FUNGAL METABOLITES. VIII[1]. STRUCTURES OF NEW SESQUITERPENES FROM *LACTARIUS SCROBICULATUS*

ROSANGELA BATTAGLIA[2], MARIA DE BERNARDI[2], GIOVANNI FRONZA[3], GIORGIO MELLERIO[2], GIOVANNI VIDARI[2] and PAOLA VITA-FINSI[2,4]

[2]*Istituto di Chimica Organica—Universita degli Studi—Viale Taramelli 10—27100 Pavia (Italy)*
[3]*Centro del CNR per lo Studio delle Sostanze Organiche Naturali—Politecnico—Piazza Leonardo da Vinci 32—20133 Milano (Italy)*

ABSTRACT.—From lots of *Lactarius scrobiculatus* extracted in different ways, in addition to ethyl linoleate and cerevisterol, nine lactarane sesquiterpenes have been isolated. The main compounds are furanosesquiterpenes, namely: furoscrobiculins A-D (**2**, **5-7**), furanethers (**3** and **4**) and the already known lactaral (**1**), and furandiol (**9a**). Furthermore a new sesquiterpene lactone with the CO group in 13, lactaroscrobiculide B (**8**), has been isolated. The structures were elucidated mainly by spectroscopic methods and by some chemical transformations. Stereochemistry of most sesquiterpenes has been determined.

Recently (1-3) we isolated from *Lactarius scrobiculatus* some sesquiterpenes with the lactarane skeleton which is peculiar to most of the known Russulaceae metabolites (4-17). At present the only mushrooms of this family containing sesquiterpenes with a completely different skeleton are *L. deliciosus* (18) (guaianes) and *L. uvidus* (19) (drimanes).

Now we report the structures of new lactarane sesquiterpenes which were isolated from a new lot of *L. scrobiculatus* extracted in a different way than in the previous work (2, 3).

DISCUSSION

L. scrobiculatus is easily distinguished not only by the morphological aspects (yellow color, big size, scrobiculated stem, pungent taste, etc.), but also by the secreted milky juice, which, for some reason not yet understood, turns quickly from white to yellow when it appears at the surface of the fruit-body. This color change could indicate enzymatic degradation reactions; therefore, in order to prevent and to check possible alterations in the sesquiterpene composition, we carried out extraction in three different ways:

a) The fruit-bodies were frozen in dry ice and acetone. The frozen material was then minced under its solvent and, after being removed by filtration, it was homogenized in ethanol and ice to extract the more polar compounds.

b) The freshly collected mushrooms were cut and put in acetone. The material was worked up in a dry-box under a N_2 stream as in a). In this case, the color change in the juice seemed to be slower but was still noticeable.

c) The fruit-bodies were cut without any precaution.

In each case acetone, not ethanol as previously described (2, 3), was used as the primary extraction solvent to prevent ethylation of hydroxy groups (3, 13).

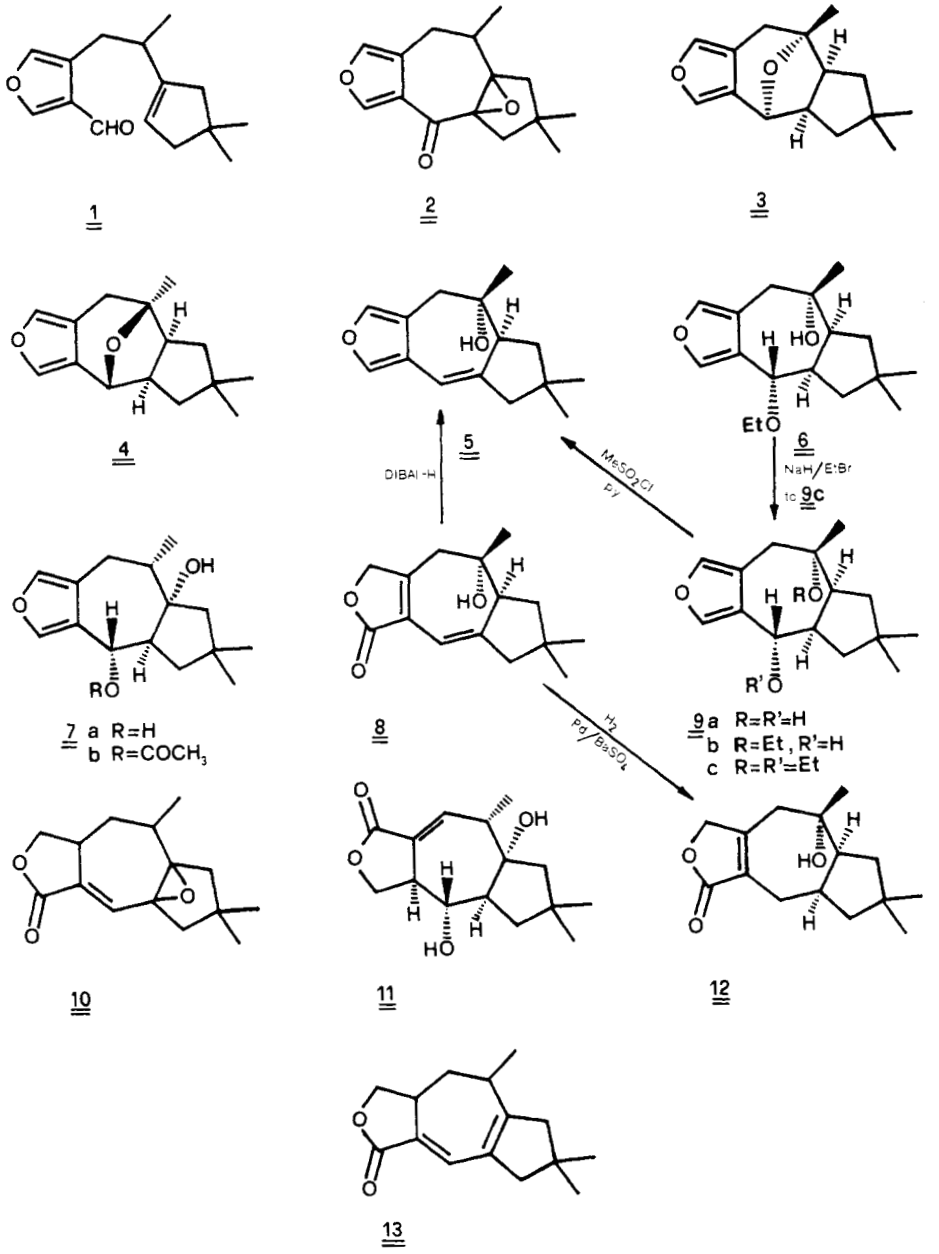
The three sesquiterpene extracts obtained as described in the Experimental section were compared by tlc and glc. The latter method of analysis gave significant results only for the less polar compounds. In fact, the polyhydroxylated sesquiterpenes were difficult to detect because of the ease of dehydration in the analytical conditions.

[1]For Part VII see M. De Bernardi, G. Fronza, G. Mellerio, G. Vidari and P. Vita-Finzi, "Stereochemistry of Blennin A and Blennin D", *Phytochemistry*, **19**, 99 (1980).

[4]To whom correspondence should be addressed.

However, comparison of the three extracts established a close qualitative similarity but a richer content in less polar compounds and furanosesquiterpenes in the a and b extracts than in the c extract.

From the combined a and b extracts after column chromatography and subsequent preparative thin layer chromatography, ethyl linoleate and cerevisterol (16, 20) were isolated in addition to nine lactarane sesquiterpenes, six of which were new compounds. The nine sesquiterpenes were lactaral (**1**) (14, 16), furo-



scrobiculin A (**2**), furanether A (**3**) (16), furanether B (3,8-enantiofuranether A) (**4**), furoscrobiculin B (**5**), furoscrobiculin C (**6**), furoscrobiculin D (**7**), lactaroscrobiculide B (**8**) and furandiol (**9a**) (21). The progressive numbering of the compounds corresponds both to the decreasing R_f in tlc and to the elution order in the cc (see Experimental section).

The chromatographic behavior, the presence of known lactarane sesquiterpenes (**9a**) which occurred in the previous work (1–3), and analysis of the spectroscopic data of the unknown compounds indicated that all had a lactarane skeleton, i.e., three methyls, two of which gave rise to a *gem*-dimethyl group, a 3,4-disubstituted cyclopentane ring and a 3,4-disubstituted furan or lactone ring.

All the isolated compounds, except **8**, were furanosesquiterpenes, as demonstrated by characteristic ir bands of furan rings (ν max 1520–1580 and 840–895 cm^{-1}); in the pmr spectra, by two signals occurring in the range of δ 7.0–7.3 attributed to the two protons of 3,4-disubstituted furan (10, 22); and in the ^{13}C-nmr spectra, by two doublets between 137–143 ppm (=CH–O) and two singlets between 118–130 ppm, (>C=) attributed to the furan carbon atoms (see in table 1 for the chemical shifts respectively for C-5, C-13 and C-6, C-7).

Lactaral (**1**), furanether A (**3**) and furandiol (**9a**) were identified by comparison of spectral data with those in the literature and with authentic samples isolated from other mushrooms: **1** (14) from *Russula sardonia* (16) and *L. pallidus* (23); **3** from *R. sardonia* (16) and **9a** (21) from *L. scrobiculatus* (2), *L. blennius* (15), *L. pallidus* (23) and *R. sardonia* (16).

Furoscrobiculin A (**2**) had mw (ms)of 246 corresponding to the $C_{15}H_{18}O_3$ formula. The ir spectrum showed, in addition to the furan absorptions, a band at 1660 cm^{-1} for a conjugated ketone and no OH bands. The pmr spectrum displayed signals for the gem-dimethyl group (δ 1.07, s, 6H), a secondary methyl (δ 0.99, d, 3H, J=7.0 Hz), two isolated methylene groups (2 ABq, 2H each, centered at δ 1.93 and δ 2.14), a–CH–CH_2– group (ABX system, at δ 2.4–2.7, 2H and at δ3.07, 1H, see assignments in experimental) and the two furan protons (δ 7.20, t, 1H and δ 8.07, d, 1H). The low-field chemical shift of the last proton (coupled only with the other furan proton), indicated that the CO group had to be conjugated to the furan ring. Decoupling experiments led to the inference that the CO was in position 8, showing that the secondary methyl group was linked to the methyne of the –CH–CH_2– group and that the CH_2 was vicinal to the furan ring and, therefore, in 4. On the basis of these data and to account for the unsaturation level of seven and the three oxygen atoms, we assigned to furoscrobiculin A the structure of the epoxyketone **2**.

The epoxy ring in position 2–9 explained the presence of two distinct AB systems in the pmr spectrum due to the two methylenes in the cyclopentane ring. The signals were assigned to the individual methylenes on the basis of the C_6D_6 solvent-induced shift which is upfield for all protons except for those lying near the plane perpendicular to the C=O double bond (see experimental section) (24, 25).

Furoscrobiculin A is the second lactarane sesquiterpene isolated from *L. scrobiculatus* with an epoxide at C-2 and C-9; the first was 2,9-epoxy-lactaroscrobiculide A (**10**) (1) which this time we could not isolate from the sesquiterpene extract.

Furanether B (**4**), which had a mw (ms) of 232 corresponding to $C_{15}H_{20}O_2$, was an isomer of **3** and showed very similar spectral data (16). The most important features which indicated the structure to be **4** were the following: the ir

spectrum showed no OH absorption and the pmr spectrum indicated the presence of three tertiary methyl groups, one of which was geminal to an oxygen atom (δ 1.38), and next to the furan ring was both a –C*H*O– group (δ 4.78, br, s, 1H), and an isolated methylene (δ 2.54 and δ 2.80, 1H each, ABq). Considering the presence of the furan ring and the molecular formula, the same oxygen had to be linked to the carbon atoms 3 and 8, and the isolated CH_2 could be placed only in position 4. This structure recalled that of **3** (16). From the Dreiding models, it was evident that the formation of an internal ether between C–3 and C–8 required the bridge-head protons at C–2 and C–9 to be *cis*. The most important difference between the pmr spectra of **3** and **4** which led to the determination of the stereochemistry of **4** was the coupling constant $J_{8-9}=0$ Hz. In fact the signal for H–8 appeared as a singlet indicating an approximate dihedral angle of 90° with H–9, as judged from the Karplus curves (26). Examination of the Dreiding models showed that H–8, H–9 and H–2 in **4** were all *syn* to each other but *anti* to the internal ether. Therefore, furanether B is the 3,8-enantio-furanether A.

Furoscrobiculin B (**5**) had a mw (ms) of 232, although isomer of the furanethers **3** and **4**, showed OH absorption (3400 cm^{-1}) in the ir spectrum. In this case the presence in the molecule of a trisubstituted double bond was clearly indicated by the ^{13}C-nmr spectrum (s at 142.2 ppm and d at 112.1 ppm) and in the pmr spectrum by a vinylic proton (δ 6.05) coupled with one aromatic and other two protons. Extensive decoupling experiments led to the inference that the double bond could be located only between C–8 and C–9, conjugated with the furan ring, and that H–8 was coupled also to two allylic protons at C–2 and C–10. The OH was tertiary and, in fact, could not be acetylated with acetic anhydride and pyridine at room temperature. It was located at C–3 owing to the lack of coupling of the C–3 methyl (s, δ 1.11).

Furoscrobiculin B was obtained, together with furanether A (**3**), by dehydration of furandiol (**9a**) with CH_3SO_2Cl and pyridine (16). We could in this way confirm the assigned structure and establish the absolute stereochemistry. However, one can observe from the Dreiding models that to make the furan and the conjugated double bond lying in the same plane, the structure undergoes a distortion from the usual conformation of lactarane sesquiterpenes (27) with the C–12 *anti* to the ring junction hydrogens; as a result the conformation will have an *endo* instead of an *exo* form. Therefore, the β–C–3 methyl, which usually is in a pseudo-equatorial position, becomes almost axial; probably for this reason, **5** showed a higher chemical shift of the C–3 methyl (20.7 ppm and δ 1.11) than the other compounds of this series with the C–3 OH *syn* to H–2.

Furoscrobiculin C (**6**) had a mw (ms) of 278 which corresponded to the formula $C_{17}H_{26}O_3$. Although hydroxy groups were indicated for **6** (band at 3400 cm^{-1} in the ir spectrum) no acetylderivative was obtained by treatment with acetic anhydride/pyridine at room temperature. The tertiary OH could be located at C–3 by the signals in the pmr spectrum of the C–3 methyl (s, δ 1.22) and of the isolated CH_2 near the furan (δ 2.79, d, $J_{4-4'}=16.5$ Hz, H–4, δ 2.94, dd, $J_{4-4'}=16.5$ Hz and $J_{4'-5}=1.5$ Hz, H–4'). The pmr as well as the ^{13}C-nmr spectrum showed the presence of an ethoxy group (δ 1.18, t, 3H, CH_3; δ 3.52, 2 dq, 2H, $J_{vic}=7.0$ Hz, $J_{gem}=9.0$ Hz, CH_2O; and q at 14.8 ppm, CH_3; t, at 63.5 ppm CH_2O) which explained the presence of two carbon atoms extra to the sesquiterpene structure. The ethoxy group was placed at C–8 by the following data: the

presence of a doublet (pmr) at δ 4.29 (1H, $J_{8-9}=4.0$ Hz), a doublet (^{13}C-nmr) at 75.4 ppm and the comparison of the ^{13}C-nmr spectrum of **6** with those of furandiol (**9a**) (2) and 3-O-ethylfurandiol (**9b**) (3) (reported in table 1). In fact, in these compounds the β- and γ-effects of the alkyl substitution (28) at C-3 OH and at C-8 OH were evident (see values of C-7, C-8 and C-9 for **6** and **9a** and of C-2, C-3 and C-4 for **9a** and **9b**).

TABLE 1. ^{13}C-nmr data of **5-9** (a-b).*

Comp.	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9
5	43.6t[a]	54.9d	71.8s	50.2t	140.8d[b]	118.2s	123.3s	112.1d	142.2s
6	45.4t[a]	51.2d	72.9s	33.6t	142.1d[b]	119.5s	122.2s	75.1d	44.1d
9a	44.9t	51.2d[d]	73.9s	33.1t	141.4d[b]	118.7s	127.1s	67.4d	46.2d[d]
9b	45.4t	49.2d	80.4s	28.2t	142.3d	118.4s	127.2s	66.7d	46.8d
7	58.0t	83.8s	43.2d	27.0t	139.1d[b]	122.6s	130.8s	67.8d	56.1d
8	44.9t[a]	53.7d	70.4s	50.1t	72.4t	152.5s[f]	122.6s	111.0d	151.8s[f]

Comp.	C-10	C-11	C-12	C-13	C-14	C-15	CH_2	CH_3
5	41.1t[a]	35.9s	20.7q	140.1d[b]	29.1q[c]	27.4q[c]		
6	44.9t[a]	36.8s	27.6q	141.5d[b]	31.3q[c]	29.7q[c]	63.5t	14.8q
9a	44.9t	36.6s	28.5q	141.2d[b]	31.5q[c]	30.3q[c]		
9b	45.2t	36.8s	25.0q	139.8d	29.8q[c]	27.6q[c]	56.8t	15.3q
7	44.2t	33.3s	17.6q	137.7d[b]	32.6q[c]	32.6q[c]		
8	43.2t[a]	36.8s	22.1q	173.9s	28.6q[c]	26.6q[a]		

*25.2 MHz, $CDCl_3$, TMS—Chemical shifts in ppm—Signal multiplicity, obtained by "off-resonance" decoupling experiments. (a), (b), (c), (d), (e) and (f) = assignments can be reversed.

Structure and absolute stereochemistry of furoscrobiculin C were definitely established by ethylation of **6** which afforded 3,8-di-O-ethylfurandiol (**9c**), identical with that obtained by ethylation of **9a** (3, 23). This compound **6**, as already reported for **9b** (3, 13), should be an artifact resulting from use of ethanol as the solvent in the last step of the mushroom extraction.

Furoscrobiculin D (**7a**) is isomeric of furandiol (**9a**) having a mw (ms) of 250 and molecular formula $C_{15}H_{22}O_3$. As **9a**, it showed OH bands in the ir spectrum (3470 and 3420 cm^{-1}) and afforded, by acetylation with acetic anhydride/pyridine at room temperature, a monoacetylderivative **7b** (ir: at 3530 cm^{-1} (OH) and 1730 cm^{-1} (CH_3COO); pmr: δ 2.14 (s, 3H, CH_3CO) and down-field shift of a C*H*OR proton from δ 4.76–4.98 to δ 6.12 compared with **7a**). Since the pmr spectrum of **7a** showed, besides two tertiary methyls (δ 1.035 and 1.205), a secondary methyl in position 3 (δ 1.01, d), a C*H*OH group in position 8 (δ 4.77) coupled to H-9 and an isolated methylene (δ 1.68, s) of the cyclopentane ring, we could locate the tertiary OH group only on C-2. This position was confirmed, in the ^{13}C-nmr spectrum, by the β-effect (about 10 ppm down-field) exerted by the OH group (29) on C-1 and C-9 signals compared with the compounds without substituents in position 2.

As far as the stereochemistry of **7** is concerned, the magnitude of the coupling constants indicated an *anti* configuration of H-8 and H-9 ($J_{8-9}=8.0$ Hz in $(CD_3)_2CO$), as for **6** and **9**, and an axial conformation of H-3 ($J_{3-4}=2.5$ Hz and $J_{3-4'}=11.5$ Hz), the C-3 methyl being, therefore, equatorial. The pyridine-induced solvent shift (30) of the C-3 CH_3 signal in **7b** ($\Delta_{CDCl_3-C_5H_5N}=-0.11$) allowed the determination of the value of about 90° for the dihedral angle between the C-2 OH and C-3 methyl, which was consistent for a *syn* relationship of the

two groups as indicated in **7**. Susbtituents and relative configuration of C–2, C–12, C–8 and C–9 in **7a** are identical to those of blennin D (**11**), a new sesquiterpene lactone recently isolated from *L. blennius* (17).

Lactaroscrobiculide B (**8**) had a mw (ms) of 248 and a molecular formula $C_{15}H_{20}O_3$. Its spectral data looked completely different from those of the just-described sesquiterpenes because of the absence of furan absorptions. The ir spectrum showed bands for an α,β-unsaturated-γ-lactone (1740 cm^{-1}, CO), for a conjugated diene (1662 and 1652 cm^{-1}) and for a tertiary hydroxyl group (3490 cm^{-1}). The hydroxyl, not acetylable by treatment with acetic anhydride/pyridine at room temperature was geminal to the C–3 methyl, as was indicated by the singlet at δ 1.16 (3H) in the pmr spectrum. The presence of three isolated methylenes, as shown in the pmr spectrum, allowed establishment of the position of the diene system. Since the CH_2O group of the lactone ring was not further coupled (δ 4.62 and δ 4.74, ABq, $J_{gem}=18.0$ Hz), the double bond conjugated with the C=O had to be between C–6 and C–7, the second double bond then being between C–8 and C–9. The other two isolated methylenes were thus in 4 (δ 2.67 and 2.92, ABq, $J_{gem}=19.0$ Hz) and in 10 (δ 2.32, br s). The ^{13}C-nmr spectrum confirmed that the diene system was formed by a tetrasubstituted double bond (two singlets at 122.6 and 152.5 (or 151.8) ppm) and by a trisubstituted double bond (a singlet at 151.8 (or 152.5) ppm and a doublet at 110.0 ppm), while the pmr spectrum showed only one vinylic proton at δ 6.09. Decoupling experiments confirmed these assignments. It was interesting to note the close similarity of the ^{13}C-nmr spectra of **8** and **5**, particularly for the chemical shifts of C–12 and C–4.

DIBAl–H reduction of **8** afforded furoscrobiculin B (**5**) and led to the establishment of the absolute stereochemistry of lactaroscrobiculide B (**8**). The last problem to be solved in the structure of **8** was determination of the lactone C=O position, which could be either in 5 or in 13. Position 13 was assumed because of spectroscopic data, i.e., a lack of homoallylic coupling constants for H–4 (3); upfield shift due to the ASIS effect [25, 31] (from $CDCl_3$ to C_6D_6) for all the signals except the C–8 vinylic signal, indicating that the C=O and the H–8 had to be on the same side of the molecule; the values of the uv absorptions were then confirmed by Pd/$BaSO_4$ hydrogenation of **8** which yielded 8,9-dihydrolactaroscrobiculide B (**12**) identical with the previously obtained 5,8-desoxylactarolide B (**3**) which had the CO in position 13.

The finding of a large amount of furanosesquiterpenes by extraction at low temperature or in absence of air clearly indicated that furans are precursors of lactones in the lactarane sesquiterpenes. Lactones are natural compounds, and it is interesting to note that the lactones with the CO in 13 seem to be mainly peculiar to *L. scrobiculatus*. Lactaroscrobiculide B is the fourth known lactone with this feature isolated from *L. scrobiculatus* along with lactaroscrobiculide A (**13**) (2), 2,9-epoxylactaroscrobiculide A (**10**) (1) and lactarolide B (**3**), which indeed was also isolated from other *Lactarius* species. It is remarkable that in the *Lactarius* species which contain lactarane sesquiterpenes, always present is furandiol (**9a**) as well as some of its dehydration products (or precursors?), for example, in this case furoscrobiculin B and furanethers.

About the stereochemistry of these compounds, it seemed again, as noticed in the *Russula sardonia* metabolites (16), that usually the configuration of C–12 is *syn* to the ring junction protons (H–2 and H–9) when C–12 is geminal to an hydrogen and *anti* when it is geminal to an hydroxy group. An exception not yet explained by this "rule" is furanether B.

EXPERIMENTAL[5]

THE COLLECTION OF PLANT MATERIAL AND PREPARATION OF EXTRACTS A, B, AND C.—a. Fruit-bodies (25 kg) of *Lactarius scrobiculatus* Scop. collected during the summer of 1975 in Val d'Aosta were frozen in acetone and dry ice and minced in the solvent. After filtration, the mushrooms were homogenized with ethanol and ice. The acetone and ethanolic solutions were evaporated under vacuum, and the remaining water solution was exhaustively extracted with ethyl acetate. The extract, after being dried and evaporated, yielded 91.5 g (0.37%) of the total extract.

b. Fruit-bodies (10 kg) of *L. scrobiculatus* collected during the summer of 1975 in Val d'Aosta were cut and put into a vessel containing acetone. The material was prepared in a dry-box under a N_2 stream. The mushrooms were worked-up as in (a) yielding 55.8 g (0.56%) of total extract.

c. Fruit-bodies (8 kg) of *L. scrobiculatus* collected during the summer of 1975 in Piemonte and Trentino regions, were extracted with acetone at room temperature without precautions. Work-up as described in a afforded 30.5 g (0.38%) of total extract.

WORK-UP OF EXTRACTS.—To remove the free fatty acids, the extracts were washed with a sodium bicarbonate solution and filtered on an Al_2O_3 (act. III) column with ethyl acetate, methanol and methanol with 7% acetic acid as eluents.

The three terpenic extracts were analyzed by tlc on silica gel (Merck GF_{254}) with mixtures of different percentages of benzene-ethyl acetate and cyclohexane-ethyl acetate.

By gc analysis the best results were afforded in the following conditions: all the stationary phases were supported on Chromsorb G(AW-DMCS) 80–100 mesh, carrier gas flow N_2 20 ml/min: (1) 2% OV-1, T from 135° to 240° (2°/min); (2) 3% OV-17, T from 100° to 280° (4°/min); (3) 2% FFAP, T from 100° (isothermal for 4 min) to 240° (4°/min).

The combined a and b extracts gave 74.5 g of the less polar sesquiterpene fraction and 6.3 g of the more polar terpene fraction.

The less polar fraction (70.8 g) was chromatographed on 1 kg of Kieselgel (Merck, 0.063–0.2 mm) and eluted with benzene and increasing percentages of ethyl acetate and ethyl acetate with increasing percentages of methanol. The six main fractions were again chromatographed on Kieselgel by elution with light petroleum ether, cyclohexane, benzene, ethyl acetate and methanol. The new fractions, after tlc analysis, were combined with those of similar composition coming from other main fractions.

ISOLATION OF COMPOUNDS.—Ethyl linoleate and lactaral (**1**) were separated on a 5% $AgNO_3$–Al_2O_3 column (petroleum ether-benzene 1:2); lactaral and furoscorbiculin A (**2**) were separated on the same column eluted with petroleum ether-benzene 1:1; furanether A (**3**) and B (**4**) were separated on an Al_2O_3 (act III) column eluted with petroleum ether-benzene from 8:1 to 6:1; furoscrobiculin B (**5**) and furoscrobiculin C (**6**) on Al_2O_3 (act III) column eluted with benzene-ethyl acetate; furoscrobiculin D (**7**) and lactaroscrobiculide B (**8**) on a Kieselgel column eluted with benzene-ethyl acetate and furandiol (**9a**) eluted with ethyl acetate and a small percentage of methanol. Cerevisterol was separated from the most polar fractions and purified by crystallization from methanol.

ETHYL LINOLEATE.—Ethyl linoleate (8.6 g) gave a pink spot on tlc, and was identical with an authentic sample.

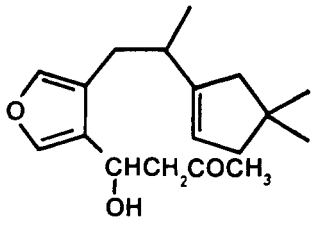
LACTARAL (**1**) (14).—Lactaral (260 mg) gave a blue spot on tlc, and was identical with an authentic sample isolated from *Russula sardonia* (16).

Besides *lactaral* (**1**), a small amount (13 mg) of a new compound of mw(ms) 290 was isolated. From the spectral data, we could determine its structure to be **14** due to condensation of **1** with acetone probably during extraction of the mushrooms.

FUROSCROBICULIN A (**2**).—Furoscrobiculin A (12 mg) gave a yellow spot on tlc. It gave $[\alpha]^{20}D = -23°$ ($CHCl_3$); uv(EtOH):λmax (log ε) = 207(3.82), 234(3.65), 276(3.19) nm. νfilm: 3140, 1582, 1527(furan), 1660(conjugated CO), 1139, 1052, 845: ms(70eV, DIS): 246(M^+, 22), 231(M–15, 34.5), 217(M–29, 19), 213(M–18–15, 5), 203 (15), 189(8), 185(6), 163(55.5), 162(28), 147(14), 145(14), 135(16), 109(25), 108(38.5), 95(20), 91(25), **83**(100), 81(27), 80(30.5), 79(24), 77(27), 71(22.5), 69(25), 67(25), 57(45), 55(50), 43(58.6), 41(55.5); δ $CDCl_3$: 0.99(d, 3H, J=7.0 Hz, H-

[5]Mps. were determined with a Fisher-Johns hot plate and are uncorrected. The pmr and ^{13}C-nmr spectra were recorded on a Varian XL-100 instrument, data are reported in ppm, TMS=0. Ir spectra were recorded on a 257 Perkin Elmer spectrophotometer, uv spectra with a Perkin Elmer 200 spectrophotometer and specific rotations were taken on an automatic Perkin Elmer polarimeter. Gc were carried out with a 5711 A Hewlett-Packard gas chromatograph equipped with a FID. Mass spectra were run on a Du Pont 21-492B instrument. Tlc was carried out on Silica-gel plates (Merck 60 GF_{254}); compounds were visualized as colored spots when the tlc plates were sprayed with a vanillin-H_2SO_4 solution and then heated at 120° for 10 min.

12), 1.07(s, 6H, H-14 and H-15), centred at 1.93(ABq, 2H, $J_{1-1'}$=14.5 Hz, H-1), centred at 2.14(ABq, 2H, $J_{10-10'}$=15.0 Hz, H-10), 2.4-2.7(m, 2H, H-3 and H-4), 3.07(dxm, 1H, $J_{4-4'}$= 14.0 Hz, $J_{4'-3}$=3.0 Hz, $J_{4'-5}$=1.5 Hz, H-4'), 7.20(t, 1H, $J_{4'-5}\simeq J_{5-13}$=1.5 Hz, H-5), 8.07(d, 1H, J_{5-13}=1.5 Hz, H-13). When the spectrum was recorded in C_6D_6 the signal at δ 1.93 (as the others) shifted up-field to δ 1.50 while the signal at δ 2.14 shifted to δ 2.22; this fact allowed assignment of the first signal to H-1 and the second to H-10.



14

FURANETHER A (3).—Furanether A (29 mg) gave a violet spot, and was identical with an authentic sample isolated from *R. sardonia* (16).

FURANETHER B (4) (3,8-ENANTIOFURANETHER A).—Furanether B (48 mg) gave a blue spot on tlc. It was a viscous oil, $[\alpha]^{20}D$ = +48.6° ($CHCl_3$); νfilm: 1554, 895 (furan), 1090, 1034 (C-O), 840, 790, 780 cm^{-1}; ms(70 eV, DIS 50°): 232(M^+, 100), 217(M-15, 53), 215(M-17, 15), 203(M-29, 34.5), 199(M-15-18, 26), 189(M-43, 84), 176(23), 175(25), 161(26), 159(15), 147(20), 145(16), 137(21), 133(27), 123(62), 119(29), 109(23), 107(32), 105(36), 95(82), 93(26.5), 91(36), 81(47.5), 79(29), 77(30), 71(11), 69(28), 67(22), 65(16), 57(20), 55(36.5), 53(21), 43(96), 41(48); δ $CDCl_3$: 0.86(s, 3H, H-14), 1.07(s, 3H, H-15), 1.38(s, 3H, H-12), 1.28(dd, 1H, J_{gem}=12.0 Hz, J_{vic}= 9.0 Hz, H-1 or H-10), 1.46 (d, 2H, J_{vic}=9.0 Hz, H-1 or H-10), 1.70(dd, 1H, J_{gem}=12.0 Hz, J_{vic}=7.5 Hz, H-1' or H-10'), 2.40-2.84(m, 2H, H-2 and H-9), 2.54 dd,1H, $J_{4-4'}$=16.0 Hz, $J_{4-5}\simeq$1 Hz, H-4), 2.80(dd, 1H, $J_{4'-4}$=16.0 Hz, $J_{4'-5}\simeq$1.5 Hz, H-4'), 4.78 (s, 1H, H-8), 7.13 and 7.16(m, 1H each, H-5 and H-13).

FUROSCROBICULIN B (5).—Furoscrobiculin B (143 mg) gave a violet spot on tlc. It was a pale yellow viscous oil, which easily decomposed: $[\alpha]D$ = +115.1° ($CHCl_3$): νfilm: 3400 br(OH), 1535 and 882(furan), 1388, 1370(-$C(CH_3)_2$), 1095, 1060(C-O), 787 cm^{-1}; ms(70 eV, DIS 80°): 232(M^+, 97), 217(M-15, 56), 215(M-17, 17), 214(M-18, 22), 203(M-29, 17), 199(M-18-15, 30), 189(M-43, 47), 176(32), 175(32), 171(21), 161(22), 159(20), 147(25), 145(21), 137(31), 133(30), 131(25), 123(15), 119(27), 115(22), 109(17), 107(23), 105(36), 95(66), 93(28), 91(35), 81(21), 79(22), 77(32), 69(27), 67(18), 65(20), 57(15), 55(35), 53(19), 51(18), **43**(100), 41(37); δ CCl_4: 1.02(s, 3H, H-14 or H-15), 1.06(s, 3H, H-15 or H-14), 1.11(s, 3H, H-12), 1.55(t, 1H, $J_{1-1'}$=12.0 Hz, J_{1-2}=12.0 Hz, H-1), 1.74 (dd, 1H, $J_{1-1'}$=12.0 Hz, $J_{1'-2}$=8.0 Hz, H-1'), 2.28(broad s, 2H, $J_{10-8}\simeq$1 Hz, H-10) 2.84(s, 2H, H-4), 3.06(br m, 1H, H-2), 6.05(ddd, 1H, J_{2-5}=3.0 Hz, $J_{8-10}\simeq$1 Hz, $J_{13-8}\simeq$3 Hz, H-8), 7.8 and 7.12(m, 1H each, H-5 and H-13). ^{13}C-nmr data are reported in table 1.

DEHYDRATION OF FURANDIOL (**9a**).—Dehydration of furandiol (**9a**) by treatment with CH_3SO_2Cl and pyridine yielded along with furanether A (**4**), furoscrobiculin B (**5**) as already published (16).

FUROSCROBICULIN C (**6**).—Furoscrobiculin C (125 mg) gave a violet spot on tlc. It gave $[\alpha]^{20}D$+12.8° ($CHCl_3$): νfilm: 3400(OH), 1542 and 885 (furan ring), 1135, 1082, 1060(C-O), 800 cm^{-1}; ms(70 eV, DIS 50°): 278 (M^+<1%), 260(M-18, <1), **232**(M-18-28,100), 217(52), 214(24), 203(19), 199(35), 189(62), 176(25), 175(23), 161(18), 147(18), 145(14), 137(19), 133(20), 123(60), 119(21), 109(11), 107(21), 105(28), 95(57), 93(19), 91(29), 81(33), 79(19), 77(23), 69(12), 67(12), 55(18), 53(13), 45(27), 43(62), 41(26); δ $CDCl_3$: 0.98(s, 3H, H-14 or H-15), 1.00(s, 3H, H-15 or H-14), 1.18(t, 3H, -OCH_2CH_3, J_{vic}=7.0 Hz), 1.22(s, 3H, H-12), 1.8-1.2(m, 4H, H-1 and H-10), 2.79(d, 1H, $J_{4-4'}$=16.5 Hz, H-4), 2.94 (dd, 1H, $J_{4-4'}$=16.5 Hz, $J_{4'-5}$=1.5 Hz, H-4'), 3.0-2.4(m, 2H, H-2 and H-9), 3.37 and 3.52 (2dxq, AB part of an ABX_3 system, 2H, J_{vic}=7.0 Hz, J_{gem}=9.0 Hz, -OCH_2CH_3), 4.29(d, 1H, J_{8-9}=4.0 Hz, H-8), 5.90(broad, shifting on heating, OH), 7.22(m, 1H, $J_{4'-5}\sim J_{5-13}$=1.5 Hz, H-5), 7.30 (d, 1H, J_{5-13}=1.5 Hz, H-13). ^{13}C-nmr data are reported in table 1.

Ethylation of **6** with NaH and ethylbromide yielded 3,8-di-O-ethylfurandiol (**9c**), which was already obtained by ethylation of **9a** (23).

FUROSCROBICULIN D (**7a**).—Furoscrobiculin D (81 mg) gave a blue spot on tlc. It gave a mp of 142-143° (Et_2O-pentane); $[\alpha]^{20}D$+10.8° ($CHCl_3$); ν KBR: 3470 and 3420(OH), 3155, 1552

(furan), 1187, 1143, 1125, 1110, 1084, 1077, 1057, 1032, 1018, 992, 980, 970, 916, 907, 898, 865, 823, 800, 790, 765, 723 cm^{-1}; ms(70 eV, DIS 70°): 250, (M^-, 8), **232**(M–18, 100), 217(M–18–15, 18), 214(M–18–18, 96), 203(M–18–29, 37.5), 199(M–18–18–15, 92), 189(8.5), 177(37.5), 176(37.5), 163(25), 161(18), 148(18), 147(30), 133(18), 123(25), 119(21), 109(80), 95(13), 91(34), 83(12.5), 81(17), 79(17), 77(21), 69(13), 67(17), 65(13), 55(21), 53(38), 51(21), 43(46), 41(12.5); δ $CDCl_3$: 1.01(d, 3H, J_{3-12}=6.5 Hz, H-12), 1.03(s, 3H, H-14 or H-15), 1.20(s, 3H, H-15 or H-14), 1.4-1.65(m, 1H, H-3), 1.68(s, 2H, H-1), 1.79–2.10(m, 3H, H-9, H-10, OH), 2.24(dd, 1H, $J_{4-4'}$=15.0 Hz, J_{4-3}=2.5 Hz, H-4), 2.61(ddd, 1H, $J_{4-4'}$=15.0 Hz, $J_{4'-3}$=11.5 Hz, $J_{4'-5}$=1.5 Hz, H-4'), 4.76–4.98(m, 1H, J_{8-9}=8.0 Hz (measured in $(CD_3)_2CO$), H-8), 7.16(t, 1H, $J_{4'-5}$=1.5 Hz~J_{5-13}, H-5), 7.30(t, 1H, J_{5-13}=1.5 Hz=J_{8-13}, H-13). ^{13}C-nmr data are reported in table 1.

ACETYLATION OF FUROSCROBICULIN D.—By treatment of **7a** with acetic anhydride/pyridine at room temperature and the usual working up of the mixture, the monoacetyl derivative **7b** was obtained in quantitative yields. It gave the following data: ν film: 3530(OH), 1730(acetate) cm^{-1}; δ $CDCl_3$: 1.00(d, 3H, J_{3-12}=7.0 Hz, H-12), 1.00(s, 3H, H-14 or H-15), 1.15(s, 3H, H-15 or H-14), 1.67(ABq, 2H, $J_{1-1'}$=13.5 Hz, H-1), 1.40–2.10(m, 3H, H-9, H-10, H-3), 2.14(s, 3H, CH_3CO), 2.23(dd, 1H, $J_{4-4'}$=14.5 Hz, J_{4-3}=2.5 Hz, H-4), 2.66(ddd, 1H, $J_{4-4'}$=14.5 Hz, $J_{3-4'}$=11.0 Hz, $J_{4'-5}$=1.5 Hz, H-4'), 6.12(dd, 1H, J_{8-9}=10.0 Hz, J_{8-13}=1.2 Hz, H-8), 7.04(t, 1H, J_{5-13}=1.5 Hz, J_{13-8}=1.2 Hz, H-13), 7.15(t, 1H, $J_{4'-5}$=J_{5-13}=1.5 Hz, H-5); δ C_5D_5N: 1.04(s, 3H, H-14 or H-15), 1.11(d, 3H, J_{3-12}=7.0 Hz, H-12), 1.22(s, 3H, H-15 or H-14), 1.64 and 2.01(d, 1H each, $J_{1-1'}$=13.5 Hz, H-1), 1.40–2.20(m, 3H, H-9, H-10, H-3), 2.28(dd, 1H, $J_{4-4'}$=14.5 Hz, J_{4-3}=2.0 Hz, H-4), 2.98(ddd, 1H, $J_{4-4'}$=14.5 Hz, $J_{4'-3}$=11.5 Hz, $J_{4'-5}$=1.5 Hz, H-4'), 6.71(dt, 1H, J_{8-9}=8.5 Hz, J_{8-13}=1.5 Hz, J_{8-10}=1.5 Hz, H-8), 7.40(t, 1H, J_{5-4}=J_{5-13}=1.5 Hz, H-5), 7.46(t, 1H, J_{13-5}=J_{13-8}=1.5 Hz, H-13).

LACTAROSCROBICULIDE B (**8**).—Lactaroscrobiculide B (125 mg) gave a green spot on tlc. It gave $[\alpha]^{28}D$ +22.3° (MeOH); mp 155–156° (Et_2O-pentane); λ max (log ε) (EtOH): 276(3.81), 208(4.06) nm; ν KBr: 3490 (OH), 1740(unsaturated γ-lactone C=O), 1662 and 1652(conjugated diene), 1152, 1045 cm^{-1}; ms(70 eV, DIS): 248(M^+, 25), 233(M–15, 38), 230(M–18, 8), 215(M–15–18, 28), 206(14), 203(7), 191(25), 174(17), 173(15), 161(6), 159(9), 153(6), 145(13), 131(8), 129(8), 119(7), 115(9), 111(5), 105(16), 95(51), 91(13), 81(8), 79(9), 77(17), 69(11), 55(10), **43**(100), 41(26); δ $CDCl_3$: 0.94 (s, 3H, H-15 or H-14), 1.16(s, 6H, H-15 or H-14 and H-12), 1.64(t, 1H, $J_{1-1'}$=J_{1-2}=12.0 Hz, H-1), 1.83(dd, 1H, $J_{1-1'}$=12.0 Hz, $J_{1'-2}$=8.0 Hz, H-1'), 2.32 (br s, 2H, allylic CH_2, H-10), 2.47(s, 1H, shifting on heating, OH), 2.67 and 2.92(ABq, 2H, $J_{4-4'}$=19.0 Hz, H-4), 3.12(dd, 1H, J_{1-2}=12.0 Hz, $J_{1'-2}$=8.0 Hz, H-2), 4.62 and 4.74(ABq, 2H, $J_{5-5'}$=18.0 Hz, H-5), 6.09 (d, 1H, J_{8-2}≃2.0 Hz, H-8); δ C_6D_6: 0.85 and 0.94(s, 3H each, H-14 and H-15), 1.00(s, 3H, H-12), 2.08(br s, 2H, H-10), 2.12 and 2.36(ABq, 2H, $J_{4-4'}$=19.0 Hz, H-4), 2.90(m, 1H, H-2), 3.84 and 3.96(ABq, 2H, $J_{5-5'}$=18.0 Hz, H-5), 6.24 (m, 1H, J_{2-8}=2.7 Hz, J_{8-10}=1.5 Hz, H-8). ^{13}C-nmr data are reported in table 1.

DIBAL-H REDUCTION OF LACTAROSCROBICULIDE B (**8**) TO FUROSCROBICULIN B (**5**).—To a solution of **8** in anhydrous THF, a few drops of a conc. DIBAl-H soln. in anhydrous THF were added at −15° with stirring and under a N_2 stream. After 45 min. at −15°, 10% H_2SO_4 was added to the reaction mixture. By extraction with ethyl ether, washing and drying over anhydrous Na_2SO_4, almost pure furoscrobiculin B was obtained.

HYDROGENATION OF LACTAROSCROBICULIDE B (**8**) TO 8,9-DIHYDROLACTAROSCROBICULIDE B (**12**).—Pd/$BaSO_4$ hydrogenation of **8** after the usual working up afforded a mixture of three products (tlc, eluent ethyl ether-acetone, 8:1) which were separated by plc. The second compound was identical in all respects with the known 5,8-desoxylactarolide B (**12**) (3). The other two products are not yet identified.

FURANDIOL (**9a**) (15, 16, 21).—Furandiol was identical with an authentic sample previously isolated from an other lot of *L. scrobiculatus* (2).

CEREVISTEROL (20).—Cerevisterol, mp 254–256°, had spectral data identical with those in literature (32). Acetylation with acetic anhydride/pyridine at room temperature afforded cerevisterol diacetate, mp 172–173° (aq. MeOH), which was identical to an authentic sample.

ACKNOWLEDGMENTS

The authors thank Mr. M. Angarano of the "Bresadola" Mycological Group (Trento) and Mr. E. Rebaudengo of the Mycological Group of Ceva for collection of part of the mushrooms. They are grateful to Professor D. H. Barton for a sample of cerevisterol acetate and to Professor E. P. Serebryakov for copies of the cerevisterol spectra. This work was supported by Consiglio Nazionale delle Ricerche (Rome) and by Ministero della Pubblica Istruzione.

Received 27 June 1979.

LITERATURE CITED

1. G. Vidari, L. Garlaschelli, M. De Bernardi, G. Fronza and P. Vita-Finzi, *Tetrahedron Letters*, 1773 (1975).
2. M. De Bernardi, G. Fronza, G. Vidari and P. Vita-Finzi, *Chim. e Ind.*, **58**, 177 (1976).
3. M. De Bernardi, G. Fronza, G. Mellerio, G. Vidari and P. Vita-Finzi, *Phytochemistry*, **18**, 293 (1979).
4. W. M. Daniewski and M. Kocór, *Bull. Acad. Polon. Sci., Ser. Chim.*, **18**, 585 (1970), **19**, 553 (1971).
5. W. M. Daniewski, M. Kocór and B. Zoxtowska, *Bull. Acad. Polon. Sci., Ser. Chim.*, **21**, 785 (1973).
6. W. M. Daniewski, M. Kocór and J. Król, *Bull. Acad. Polon. Sci., Ser. Chim.*, **23**, 637 (1975).
7. W. M. Daniewski, M. Kocór and J. Król, *Rocz. Chem.*, **50**, 2095 (1976).
8. W. M. Daniewski, M. Kocór and S. Thorén, *Heterocycles*, **5**, 77 (1976).
9. W. M. Daniewski, M. Kocór and J. Król, *Rocz. Chem.*, **51**, 1395 (1977).
10. G. Magnusson, S. Thorén and B. Wickberg, *Tetrahedron Letters*, 1105 (1972).
11. G. Magnusson and S. Thorén, *Acta. Chem. Scand.*, **27**, 1573 (1973).
12. G. Magnusson, S. Thorén and T. Drakenberg, *Tetrahedron*, **29**, 1621 (1973).
13. G. Magnusson, S. Thorén, J. Dahmén and K. Leander, *Acta Chem. Scand. B*, **28**, 841 (1974).
14. G. Magnusson and S. Thorén, *Tetrahedron*, **30**, 1431 (1974).
15. G. Vidari, M. De Bernardi, P. Vita-Finzi and G. Fronza, *Phytochemistry*, **15**, 1953 (1976).
16. D. Andina, M. De Bernardi, A. Delvecchio, G. Fronza, G. Mellerio, G. Vidari and P. Vita-Finzi, *Phytochemistry*, **19**, 93 (1980).
17. M. De Bernardi, G. Fronza, G. Mellerio, G. Vidari and P. Vita-Finzi, *Phytochemistry*, **19**, 99 (1980).
18. K. Vokač, Z. Samek, V. Herout and F. Sǒrm, *Coll. Cvech. Chem. Commun.*, **35**, 1296 (1970).
19. M. De Bernardi, G. Mellerio, G. Vidari, P. Vita-Finzi and G. Fronza, *J. Chem. Soc., Perkin I*, 221 (1980).
20. G. H. Alt and D. H. Barton, *J. Chem. Soc.*, 1356 (1954).
21. S. Nozoe, H. Matsumoto and S. Urano, *Tetrahedron Letters*, 3125 (1971).
22. S. Gronowitz, G. Sörlin, B. Gestblom and R. H. Hoffman, *Ark. Kemi*, **19**, 483 (1963).
23. M. De Bernardi, G. Fronza, G. Mellerio, G. Vidari and P. Vita-Finzi, unpublished data on *Lactarius pallidus*.
24. J. D. Connolly and R. McCrinde, *Chem. Ind.*, **379**, 2066 (1965).
25. N. S. Baccha and D. H. Williams, "Applications of N.M.R. Spectroscopy in Organic Chemistry, Illustrations from Steroid Field", Holden Day, San Francisco (1964).
26. L. M. Jackman and S. Sternhell, "Applications of N.M.R. Spectroscopy in Organic Chemistry", Pergamon Press, Oxford (1969).
27. J. Froborg and G. Magnusson, *J. Am. Chem. Soc.*, **100**, 6728 (1978).
28. N. K. Wilson and J. B. Stothers, *Topics in stereochemistry*, **8**, 1 (1974).
29. J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York (1972).
30. P. V. DeMarco, E. Farkas, D. Doddrell, B. L. Mylari and E. Wenkert, *J. Am. Chem. Soc.*, **90**, 5480 (1968).
31. E. M. Engler and P. Laszlo, *J. Am. Chem. Soc.*, **93**, 1317 (1971).
32. E. P. Serebryakov, A. V. Simolin, V. F. Kucherov and B. V. Rosynov, *Tetrahedron*, **26**, 5215 (1970).