FUNGAL EXTRACTIVES-IV*

STRUCTURE OF A NOVEL SESQUITERPENE DIALDEHYDE FROM LACTARIUS BY SPECTROSCOPIC METHODS

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Abstract – The complete structure of a sesquiterpene dialdehyde ("velleral"), isolated from *Lactarius* vellereus and *L. pergamenus*, has been elucidated by means of IR, UV, ¹³C-NMR spectroscopy, NOE measurements and computer simulation of the ¹H-NMR spectrum.

In recent publications we reported the structures for a pungent-tasting sesquiterpene dialdehyde, "isovelleral" (2),¹ and two sesquiterpene lactones.² In addition, a related crystalline, pungent-tasting dialdehyde, "velleral", was isolated¹ from *Lactarius vellereus* and *L. pergamenus* (Russulaceae), other workers³ having isolated it from *L. vellereus*. We have now determined that velleral has the structure and relative configuration 1 (see Table 1) and the preferred conformation 1a (see Fig 1).

Elemental analysis supplemented by ¹³C-NMR (15 C; 20 H) and mass spectrometry (M⁺ 232) gave the molecular formula for velleral $(C_{15}H_{20}O_2)$. The presence of two conjugated aldehydic groups was indicated by CO stretching frequencies in the IR spectrum, (CCl₄, ν 1705 and 1693 cm⁻¹), and was supported by two aldehydic ¹H-NMR doublets at 9.49 (J = 0.55 Hz) and 9.47 (J = 0.55Hz) ppm and two broad olefinic doublets at 7.02 (J = 5.7 Hz) and 7.07 (J = 2.7 Hz) ppm. Double irradiation experiments showed that each aldehydic proton appeared to be coupled to one only of the olefinic protons. The small long-range coupling constant (0.55 Hz) clearly excludes systems in which vinylic protons are alpha to the aldehydic groups.

The UV spectrum (EtOH)(λ_{infl} 245 nm, ϵ 11,600 and λ_{max} 220 nm, ϵ 23,000) requires a more extended conjugation than is furnished by two isolated α,β -unsaturated aldehyde groups. In the ¹³C-NMR spectrum of velleral the sp²-carbons have similar chemical shifts in pairs reflecting a

highly symmetrical system.⁴ These facts (UV, NMR) and the fact that there is no apparent coupling between the olefinic protons leaves only one reasonable possibility for connecting the α , β -unsaturated aldehyde groups (1).

The fragmentation pattern in the mass spectrum of velleral shows a close resemblance to that of isovelleral (2) from the same species. Furthermore the ¹³C-NMR spectra of these two dialdehydes have obvious similarities for the saturated portions of the molecules (Table 1). These observations are consistent with structure 1 for velleral.

In order to confirm structure 1 and to determine the stereostructure, a complete analysis of the seven proton spin system in the region 1.20-3.15 ppm was carried out. On irradiation of the vinylic protons the complex pattern in the allylic spectral region was simplified (Fig 1, I and II; H₆ and H₇ region). The two allylic protons were differentiated by irradiating the doublet of the Me group on the 7-membered ring (CH_3 -13) to obtain the values $J_{6-8} = 2.7 \text{ Hz}$ and $J_{7-9} = 5.7 \text{ Hz}$ (cf above). The coupling constant $J_{6-8} = 2.7$ Hz requires a dihedral angle between H₆ and H₈ close to 90°. Inspection of Dreiding models of 1 in different conformations of cis- and trans-fused ring isomers revealed that this requirement is realized only for the cis-fused ring isomers in the conformation 1a. The coupling constants for the seven proton spin system (H1- H_7 , 1a) were estimated from bond angles measured in Dreiding models of velleral and also from known NMR data for the aforementioned closely related substance isovelleral (2).¹ These data together with approximate chemical shift values estimated

^{*}Part III, see Ref. 2.

Table 1. ¹³C-NMR data for velleral (1) and isovelleral (2) (25.2 MHz, CDCl₃)



Chemical shift (ppm from TMS)	Signal multiplicity ^a	Group	<i>J</i> _{С-Н} (Hz)	Assignment (carbon nr.)
190.0	s	СНО	187)	.
190-0	S	—СНО	187	14, 15
162.0	d	C==CH	155	6.0
161.0	d	C=CH-	155)	0,9
137-1	S	c=c-	}	7,8
136-7	s	c=ć-	J	
59·2 48·3 47·3	d t t	↓ −CH− −CH₂− −CH₂−	133 128} 126]	4 or 5 or 10 1, 3
40.7		١	(a a)	
40-7 37-7	d d	—Сн— —Сн—	130	4, 5 or 4, 10 or 5, 10
37.7	s	$-\dot{\mathbf{c}}$,	2
29-4	q		125)	
27.2	q	-CH ₃	128)	11, 12
18.3	q	CH ₃	128	13
	c	$HC^{-8}_{15} + H^{-13}_{15} + H^{1$	$\frac{3}{12}$	
197.3	d	CHO	180	14
191.9	ď	СНО	177	15
153-3	đ	C = CH -	158	9
140.0	S	C = C - C		8
46.9	t	CH₂	128	1,3
43.3	τ		124J	
41.7	ď	CH	127	4, 10
39.4	đ	—ċн—	123	1, 20
37.4	S	¢		

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Chemical shift (ppm from TMS)	Signal multiplicity ^a	Group	J _{с-н} (Hz)	Assignment (carbon nr.)
34•4	S	-¢-	ļ	2, 5, 7
34.1	S			
31.5 31.0 26.8 18.6	q q t	CH ₃ CH ₃ CH ₂ CH ₂	125) 125) 162 127	11, 12 6 13

Table 1. (Continued)

 $a_s = singlet$, d = doublet, t = triplet, q = quartet; obtained by "off-resonance" decoupling.



Fig 1. Partial ¹H-NMR spectrum (100 MHz) of velleral. I: normal spectrum. II: irradiation at H₈ and H₉. III: simulated spectrum.

*The coupling between H_7 and the methyl group protons could not be acommodated by the computer program (max. seven spins).

from the experimental spectrum were used as initial parameters in a theoretical simulation of the seven proton spectral region using the LAOCN 3 computer program.⁵ Successive iterations resulted in fairly good agreement between the experimental (vinylic protons decoupled) and theoretical spectra (Fig 1, II and III). The calculated coupling constants agreed well with the expected values. Similar simulation of 60 MHz spectrum also showed good agreement with the experimentally obtained spectrum (same coupling constants; chemical shifts reduced with the factor 0.6), thereby providing further assurance for the coupling interpretation.

The endo position of the Me-13 group was deduced from the coupling constant $J_{5-7} = 6.5$ Hz. The hypothetical exo position of the Me group would require a larger coupling constant (H₅ and H_7 antiperiplanar). The configuration at C-5 was confirmed by measuring NOE; complete saturation of the Me-13 protons resulted in increased integrated intensity for the vinylic protons (H₈: 13% NOE; H₉: 10% NOE) only consistent with the endo epimer. Irradiation of the vinylic protons gave 40% NOE for both aldehydic protons. This unusually large NOE effect is consistent with a state of rapid rotation between the two possible and approximately equally populated, intramolecularly H-bonded (6-membered, quasi planar ring) aldehyde group rotamers, where the aldehydic protons are sterically remote from other protons in the molecule. In order to verify these fine structural features, theoretical calculations of the NOE's were made under conditions for saturated Me-13.6 All protonproton distances for different aldehyde group and Me-13 rotamers were estimated from Dreiding models. In all cases the theoretical NOE for H₈ was too large and for H₉ too small compared with the experimental values. This discrepancy between theoretical and experimental values might be due to inversion of the heptadiene ring resulting in a conformation (present to a minor degree) in which the Me-13 group is bent away from the H_8 proton.

In conclusion it is significant that the relative configuration at C-5 in velleral is the same as that in isovelleral (2). The *cis*-ring junction is found not only in isovelleral but also in several characterised sesquiterpenoids of basidiomycetes origin.^{7.8}

EXPERIMENTAL

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

Isolation of velleral (1). Fresh 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, evaporated, and the residue crystallised from hexane to remove stearic acid. The mother liquor was concentrated and chromatographed (silica gel, methylene chloride) to obtain a crude fraction which was rechromatographed (silica gel, methylene chloride) yielding almost pure velleral.

Velleral (1), m.p. $86 \cdot 5 - 87 \cdot 5^{\circ}$ (from hexane); $[\alpha]_{19}^{19} - 25^{\circ} (c 2 \cdot 1 \text{ in CHCl}_3)$; UV (EtOH): $\lambda_{\text{infl}} 245 \text{ nm} (\epsilon 11,600)$, $\lambda_{\text{max}} 220 \text{ nm} (\epsilon 23,000)$; IR (CCl₄): ν 2720, 1705, 1693 (CHO), 1605 (C=C), 1360, 1355 (gem Me), 1207, 856, 714 cm⁻¹; NMR (CDCl₃, TMS): δ 9.49 (1H, d, J = 0.55 Hz; OHC—C=CH—), 9.47 (1H, d, J = 0.55 Hz; OHC—C=CH—), 7.07 (1H, d broad, J = 2.7 Hz; OHC—C=CH—, 7.07 (1H, d broad, J = 5.7 Hz; OHC—C=CH—CH—), 7.02 (1H, d broad, J = 5.7 Hz; OHC—C=CH—CHCH₃—), 1.20–3.15 (7H, m; for interpretation see Fig 1), 1.17 (3H, d, J = 6.6 Hz; —CH—CH₃), 1.13 (3H, s; —C—CH₃), 0.98 (3H, s; —C—CH₃) ppm; ¹³C-NMR data see Table 1; MS (70 eV) m/e: 232 (M⁺, 11%) (C₁₅H₂₀O₂), 119 (28%), 105 (54%), 91 (83%), 79 (51%), 77 (67%), 41 (100%; base peak). (Found: C, 77.7; H, 8.7. C₁₅H₂₀O₂ requires: C, 77.5; H, 8.7%).

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