830, 815, (>C=CH—). PMR: δ 0.54 (3H, *s*, C-18), 0.84 (3H, *s*, C-19), 2.05 (3H, *s*, C-3β-OAc), 0.85 (3H, *d* = 6.1 Hz, C-30), 0.95 (3H, *d*, *J* = 6.5 Hz, C-21), 0.98 (6H, *d*, *J* = 7.1 Hz, C-26, C-27), 1.59(3H, *d*, *J* = 6.2 Hz, C-29), 5.11 (1H, *q*, *J* = 6.6 Hz, C-28), 2.83 (1H, *hept.*, *J* = 6.9 Hz, C-25), 4.51 (1H, *m*, $W_{1/2}$ = 25 Hz, C-3α), 5.18 (1H, *m*, $W_{1/2}$ = 11 Hz, C-7). MS *m/e*: 468 (M^+), 370, 327 (base peak), 310, 295, 269, 267, 242, 227. The GLC and spectral data were identical with those of authentic **15**-acetate isolated from wheat germ oil [50]. The fraction (38 mg) from band 4 was a mixture of two components with RR_t 1.78 (75%) and 2.23 (25%) in GLC. The component with RR_t 1.78 was tentatively identified as **14**-acetate by the comparison of the GLC and GC–MS (M^+, *m/e* 456) data with those of authentic **14**-acetate isolated from wheat germ oil [50]. The remaining one minor component (RR_t 2.23) is as yet unidentified.

Isolation of **14** *from* N. tabacum *seeds.* The fraction (16 mg) from the more polar division of the 4-monomethylsterol band on Si gel PLC of the unsaponifiable matter (331 mg), on acetylation, afforded to acetate (15 mg). The acetate was sepd into three major bands on argentation PLC. The most polar band yielded **14**-acetate (*ca* 1 mg, RR_t 1.78), mp 133–135° (lit. [48] mp 134°, [50] mp 132–134°). IR $\nu_{max}cm^{-1}$: 1720, 1253 (OAc), 823, 816 (>C=CH—), 3080, 1637, 895 (>C=CH_2). PMR: δ 0.54 (3H, *s*, C-18), 0.84 (3H, *s*, C-19), 2.05 (3H, *s*, C-3β-OAc), 0.85 (3H, *d*, *J* = 6.3 Hz, C-30), 0.96 (3H, *d*, *J* = 6.6 Hz, C-21), 1.02 (6H, *d*, *J* = 6.7 Hz, C-26, C-27), 4.69 (2H, broad *d*, *J* = 4.8 Hz, C-28), 4.40 (1H, *m*, $W_{1/2}$ = 28 Hz, C-3α), 5.18 (1H, *m*, $W_{1/2}$ = 10.6 Hz, C-7). MS *m/e*: 454 (M^+), 439, 379, 370, 327 (base peak), 302, 287, 267, 227. The GLC and spectral data were indistinguishable from those of **14**-acetate.

Isolation of **13**, **15** *and* **17** *from* S. melongena *seeds.* The fraction (622 mg) from the more polar section accounting for 3/4 in area of the 4-monomethylsterol band on Si gel PLC of the unsaponifiable matter (4.5 g), on acetylation after rechromatography, gave the acetate (598 mg). The acetate fraction was sepd into 4 bands (referred to bands 1–4 in the order of polarity, beginning with the least) on argentation PLC. Rechromatography on argentation PLC of the less polar section of band 1 afforded **13**-acetate (160 mg), mp 153–155°. IR $\nu_{max}cm^{-1}$: 1735, 1238 (OAc), 830, 818 (>C=CH—). PMR: δ 0.53 (3H, *s*, C-18), 0.84 (3H, *s*, C-19), 2.05 (3H, *s*, C-3β-OAc), 0.81 (3H, *d*, *J* = 6.1 Hz, C-27) [21], 0.84 (3H, *d*, *J* = 6.9 Hz, C-26) [21], 0.92 (3H, *d*, *J* = 6.9 Hz, C-21), 0.84 (3H, *t*, *J* = 7 Hz, C-29) [21, 22], 4.40 (1H, *m*, $W_{1/2}$ = 26 Hz, C-3α), 5.17 (1H, *m*, $W_{1/2}$ = 10 Hz, C-7). MS *m/e*: 470 (M^+, base peak), 455, 410, 395, 329, 302, 287, 269, 243, 227. The GLC and spectral data were identical with those of authentic **13**-acetate (24*RS* mixture, mp 151–153°) prepared from **15**-acetate by hydrogenation. Band 2 afforded **17**-acetate accompanied with a trace amount of **16**-acetate (6 mg), mp 156–158°. IR ν_{max} cm^{-1}: 1730, 1238, 820, 815. PMR: δ 0.54 (3H, *s*), 0.84 (3H, *s*), 1.63 (6H, *s*), 2.05 (3H, *s*), 0.85 (3H, *d*, *J* = 6.7 Hz), 0.93 (3H, *t*, *J* = 7.2 Hz), 4.41 (1H, *m*, $W_{1/2}$ = 26 Hz), 5.17 (1H, *m*, $W_{1/2}$ = 11 Hz). MS *m/e* (rel. int.): 468 (M^+, 16), 453 (11), 408 (2), 393 (5), 370 (52), 327(100), 310 (5), 269 (13), 227 (10). These data as well as RR_t (2.60) in GLC were identical with those of authentic **17**-acetate. Band 3 afforded **15**-acetate (30 mg, mp 147–149°) and band 4 yielded a mixture (12 mg) of the acetates of **14** and an unidentified component with RR_t 2.26.

Identification of **6** *and* **7** *in* D. metel *seeds.* Rechromatography of the fraction (117 mg) from the less polar division of the band corresponding to 4-monomethylsterols on Si gel PLC of the unsaponifiable matter (4.0 g) afforded a fraction (62 mg), which on acetylation, yielded its acetate (69 mg). The acetate fraction was sepd into 5 bands by argentation PLC. The second faint band from the solvent front afforded a mixture (1 mg) constituted of a component with RR_t 1.79 and several other unidentified components. The component with RR_t 1.79 showed ions in GC–MS at *m/e* 468 (M^+), 435 (base peak), 408, 393, 384, 369, 341, 301, 297, 287, 241 and 227. The GLC and MS data were identical with those of authentic **7**-acetate [38]. Hence the component was regarded as **7**-acetate. The fraction (17 mg)

from the most polar band gave 6-acetate accompanied with a minor quantity of **3**-acetate (7%), mp 110–112° (authentic material, mp 106–108°), IR ν_{max} cm^{-1}: 1735, 1252 (OAc), 3080, 1640, 885 (>C=CH$_2$). PMR: δ 0.71 (3H, *s*, C-18), 0.89 (3H, *s*, C-32), 0.99 (3H, *s*, C-19), 2.05 (3H, *s*, C-3β-OAc), 0.86 (3H, *d*, J = 6 Hz, C-30), 0.93 (3H, *d*, J = 7 Hz, C-21), 1.03 (6H, *d*, J = 7 Hz, C-26, C-27), 4.70 (3H, *br d*, J = 4.5 Hz, C-28), 4.38 (1H, *m*, $W_{1/2}$ = 26 Hz, C-3α). The GLC and the spectral data are indistinguishable from those of authentic **6**-acetate.

Prepn of authentic material Authentic **4** and **8** were prepared from **1** by HCl isomerization in a similar manner as described earlier [24, 51–53]. A soln of **1** acetate (8 mg) in $CHCl_3$ (5 ml) was treated with a vigorous stream of dry HCl at room temp. for 30 min. After evapn of $CHCl_3$, the residue was chromatographed on argentation PLC to yield two distinct bands. The fraction (*ca* 1 mg) from the less polar division of the band closer to the solvent front was a mixture of the Δ^8- and Δ^7-isomers of **1**-acetate [24, 52, 53], *i.e.* **4**-acetate (RR_t 1.10, 60%) and 4α,14α-dimethyl-5α-cholest-7-en-3β-yl acetate (RR_t 1.31, 40%). The mixture was used without further purification for the identification of **4**-acetate isolated in this study. The band closer to the starting line afforded the $\Delta^{9(11)}$-isomer of **1**-acetate [24, 52, 53], *i.e.* **8**-acetate (RR_t 1.26, 2 mg), mp 111–113°. The HCl isomerization was also performed for the preparation of authentic **9**-acetate from 24(28)-dihydrocycloeucalenyl acetate (95 mg), which was obtained by hydrogenation of **3**-acetate. The isomerization gave a mixture of Δ^7-, Δ^8- and $\Delta^{9(11)}$-isomers. The $\Delta^{9(11)}$-isomer (**9**-acetate, 24*RS* mixture, 32 mg) isolated from the mixture by argentation PLC showed mp 123–125° (lit. [53] mp 117.5–118.5°). IR ν_{max} cm^{-1}: 1730, 1240 (OAc), 3050, 812 (>C=CH—). PMR: δ 0.65 (3H, *s*, C-18), 0.75 (3H, *s*, C-32), 1.00 (3H, *s*, C-19), 2.05 (3H, *s*, C-3β-OAc), 0.79 (6H, *d*, J = 7 Hz, C-26, C-27) [20], 0.84 (3H, *d*, J = 6 Hz, C-30), 0.86 (3H, *d*, J = 7 Hz, C-21) [20], 4.37 (1H, *m*, $W_{1/2}$ = 25 Hz, C-3α), 5.29 (1H, *m*, $W_{1/2}$ = 10 Hz, C-11). MS *m/e*: 470 (M$^+$). Authentic **10**-acetate was prepared by hydrogenation of 4α-methylzymosteryl acetate isolated by argentation PLC from the acetylated 4-monomethylsterol fraction of a bakery yeast, *Saccharomyces* sp. The yeast was kindly supplied by Dr. T. Shimomura (Kanegafuchi Chemical Industry Co., Hyogo, Japan). 4α-Methylzymosteryl acetate, mp 144–146° (lit. [54] mp 143–145.5°). IR ν_{max} cm^{-1} 1730, 1242 (OAc), 842, 825 (>C=CH—). PMR: δ 0.61 (3H, *s*, C-18), 0.98 (3H, *s*, C-19), 1.60 (3H, *br s*, C-26) [55], 1.68 (3H, *br s*, C-27) [55], 2.05 (3H, *s*, C-3β-OAc), 0.85 (3H, *d*, J = 6 Hz, C-30), 0.95 (3H, *d*, J = 7 Hz, C-21), 5.09 (1H, *t*, J = 7 Hz, C-24), 4.37 (1H, *m*, $W_{1/2}$ = 26 Hz, C-3α). MS *m/e*: 440 (M$^+$, base peak), 425, 380, 365, 327, 287, 269, 243, 241, 227. **10**-Acetate, mp 95–102° (lit. [56] mp 106.5–108.5°). IR ν_{max}cm^{-1} 1730, 1240 (OAc). PMR: δ 0.61 (3H, *s*, C-18), 0.98 (3H, *s*, C-19), 2.05 (3H, *s*, C-3β-OAc), 0.85 (3H, *d*, J = 6 Hz, C-30), 0.86 (6H, *d*, J = 6 Hz, C-26, C-27), 4.39 (1H, *m*, $W_{1/2}$ = 26 Hz, C-3α). MS *m/e*: 442 (M$^+$, base peak), 427, 382, 367, 329, 287, 269, 243, 227. Authentic **16**-acetate was prepared from **14**-acetate by isomerization of the side chain double bond with H_2SO_4 in the same way as described previously [38, 57] with one exception that a mixture of EtOAc and IPA instead of IPA alone was used as the solvent. A soln of **14**-acetate (30 mg) in EtOAc (30 ml), IPA (100 ml) and H_2SO_4 (3.7 g) was refluxed for 6 hr. The reaction mixture was concd under red. pres., added with an excess amount of H_2O and was extracted with Et_2O. The usual work-up of the extract followed by PLC on Si gel afforded the isomerized mixture (8 mg), which on argentation PLC was sepd into four bands. The least polar band afforded **16**-acetate (*ca* 1 mg), mp 168–170°. IR ν_{max} cm^{-1} 1735, 1240 (OAc), 822, 816 (>C=CH—). PMR: δ 0.53 (3H, *s*, C-18), 0.84 (3H, *s*, C-19), 1.62 (9H, *s*, C-26, C-27, C-28), 2.05 (3H, *s*, C-3β-OAc), 0.85 (3H, *d*, J = 6 Hz, C-30), 0.96 (3H, *d*, J = 6 Hz, C-21), 4.40 (1H, *m*, $W_{1/2}$ = 21 Hz, C-3α), 5.18 (1H, *m*, $W_{1/2}$ = 10 Hz, C-7). MS *m/e*: 454(M$^+$). Authentic **17**-acetate was prepared from **15**-acetate also by H_2SO_4 isomerization in the same way as above. Isomerization and subsequent PLC on Si gel of **15**-acetate (100 mg) afforded the isomerized mixture (32 mg) which was then sepd into 3 bands on argentation PLC. The least polar band afforded **17**-acetate (2 mg, mp 156–158°) and the most polar band gave unisomerized **15**-acetate (19 mg). The fraction (6 mg) from the middle band was a mixture of two unidentified components. The following PMR data for the skeletal methyl and methine signals of two tetracyclic triterpene acetates [24, 58] were used as the references for the assignment of the PMR signals of the $\Delta^{9(11)}$ 4α-methylsterols described in this paper. Lanost-9(11)-enyl acetate, PMR: δ 0.64 (3H, *s*, C-18), 0.74 (3H, *s*, C-32), 0.87 and 0.89 (each 3H, *s*, C-30 and C-31), 1.07 (3H, *s*, C-19), 5.28 (1H, *m*, $W_{1/2}$ = 11 Hz, C-11). Lanost-7-enyl acetate, PMR: δ 0.64 (3H, *s*, C-18), 0.87 (3H, *s*, C-30 or C-31), 0.89 (6H, *s*, C-19 and either C-30 or C-31), 0.97 (3H, *s*, C-32), 5.19 (1H, *m*, $W_{1/2}$ = 9 Hz, C-7).

Nomenclature. 31-Norcycloartanol = 4α,14α-dimethyl-9β,19-cyclo-5α-cholestan-3β-ol (**1**); 31-norcycloartenol = 4α,14α-dimethyl-9β, 19-cyclo-5α-cholest-24-en-3β-ol (**2**); cycloeucalenol = 4α,14α,24-trimethyl-9β,19-cyclo-5α-cholest-24(28)-en-3β-ol (**3**); 31-norlanost-8-enol = 4α,14α-dimethyl-5α-cholest-8-en-3β-ol (**4**); 31-norlanosterol = 4α,14α-dimethyl-5α-cholesta-8, 24-dien-3β-ol (**5**); obtusifoliol = 4α,14α, 24-trimethyl-5α-cholesta-8, 24(28)-dien-3β-ol (**6**), 4α,14α,24-trimethylcholesta-8,24-dienol = 4α,14α,24-trimethyl-5α-cholesta-8,24-dien-3β-ol (**7**); 31-norlanost-9(11)-enol = 4α,14α-dimethyl-5α-cholest-9(11)-en-3β-ol (**8**); 24-methyl-31-norlanost-9(11)-enol = 4α,14α,24ξ-trimethyl-5α-cholest-9(11)-en-3β-ol (**9**); 4α-methylcholest-8-enol = 4α-methyl-5α-cholest-8-en-3β-ol (**10**); lophenol = 4α-methyl-5α-cholest-7-en-3β-ol (**11**); 24-methyllophenol = 4α,24ξ-dimethyl-5α-cholest-7-en-3β-ol (**12**); 24-Ethyllophenol = 4α-methyl-24ξ-ethyl-5α-cholest-7-en-3β-ol (**13**); gramisterol = 4α,24-dimethyl-5α-cholesta-7,24(28)-dien-3β-ol (**14**); citrostadienol = 4α-methyl-24-ethyl-5α-cholesta-7,*Z*-24(28)-dien-3-β-ol (**15**); 4α,24-dimethylcholesta-7,24-dienol = 4α,24-dimethyl-5α-cholesta-7,24-dien-3β-ol (**16**); 4α-methyl-24-ethylcholesta-7,24-dienol = 4α-methyl-24-ethyl-5α-cholesta-7,24-dien-3β-ol (**17**); cholesterol = cholest-5-en-3β-ol; 4α-methylzymosterol = 4α-methyl-5α-cholesta-8,24-dien-3β-ol; 24(28)-dihydrocycloeucalenol = 4α,14α,24ξ-trimethyl-9β,19-cyclo-5α-cholestan-3β-ol, lanost-9(11)-enol = 5α-lanost-9(11)-en-3β-ol; lanost-7-enol = 5α-lanost-7-en-3β-ol.

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