necting it to an O4 atom in a symmetry-related molecule. The O···O distance is 2.847 (9) Å and this fact further confirms the assignment of atomic labels to the epoxide atoms, which was first based on the heights of peaks in the E map, then on the fact that the assignment produced very similar thermal factors for the external atoms O4 and C30, and finally on the detection of peaks attributable to H atoms at the calculated positions for C30 (full-matrix least-squares refinement, heavy atoms anisotropic, XTAL[14] refinement program, final R factor = 0.07). Fuller experimental data, tables of observed and calculated structure factors, and refinement parameters are available as Supplementary Material.

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Supplementary Material Available: A packing diagram, full refinement parameters, and a table of molecular dimensions (5 pages); observed and calculated structure factors (13 pages). Ordering information is given on any current masthead page.

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Larreantin, a Novel, Cytotoxic Naphthoquinone from *Larrea tridentata*

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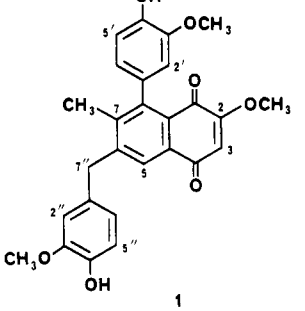
The structure of larreantin (1), a novel, cytotoxic naphthoquinone derivative from the roots of *Larrea tridentata* (Zygophyllaceae), has been deduced through a combination of spectroscopic techniques, with particular use being made of the NOE difference and CSCM 1D and selective INEPT techniques. Larreantin represents a new class of natural products in which the naphthoquinone ring may be formed through oxidative cyclization of two phenylpropene units with a preformed benzoquinone.

In previous work we have described the isolation of several new triterpenes[1] and lignans[2,3] from the stems and leaves of the creosote bush *Larrea tridentata* (DC) Coville (Zygophyllaceae), a plant under investigation for its fertility regulating principles. With a view to isolating additional quantities of nor-3′-demethoxyisoguaiacin, the active antifertility principle[3] from the above ground parts, a phytochemical investigation of this material was initiated. At the same time we also examined the methanol extract of the root of this plant for its cytotoxic potential in both the KB and P-388 assays.[4,5] The methanol extract of the plant was subjected to flash chromatography on Celite, eluting with $CHCl_3$/MeOH (98:2) and a portion of the resulting cytotoxic (P-388, ED_{50} 0.57 μg/mL) fraction was chromatographed on silica gel to afford nine fractions, all of which displayed cytotoxic activity in the P-388 test system. Further chromatography of one of these fractions (ED_{50} 0.62 μg/mL) by LPLC on silica gel afforded 16 fractions, the most polar of which gave larreantin on crystallization.

Larreantin, mp 204–206 °C, displayed a molecular ion at m/z 460, analyzing for $C_{27}H_{24}O_7$, and only the fragment ions at m/z 429 ($M^+ - 31$) and 137 were of any significance in the EIMS. The UV spectrum displayed maxima at



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241.5, 259, 286, and 348 nm, and the IR spectrum showed strong hydroxyl group absorption at 3400 cm^{-1}, together with carbonyl bands at 1687 and 1649 cm^{-1}. Other strong absorption bands were observed at 1619, 1514, 1340, 1284, 1255, 1239, 1212, and 1088 cm^{-1}.

The ^1H NMR spectrum indicated the presence of an aromatic methyl group (δ 2.024), a benzylic methylene (δ 4.057), three aromatic methoxy groups at δ 3.792, 3.818, and 3.837, and eight aromatic protons. Six of these protons were observed in two 1,2,4-(or 1,3,4-)trisubstituted aromatic systems, with two singlet aromatic protons at δ 6.083 and 7.983. The final two protons were observed as exchangeable phenolic protons at δ 5.697 and 5.781. The interrelationships of the aromatic protons were determined through a homonuclear COSY experiment. Thus the ortho-coupled doublet at δ 6.954 was coupled to a doublet of doublets (J = 2.1, 7.7 Hz) at δ 6.499, which was itself coupled to a doublet (J =2.1 Hz) at δ 6.539. The corresponding coupled protons in the second aromatic unit

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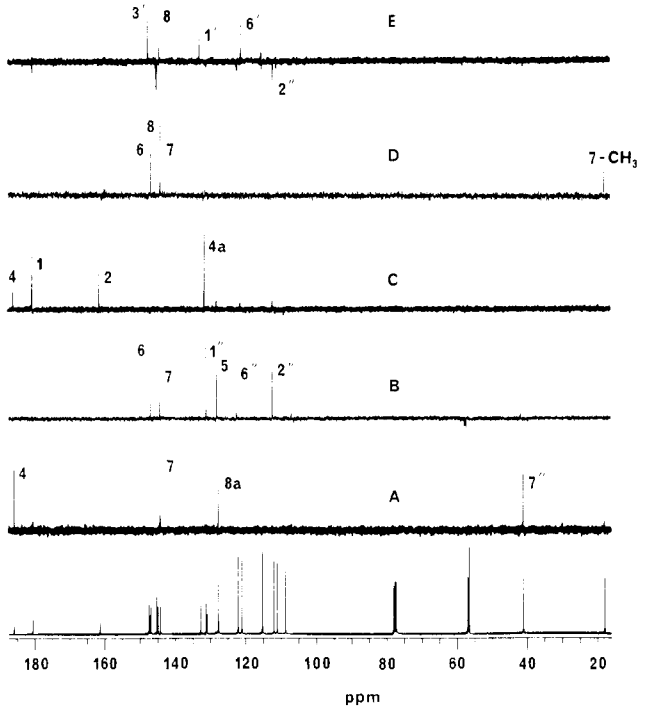

Figure 1. Examples of selective INEPT experiments on larreantin (1). Irradiation of (A) H-5, (B) H-7″, (C) H-3, (D) 7-CH_3, and (E) H-2′.

appeared at δ 6.839, 6.620, and 6.680, respectively.

A COSY spectrum enhancing the long-range couplings established that the three aromatic methoxy groups were each coupled to *different*, single aromatic protons. Thus the methoxy group at δ 3.837 was adjacent to the doublet (J = 2.1 Hz) at δ 6.680, the methoxy group at δ 3.818 was adjacent to the doublet (J = 2.1 Hz) at δ 6.539, while the third methoxyl group at δ 3.797 was adjacent to the singlet at δ 6.083.

In the carbon-13 NMR spectrum all of the individual resonances were revealed and the APT spectrum permitted the observation of 14 quaternary aromatic carbons, in addition to the protonated carbons anticipated from the ^{1}H NMR spectrum. Two carbonyl carbons were observed at δ 185.78 and 180.44, and this formation, together with an analysis of the carbon framework requirements, suggested that the central nucleus was a naphthoquinone, substituted by a methoxy, a methyl, a 4-hydroxy-2(or 3)-methoxyphenyl and a (4-hydroxy-2(or 3)-methoxyphenyl)methyl group. The arrangement of these groups on the naphthalene nucleus forms the body of this report together with the unambiguous assignment of both the proton and carbon-13 spectra of this novel isolate. Crucial in this work was the powerful combination of the NOE difference and CSCM 1D[6] and the selective INEPT techniques[7] which we have used previously.[8-16]

The only assumption that was made, and later confirmed, was that the singlet aromatic proton at δ 7.983 was peri to one of the carbonyl groups. Selective INEPT irradiation (Figure 1) of this proton (Figure 1a) with $^3J_{CH}$ = 6 Hz enhanced three quaternary carbons at δ 185.28, 143.49, and 127.09 which should be C-4, C-8a, and C-7. The latter two signals could not be further assigned at this time. Irradiation of the methylene group at δ 4.057 (Figure 1b) led to the enhancement of six signals, δ 146.20, 143.49, 130.33, 127.23, 121.50, and 111.37. The signal at δ 127.23 was assigned to C-5, and the latter two to C-6″ and C-2″, respectively, through appropriate CSCM 1D irradiations.

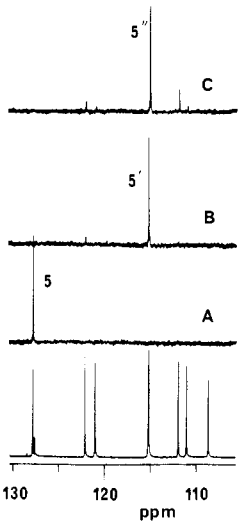

Figure 2. Examples of CSCM 1D experiments on larreantin (1). Irradiation of downfield satellite of (A) H-5, (B) H-5′, and (C) H-5″.

The irradiation of H-3 provided extremely valuable structure information. Under NOE conditions one of the methoxyl groups (δ 3.792) was enhanced 2.3% as expected, and under selective INEPT conditions (Figure 1c), it was apparent that the carbonyl carbon at δ 179.93 was also enhanced, thereby placing the methoxyl group at C-2. The COSY spectrum enhancing the long-range couplings also revealed another important structure feature, namely, the coupling of the aromatic methyl group with both the benzylic methylene protons and H-5, suggesting that the methyl group was at C-7 and the benzylic group was at C-6 or C-8. A distinction between the latter two possibilities was achieved when H-5 was irradiated under selective INEPT conditions (Figure 1a), where it was observed that the signal at δ 40.93 was also enhanced. The benzylic group is therefore at C-6.

Consequently, it remained to define the relationship of the hydroxy and methoxy groups on the two phenolic rings, given that the substitution a priori could be either 2,4 or 3,4. The long-range coupling enhanced COSY spectrum eliminated the possibility of the two methoxy groups both being on the same ring, as did an interpretation of the fragment ion at m/z 137. When the 7-CH_3 was irradiated, NOE effects were observed for the 2′- and the 6′-protons, indicating the presence of a 3,4-substituted system in ring C where the 3-substituent is a methoxyl group. For the D ring, irradiation of either H-5 or the benzylic methylene group enhanced the meta-coupled protons (i.e. H-2″ and H-6″), indicating that ring D has the same substitution as ring C. On this basis the methoxy group is placed at C-2, the substituted benzyl group at C-6, the methyl group at C-7, and the substituted phenyl moiety at C-8. Larreantin therefore has the structure 1.

Selective INEPT experiments provided internally consistent confirmatory evidence for this structure proposal.

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Table I. Proton[a] and Carbon-13[b] NMR Data for Larreantin (1)

C	δ_C	δ_H	mult, J (Hz)
1	179.93		
2	160.75		
3	108.04	6.083	s
4	185.28		
4a	130.63		
5	127.23	7.983	s
6	146.20		
7	143.49		
8	143.57		
8a	127.09		
1′	132.12		
2′	110.46	6.539	d 2.1
3′	146.86		
4′	144.62		
5′	114.52	6.954	d 7.7
6′	120.33	6.499	dd 2.1, 7.7
1″	130.33		
2″	111.37	6.680	d 2.1
3″	146.74		
4″	144.29		
5″	114.59	6.839	d 7.7
6″	121.50	6.620	dd 2.1, 7.7
7″	40.35	4.057	s
2-OCH_3	55.90	3.792	s
3′-OCH_3	55.95	3.816	s
3″-OCH_3	56.34	3.837	s
7-CH_3	17.29	2.024	s
4′-OH		5.781	s
4″-OH		5.697	s

[a] Recorded at 360 MHz in $CDCl_3$, δ_{TMS} = 0 ppm. [b] Recorded at 90.54 MHz in $CDCl_3$, δ_{TMS} = 0 ppm.

Thus irradiation of the 7-CH_3 (Figure 1d) led to the enhancement of the quaternary carbon signals at δ 146.20, 143.57, and 143.49. Irradiation of the 2′-(Figure 1e) or 6′-protons also enhanced the resonance at δ 143.57, indicating that it should be C-8. Irradiation of H-5 (Figure 1a) enhanced the signals at δ 143.49 and 127.09 (C-8a), and consequently the signal at δ 143.49 could be attributed to C-7, while the signal at δ 146.20 should be C-6. The resonance for C-7 was also enhanced through the irradiation of the 7″-H_2.

All of the protonated carbons, including the three methoxyl carbons, could be assigned through the use of the CSCM 1D technique (Figure 2). But there remained several quaternary carbons in the phenyl rings to be assigned. For example, the signals at δ 130.33 and 132.12 should be C-1′ and C-1″, and those at δ 144.29, 144.59, 146.74, and 146.86 should be C-3′, C-3″, C-4′, and C-4″. Selective INEPT irradiation of H-5″ (δ 6.839) enhanced the signals at δ 130.33, 144.29, and 146.74, permitting a distinction between the two ring systems, and irradiation in the methoxyl group region led to enhancement of the signals at δ 160.75 (C-2), 146.86, and 146.74. The latter signals can therefore be assigned to C-3′ and C-3″, respectively, while those at δ 144.59 and 144.29 are C-4′ and C-4″, respectively. In this way all of the carbon atoms in lareantin could be attributed unambiguously.

Larreantin (1) was evaluated in the P-388 cytotoxicity assay where an ED_{50} value of 0.38 μg/mL was observed. Compounds displaying an ED_{50} 4 μg/mL are regarded as active.[4] Studies of the remaining active compounds present in the plant are in progress.

Larreantin represents a new class of natural products in which it would appear biogenetically that two isomeric phenylpropene units have combined with a preformed benzoquinone to afford an intermediate which can undergo oxidation to the naphthoquinone nucleus. We know of no such precedent in Nature.

Experimental Section

Melting points were determined on a Kofler-type hot-stage apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Ultraviolet spectra were recorded with a Beckman Model DU-7 spectrophotometer and infrared spectra were obtained with a Nicolet MX-1 interferometer. Mass spectra were determined with a Varian MAT 112S double focusing mass spectrometer at 80 eV. The ^1H NMR spectra were obtained with either a Nicolet NMC 360 instrument operating at 360 MHz or a Varian XL-300 instrument operating at 300 MHz. Tetramethylsilane (TMS) was used as the internal standard and chemical shifts are reported in δ downfield from TMS.

Homonuclear COSY spectra were recorded at 1 K with a Varian XL 300 spectrometer. Standard Varian pulse sequences were used. NOE difference spectra were measured on a Nicolet NMC 360 spectrometer. The samples were degassed by using a repeated freeze–pump–thaw cycle and then closed under nitrogen. Data sets of 16K covering a spectral width of 2000 Hz were acquired. A 2.0-Hz line broadening was applied to the data prior to Fourier transformation.

The one-dimensional heteronuclear ^1H-^{13}C shift correlation (CSCM 1D) and selective INEPT experiments were performed on a Nicolet NMC 360 spectrometer. Data sets of 16K covering a spectral width of 10 MHz were acquired. Proton pulse widths were calibrated by using a sample of acetic acid in 10% C_6D_6 (^1J = 6.7 Hz) in a 5-mm NMR tube.[17] The radio frequency field strength for the soft proton pulse was on the order of 25 Hz for these experiments.

Plant Material. The roots of *L. tridentata* were collected by Wendy Hodgson, Desert Botanical Garden, Phoenix, AZ, in the vicinity of Phoenix, AZ, in July, 1986. The sample was identified by W. H. and also by Dr. D. D. Soejarto of this laboratory. Herbarium specimens representing the collection are deposited in the herbarium of the Desert Botanical Garden and in the John G. Searle Herbaium, Field Museum of Natural History, Chicago, IL.

Isolation of Larreantin (1). A sample (10 kg) of the root plant part was exhaustively extracted with petroleum ether and the marc extracted with MeOH. The residue after evaporation of the MeOH was chromatographed on Celite and the fraction (400 g, ED_{50} 0.57 μg/mL) eluting with $CHCl_3$/MeOH (4:1) was chromatographed on silica gel to afford 14 fractions. One of these fractions eluted with $CHCl_3$/MeOH (49:1) (ED_{50} 0.62 μg/mL) was further chromatographed on silica gel to afford 16 fractions. From the most polar of these, larreantin (1) was obtained as yellow needles having the following physical and spectroscopic properties: 650 mg, 0.0065%; mp 204–206 °C; IR ν_{max} (KBr) 3400, 1687, 1649, 1618, 1514, 1340, 1285, 1234, 1212, 1088 cm^{-1}; UV λ_{max} (MeOH) (log ϵ) 241.5 (4.27), 259 (4.40), 286 (4.42), 348 (3.61); ^1H NMR, see Table I; ^{13}C NMR, see Table I; mass spectrum, m/z (rel. intensity) 460 (M^+, 100), 445 (M^+ – 15, 8), 443 (M^+ – 17, 5), 429 (M^+ – 31, 12), 305 (2), 304 (3), 230 (5), 137 (11).

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