the ratios of shifts in parameters to estimated standard deviations were all less than 0.2. The refinement was based on F_o, the quantity minimized being $\Sigma w(F_o - F_c)^2$. Unit weights were used. The scattering factors used were those of Hanson, Herman, Lea, and Skillman.[16]

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Supplementary Material Available. Tables of temperature factors and bond distances and angles involving hydrogens (3 pages). Ordering information is given on any current masthead page.

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(18) Of the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md.
(19) Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. ^{1}H NMR, ir, uv, and mass spectra were determined using a Varian T-60 spectrometer, a Beckman IR-33, a Beckman DBG, and a Hitachi Perkin-Elmer double focusing spectrometer (Model RMU-6-E), respectively. The melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

Antitumor Agents from *Jatropha macrorhiza* (Euphorbiaceae). II. Isolation and Characterization of Jatrophatrione[1]

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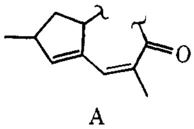
As a result of the continuing search for plants having tumor-inhibiting constituents, the chloroform extract of the roots of *Jatropha macrorhiza* Benth. (Euphorbiaceae)[2] was found to possess inhibitory activity toward the P-388 (3PS) lymphocytic leukemia test system.

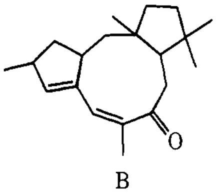
Discussion

One constituent of the chloroform extract of *Jatropha macrorhiza* Benth. roots is the new diterpene jatrophatrione, $C_{20}H_{26}O_3$, subsequently shown to be I. The initial spectral data (ir, uv, ^{1}H NMR) of jatrophatrione immediately led to the conclusion that jatrophatrione was structurally related to jatrophone (II, $C_{20}H_{24}O_3$), previously isolated from *Jatropha gossypiifolia*.[4] Specifically, the partial structure A appeared to be a common feature of the two diterpenes. Table I shows the nearly identical spectral data for jatrophatrione (I) and jatrophone (II) generated by partial structure A.



A

Assuming a close biogenetic and structural relationship to jatrophone (II), the rest of the constitution of jatrophatrione (I) was deduced. The two double bonds in partial structure A accounted for all of the vinylic carbon atoms (^{13}C NMR), and with two more carbonyl groups (ir, ^{13}C NMR) there had to be one more ring. That the latter is five membered could be seen in the infrared spectrum at 1746 cm^{-1} (cyclopentanone) and a quaternary methyl group in the ^{1}H NMR spectrum (δ 1.47) dictated that the closure of this ring be as shown in partial structure B. The downfield position of this angular methyl group suggested that it is flanked by both remaining carbonyl groups, as in structure I. Scheme I shows how the tricyclic structures I (jatrophatrione) and II (jatrophone) may be derived in nature from bicyclic precursor III; steps to this precursor from geranylgeranyl pyrophosphate via casbene oxidation product IV are readily imagined (cf. ref 5).



B

An x-ray study on jatrophatrione (I) confirmed the proposed constitution and showed the relative configurations to be as depicted. The absolute configuration was not determined crystallographically but is based on the assumption that jatrophatrione (I) and jatrophone (II) possess the same configuration at C2.[4] Bond distances are given in Table II and bond angles in Table III.

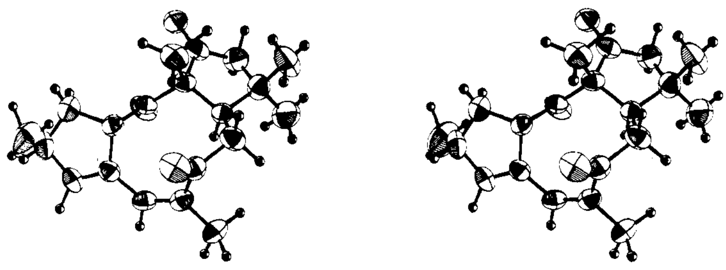


Figure 1. Stereoscopic view of jatrophatrione (I).

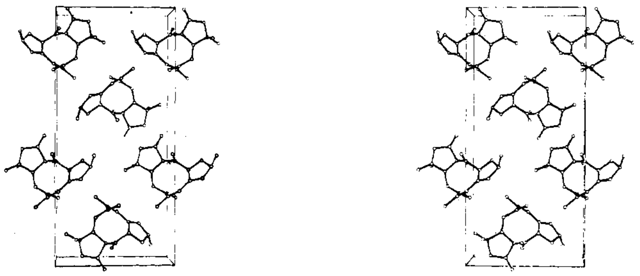


Figure 2. Stereoscopic view of a unit cell, *a* axis projection, with the *c* axis vertical and the *b* axis horizontal.

Scheme I. Possible Biological Route to Jatrophatrione (I) and Jatrophone (II) from Bicyclic Precursor III

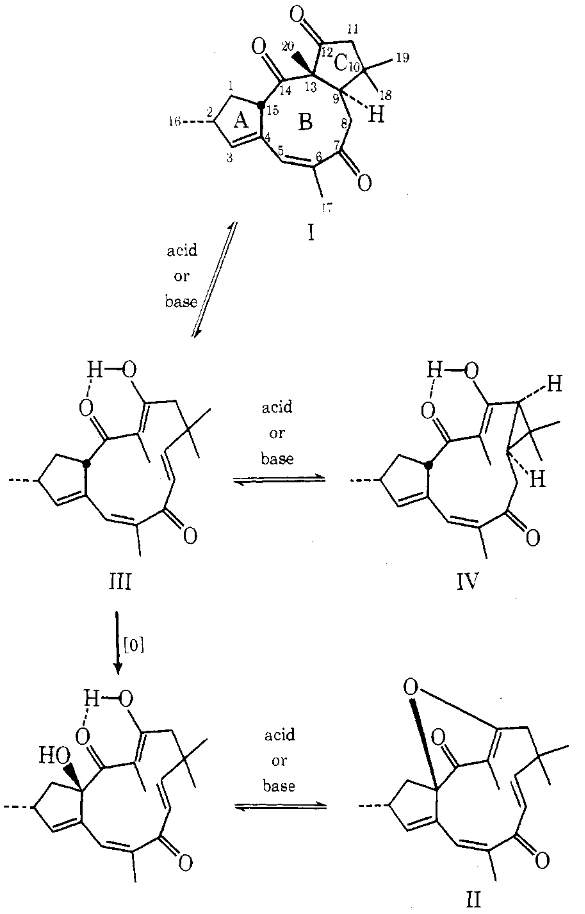


Table I. A Comparison of Some Spectral Data for Jatrophatrione and Jatrophone

	Jatrophatrione	Jatrophone
Ir	1690 cm^{-1}	1690 cm^{-1}
Uv	280 nm	285 nm
^1H NMR	Vinyl protons δ 5.82, 6.08	δ 5.86, 6.04
	Vinyl methyl δ 1.93	δ 1.83

Table II. Intramolecular Bond Distances, with Estimated Standard Deviations in Parentheses

Bond	Distance, Å
O1–C14	1.200 (6)
O2–C12	1.200 (7)
O3–C7	1.226 (7)
C1–C2	1.499 (9)
C1–C15	1.557 (7)
C2–C3	1.476 (8)
C2–C16	1.547 (11)
C3–C4	1.319 (7)
C4–C5	1.474 (7)
C4–C15	1.524 (7)
C5–C6	1.327 (8)
C6–C7	1.503 (9)
C6–C17	1.510 (8)
C7–C8	1.504 (8)
C8–C9	1.514 (7)
C9–C10	1.563 (8)
C9–C13	1.560 (7)
C10–C11	1.529 (8)
C10–C18	1.496 (10)
C10–C19	1.541 (11)
C11–C12	1.460 (8)
C12–C13	1.535 (8)
C13–C14	1.540 (8)
C13–C20	1.564 (8)
C14–C15	1.523 (7)

The molecular conformation is depicted in Figure 1. The most striking feature is the lack of conjugation between the diene system and the C7–O3 carbonyl group: torsion angle C5-C6-C7-O3 is 61.0°. Apparently nonbonded steric interactions and angle strain cause conformations which would provide better overlap between these π systems to be of higher energy. A similar torsion angle is observed between the corresponding groups in jatrophone (II).[6] The conjugated diene system in I is approximately transoid with a twist of 15.5° from coplanarity, based on the C3–C4–C5–C6 torsion angle. Torsion angles around the rings (Table IV) show a nearly planar A ring, an irregularly puckered B ring, and an envelope conformation[7] with C10 at the point for the C ring. Figure 2 shows the molecular packing in the crystal.

Jatrophatrione (I) demonstrated activities of 130% test/control (T/C) and 141% T/C at 1 and 0.5 mg/kg, respectively, in the 3PS system. Activity in the 3PS is defined as an increase in the survival of treated animals over that of controls resulting in a T/C ⩾ 125%.[8]

Jatrophatrione (I) is thus active, even though it lacks the C8–C9 double bond to which thiols add nucleophilically in jatrophone (II).[9] An alternative conjugate addition at C3 or C5 is rendered unlikely in view of the lack of conjugation between the diene system and the attached carbonyl group (see above); indeed, under conditions under which *n*-propyl mercaptan adds in good yield to the C8–C9 double bond of jatrophone (II),[9] *n*-butyl mercaptan does not react appreciably with jatrophatrione (I). Possibly the activity of I is due to its ability to undergo reverse Michael addition to give III, which can undergo addition similar to that of II.

Experimental Section[3]

Extraction Procedure. The fresh ground roots (200 lb) of *Jatropha macrorhiza* were extracted exhaustively in a Lloyd-type extractor with ethanol. The solvent from the ethanol extract was removed in air and the residue partitioned between chloroform and water (1:1). After the layers had been separated, the chloroform was removed in air and this resulted in 130.3 g of residue.

Isolation of Jatrophatrione (I). The residue from the chloroform phase (above, 100 g) was stirred vigorously with ether (1.4 l.) and filtered. This provided 7 g of ether-insoluble residue, and after removal of the solvent in vacuo, 92 g of ether-soluble material. The ether-soluble fraction (50 g) was then chromatographed over silica gel 60 (E. Merck, 850 g, 55 × 840 mm). Elution of the column was begun with benzene and continued with increasing amounts of chloroform in

Table III. Bond Angles with Estimated Standard Deviations in Parentheses

Atoms	Angle, deg
C2–C1–C15	108.3 (5)
C1–C2–C3	104.2 (5)
C1–C2–C16	113.9 (5)
C3–C2–C16	112.7 (5)
C2–C3–C4	113.6 (4)
C3–C4–C5	124.0 (4)
C3–C4–C15	111.8 (4)
C5–C4–C15	124.0 (4)
C4–C5–C6	130.6 (4)
C5–C6–C7	123.1 (5)
C5–C6–C17	122.5 (4)
C7–C6–C17	114.3 (4)
C6–C7–C8	118.1 (5)
C6–C7–O3	119.6 (4)
C8–C7–O3	122.4 (4)
C7–C8–C9	111.8 (4)
C8–C9–C10	114.9 (4)
C8–C9–C13	116.5 (4)
C10–C9–C13	105.5 (4)
C9–C10–C11	101.9 (5)
C9–C10–C18	109.9 (5)
C9–C10–C19	113.1 (5)
C11–C10–C18	112.5 (6)
C11–C10–C19	111.7 (5)
C10–C11–C12	106.9 (5)
C11–C12–C13	110.0 (4)
C11–C12–O2	128.3 (5)
C13–C12–O2	121.7 (4)
C12–C13–C14	109.1 (4)
C12–C13–C20	106.7 (4)
C14–C13–C20	110.3 (4)
C12–C13–C9	103.5 (4)
C14–C13–C9	109.0 (4)
C20–C13–C9	117.7 (4)
C13–C14–C15	121.4 (4)
C13–C14–O1	118.2 (4)
C15–C14–O1	120.3 (4)
C14–C15–C1	110.5 (4)
C14–C15–C4	111.6 (4)
C1–C15–C4	101.6 (4)

Table IV. Torsion Angles in the Rings of Jatrophatrione (I)

	Atoms	Angle, deg
Ring A	C1–C2–C3–C4	5.3
	C2–C3–C4–C15	−1.7
	C3–C4–C15–C1	−2.4
	C4–C15–C1–C2	5.5
	C15–C1–C2–C3	−6.5
Ring B	C4–C5–C6–C7	9.5
	C5–C6–C7–C8	−120.8
	C6–C7–C8–C9	61.2
	C7–C8–C9–C13	62.0
	C8–C9–C13–C14	−93.6
	C9–C13–C14–C15	96.0
	C13–C14–C15–C4	−121.1
	C14–C15–C4–C5	54.7
	C15–C4–C5–C6	21.2
Ring C	C9–C10–C11–C12	33.6
	C10–C11–C12–C13	−21.1
	C11–C12–C13–C9	−0.7
	C12–C13–C9–C10	21.6
	C13–C9–C10–C11	−33.7

benzene. Elution with 100% chloroform produced a semicrystalline fraction (7.0 g) which was recrystallized from ether–hexane. This gave jatrophatrione (I, 450 mg) and another recrystallization resulted in pure material as colorless spears, mp 148–150 °C (starts to sweat at 137 °C). Jatrophatrione (I) is optically active, $[\alpha]^{25}\mathrm{D}$ −187° (*c* 0.2, $CHCl_3$). The infrared [($CHCl_3$) 1746, 1690, 1640, and 1632 cm^{-1}], ultraviolet [(EtOH), λ_{max} 280 (log ϵ 3.49), 244 (shoulder, 3.95), and 223 nm (4.04)], ^1H NMR [($CDCl_3$) δ 0.91 (3 H, s), 1.11 (3 H, d, J = 7 Hz), 1.23 (3 H, s), 1.47 (3 H, s), 1.93 (3 H, br s), 2.2–3.0 (8 H, complex), 4.08 (1 H, br t, J = 7 Hz), 5.82 (1 H, m), and 6.08 (1 H, m)], ^{13}C NMR [($CDCl_3$) δ 13.0 (q), 19.2 (2 carbons, both q), 21.6 (q), 25.6 (q), 34.1 (t), 34.5 (t), 35.2 (s), 36.6 (d), 46.8 (d), 47.2 (d), 51.0 (t), 59.1 (s), 116.7 (d) 124.5 (s), 126.8 (s), 131.8 (d), 193.8 (s), 196.2 (s) and 198.8 (s)], and mass [*m/e* 314 (M^+), 286, 256, 162 (base), 147, 120, 105, 91, 79, 69, and 55] spectra were in complete agreement with structure I.

Anal. Calcd for $C_{20}H_{26}O_3$: C, 76.40; H, 8.33. Found: C, 76.49; H, 8.53.

Crystallographic Study of Jatrophatrione (I). Colorless crystals of I were grown from ether–petroleum ether. A needle 0.1 × 0.2 × 1.2 mm was mounted with the *a* axis parallel to the goniostat ϕ axis. The space group was found to be $P2_12_12_1$ by a combination of film and counter methods. The cell lengths were found using nine reflections on a Picker FACS-I diffractometer (Cu Kα, λ = 1.54178 Å, graphite monochromator) to be a = 6.731 (2), b = 11.149 (4), c = 24.188 (9) Å. The crystal density was measured by flotation in aqueous KI as 1.13 g/ml, in agreement with a calculated density of 1.15 g/ml assuming four molecules in the unit cell. Intensity data were collected using a scintillation counter with pulse-height analyzer, θ–2θ scan technique, 2°/min scan rate, 10-s background counts, attenuators when the count rate exceeded 10^4 counts/s, and 2° scan range with a dispersion factor allowing for α_1–α_2 splitting at large 2θ values. Of 1644 independent reflections measured, 1267 > $3\sigma(I)$ were considered observed. No decrease in intensity of standard reflections was observed, and no correction was made for absorption.

The structure was solved using MULTAN.[10] All nonhydrogen atoms were located on the first E map. Full-matrix least-squares refinement with isotropic thermal parameters reduced $R = [\Sigma w(F_o - |F_c|)^2/\Sigma w F_o^2]^{1/2}$ to 0.128, and with anisotropic, to 0.096. A difference map revealed all of the hydrogen positions. Further refinement using anisotropic temperature factors for nonhydrogen atoms and isotropic temperature factors (of nonhydrogen atoms to which they were attached) for hydrogens reduced R to its final value of 0.048. Refinement was based on F_o, the quantity minimized being $\Sigma w(F_o - |F_c|)^2$, with unit weights. The scattering factors used were those of Hanson, Herman, Lea, and Skillman.[11]

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Registry No.—I, 58298-76-3.

Supplementary Material Available. Tables of atomic coordinates and temperature factors (3 pages). Ordering information is given on any current masthead page.

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(2) Identification was confirmed by R. M. Wiedhopf, College of Pharmacy, and Dr. Charles T. Mason, Botany Department, University of Arizona, Tucson, Ariz. A reference specimen was deposited in the University of Arizona Herbarium.

(3) Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. Mass, ^{13}C NMR, ^1H NMR, uv, and ir spectra were recorded using a Hitachi Perkin-Elmer double focusing spectrometer (Model RMU-6E), a Bruker WH-90 (22.6 MHz) spectrometer, a Varian T-60 spectrometer, a Beckman DB-G spectrophotometer, and a Beckman IR-33, respectively. The optical rotation was obtained on a Rudolph Model 70 polarimeter and melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

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