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The Isolation of New Sesquiterpene Aldehydes from Injured Fruit Bodies of *Lactarius scrobiculatus*

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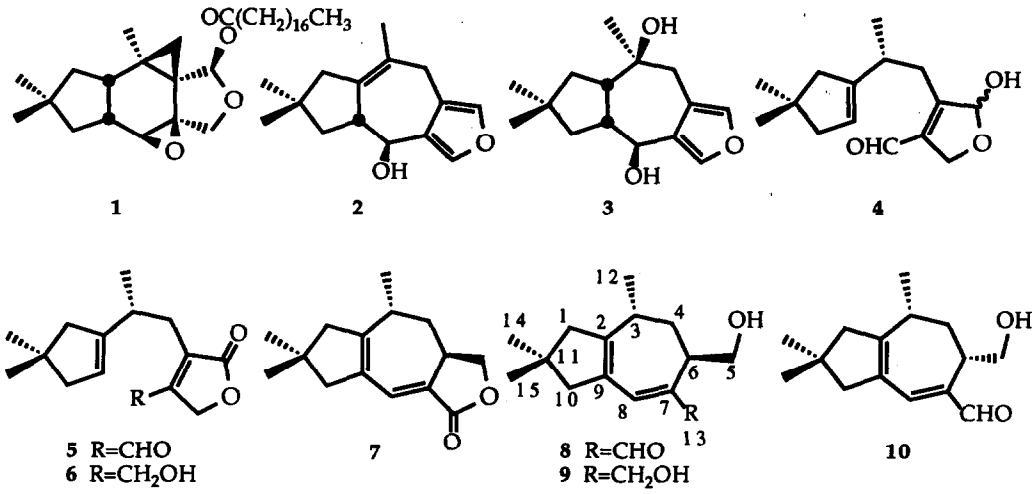
Abstract: The enzymatic conversion products of stearoylvelutinal in injured fruit bodies of *Lactarius scrobiculatus* have been investigated. Two sesquiterpene aldehydes not previously reported as natural products were isolated in addition to known sesquiterpenes. The structures were determined by spectroscopy combined with molecular mechanics calculations, and chemical transformation into known compounds.

The pungent taste of the fruit bodies of several species belonging to the Russulaceae family of fungi (genera *Lactarius* and *Russula*), has been shown to be caused by the rapid enzymatic formation of sesquiterpenoid unsaturated dialdehydes in the injured fruit body.[1] The dialdehydes are subsequently reduced enzymatically in the mushroom tissue to monoaldehydes. At least in some species, furan and lactone sesquiterpenes are formed in addition to the aldehydes. The precursor to the enzymatically formed Russulaceae sesquiterpenes is velutinal, present as a fatty acid ester (e.g. stearoylvelutinal **1**) in the intact fruit bodies. The velutinal esters are stable in the intact fruit bodies, but they are chemically labile and rapidly degraded in reagent grade solvents or during chromatography, to dihydrohydroxyfurans and furans *via* cations formed from acid catalysed opening of the epoxide ring.[2]

Fruit bodies of *Lactarius scrobiculatus* have been extensively investigated previously,[3] and a number of furan and lactone sesquiterpenes have been identified. However, non of these appears to be responsible for the pungency of the mushroom, and in view of the conversions of sesquiterpenes induced in the fruit bodies of other *Lactarius* species by physical injury, a reinvestigation of the contents of intact as well as injured fruit bodies of *L. scrobiculatus* was motivated. When fresh, undamaged fruit bodies of *L. scrobiculatus* were extracted with ethyl acetate in a mixer (no cutting of the mushroom prior to the mincing in the solvent), the crude extract contained stearoylvelutinal (**1**) as the only sesquiterpenoid detectable by analysis with TLC and ^{1}H NMR. In an ethyl acetate extract of fruit bodies of *L. scrobiculatus* that had been ground in a meat grinder 15 minutes prior to extraction, no trace of stearoylvelutinal (**1**) were detected. Instead, the presence of a number of other compounds, not present in the original extract, was indicated by TLC analysis of the extract, and these were isolated by preparative chromatography on silica gel.



1 2 3 4 5 R=CHO 6 R=CH_2OH 7 8 R=CHO 9 R=CH_2OH 10

Scheme 1.

From the ethyl acetate extract of the injured fruit bodies, the two lactarane furans **2** (11 mg) and **3** (58 mg), the secolactaranes **4** (3 mg), **5** (13 mg) and **6** (23 mg), as well as the lactone **7** (45 mg) and the aldehyde **8** (26 mg) were isolated. The furans have been isolated from virtually all pungent *Lactarius* species investigated, both may be formed by chemical transformation of stearoylvelutinal **1**[2] but have also been suggested to be products of enzymatic conversions.[1b] Lactardial (**4**), formally a 1,4-dialdehyde possessing pungent taste and antimicrobial activity,[4] is a conversion product also in other species.[1] The aldehyde **5** is new as a natural product, but has previously been prepared synthetically from blennin C (**6**).[5] The spectral data of compound **5** isolated in this investigation are identical to those reported previously, and reduction with $NaBH_4$ in ethanol yielded blennin C (**6**).[6] The lactone **7** was identified, but no trace of the 2,9-epoxy derivative of **7** previously co-isolated from *L. scrobiculatus*[3b] was detected. The position of the lactone carbonyl group on C-13 in lactone **7** is remarkable, as most sesquiterpene lactones isolated from *Lactarius* species have the carbonyl function on C-5. However, the counterparts of the new aldehyde **8** isolated from other *Lactarius* species have C-5 as an aldehyde and C-13 as a primary alcohol, and the differences in the C-5/C-13 oxidation pattern probably reflects differences between the enzymatic systems of various *Lactarius* species.

The structure of scrobicalol (**8**, name proposed by us) was determined by high resolution NMR and mass spectrocopy, while its relative stereochemistry was determined by NOE experiments and the comparison of actual and calculated $^3J_{HH}$ NMR coupling constants. For the latter, a conformational analysis of the two possible isomers **8** and **10** was carried out by molecular mechanics calculations (with full rotamer analysis), and the average vicinal coupling constants for the conformers of **8** and **10** with less than 3 kcal/mol higher steric energy compared with the most stable conformer were estimated using Osawa's 3JHH program.[7] (See

reference 8 for a more detailed description of how the technique was used to solve a similar problem.)

Table 1. Some Observed and Calculated $^3J_{HH}$ NMR Coupling Constants for Compounds **8** and **10**.

	Observed $^3J_{HH}$ in $CDCl_3$	Calculated for **8**	Calculated for **10**
$^3J_{3\text{-}4\alpha}$ (Hz)	11.5	12.0	9.0
$^3J_{3\text{-}4\beta}$ (Hz)	4.2	3.5	4.9
$^3J_{4\alpha\text{-}6}$ (Hz)	2.0	1.7	8.8
$^3J_{4\beta\text{-}6}$ (Hz)	6.1	5.7	1.5

It is obvious that the estimated coupling constants for isomer **8** are in better agreement with the experimental data, and structure **8** is also supported by NOE experiments. NOE:s were observed between 5-H_2 and 3-H (large when 5-H_2 was irradiated) as well as 4-Hβ (small when 5-H_2 was irradiated), between 3-H and 4-Hβ, between 13-H and 8-H, and between 8-H and 10-H_2, and are all in support with the most stable conformer of scrobicalol (**8**). Lactone **7** and scrobicalol (**8**) were correlated to each other by the reduction of both compounds to the same diol **9**, and this establishes the relative stereochemistry of lactone **7**. Unfortunately it was not possible to correlate either compound to any sesquiterpene with known C-3 configuration, and the stereochemistry indicated for compounds **7** and **8** is therefore only relative.

Because the limited amounts of fresh fruit bodies available, it was not possible to investigate the kinetics of the conversions in the mushroom tissue, e.g. whether scrobicalol (**8**) is formed prior to the lactone **7** or not. In addition, the possibility that the dialdehyde corresponding to scrobicalol (**8**) (the 5,13-dicarbaldehyde) is formed as a short-lived intermediate, similar to the situation in injured fruit bodies of other pungent Lactarius species, should be investigated.

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EXPERIMENTAL: The fruit bodies (approximately 1 kg) were collected on the island of Gotland (Sweden), and arrived to the laboratory in Lund approximately 24 hours after collection. Some specimen were extracted directly with ethyl acetate in a blender, to analyse the sesquiterpenoid contents of intact fruit bodies, while others were ground in a meat grinder and left 15 minutes at room temperature as a mush before extraction with ethyl acetate. The pure compounds were isolated by chromatography on silica gel with different mixtures of ethyl acetate and heptane as an eluent. Mass spectra were obtained with a Jeol SX102 spectrometer, while NMR spectra were obtained with a Varian XL300 spectrometer. The molecular mechanics calculations were carried out with "MacMimic", obtained from InStar Software AB, Lund (Sweden), on an Apple Macintosh IIfx computer.

Scrobicalol **8** (26 mg) were obtained as a colourless oil. RF 0.25 (ethyl acetate:heptane 1:2) $[\alpha]_D^{22}$=+37 ° (c 0.1 in chloroform). UV (ethanol) λmax (log ε): 315 nm(4.15). IR (KBr): 3400, 2950, 1685, 1595, 1180, 1045 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$; δ in ppm and *J* in Hz): 9.31, s, C(13)H; 6.60, s, C(8)H; 3.59, dd, $J_{5a\text{-}5b}$=10.4, $J_{5a\text{-}6}$=7.4, C(5)Ha; 3.54, dd, $J_{5a\text{-}5b}$=10.4, $J_{5b\text{-}6}$=7.4, C(5)Hb; 3.30, m, C(6)H; 2.62-2.45, m, C(1)Ha, C(3)H and C(10)Ha; 2.34-2.20, m, C(1)Hb and C(10)Hb; 2.07, ddd, $J_{3\text{-}4\beta}$=4.2, $J_{4\alpha\text{-}4\beta}$=14.1, $J_{4\beta\text{-}6}$=6.1, C(4)Hβ; 1.49, ddd, $J_{3\text{-}4\alpha}$=11.5, $J_{4\alpha\text{-}4\beta}$=14.1, $J_{4\alpha\text{-}6}$=2.0, C(4)4α; 1.12 and 1.04, s, C(14)H_3 and C(15)H_3; 1.10, d, $J_{3\text{-}12}$=6.9, C(12)H_3. ^{13}C NMR (75MHz, CDCl3): 194.3 C-13; 156.6 C-2; 146.9 C-8; 142.8 C-7; 129.2 C-9; 63.5 C-5; 53.5 and 52.8 C-1 and C-10; 37.0 C-6; 36.5 C-11; 34.4 C-4; 33.4 C-3; 29.3 and 28.9 C-14 and C-15; 20.2 C-12. MS [EI 70ev, m/z (% rel int.)]: 234.1639 (M^+, 100%, calculated for $C_{15}H_{22}O_2$ 234.1620), 216(55), 201(67), 187(87), 173(53), 145(48), 105(58), 91(69).

Compound **9** was obtained by reduction of the lactone **7** with $LiAlH_4$ in ether, or scrobicalol **8** with $NaBH_4$ in ethanol. RF 0.12(ethyl acetate:heptane 1:2) $[\alpha]_D$ =+67° (c 0.1 in chloroform). UV (ethanol) λmax (log ε): 268 nm (4.11). IR (KBr): 3380, 2961, 1100, 1010 cm-1. ^1H NMR (300 MHz, CDCl3; δ in ppm and *J* in Hz): 5.77, s, C (8); 4.13 and 4.05, 2d, $J_{13a\text{-}13b}$=12.0, C(13)H_2; 3.74, dd, $J_{5a\text{-}5b}$=9.9, $J_{5a\text{-}6}$=8.7, C(5)Ha; 3.67, dd, $J_{5a\text{-}5b}$=9.9, $J_{5b\text{-}6}$=5.3, C(5)Hb; 2.68, m, C(6)H; 2.44-2.34, m, C(1)Ha, C(3)H and C(10)Ha; 2.20-2.02, m, C(1)Hb and C(10)Hb; 1.96, ddd, $J_{3\text{-}4a}$=5.1, $J_{4a\text{-}4b}$=13.7, $J_{4a\text{-}6}$=6.4, C(4)Ha; 1.63, ddd, $J_{3\text{-}4b}$=11.7, $J_{4a\text{-}4b}$=13.7, $J_{4b\text{-}6}$=1.5, C(4)4b; 1.07 and 1.02, s, C(14)H_3 and C(15)H_3; 1.03, d, $J_{3\text{-}12}$=7, C(12)H_3. ^{13}C NMR (75MHz, CDCl3): 144.3, 143.0 and 128.8 C-2, C-7 and C-9; 124.7, C-8; 69.1 and 63.7 C-5 and C-13; 53.5 and 52.6 C-1 and C-10; 42.8 C-6; 37.0 C-4; 36.2 C-11; 33.1 C-3; 29.5 and 29.2 C-14 and C-15; 20.1 C-12. MS [EI, 70 ev m/z (% rel. int.)]: 236 (M^+, 3%), 218.1677 (M^+-H_2O, 69%, calculated for $C_{15}H_{22}O$ 218.1670), 203 (21), 187 (100), 173 (26), 145 (31), 105 (37), 91 (39).

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