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Fungal Extractives. VIII.* Two Sesquiterpene Furans from *Lactarius*

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The structures and relative configurations of two sesquiterpene furans (*8* and *9*) isolated from *Lactarius vellereus*, *L. pergamenus*, and *L. helvus* have been determined with the help of standard spectroscopic methods and computer analysis of lanthanide-induced chemical shifts. Evidence that *8* and *9* are artifacts formed during the isolation procedure is presented.

Hydroazulenic sesquiterpenes with a *gem*-substituted cyclopentane ring were reported (without stereochemical details) for the first time by Nozoe *et al.*[2] (compounds *1* and *2*). Five more compounds with this carbon skeleton have since been reported: Velleral[3] (*3*) and two lactones[4,5] (*4* and *5*) from *Lactarius vellereus* and *L. pergamenus*; lactarorufins A[6] (*6*) and B[7] (*7*) from *L. rufus*. The hydroazulene *2* has also been found in *L. necator* and its relative configuration determined (Fig. 1).[8]

* Part VII, see Ref. 1.

We now report the structures and relative configurations of two sesquiterpene furans (*8* and *9*) from *L. vellereus*, *L. pergamenus*, and *L. helvus* (Russulaceae).

Compound *8* was shown by ^{13}C NMR (15 C and 19 H) and mass spectrometry (M$^+$ at m/e 232) to have the molecular formula $C_{15}H_{20}O_2$. Its IR spectrum revealed the presence of a hydroxyl group, a *gem*-dimethyl group (ν_{max} 1390 and 1385 cm^{-1}) and a furan ring (ν_{max} 1540 and 880 cm^{-1}). The ^{1}H NMR spectrum showed that the furan ring is disubstituted with substituents in the 3 and 4 positions (signals at δ 7.37 and 7.10 ppm)[9,10] and that the hydroxyl group is attached to a tertiary carbon atom next to the furan ring (two doublets centered at δ 4.34 ppm with $J_1 = 11.0$ and $J_2 = 1.4$ Hz). In addition to the signals from the *gem*-dimethyl group there was a broadened three-proton signal (δ 1.71 ppm), which was assigned to a methyl

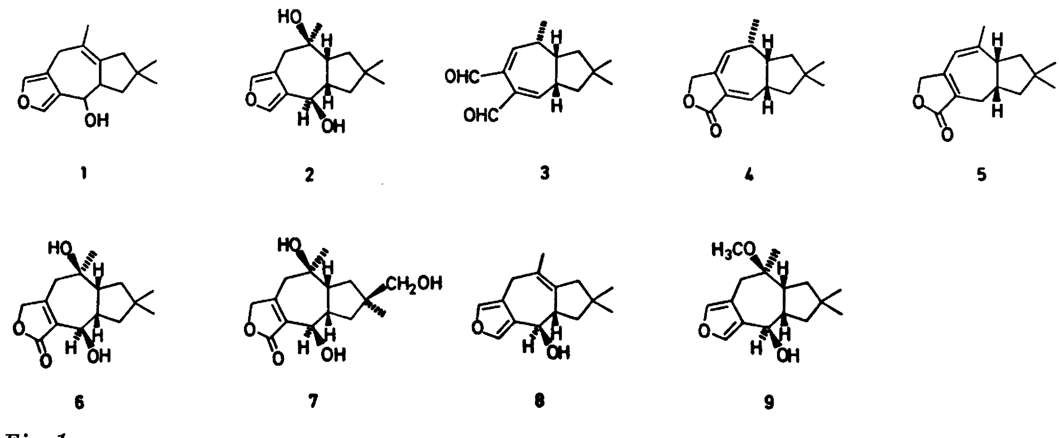


Fig. 1.

group situated on a double bond. From the ^{13}C NMR data (Table 1) it was established that *8* is a tricyclic compound with a tetrasubstituted double bond, with three primary, three secondary, two tertiary and one quaternary carbon atoms and, in addition, with furan ring carbon atoms. Extensive ^{1}H NMR decoupling experiments established the structure and relative configuration of *8* (Fig. 2). The ^{1}H NMR shifts

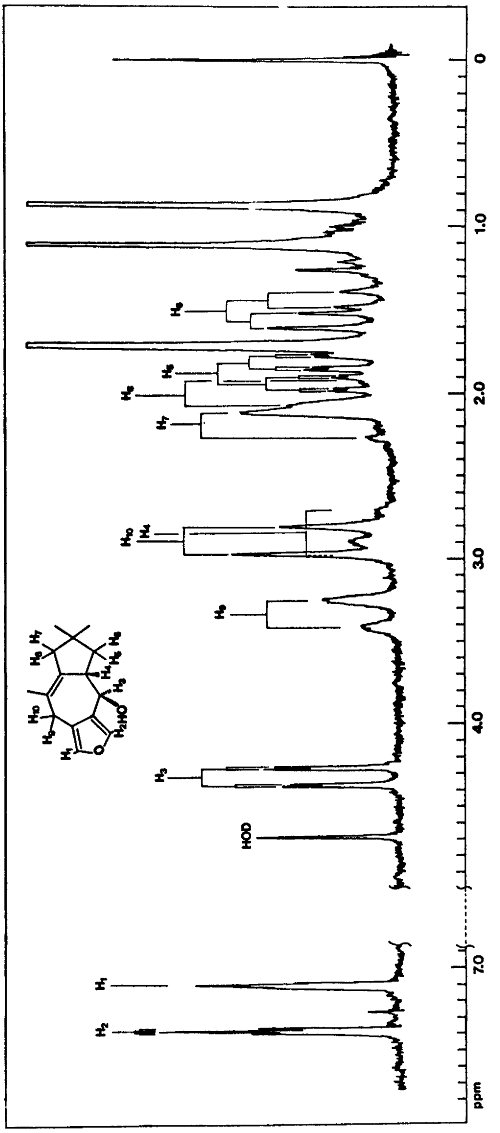
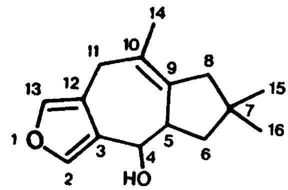


Fig. 2. ^{1}H-NMR spectrum of compound *8* (100 MHz; $CDCl_3/D_2O$).

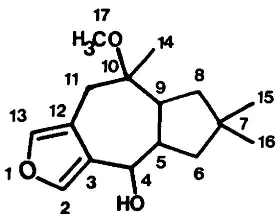
Table 1. ^{13}C NMR data for compounds *8* and *9* (25.2 MHz; $CDCl_3$).

Chemical shift (ppm from TMS)	Signal multiplicity[a]	Assignment (carbon No.)



8

140.4	d	2, 13
137.7	d	
136.7	s	9 or 10
128.8	s	3, 12
127.5	s	
120.7	s	9 or 10
70.4	d	4
48.4	d	5
46.3	t	6, 8
45.6	t	
37.0	s	7
29.5	t	11
29.5	q	15, 16
26.6	q	
21.9	q	14



9

141.8	d	2, 13
139.7	d	
127.3	s	3, 12
127.3	s	
80.2	s	10
66.7	d	4
48.8	q	17
46.8	d	5, 9
46.8	d	
45.3	t	6, 8
45.0	t	
36.6	s	7
29.9	q	15 or 16
28.0	t	11
27.7	q	15 or 16
24.4	q	14

[a] s=singlet, d=doublet, t=triplet, q=quartet; obtained by "off-resonance" decoupling.

reported here for *8* are in close agreement with those reported for *1*[2], except for the furan protons which appear at δ 7.37 and 7.10 ppm (in $CDCl_3$) instead of at 7.72 and 7.04 ppm (solvent not reported). The specific rotation for *8* is +123° (in methanol) instead of +69.5° (solvent not reported) for *1*.[2] In spite of these differences it seems probable that the compounds *1* and *8* are identical.

Compound *9* was shown by the same techniques as used for *8* to be a tricyclic molecule with a 3,4-disubstituted furan ring and a secondary alcohol group next to this ring. The molecular formula ($C_{16}H_{24}O_3$) implied a formal addition of one molecule of methanol to *8*. Compound *9* is devoid of sp^2 carbon atoms other than those constituting the furan ring (^{13}C NMR data in Table 1) and possesses four primary, three secondary, three tertiary, and two quaternary carbon atoms. From these data, including ^1H NMR chemical shifts and integrals, a probable structure could be constructed. There are eight configurational isomers I–VIII (Fig. 3) of this structure to be considered.

Extensive decoupling experiments did not solve the stereostructure of *9*, but revealed two important facts: The protons H_3 and H_4 (see formula I in Table 2) showed *vicinal* coupling of $J = 4.0$ Hz reflecting an approximate dihedral angle of either 50° or 135° as judged from the Karplus curves;[11] the protons H_3 and H_2 were coupled with $J < 0.5$ Hz. The dihedral angle in this *cis*-allylic coupling system should then be close to 0°.[12,13] (The decoupling experiments were run in $CDCl_3$ with D_2O added. The coupling constants were unchanged in CCl_4 solution). The IR spectrum of *9* (0.0027 M solution in CCl_4) showed only one band (3430 cm^{-1}) in the hydroxyl stretching region. This indicates an intramolecular $OH \cdots OCH_3$ bond and thus that the hydroxy and methoxy groups are on the same side (*cis*) of the seven-membered ring. The intramolecular hydrogen bond in combination with the two possible dihedral angles between H_3 and H_4 requires the bridgehead hydrogens to be *cis*. This still, however, leaves two possible isomers, compounds I and IV.

Another approach to the stereochemical problem was tried. A ^1H NMR spectrum of *9* in CCl_4 containing $Yb(fod)_3$, (fod = 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionate), showed induced chemical shifts but these did not establish the relative configuration of *9*. A newly developed computer program [14] (*cf.* also Ref. *15*) was used to calculate the expected lanthanide-induced chemical shifts for isomers I–VIII. Since conformational changes may occur in *9* in the presence of the $Yb(fod)_3$ complex, no assumptions regarding hydrogen bonding or dihedral angles were made. From Dreiding models the Cartesian coordinates in an arbitrary coordinate system of the hydrogens $H_1 - H_{23}$ were determined. Twenty-eight cases were included (four conformers of each of the *cis*-ring junction isomers I–IV, and three of each *trans*-ring junction isomer V–VIII). These sets of figures together with the experimental lanthanide-induced chemical shift values

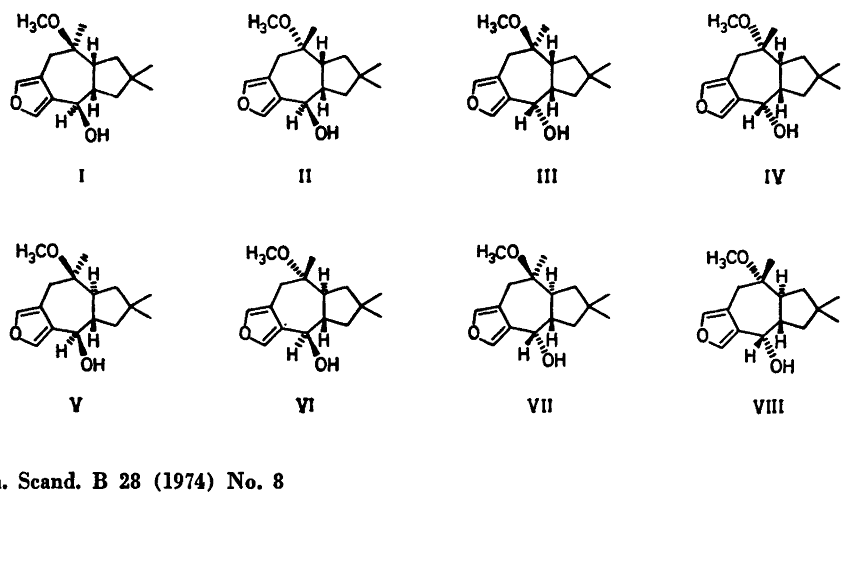


Fig. 3.

were used as input parameters in the program. Association of the shift reagent with the furan oxygen was neglected [16] and an association with the methoxy oxygen could be ruled out by the relatively small observed shift difference for the methyl group ($H_{12}-H_{14}$). The shifts of the methyls and the methoxy group were calculated as mean values of their proton shifts and a single position was chosen for the freely rotating methoxy group. Unequivocal assignments of the protons H_4 and H_9, H_{10} and H_{11}, and of the methyl group protons $H_{15}-H_{17}$ and $H_{18}-H_{20}$ were not possible and permutations of these three assignment pairs were therefore made. Only approximate values for the experimentally-induced chemical shifts of the four methylene protons (H_5-H_8) of the cyclopentane ring could be estimated and these were used with reduced weight in the computer program. A threefold potential barrier for oxygen – carbon rotation was assumed (in analogy with the calculations in Ref. 15) and the relative populations of the three rotamers of the hydroxy group were adjusted to best fit.

Four isomers (III, IV, VII, VIII) could be excluded in the initial calculations. Cases which gave agreement factors (R) [17] higher than 25 %, and cases with R-values between 20 % and 25 % and ytterbium – oxygen distances (d) far outside ($d > 5.77 \ldots\ldots 1.87 > d$ Å) reasonable limits (*ca.* 3.2 – 2.2 Å)[18] were rejected. There remained eight cases, two conformers of each isomer I, II, V, and VI, with R-values in the interval 13.6 – 19.5 % and with acceptable Yb – O distances. It was realised that there need not necessarily be only a single conformer present even though this was the case in the absence of the Yb complex (^{1}H NMR, ^{13}C NMR, IR). Mixed conformer populations of the four isomers were therefore used in the second calculation. One isomer (I) gave a very low agreement factor ($R = 6.0$ %; $d = 2.22$ Å) for a mixture of the boat and chair conformations in a ratio of *ca.* 55:45 (Fig. 4). Agreement factors for the three other isomers (II, V, VI) did not improve in this second calculation and could be rejected with high statistical significance (> 99.5 %).[17,19] Experimental and calculated shifts for I are presented in Table 2. Isomer I is one of the two derived by the independent reasoning above. In view of the complexity of the twentythree proton system some uncertainty in the calculation results cannot be excluded. However, in combination with the spectroscopic evidence the calculations firmly establish the relative configuration of the furan alcohol *9*.

Interestingly the stereostructure found for *9*, with the *cis*-fused hydroazulene ring system and

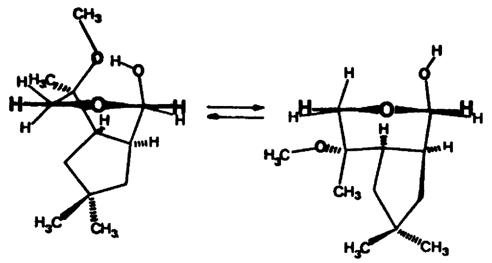
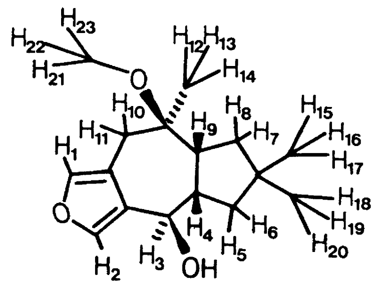


Fig. 4.

Table 2. Yb(fod)$_3$-induced and calculated chemical shifts of I.



1

Proton No.	Induced chemical shifts (Hz) Observed	Calculated
1	60	71
2	86	85
3	430	424
4	230	232
5	75[a]	57
6	75[a]	126
7	75[a]	31
8	75[a]	73
9	40	40
10	90	82
11	103	111
12 – 14	57	52
15 – 17	27	13
18 – 20	36	52
21 – 23	79	78

[a] Only roughly estimated shifts (± 50 Hz).

the methyl group $[-CH_{3(12-14)}]$ *trans* to the bridgehead hydrogens is the same as in other basidiomycete sesquiterpenes (see, *e.g.*, Fig. 1) and has the same relative configuration of the hydroxyl bearing carbon as in compounds *2*, *6*, *7*, and *8*.

It should be pointed out that compounds *8* and *9* may not be native to the *Lactarius* species investigated. The procedure used for the isolation of *8* and *9* was different from that used for the dialdehydes velleral (*3*), isovelleral,[10] and the two lactones *4* and *5*. *Methanol* was used instead of hexane [3] for extraction. Carbon tetrachloride extraction of the methanolic phase gave *8* and *9*, but neither the two dialdehydes (*cf.* Ref. 20) nor the two lactones were detected (TLC). On the other hand *8* and *9* were not obtained when hexane was used for extraction. It thus seems probable that *8* and *9* were formed during the work-up process. In order to test this possibility the mushrooms were ground with *ethanol* instead of methanol. From this extract there were isolated *8* and an *ethyl* ether (*10*), homologous to *9*. The methyl ether *9* was not detected. This finding is a strong indication that compound *9* at least, and probably also *8* are artifacts. Their formation by an enzymatically-assisted reaction sequence would seem more plausible than a pure chemical one since no stereoisomers of *8* and *9* were detected. The nature of the precursors of these compounds is of course important but, at this stage it is premature to consider this topic.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Varian XL-100 instrument with ^{13}C NMR capability and Fourier transform equipment. Mass spectra were recorded on an LKB 1100 instrument.

Isolation procedure. Fresh fungi (*Lactarius vellereus, L. pergamenus, L. helvus*) were ground with methanol and the mixture was pressed with Celite in a fruit press (Hafico). The aqueous filtrate was evaporated at room temperature to half its volume, diluted with water to the original volume and then extracted with three portions of carbon tetrachloride. The residue obtained on evaporation of the solvent was chromatographed on a silica gel column. Elution with benzene – ether (9:1) and then light petroleum – ether (2:1) gave the furan compounds *8* and *9*.

Furan alcohol 8. Recrystallisation from hexane at −20 °C gave *8*, m.p. 34 – 44 °C; $[\alpha]_D^{22}$ +123° (*c* 0.6, methanol), $[\alpha]_D^{22}$ +123° (*c.* 0.6, chloroform); IR: ν_{max} ($CHCl_3$) 3600, 1535 (furan), 1385 and 1368 (*gem*-CH_3), 1049 and 875 (furan) cm^{-1}; UV, nm (ε): λ_{max} (ethanol) 208.5 (ε 8200); ^{1}H NMR: δ_{TMS} ($CDCl_3/D_2O$) 7.37 (1H, d of d J_1 = 1.7 and J_2 = 1.4 Hz; −CHOD − fur − *H*), 7.10 (1 H, m; $-CH_2-$fur − *H*), 4.34 (1 H, d of d *J* = 11.0 and 1.4 Hz; fur − C*H*OD − CH −), 3.35 (1 H, d broad *J* = 17.0 Hz; fur − *H*CH − C = C −), 2.91 (1 H, d *J* = 17.0 Hz; fur − HC*H* − C = C −), 2.86 (1 H, m; −CHOD − C*H* −), 2.19 2.01)1 H each, d of d broad *J* = 16.0 Hz; = C − *H*C*H* − C −), 1.88 (1 H, m *J* = 13.0, 8.0 and 1.5 Hz; −CH − *H*CH − C −), 1.51 (1 H, d of d *J* = 13.0 and 9.0 Hz; −CH − HC*H* − C −), 1.71 (3 H, s broad; CH_3 − C = C −), 1.11 0.87 (3 H each, s; *gem*-CH_3-CH_3) ppm. ^{13}C NMR data see Table 1. MS (70 eV): *m/e* 232 (M+ 1 %; $C_{15}H_{20}O_2$), 214 (100), 199 (80), 197 (28), 158 (57), 129 (29), 128 (30).

Furan alcohol 9. Recrystallisation from ether at −20 °C gave *9*, m.p. 65 – 66 °C; $[\alpha]_D^{22}$ +6.0° (*c* 0.6, methanol); IR: ν_{max} ($CHCl_3$) 3360, 1538 (furan), 1390 and 1375 (*gem*-CH_3), 1110, 1060, 880 (furan) cm^{-1}; UV, nm (ε): λ_{max} (ethanol) 216 (4400); ^{1}H NMR: δ_{TMS} ($CDCl_3/D_2O$) 7.38 (1 H, d *J* = 1.6 Hz; −CHOD − fur − *H*), 7.17 (1 H, m $-CH_2-$fur − *H*), 4.61 (1 H, d *J* = 4.0 Hz; fur − C*H*OD − CH −), 3.21 (3 H, s; $-OCH_3$), 2.97 (1 H, m t *J* = 18.0, 1.2 and 1.0 Hz; fur − *H*CH − C −), 2.78 (1 H, d of d *J* = 18.0 and 1.4 Hz; fur − HC*H* − C −), 2.80 2.68 (1 H each, m; bridgehead protons), 1.70 (1 H, d of d *J* = 10.5 and 8.0; −CH − *H*CH − C −), 1.10 – 1.55 (3 H, m), 1.19 (3 H, s; $CH_3-C(OCH_3)-$), 1.06 (6 H, s; *gem*-CH_3-CH_3) ppm. ^{13}C NMR data, see Table 1. MS (70 eV): *m/e* 246 ($C_{16}H_{24}O_3$, M+ $-H_2O$ 63 %), 231 (36), 215 (63), 214 (100), 199 (100), 158 (72).

Furan alcohol 10. Fresh fungi (*L. vellereus*) were ground with ethanol and the mixture was treated as described under *Isolation procedure.* Compound *10* was isolated by chromatography on a silica gel column with light petroleum – ether (3:1) as eluent. *10* was obtained as a viscous oil, $[\alpha]_D^{22}$ +5.6° (*c* 0.7, ethanol) with physical properties similar to those described for *9*, except for changes due to the ethoxy group.

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