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The Structure Determination of Panellon and Panellol, two 14-Noreudesmanes Isolated from Resupinatus Leightonii

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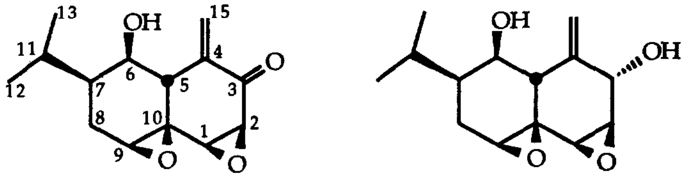
Abstract: The structures of two fungal norsesquiterpenes containing a diepoxide functionality, isolated from cultures of the basidiomycete *Resupinatus leightonii*, were elucidated by a combination of X-ray analysis, NMR spectroscopy and molecular mechanics calculations.

The noreudesmane panellon (**1**) was recently isolated as the active principle from the culture filtrate of the basidiomycete *Resupinatus leightonii.*[1] It exhibits strong antimicrobial, cytotoxic and phytotoxic activities, while the reduced derivative panellol (**2**) isolated from the same fungus was practically devoid of biological activities.[1]



1 **2**

Figure 1.

The structure of panellol (**2**) was determined by X-ray analysis (see Figure 2, Table 1 and the experimental section). An assignment of the absolute configuration was not possible, and Figure 2 displays orbitrarily the R-C(6) configuration. Comparison of the NMR data of the two compounds suggested that they are very similar, although at different oxidation levels. 1D and 2D experiments confirmed that panellon (**1**) is a 14-noreudesmane oxidised at the same carbons as panellol (**2**), but with a ketone function replacing the secondary

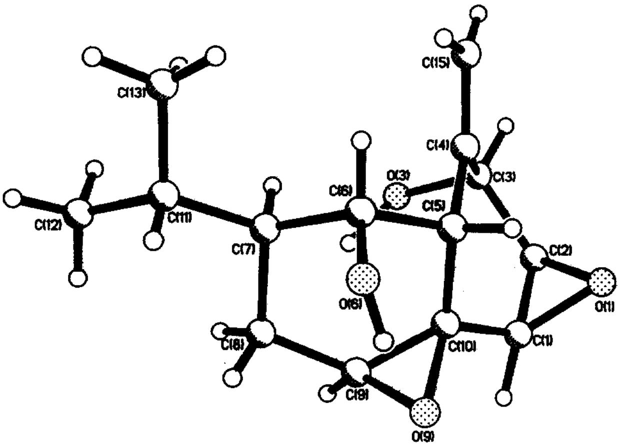


Figure 2. Molecular structure of panellol (**2**).

Table 1. Atomic Coordinates and Equivalent Isotropic Displacement Coefficients ($Å^2x10^3$).

Atom	x	y	z	U(eq)
C(1)	-0.1406 (12)	0.2341 (12)	0.0585 (2)	67 (3)
O(1)	-0.0622 (8)	0.2672 (9)	0.0179 (1)	90 (2)
C(2)	-0.1756 (13)	0.4273 (14)	0.0369 (2)	69 (3)
C(3)	-0.0654 (13)	0.6181 (12)	0.0530 (2)	63 (3)
O(3)	-0.1759 (8)	0.6836 (8)	0.0893 (1)	70 (2)
C(4)	0.1573 (12)	0.5688 (12)	0.0642 (2)	55 (3)
C(5)	0.2032 (10)	0.3590 (11)	0.0839 (2)	51 (2)
C(6)	0.3401 (11)	0.3762 (11)	0.1227 (2)	53 (2)
O(6)	0.4331 (7)	0.1793 (8)	0.1327 (2)	73(2)
C(7)	0.2228 (12)	0.4642 (12)	0.1600 (2)	58 (2)
C(8)	0.0478 (12)	0.3167 (14)	0.1725 (2)	75 (3)
C(9)	-0.0641 (12)	0.2169 (13)	0.1359 (2)	69 (3)
O(9)	0.0396 (8)	0.0412 (8)	0.1156 (2)	74 (2)
C(10)	0.0083 (11)	0.2383 (10)	0.0937 (2)	57 (2)
C(11)	0.3580 (14)	0.5216 (14)	0.1976 (2)	79 (3)
C(12)	0.2360 (19)	0.6073 (17)	0.2340 (2)	117 (5)
C(13)	0.5310 (15)	0.6655 (17)	0.1870 (3)	106 (4)
C(15)	0.2971 (13)	0.7128 (13)	0.0555 (2)	70 (3)

alcohol at C-3. Panellon (**1**) has previously been isolated as an antibiotic constituent of the fungus *Panellus violaceofulvus*,[2a,b] although its stereostructure was not determined. All the spectral data reported for panellon (**1**)[2b] are identical with those obtained in this investigation, and the NMR data are included in Table 2 only in order to facilitate the comparison of the two compounds.

An attempt to correlate the two compounds chemically by careful reduction of panellon (**1**) with KBH_4 in ethanol failed. Instead, the stereostructure of panellon was determined by NMR techniques, especially NOE measurements (1D and NOESY 2D experiments), and by comparing actual and calculated ^{1}H NMR coupling constants of the two compounds. The large coupling constant between 6-H and 6-OH (11.4 Hz in both compounds) and the IR absorption at 3500 cm^{-1} indicate that the C-6 hydroxyl group is axial and hydrogen bonded to the 9,10 epoxy oxygen. The NOE observed between 1-H and 9-H in panellon (**1**) (8% enhancement of 1-H when 9-H was irradiated) shows that also the 1,2 epoxy group is situated on the β-side, as indeed is the case for panellol (**2**). The stereochemistry of C-6 and C-7 in panellon (**1**) was more difficult to assign from the NMR data, although the NOE:s observed between 6-H and 15-Hb (16% enhancement of 6-H when 15-Hb was irradiated), between 6-H and 7-H (7% enhancement of 7-H when 6-H was irradiated), between 6-OH and 5-H (9% enhancement of 5-H when 6-OH was irradiated), and between the two methyl groups and 6-H as well as 8-H_2 suggest that the relative stereochemistry of panellon (**1**) is as depicted in Figure 1. In order to confirm this, an investigation of the geometries of the two compounds in their most stable conformation was made by molecular mechanics calculations,[3] and the theoretical ^{1}H NMR coupling constants of these structures, obtained using Osawa's 3JHHM program,[4] were compared with the experimental data (see Table 4).

Table 2. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR Data for Panellon (**1**) and Panellol (**2**) ($CDCl_3$, Shifts Given in ppm Relative TMS, Coupling Constants J in Hz).

	1 δ ^{1}H; mult.; *J*	**2** δ ^{1}H; mult.; *J*	**1** δ ^{13}C; mult.	**2** δ ^{13}C; mult.
1	3.21; d; 4.4	2.88; d; 4.3	58.1; d	57.2^1; d
2	3.62; d; 4.4	3.43; dd; 2.7, 4.3	55.9; d	55.4^1; d
3	-	4.63; dd; 1.1, 2.7	193.2; s	70.8^2; d
4	-	-	139.1; s	144.3; s
5	3.58; ddd; 2, 2.9, 3.3	3.13; ddd; 2.2, 2.4, 2.4	38.1; d	40.1^3; d
6	4.13; ddd; 2, 2, 11.4	3.94; dd; 2, 2.4, 11.4	68.3; d	70.4^2; d
7	1.00; dddd; 2, 7, 9, 12	1.21; dddd; 1.5, 7.1, 8.9, 11.8	39.3; d	39.6^3; d
8a	2.15; ddd; 5.4, 7.2, 16.1	2.19; ddd; 5.7, 7.1, 16.0	23.4; t	23.7; t
8b	1.88; dd; 12.0, 16.1	1.82; dd; 11.8, 16.0	-	-
9	3.45; d; 5.4	3.40; d; 5.7	56.5; d	54.8^1; d
10	-	-	60.3; s	61.3; s
11	1.66, dh; 6.6, 9.1	1.64; dh; 6.6, 9	29.6; d	29.6; d
12	0.92; d; 6.6	0.92; d; 6.6	20.4; q	20.4^4; q
13	0.88; d; 6.6	0.89; d; 6.6	20.3; q	20.6^4; q
15a	6.46; d; 3.3	5.38; dd; 1.1, 2.4	125.7; t	116.0; t
15b	5.47; d; 2.9	5.11; d; 2.2	-	-
6-OH	3.10; d; 11.4	2.87; d; 11.4	-	-

$^{1, 2, 3, 4}$ Interchangable

Conformational analyses of the two compounds shows that the geometries of the most stable conformers are very similar to the X-ray structure of panellol (**2**) (pertinent dihedral angles are given in Table 3). Superimposition of the X-ray and MM2 structures of panellol (**2**) using all ring atoms gave an RMS (ratio of mean squares) of fit of 0.078 Å, i.e. the calculated structure of panellol (**2**) is almost identical to that obtained by X-ray analysis (which in turn indicates that the parameters used for the molecular mechanics calculations are valid). It is also evident that the C6-C7-C8-C9 part of the two compounds is very similar.

Table 3. Dihedral Angles (degrees) in X-ray and MM2 Minimised Structures of Panellon (**1**) and Panellol (**2**).

Compound:	**1** MM2	**2** MM2	**2** X-ray
C1-C2-C3-C4	12.6	34.5	43.1
C2-C3-C4-C5	2.4	-38.7	-38.0
C3-C4-C5-C6	-158.9	-127.4	-132.2
C4-C5-C6-C7	69.7	71.2	73.7
C5-C6-C7-C8	63.6	62.2	62.9
C6-C7-C8-C9	-39.4	-38.2	-39.4
C7-C8-C9-C10	9.0	8.3	8.2
C8-C9-C10-C1	-154.3	-149.9	-151.1
C9-C10-C1-C2	117.4	100.0	109.7
C10-C1-C2-C3	4.2	8.1	-2.5

A comparison between experimental and calculated vicinal ^1H-^1H coupling constants is made in Table 4, and the good agreement together with the observed NOE:s (*vide supra*) determines the structure of panellon (**1**).

Table 4. Experimental and Calculated Vicinal ^1H-^1H Coupling Constants $^3J_{HH}$ in Hz for Panellon (**1**) and Panellol (**2**).

Compound:	**1**		**2**	
	Exp.	Calc.	Exp.	Calc.
5-6	2	2.8	2.4	2.5
6-7	2	0.8	1.5	1.2
7-8a	7.2	5.9	7.1	6.1
7-8b	12.0	11.4	11.8	11.2
8a-9	5.4	4.9	5.7	4.9
8b-9	<2	1.5	<2	1.5

When the concentrations of panellon and panellol in the culture broth were monitored by HPLC analysis during 15 days of fermentation (in potato-dextrose broth),[1] panellon (**1**) was detected between days 8 and 13 at concentrations below 5 mg/l broth. This is also the period when the biological activity (measured as the activity of the broth against *Nematospora coryli*) is highest[1]. The amounts of panellol (**2**) are increasing continuously from day 8, and its concentration is 25 mg/l broth at the end of the fermentation. This suggests that panellol (**2**) is formed from panellon (**1**), possibly by a detoxification process that protects the mycelium itself. The loss of biological activity upon reduction of the keto function indicates that the biological activity of panellon (**1**) is linked to its electrophilicity, possessing reactive α,β-unsaturated keto functionality and an activated epoxide. The reaction between panellon (**1**) and cysteine has been shown to be rapid, while panellol (**2**) does not seem to react at all.[1]

EXPERIMENTAL

The compounds were isolated by chromatography on silica gel with different mixtures of cyclohexane and ethyl acetate as eluent from an extract of the culture filtrate of a potato dextrose medium culture of the fungus, as described previously[1]. UV spectra were obtained with a Perkin Elmer λ 16, IR spectra with a Bruker IFS 48, mass spectra (direct inlet, CI (NH_3) ionisation) with a Jeol SX102, and NMR spectra were obtained with a Varian XL300 spectrometer.

Panellol[8] (**2**) was obtained as white crystals, mp. 154-156 °C (uncorrected). $[\alpha]_D$ +44° (c 0.5 in $CHCl_3$). UV (methanol) λ_{max} (ε): 270 nm (2845). IR (KBr): 3500, 3390, 2960, 2900, 1430, 1000, and 890 cm^{-1}. For NMR data, see Table 2. MS, m/z: 270 ($M+NH_4^+$, 100%), 253 (35%), 252 (41%), 235 (79%).

Computational methods:

Conformational energies and energy-minimized structures were calculated with the MM2(91) molecular mechanics program developed by Allinger and co-workers.[3,5] In addition to the standard force field parameters of MM2(91), the parameters for epoxides given in Table 5 were used. The added parameters were estimated from analogous MM2(91) parameters for three membered rings, and tested by comparing the energy minimized structures of anti- as well as syn-2,3,4,5-diepoxy-12-oxa(4.4.3)propellane[6], and 3α,4α,5β,6β-diepoxyandrostan-17-one[7] to their experimentally determined crystal geometries. Least squares fits resulted in RMS values in the range of 0.029 to 0.037Å. The energy minimized geometry of panellol (**2**) was not sensitive to the magnitude of the V1, V2, and V3 torsional constants, and changes of the torsional constants with ± 1 kcal/mol yielded virtually identical geometries.

Table 5. Additional MM2 Force Field Parameters.

Torsional constants:

Angle	V1	V2	V3	Angle	V1	V2	V3	kcal/mol
6-1-1-22	0.10	0.10	0.18	3-22-49-22	0.00	0.00	1.21	
22-1-2-3	0.00	0.00	0.46	2-3-22-49	0.00	0.00	0.00	
1-2-3-22	2.60	15.00	0.00	7-3-22-49	0.00	0.00	0.00	
2-2-3-22	-2.30	11.10	0.00	2-1-22-49	0.00	0.00	0.53	
2-3-22-5	-0.30	1.60	-0.50	6-1-22-22	0.00	0.00	0.00	
2-3-22-22	-0.44	0.24	0.06	22-1-6-21	0.80	0.00	0.09	
22-22-22-49	0.00	0.00	0.00	22-1-6-20	0.00	0.00	0.00	
22-22-49-22	0.00	0.00	1.21	6-1-22-49	0.00	0.00	0.00	
49-22-22-49	-0.52	0.00	0.16	6-1-22-5	0.00	0.00	0.18	
3-22-22-49	0.00	0.00	0.53					

Table 5 (cont.)

Bending parameters:

Angle	k_b, mdyn Å^{-1} rad^{-2}	θ_0, deg
3-22-22	0.60	118.20
2-3-22	0.38	121.00
3-22-49	0.56	115.00
22-22-49	0.35	119.14
6-1-22	0.70	107.50

Three membered ring stretching constant:

Bond type	k_s, mdyn Å^{-1}	l_0, Å
22-22	4.40	1.467

Hydrogen bond:

Atom pair	EPS, kcal/mol	Sum of radii, Å
21-49	1.30	1.830

Vicinal proton-proton coupling constants were calculated with the 3JHHM extended Karplus program developed by Osawa and co-workers.[4,5] MM2(91) minimized structures were used as input to 3JHHM, and the default parameter set of 3JHHM was used to cal-culate coupling constants with the unparameterized epoxide oxygens considered to be sp^3 carbons. The construction and superimposition of structures was performed with the molecular modelling system MacMimic.[5]

X-ray structure analysis:

Panellol (**2**) crystallises in the ortorhombic space group $P2_12_12_1$ (No. 19) with a= 6.554(1), b=389(1), c=31.995(6) Å, V=1339,7(4) Å^3, Z=4, D_c=1.25g·cm^{-3}, F(000)=544. The structure was refined to R=0.070, wR=0.071 for 899 observed reflections with $E_0 > 4\sigma(E_0)$ (MoKα radiation, $2\theta_{max}$=55°, 1729 independent reflections measured, crystal size 0.25 x 0.45 x 0.60 mm). Anisotropic temperature factors were introduced for the nonhydrogen atoms; hydrogen atoms were included at geometrically calculated positions.

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