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The Crystal Structure of Biatractylolide, an 8,8′ (C–C) Linked Dimeric 12,8-Eudesmanolide from the Resin of *Trattinickia rhoifolia* Willd.

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A symmetrical dimeric sesquiterpenoid, biatractylolide (1), was isolated from the resin of *Trattinickia rhoifolia* Willd. The structure of compound 1 was elucidated by one- and two-dimensional NMR techniques and electron impact-mass spectra (EI-MS) data, and confirmed by X-ray crystallographic analysis.

Key words *Trattinickia rhoifolia*; bis-sesquiterpene lactone; bis-eudesmanolide; biatractylolide

In previous papers[1,2] we have described several compounds, mainly sesquiterpenoids, isolated from the resin of *Trattinickia rhoifolia* Willd. (Burseraceae). In this paper we report the isolation and structure elucidation by spectroscopic methods and X-ray diffraction analysis of biatractylolide (**1**).

Results and Discussion

To the solution of resin of *T. rhoifolia* in methylene chloride, *n*-hexane was added to selectively precipitate and separate the insoluble material; the soluble part was taken to dryness and redissolved in diethyl ether, then washed with aqueous sodium bicarbonate (5% w/v) to remove the acidic compounds. Elimination of the solvent yielded an oily mixture, which was resolved by silica gel column chromatography using increasing gradient *n*-hexane–ethyl acetate mixtures as eluent. After column rechromatography on silica gel using an increasing gradient of *n*-hexane–methylene chloride as eluent, low polarity fractions afforded, along with other previously reported compounds,[3] the bis[8-eudesma-4(15),7(11)-dien-12,8α-olide] (biatractylolide; **1**).

Compound **1** was obtained as a colorless crystalline solid. Its molecular formula $C_{30}H_{38}O_4$, deduced from its molecular weight, (M^+, *m*/*z*: 462), contrasted with the appearance of only fifteen signals in its ^{13}C-NMR spectrum, thus revealing a symmetrical dimer structure. Its IR spectrum showed absorptions of α,β unsaturated γ-lactone (1759, 1150, 1105 cm^{-1}), and exocyclic methylene (3079, 1647, 889 cm^{-1}) functions. The ^{1}H-NMR spectrum (see Table 1) showed one singlet assigned to the angular methyl groups [δ: 1.07 (6H), s, H-14(14′)], five multiplet methylene signals [δ: 1.17 (2H), ddd, H-1$_{ax}$ (H-1$'_{ax}$)], [δ: 1.35 (2H), d, H-9$_{ax}$ (H-9$'_{ax}$)], [δ: 1.54 (2H), d(br), H-1$_{eq}$ (H-1$'_{eq}$)], [δ: 1.56 (2H), m, H-2$_{ax}$ (H-2$'_{ax}$)], and [δ: 1.59 (2H), d(br), H-2$_{eq}$ (H-2$'_{eq}$)], one methine absorption [δ: 1.67 (2H), dd, H-5$_{ax}$ (H-5$'_{ax}$)], one singlet for the methyl group attached to an olefin C=C carbons [δ: 1.68 (6H), s, H-13 (13′)], five absorptions corresponding to ten methylene protons [δ: 1.88 (2H), dd, H-3$_{ax}$ (H-3$'_{ax}$)], [δ: 2.29 (2H), d(br) , H-3$_{eq}$ (H-3$'_{eq}$)], [δ: 2.58 (2H), dd, H-6$_{eq}$ (H-6$'_{eq}$)], [δ: 2.71 (2H), t, H-6$_{ax}$(H-6$'_{ax}$)], [δ: 2.76 (2H), d, H-9$_{eq}$

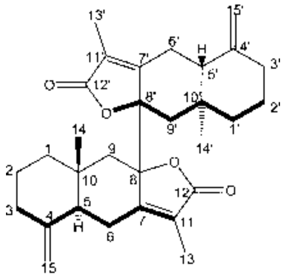

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Compound **1**

C[a)]	δ_C	H	δ_H, multiplicity (*J*, Hz)
C-1 (25)	42.2	H-1$_{ax}$	1.17, ddd (13.0; 12.2; 2.2)
C-1′ (10)		H-1$_{eq}$	1.54, d(br) (13.0)
C-2 (24)	22.4	H-2$_{ax}$	1.56, q(br) (12.2)
C-2′ (9)		H-2$_{eq}$	1.59, m
C-3 (23)	36.0	H-3$_{ax}$	1.88, dd (12.6; 9.0)
C-3′ (8)		H-3$_{eq}$	2.29, d(br) (12.6)
C-4 (21)	148.0	—	—
C-4′ (6)			
C-5 (20)	53.0	H-5$_{ax}$	1.67, dd (13.1; 3.3)
C-5′ (5)			
C-6 (27)	28.0	H-6$_{ax}$	2.71, t (13.1)
C-6′ (4)		H-6$_{eq}$	2.58, dd (13.1; 3.3)
C-7 (18)	164.7	—	—
C-7′ (3)			
C-8 (29)	89.5	—	—
C-8′ (14)			
C-9 (28)	49.8	H-9$_{ax}$	1.35, d (14.5)
C-9′ (13)		H-9$_{eq}$	2.76, d (14.5)
C-10 (19)	37.1	—	—
C-10′ (11)			
C-11 (16)	124.5	—	—
C-11′ (1)			
C-12 (30)	172.1	—	—
C-12′ (15)			
C-13 (17)	8.5	H-13	1.68, s
C-13′ (2)			
C-14 (26)	17.3	H-14	1.07, s
C-14′ (12)			
C-15 (22)	107.5	H-15a	4.58, s
C-15′ (7)		H-15b	4.80, s

a) In parentheses, X-ray molecular structure numeration.

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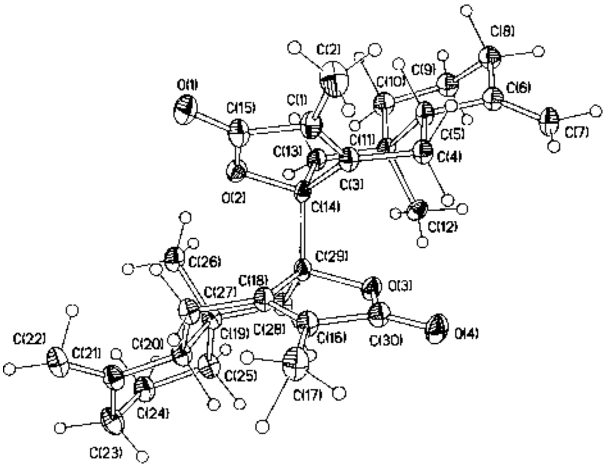

Fig. 1. Molecular Structure of **1** Obtained by X-Ray Diffraction

(H-$9'_{eq}$)] and those of four olefinic methylene protons [δ: 4.58 (2H), s, H-15a (H-15′a)], and [δ: 4.80 (2H), s, H-15b (H-15′b)].

One- and two-dimensional NMR data (see Experimental) were in agreement with those reported by Lin and coworkers for this compound, isolated from *Atractylodes macrocephala* and called biatractylolide[3]; however, no discussion was made by these authors about the C-8 and C-8′ configuration assignations. We describe here the absolute configuration of **1** finally established by X-ray crystallographic analysis as shown in Fig. 1.

Experimental

General Experimental Procedures Optical rotations were recorded on an AUTOPOL III-589-10 Rudolph Research Polarimeter using $CHCl_3$ as solvent. IR spectra were recorded as film on a Perkin Elmer FT-IR spectrometer 1725X. NMR spectra were run on a Bruker ARX 400 using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard.

The X-ray crystallographic data were collected at 273 K on a SEIFERT XRD 3003 SC diffractometer using Cu radiation (λ=1.54180 Å).

Plant Material Resin from *T. rhoifolia* was collected from Mucujepe (400 m.o.s.l.), Mérida State, Venezuela, in March 1995. Voucher specimens were deposited at the herbarium of Facultad de Ciencias Forestales, Universidad de los Andes, Mérida, Venezuela (MER Nava 1–2).

Extraction and Isolation Resin of *T. rhoifolia* (193 g) was dissolved in 300 ml of methylene chloride and then diluted with *n*-hexane (5.7 l) to selectively precipitate the insoluble material. Methylene chloride extract was taken to dryness to give a major soluble fraction (174.2 g) that was redissolved in ether and then washed with an aqueous $NaHCO_3$ 5% solution. The non-acidic fraction was concentrated *in vacuo* to yield an oil mixture that was chromatographed on silica gel using an increasing gradient of *n*-hexane–ethyl acetate eluent. The fraction eluted with *n*-hexane–EtOAc (9 : 1) percentage was rechromatographed on silica gel, using an increasing gradient of *n*-hexane–methylene dichloride to afford compound **1** (87 mg), in addition to other previously described compounds.[2]

bis-[8-Eudesma-4(15),7(11)-dien-12,8α-olide], **1**: Colorless prism, mp 211—212 °C (CH_2Cl_2) [reported[3]: 210—212 °C], $[\alpha]_D^{20}$ +255.8° (c=0.122, $CHCl_3$) [reported[3]: +256.40° (c=0.02, $CHCl_3$)]. IR (KBr) 3497, 3079, 2931, 2868, 2849, 1759, 1674, 1649, 1442, 1385, 1269, 1105, 1042, 1011, 889, 737 cm^{-1}. ^1H- and ^{13}C-NMR ($CDCl_3$), see Table 1. Electron impact-mass spectra (EI-MS) *m/z*: 462 (<1) [M^+], 230 (6), 231 (9), 153 (16), 136 (15), 107 (18), 95 (19), 93 (15), 89 (20), 83 (16), 81 (45), 79 (17), 78 (17), 77 (39), 73 (20), 71 (18), 70 (18), 69 (100), 68 (22), 67 (26), 60 (25), 57 (35), 56 (19), 53 (18), 51 (18).

Table 2. Crystal Data and Structure Refinement for **1**

Empirical formula	$C_{30}H_{38}O_4$
Formula weight	462.60
Temperature	293 (2) K
Wavelength	1.54180 Å
Crystal system	Orthorhombic
Space group	*P*2 (1) 2 (1) 2 (1)
Unit cell dimensions	a=23.261 (2) Å α=90°
	b=9.791 (2) Å β=90°
	c=10.947 (4) Å γ=90°
Volume	2493.2 (3) $Å^3$
Z	4
Calculated density	1.232 g/cm^3
Absorption coefficient	0.632 mm^{-1}
F(000)	1000
Crystal size	0.06×0.06×0.08 mm
θ range for data collection	1.90 to 59.66°
Limiting indices	$0\,h\,24$, $0\,k\,10$, $\bar{1}1\,l\,11$
Reflections collected/unique	3626/3626
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	3626/0/441
Goodness-of-fit on F^2	1.160
Final *R* indices [$I>2\sigma(I)$]	R_1=0.0796, wR_2=0.2089
R indices (all data)	R_1=0.1068, wR_2=0.2594
Largest diff. peak and hole	0.354 and −0.310 e·$Å^{-3}$

X-Ray Crystal Structure Analysis of 1 A colourless crystal of **1** with dimensions 0.06×0.06×0.08 mm was selected for X-ray analysis. Structure analysis was made using the SHELXTL v. 5.0 program. Experimental and crystal data are given in Table 2. The compound crystallised in the orthorhombic system and the space group *P*2 (1) 2 (1) 2 (1). A total of 3626 frames were collected for the analysis, and an empirical absorption correction was applied. The structure was solved by the direct method and refined by full-matrix least-squares on F^2 values. Non-hydrogen atoms were anisotropically refined. The hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R_1=0.0796, wR_2=0.2089 with goodness-of-fit on F_2=1.160. Scattering factors were taken from the *International Tables for X-Ray Crystallography*.

Supporting Information Available X-Ray crystallographic data for the structure **1** have been deposited at the Cambridge Crystallographic Data Center under code CCDC 179899.

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