The Structure of a Novel Fungal Sesquiterpene, Elucidated by Spectral and Computational Methods

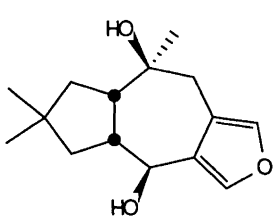
Olov Sterner, Ola Wik and Robert E. Carter

Organic Chemistry 2, Lund Institute of Technology, P.O. Box 124, S-221 00 Lund, Sweden

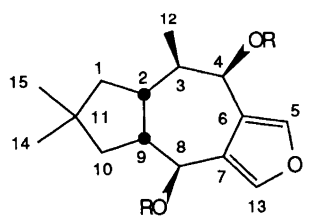
Sterner, O., Wik, O. and Carter, R. E., 1988. The Structure of a Novel Fungal Sesquiterpene, Elucidated by Spectral and Computational Methods. – Acta Chem. Scand., Ser. B 42: 43–47.

A novel dihydroxyfuran sesquiterpene has been isolated in small amounts from ethyl acetate extracts of fruit bodies of several *Lactarius* species (mushrooms). The chemical structure could be established by examination of its spectral data, while the determination of the relative configuration is based on the comparison of experimental ^{1}H NMR data with theoretical data (obtained by computer calculations) for all possible stereoisomers. The compound is a minor product of the enzymatic conversions of sesquiterpenes that take place in the fruit bodies as a response to injury.

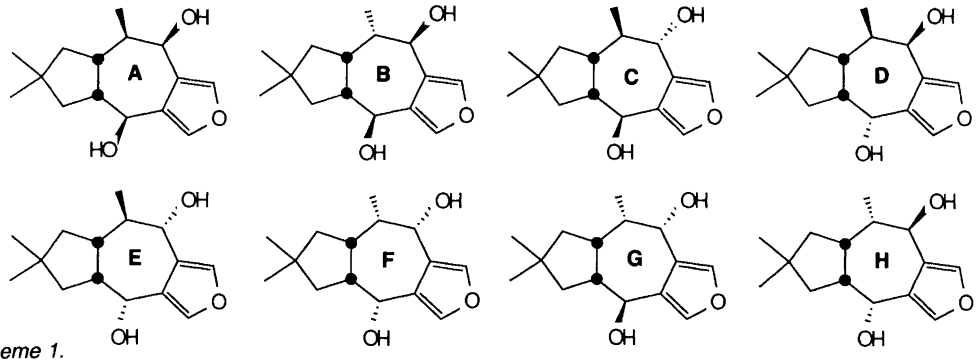
Recent investigations have suggested that the fruit bodies of many *Lactarius* and *Russula* species (family Russulaceae of the Basidiomycotina subdivision of Fungi), are armed with a chemical defense system that protects them from parasites and microorganisms.[1,2] As a response to injury to the fruit bodies, fatty acid esters of the sesquiterpene velutinal are enzymatically converted to a number of unsaturated dialdehyde sesquiterpenes with antifeedant and antimicrobial activities. In addition to such proposed defense compounds, small amounts (1–2 mg per kg fresh mushrooms) of the two dihydroxyfuran sesquiterpenes (**1**[3] and **2a**) could also be isolated from ethyl acetate extracts of injured fruit bodies of *Lactarius piperatus* (Scop. ex Fr.),[2] *L. torminosus* (Schff. ex Fr.)[2] and *L. necator* (Pers. ex Fr.). Their furanoid nature initially made us suspicious of their origin, as the velutinal esters originally present in the fruit bodies previously have been found to be chemically degraded to a number of furans under conditions involving traces of acid.[4] 3,8-Dihydroxyfuran (**1**) is actually a major product of such degradation, and has consequently been regarded as an extraction artifact. However, except for **1**, none of the numerous other degradation products were found, which suggests that **1** can also be formed by another route. 4,8-Dihydroxyfuran (**2a**), a new compound whose structure is dicussed below, has never been observed as a degradation product. It is therefore probable that the compounds **1** and **2a** are formed as by-products during the enzymatic conversions of sesquiterpenes that take place in injured fruit bodies of *L. piperatus, L. torminosus* and *L. necator*.



1



2a R=H, **2b** R=$COCH_3$



Scheme 1.

Results and discussion

Spectral data for compound **2a** suggested that its molecular composition is $C_{15}H_{22}O_3$. All signals in the ^1H NMR spectrum recorded in $CDCl_3$ are well resolved, and only one structure is in accordance with the results obtained from double resonance experiments (including long-range couplings between H-4 and H-5, and between H-8 and H-13). In order to determine the stereostructure of furan **2a**, extensive NOE experiments were performed. In $CDCl_3$, these only revealed that H-2 and H-9 are *cis,* but in CD_3OD the proximity of H-3 to H-8 was also indicated (8% enhancement of H-3 when H-8 was irradiated and 12% enhancement of H-8 when H-3 was irradiated). Together with the observed small coupling constant between H-3 and H-4 (1 Hz), this provided evidence for the relative stereochemistry shown in formula **2a**. It is possible that compound **2a** exists in different conformations in $CDCl_3$ and CD_3OD, which would explain why different results were obtained when NOE experiments were performed in the two solvents.

In order to investigate this possibility, and to determine unambiguously the relative stereochemistry of compound **2a**, a computational conformational analysis of the eight possible isomers with H-2 and H-9 *cis* (shown in Scheme 1) was carried out using the MM2(85) program.[5,*] The geometries and relative steric energies obtained from the molecular mechanics (MM) calculations were then used to estimate the average vicinal coupling constants, using Osawa's 3JHH program.[6,§] The theoretical coupling constants were compared with the experimental (obtained in $CDCl_3$); and the root-mean-square (RMS) deviation was calculated for all isomers (see Table 1).

It is clear that the best agreement is obtained for stereoisomer **A**. When the conformational analysis of isomer **A** was carried out including all possible OH rotamers, the RMS deviation improved slightly, vis. to 1.4 Hz. Generally, as could be shown with similar sesquiterpenes, essentially no geometrical information was lost if the OH groups were oriented manually in the most favourable position for hydrogen bonding, and to save computer time no full OH rotamer analysis was made for isomers **B–H**. The complete OH rotamer conformational analysis of **A** indicated a mixture of three conformers (shown in Fig. 1). Conformers I (50%) and II (10%) differ mainly in a twist of the cyclopentane ring.

*MM2(85) is available from the Quantum Chemistry Program Exchange, University of Indiana, Bloomington, Indiana 47405, and from Molecular Design Ltd., 2132 Farallan Drive, San Leandro, California 94577, USA. The parameter set available in MM2(85) lacked torsional parameters for the structural fragment $C(sp^3)$–C(alkene)–C(alkene)–O(furan). As an approximation the fragment was assigned a two-fold torsional constant, *V*2, of 16 kcal mol^{-1}. The different conformations of stereoisomers **A–H** have almost identical geometries in this fragment, due to the flatness of the furan ring. Consequently, the errors introduced by this approximation have the same magnitude and the error in weighting between different conformations becomes negligible.

§An updated and corrected version of the program was kindly provided by Prof. E. Osawa, Hokkaido University, Japan.

Table 1. Comparison of calculated and experimental ^{1}H NMR coupling constants (Hz) for the 8 possible stereoisomers of compound **2a** at C-3, C-4 and C-8 (H-2 and H-9 *cis*). MM calculations were made without full OH rotamer analysis (see text).

Stereoisomer	RMS deviation	Maximal individual deviations
A	1.6	2.9
B	5.0	6.6
C	4.4	9.1
D	3.9	6.8
E	4.8	7.7
F	4.9	7.8
G	4.1	7.0
H	5.4	9.5

Conformer III (40 %) has an entirely different folding of the 7-membered ring, which facilitates the formation of an intramolecular hydrogen bond across that ring. The computational method performed somewhat less satisfactorily in the case of sesquiterpenes containing furan structures; in this connection it should be remembered that MM calculations refer to a molecule in the gas phase. Consequently, it is reasonable to expect that the calculated conformational mixture will differ slightly from that actually present in $CDCl_3$ solution. With a conformational mixture of 40 % I and 60 % III, optimum coincidence between experimental and calculated coupling constants is achieved (RMS deviation 0.6 Hz) (Table 2). This corresponds roughly to a lowering

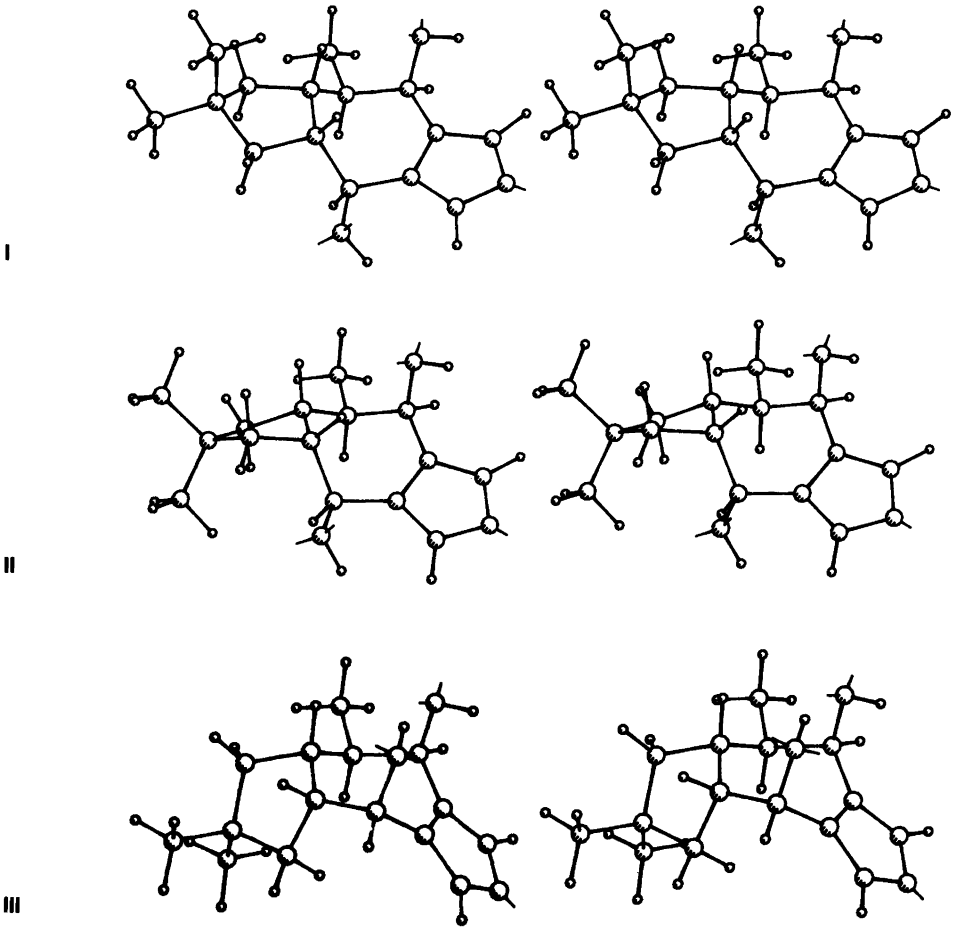


Fig. 1. Stereoscopic drawings of conformations I, II and III of compound **2a**.

of the relative steric energy of conformer III by 0.5 kcal mol^{-1}.

The possibility of the occurrence of an intramolecular hydrogen bond in $CDCl_3$, but not in CD_3OD, thus explains the different results obtained in the ^1H NMR NOE experiments. In order to eliminate this hydrogen bond between the C-4 and C-8 hydroxy groups, the acetylated derivative **2b** was prepared. Unfortunately, because of signal overlap in the ^1H NMR spectrum recorded in $CDCl_3$, it is difficult to interpret the observed NOE's. However, the coupling constants between H-8 and H-9 (6.8 Hz in $CDCl_3$ and 9.5 Hz in CD_3OD for compound **2a**, and 11.5 Hz for compound **2b** in $CDCl_3$) show that acetylation of **2a** significantly influences its conformations. In addition, an unexpectedly large difference between the optical rotations of compounds **2a** and **2b** is observed, which also suggests that there are major conformational differences between the compounds.

Experimental

The fruit bodies were collected near Lund in the autumns of 1984 and 1985, and ethyl acetate extracts were prepared and worked up as described previously.[1,2] ^1H NMR spectra were recorded on a Varian XL-300 spectrometer (300 MHz). The coupling constants (J) are given in Hz. ^{13}C NMR spectra, proton noise-decoupled and coupled, were recorded on the same spectrometer (76 MHz). Deuteriochloroform ($CDCl_3$) was used as solvent unless stated otherwise, and chemical shifts are reported in ppm with tetramethylsilane as internal standard. Melting points were obtained with a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 257 spectrophotometer, UV spectra were recorded on a Cary 219 spectrophotometer, while mass spectra were recorded on a Varian MAT 112.

4,4a,5,6,7,7a,8,9-Octahydro-4,9-dihydroxy-6,6,8-trimethylazuleno[5,6-c]furan (**2a**) was obtained as a colourless oil (7 mg) by SiO_2 and Al_2O_3 chromatography of EtOAc extracts of *L. piperatus, L. torminosus* and *L. necator*. Insufficient amounts were obtained for elemental analysis. $[\alpha]_D^{24}$ +4° (*c* 0.7 in chloroform); UV (ethanol): no maximum above 220 nm; IR (KBr): 3350, 2960, 1460, 1050 and 890 cm^{-1}. ^1H NMR: 7.34, m, C(5)H; 7.31, m, C(13)H; 4.66, d, C(8)H, J_{8-9} = 6.8; 4.56, d, C(4)H, J_{3-4} = 1; 2.51, dddd, C(9)H, J_{2-9} = 6.5, J_{8-9} = 6.8, J_{9-10a} = 6.6, J_{9-10b} = 12; 2.36, m, C(2)H; 1.76, ddd, C(3)H, J_{2-3} = 11, J_{3-4} = 1, J_{3-12} = 6.5; 1.68, dd, C(1)Ha, J_{1a-1b} = 13.1, J_{1a-2} = 7.1; 1.51, dd, C(1)Hb, J_{1a-1b} = 13.1, J_{1b-2} = 4.7; 1.48, dd, C(10)Ha, J_{9-10a} = 6.6, $J_{10a-10b}$ = 12.4; 1.23, dd, C(10)Hb, J_{9-10b} = 12, $J_{10a-10b}$ = 12.4; 1.14, d, $C(12)H_3$, J_{3-12} = 6.5; 1.04 and 1.02, s, $C(14)H_3$ and $C(15)H_3$. ^{13}C NMR: 140.3 and 140.2, C(5) and C(13); 127.3 and 126.3 C(6) and C(7); 69.7 and 67.3, C(4) and C(8); 48.5, 41.3 and 39.1, C(2), C(3) and C(9); 46.4 and 43.2, C(1) and C(10); 36.0, C(11); 32.1, 31.6 and 19.4, C(12), C(14) and C(15). MS [*m/z* (% rel. int.)]: 250 (M^+, 13), 232 (68), 214 (40), 199 (34), 136 (100) and 123 (58).

The diacetylated derivative **2b** was prepared by acetylation of compound **2a** with acetic anhydride in pyridine. It was obtained as a white solid, m.p. 70–75 °C, after silica gel chromatography. $[\alpha]_D^{24}$ +56° (*c* 0.6 in chloroform). ^1H NMR: 7.48, dd, C(5)H, J_{4-5} = 0.5, J_{5-13} = 1.6; 7.03, dd, C(13)H, J_{5-13} = 1.6, J_{8-13} = 1.5; 5.82, dd, C(4)H, J_{3-4} = J_{4-5} = 1; 5.82, dd, C(8)H, J_{8-9} = 11.5, J_{8-13} = 1.5; 2.58, m, C(9)H; 2.46, m, C(2)H; 2.17 and 2.06, s, Ac; 2.01, ddd, C(3)H, J_{2-3} = 10.4, J_{3-4} = 1.2, J_{3-12} = 6.8; 1.67, m, C(1)Ha and C(10)Ha; 1.40, dd, C(1)Hb, J_{1a-1b} = J_{1b-2} = 12; 1.36, dd, C(10)Hb, J_{9-10b} = 6.4, $J_{10a-10b}$ = 13.7; 1.04, d, $C(12)H_3$, J_{3-12}

Table 2. Experimental and calculated ^1H NMR coupling constants (Hz) for isomer **A** of compound **2a**.

Bond fragment	Expt.	Calc.[a]	Calc.[b]	Calc.[c]
1a–2	4.7	7.6	7.2	5.0
1b–2	7.1	6.9	7.0	7.2
2–3	11	11.8	11.9	12.1
2–9	6.5	7.7	7.6	6.8
3–4	1	1.2	1.2	1.2
8–9	6.8	8.3	8.0	6.5
9–10a	12	9.2	9.6	11.0
9–10b	6.6	6.8	6.7	6.0
RMS deviation:		1.6	1.4	0.6

[a]Without full OH rotamer analysis. [b]With full OH rotamer analysis. [c]Optimal conformational mixture (40 % of conformation I and 60 % of conformation III; see Fig. 1).

= 6.8; 1.07 and 1.02, s, C(14)H_3 and C(15)H_3. MS [*m/z* (% rel. int.)]: 334 (M^+, 2), 274 (15), 232 (78), 214 (93), 110 (52) and 43 (100).

Acknowledgements. Financial support from the National Swedish Board for Technical Development (STU project No. 86-3033), and the Swedish Natural Science Research Council (NFR grant No. K-KU 2070–104) is hereby gratefully acknowledged. The authors thank Dr. Tommy Liljefors, Lund, for valuable discussions.

References

1. Sterner, O., Bergman, R., Kihlberg, J. and Wickberg, B. *J. Nat. Prod. 48* (1985) 279.
2. Sterner, O., Bergman, R., Franzen, C. and Wickberg, B. *Tetrahedron Lett. 26* (1985) 3163 and references therein.
3. Nozoe, S., Matsumoto, H. and Urano, S. *Tetrahedron Lett.* (1971) 3125.
4. Sterner, O., Bergman, R., Kihlberg, J., Oluwadiya, J., Wickberg, B., Vidari, G., De Bernardi, M., De Marchi, F., Fronza, G. and Vita Finzi, P. *J. Org. Chem. 50* (1985) 950.
5. Burkert, U. and Allinger, N. L. *Molecular Mechanics,* American Chemical Society, Washington DC, USA (1982).
6. Osawa, E. and Jamie, C. *QCPE Bull. 3* (1983) 66; QCPE program No. 461.

Received September 22, 1987.