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ISOLATION AND X-RAY STRUCTURE DETERMINATION OF A NEOLIGNAN FROM *CLERODENDRON INERME* SEEDS

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Key Word Index—*Clerodendron inerme*; Verbenaceae; seeds; neolignans; 5,8-epoxy-6,7-dimethyl 2′,3′,2″,3″-dimethylenedioxy-4′,1″-dimethoxy-1,2:3,4-dibenzo-1,3-cyclooctadiene; X-ray study.

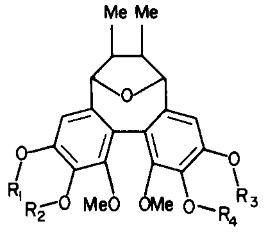
Abstract—A new neolignan, 5,8-epoxy-6,7-dimethyl 2′,3′,2″,3″-dimethylenedioxy-4′,1″-dimethoxy-1,2:3,4-dibenzo-1,3-cyclooctadiene, from the petrol extract of *Clerodendron inerme* seeds, was characterized by spectroscopic and X-ray crystallographic methods. This compound makes up *ca* 5% by wt of the seeds.

INTRODUCTION

Clerodendron is a genus of tropical and subtropical shrubs of the Verbenaceae. The seeds of *C. inerme* investigated were collected in Thailand. TLC of the petrol extract indicated that triacylglycerols were the major components, along with small amounts of hydrocarbons, free acids and partial acylglycerols, but two spots were not readily identifiable as normal seed oil constituents. These compounds are now identified as new neolignans **1** and **2**, the first, to our knowledge, to exhibit both the furanoid and the cyclooctadiene ring systems [1].

RESULTS AND DISCUSSION

Compound **1** was easily isolated by chromatography on silica and purified by recrystallization from EtOH. Its molecular formula, as derived by high-resolution mass spectrometry, indicated 12 rings and/or double bonds. The ^{1}H NMR spectrum showed two methylenedioxy and two OMe groups, a doublet representing two Me groups and two aromatic protons accounting for 18 of the 22 protons. Structure **1** includes environments for the four methine protons that are in reasonable agreement with the rest of the NMR data. Shifts of the protons on C-2 and C-3 (see Fig. 1 for numbering) are indicative of *cis* substitution since *trans*-3,4-dimethyltetrahydrofurans have pseudoaxial protons at lower shift values than those found for pseudoequitorial *cis* protons [2]. The proton at C-1 appears as a doublet coupled to the C-2 proton, whereas the proton at C-4 appears as a singlet.



1 $R_1 + R_2 = R_3 + R_4 = \text{—}CH_2\text{—}$
2 $R_1 + R_2 = \text{—}CH_2\text{—}$; $R_3 = R_4 = Me$
or $R_1 = R_2 = Me$; $R_3 + R_4 = \text{—}CH_2\text{—}$

Although the NMR and mass spectra provided enough data to suggest tentative structures such as **1**, definitive information about the aromatic substitution pattern and the stereochemistry of the rings was not forthcoming. These data were determined by an X-ray structure analysis on a single crystal of **1**.

Compound **1** crystallizes in the monoclinic space group $P2_1/c$ with $a = 9.520(3)$ Å, $b = 14.765(6)$ Å, $c = 13.618(6)$ Å and $\beta = 91.4(1)°$ with 4 molecules/unit cell. A total of 2499 independent reflections were collected on a Nicolet P3F automatic diffractometer using CuKα radiation and a graphite monochromator out to a $2\theta_{max}$ of 112°. The structure was solved by the symbolic addition procedure for centrosymmetric crystals [3]. All 29 nonhydrogen atoms in the asymmetric unit were located in one E-map computed with the 745 highest E-values. Full-matrix least squares refinement, minimizing the function $\Sigma w(|F_o| - |F_c|)^2$, was performed. All 22 hydrogen atoms were found in a difference map calculated at an intermediate stage in the refinement. The heavy atoms were refined with anisotropic thermal parameters, but only positional parameters were refined for the hydrogen atoms. Final R-factors (agreement between observed and calculated structure factors) were $R = 0.053$ and $R_w = 0.055$. Coordinates and B_{eq} values for the nonhydrogen atoms are listed in Table 1. Anisotropic thermal parameters for the heavy atoms, hydrogen coordinates and a listing of the final structure factors are available.* Figure 2 is a computer-generated stereodiagram [4] illustrating the final results of the X-ray analysis. It is important to note that **1** is a *meso* compound. However, steric requirements for 06 and 07 preclude mirror symmetry relating the two sides of the

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* Data available upon request from Dr. Flippen-Anderson, Naval Research Laboratory.

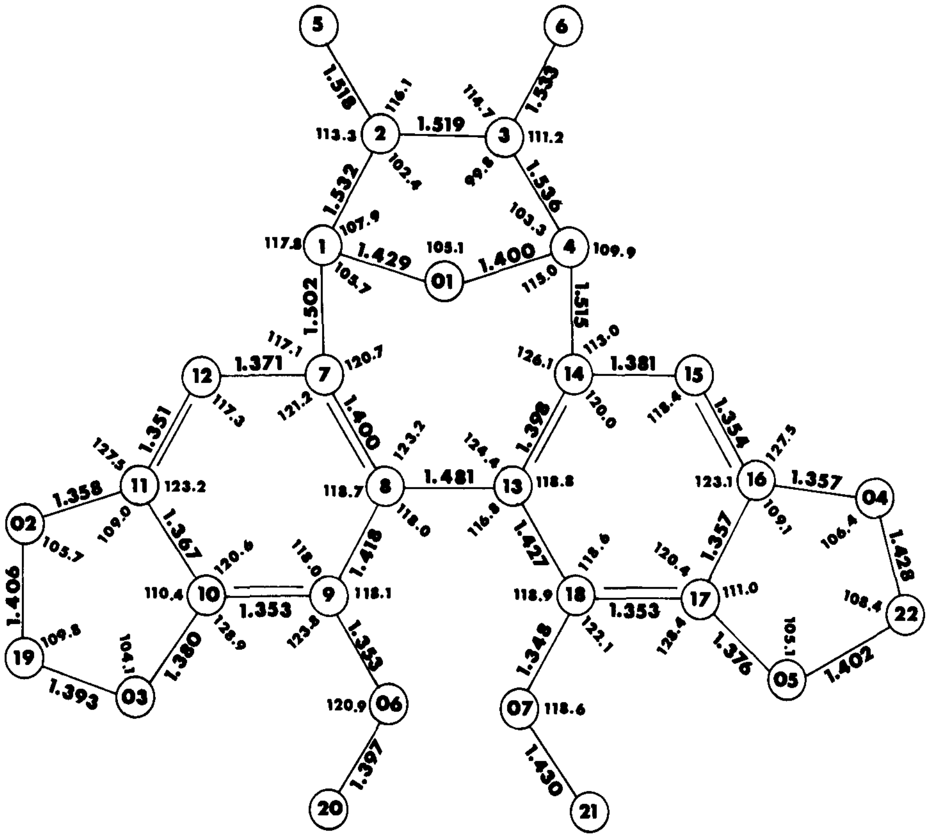

Fig. 1. Bond lengths and angles for neolignan **1**. Based soelely on least-squares results, s.d. values are of the order of 0.003 Å for bond lengths and 0.2° for bond angles.

molecule. It must be twisted about the C8–C13 bond to allow enough space between the two oxygens. A measure of this twist is the torsion angles about the C2–C3 (C5–C2–C3–C6 at 34.8° and C1–C2–C3–C4 at 29.7°) and C8–C13 (C9–C8–C13–C18 at 45.2°) bonds. All three of these torsion angles would have to be 0° for **1** to have mirror symmetry. Bond lengths and angles for the heavy atoms in **1** are illustrated in Fig. 1. The average C–H bond length is 0.99(3) Å. Effects of conjugation are evident in all bonds which are contiguous to the two aromatic rings. The average C–O bond length adjacent to an aromatic ring is 1.362(3) Å, whereas the average for the remaining C–O bonds is 1.410(3) Å. The C8–C13 bond at 1.481(3) Å shows the largest effect of the conjugation since it is affected by both aromatic rings. Similar effects have been noted in other biphenyl compounds. For example, spiro (2H-dibenzo[f, h]-3-4-dihydro-1,5-dioxacyclononene-3,1′-cyclopropane) [5] also has a biphenyl ring system with oxygen atoms substituted in the same relative positions as are O6 and O7 in **1**. In the spiro compound, the C–C bond length between the phenyl rings is 1.480 Å and the C–C–C–C torsion angle is 58°, with an intramolecular O...O approach of 2.925 Å. In **1** the comparable C–C–C–C angle is only 45.2° and the O...O distance is correspondingly less at 2.663 Å. Packing is influenced only by Van der Waals' forces, with the closest C–C intermolecular approach being C17...C27 at 3.44 Å and the closest C...O intermolecular approach is O1...C25 at 3.13 Å.

The compounds represented by structure **2** were inseparable by the chromatographic techniques employed and they could not be purified by

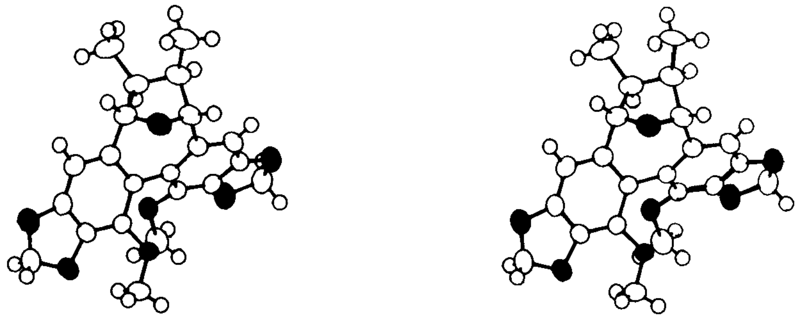

Fig. 2. Stereodiagram of the structure of neolignan **1**, including hydrogen atoms as determined by X-ray diffraction analysis. Black atoms represent oxygens.

Table 1. Fractional co-ordinates* and thermal parameters†

Atom	x	y	z	B_{eq}
C1	0.5593(2)	0.1959(2)	0.3439(2)	4.0
C2	0.6016(3)	0.2359(2)	0.2447(2)	4.4
C3	0.5446(3)	0.3338(2)	0.2494(2)	4.8
C4	0.5553(2)	0.3501(2)	0.3608(2)	4.1
C5	0.5516(4)	0.1781(3)	0.1576(2)	6.2
C6	0.3929(4)	0.3449(3)	0.2108(3)	6.7
C7	0.6728(2)	0.1545(2)	0.4085(2)	3.7
C8	0.7760(2)	0.2105(2)	0.4529(2)	3.1
C9	0.8518(2)	0.1762(2)	0.5358(2)	3.3
C10	0.8377(2)	0.0857(2)	0.5583(2)	3.9
C11	0.7441(3)	0.0313(2)	0.5071(2)	4.4
C12	0.6564(3)	0.0641(2)	0.5071(2)	4.6
C13	0.8079(2)	0.3053(1)	0.4189(2)	3.0
C14	0.7062(2)	0.3705(2)	0.3912(2)	3.5
C15	0.7455(3)	0.4605(2)	0.3720(2)	4.0
C16	0.8841(2)	0.4815(2)	0.3740(2)	3.6
C17	0.9865(2)	0.4179(2)	0.3915(2)	3.3
C18	0.9528(2)	0.3295(1)	0.4130(2)	3.1
C19	0.8541(4)	−0.0541(2)	0.6187(3)	5.7
C20	1.0157(4)	0.2067(2)	0.6677(3)	6.2
C21	1.1856(3)	0.2845(2)	0.4618(3)	5.2
C22	1.0925(3)	0.5477(2)	0.3517(2)	4.9
O1	0.4998(2)	0.2689(1)	0.4000(1)	4.1
O2	0.7459(2)	−0.0549(1)	0.5466(2)	6.7
O3	0.9020(2)	0.0360(1)	0.6333(1)	5.9
O4	0.9444(2)	0.5635(1)	0.3515(1)	4.7
O5	1.1174(2)	0.4552(1)	0.3771(1)	4.6
O6	0.9266(2)	0.2371(1)	0.5914(1)	4.0
O7	1.0505(2)	0.2623(1)	0.4202(1)	3.7

* Standard deviations, given in parentheses, are based solely on least-squares parameters.

† The B_{eq} values are calculated according to the equation

$$B_{eq} = \frac{4}{3}\sum_i \sum_j \beta_{ij}\bar{a}_i \cdot \bar{a}_j$$

where the β_{ij} values are the final refined anisotropic thermal parameters.

crystallization. The basis for these structures lies in the mass and NMR spectra. The ^1H NMR spectrum has, compared with that of **1**, two more OMe groups and one less methylene dioxy group. Also, some of the signals for OMe and aromatic protons appear at slightly different shifts (δ 3.45 and 3.47, 3.86 and 3.87, 3.91 and 3.92, 4.91 and 4.95, 6.43 and 6.49). The most logical explanation for these observations is that **2** consists of a nearly equal mixture of the two isomers shown and that these signals are truly singlets and not doublets. It is possible, however, that stereoisomers are also present, in which case coupling could be occurring.

Quantitation of **1** and **2** by GC with an internal standard showed them to be present at 16% and 28%, respectively, in the extract or 5% and 9% in the seeds.

The major portion of the extract consisted of triacylglycerols, which were analysed for fatty acid composition (as Me esters). The fatty acid profile is: 12:0–0.2%, 14:0–0.7%, 16:0–13%, 18:0–7.3%, 18:1–20%, 18:2–57%, 18:3–0.2%, 20:0–0.3% and traces of odd-numbered chain fatty acids.

EXPERIMENTAL

C. inerme oil Gaertn. was extrd from finely ground seeds (32% dry wt) with petrol (bp 35–60°). The first fractionation of the oil was done chromatographically on a 35 × 1 cm Si gel column which was sequentially eluted with: 500 ml of hexane–Et_2O (19:1), 250 ml of hexane–Et_2O (9:1), 250 ml of hexane–Et_2O (4:1) and 250 ml of hexane–Et_2O (3:2). Further purifications were made by prep. TLC on 1-mm layers of Si gel developed in hexane–Et_2O–HOAc (70:30:1).

Mps were determined on a Fisher–Johns apparatus and are uncorr. ^1H NMR spectra were measured in $CDCl_3$ soln at 90 MHz relative to TMS.

Preparation of Me esters of and analysis of the fatty acids by GC has been described previously [6, 7]. Quantitation of the neolignans was made by GC with fluorescein diacetate as an int. standard [8] on a 3% OV-1 (90 × 0.2 cm) column that was programmed from 200 to 300°. Chromatographic responses of the compounds were considered to be equal.

Compound **1** was eluted from the Si gel column with hexane–Et_2O (9:1) and purified by recryst from EtOH. Mp 175–177°; $[\alpha]_D^{25}$ 0°; (c 3.1 $CHCl_3$); MS (probe) 70 eV m/z 398.1340 (100%), $C_{22}H_{22}O_7$ requires 398.1366 ^1H NMR (90 MHz, $CDCl_3$): δ 1.04 (6 H, *d*, J = 7 Hz, C-5 and C-6), 2.05 (1 H, *m*, C-2), 2.66 (1 H, *m*, C-3), 3.77 (3 H, *s*, C-20 or C-21), 3.85 (3 H, *s*, C-20 or C-21), 4.31 (1 H, *d*, J = 7 Hz, C-1), 4.89 (1 H, *s*, C-4), 5.94 (4 H, *q*, C-22 and C-19), 6.31 (1 H, *d*, J = 0.5 Hz, C-12), 6.43 (1 H, *s*, C-15).

Compound(s) **2**, which were eluted from the Si gel column with hexane–Et_2O (4:1), were purified by prep. TLC. MS (probe) 70 eV m/z 414.1656, $C_{23}H_{26}O_7$ requires 414.1679. ^1H NMR (90 MHz, $CDCl_3$): δ 1.04 (6 H, *d*, J = 7 Hz), 2.06 (1 H, *m*), 2.66 (1 H, *m*), 3.45 and 3.47 (3 H, *s*), 3.82 (3 H, *s*), 3.86 and 3.87 (3 H, *s*), 3.91 and 3.92 (3 H, *s*), 4.34 (1 H, *d*, J = 7 Hz), 4.91 and 4.95 (1 H, *s*), 5.94 (2 H, *q*), 6.33 (1 H, *d*, J = 2 Hz), 6.43 and 6.49 (1 H, *s*).

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