

CITLALITRIONE, A NEW DITERPENE FROM *JATROPHA DIOICA* VAR. *SESSILIFLORA*

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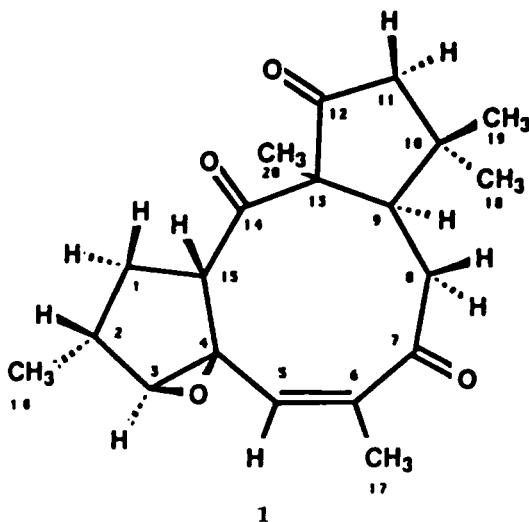
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ABSTRACT.—Solvent partitioning and column chromatography of MeOH root extracts of *Jatropha dioica* yielded four pure compounds, β -sitosterol, jatropholone B, a previously unknown diterpene epoxytrione given the trivial name citlalitrione [1], and riolozatrione. Structures were determined using physical and spectral techniques and X-ray crystallography.

Extracts of roots and stems of *Jatropha* species (Euphorbiaceae) have often been used in folk medicine, and studies of active materials from such extracts have resulted in several recent reports of biologically active diterpenoid natural products. The tricyclic dione alcohol jaherin from *Jatropha zeyberi* has antimicrobial properties (1). *Jatropha curcus*, which is used in Africa to prepare seed oil purgative mixtures, produces phorbol and lathyrane materials (2,3). *J. gossypifolia* is the source for the tumor inhibitors jatrophone (4) and its epoxy derivatives (5), as well as the inactive related compounds jatropholones A and B (6). *Jatropha macrorhiza* has been found to produce the interesting tricyclic diterpenoid jatrophatrione, which is inhibitory toward lymphocytic leukemia (7). Other Euphorbiaceae have been shown to have irritant and cocarcinogenic activity, due mainly to the presence of diterpene esters (8).

Our studies were of the species *Jatropha dioica* var. *sessiliflora* (Hook.) McVaugh, called "tlapelex patli" by the Aztecs. In northeastern Mexico extracts of this plant have long been used to treat toothache and skin cancer. In earlier works, the tricyclic diterpene riolozatrione (9) was isolated from root extracts of this plant and identified



through spectral and X-ray crystallographic data. We here report further research which resulted in identification of the new diterpene epoxide citlalitrione.

Chromatographic separation of MeOH extracts of macerated *J. dioica* roots yielded three products identified as β -sitosterol, jatropholone B, and riolozatrione by comparison of their physical and spectral qualities with those of authentic samples, together with citlalitrione (7). The structure of compound **1** was elucidated by the following spectral and X-ray crystallographic studies.

Eims indicated a possible molecular formula of $C_{20}H_{26}O_4$. Peaks produced by three carbonyl groups were present in the ir spectrum. 1H -nmr spectroscopy showed the presence of one vinyl proton, 4 singlet and 1 doublet methyl groups, and several methylene and methine protons whose coupling patterns are given in the Experimental. ^{13}C nmr revealed 20 non-equivalent carbons, including 3 ketone groups, 1 double bond, and possibly 1 epoxy function. A DEPT experiment revealed 5 CH_3 groups, 3 CH_2 groups, 5 CH groups, and 7 quaternary carbons. From 1H nmr it was determined that one singlet methyl group was located on a double bond, which from uv evidence was conjugated with one of the carbonyl groups. The vinyl proton chemical shift and the uv extinction coefficient indicated that physical constraints prevent full resonance. Data from the 1H spectra and COSY experiments were used in computer simulations that allowed coupling constants to be assigned. The structure **1**, an epoxy derivative of the previously identified diterpene jatrophaatrione (7), was tentatively assigned and then confirmed by X-ray crystallographic data. The molecular structure is illustrated in Figure 1. Further studies of the biological activity of the new compound are in progress.

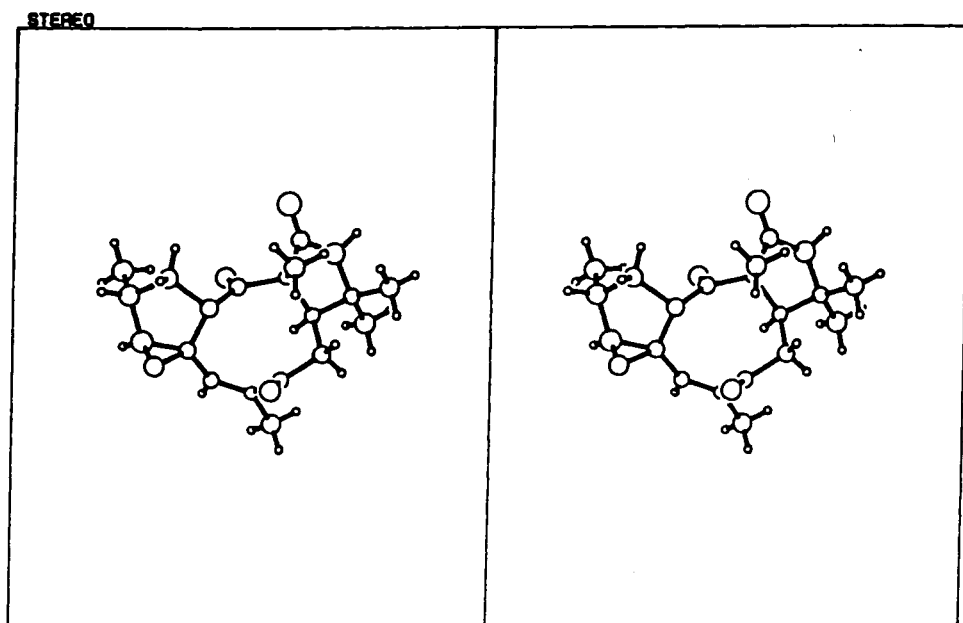


FIGURE 1. Stereo representation of citlalitrione [1].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected; ir spectra were recorded on a Beckman model 4244 instrument using KBr pellets; uv spectra were recorded on a Perkin-Elmer Lambda 3 instrument in EtOH solution; 1H - and ^{13}C -nmr spectra were recorded at 500 and 125 MHz on a Bruker AM 500 instrument in $CDCl_3$, Me_2CO-d_6 , or C_6H_6 as noted. Mass spectra were recorded on a VG Instruments VG 70C spectrometer with electron impact ionization at 70 eV. Analytical tlc was performed

on SiO₂-G (Merck) plates and visualized using uv light or CoCl₂. X-ray crystallography was performed using a Nicolet R3/m diffractometer.

PLANT MATERIAL.—*J. dioica* var. *sessiliflora* was collected in Reynosa, Tamaulipas, México during July 1986. Voucher specimen #7651 is deposited at the ITESM Herbarium.

EXTRACTION AND FRACTIONATION PROCEDURES.—Dried ground roots of *J. dioica* were extracted for 7 days with MeOH using a Soxhlet apparatus. On evaporation, the residue (30 g) was partitioned between CH₂Cl₂ (90 ml) and H₂O (30 ml). The CH₂Cl₂ solution was dried (Na₂SO₄) and evaporated, and the residue (15 g) was subjected to cc [Si gel (Merck), 50 × 5 cm]. Elution with hexane-CH₂Cl₂ (8:2) gave β-sitosterol: 45 mg, 0.009%, mp 138°, *R_f* 0.45 [C₆H₆-HOAc (9:1)]. Elution with hexane-CH₂Cl₂ (7.5:2.5) gave jatropholone B: 65 mg, 0.13%, recrystallized from MeOH/hexane, mp 220°, *R_f* 0.60 [C₆H₆-HOAc (9:1)]; [α]²⁵ [λ (α)] 589 (230.8), 578 (6.8), 546 (30.2), 436 (269.4) (*c* = 5 mg/ml, CHCl₃); uv λ max, (ε) 206 (44,400), 230 (15,984), 270 (9324), 310 (3996); ir 3290, 1685, 1595, 1570, 875, 860 cm⁻¹; ¹H nmr δ 0.81 (s, 3H), 1.25 (s, 3H), 1.30 (d, 3), 2.29 (s, 3H), 4.68 and 5.08 (s, 2H), 5.24 (s, 1H). Elution with hexane-CH₂Cl₂ (7:3) gave citralitrone [1] (136 mg, 0.02%) and riolozatrione { from hexane, colorless needles, mp 118–119°; *R_f* 0.46 [C₆H₆-Me₂CO (9:1)] (95 mg, 0.019%) lit. (9) }.

Citralitrone [1].—Recrystallized from MeOH/hexane, white needles, mp 194–196°, *R_f* 0.60 [C₆H₆-Me₂CO (9:1)], positive 2,4 DNP test, [α]²⁵ [λ (α)] 589 (−146.26), 578 (−0.40), 546 (−14.93), 436 (−195.46), 365 (−225.06) (*c* = 7.5 mg/ml, CHCl₃); uv λ max, (ε) 200 (42,075), 228 (21,450); ir 1740, 1700, 1685, 1380–1370, 1265–1050, 970–810 cm⁻¹; ms *m/z* (%) 315 (0.8), 302 (4.1), 206 (7.7), 178 (29.4), 164 (22.0), 121 (32.8), 108 (100), 41 (39.6); ¹H nmr (Me₂CO-*d*₆) δ 1.21 (H-1α), 1.85 (H-1β), 2.23 (H-2), 3.09 (H-3), 5.48 (H-5), 2.47 (H-8α), 2.50 (H-8β), 3.16 (H-9), 2.30, 2.37 (H-11α, H-11β), 3.55 (H-15), 1.10 (Me-16), 1.94 (Me-17), 0.96 and 1.30 (Me-18, Me-19), 1.37 (Me-20); *J*_{1α,1β} = −13.7, small (1α,2), 9.4 (1β,15), small (1β,16), small (2,3), 1.4 (2,15), 7.5 (2,16), small (5,15), 1.8 (5,17), −13.9 (8α,8β), 2.8 (8α,9), 14.1 (8β,9), −17.4 (11α,11β), small (11β,19); ¹³C nmr (C₆H₆-*d*₆) δ 216.8 (C-12 or C-14), 215.1 (C-12 or C-14), 208.1 (C-7), 144.9 (C-6), 128.9 (C-5), 72.5 (C-3), 67.9 (C-4 or C-13), 65.7 (C-4 or C-13), 55.5 (C-11), 52.9 (C-9), 46.8 (C-15), 38.1 (C-8), 37.1 (C-10), 35.0 (C-1), 34.3 (C-2), 27.8 (C-18 or C-19), 23.3 (C-18 or C-19), 20.3 (C-17), 16.2 (C-16), 14.9 (C-20).

TABLE 1. Atomic Coordinates (× 10⁴) and Equivalent Isotropic Displacement Parameters (Å² × 10³) for 1.

Atom	<i>x</i>	<i>y</i>	<i>z</i>	U(eq) ^a
O-1	12665(8)	9175(8)	8735(7)	36(3)
O-2	10257(8)	8993(8)	10675(7)	39(3)
O-3	7191(10)	7775(10)	6906(9)	56(3)
O-4	9352(9)	9450(9)	7094(7)	40(3)
C-1	11429(13)	7808(13)	7512(1)	35(4)
C-2	12366(14)	8626(13)	7007(11)	40(4)
C-3	12468(14)	8557(14)	7754(12)	44(4)
C-4	11453(12)	9559(12)	8474(10)	28(4)
C-5	11009(12)	10518(12)	8951(11)	24(3)
C-6	10167(10)	10595(11)	9678(8)	17(3)
C-7	9601(12)	9657(11)	10183(10)	25(3)
C-8	8234(11)	9457(12)	10105(10)	28(3)
C-9	7796(11)	9481(11)	9023(9)	22(3)
C-10	6399(11)	9616(11)	8904(10)	25(3)
C-11	6241(14)	9258(14)	7801(12)	46(4)
C-12	7226(13)	8465(12)	7551(11)	32(4)
C-13	8221(11)	8500(11)	8344(10)	20(3)
C-14	9441(12)	8861(12)	7831(11)	28(3)
C-15	10633(12)	8498(12)	8287(10)	29(4)
C-16	11994(14)	9087(13)	5986(11)	44(4)
C-17	9760(13)	11722(11)	10091(11)	38(4)
C-18	5683(13)	8832(12)	9542(11)	35(4)
C-19	5983(14)	10812(12)	9084(12)	42(4)
C-20	8337(13)	7401(12)	8842(11)	37(4)

^aEquivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.

TABLE 2. Bond Lengths (Å) and Angles (°) for **1**.

Bond lengths			
O-1-C-3	1.415(18)	C-1-C-4	1.445(15)
O-2-C-7	1.264(15)	O-3-C-12	1.205(16)
O-4-C-14	1.225(16)	C-1-C-2	1.578(19)
C-1-C-15	1.593(19)	C-2-C-3	1.514(21)
C-2-C-16	1.537(20)	C-3-C-4	1.472(19)
C-4-C-5	1.412(19)	C-4-C-15	1.585(19)
C-5-C-6	1.345(17)	C-6-C-7	1.459(18)
C-6-C-17	1.539(18)	C-7-C-8	1.515(18)
C-8-C-9	1.533(17)	C-9-C-10	1.542(18)
C-9-C-13	1.568(18)	C-10-C-11	1.555(20)
C-10-C-18	1.499(18)	C-10-C-19	1.538(19)
C-11-C-12	1.479(19)	C-12-C-13	1.523(18)
C-13-C-14	1.562(18)	C-13-C-20	1.495(18)
C-14-C-15	1.504(18)		
Angles			
C-4-O-1-C-3	61.9(9)	C-15-C-1-C-2	107.8(11)
C-3-C-2-C-1	103.2(12)	C-16-C-2-C-1	116.2(1)
C-16-C-2-C-3	110.0(12)	C-2-C-3-O-1	112.8(13)
C-4-C-3-O-1	60.0(9)	C-4-C-3-C-2	112.5(13)
C-3-C-4-O-1	58.0(8)	C-5-C-4-O-1	117.9(12)
C-5-C-4-C-3	123.9(14)	C-15-C-4-O-1	107.1(11)
C-15-C-4-C-3	108.6(12)	C-15-C-4-C-5	123.0(11)
C-6-C-5-C-4	128.4(13)	C-7-C-6-C-5	125.0(12)
C-17-C-6-C-5	121.4(12)	C-17-C-6-C-7	113.6(10)
C-6-C-7-O-2	119.9(11)	C-8-C-7-O-2	119.5(12)
C-8-C-7-C-6	120.6(12)	C-9-C-8-C-7	111.6(11)
C-10-C-9-C-8	114.1(11)	C-13-C-9-C-8	116.6(11)
C-13-C-9-C-10	108.2(11)	C-11-C-10-C-9	100.3(12)
C-18-C-10-C-9	112.9(12)	C-18-C-10-C-11	108.2(12)
C-19-C-10-C-9	112.0(12)	C-19-C-10-C-11	112.4(12)
C-19-C-10-C-18	110.6(11)	C-12-C-11-C-10	108.5(13)
C-11-C-12-O-3	126.2(14)	C-13-C-12-O-3	123.1(14)
C-13-C-12-C-11	109.9(12)	C-12-C-13-C-9	102.7(10)
C-14-C-13-C-9	107.3(10)	C-14-C-13-C-12	107.8(11)
C-20-C-13-C-9	116.0(11)	C-20-C-13-C-12	110.4(12)
C-20-C-13-C-14	112.0(11)	C-13-C-14-O-4	117.0(13)
C-15-C-14-O-4	124.6(13)	C-15-C-14-C-13	118.4(11)
C-4-C-15-C-1	102.8(10)	C-14-C-15-C-1	110.9(11)
C-14-C-15-C-4	108.4(11)		

X-RAY DATA¹.—A box-shaped, colorless crystal (0.2 mm × 0.3 mm × 0.4 mm) was mounted on a glass fiber in a random orientation. Preliminary examination and data collection were performed with MoK α radiation on a NICOLET R3m diffractometer ($\lambda = 0.71073 \text{ \AA}$). Cell constants were obtained from the least-squares refinement of the setting angles of 25 reflections ($2\theta_{\text{avg}} = 19.87^\circ$). Omega scans of several intense reflections were less than 0.3° at half-height, indicating good crystal quality. Examination of the cell constants indicated an orthorhombic system. Axial photos verified the Laue group assignment of mmm. Systematic absences indicated that the space group was $P2_12_12_1$. The data were collected at $-100 \pm 1^\circ$ using θ - 2θ scanning techniques with a variable scan rate. The scan range was determined as a

¹Atomic coordinates for compound **1** have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

function of $\theta_{K\alpha 1}$ and $\theta_{K\alpha 2}$. The diameter of the beam collimator was 1.5 mm. A total of 1869 reflections were collected of which 65 reflections were standards. Lorentz and polarization corrections were applied to the data. No absorption correction was applied. The intensity of the data decayed 20% during data collection, and data were adjusted by fitting to a correction curve based upon the intensity of three standard reflections which were collected every 97 reflections.

The molecular structure was solved by direct methods [SHELXTL-PLUS¹] employing 1785 unique reflections of which 959 were flagged as observed (minimum E of 1.2) and 3453 triple phase relationships. All atoms were located in the resulting E-map and were included for future refinement. Hydrogen atoms were placed in idealized positions and their individual isotropic thermal motion was fixed at 0.05. The structure was refined by full-matrix least squares methods. All non-hydrogen atoms were refined isotropically. Scattering factors were taken from Cromer and Waber (10). Only 960 reflections with structural factors 4.0 times their standard deviation were employed in the least squares calculations. The weight assigned to each reflection was based upon the equation $w = 1/[\sigma^2(F) + g(F^2)]$ where g was fixed at 0.001. The largest peak in the final Fourier map was $0.6 e^{-\text{\AA}^{-3}}$ while the smallest peak was $-0.4 e^{-\text{\AA}^{-3}}$. The atomic coordinates for non-hydrogen atoms are listed in Table 1. Table 2 lists bond lengths and angles.

Details of the crystallographic experiment and computation are as follows: $C_{20}H_{26}O_4$, MW 330.4 g/mol; crystal system orthorhombic; space group $P2_12_12_1$; lattice constants (a, b, c) 10.910(2), 12.107(4), 13.453(4) Å; V 1776(1) Å³; Z 4; ρ (calculated) 1.23 g/cm³; radiation MoK α ($\lambda = 0.71073$ Å); graphite monochromator; μ 0.37 cm⁻¹; scan type θ - 2θ ; bisecting geometry; scan speed variable 2.0–40.0°/min; 2θ range 3.5–50.0°; index restrictions $-13 \leq h \leq 10$, $-15 \leq k \leq 0$, $-16 \leq l \leq 0$; total reflections 1869, unique observed reflections 960; observed criterion $|F| > 4.0\sigma|F|$; final data parameter ratio 8.5; $R = 0.110$; $wR = 0.119$; $s = 2.02$; $g = 0.001$ (fixed).

Assignment of the absolute configuration shown is by analogy (4,7) and awaits experimental confirmation.

ACKNOWLEDGMENTS

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¹Software used for diffractometer operations was provided with the NICOLET R3/m diffractometer. All crystallographic computations were carried out using the SHELXTL-PLUS program library (written by G.M. Shellrick and supplied by NICOLET XRD for the micro VAXII computer) in the crystallographic laboratory at Texas A&M University.