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Fungal Extractives

III.* Two Sesquiterpene Lactones from *Lactarius*

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Two sesquiterpene lactones, isolated from *Lactarius vellereus* and *L. pergamenus*, have been shown to possess structures *1* and *2*.

In a recent publication[2] we reported the structure of a sesquiterpene dialdehyde ("isovelleral") and the isolation of two other C_{15}-aldehydes and lactones from *Lactarius vellereus* and *L. pergamenus* (Russulaceae). These terpenoids were obtained from fresh fungus extract by repeated column chromatography on silica gel. We have now shown that the two lactones have structures *1* and *2*, respectively (Fig. 1).

^{13}C-NMR spectroscopy (C-15; H-20) in combination with mass spectrometry (M^+ 232) gave the same molecular formula $C_{15}H_{20}O_2$ for both lactones, and a close structural relationship was shown by the similarity of their mass spectra and by conversion of lactone *1* to lactone *2* on heating.

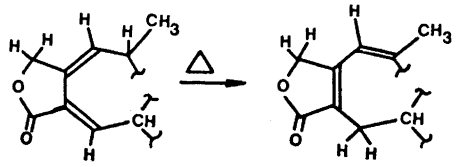
In lactone *1* the IR band at 1772 cm^{-1} suggested the presence of a γ-lactone and bands at 1675 and 1652 cm^{-1} indicated one or more double bonds. The ^{13}C-NMR showed signals from a carbonyl carbon, from two disubstituted and two monosubstituted vinyl carbons and from a methylene carbon attached to oxygen (Table 1). The UV absorption maximum at 282 nm (ε 4300) required the double bonds and the carbonyl group to be in the same conjugated system, and this was further supported by the ^{1}H-NMR spectrum which showed low-field signals at 6.99 and 5.61 ppm from two olefinic protons. The signal at 6.99 ppm appeared as a broadened doublet ($J = 7$ Hz) reflecting a vicinal interaction with a secondary allylic proton and a long-range coupling. The two protons of the $-O-CH_2-$ group almost coincided at 4.72 ppm (outer AB signals negligible) and were both split by allylic coupling ($J = 3$ Hz) with the proton at 5.61 ppm (broad singlet). A methyl doublet appeared at 1.12 ppm ($J = 7$ Hz).

The IR spectrum of lactone *2* had bands at 1760 cm^{-1} and at 1670 and 1650 (C=C) cm^{-1}. The ^{13}C-NMR spectrum of *2* had signals for carbonyl carbon

* Part II, see Ref. 1.

and for methylene carbon attached to oxygen and showed three disubstituted and one monosubstituted vinyl carbon atom signals (Table 1). The ^{1}H-NMR spectrum of *2* showed, correspondingly, only one olefinic proton (5.98 ppm, broad singlet). The $-O-CH_2-$ group appeared as a broad singlet (4.68 ppm) and the methyl group was situated on a double bond (broadened singlet at 1.94 ppm). The UV spectrum had a maximum at 270 nm (ε 6100), characteristic for α,β-γ,δ-unsaturated butyrolactones [3] though the intensity was rather low.

The spectral data above suggested the partial structures *1a* and *2a*.



1a 2a

The molecular formulae gave an unsaturation number of six. The partial structures *1a* and *2a* both contained all three double bonds (^{13}C-NMR) and one ring, which left two rings to be accounted for. The ^{13}C-NMR spectrum of lactone *1* showed, in addition to the functional group carbons discussed above, a further nine carbon atoms – three methyl, two methylene, three methine, and one quaternary (Table 1).

Two methyl singlets at 1.02 and 0.98 ppm in the ^{1}H-NMR and a doublet at 1380 cm^{-1} in the IR spectrum established a quaternary *gem*-dimethyl group. The spectral findings and analogies with known sesquiterpenoids from basidiomycetes suggested structure *1* for this compound. The three one-proton multiplets at 3.01, 2.61, and 2.13 ppm could then be assigned to the allylic and homoallylic bridgehead protons, respectively.

Analogous interpretation of the spectral data (see Table 1 and Experimental) combined with sesquiterpenoid analogies suggested structure *2* for the second lactone.

The best analogy on which these structures can be based is "isovelleral" [2] (*9*) from the same species, but other sesquiterpenoids that have been found in basidiomycetes, *e.g.* marasmic acid,[4] the illudins,[5] hirsutic acid,[6] and fomannosin,[7] all have a 3,3-disubstituted cyclopentane ring. In two sesquiterpenoids recently isolated from *Fomitopsis* [8] (*e.g. 10*) the entire carbon skeleton has been found to be the same as is suggested here for lactones *1* and *2*. It also appears that the lactarorufins A and B found by Daniewski and Kocór in *L. rufus* [9] may well have closely related structures.*

To establish the aliphatic portion of the molecule and the position of the double bonds, lactone *1* was ozonized in methanol solution. Reductive work-up of the hydroperoxides with dimethyl sulphide [10] gave the dialdehyde *3* which was precipitated as the *bis*-dinitrophenylhydrazone *4*. Ring closure and aromatisation of the crude *bis*-DNP was effected in boiling acetic acid-conc. hydro-

* *Added in proof.* For the structure of lactarorufin A, see Barauowska, E. and Daniewski, W. M. *Bull. Acad. Pol. Sci. Ser. Chim.* **20** (1972) 313 and references cited therein.

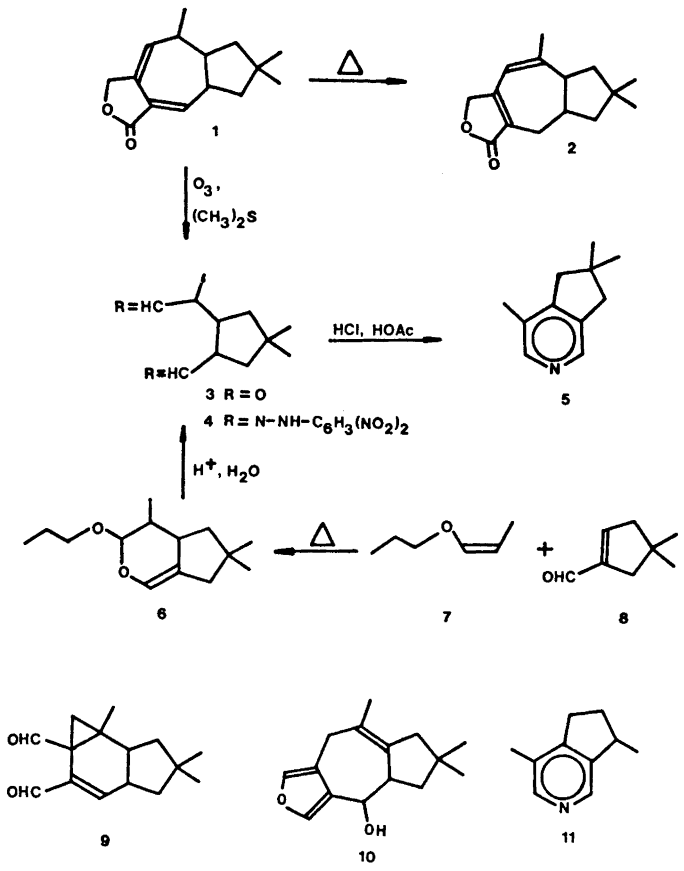


Fig. 1.

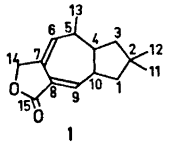
chloric acid according to the method used by Cavill and Ford in their preparation of actinidine (*11*) from iridodial.[11] The solution was made alkaline and steam-distilled. This gave the pyridine *5* in low yield. The structure of compound *5* was unequivocally ascertained by synthesis in the following way. *cis*-Propenyl propyl ether[12] (*7*) was reacted with the cyclopentenecarboxaldehyde *8*, prepared from dimedone by a newly developed procedure.[13] The Diels-Alder adduct[14] obtained (*6*) was hydrolysed and the product was reacted with dinitrophenylhydrazine. The *bis*-DNP mixture (*4*) was apparently a mixture of isomers not identical with that obtained from ozonisation (TLC). The ^{1}H-NMR spectrum of the pyridine obtained on treatment of the crude bis-DNP as above was identical with that obtained from lactone *1*. The two pyridines gave identical picrates (m.p. and mixed m.p.). The structure of the pyridine was further confirmed by elemental analysis of the picrate and by IR (ν_{max} C=N 1590 cm^{-1}), ^{1}H-NMR and UV [λ_{max} (EtOH) 261 nm (ε 3200) and 269 nm (ε 2970)]; [*cf.* Refs. 11 and 15 for the closely related alkaloid acti-

nidine *11*: λ_{max} (EtOH) 262 nm (ε 2590), 270 nm (ε 2310) and λ_{max} (EtOH) 262 nm (ε 2400), respectively].

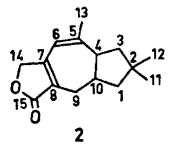
The chemical degradation of lactone *1* together with the interrelation with lactone *2* and the spectroscopic data firmly established the structures given for the two compounds.

Work on the stereochemistry of the lactones is in progress.

Table 1. ^{13}C-NMR data for lactones *1* and *2* ($CDCl_3$/TMS).



1



2

Chemical shifts ppm	Assignment carbon atom number	Chemical shifts ppm	Assignment carbon atom number
171.2	15	173.5	15
144.8	6,9	158.2	5,8
131.1		146.6	
127.8	7,8	122.9	7
123.4		112.7	6
69.0	14	71.2	14
47.2	1 or 3	50.5	4
46.4	4 or 10	48.6	1,3
44.6	1 or 3	47.7	
42.8	4 or 10	37.5	10
35.1	2	34.4	2
34.6	5	32.2	11,12
32.5	11,12	31.1	
31.7		30.9	9
19.4	13	26.4	13

EXPERIMENTAL

Melting points are uncorrected. The NMR spectra were recorded on a Varian XL-100 and a Varian T-60 spectrometer. Mass spectra were recorded on an LKB 1100 instrument and on a high resolution AEI MS902 instrument equipped with a AEI DS30 data system. (Dr. G. Hvistendahl, Kjemisk Institutt, University of Oslo, Norway.)

Isolation of lactones 1 *and* 2. Freshly collected fungi (*Lactarius vellereus* or *L. pergamenus*, Russulaceae) were ground with hexane and the mixture was pressed in a fruit press (Hafico). The hexane phase was separated, dried and evaporated and the residue crystallized from hexane to remove *stearic acid*. The mother liquor was partitioned between hexane and aqueous methanol (90 %). The methanol phase was diluted with water to 50 % and repeatedly extracted with chloroform. The residue from the chloroform phase was chromatographed on a silica gel column with ether (10 %) in benzene which gave "isovelleral",[2] another C_{15}-dialdehyde* and a mixture of the lactones *1* and *2* (0.015 % of the fresh fungus). The lactone fraction was rechromatographed on silica gel with methylene chloride.

* Magnusson, G., Thorén, S. and Drakenberg, T. *Tetrahedron* **29** (1973) 1621 (*added in proof*).

Lactone 1. The compound, a bright yellow oil, had $[\alpha]_D^{25}$ +364° (*c* 1.9); λ_{max} (EtOH) 282 nm (ε 4300); ν_{max} (neat) 1772 (strong) (C=O), 1730 (weak), 1675 1652 (C=C), 1385 1368 (*gem*-dimethyl), 1220 1200 1180 (ester bands), 1035 (C–O), 755 cm^{-1}; ^{1}H-NMR: δ_{TMS} ($CDCl_3$) 6.99 (1H, d J=7.0 Hz; –OCO–C=C*H*–CH–), 5.61 (1H, s broad; –O–CH_2–C=C*H*–CHCH_3–), 4.72 (2H, pair of d J=3.0 Hz; –O–CH_2–C=CH–), 3.01 2.61 2.16 (1H each, m; allylic and homoallylic protons), 1.12 (3H, d J=7.0 Hz; CH_3–CH–), 1.02 0.98 (3H each, s; –CH_3–CH_3) ppm. ^{13}C-NMR data, see Table 1. MS (70 eV): *m/e* 232 (M^+ 95 %; $C_{15}H_{20}O_2$), 217 (80 %), 122 (100 %), 91 (80 %). (Found: M. wt. 232.1465. Calc. for $C_{15}H_{20}O_2$: M. wt. 232.1463.)

Lactone 2. The compound was obtained as an oil which crystallized slowly from benzene-hexane, m.p. 41–44°; $[\alpha]_D^{25}$ –73° (*c* 0.9); λ_{max} (EtOH) 270 nm (ε 6100); ν_{max} (neat) 1760 (C=O), 1670 1650 (C=C), 1380 1370 (*gem*-dimethyl), 1118 1035 (C–O), 772 cm^{-1}; ^{1}H-NMR: δ_{TMS} ($CDCl_3$) 5.98 (1H, s broad; –C=C*H*–C=C–COO–), 4.68 (2H, s; =C–CH_2–OCO–), 2.92–2.17 (4H, m; allylic and homoallylic protons), 1.94 (3H, s broad; CH_3–C=CH–C=C–), 1.13 1.08 (3H each, s; –CH_3–CH_3) ppm. ^{13}C-NMR data, see Table 1. MS (70 eV): *m/e* 232 (M^+ 100 %; $C_{15}H_{20}O_2$), 217 (58 %, 187 (37 %), 122 (79 %). (Found: C 77.3; H 8.7. Calc. for $C_{15}H_{20}O_2$: C 77.5; H 8.7.)

Isomerization of lactone 1 *to lactone* 2. Lactone *1* was heated at 140° for 1 h under partial vacuum and then distilled, $b.p._{0.05}$ 125°. The distillate consisted of almost pure lactone *2*, identified by IR and NMR spectroscopy. It had $[\alpha]_D^{25}$ –53° (*c* 2.6).

Ozonisation of lactone 1. Lactone *1* (458 mg) was ozonized in methanol (6 ml) at –70°. The yellow colour of the solution had disappeared after 3.5 h and the ozonisation was continued for another 4 h. Dimethyl sulphide [10] (0.4 ml) was then added. The solution was stirred at –10° for 1 h, at 0° for 1 h, and finally at room temp. for 1 h. The solvents were evaporated under reduced pressure and the remaining oil was dissolved in ethanol (0.5 ml) and added to a solution of dinitrophenylhydrazine (1800 mg) in conc. sulphuric acid (3.5 ml) and ethanol (25 ml). The reaction mixture was stirred at room temp. for 1 h, and then water (75 ml) was added to complete the precipitation. The bis-dinitrophenylhydrazones were extracted with ethyl acetate-hexane (9:1) (4 × 50 ml) and the residue from the organic phase was dissolved in boiling benzene and filtered hot. The filtrate was evaporated and chromatographed on silica gel (100 g). Elution with ethyl acetate-ligroin (1:2) gave crude dinitrophenylhydrazones of *4* (408 mg) [TLC silica gel, ethyl acetate-ligroin (1:2) visualized by 0.2 % $K_3Fe(CN)_6$ in 2 M hydrochloric acid [16]]. The crude product was refluxed with conc. hydrochloric acid (2 ml) in acetic acid (10 ml) for 2 h,[11] cooled, and the pH of the solution was adjusted to 9 with 6 M sodium hydroxide and steam-distilled. The distillate (250 ml) was extracted with chloroform (3 × 25 ml), the organic phase was dried (Na_2SO_4) and the solvent was distilled through a 30 cm Vigreux column in a slow stream of dry nitrogen, the last traces of chloroform being distilled away by adding dry ether. The residue (22 mg) consisted of almost pure *2',2',5-trimethyl-3,4-cyclopentanopyridine* (*5*). The ^{1}H-NMR spectrum of *5* was superimposable on that of a synthetic sample (*vide infra*). The *picrate* was recrystallized from ethanol and had m.p. 145.5–147.0° (*cf.* synthetic sample, m.p. and mixed m.p. 145.5–147.0°). (Found: N 13.9. $C_{17}H_{18}N_4O_7$ requires: N 14.3.)

3-Propyloxy-4,6,6-trimethyl-3,4,4a,5,7-pentahydro-cyclopenta[c]pyran (6). 4,4-Dimethylcyclopentenecarboxaldehyde (*8*) [12] (3.10 g; 0.025 mol) was heated in a sealed tube under nitrogen at 170° for 65 h with the *cis*-propenyl ether *7* [12] (3.00 g; 0.03 mol) and some crystals of hydroquinone. The *cis*-olefin was contaminated by smaller amounts of the *trans* isomer. After cooling, the reaction mixture was distilled, which gave a fore-run of unreacted aldehyde, $b.p._{11}$ 57–58° (1.35 g), and as main fraction an isomer mixture (^{1}H-NMR) of *pyranyl ethers* (*6*) (63 % yield calculated on reacted aldehyde), $b.p._{10}$ 118–123°; n_D^{23} 1.4638; ν_{max} (neat) 3080, 1685 (C=C), 1380, 1370 (*gem*-dimethyl), 1125, 1085 (C–O) cm^{-1}; ^{1}H-NMR: δ_{TMS} ($CDCl_3$) 6.20 (1H; –O–C*H*=C), 4.54 (1H; –O–C*H*–O–), 3.66 (2H; –O–CH_2–CH_2–) ppm; MS (70 eV): *m/e* 224 (M^+; $C_{14}H_{24}O_2$). (Found: C 74.9; H 10.7. $C_{14}H_{24}O_2$ requires: C 74.9; H 10.8.)

2',2',5-Trimethyl-3,4-cyclopentanopyridine (5). The pyranyl ether mixture (*6*) (448 mg) was converted to *bis*-dinitrophenylhydrazones *4* by adding it to a solution of dinitrophenylhydrazine (900 mg) in conc. sulphuric acid (2 ml), ethanol (12 ml) and water (2 ml) at room temp. After 1 h, water (15 ml) was added and the crystals were filtered off, giving a quantitative yield of isomeric *bis*-hydrazones (TLC, see above). The hydrazone mixture (400 mg) was refluxed for 2 h in conc. hydrochloric acid (2 ml) and acetic

acid (10 ml) [11] and processed as described above. The brown odorous residue from evaporation of the chloroform was distilled under reduced pressure to give the *pyridine 5* in 60 % yield (71 mg). B.p.$_{12}$ 112°; n_D^{22} 1.5114; λ_{max} (EtOH) 261 nm (ε 3200), 269 nm (ε 2970); ν_{max} (neat) 3030, 1600, 1590, 1390 1370 (*gem*-dimethyl), 840, 720 cm^{-1}; ^{1}H-NMR: δ_{TMS} ($CDCl_3$) 8.27 8.22 (1H each, s broad; pyridine protons), 2.75 2.67 (2H each, s; pyr$-CH_2-$), 2.22 (3H, s; pyr-CH_3), 1.15 (6H, s; $-CH_3-CH_3$) ppm. *Picrate*: M.p. 145.5 – 147.0°. (Found: C 52.3; H 4.7; N 14.2. $C_{17}H_{18}N_4O_7$ requires: C 52.3; H 4.7; N 14.3.)

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