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NEW METABOLITES OF *FUSARIUM MONILIFORME* SHELD

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Abstract—Culture filtrates of *Fusarium moniliforme* contain 16α-hydroxy-(−)-kauran-19-al (**8**) and 16α-hydroxy-(−)-kauran-19-oic acid (**9**)—two diterpenoids related to the intermediates in the biosynthesis of gibberellins. The previously described 7,13-dihydroxykaurenolide (**10**) is an endogenic metabolite of the fungus. From the mycelium extracts three C_{28}-sterols were isolated including the hitherto unknown 5α, 8α-epidioxyergosta-6,9(11), 22-trien-3β-ol-12-one (**19**). The content of the oxidized kaurene derivatives in the mycelium was much lower than in the culture filtrate. Among the fatty acids of the mycelium a new compound, 4-oxododecanedioic acid (**21**) was isolated.

THE fungus *Fusarium moniliforme* Sheld. produces a number of diterpenoids belonging to the (−)-kaurane group.[1–4] Some are of particular interest as intermediates in the biosynthesis of gibberellins both in the fungus itself[4–8] and in higher plants.[8–10] In this communication we report the occurrence of kaurane diterpenoids in the culture filtrates and in the mycelium cells of *F. moniliforme*. We analysed both the neutral fraction of metabolites extracted from the culture filtrate and the ethanolic extracts of the mycelium. The fermentations were carried out with the strain PG-7 grown on the medium recommended by Fusca *et al.*[11]

The culture filtrate from a large-scale fermentation (500 l.) afforded a neutral gum which was collected after the following operations: (a) adsorbtion on the charcoal; (b) filtration; (c) elution of the adsorbate by aqueous acetone; (d) concentration of the eluate and acidification to pH 2·5; (e) extraction with ethyl acetate; (f) re-extraction of acidic products with phosphate buffer at pH 6·8; (g) washing and concentration of the organic layer. The gum was chromatographed on silica gel, the adsorbate/adsorbent ratio being only 1:6. The rough separation thus achieved in most cases was sufficient for direct isolation of metabolites. The known diterpenoids were represented by (−)-kaurene (**1**), (−)-isokaurene (**2**), (−)-kauran-16α-ol (**3**), 7-hydroxy-kaurenolide (**4**), 7,18-dihydroxykaurenolide (**5**), fujenal (**6**) and fujenic acid (**7**). All these were identified by comparison with known samples[1] (constants and IR spectra as well as mass spectra).

The fractions directly preceeding the elution of 7-hydroxykaurenolide **4** from the column contained a new substance which was separated from **4** by rechromatography on alumina. This compound, $C_{20}H_{32}O_3$ (**8**), had m.p. 169–173° and $[\alpha]_D$ −68°. Another new substance, $C_{20}H_{32}O_4$ (**9**), m.p. 281–283°, $[\alpha]_D$ −92°, was isolated from the fractions which followed after the elution of **4**. Finally, the elution of the column

with more polar solvent mixtures afforded a third new substance, $C_{20}H_{28}O_4$ (**10**), which was later identified as 7,13-dihydroxykaurenolide, a diterpenoid formerly obtained from the microbiological oxidation of steviol in the culture of *F. moniliforme*.[12]

The IR spectrum of **8** displays characteristic bands of an aldehyde (2720 and 1720 cm^{-1}) and tertiary OH group (3330 and 1130 cm^{-1}) but no C=C absorption. The NMR spectrum of **8** places the aldehyde group in a tertiary position (1H singlet at δ 9·65 ppm) and shows that one of three CH_3—C groups must be adjacent to the tertiary OH (3H singlet at δ 1·31 ppm). The acetylation of **8** with Ac_2O in pyridine proceeds very slowly.

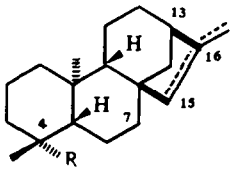
Compound **9**, as follows from its IR spectrum, must be a hydroxy acid (3300–2900, 1710 and 1130 cm^{-1})—again with a tertiary OH-group. It gives a methyl ester, $C_{21}H_{34}O_3$ (**9a**), but cannot be acetylated under the usual conditions. The IR spectrum of **9a** retains the band of the tertiary OH at 1130 cm^{-1}; a 3H-singlet in the NMR spectrum of **9a** (δ 1·31 ppm) shows the presence of a $CH_3-\overset{|}{\underset{|}{C}}-OH$ grouping.

The NMR spectra of **8** and **9a** display the signals of two angular Me groups (δ 0·84 and 0·92 ppm for **8** and 0·78 and 1·12 ppm for **9a**) which closely resemble those of C_4—CH_3 and C_{10}—CH_3 in the derivatives of (−)-kaurene with an axial carbonyl-containing function at C_4.[13] All these data suggested the close relationship between **8** and **9**. In fact, the hydroxy aldehyde **8** on oxidation with chromic acid gave the hydroxy acid **9** as the main product. The LAH reduction of its methyl ester, **9a**, afforded the previously described[13] (−)-kauran-16α,19-diol (**11**), identified by its m.p., $[\alpha]_D$, IR spectrum and mass spectrum. Dehydration of **9** with $POCl_3$ in pyridine gave rise to a mixture of (−)-kaur-16-en-19-oic acid (**12**) and its Δ^{15}-isomer (**13**) which were tentatively identified as the corresponding methyl esters (**12a** and **13a**) by GLC and by the IR spectrum of the mixture.[2, 13] Consequently, **8** must be 16α-hydroxy-(−)-kauran-19-al while **9** is 16α-hydroxy-(−)-kauran-19-oic acid.

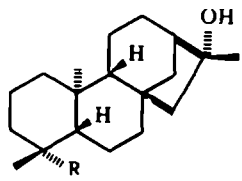
These assignments were confirmed by the mass spectra of **8**, **9** and **9a** as compared with those of **3** and **11**.* In all cases strong peaks M—H_2O, M—H_2O—CH_3, M—R and M—H_2O—R are observed (R represents an axial substituent at C_4) together with a prominent ion M—58 which corresponds to the splitting of the ring D with its oxygen function at C_{16}. In the region of lower masses abundant ions at *m/e* 123 and 109 are common to all five compounds; they are believed to originate from the ring A (ions *a* and *b*, respectively).

The third compound, **10**, had m.p. 259–262° and $[\alpha]_D$ −1·7 ±3°. Its IR spectrum shows a γ-lactone (1758 cm^{-1} in KBr-pellets or 1765 cm^{-1} in dioxan), a double bond (1655 and 905 cm^{-1}) and two OH groups (3600–3050, 1130 and 1060 cm^{-1}). The NMR spectrum of this lactone (in pyridine) reveals the presence of two angular Me groups (δ 0·77 and 1·13 ppm) and a terminal $-\overset{|}{C}=CH_2$ grouping (δ 5·17 ppm). On prolonged acetylation **10** gave a diacetate, $C_{24}H_{32}O_6$ (**10a**), which was identical with the previously described sample[12] according to its IR and NMR spectra.

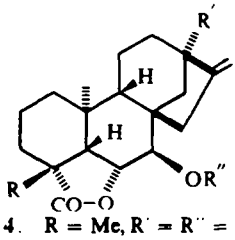
* For details see A. I. Kalinovsky, E. P. Serebryakov, B. M. Zolotarev, A. V. Simolin, V. F. Kucherov and O. S. Chizhov, *Organic Mass Spectrometry* in press.



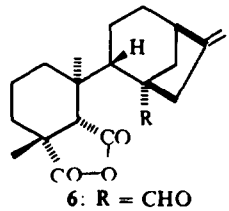
1: R = Me, Δ^{16}
2: R = Me, Δ^{15}
12: R = COOH, Δ^{16}
12a: R = COOMe, Δ^{16}
13: R = COOH, Δ^{15}
13a: R = COOMe, Δ^{15}
15: R = CHO, Δ^{16}
17: R = CH_2OH, Δ^{16}



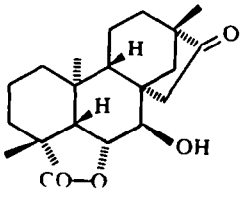
3: R = Me
8: R = CHO
9: R = COOH
9a: R = COOMe
11: R = CH_2OH



4: R = Me, R′ = R″ = H
5: R = CH_2OH, R′ = R″ = H
10: R = Me, R′ = OH, R″ = H
10a: R = Me, R′ = OAc, R″ = Ac



6: R = CHO
7: R = COOH



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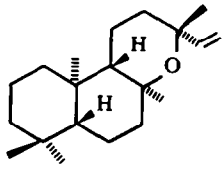

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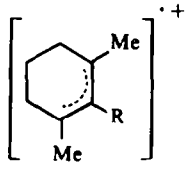


ion a: R = Me
ion b: R = H

The treatment of **10** with dilute H_2SO_4 gave a hydroxy ketone (m.p. 179–182°) with the CO group in the 5-membered ring (ν_{max}^{KBr} 1741 cm^{-1}). This compound must be identical with the formerly obtained[12] ketone **14**. The mass spectrum of **10** (M^+ 332) contains an intense ion at *m/e* 109 (ion *b*) which is also observed in the mass spectra of 7-hydroxykaurenolide **4** and its derivatives. This suggests the absence of additional substituents in the ring A of the dihydroxy lactone **10** as compared with **4**. All this evidence proves the identity of **10** with the previously isolated[12] 7,13-dihydroxy-kaurenolide.

From the biogenetic point of view the hydroxy aldehyde **8** and the hydroxy acid **9** represent the hydrated forms of (−)-kaur-16-en-19-al (**15**) and (−)-kaur-16-en-19-oic acid (**12**), which were shown[6, 8] to be intermediates in the process of transformation of (−)-kaurene **1** into gibberellins. However, at the moment of writing neither **15** nor **12** have been isolated from the culture filtrates—possibly, because of the high rate of metabolisation of these compounds in the culture of *F. moniliforme*. The hydration

of the Δ^{16}-double bond seems to make the substrates **15** and **12** unsuitable for further enzymic reactions and thus brings about relative accumulation of the corresponding hydrated forms, **8** and **9**, in the culture filtrates. The formation of 7,13-dihydroxy-kaurenolide **10**, presumably from **4**, is analogous to the formation of gibberellins A_1 and A_3 from A_4 and A_7. However, in the series of kaurenolides this type of hydroxylation is much less pronounced than the alternative transformation of **4** into **5**.

The analysis of diterpenoids and other metabolites of mycelium was carried out in accordance with the procedure of Cavell and MacMillan.[2] For this purpose the strain PG-7 was grown on Fusca's medium and the fresh mycelium was extracted with ethanol. The extract was divided into three acidic (bicarbonate-, carbonate- and alkali-soluble) fractions and one neutral fraction. This fractionation was rendered difficult by the high content of surface-active substances. The bulk of mycelial products was found in the alkali-soluble part of the acidic fraction and in the neutral fraction, both of which were subject to column chromatography on silica gel.

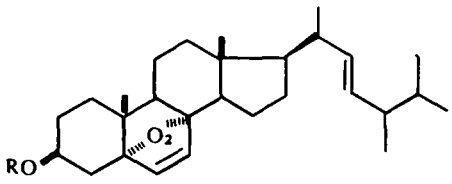
The main diterpenoids of the mycelium were (−)-kaurene **1** and 13-epi-(−)-manoyl oxide (**16**), the former representing about 5% of the total extract. Other diterpenoids were isolated in much smaller amounts. They were represented by (−)-kauran-16α-ol **3**, fujenal **6** and 16α-hydroxy-(−)-kauran-19-oic acid **9**. We were unable to isolate the acid **12** (its presence in the alkali-soluble acidic fraction was only detected by GLC analysis) or the corresponding alcohol, (−)-kaur-16-en-19-ol (**17**), which was recently found in the mycelium of *F. moniliforme*.[14] These results as well as those of Cavell and MacMillan[2] prompt us to suggest that only early and intermediate stages of the gibberellin-kaurenolide biosynthesis take place inside the mycelium cells (e.g. the alternative cyclization of geranyl-geranyl pyrophosphate into **1** or **16**) while the later oxidative and skeletal transformations are performed by the enzymic systems on the outer side of the cell walls.

Another direction of the mevalonate metabolism in the mycelium of *F. moniliforme* is represented by C_{28}-sterols.[14, 15] In our case three sterols of the ergostane group were isolated all of which contained the oxygenated ring B. The less polar sterol, $C_{28}H_{44}O_3$ (**18**) was identified as 5α,8α-epidioxy-5α-ergosta-6,22-dien-3β-ol ("ergosterol peroxide") on the basis of IR, NMR and mass spectra both of the sterol itself and its acetate (**18a**). As the m.p. and $[\alpha]_D$ of **18** differed slightly from those in the literature,[16, 17] we carried out photosensitized oxygenation of ergosterol[17] which gave a sample of ergosterol peroxide identical with **18** in all respects.

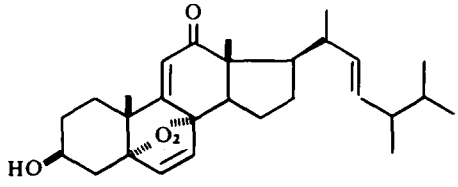
The second, more polar sterol was isolated in much smaller amounts. On the basis of its mass spectrum (M^+ 440) this sterol was assigned the brutto-formula $C_{28}H_{40}O_4$ (**19**). A group of intense peaks belonging to M—C_9H_{17} suggested that **19** should be placed into the Δ^{22}-ergostene series, since this type of fragmentation (rather than M—side chain—2H) is observed in Δ^{22}-sterols.[18] The IR spectrum of **19** contains the bands corresponding to an OH group (3440 and 1080 cm^{-1}, disappear on acetylation), a conjugated ketone (1680 cm^{-1}) and olefinic unsaturation (1640, 970 and 945 cm^{-1}). The presence of a C=C—C=O chromophore was confirmed by the UV spectrum of **19** (λ 232 and 313 nm). The position of the π,π^* band suggested that sterol **19** is either a Δ^1-en-3-one or Δ^5-en-7-one or else $\Delta^{9(11)}$-en-12-one since these systems exhibit a maximum between 230 and 235 nm.[19] The nature of the two remaining O atoms could not be deduced from the IR, UV and mass spectra and hence it was supposed that these atoms form a peroxide bridge between C_5 and C_8. In order to check this assump-

tion **19** was hydrogenated over Adams catalyst in AcOEt—AcOH medium,[20] upon which the TLC analysis revealed the presence of three new substances. The main product, isolated by plate chromatography was identical with the previously described[21] 5α,8α-epidioxy-5α-ergost-9(11)-en-3β-ol-12-one by its m.p., R_f, UV and IR spectra. Therefore, the most probable structure for sterol **19** is 5α,8α-epidioxy-5α-ergosta-6,9(11),22-trien-3β-ol-12-one.*

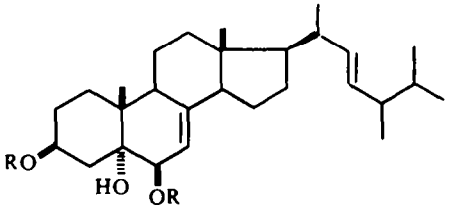
Finally, the most polar and the most abundant of three mycelium sterols, $C_{28}H_{46}O_3$ (**20**), was identified as cerevisterol[22] on the basis of its m.p., $[\alpha]_D$, IR, NMR and mass spectra; its diacetate (**20a**) was identical with an authentic specimen of cerevisterol diacetate. The common feature of all three sterols (**18**, **19** and **20**) is the prominence of the M—C_9H_{17} peaks in their mass spectra.



18: R = H
18a: R = Ac

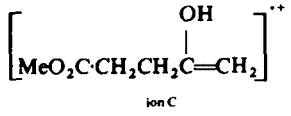


19



$RO_2C{\cdot}CH_2CH_2CO(CH_2)_7{\cdot}COOR$

21: R = H
21a: R = Me



The acidic fraction of the mycelium extract contains a number of common fatty acids. We found among them lauric, myristic, palmitic, stearic, oleic and linoleic acids which were identified as the corresponding methyl esters upon GLC analysis. The main lipid component of the mycelium is palmitic acid (about 40% of the total extract). We could not detect the presence of behenic, tetracosanoic and cerotic acids which were found in the mycelium of the ACC-917 strain.[2] Presumably, the nature of the fatty acid utilized to form the cell walls is morphologically unimportant for *F. moniliforme* and can vary from one strain to another or depends on the conditions of cultivation.

In addition to monobasic fatty acids we isolated in considerable amounts aselaic and suberic acids, identified by their m.p.p., IR spectra and retention times of the

* The fractions, containing **19**, on TLC-analysis showed the presence of a compound whose R_f coincided with that of 5α,8α-epidioxy-5α-ergosta-6,9(11),22-trien-3β-ol[20] in several systems.

methyl esters. They are likely to originate from oleic acid as a result of its oxidation by the fungus. The third dicarboxylic acid, $C_{12}H_{20}O_5$ (**21**), m.p. 111–112·5°, $[\alpha]_D = 0°$, ν_{max} 1710 and 940 cm^{-1}, was isolated in smaller quantities. The IR and UV spectra of the corresponding dimethyl ester (**21a**) revealed the presence of a keto group (ν 1705 cm^{-1}, λ_{max} 278 nm). In the mass spectrum of **21a** (M^+ 272) the base peak is at *m/e* 130; this peak (ion *C*) must be due to the McLaferty cleavage which is very typical of fatty keto acids.[23] The NMR spectrum of **21a** displays no peaks corresponding to CH_3—C groups and thus excludes the possibility of branching. Consequently, **21** is formulated as 4-oxododecanedioic acid.

The Fusca's medium used in the cultivation of the PG-7 strain contained soy flour which is a good source both of fatty acids and sterols. However, the bulk of soy fatty acids are unsaturated species (more than 85% of the total acid content) while palmitic acid is reduced to some 10% and that of lauric and myristic acids does not exceed 0·1%.[24] Hence it can be safely assumed that the greater part of saturated C_{12}—C_{18} acids found in the mycelium of *F. moniliforme* are produced by the fungus. Sterols **18**, **19** and **20** are believed to be endogenic metabolites of *F. moniliforme* since none of them were ever found during the careful analysis of soy sterols.[25] It may be, however, that ergosterol peroxide **18** is a fungal artefact as in the case of other pigmented fungi.[26]

EXPERIMENTAL

All m.ps were determined on a Bočtius hot plate, $[\alpha]_D$ values: with a Hilger polarimeter (manually). IR spectra: UR 10 instrument (Zeiss, Iena), in KBr pellets unless stated otherwise. UV spectra: Unicam SP700 instrument, in EtOH. NMR spectra: Varian A-60 instrument. Mass spectra: MX 1303 instrument, all-glass inlet system, temp range 130–190°. Column chromatography: silica gel KSK (60–100 mesh) and neutral alumina grade II and III/IV. GLC: (a) all-glass column, 1 = 2 m, 10% SE-30 +0·5% neopentylglycol succinate on silanized chromosorb W, H_2/N_2 p = 2·5 atm, t = 170–180° (for **1**, **2** and **16**) or 220–225° (for **3**, **8**, **9a**, **12a** and **13a**); (b) LKhM-8 stainless steel instrument, 1 = 2 m, 17% polyethyleneglycol succinate on chromosorb W, H_2/N_2 p = 3 atm, t = 185° (for fatty acids). Light petroleum: boiling range 40–70.

Fermentation, extraction and fractionation

Fusarium moniliforme (strain PG-7) was cultivated in aerated fermentation tanks at 26° until maximum gibberellin formation (7 days). The medium:[11] sucrose: 50 g, soy flour: 10 g, $(NH_4)_2HPO_4$: 400 mg, $(NH_4)_2SO_4$: 170 mg, K_2CO_3: 400 mg, $ZnSO_4$: 50 mg, $FeSO_4$: 50 mg, water: 1 l., initial pH 3·5.

The mycelium was separated from the culture filtrate and washed with water. The culture filtrate was treated as described to give 126 g of dark-brown neutral gum. Part of the mycelium (22 kg) was extracted at 20° with three portions of EtOH (40, 30 and 25 l., 3 × 24 hr) in an enamelled reactor with stirrer. The extracts were combined and concentrated to 1·5 l., diluted with water (1·5 l.) and the aqueous residue was extracted with ether (5 l. + 9 × 0·5 l.). The combined ether extract after concentration to 1·3 l. was successively treated with 2% HCl (3 × 0·15 l.), 5% $NaHCO_3$ (5 × 0·15 l.), 5% Na_2CO_3 (5 × 0·2 l.) and, finally, with 10% NaOH (5 × 0·5 l.). For better separation of emulsions the extraction with NaOH was alternated with washing of the organic layer with sat NaCl aq (5 × 0·2 l.). The organic layer gave on evaporation 106 g of brown gum which on standing deposited 58 g of waxy matter consisting mainly of palmitic acid. The rest was considered as "mycelium neutrals". Bicarbonate, carbonate and alkaline layers were acidified to pH 2·0 each and extracted with ether (5 × 0·2 l., 5 × 0·2 l. and 5 × 0·3 l. respectively) to give three acidic gums: bicarbonate-soluble (10·3 g), carbonate-soluble (3·5 g) and alkali-soluble (50·1 g).

Chromatography and isolation of metabolites

A. *Culture filtrate, neutral gum.* The gum (126 g) was mixed with 130 g silica gel and placed on the top of a column packed with 650 g silica gel. Fractions volume: 0·5 l.

Fractions 2–11 (light petroleum; 4·72 g) gave a crystalline mixture of **1** and **2** in proportion ~4:6.

Fractions 18–28 (light petroleum; 430 mg) after rechromatography on alumina gave **16**, m.p. 94–96°.

Fractions 38 and 39 (light petroleum + 5% AcOEt; 3·15 g) afforded 410 mg of pure **6**, m.p. 166–168°.

Fractions 40–46 (light petroleum + 5% AcOEt; 4·70 g) after fractional crystallization gave **3**, m.p. 213–216° (210 mg) and **7**, m.p. 213–217° (11 mg).

Fractions 56–60 (light petroleum + 10% AcOEt: 3·71 g) gave a crystalline mixture, m.p. 176–182°. It was re-chromatographed on 150 g of alumina (fractions volume 200 ml). Fractions 8′–12′ (light petroleum + 7·5% AcOEt) gave 470 mg of crystals, m.p. 168–176°. Two recrystallizations from AcOEt–hexane afforded pure **8**, m.p. 169–173° (needles), $[\alpha]_D^{22}$ −68° (*c* 0·56 in acetone); ν_{max}: 3330, 2720, 1720, 1130 cm^{-1}; δ (in $CDCl_3$): 0·84 (3H, s), 0·92 (3H, s), 1·31 (3H, s) and 9·65 ppm (1H, s); mass spectrum: M^+ 304, *m/e* 286, 275, 271, 257, 246, 217, 123, 109. (Found: C, 78·59; H, 10·20. $C_{20}H_{32}O_2$ requires: C, 78·95; H, 10·51%).

Fractions 15′–25′ (light petroleum + 10% AcOEt) gave 2·05 g of pure **4**, m.p. 187–189°.

Fractions 61–69 (light petroleum + 10% AcOEt; 6·90 g) gave 3·72 g of pure **4**, m.p. 187–190°.

Fractions 70–77 (light petroleum + 10% AcOEt) after fractional crystallization from acetone gave **4** (456 mg, m.p. 185–189°) and **9** (14 mg, m.p. 272–277°).

Fractions 78–101 (light petroleum + 15% AcOEt; 10·21 g) were viscous dark-orange gums which deposited crystals on standing. Two recrystallizations from acetone gave pure **9** (135 mg), m.p. 281–283°, $[\alpha]_D^{22}$ −92° (*c* 0·51 in MeOH); ν_{max}: 3300–2900, 1710, 1130 cm^{-1}; mass spectrum: M^+ 320, *m/e* 302, 287, 275, 262, 247, 217, 123, 109. (Found: C, 75·12; H, 10·21. $C_{20}H_{32}O_3$ requires: C, 75·01; H, 10·00%). The methyl ester (**9a**) was obtained with freshly distilled CH_2N_2, m.p. 153–156°; ν_{max}: 3360, 1720, 1130 cm^{-1}; δ (in $CDCl_3$): 0·78 (3H, s), 1·12 (3H, s), 1·31 (3H, s) and 3·69 ppm (3H, s); mass spectrum: M^+ 334, *m/e* 316, 301, 276, 275, 257, 217, 123, 109.

Fractions 126–140 (light petroleum + 25% AcOEt; 14·62 g) were recrystallized from AcOEt to give pure **5**, m.p. 215–217° (9·05 g).

Fractions 183–186 (light petroleum + 70% AcOEt; 2·81 g) partly solidified on standing. Recrystallization from AcOEt gave 36 mg of pure **10**, m.p. 259–262°, $[\alpha]_D^{19}$ −1·7 ±3° (*c* 1·51 in pyridine); ν_{max}: 3600–3050 (centred at 3260), 1758, 1650, 1220, 1130, 1060 and 905 cm^{-1} or 3530 and 1765 cm^{-1} (in dioxan); δ (in pyridine): 0·77 (3H, s), 1·13 (3H, s), 4·40 (1H, t?) and 5·17 (2H, m); mass spectrum: M^+ 332, *m/e* 314, 299, 296, 286, 271, 268, 253, 137, 109. Diacetate **10a** (Ac_2O–pyridine, 20°, 16 days) was separated from the more polar product on a silica gel chromatoplate (loose layer, benzene–AcOEt 9:1), m.p. 213–216° (from acetone–hexane); ν_{max}: 1762, 1728, 1655, 897 cm^{-1}; δ ($CDCl_3$): 0·97 (3H, s), 1·27 (3H, s), 2·01 (3H, s), 2·12 (3H, s), 4·70 (1H, t, J = 6 c/s), 5·05 (1H), 5·19 (1H) and 5·82 ppm (1H, d, J = 6 c/s). Lit.:[12] m.p. 261–263° for **10** and 216–217° for **10a**.

B. *Mycelium extract, neutral gum.* Brown gum recovered after separation of the solidified palmitic acid (47·5 g) and 150 g silica gel were mixed and placed on the top of a column packed with 580 g silica gel. Fractions volume: 1 l.

Fractions 1 and 2 (light petroleum; 5·70 g) gave pure **1**, m.p. 48·5–49·5°, $[\alpha]_D$ −78°.

Fractions 10–18 (light petroleum + 10% benzene; 2·60 g) were re-chromatographed on alumina to give 515 mg of pure **16**, m.p. 94–97°, $[\alpha]_D$ −35°.

Fractions 49–57 (benzene; 1·59 g) gave a wax consisting of palmitic and oleic acid in proportion ~7:3.

Fractions 68–70 (benzene + 10% AcOEt; 1·05 g) were re-chromatographed on alumina to give 93 mg of **18**, m.p. 177–182° (from AcOEt–hexane), $[\alpha]_D^{22}$ −25·7° (*c* 2·04 in acetone); ν_{max}: 3525, 3410, 1380, 1050, 980 cm^{-1}; δ (in $CDCl_3$): 0·75–1·02 (18H), 3·91 (1H, m), 5·10–5·23 (2H, m), 6·22 and 6·48 ppm (2H, AB-quartet, J_{AB} = 8·5 c/s); mass spectrum: M^+ 428, *m/e* 410, 395, 376, 303, 285, 243, 213. Lit.:[16, 17] m.p. 178°, $[\alpha]_D$ −36°. Acetate, **18a** (Ac_2O–pyridine, 20°, 2 days), m.p. 201–203° (from aq MeOH), ν_{max}: 1738, 1645, 1380, 1247 cm^{-1}; δ (in $CDCl_3$): 0·75–1·05 (18H), 2·13 (3H, s), 4·88 (1H, m), 5·10–5·26 (2H, m), 6·22 and 6·48 ppm (2H, AB-quartet, J_{AB} = 9 c/s).

Fractions 90–92 (benzene + 40% AcOEt; 280 mg) on standing deposited a little of crystalline material. Recrystallization from aq EtOH afforded **19** (4·8 mg), m.p. 242–246° (needles); ν_{max}: 3440, 3350, 1680, 1640, 1390, 1370, 1290, 1080, 980 and 945 cm^{-1}; λ_{max}: 232 and 313 nm (ε 13,700 and 130, respectively); mass spectrum: M^+ 440, *m/e* 425, 422, 407, 315, 297, 273, 217, 69. Acetate (Ac_2O–pyridine, 20°, 2 days) was eluted from an alumina chromatoplate as chromatographically pure oil, ν_{max}: 1741, 1680, 1643, 1247 cm^{-1}; mass spectrum: M^+ 482, *m/e* 422.

Fractions 104–113 (benzene + 70% AcOEt; 0·92 g) after two recrystallizations from MeOH gave 255 mg of pure cerevisterol, **20**, m.p. 254–256°, $[\alpha]_D^{21}$ −79·9° (*c* 1·35 in pyridine); ν_{max}: 3430, 1660, 1390, 1380, 1170, 1050, 975 and 945 cm^{-1}; δ (in pyridine): 0·55–1·02 (18H), 4·22 (1H, m), 4·88 (1H, t, J = 2 c/s) and 5·12 ppm (2H, m); mass spectrum: M^+ 430, *m/e* 412, 397, 394, 305, 287, 269, 251, 215. Lit.:[22] m.p. 256–259°, $[\alpha]_D$ −83°. Diacetate **21a** (Ac_2O–pyridine, 20°, 5 days) m.p. 168–170° (aq MeOH), ν_{max}: 3435, 1730, 1705, 1255 and 1235, was fully identical with an authentic sample of cerevisterol diacetate.

C. *Mycelium extract, alkali-soluble acidic gum.* The gum (50·1 g) and 160 g silica gel were mixed and placed on the top of a column packed with 500 g silica gel (unbuffered). Fractions volume: 0·5 l.

Fractions 1–4 (light petroleum) gave 430 mg of pure **1**. Fractions 22–28 (benzene; 8·12 g) gave a wax which was treated with CH_2N_2 and GLC analysed. By comparison with authentic samples the following acids were identified: lauric (~5%), myristic (5–10%), palmitic (40–45%), oleic (20–25%), stearic (~10%) and linoleic (5–10%).

Fractions 29–34 (benzene; 1·20 g) deposited 36 mg of crystals which were recrystallized from AcOEt–hexane to give pure **6**, m.p. 165–168°. Fractions 35–37 (benzene + 5% AcOEt; 890 mg) gave 24 mg of pure **3**, m.p. 212–216°.

Fractions 53–70 (benzene + 10% AcOEt; 6·8 g) after one crystallization from acetone gave azelaic acid, m.p. 106–108°. Fractions 75 and 76 in the same manner afforded 32 mg of pure suberic acid.

Fraction 80 (benzene + 25% AcOEt; 380 mg) was partly crystalline. The crystals were recrystallized from AcOEt to give 45 mg of pure **21**, m.p. 111–112·5°, $[\alpha]_D^{19} = 0°$; ν_{max}: 3580, 3040, 1710, 1235, 1080, 940 cm^{-1}. (Found: C, 59·05; H, 8·40. $C_{12}H_{20}O_5$ requires: C, 59·01; H, 8·20%). Treatment of **21** with freshly distilled CH_2N_2 gave the dimethyl ester, m.p. 35–36°, ν_{max}: 1730 and 1705 cm^{-1}; λ_{max}: 278 nm (ε 38·5); δ (in $CDCl_3$): 3·61 (6H, s); mass spectrum: M^+ 272, *m/e* 241, 209, 185, 157, 130, 115, 98, 87.

Fractions 114 and 115 (benzene + 70% AcOEt; 105 mg) after two recrystallizations from MeOH gave **20**, m.p. 252–253°.

Acetylation of **8** *and* **9**. The hydroxy aldehyde **8** (20 mg) was treated with Ac_2O (1 ml) and pyridine (0·2 ml) and left at room temp for 16 days. Usual working up and purification on 1 g of alumina gave 3 mg of crystalline acetate, m.p. 137–142°, ν_{max}: 2710, 1730, 1725 and 1265 cm^{-1}.

The hydroxy acid **9** under similar conditions remains unaffected.

Oxidation of **8** *to* **9**. The hydroxy aldehyde **8** (31 mg) in pure acetone (2 ml) was treated with slight excess of 8N CrO_3 in H_2SO_4 (20°, 3 hr). MeOH was then added and the soln concentrated in vacuum. The reaction product, precipitated with water, had m.p. 229–237°. Two recrystallizations from acetone gave 14 mg of pure **9**, m.p. 278–281°.

LAH-reduction of **9a**. A mixture of **9a** (30 mg) and LAH (200 mg) in 30 ml abs ether was heated for 2 hr, and the excess of LAH was destroyed with MeOH. The solvents were evaporated, the residue diluted with 5% HCl (50 ml) and extracted with ether (3 × 50 ml). The extract gave 26 mg of solid which was chromatographed on 1·2 g silica gel. Elution with benzene–AcOEt (4:1) afforded 15·4 mg of **11**, m.p. 199–203° (from benzene), $[\alpha]_D^{23}$ −41° (*c* 1·5 in EtOH), ν_{max}: 3620, 3585 and 1120 cm^{-1}; mass spectrum: M^+ 306, *m/e* 288, 248, 123, 109. Lit:[13] m.p. 200–201°, $[\alpha]_D$ −43°.

Dehydration of **9** *with* $POCl_3$. The acid **9** (100 mg) was treated with $POCl_3$ (0·5 ml) in 2 ml pyridine and the mixture was heated 1 hr at 60°. The volatile products were removed in vacuum and the residue was dissolved in 50 ml AcOEt and washed with 2N HCl (3 × 5 ml) and sat NaCl aq (3 × 5 ml). The crude product was purified on a silica gel chromatoplate to give 26 mg of amorphous solid, m.p. 90–95°. When treated with CH_2N_2 it gave a mixture of **12a** and **13a** in proportion ~4:6 (GLC-data); ν_{max}: 1742, 1660, 930 and 875 cm^{-1}.

Acid-catalyzed rearrangement of **10**. The lactone **10** (10 mg) was dissolved in 1 ml EtOH and then refluxed with 0·1 ml dil (1:5) H_2SO_4 and 2 ml water for 1 hr. The soln was slightly concentrated in vacuum and extracted with AcOEt (3 × 10 ml). Purification of the crude reaction product on a silica gel chromatoplate ($CHCl_3$ + 5% MeOH) gave **14** (2·1 mg), m.p. 179–182°, ν_{max}: 3430–3380, 1752 (sh), 1741 and 1060 cm^{-1}. Lit:[12] m.p. 186–187°.

Photosensitized oxygenation of ergosterol. The reaction was carried out in EtOH with O_2 and eosin as described.[17] From 396 mg ergosterol 215 mg ergosterol peroxide were obtained with m.p. 176–184°. This compound was identical with **18** (m.p. R_f, IR-spectrum).

Hydrogenation of **19**. The reaction was carried out as described[20] in an apparatus for mycrohydrogenation. Sterol **19** (3·1 mg) and Adams platinic oxide (5 mg) in 2·5 ml AcOEt–AcOH mixture (19:1) were shaken under H_2 for 12 hr. The catalyst was removed and the reaction products were separated on a small alumina chromatoplate (benzene + 15% AcOEt). The thickest of three zones (R_f 0·15–0·25) was eluted to give 1·7 mg of crystals, m.p. 179–183° (from aq MeOH), ν_{max}: 3470, 1680, 1640, 1370 and 1060 cm^{-1}; λ_{max} 232 and 315 nm (ε 12,900 and 95, respectively). This compound was identical with an authentic sample of 5α,8α-epidioxy-5α:ergost-9(11)-en-3β-ol-12-one[21] prepared on alkaline saponification of the corresponding acetate.

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