minor companion of 24-methylenecycloartanol in the 4,4-dimethylsterol fraction from the seed oil of *B. napus*.

Significance. This appears to be the first record of the detection of 24-methylenelanost-8-en-3β-ol in higher plants. This triterpene alcohol has so far been identified only in fungi [8,10,11] among the plant kingdom. The co-occurrence of the Δ^8-triterpene with the 9β,19-cyclopropyl isomer, 24-methylenecycloartanol, in the seed of *B. napus* suggests that the enzymatic opening of the 9β,19-cyclopropane ring may occur at the 4,4-dimethylsterol level. The presence of lanosterol and 24-dihydrolanosterol was recently demonstrated in the seeds of *Capsicum annuum*, Solanaceae [12]. These facts may suggest that the tetracyclic Δ^8-triterpene alcohols are of wide occurrence in higher plants though mostly as the minor triterpene consitiuents.

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CONSTITUENTS OF THE BASIDIOMYCETE *SCLERODERMA AURANTIUM*

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Key Word Index—*Scleroderma aurantium*; Basidiomycetes; fatty acids; steroids; lanostanediols.

Abstract—The following compounds were isolated from the extract of the fresh peridium of *Scleroderma aurantium*: palmitic acid, linoleic acid, a mixture of triglycerides, an ester of a steroidal diol, ergosterol, ergosterol peroxide, 9(11)-dehydroergosterol peroxide, lanosta-8,23-dien-3β,25-diol, lanosta-8,24-diene-3β,23-diol, and mannitol.

INTRODUCTION

The metabolites of the Basidiomycete *Scleroderma aurantium* have been examined by several authors. Zellner [1] reported the isolation of fumaric acid, glycerol, and mannitol. In the extract of the dried peridium, Entwistle and Pratt [2,3] identified a previously reported crystalline substance [1,4] as (23*S*)-lanosta-8,24-diene-3β,23-diol. This diol is labile towards acids and its side chain undergoes allylic rearrangement with the formation of lanosta-8,23-diene-3β,25-diol [5]. The minor components of the extract are assumed to be artefacts formed by the action of acids [2].

RESULTS AND DISCUSSION

In this paper we report the isolation and identification of constituents of the fresh peridium collected in the neighbourhood of Soběslav in Bohemia (Czechoslovakia). Young peridium was used which had a white to light purple belly. As indicated by TLC of the acetone extract, more than the four components previously reported [2] were present and the content of the main component was lower than the 98% previously claimed. In order to prevent the potential formation of artefacts by the action of the acids present, the crude extract was treated with diazomethane to esterify the acids. The methylated extract was concentrated and separated by a combination of column chromatography on Si gel, PLC on Si gel, and GLC. The isolated compounds are considered below according to their increasing polarity on Si gel TLC.

As indicated by MS and GLC, the least polar fraction represented a 3:7 mixture of methyl palmitate and methyl linoleate.

The subsequent oily fraction exhibited in the IR spectrum ester bands (1730 and 1168 cm^{-1}) and C=C bands

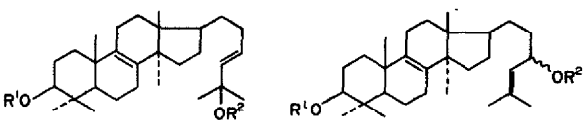
(1670 cm^{-1}); according to MS, this fraction was a mixture of triglycerides.

All the remaining compounds showed a positive Liebermann–Burchard reaction. The first was a non-crystalline ester of a steroidal diol with a C_{18} acid with two double bonds ($C_{46}H_{74}O_4$) but this ester has not been identified in detail. The IR spectrum exhibited ester bands (1730 and 1172 cm^{-1}) and a band (723 cm^{-1}) characteristic of $(CH_2)_n$. In the MS, the ester was split into a pair of complementary fragments, *m/e* 410 ($C_{28}H_{42}O_2$) and *m/e* 280 ($C_{18}H_{32}O_2$).

The next steroidal compound, $C_{28}H_{44}O$, mp 160–161°, was identified by its spectra as ergosterol.

Elution of ergosterol was followed by elution of two crystalline compounds (60% by weight of the extract; ratio, 1:6) with very close R_f values on TLC and identical mps (189–190°). MS of these two isomeric triterpenic compounds ($C_{30}H_{50}O_2$) were almost identical, differing only a little in the peak intensities. Acetylation of the less polar compound **1** afforded the monoacetate **1a**, $C_{32}H_{52}O_3$ (MS), mp 173° (IR:980, 1030, 1725, 3600 cm^{-1}) while the more polar compound **2** gave a diacetate (**2a**), $C_{34}H_{54}O_4$ (MS), mp 138° (IR:940, 1240, 1645, 1730 cm^{-1}).

The NMR spectra of the two diols exhibited signals for the five tertiary methyl groups of the triterpene skeleton (singlets at δ 0.70, 0.81, 0.87, 0.98, 1.00) and of the –CH–O– proton (multiplet at δ 3.23, W = 15 Hz) characteristic of 3β-hydroxytriterpenes. Some differences were observed in the signals of the side-chain protons. In addition to a three-proton doublet (δ 0.89, J = 6 Hz) attributable to the C-21 methyl group, **1** exhibited a six-proton singlet of two equivalent tertiary methyl groups (δ 1.31) shifted downfield apparently because of the presence of a tertiary non-acetylated hydroxyl function in the $-C(Me)_2-OH$ fragment. The occurrence of this group was also confirmed by the spectrum of the ethoxy derivative **1b** (vide infra) which differed from that of the diol only in the position of the signal of these methyl groups (δ 1.26). The region of the signals due to olefinic protons exhibited a two-proton complex multiplet (δ 5.59) which remained intact in the monoacetate. More detailed information on the position of the double bond in the side chain was obtained from the NMR spectrum of the monoacetate in the presence of the lanthanide shift reagent ($Eu(FOD)_3$). The olefinic protons afforded well separated multiplets, namely, a doublet with J = 16 Hz and a doublet of triplets with vicinal coupling constants J = 16, 7 and 7 Hz. The observed fission, along with high induced shift values of the two methyl groups and olefinic protons, established the presence of a *trans* disubsti-



(1) $R^1 = R^2 = H$ (2) $R^1 = R^2 = H$

(1a) $R^1 = Ac$; $R^2 = H$ (2a) $R^1 = R^2 = Ac$

(1b) $R^1 = OH$; $R^2 = C_2H_5$ (2b) $R^1 = H$; $R^2 = C_2H_5$

tuted double bond in the $-CH_2-CH=CH-C(Me)_2-OH$ portion of the sidechain. Since both NMR and IR spectra excluded the presence of a cyclopropane ring, an additional tetrasubstituted double bond must be present at position $\Delta^{8(9)}$. Compound **1** was thus lanosta-8,23-diene-3β,25-diol.

It was inferred from the NMR spectrum of the more polar compound **2**, that the side chain contained a secondary C-21 methyl group (δ 0.98, J = 6 Hz), another secondary hydroxyl function (a multiplet for the –CH–O– proton at δ 4.46), and a trisubstituted double bond (broadened singlets of two geminal sp^2-methyls at δ 1.67 and 1.68, and a broad doublet of the olefinic hydrogen at δ 5.20). NMR decoupling experiments established the presence of a $-CH_2-CH(OH)-CH=C(Me)_2$ structure. The diacetate exhibited singlets for two acetyl group protons (δ 2.00 and 2.04) and multiplets for –CH–O– protons (δ 4.51 and 5.62). Compound **2** was thus lanosta-8,24-diene-3β,23-diol. Our NMR data summarised in Table 1 agree within the limits of experimental error with the NMR data of the diol **2** as reported by Entwistle and Pratt [2].

Since the physical constants of our isolated compounds and their derivatives differed from the reported data (see Table 2), an acidic isomerisation of the lanosta-8,24-diene-3β,23-diol was performed in ethanol according to the procedure of Entwistle and Pratt [5]. The lanosta-8,23-diene-3β,25-diol so obtained by the isomerisation was identical with compound **1** isolated from the naturally occurring material.

The occurrence of lanosta-8,23-diene-3β,25-diol in Nature was established as follows. Two extracts were prepared, one of which was treated with diazomethane to remove acids as methyl esters. Both extracts were then kept in ethanol. After one week, the original (untreated) extract containing acids was subjected to PLC to afford the 25-ethoxy and 23-ethoxy derivatives previously obtained by Entwistle and Pratt [5] as by-products of the isomerisation of lanosta-8,24-diene-3β,23-diol in ethanol, namely, 25-ethoxylanosta-8,23-diene-3β-ol (**1b**), mp 150–151° (reported [5], mp 145–146°) and 23-ethoxy-

Table 1. Chemical shifts in the NMR spectra of lanostadiols in $CDCl_3$

Compound	C-30*	C-31*	C-19*	C-18*	C-32*	C-21†	C-26	C-27	C-3	C-23	C-24	-OR
1	1.00	0.81	0.98	0.70	0.87	0.89	1.31*	1.31*	3.23[c]	5.59[d]	5.59[d]	1.57
1a	0.89	0.89	1.02	0.71	0.89	0.89	1.31*	1.31*	4.49[c]	5.59[d]	5.59[d]	2.04
1b	1.01	0.82	0.99	0.71	8.88	0.90	1.26*	1.26*	3.24[c]	5.57[d]	5.57[d]	1.14; 3.34
2	1.00	0.81	0.98	0.72	0.87	0.98	1.67[e]	1.68[e]	3.23[c]	4.46[f]	5.20[g]	1.55
2a	0.89	0.89	1.01	0.69	0.88	0.95	1.70[e]	1.73[e]	4.51[c]	5.62[f]	5.11[g]	2.00; 2.04
2b	1.01	0.82	1.00	0.72	0.88	0.96	1.67[e]	1.72[e]	3.26[c]	4.05[f]	5.09[g]	1.16; 3.47

* Singlet; † Doublet with $J_{21,20}$ = 6 Hz; [c] Multiplet, W = 15 Hz; [d] Complex unresolved multiplet. After the adition of $Eu(FOD)_3$ to **1b** J s: $J_{23,24}$ = 16 Hz; $J_{23,22}$ = 7 and 7 Hz were obtained. [e] Broadened signals with $J_{26,24} = J_{27,24}$ = 1.3 Hz; [f] Doublet of triplets, $J_{23,22}$ = 3 and 9 Hz, $J_{23,24}$ = 9 Hz; [g] Broad doublet, $J_{24,23}$ = 9 Hz; $J_{24,26} = J_{24,27}$ = 1.3 Hz.

Table 2. Physical constants of lanostadiols and their acetates

Compound	Literature [2,5] mp	$[\alpha]_D$	This work. mp	$[\alpha]_D$
Lanosta-8,23-diene-3β,25-diol	160–164°	+43°	189–190°	+60.3°
25-Hydroxylanosta-8,23-dien-3β-yl acetate	122–124°	+40°	173°	+50.2°
Lanosta-8,24-diene-3β,23-diol	158–160,5°	+66°	189–190°	+37,4°
Lanosta-8,24-dien-3β,23-yl diacetate	165–166°	+55°	138°	+38,4°

lanosta-8,24-diene-3β-ol (**2b**), mp 123–125° (reported [5], mp 115–115.5° for the 23*S*-isomer and mp 125–127° for the 23*R*-isomer). No ethoxy derivatives were present even after two months in the extract treated with diazomethane. In both the origin extract and that treated with diazomethane, the ratio of compounds **1** and **2** remained the same.

The most polar steroidal component (mp 178–179°) was identified by its spectra as a mixture of ergosterol peroxide ($C_{28}H_{44}O_3$) and 9(11)-dehydroergosterol peroxide ($C_{28}H_{42}O_3$).

The last constituent of the extract was identified by comparison with an authentic material as mannitol.

EXPERIMENTAL

Mp's were measured on a Kofler block. TLC was performed on Si gel G. Optical rotations were measured in MeOH, IR spectra in KBr micropellets. High resolution MS were measured at 70 eV, and the NMR spectra on a 100 MHz instrument.

Extraction. Fresh peridia of *Scleroderma aurantium* (Waill) Pers. (4.5 kg) were disintegrated in Me_2CO and the extract was conc. The concentrate was distributed between 60% aq. EtOH and C_6H_6. The C_6H_6 layer was conc and kept at room temp. with ethereal CH_2N_2 for 1 hr to convert the free acids (if any) to their methyl esters. The mixture was evaporated and the residue repeatedly chromatographed on Si gel (15% H_2O) using C_6H_6 to C_6H_6-Me_2CO (9:1).

Palmitic acid and linoleic acid. Isolated in the form of methyl esters, MS: $C_{17}H_{34}O_2$ and $C_{19}H_{34}O_2$, identified by fragmentation as methyl palmitate and methyl linoleate [6] in the ratio 3:7 (GLC on QF-1).

Triglycerides. The mixture consisted (MS) of triesters of $C_{18:1}$ or $C_{18:2}$ acids (M^+ 878, 880, 882, and 884) and triesters in which one C_{18} acid was relaced by a $C_{16:0}$ or a $C_{16:1}$ acid (M^+ 858, 856, 854, and 852). Fragment ions: M-RCOO at *m/e* 603, 601, 599, 577, 573; RCO + 128 at *m/e* 393, 391, 367, 365; RCO + 74 at *m/e* 339, 337, 313, 311; RCO at *m/e* 265, 263, 239, 237 [7].

Ester of the steroidal diol. IR ν_{max}^{KBr} cm^{-1}: 1730, 1172, 772; MS: M^+ 690 ($C_{46}H_{74}O_4$), *m/e* 410 ($C_{28}H_{42}O_2$) and *m/e* 280 ($C_{18}H_{32}O_2$).

Ergosterol. Mp 160–161°; MS: M^+ 396 ($C_{28}H_{44}O$) identical with the reported [6] MS.

Lanosta-8,23-diene-3β-25-diol **1**. mp 189–190°; $[\alpha]_D^{21}$ + 60.3°. IR ν_{max}^{KBr} cm^{-1} 3380, 1635, 1090; MS *m/e*: M^+ 442, 427 ($C_{29}H_{47}O_2$), 424 ($C_{30}H_{48}O$), 409 ($C_{29}H_{45}O$), 391 ($C_{29}H_{43}$), 327 ($C_{23}H_{35}O$), 309 ($C_{23}H_{33}$), 255 ($C_{19}H_{27}$).

25-Hydroxylanosta-8,23-dien-3β-yl acetate **1a**. C_5H_5N–Ac_2O, mp 173°; $[\alpha]_D^{21}$ + 50.2°; IR ν_{max}^{KBr} cm^{-1}: 3400, 1730, 1090; MS *m/e*: M^+ 484, 466, 451, 391.

Lanosta-8,24-dien-3β,23-diol **2**. mp 189–190°; $[\alpha]_D^{21}$ + 37.4°; IR ν_{max}^{KBr} cm^{-1}: 3400, 1635, 1090; MS *m/e*: M^+ 442, 427 ($C_{29}H_{47}O_2$), 424 ($C_{30}H_{48}O$), 409 ($C_{29}H_{45}O$), 391 ($C_{29}H_{43}$), 327 ($C_{23}H_{35}O$), 309 ($C_{23}H_{33}$), 255 ($C_{19}H_{27}$).

Lanosta-8,24-dien-3β,23-yl diacetate **2a**. C_5H_5N–Ac_2O, mp 165–166°; $[\alpha]_D^{21}$ + 38.4°; IR ν_{max}^{KBr} cm^{-1}: 1640, 1730 cm^{-1}; MS: M^+ 526, 466, 451, 391, 109.

Acidic isomerisation of lanosta-8,23-diene-3β,23-diol. Performed by a reported [5] procedure. 25-Ethoxylanosta-8,23-dien-3β-ol **1b**, mp 150–151°; IR ν_{max}^{KBr} cm^{-1}: 3380, 3080, 1635, 1372, 1090; MS *m/e*: 470, 424, 409, 391, 113. 23-Ethoxylanosta-8,24-dien-3β-ol **2b**, mp 123–125°; IR ν_{max}^{KBr} cm^{-1}: 3470, 1660, 1062. 842; MS: *m/e*: 470, 424, 409, 391, 109.

Ergosterol peroxide and 9(11)-dehydroergosterol peroxide. Isolated as a mixture, mp 178–179°, $[\alpha]_D^{21}$ − 17.8°; MS: 428 ($C_{28}H_{44}O_3$), 426 ($C_{28}H_{42}O_3$). Physical constants were in accord with lit. [8].

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