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The stereochemistry of ledol from *Renealmia chrysotrycha*: an NMR study

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Abstract

From the leaves of *Renealmia chrysotrycha* (Zingiberaceae), we isolated a sesquiterpene alcohol for which spectral data suggested the structure of a 10-aromadendranol. A meticulous NMR investigation, based mainly on vicinal proton–proton coupling constants and NOE interactions, and confirmed by a molecular mechanics calculation, established its relative stereochemistry as that of ledol. This finding resolves several recent literature ambiguities. Three known compounds (aromadendrene, *cis*-calamenene and palustrol) were also isolated.

Keywords: *Renealmia chrysotrycha*; Zingiberaceae; Ledol; Sesquiterpene alcohol; NMR determination of stereochemistry

1. Introduction

Renealmia chrysotrycha Petersen (Zingiberaceae), known locally as pacová (in the Tupi language: "leaf which is rolled") (Maas, 1977; Dahlgren, 1980), is widespread in the humid forests of southeastern Brazil, and is used in the local pharmacopoeia (Pugialli, 1998): the oil of the seeds is employed as a vermifuge and a decoction of the rhizomes is considered a carminative and a stimulant. The latter, together with the bark, is also used topically to treat inflammations and contusions, to disinfect wounds and promote healing. A tea made from the rhizomes serves for the treatment of Herpes zoster.

From the leaves of *R. chrysotrycha* we have isolated four compounds **1–4**. Of these, three are known sesquiterpenes: aromadendrene, **2** (Dolejs et al., 1959, 1960b; Büchi et al., 1969), *cis*-calamenene, **3** (Andersen et al., 1977) and palustrol, **4** (Dolejs et al., 1960a), as well as an alcohol for which spectral data suggested the structure of a 10-aromadendranol. In view of the very confusing literature record for identifying the stereoisomers of the latter (see Section 3), we decided to submit this substance to a meticulous NMR spectroscopic investigation, and were able to establish its relative stereochemistry as for **1**. In most of the literature (vide infra), the material as drawn is named ledol.

2. Results

The best strategy for the determination of stereochemistry by NMR spectroscopy is to rely on proton–proton coupling constants, which provide information on dihedral angles, as well as on NOE-derived spatial relationships. Both techniques require a full assignment of the proton signals and minimal spectral overlap. In the case of **1**, even at 600 MHz (see e.g. Fig. 1) and with the aid of two-dimensional experiments such as COSY and HMQC (gradient-assisted one-bond $^{13}C-^{1}H$ correlation), this was not a trivial task. As can be seen in Table 1, the spectra, in each of three different deuterated solvents, include several overlapping resonances. Nonetheless, by collating data from the three solutions, we can get identifiable, separatable signals for most protons in the molecule. When coupling constants can be measured in more than one of the three spectra, they are of similar value, indicating that the conformation is essentially conserved and that the obtained splittings are not significantly affected by second-order effects.

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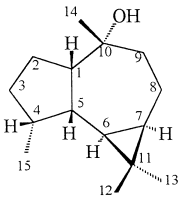

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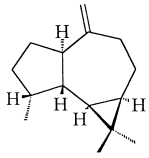

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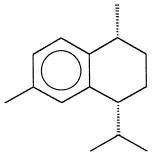

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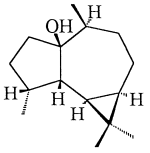

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Similarly, through-space relationships were obtained from NOESY spectra taken in each of the three solvents. A summary of all the observed interactions is presented in Table 2. With the coupling and NOE data in hand, we can establish the stereochemistry through the following logical sequence:

The ring-junction between the seven and the three-membered rings is *cis*. This, of course, is to be expected on energy grounds, but is confirmed by the 9 Hz coupling constant between H-6 and H-7, and by the observation that one of the *gem*-dimethyl groups on the cyclopropane ring (13, which appears at low field in all solvents) has NOE interactions only with the two cyclopropane hydrogens, i.e. H-6 and 7. The other, 12, has NOE interactions with H-5 and one of the protons on the 8-methylene, which must be therefore on the β face of the molecule.

As might be predicted from the latter, H-5 is *anti* and *trans* to H-6, as they show a very weak NOE and $J_{5,6} = 10.5$ Hz.

The ring-junction between the seven and the five-membered rings is *cis*, since the two ring-junction protons show a measurable NOE and $J_{1,5} = 6.5$ Hz (consistent with a *gauche* relationship). We also observe an NOE interaction between H-6 and one proton on each of carbons 2 and 3; these must be located on the α side of the molecule, which is therefore somewhat concave. A

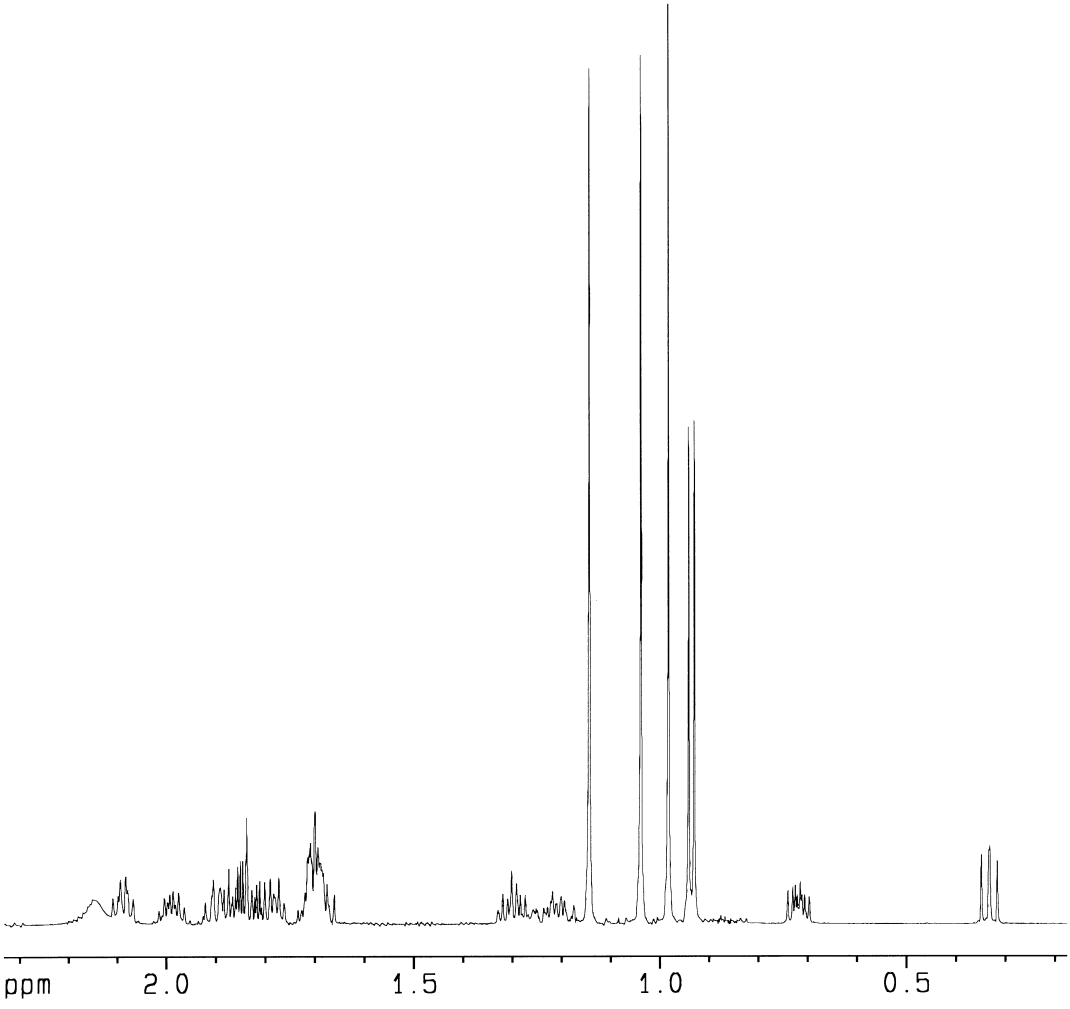


Fig. 1. 600 MHz ^{1}H NMR spectrum of ledol (**1**) in $CDCl_3$.

Table 1
NMR spectral data for **1** in various solvents

	$CDCl_3$			C_6D_6			$(CD_3)_2CO$		
	δ_C	δ_H	Mult.	δ_C[a]	δ_H	Mult.	δ_C[a]	δ_H	Mult.
1	53.79	2.09	*td* 9.5, 6.5	54.4	1.86	*m*	55.8	2.02	*m*
2α	24.63	1.90	*m*	25.2	2.02	*m*	24.6	1.83	*m*
2β		1.69	*m*		1.71	*m*		1.73	*m*
3α	30.80	1.30	*m*	31.3	1.30	*m*	30.4	1.27	*m*
3β		1.70	*m*		1.69	*m*		1.70	*m*
4	38.44	1.99	*dsxt*[b] 10.5, 6.5	38.9	1.86	*m*	38.8	2.01	*m*
5	40.79	1.78	*dt* 10.5, 6.5	41.1	1.53	*dt* 10.5, 6.5	40.9	1.69	*dt* 9.5, 5.5
6	23.41	0.33	*dd* 10.5, 9	23.8	0.18	*dd* 10.5, 9	23.5	0.32	*t*, 9.5
7	25.03	0.72	*ddd* 11, 9,6	25.6	0.54	*ddd* 11.5, 9, 6	26.5	0.71	*ddd* 11, 9.5, 6.5
8α	20.30	1.83	*m*	20.7	1.71	*m*	20.5	1.77	*m*
8β		1.21	*m*		1.03	*dtd* 14.5, 11, 5.5		1.10	*dtd* 14.5, 11.5, 4
9α	39.21	1.86	*m*	39.8	1.83	*ddd* 14, 11, 5.5	39.1	1.81	*m*
9β		1.69	*m*		1.55	*ddd* 14, 5.5, 4		1.59	*dddd* 13.5, 5, 4, 1
10	74.59	–	–	a	–	–	a	–	–
11	19.19	–	–	a	–	–	a	–	–
12	15.41	0.98	*s*	15.7	0.88	s	15.4	0.97	*s*
13	28.66	1.04	*s*	28.8	0.92	s	28.4	1.03	*s*
14	30.52	1.14	*s*	31.3	1.16	s	30.9	1.13	*s*
15	15.99	0.94	d, 7	16.4	0.93	*d*, 7	16.0	0.94	*d*, 7
OH	–	–	–	–	1.82	*bs*	–	2.92	*s*

[a] Measured indirectly from the HMQC spectrum, and therefore non-protonated carbons are not observed.
[b] Doublet of sextets.

Table 2
Observed NOE relationships

Between H-	and H-
1	2β, 5, 14
2α	2β, 3α, 3β, 6, 14
2β	1, 2α
3α	2α, 3β, 6, 15
3β	2α, 3α, 4
4	3β, 5, 15
5	1, 4, 6, 8β, 12
6	2α, 3α, 5, 7, 13, 15, OH
7	6, 8α, 13, OH
8α	7, 8β, 9α
8β	5, 8α, 9β, 12
9α	8α, 9β, OH
9β	8α, 8β, 9α, 14, OH
12	5, 8β
13	6, 7
14	1, 2α, 9β, OH
15	3α, 4, 6
OH	6, 7, 9α, 14

trans-ring junction would imply a much more rigid, quasi-planar bicyclic system.

The methyl group on C-4, i.e. 15, is α, as it shows an NOE with H-6. Conversely, H-4 is β-oriented and interacts with H-5; these two protons are *gauche*, as indicated by their coupling constant, $J_{4,5} = 6.5$ Hz.

The seven-membered ring has a boat-like shape, with the concave part pointing towards the β side of the molecule. This can be seen by the mutual NOE interactions between the 12 methyl, H-5 and 8β (vide supra).

The stereochemistry at the tertiary alcohol site (C-10) was unambiguously established by (i) the fact that Me-14 has an NOE with H-1, which we have shown to be on the β side of the molecule; and since (ii), the OH hydrogen (which can be clearly seen in the d_6-acetone solution as a sharp singlet at δ 2.92) has NOE interactions with the two cyclopropane protons, H-6 and H-7. Since these pairs of hydrogens are separated by six chemical bonds, this is only possible if the OH points inwards on the α side of the molecule.

In order to better visualise the conformation of ledol we calculated the energy-minimised structure of **1**, using an MM2-based programme (PCMODEL, Serena Software, Box 3076, Bloomington, IN 47402-3706, USA). The results are shown in Fig. 2 and in Table 3. The latter indicates reasonable agreement for the vicinal coupling constants (and, therefore, geometry); it should be noticed that the main deviations from the experimental values occur for dihedral angles of ca. 45°, where the Karplus relationship is at its steepest. For instance, the torsional angle between the 7- and 8-methylenes is calculated as 34°, while the experimental data are more consistent with a value near 50°. The overall shape in Fig. 2, nonetheless, reproduces qualitatively the observed NOE interactions, which all refer to pairs of protons which are less than 3.1 A apart (except for the two involving the OH group, which is quite free to rotate).

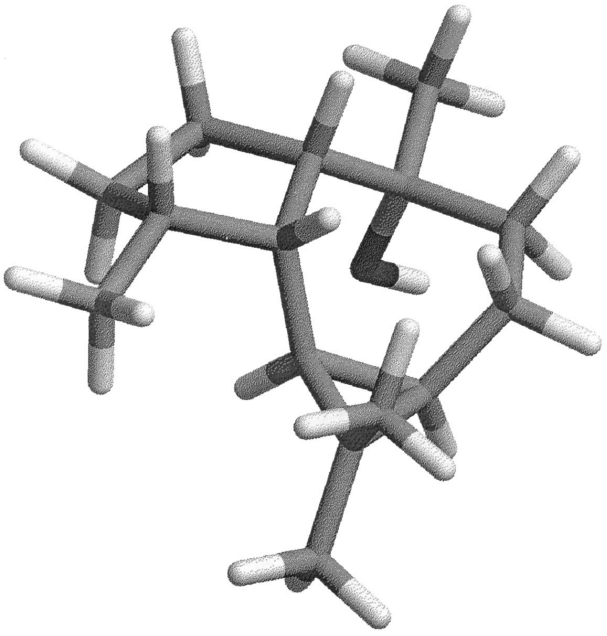


Fig. 2. Molecular mechanics-minimised structure of ledol (**1**).

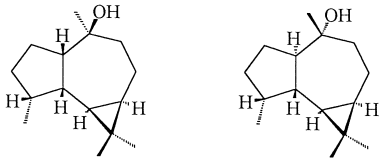
Table 3
Calculated vs experimental vicinal coupling constants

H–C–C–H		Calculated[a]		Experimental
		Dihedral angle	$^3J_{HH}$	$^3J_{HH}$
1	2α	132	6.5	6.5
1	2β	13	10.0	9.5
1	5	27	8.5	6.5
3α	4	166	11.8	10.5
3β	4	46	5.5	6.5
4	5	42	5.7	6.5
5	6	168	12.0	10.5
6	7	1	10.6	9
7	8α	54	4.2	6
7	8β	168	11.9	11
8α	9α	35	8.2	5.5
8α	9β	79	0.5	4
8β	9α	147	9.9	11
8β	9β	33	8.4	5.5

[a] With a molecular mechanics programme (see text).

3. Discussion

The tertiary sesquiterpene alcohol ledol was first isolated, from *Ledum palustre*, by Kir'yalov (1948, 1949, 1951). The stereochemistry of the various asymmetric centres in the molecule was discussed in subsequent papers (e.g. Dolejs et al., 1959); eventually, Büchi et al. (1969, and references therein) proposed **1**, but other stereoisomers (viridiflorol, **5** and globulol, **6**) are also known as natural products, and the identification of ledol may not be trivial. We have collated typical values for melting points and optical rotation values in Table 4, but we believe the easiest criterion for differentiating the various possible isomers is the use of the chemical shifts of the cyclopropane ring protons (the NMR spectroscopic data in Table 4 refer to $CDCl_3$ solutions).



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Conflicting reports have appeared recently in the literature. For instance, Koul et al. (1993) describe the isolation of the enantiomeric (−) ledol ($[\alpha]_D = -6.3^\circ$ in $CHCl_3$). Their NMR data are virtually identical to ours (Table 1), including the signals at δ 0.33 and 0.72; the structure is drawn, however, with the 15-methyl group oriented *cis* to the cyclopropane ring. We have, anyway, doubts regarding the identification of this as an enantiomeric sesquiterpene. Optical rotation measurements are usually performed for ledol in alcohol solvents (e.g. Kir'yalov, 1949; Pakrashi et al., 1980); significantly, Naves (1959) reports $[\alpha]_D = +2.6^\circ$ in EtOH, but -5.6° in $CHCl_3$!

Miyazawa et al. (1994) use commercial "ledol" as a substrate for microbiological oxidation; however, this material had cyclopropane proton absorptions at δ 0.11 and 0.61. Finally, Wu et al. (1996) claim to have isolated (−) ledol, giving its structure as the mirror image of **1** ($[\alpha]_D = -3.7^\circ$ in $CHCl_3$). These authors use only the observation of an NOE interaction between H-1 and the 14 methyl to validate their assumption — but their cyclopropane proton absorptions are δ 0.11 and 0.61, and therefore their sesquiterpene may be identical to that of Miyazawa et al. (1994), but not to ours. Since we also observe an NOE interaction between these two protons, this observation by itself cannot be sufficient. We believe that our evidence in favour of the stereochemistry in **1** for the isomer with δ 0.33 and 0.72 for the cyclopropane protons is unambiguous.

4. Experimental

4.1. General

Melting points were determined with a Kofler apparatus, optical rotations on a Perkin–Elmer 141 polarimeter, IR spectra on a Nicolet 205 FT-IR instrument, and Mass spectra on a VG Autospect (at 70 eV).

NMR spectra were run on a Bruker DMX-600 instrument, at 600.1 and 150.9 MHz for ^1H and ^{13}C, respectively. All chemical shifts are reported in ppm downfield from internal TMS, and coupling constants

Table 4
Characteristic physical constants for stereoisomers **1**, **5** and **6**

		mp	$[\alpha]_D$	Cyclopropane	δ_H	Reference
1	Ledol	103°C	+2°	0.33	0.72	Pakrashi et al. (1980)
5	Viridiflorol	74°C	+2°	0.11	0.61	San Feliciano et al. (1989) Faure et al. (1991)
6	Globulol	87°C	−43°	0.51	0.59	Büchi et al. (1959) Faure et al. (1991)

in Hz. NOESY spectra were recorded with mixing times of 1–2 s.

Chromatographic separations were performed on silica gel 60 (Merck), 70–270 mesh and the fractions were analysed on Merck 60G silica gel plates, visualised with 254 and 336 nm UV light; with 2% cerium sulphate solutions in H_2SO_4; and with thymol in methanol/H_2SO_4, followed by heating. All solvents were analytical grade.

4.2. Plant materials

Six hundred and ninety grams of dried leaves of *Renealmia chrysotrycha* Petersen were collected within the Atlantic Forest ecosystem, in the Ecological Reserve of Macae de Cima, Municipio de Nova Friburgo, RJ (between 22°21′ and 22°28′ S and 42°27′ and 42°35′ W) at an altitude of 1000 m. A voucher specimen is deposited at the Herbarium of the Jardim Botanico do Rio de Janeiro (RJ) under the registry number 3321821. 500 g of dried and ground leaves were macerated with hexane at room temp. The extract was concentrated (30 g) and 18 g thereof were subjected to silica gel column (50 g) chromatography eluted with a gradient of hexane-AcOEt; fractions 2 (0% AcOEt, 141 mg), 3 (0% AcOEt, 59 mg) and 17 (2.5% AcOEt, 92 mg) were identified as aromadendrene, **2** (Dolejs et al., 1959; Dolejs et al., 1960b; Büchi et al., 1969), *cis*-calamenene, **3** (Andersen et al., 1977) and palustrol, **4** (Dolejs et al., 1960a), respectively. Fractions 22–25 (10% EtAcO, 301 mg after recrystallisation from EtAcO) yielded **1** as colourless crystals, mp 98° (uncorr.); $[\alpha]_D$ +2° (MeOH; c 1.4); MS (EI) *m*/*z* (rel. int.): 204 [M^+] (7), 189 (6), 161 (19), 147 (14), 133 (10), 122 (44), 109 (53), 95 (30), 81 (36), 69 (44), 55 (28), 43 (100); IR (ν_{max}, cm^{-1}, KBr): 3350, 2925, 2866, 1466, 1376, 1112, 990, 941, 887, 682; NMR: see Table 1.

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