Structure and Stereochemistry of Jatrophone, a Novel Macrocyclic Diterpenoid Tumor Inhibitor[1,2]

S. Morris Kupchan,* Carl W. Sigel, Marilyn J. Matz, Christopher J. Gilmore, and Robert F. Bryan*

Contribution from the Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901. *Received July 29, 1975*

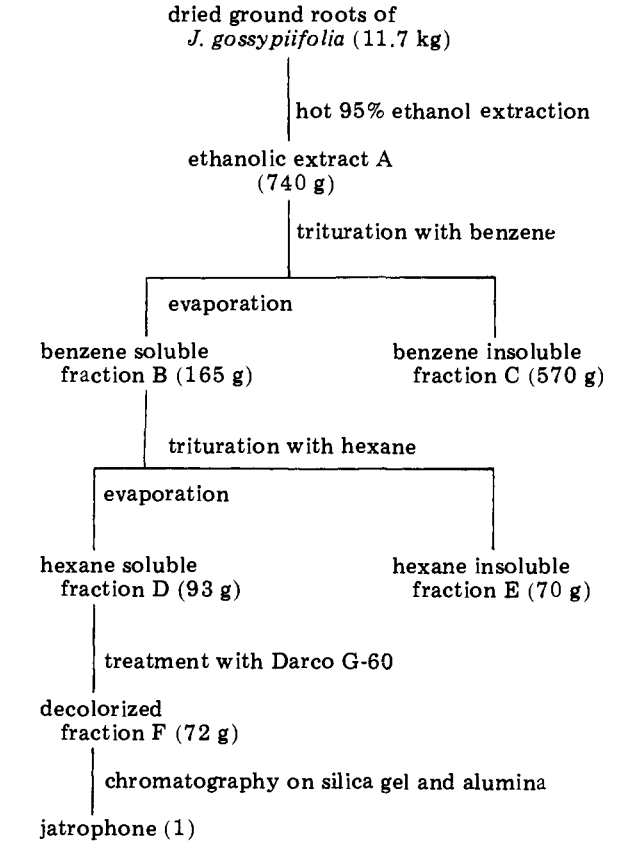
Abstract: The isolation and elucidation of the structure and stereochemistry of an antileukemic macrocyclic diterpenoid, jatrophone (**1**), are reported. Mass spectrometry and elemental analysis supported a $C_{20}H_{24}O_3$ formula. Ketalization of **1** afforded ketal **3** which established the presence of a 2,3-dihydrofuran-3-one ring system in **1**. On treatment with dry hydrobromic acid in acetic acid, **1** yielded the bis(hydrogen bromide) adduct **6** which on treatment with alumina was converted back to **1**. The structure and absolute stereochemistry of **6** were established by x-ray crystallographic analysis. From the spectral properties of jatrophone and its reversible interrelation with **6**, structure **1**, with uncertainty only about the stereochemistry of the 5,6-double bond, could be derived for jatrophone. The probable stereoelectronic course of formation of adduct **6** (via **9** → **11**) favored assignment of the cis configuration to the 5,6-double bond. This stereochemistry and the absolute configuration of jatrophone were established by direct single-crystal x-ray analysis. The reaction of jatrophone with thiols and the possible significance of this reaction for the mechanism of action of jatrophone are discussed.

Extracts of *Jatropha gossypiifolia* L. (Euphorbiaceae) and related species have been used for many years to treat cancerous growths.[3] In the course of a continuing search for tumor inhibitors of plant origin, we found that an alcoholic extract of *J. gossypiifolia*[4] showed significant inhibitory activity in vitro against cells derived from human carcinoma of the nasopharynx (KB) and in vivo against four standard animal tumor systems.[5] We report here in detail the systematic fractionation of the active extract and the isolation and characterization of jatrophone (**1**), a novel macrocyclic diterpenoid tumor inhibitor.[6,7] In addition, the direct x-ray crystallographic analysis of jatrophone is described.

Fractionation of the ethanol extract, summarized in Chart I, was guided by the KB assay.[5] Results of a preliminary fractionation suggested that the active principle might be unstable, and, as a result, mild procedures were subsequently followed. Trituration of the alcoholic extract with benzene followed by trituration of the benzene solubles with hexane afforded a cytotoxic hexane-soluble fraction. Treatment with Darco G-60 followed successively by rapid chromatography on silica gel and neutral alumina (activity III) yielded a fraction which crystallized from hexane to afford jatrophone (**1**).

On the basis of elemental analyses and high resolution mass spectrometry, jatrophone was assigned the molecular formula $C_{20}H_{24}O_3$. The ultraviolet spectrum exhibited absorption at 285 nm, and the infrared spectrum showed bands at 5.90 and 6.05 μ which were suggestive of a highly conjugated carbonyl system. The infrared spectrum also showed a very strong band at 6.20 μ, indicative of an enolic double bond. To obtain more information about the oxygen moieties, several exploratory reactions were tried. Attempts to make the usual carbonyl derivatives (e.g., hydrazones and semicarbazones) were unsuccessful, and it soon became apparent that alkaline treatment converted jatrophone into intractable mixtures. In contrast, the compound appeared to be reasonably stable in acidic media. Ketalization with ethylene glycol and *p*-toluenesulfonic acid proceeded slowly, and optimum yields of the two ketals **2** and **3** were obtained only after heating under reflux for 4 days. Ketal **2** showed spectral characteristics very similar to **1**, with the additional signals in the NMR spectrum assignable to an ethylenedioxy group. Since the ultraviolet spectrum still showed the presence of the 285 nm chromophore, the highly conjugated ketone remained, indicative that a second ketone was present in jatrophone. The spectral data for ketal **3**

Chart I. Fractionation of the Cytotoxic Extract from *Jatropha gossypiifolia*

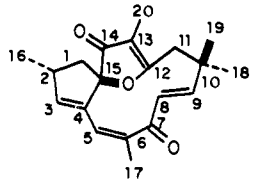


were markedly different from those of jatrophone and were suggestive of a rearranged carbon skeleton. The infrared and ultraviolet spectra of **3** no longer showed bands for the highly conjugated carbonyl system. The infrared spectrum showed only one carbonyl band at 5.70 μ, indicative of a ketone in a five-membered ring. The most likely precursor for this system was considered an α,β-unsaturated ketone in a five-membered ring which had become saturated during an acid-catalyzed ring closure.

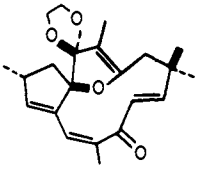
Further studies of the reactions of jatrophone under acidic conditions led to the preparation of several crystalline derivatives. Treatment of jatrophone (**1**) with a solution of glacial acetic acid saturated with dry HCl afforded the

bis(hydrogen chloride) adducts **4** and **5**. Realizing the potential of this reaction for providing a heavy atom x-ray derivative, jatrophone was treated with dry HBr in glacial acetic acid, and the adduct **6** was obtained in good yield.

Elemental analysis and mass spectrometry supported a $C_{20}H_{26}Br_2O_3$ formula for **6**, which substantiated that it was a bis(hydrogen bromide) adduct. Spectral characterization of the adduct led to the conclusion that extensive rearrangement of the jatrophone skeleton had occurred. All of the methyl group resonances were found to be higher than τ 8.6 and only one olefinic proton signal was observed in the NMR spectrum. The infrared spectrum of **6** showed a band at 5.85 μ which suggested the presence of a six-membered ring ketone, and a band at 2.75 μ indicated that a carbonyl group in **1** had been converted to a hydroxyl group.



1



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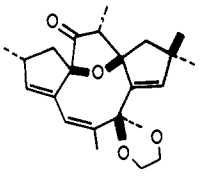

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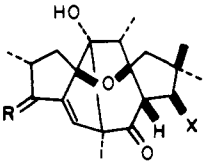
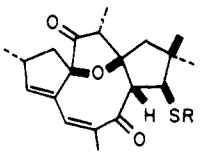


4, X = Cl; R = 

5, X = Cl; R = 

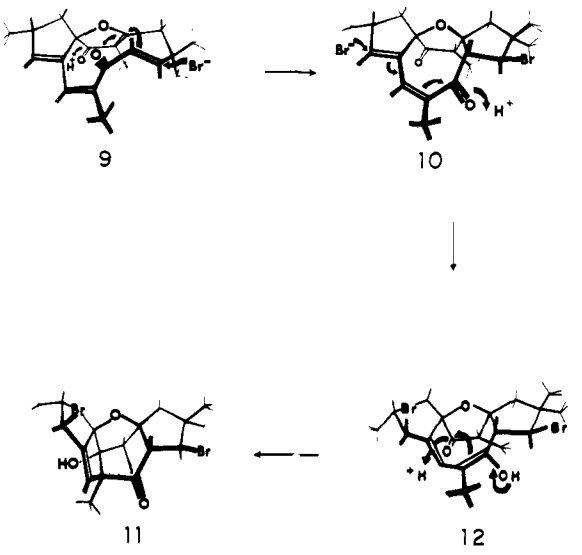
6, X = Br; R = 



7, R = $CH_2CH_2CH_3$
8, R = protein

The ultraviolet spectrum of **6** in ethanol no longer showed bands for the highly conjugated system of jatrophone, but when a drop of sodium hydroxide solution was added to the uv cuvette, the 285-nm chromophore present in jatrophone reappeared immediately. It was found in a subsequent experiment that when a solution of **6** was stirred with neutral alumina, jatrophone could be isolated in good yield. The reversible interrelation of jatrophone and the bis(hydrogen bromide) adduct made the latter derivative an attractive target for x-ray crystallographic analysis, and its structure was established as **6** by that method.[7,8]

On the bases of the spectral properties of jatrophone and its reversible interrelation with **6**, structure **1**, with uncertainty only about the stereochemistry of the 5,6-double bond, could be derived for jatrophone. The ready formation of the bis(hydrogen bromide) adduct was initially envisioned as a result of two transannular conjugate addition reactions: protonation of the C(14) ketone with nucleophilic attack by bromide ion at C(9) to form the 8,12-bond, followed by attack of a second bromide ion at C(3) in an acid-catalyzed ring closure of the 6,14-bond.[7] However, subsequent critical reexamination of molecular models led to the proposal of the stereoelectronic course represented in Figure 1,[9,10] and involving the 5,6-*cis* structure **9**. In this structure, nucleophilic attack by bromide ion at C(9) drives C(8) across the open top face of the molecule to a position favoring ring closure to form the monoadduct **10**. Stereochemical considerations favor the subsequent conjugate addition of hydrogen bromide to the 3,7-dienone system and transannular carbon–carbon bond formation via the enol **12**.



9 10 11 12

Figure 1. Stereoelectronic course of addition of hydrogen bromide to jatrophone.

Nuclear magnetic double resonance studies on jatrophone provided further support for the structure **1** and made it possible to assign all of the signals in the NMR spectrum (see Table I). Irradiation of the multiplet (C(2) H) at τ 7.09 caused collapse of the following signals: two doublets of doublets (C(1) protons), at τ 7.87 (J = 7, 12 Hz) and at τ 7.99 (J = 5.5, 12 Hz), a doublet (C(16) methyl) at τ 9.07, and a multiplet (C(3) H) at τ 4.14. Irradiation of the olefinic proton (C(5) H) at τ 3.96 caused the doublet at τ 8.17 (C(17) methyl) to collapse. Finally, irradiation of the doublet at τ 7.12 (J = 14 Hz) caused collapse of the doublet at τ 7.61 (J = 14 Hz). These two signals represent the geminally coupled C(11) protons.

The formation of ketal **3** is envisioned as occurring by a mechanism similar to that for the generation of adduct **6**. Protonation of the C(14) ketone with nucleophilic attack by ethylene glycol at C(9) and 8,12-bond formation is followed by a β-elimination of the glycol and ketalization. The assignment of the diene system to the Δ^3,Δ^5 positions appears most consistent with the NMR spectrum. The signal assigned to the C(2) proton is a very broad multiplet which appears to be coupled to protons in addition to the C(16) methyl and C(3) olefinic protons. This point has not been proven unambiguously, since the protons assigned to the C(1) position are overlapped with other signals in the methine region, and double resonance studies have failed to clear up this region. At any rate, the important structural feature of the ketal **3** is the presence of the tetrahydrofuran-3-one ring system, which supports the presence of a 2,3-dihydrofuran-3-one ring system in jatrophone.

The structures of the bis(hydrogen chloride) adducts have been assigned by comparison of the NMR spectra with that of **6**. The spectrum of the more polar adduct **4** is almost superimposable with that of **6**, and **4** is assumed to have an analogous structure. The NMR spectrum for the less polar adduct is consistent with the C(3) epimeric structure **5** (see Table I).

Although the cis configuration was favored for the 5,6-

Table I. Nuclear Magnetic Resonance Data[a]

Compd	C(1)	C(2)	C(3)	C(5)	C(8)	C(9)	C(11)	C(16)	C(17)	C(18) or C(19)	C(20)	Other
1[b]	7.87 dd(7, 12) 7.99 dd(5.5, 12)	7.09 m	4.14 m	3.96 m	3.74 d(15)	3.25 d(15)	7.12 d(14) 7.61 d(14)	9.07 d(7)	8.17 d(2)	8.67 s 8.80 s	8.18 s	
2[c]		6.95 m	4.18 m	4.10 m	4.40 d(16)	3.92 d(16)	7.75 d(12) 8.13 d(12)	9.04 d(7)	8.28 d(1.5)	8.95 s 9.09 s	8.42 s	6.47 m, ethyl enedioxy
3[d]		7.2 m	4.03 brd(4)	4.15 brs		4.22 s	7.75 d(14) 7.90 d(14)	8.73 d(7)	8.21 brs	8.82 s 8.85 s	8.90 d(7)	6.25 m, ethyl enedioxy
4[c]			5.14 dd(8,2)	4.46 d(2)	6.38 d(11)	6.26 d(11)		9.00 d(7)	8.75 s or 9.15 s	8.75 s(6H) or 9.15 s	9.55 d(7)	
5[c]		7.10 m	6.13 d(5)	5.14 s	6.31 d(11)	5.90 d(11)		9.18 d(7)	8.70 s or 8.74 s or 9.08 s	8.70 s or 8.74 s or 9.08 s	9.49 d(7)	
6[c]		7.00 m	6.06 d(5)	5.20 s	6.28 d(11)	5.75 d(11)		9.18 d(7)	8.62 s or 8.78 s or 9.09 s	8.62 s or 8.78 s or 9.09 s	9.72 d(7)	
7			3.73 m	4.30 brs	6.70 d(13)	6.01 d(13)		8.87 d(7) or 9.13 d(7)	8.10 brs	8.85 s or 8.89 s	8.87 d)7) or 9.13 d(7)	

[a] Spectra were determined on a Varian HR-100 spectrometer in deuteriochloroform unless otherwise indicated. Values are given in τ units relative to tetramethylsilane as internal standard. Multiplicity of signals is designated as follows: s, singlet; brs, broad singlet; d, doublet; dd, doublet of doublets; m, multiplet. Numbers in parentheses denote coupling constants in hertz. [b] Pyridine-d_5. [c] Benzene-d_6. [d] C(13) H appeared at τ 7.58 (q, J = 7 Hz).

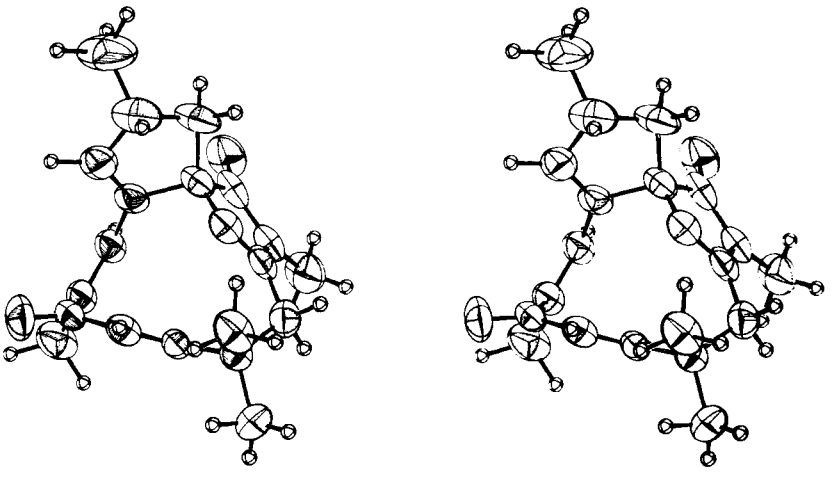


Figure 2. Stereoscopic view of the molecule of jatrophone as found in the crystal. Thermal ellipsoids for C and O are drawn to the 50% probability level as boundary surface.

double bond by the stereoelectronic considerations discussed above, it was deemed desirable to seek confirmation of structure **1**. The stereochemistry and absolute configuration of jatrophone have been established by direct single-crystal x-ray analysis. A stereoscopic view of the molecular conformation as found in the crystal is shown in Figure 2, and the torsion angles defining the conformations of the individual ring systems are shown in Figure 3. Atomic parameters are given in the microfilm edition. The configuration at the C(5)–C(6) double bond is cis and at C(8)–C(9) is

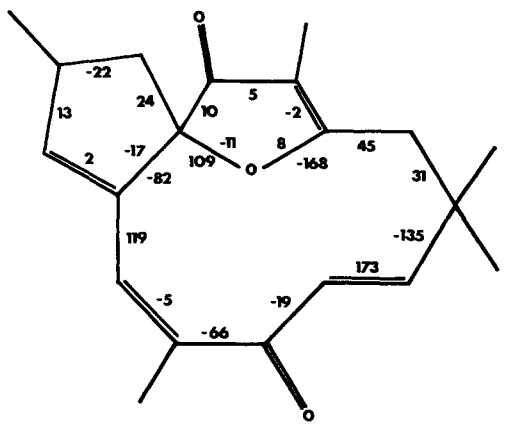


Figure 3. Selected endocyclic torsion angles for jatrophone.

Table II. Molecular Geometry in Jatrophone

	(a) Bond Lengths (Å)		
C_{sp^3}-C_{sp^3}			
C(1)-C(2)	1.541 (11)	C(2)-C(19)	1.540 (12)
C(1)-C(15)	1.539 (10)	C(10)-C(11)	1.568 (8)
C(10)-C(16)	1.540 (9)	C(10)-C(17)	1.553 (9)
Mean 1.547			
C_{sp^3}-C_{sp^2}			
C(2)-C(3)	1.528 (11)	C(10)-C(9)	1.527 (8)
C(11)-C(12)	1.496 (9)	C(15)-C(14)	1.520 (9)
C(15)-C(14)	1.547 (9)	C(18)-C(13)	1.513 (9)
C(20)-C(6)	1.514 (9)	Mean 1.521	
C_{sp^2}-C_{sp^2} single			
C(4)-C(5)	1.486 (9)	C(6)-C(7)	1.522 (8)
C(7)-C(8)	1.472 (9)	C(13)-C(14)	1.434 (9)
Mean 1.479			
C_{sp^2}-C_{sp^2} double			
C(3)-C(4)	1.325 (9)	C(5)-C(6)	1.317 (7)
C(8)-C(9)	1.335 (8)	C(12)-C(13)	1.346 (7)
Mean 1.331			
C=O			
C(7)-O(2)	1.210 (8)	C(14)-O(3)	1.220
Mean 1.215			
C_{sp^3}-O		C_{sp^2}-O	
1.456 (7)		1.366 (7)	
	(b) Bond Angles (deg)		
C(2)-C(1)-C(15)	103.7 (6)	C(1)-C(2)-C(19)	112.6 (6)
C(1)-C(2)-C(3)	103.3 (6)	C(3)-C(2)-C(19)	110.3 (8)
C(2)-C(3)-C(4)	112.3 (6)	C(3)-C(4)-C(5)	127.9 (6)
C(3)-C(4)-C(15)	109.8 (6)	C(5)-C(4)-C(15)	122.3 (6)
C(4)-C(5)-C(6)	126.3 (6)	C(5)-C(6)-C(7)	121.9 (6)
C(5)-C(6)-C(20)	122.8 (6)	C(7)-C(6)-C(20)	115.3 (5)
C(6)-C(7)-C(8)	116.8 (5)	C(6)-C(7)-O(2)	121.1 (6)
C(8)-C(7)-O(2)	122.1 (6)	C(7)-C(8)-C(9)	121.3 (6)
C(8)-C(9)-C(10)	126.4 (5)	C(9)-C(10)-C(11)	106.8 (5)
C(9)-C(10)-C(16)	109.6 (5)	C(9)-C(10)-C(17)	111.9 (5)
C(11)-C(10)-C(16)	106.9 (5)	C(11)-C(10)-C(17)	110.6 (5)
C(16)-C(10)-C(17)	110.8 (5)	C(10)-C(11)-C(12)	114.5 (6)
C(11)-C(12)-C(13)	130.9 (6)	C(11)-C(12)-O(1)	114.8 (5)
C(12)-C(13)-C(14)	108.4 (6)	C(12)-C(13)-C(18)	128.3 (6)
C(14)-C(13)-C(18)	123.1 (6)	C(13)-C(14)-C(15)	105.0 (5)
C(13)-C(14)-O(3)	131.1 (7)	C(15)-C(14)-O(3)	123.9 (6)
C(14)-C(15)-C(1)	117.1 (5)	C(14)-C(15)-C(4)	112.7 (5)
C(14)-C(15)-O(1)	103.8 (5)	C(1)-C(15)-C(4)	104.8 (6)
O(1)-C(15)-C(4)	107.9 (4)	C(1)-C(15)-O(1)	110.4 (5)
C(12)-O(1)-C(15)	107.3 (4)		

trans. The overall conformation closely matches that predicted earlier for **9**.[9]

The principal bond lengths and angles in the molecule are given in Table II. The mean values of the various bond types agree with accepted values, and deviations from the mean are acceptable save that C(10)-C(11), 1.568(8) Å, is longer and C(11)-C(12), 1.496(9) Å, shorter than expected suggesting a possible error of about 0.02 Å (3σ) in the position of C(11). The bond C(6)-C(7), 1.522(8) Å, is longer than expected for a C_{sp^2}-C_{sp^2} single bond, and reflects a restriction of conjugation between the C(5)-C(6) and C(7)-O(2) groups as implied by the 66° torsion angle about C(6)-C(7). Conversely, the short C(13)-C(14) bond distance, 1.434(9) Å, indicates some delocalization between C(14)-O(3) and C(12)-C(13).

Both the cyclopentene and dihydrofuranone rings are envelopes with flaps at C(1) and C(15) opposite the endocyclic double bonds. The displacement of C(1) from the least-squares mean plane through the remaining four atoms of the ring is 0.39 Å, and that of C(15) from the corresponding plane in the oxide ring is 0.17 Å.

Within the macrocycle, the two four-atom olefin planes are inclined to one another at an angle of 104° and the four-atom cisoid olefin group makes an angle of 66° with the least-squares mean plane through C(2), C(3), C(4), and C(15). The atoms C(9), C(10), C(11), and C(12) are roughly coplanar in the crystal conformation as indicated by the 31° torsion angle about C(10)-C(11). C(8), which is involved in ring closure with C(12) in formation of the dihydrobromide, is well removed from this plane and is 3.68 Å from C(12), 3.26 Å from O(1).

The molecules are held together in the crystal by normal van der Waals interactions. A list of shorter intermolecular contacts and a view of the molecular packing in the crystal, seen in projection down the *c* axis, are given in the microfilm edition. The ketonic O(3) and a hydrogen atom attached to the C(20) methyl group of a neighbor approach within 2.33 Å, and two hydrogen atoms come within 2.07 Å of one another. Other contacts have normal values.

In view of the recent demonstration of the potential importance of nucleophilic additions to unsaturated systems for the tumor-inhibitory activity of other natural products,[11] it appeared of interest to investigate the reactivity of jatrophone with model biological nucleophiles and, in particular, with thiols.

As expected, jatrophone reacted quickly with thiophenols in neutral solution to give single-component adducts. Attempts to isolate the adducts were unsuccessful, however, as they were unstable and underwent retro-reactions readily. Jatrophone also underwent reactions with cysteine, as evidenced by the change in the ultraviolet chromophore at 285 nm upon addition of **1** to a solution of cysteine hydrochloride in pH 7.4 phosphate buffer.[12]

The reaction between jatrophone and *n*-propylthiol in borate buffer afforded the stable adduct **7**. High resolution mass spectrometry supported a $C_{23}H_{32}O_3S$ formula, which substantiated that **7** was a monoadduct. The ultraviolet spectrum showed a chromophore at 291 nm, which indicated that the C(7) ketone was conjugated with the diene. The infrared spectrum showed a carbonyl band at 5.72 μ which was indicative of the presence of the tetrahydrofuran-3-one ring system in **7**. All of these data were supported by the NMR spectrum and were consistent with the attack of *n*-propylthiol at C(9) followed by 8,12-bond formation to give the structure **7**. The doublets at τ 6.01 (J = 13 Hz) and 6.70 (J = 13 Hz) were assigned to the C(9) and C(8) protons, respectively, and the large coupling constant was indicative of a trans-diaxial arrangement which defined the stereochemistry of the thiol ether group as β.

In a subsequent study, jatrophone was found to react also with thiol groups on proteins such as bovine serum albumin and DNA-dependent RNA polymerase from *Escherichia coli*.[13] When bovine serum albumin was treated with jatrophone, all of the sulfhydryl groups in the albumin molecule had reacted after 15 h. Gel filtration afforded a good separation between protein and excess jatrophone. The separated protein contained virtually no free sulfhydryl groups,

and the ultraviolet spectrum was in agreement with a monoadduct product of type **8**. When a portion of the jatrophone-treated protein was precipitated with trichloroacetic acid, no absorbing material was detected in the supernatant, indicative that the jatrophone was covalently linked to the albumin.

The reaction between jatrophone and protein sulfhydryl groups was further investigated with RNA polymerase from *E. coli*.[13] After 4 h, approximately 50% of the activity had been lost, and approximately eight sulfhydryl groups on the enzyme had reacted with jatrophone. Further incubation up to 22 h resulted in almost complete loss of activity. At that point 11 sulfhydryl groups of the enzyme had reacted. When a sample of the polymerase which had been allowed to react with jatrophone for 4 h was subjected to gel filtration, the spectrum of the treated polymerase was similar to that of jatrophone-treated bovine serum albumin. Evidently, in this case also, a monoadduct of type **8** had been formed.

The significance of the reactions of jatrophone and other electrophilic tumor inhibitors with thiols remains speculative but the results discussed herein support further the hypothesis that these agents may act by selective alkylation of growth-regulatory biological macromolecules.

Experimental Section

Melting points were determined on a Mettler FP2 melting point-boiling point determinator and are corrected. Infrared spectra were determined on Perkin-Elmer 257 and 337 recording spectrophotometers. Ultraviolet absorption spectra were determined on a Coleman Model EPS-3T recording spectrophotometer. Specific rotations were determined on a Zeiss-Winkel polarimeter. Evaporations were carried out at temperatures less than 40° under reduced pressure. Thin layer chromatography was carried out on silica gel plates (E. Merck). Plates were developed in 25% ethyl acetate–cyclohexane and were visualized by spraying with vanillin–H_2SO_4 solution followed by heating until colored spots appeared. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich.

Extraction and Preliminary Fractionation. The dried ground roots (11.7 kg) were extracted continuously with 95% ethanol until the extracts were colorless. The ethanol extract was evaporated under reduced pressure to yield a dark brown gum (A, 740 g). The extract was triturated with 500-ml portions of benzene until the benzene-soluble materials were all dissolved. The benzene solubles were combined and evaporated to afford a cytotoxic fraction (B, 165 g). The benzene insolubles (C) were inactive and were discarded. Fraction B was triturated with seven 250-ml portions of hexane to afford a cytotoxic hexane-soluble fraction (D, 93 g) and an inactive hexane-insoluble fraction (E). Fraction D was treated successively with three portions (30 g) of Darco G-60 activated carbon to afford a light yellow oily fraction (F, 72 g).

Fraction F was further fractionated by absorption chromatography on silica gel (E. Merck, 0.05–0.2 mm, 2.5 kg) by eluting rapidly with dichloromethane (16 l.) followed by 2% methanol in dichloromethane (19.5 l.). Fractions were collected in 1-l. portions and immediately concentrated, and the residues were assayed by the differential agar-diffusion technique for KB cytotoxicity.[5] Fractions 19–21 were found to be active and were combined to afford fraction G (48 g).

Isolation of Jatrophone (1). Fraction G was rechromatographed on neutral aluminum oxide (Woelm, activity grade III, 3.5 kg) by eluting rapidly with benzene (22 l.) and 10% dichloromethane in benzene (13 l.). After the first 20 l. of eluent, fractions were collected in 100-ml portions and evaporated, and the residues were assayed for 9KB cytotoxicity. Fractions 109–146 were found to be active, and they were combined to afford fraction H which crystallized from hexane to yield colorless needles of jatrophone (**1**, 1.37 g); mp 152–153°; $[\alpha]^{24}D$ +292° (*c* 1.23, C_2H_5OH); uv max (95% C_2H_5OH) 285 (ϵ 10 200), 225 nm (sh); ir (KBr) 3.35, 3.43, 3.46, 5.90, 6.05, 6.20, 7.10, 7.35, 8.07, 8.15, 10.10 μ; mass spectrum, M^+ at *m/e* 312.1725 (calcd for $C_{20}H_{24}O_3$: 312.1724), 297, 284, 269, 242, 227, 213, 199, 188, 173, 160, 147.

Anal. Calcd for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 77.11; H, 7.73.

Ketalization of Jatrophone. To a solution of jatrophone (**1**, 60 mg) in 50 ml of dry benzene were added *p*-toluenesulfonic acid (3 mg) and ethylene glycol (0.5 ml). The solution was heated under reflux, and the water removed by azeotropic distillation. After 24 h, thin layer chromatographic examination of an aliquot indicated a new high-R_f component was present in the reaction mixture. Additional *p*-toluenesulfonic acid (3 mg) and ethylene glycol (0.5 ml) were added, and the azeotropic distillation was continued. After 4 days, the reaction mixture was cooled, and chloroform (20 ml) and pyridine (0.25 ml) were added. The organic solution was washed with 2 N KOH (10 ml), distilled water, and saturated salt solution, dried (Na_2SO_4), and evaporated. TLC of the residue showed two major products, R_f 0.32 and 0.43. The two materials were separated by preparative TLC to afford an oily ketal **2** (3 mg): uv max (95% C_2H_5OH) 285 nm (ϵ 8800); ir ($CHCl_3$) 5.91, 6.20 μ; mass spectrum, M^+ at *m/e* 356.1980 (calcd for $C_{22}H_{28}O_4$: 356.1987), 341, 328, 313, 272; 140; and a high-R_f ketal **3** (14.2 mg) which was crystallized from 95% C_2H_5OH to afford colorless needles (10 mg): mp 140–141°; uv max (95% C_2H_5OH) 255 nm (ϵ 20 000); ir (KBr) 5.70 μ; mass spectrum, M^+ at *m/e* 356.1975 (calcd for $C_{22}H_{28}O_4$: 356.1987), 341, 328, 313, 269, 244, 207, 193.

Anal. Calcd for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.19; H, 7.96.

Jatrophone–Bis(hydrogen chloride) Adducts (4 and 5). To 0.3 ml of glacial HOAc saturated with dry HCl and cooled to 10° was added jatrophone (**1**, 20 mg). The mixture was kept at 10° for 15 min and allowed to warm to room temperature over 4 h. The reaction mixture was applied directly to four 20 × 20 cm silica gel plates (E. Merck, 0.25 mm), the acetic acid was evaporated with a stream of warm air, and the TLC plates were developed with 25% ethyl acetate in cyclohexane. Two bands (R_f 0.15 and 0.28) were removed. The high-R_f compound (3 mg) was crystallized from cyclohexane to afford colorless needles of bis(hydrogen chloride) adduct **4** (2.5 mg): mp 170–174°; ir (KBr) 2.82, 5.82 μ; mass spectrum, M^+ at *m/e* 384.1263 (calcd for $C_{20}H_{26}Cl_2O_3$: 384.1257). The low-R_f compound (14 mg) was crystallized from ethyl acetate–cyclohexane to afford colorless needles of bis(hydrogen chloride) adduct **5** (14 mg): mp 194–198°; uv max (95% C_2H_5OH) 229 nm (ϵ 3100), 297 (1700); ir (KBr) 2.79, 5.85 μ; mass spectrum, M^+ at *m/e* 384, 351, 350, 349, 333, 331, 313, 295, 212, 205, 199, 194, 193, 175, 163, 159, 149, 147, 122.

Anal. Calcd for $C_{20}H_{26}Cl_2O_3$: C, 62.36; H, 6.78; Cl, 18.44. Found: C, 62.41; H, 6.86; Cl, 18.29.

Jatrophone–Bis(hydrogen bromide) Adduct (6). To 0.6 ml of glacial HOAc saturated with dry HBr and cooled to 0° was added jatrophone (**1**, 22 mg). The mixture was allowed to warm to room temperature over a 2-h period and the single major product (R_f 0.22) isolated in the same manner as for the bis(hydrogen chloride) adducts. The crude adduct (23 mg) was crystallized from ethyl acetate–cyclohexane to afford colorless needles (19 mg). Recrystallization twice from 95% C_2H_5OH afforded needles: mp 150–152° dec; uv max (95% C_2H_5OH) 232 nm (ϵ 4700), 300 (1200); ir (KBr) 2.75, 5.85 μ.

Anal. Calcd for $C_{20}H_{26}Br_2O_3$: C, 50.63; H, 5.49; Br, 33.74. Found: C, 50.73; H, 5.45; Br, 33.67.

Dehydrobromination of 6. To a chloroform solution of **6** (1 mg) was added neutral alumina (Woelm, activity grade I, 12 mg). The suspension was stirred at room temperature for 18 h. The alumina was removed by filtration and the solvent evaporated. The residue was purified by TLC to afford a material which was shown to be identical with jatrophone (**1**) by TLC on silica gel and alumina and by infrared spectral comparison.

Jatrophone–*n*-Propylthiol Adduct (7). To a solution of **1** (28 mg) in THF (0.75 ml) were added pH 9.2 borate buffer (0.5 ml) and *n*-propylthiol (0.25 ml). The mixture was stirred at room temperature for about 30 min and poured into H_2O (10 ml). The aqueous solution was washed with ether. The ether extract was dried (Na_2SO_4) and evaporated with a steam of N_2. The residue was purified by TLC to afford a spectrally homogeneous light yellow oil (24 mg): ir (KBr) 5.72, 6.08 μ; uv max (CH_3OH) 291 nm (ϵ 12 000); mass spectrum, M^+ at *m/e* 388.2088 (calcd for $C_{23}H_{32}O_3S$: 388.2071), 360, 345, 318, 313, 312, 297, 289, 285, 279, 197, 196, 175, 165, 135.

Crystal Structure Determination. Crystals of jatrophone have or-

thorhombic symmetry, and from the observed systematic absences $h00$ with h odd, $0k0$ with k odd, and $00l$ with l odd are assigned to space group $P2_12_12_1$. The unit cell dimensions, found from a least-squares fit to the observed diffractometer values of $\pm 2\theta$ for 18 general reflections having $2\theta > 45°$, and assuming λ 1.5418 Å, are: a = 17.527 (2), b = 10.826 (1), and c = 9.659 (1) Å. The observed density, found by flotation with aqueous ZnI_2, is 1.13 g cm^{-3}, identical with that calculated for $Z = 4$.

Intensity measurements were made from a single crystal mounted with the $(\bar{h}0h)$ reciprocal axis parallel to the instrumental ϕ axis of a Picker diffractometer controlled by an XDS Sigma 2 computer. Cu Kα radiation, made monochromatic by Bragg reflection from the 002 planes of a highly oriented graphite crystal, was used with scintillation counting and pulse-height analysis. A single octant of reciprocal space was surveyed to sin $\theta/\lambda = 0.561$, and scattered intensity significantly above background $[I > 3\sigma(I)]$ measured at 1319 of the 1620 locations examined. The θ-2θ scan method was used with a scan range of 3° in 2θ and a scan speed of 2° min^{-1}. Background intensities were derived from a carefully premeasured table of intensity vs. scattering angle. No significant variation was detected in the intensities of two reference reflections monitored after every 50 measurements, and a single scale factor was used. The absorption coefficient for Cu Kα radiation is 6 cm^{-1} yielding calculated transmission factors for the crystal used of between 0.75 and 0.79. No absorption correction was made.

Structure amplitudes and normalized structure amplitudes were derived in the usual ways and the phase problem solved by use of the multisolution tangent formula method.[14] Of the 32 solutions generated, that of lowest residual showed the locations of all 23 nonhydrogen atoms in the asymmetric unit in an E-map free from spurious peaks ($R = 0.32$). The parameters were refined by block-diagonal least-squares methods. Isotropic thermal parameters were first assumed ($R = 0.15$) and then anisotropic ($R = 0.09$). Hydrogen atoms were unambiguously located from a three-dimensional ($\rho_o - \rho_c$) map. Contributions from these atoms in fixed idealized positions and with B values of 3.0 Å^2 were included in the least-squares calculation. At convergence, with a conventional weighting scheme,[15] the normal unweighted and weighted residuals were 0.065 and 0.069 for the 1319 observed structure amplitudes with the standard deviation of an observation of unit weight being 1.21. No obvious systematic trends could be found in the distribution of ΔF. In the final cycle of refinement the maximum calculated shift to error ratio was 0.2 and the average 0.05.

Atomic scattering factors for C and O were taken from Hanson et al.[16] and for hydrogen from Stewart et al.[17] A listing of the observed and calculated structure amplitudes is given in the microfilm edition. Except for the calculation of phase sets, which was carried out on a CDC 6400 computer, all calculations were made with programs written in this laboratory for the XDS Sigma 2 computer also used to control the diffractometer.

Determination of Absolute Configuration. When allowance was made for the anomalous dispersion terms for oxygen[18] in separate structure factor calculations, residuals of 0.0646 and 0.0648 were obtained for the two enantiomeric structures. The ratio of these two quantities indicates a preference for one enantiomer over the other at the 97.5% confidence level.[19] This indication was strengthened by considering the ten reflections for which the calculated difference in structure factor for the different enantiomers exceeded 0.2. The intensities of the corresponding Bijvoet pairs of reflections were then carefully measured in all eight octants and appropriately averaged. In all ten cases the sign of the observed difference in intensity was the same as that calculated.[20] The absolute configuration thus indicated is consistent with that found for jatrophone dihydrobromide.[8]

Supplementary Material Available: A listing of the atomic parameters, the observed and calculated structure amplitudes, and selected intermolecular approach distances (13 pages). Ordering information is given on any current masthead page.

References and Notes

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