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24-METHYL-*E*-23-DEHYDROLOPHENOL, A NEW STEROL AND TWO OTHER 24-METHYL-*E*-Δ^{23}-STEROLS IN *ZEA MAYS* GERM OIL

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Key Word Index—*Zea mays*; Gramineae; germ oil; 24-methyl-*E*-Δ^{23}-sterols; ^{1}H and ^{13}C NMR spectra; 24-methyl-*E*-23-dehydrocycloartanol; cyclosadol; 24-methyl-*E*-23-dehydrolophenol; 24-methyl-*E*-23-dehydrocholesterol.

Abstract—The configuration of the Δ^{23}-bond of cyclosadol (24-methyl-23-dehydrocycloartanol) was determined as *E* based on the ^{1}H and ^{13}C NMR comparisons with model olefins. This sterol and two other 24-methyl-*E*-Δ^{23}-sterols, 24-methyl-*E*-23-dehydrolophenol and 24-methyl-*E*-23-dehydrocholesterol, of which the former is considered to be a new sterol from natural sources, were detected as the minor sterols in the unsaponifiable lipid of maize germ oil.

INTRODUCTION

Sterols of maize (*Zea mays*) have been much studied and several usually occurring compounds have been identified in the unsaponifiable lipid of the germ oil [1–6] as well as in other plant materials [7–11]. In addition, maize has been reported to contain three 24-methyl-Δ^{23}-sterols. They are cyclosadol (**1a**, 24-methyl-23-dehydrocycloartanol) isolated from the unsaponifiable lipid of the germ oil [12], and 24-methyl-23-dehydrocholesterol (**3a**) and 24-methyl-23-dehydrolathosterol isolated from the etiolated coleoptiles [13], but the configuration of the Δ^{23}-bond of these sterols remained undetermined. This paper describes a further study on the sterols of maize germ oil resulting in the detection of a new 24-methyl-Δ^{23}-sterol, 24-methyl-23-dehydrolophenol (**2a**), besides two of the above 24-methyl-Δ^{23}-sterols, **1a** and **3a**. The configuration of the Δ^{23}-bond of these three sterols was established as *E* based on the GLC correlation with synthetic **1a**, the Δ^{23}-bond of which was determined as the *E*-configuration by the ^{1}H and ^{13}C NMR comparisons with model olefins.

Nomenclature: Cyclosadol (24-methyl-*E*-23-dehydrocycloartanol) = 24-methyl-9β,19-cyclo-5α-lanost-*E*-23-en-3β-ol; cycloartenol = 9β,19-cyclo-5α-lanost-24-en-3β-ol; cycloartanol = 9β,19-cyclo-5α-lanostan-3β-ol; 24-methylenecycloartanol = 24-methyl-9β, 19-cyclo-5α-lanost-24(28)-en-3β-ol; 24-methylcycloartanol = 24ξ-methyl-9β,19-cyclo-5α-lanostan-3β-ol; α-amyrin = 5α-urs-12-en-3β-ol; β-amyrin = 5α-olean-12-en-3β-ol; 24-methyl-*E*-23-dehydrolophenol = 4α,24-dimethyl-5α-cholesta-7,*E*-23-dien-3β-ol; lophenol = 4α-methyl-5α-cholest-7-en-3β-ol; 24-methyllophenol = 4α,24ξ-dimethyl-5α-cholest-7-en-3β-ol; 24-ethyllophenol = 4α-methyl-24ξ-ethyl-5α-cholest-7-en-3β-ol; gramisterol = 4α,24-dimethyl-5α-cholesta-7,24(28)-dien-3β-ol; citrostadienol = 4α-methyl-24-ethyl-5α-cholesta-7,*Z*-24(28)-dien-3β-ol; obtusifoliol = 4α, 14α,24-trimethyl-5α-cholesta-8,24(28)-dien-3β-ol; cycloeucalenol = 4α,14α,24-trimethyl-9β,19-cyclo-5α-cholest-24(28)-en-3β-ol; 24-methyl-*E*-23-dehydrocholesterol = 24-methylcholesta-5,*E*-23-dien-3β-ol; 24-methyl-*E*-23-dehydrolathosterol = 24-methyl-5α-cholesta-7,*E*-23-dien-3β-ol; fucosterol = 24-ethylcholesta-5,*E*-24(28)-dien-3β-ol; isofucosterol = 24-ethylcholesta-5,*Z*-24(28)-dien-3β-ol; cholesterol = cholest-5-en-3β-ol; 24-methylcholesterol = 24ξ-methylcholest-5-en-3β-ol; 24-ethylcholesterol = 24ξ-ethylcholest-5-en-3β-ol; 24-ethylcholesta-5,*E*-22-dienol = 24ξ-ethylcholesta-5,*E*-22-dien-3β-ol; 24-methylenecholesterol = 24-methylcholesta-5,24(28)-dien-3β-ol.

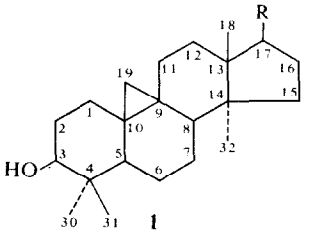
RESULTS AND DISCUSSION

The 4,4-dimethyl- (60 mg), 4-monomethyl- (84 mg) and 4-desmethyl-sterols (950 mg) separated by Si gel TLC from the unsaponifiable lipid (2 g) of crude maize germ oil (170 g) were acetylated. The 4,4-dimethylsteryl acetates (56 mg) were separated into seven bands by $AgNO_3$-Si gel TLC in a similar manner to that already described [14]. Band 1 (R_f 0.69, 1 mg) contained α-amyrin and β-amyrin acetates, band 2 (R_f 0.64, 1 mg) contained several unidentified components, band 4 (R_f 0.31, 18 mg) gave cycloartenyl acetate, band 5 (R_f 0.17, 16 mg) afforded 24-methylenecycloartanol (**1c**) acetate, and band 6 (R_f 0.10, 1 mg) and band 7 (R_f 0.06, 2 mg) contained several unidentified components. Band 3 (R_f 0.40, 2 mg) afforded a fraction rich in a steryl acetate (RR_t: OV-17, 2.08; OV-1, 2.00). The mass spectrum of the steryl acetate showed that it was an acetate of a C_{31}-sterol with two double bonds (m/z 482, M^+) of which one was located in the side chain (m/z 295, M − HOAc − C_9H_{17}[SC] − 2H) [15]. The fragment ions at m/z 255 (M − HOAc − SC − C_3H_6) and 241 (m/z 255 − CH_2) indicated the presence of an additional C-32 methyl group in the ring system [16]. A prominent fragment ion at m/z 325 (M − HOAc − C_7H_{13}) suggested that the side chain double bond was located at C-23 in order to facilitate cleavage at the C-20, C-22 bond [13]. Furthermore, an ion at m/z 300 (M − $C_{10}H_{16}O_2$ − Me) was probably due to the presence of a 9β,19-cyclopropyl group rather than the double bond in the ring system [17]. The mass spectral data thus suggest that the sterol possessed a 9β,19-cyclolanostane ring

system (**1**) with a 24-methyl-Δ^{23} side chain (**a**), and the GLC and mass spectral data were consistent with those of authentic cyclosadol (**1a**) acetate, which has been isolated previously from maize germ oil [12]. The sterol detected here was therefore identified as **1a**.

The configuration of the Δ^{23}-bond of **1a** remained undetermined [12], and hence in order to establish the configuration, the ^{1}H NMR and ^{13}C NMR signals arising from the side chain of **1a** were compared with those of two model olefins, 3,4-dimethyl-*E*-2-pentene (**4**) and its *Z*-isomer (**5**), either representing part of the side chain of **1a**. A sufficient amount of **1a** was then prepared following isomerization of 24-methylenecycloartanol (**1c**) with *N*-lithioethylenediamine in ethylenediamine under reflux [18, 19]. Synthetic **1a**-acetate, which showed identical GLC and mass spectral data with those of authentic **1a**-acetate, afforded the ^{1}H NMR signals for the ring system protons identical with those for **1c**-acetate [14]. The ^{1}H NMR spectrum of **1a**-acetate showed a singlet at δ 1.55 and a septet at δ 2.18 corresponding to the C-28 methyl and C-25 methine protons, respectively. The chemical shifts of these signals are consistent with those for the C-7 methyl protons (*s*, δ 1.56) and the C-4 methine proton (*septet*, δ 2.22) of the *E*-isomer of 3,4-dimethyl-2-pentene (**4**), whereas they differed sufficiently for differentiation from those for its *Z*-isomer (**5**) which showed the C-7 methyl signal at δ 1.59 and the C-4 methine signal at δ 2.83. The Δ^{23}-bond of **1a** was therefore concluded to have the *E*-configuration. The ^{1}H NMR spectral correlation for the allylic methine signals was consistent with that previously observed for the C-24 isomeric pair of 24-ethylidene sterols: fucosterol (24*E*-isomer; C-25 methine, δ 2.2, *septet*) and isofucosterol (24*Z*-isomer; C-25 methine, δ 2.8, *septet*) [20, 21].

The ^{13}C NMR correlation of **1a** with **4** and **5** further supported the *E*-configuration of the Δ^{23}-bond of **1a**. In the ^{13}C NMR spectrum of **1a**, the chemical shifts of the signals arising from the ring system carbons (C-1 through C-19, and C-30 through C-32), and C-20 and C-21 carbons were consistent with the corresponding signals of the literature data of cycloartanol [22]. Among the other side chain signals of **1a**, the C-25 through C-28 signals were shown to have almost identical chemical shifts with those of the C-4 through C-7 signals, respectively, of **4**, the *E*-isomer of the model olefins, i.e. **1a**: C-25 (δ 37.1), C-26 (21.6), C-27 (21.6) and C-28 (13.5); and **4**: C-4 (δ 36.8), C-5 (21.4), C-6 (21.4) and C-7 (13.2 or 13.0, of which the former is preferable). However **5**, the *Z*-isomer of the model olefins, showed strikingly different resonances from those of its *E*-isomer, i.e. **5**: C-4 (δ 28.1), C-5 (20.7), C-6 (20.7) and C-7 (18.1). Though the *Z*-isomer of **1a** was unavailable, it seems probable that each of the isomeric pair of Δ^{23}-sterols would elute separately on dimethyl silicone (OV-1) and on phenyl methyl silicone (OV-17)



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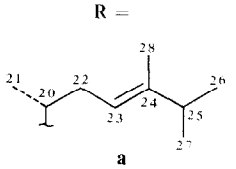

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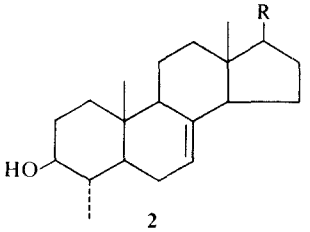

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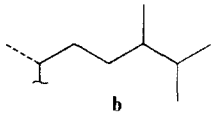

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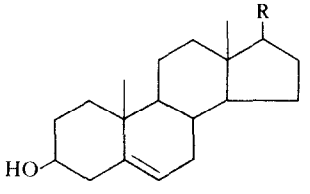

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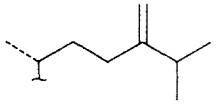

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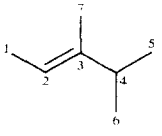

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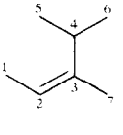

5

stationary phases in GLC as do each of the isomeric pairs of Δ^{22}- [23] and Δ^{24}- [24, 25] sterols, respectively, and the natural cyclosadol was therefore considered to be the *E*-counterpart, i.e. **1a**.

The 4-monomethylsteryl acetates (80 mg) separated into five bands by $AgNO_3$–Si gel TLC in a similar way to that already described [24]. Band 1 (R_f 0.56, 3 mg) contained lophenyl, 24-methyllophenyl and 24-ethyllophenyl acetates; band 2 (R_f 0.41, 2 mg) contained several unidentified components; band 4 (R_f 0.22, 19 mg) contained obtusifoliyl and cycloeucalenyl acetates; and band 5 (R_f 0.15, 16 mg) gave gramisteryl acetate. Band 3 (R_f 0.32, 25 mg) contained citrostadienyl acetate (*ca* 85%) and an unknown steryl acetate (*ca* 15%; RR_t: OV-17, 1.79; OV-1, 1.63). The mass spectrum of the steryl acetate showed that it was an acetate of a C_{29}-sterol with two double bonds (*m/z* 454, M^+). A prominent fragment ion at *m/z* 297 (M − HOAc − C_7H_{13}) suggested that one of the double bonds was located in the side chain, most probably at C-23 as in the case of **1a**, in order to facilitate cleavage at the C-20, C-22 bond [13]. The other double bond was probably located at C-7 since a fragment ion at *m/z* 327 (base peak, M − SC − 2H), characteristic of the Δ^7-bond in addition to a double bond in the side chain [15, 26] was observed. That the sterol possessed the lophenol (4α-methyl-Δ^7) ring system (**2**) and an extra methyl group at C-24 was demonstrated by the formation of 24-methyllophenol (**2b**) acetate (RR_t: OV-17, 1.72; OV-1, 1.66), which was identified by GLC and MS, on partial hydrogenation of its acetate. Furthermore, the following GLC correlation provided evidence for the *E*-configuration of the Δ^{23}-bond of the sterol. The separation factors between the steryl acetate vs **2b**-acetate were calculated as 1.04 (OV-17) and 0.98 (OV-1), which were consistent with the 24-methyl *E*-Δ^{23}/24-methyl saturated side chain separation factors calculated from the retention data of **1a**-acetate vs 24-methylcycloartanol (**1b**) acetate (RR_t: OV-17, 2.00; OV-1, 2.04), respectively. Thus it was concluded that the sterol had the structure 24-methyl-*E*-23-dehydrolophenol (**2a**).

The 4-desmethylsteryl acetates (100 mg) separated into five major bands by $AgNO_3$–Si gel TLC in a similar way to that previously described [25]. Band 1 (R_f 0.46, 66 mg) contained 24-methylcholesterol (**3b**) acetate (RR_t: OV-17, 1.31; OV-1, 1.29) and 24-ethylcholesteryl acetate, band 2 (R_f 0.40, 5 mg) gave 24-ethylcholesta-5,*E*-22-dienyl acetate, band 4 (R_f 0.20, 12 mg) afforded isofucosteryl acetate and band 5 (R_f 0.15, 2 mg) contained 24-methylenecholesteryl acetate accompanied by other minor components. Band 3 (R_f 0.29, 2 mg) contained a steryl acetate (RR_t: OV-17, 1.36; OV-1, 1.26) and other components. The mass spectrum of the steryl acetate showed that it was an acetate of a C_{28}-sterol with two double bonds (*m/z* 380, M − HOAc) of which one was in the side chain (*m/z* 253, M − HOAc − SC − 2H) [15], probably located at C-23 (*m/z* 283, base peak, M − HOAc − C_7H_{13}) [13] as in the cases of **1a** and **2a**. The other double bond was most probably located at C-5 since no molecular ion peak could be observed at *m/z* 440 [24]. The mass spectral fragmentation pattern was identical with that reported for 24-methyl-23-dehydrocholesterol (**3a**) acetate [13] and the sterol was therefore regarded as **3a**. The separation factors in GLC of the steryl acetate vs **3b**-acetate were calculated to be 1.04 (OV-17) and 0.98 (OV-1), which were identical with the 24-methyl *E*-Δ^{23}/24-methyl saturated side chain separation factors described above, and consequently the sterol was considered to have the structure 24-methyl-*E*-23-dehydrocholesterol (**3a**).

Three sterols, **1a**, **2a** and **3a**, detected as the minor sterols in the unsaponifiable lipid of maize germ oil, were thus demonstrated to have the 24-methyl-*E*-Δ^{23} side chain, and among which **2a** was considered to be a new sterol from natural sources. The *E*-configuration of the Δ^{23}-bond of **1a** isolated previously from maize germ oil [12] was also verified here by the evidence from the GLC and mass spectral data as described above. Furthermore, though the configuration of the Δ^{23}-bond of **3a** and 24-methyl-23-dehydrolathosterol isolated previously from maize coleoptiles [13] was not determined, the ^1H NMR signals of the C-28 methyl and C-25 methine protons for the acetates of **3a** (C-28, δ 1.544, *s*; C-25, δ 2.232, *septet*) and 24-methyl-23-dehydrolathosterol (C-28, δ 1.547, *s*; C-25, δ 2.234, *septet*) cited in the literature were consistent with the corresponding signals of synthetic **1a**-acetate, and hence these sterols also have the *E*-configuration of their Δ^{23}-bond.

Although 24-methyl-Δ^{23}-sterols are known to be produced from 24-methylene-sterols by isomerization either with iodine in benzene under reflux [13] or with *N*-lithioethylenediamine complex under reflux as described in the Experimental, **1a**, **2a** and **3a** detected in this study are considered to be the natural products rather than artefacts produced from the corresponding 24-methylene-sterols during the extraction and separation procedures because the isomerization could only be achieved under chemically drastic conditions. The intermediacy of Δ^{23}-sterols in the biosynthesis of 24-alkyl sterols has been proposed previously [13, 27] and the three 24-methyl-*E*-Δ^{23}-sterols now found might be considered to participate in the biosynthesis of sterols in maize germ.

EXPERIMENTAL

Materials. Plant material used in this work was the crude oil extracted commercially from maize germ by hexane and generously supplied by Nihon Shokuhin Kako Ltd. (Fuji-shi, Shizuoka). Authentic cyclosadol (**1a**) was courteously supplied by Dr. Pinhas (Recherche Laroche Navarron, Montlhéry, France). 24-Methyllophenol (**2b**) [24], 24-methylcycloartanol (**1b**) [28] and 24-methylenecycloartanol (**1c**) [28] were used in this study. The *E*- (**4**) and *Z*- (**5**) isomers of 3,4-dimethyl-2-pentene were purchased from Chemical Samples Co. (Colombus, Ohio, U.S.A.).

General. Most of the techniques used in this study have been described previously [14, 23, 25]. GLC was performed either on OV-17 (column 260°) or on OV-1 (column 255°) SCOT glass capillary column (30 m × 0.3 mm i.d.). The RR_t of steryl acetates is given relative to cholesteryl acetate. MS (70 eV, >*m/z* 100) were taken with a GC/MS (2% OV-17 column). ^1H NMR (100 MHz) and ^{13}C NMR (25.05 MHz) spectra were recorded in $CDCl_3$ and the chemical shifts (δ) are expressed in ppm relative to internal TMS. ^{13}C FT NMR measurement conditions were as follows: spectral width 5 kHz, pulse width 6 μsec, acquisition time 2.5 sec, and number of data points 8192. Identification of the sterols not described below was performed by GLC on the 2 columns and $AgNO_3$-Si gel TLC as the acetates.

Preparation of cyclosadol (**1a**) *acetate from 24-methylenecycloartanol* (**1c**) *acetate with N-lithioethylenediamine.* **1c**-Acetate (900 mg) was added to a stirred soln of *N*-lithioethylenediamine complex (Li 1.5 g, ethylenediamine 45 ml; N_2) [18, 19] under reflux and the mixture further refluxed for 6 hr. The product

obtained after usual work-up was acetylated (pyridine–Ac_2O) to give a product (530 mg) of which upon $AgNO_3$-Si gel TLC separated into 4 bands. The fraction recovered from the 3rd band (R_f 0.40) from the solvent front upon further $AgNO_3$-Si gel TLC gave **1a**-acetate (40 mg), mp 115.5–118.5°. IR ν_{max}^{KBr} cm^{-1}: 3050, 1020 (cyclopropyl), 830, 821, 815 (>C=CH—), 1735, 1242 (OAc). ^1H NMR: δ 0.85 (3 H, *s*, C-30), 0.90 (6 H, *s*, C-31, C-32), 0.96 (3 H, *s*, C-18), 1.55 (3 H, *s*, C-28), 2.05 (3 H, *s*, C-3β-OAc), 0.84 (3 H, *d*, J = 5.6 Hz, C-21), 0.99 (6 H, *d*, J = 6.9 Hz, C-26, C-27), 0.34, 0.58 (each 1 H, *d*, J = 4 Hz, C-19), 2.18 (1 H, *septet*, J = 6.8 Hz, C-25), 5.16 (1 H, *t*, J = 6 Hz, C-23), 4.54 (1 H, *m*, $W_{1/2}$ = 20 Hz, C-3α). MS *m/z* (rel. int.): 482 (13, M^+), 467 (9), 422 (68), 407 (60), 385 (9), 379 (25), 353 (20), 325 (53), 300 (26), 297 (18), 295 (11), 255 (10), 241 (10), 229 (25), 203 (74), 173 (59), 161 (54), 147 (74), 121 (86), 109 (89), 107 (100). Hydrolysis of **1a**-acetate gave free **1a**, mp 120–124°. ^{13}C NMR of **1a**: C-1 (δ 31.9), C-2 (30.3), C-3 (78.8), C-4 (40.4), C-5 (47.0), C-6 (21.1), C-7 (28.2), C-8 (47.9), C-9 (20.0), C-10 (26.0), C-11 (26.0), C-12 (35.6), C-13 (45.3), C-14 (48.7), C-15 (32.8), C-16 (26.4), C-17 (52.4), C-18 (18.0), C-19 (29.9), C-20 (36.9), C-21 (18.3), C-22 (34.3), C-23 (120.8), C-24 (141.3), C-25 (37.1), C-26 (21.6), C-27 (21.6), C-28 (13.5), C-30 (25.4), C-31 (14.0), C-32 (19.3).

3,4-Dimethyl-*E*-2-pentene (**4**): ^1H NMR: δ 0.98 (6 H, *d*, J = 7.1 Hz, C-5, C-6), 1.56 (3 H, *s*, C-7), 1.56 (3 H, *d*, J = 4.9 Hz, C-1), 2.22 (1 H, *septet*, J = 6.9 Hz, C-4), 5.21 (1 H, *m*, $W_{1/2}$ = 17 Hz, C-2); ^{13}C NMR: C-1 (δ 13.0 or 13.2, of which the former is preferable), C-2 (115.7), C-3 (141.6), C-4 (36.8), C-5 (21.4), C-6 (21.4), C-7 (13.0 or 13.2, of which the latter is preferable). 3,4-Dimethyl-*Z*-2-pentene (**5**): δ 0.96 (6 H, *d*, J = 7.1 Hz, C-5, C-6), 1.56 (3 H, *d*, J = 4.9 Hz, C-1), 1.59 (3 H, *s*, C-7), 2.83 (1 H, *septet*, J = 6.9 Hz, C-4), 5.13 (1 H, *m*, $W_{1/2}$ = 17 Hz, C-2); ^{13}C NMR: C-1 (δ 12.7), C-2 (117.4), C-3 (141.4), C-4 (28.1), C-5 (20.7), C-6 (20.7), C-7 (18.1). Assignment of the ^{13}C NMR signals of **4** and **5** was facilitated by the off-resonance decoupling experiment and comparison of related olefins with the literature data [29].

24-Methyl-*E*-23-dehydrolophenol (**2a**) acetate: MS *m/z* (rel. int.): 454 (13, M^+), 439 (10), 394 (4), 379 (5), 357 (19), 327 (100), 297 (41), 267 (10), 227 (13), 215 (13), 201 (11), 121 (31), 119 (32), 107 (35). 24-Methyllophenol (**2b**) acetate: MS *m/z* (rel. int.): 456 (100, M^+), 441 (16), 396 (14), 381 (20), 329 (9), 302 (7), 287 (7), 269 (47), 243 (15), 227 (28), 173 (9), 161 (19), 147 (16), 135 (12), 109 (22), 107 (19). 24-Methyl-*E*-23-dehydrocholesterol (**3a**) acetate: MS *m/z* (rel. int.): 380 (97, M^+ – HOAc), 365 (11), 296 (8), 283 (100), 259 (6), 255 (11), 253 (42), 227 (8), 217 (6), 215 (12), 213 (13), 159 (33), 133 (65).

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