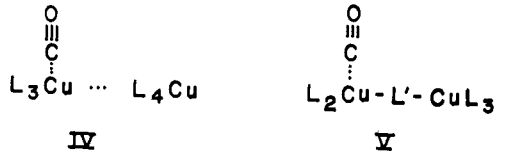
as unlikely possibilities by Williams,[8] in order to explain the stoichiometry of binding CO in carboxyhemocyanin, would require lower stretching frequencies and are inconsistent with the data. Structure III, in which carbonyl π_x and π_y bonds coordinate with copper, is not tested by these data but is unknown in copper chemistry and will not be considered further. The probable structures for carboxyhemocyanin are therefore IV or V. In structure IV the second copper



IV V

does not take part in binding CO but may stabilize the protein, whereas in structure V the second copper may influence CO binding through a bridging ligand or metal–metal bond. The infrared data indicate that CO is coordinated to only one copper per binding unit in hemocyanin. It is probable that oxygen is similarly coordinated to only one copper in oxyhemocyanin.

Acknowledgments. This work was supported in part by Grant GM-11067 from the National Institute of General Medical Sciences. The Perkin-Elmer Model 102 infrared spectrometer was made available under a contract with the Air Force Materials Laboratory, Air Force Systems Command, U. S. Air Force. This support is gratefully acknowledged.

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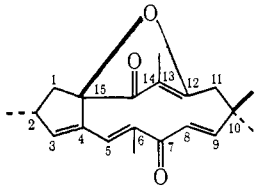
Jatrophone, a Novel Macrocyclic Diterpenoid Tumor Inhibitor from *Jatropha gossypiifolia*[1,2]

Sir:

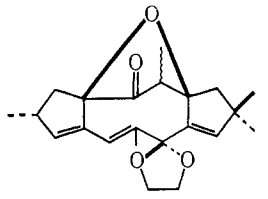
Extracts of *Jatropha gossypiifolia* L. (*Euphorbiaceae*) and related species have been used for many years to treat cancerous growths.[3] In the course of a continuing search for tumor inhibitors of plant origin[4] we found that an alcoholic extract of *J. gossypiifolia*[5] showed significant inhibitory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

Fractionation of the ethanol extract was guided by the KB assay.[6] 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 chromatography on silica gel and neutral alumina (activity III) yielded jatrophone (**1**): $C_{20}H_{24}O_3$;[8] mp 152–153°; $[\alpha]^{24}D$ +292° (*c* 1.23, C_2H_5OH); uv max (95% C_2H_5OH) 285 (ϵ 10,200), 225 mμ (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; nmr (C_6D_6) τ 3.25 (1 H, d, J = 15 Hz), 3.74 (1 H, d, J = 15 Hz), 3.96 (1 H, m), 4.14 (1 H, m), 7.09 (1 H, m), 3.12 (1 H, d, J = 14 Hz), 7.61 (1 H, d, J = 14 Hz), 8.17 (3 H, d, J = 2 Hz), 8.18 (3 H, s), 8.67 (3 H, s), 8.80 (3 H, s), 9.07 (3 H, d, J = 7 Hz).

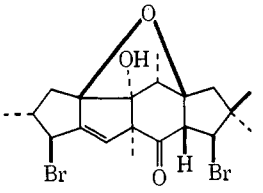
Jatrophone was converted to intractable mixtures when treated under alkaline conditions, but was quite stable in acid media. Treatment of **1** with ethylene glycol and *p*-TsOH afforded an oily C-14 ketal, $C_{22}H_{28}O_4$ [uv max 285 mμ (ϵ 8800); ir ($CHCl_3$) 5.91, 6.20 μ; nmr spectrum similar to that of **1**, with additional signals for the ethylenedioxy group], and crystalline ketal **2**, $C_{22}H_{28}O_4$ [mp 140–141°; uv max 255 mμ (ϵ 20,000); ir (KBr) 5.70 μ; nmr ($CDCl_3$) τ 4.02 (1 H, d, J = 4 Hz), 4.15 (1 H, m), 4.22 (1 H, s), 6.25 (m, ethylenedioxy), 8.21 (3 H, br s), 8.73 (3 H, d, J = 7 Hz), 8.82 (3 H, s), 8.85 (3 H, s), 8.90 (3 H, d, J = 7 Hz)]. The nature of the two ketals indicated that jatrophone possesses two ketone groups, one of which is in a five-membered ring.



1



2



3

Treatment of **1** in glacial acetic acid with dry hydrogen bromide afforded the dihydrobromide **3**: $C_{20}H_{26}Br_2O_3$; mp 154–156° dec; uv max 300 (ϵ 1200), 232 mμ (ϵ 4700); ir (KBr) 2.75, 5.85 μ; nmr (C_6D_6) τ 5.20 (1 H, s), 5.75 (1 H, d, J = 11 Hz), 6.06 (1 H, d, J = 5 Hz), 6.28 (1 H, d, J = 11 Hz), 7.00 (1 H, m), 8.62 (3 H, s), 8.78 (3 H, s), 9.09 (3 H, s), 9.18 (3 H, d, J = 7 Hz), 9.72 (3 H, d, J = 7 Hz). Dehydrobromination of **3** to afford jatrophone in high yield was effected by stirring a chloroform solution of **3** with a suspension of neutral alumina. The reversible interrelation of jatro-

(1) Tumor Inhibitors. LIX. Part LVIII: S. M. Kupchan, M. Takasugi, R. M. Smith, and P. S. Steyn, *Chem. Commun.*, in press.

(2) Supported by grants from the National Cancer Institute (CA-04500 and CA-11718) and the American Cancer Society (T-275), and a contract with Chemotherapy, National Cancer Institute, National Institutes of Health (PH-43-64-551).

(3) J. L. Hartwell, *Lloydia*, 32, 153 (1969).

(4) S. M. Kupchan, *Trans. N. Y. Acad. Sci.*, 32, 85 (1970).

(5) The roots were collected in Costa Rica by Professor J. A. Saenz Renauld, Department of Biology, University of Costa Rica, San Jose, in Jan 1961 and Dec 1967.

(6) Significant inhibitory activity was noted against sarcoma 180, Lewis lung carcinoma, and P-388 lymphocytic leukemia in the mouse and the Walker 256 intramuscular carcinosarcoma in the rat. Cytotoxicity and *in vivo* activity were assayed under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, by the procedures described in *Cancer Chemother. Rep.*, 25, 1 (1962). Cytotoxicity was also assayed by differential agar diffusion by Professor D. Perlman, University of Wisconsin; *cf. J. Pharm. Sci.*, 58, 633 (1969).

(7) Jatrophone showed significant antileukemic activity against P-388 lymphocytic leukemia at 27 and 12 mg/kg, and cytotoxicity (ED_{50}) against KB cell culture at 0.17 μg/ml.[6]

(8) Elemental formula confirmed by high-resolution mass spectrometry. We cordially thank Dr. D. Rosenthal, Research Triangle Institute, and Drs. W. E. Baitinger and W. L. Budde, Purdue University, for the mass spectra. All crystalline compounds have also been characterized by concordant elemental analyses.

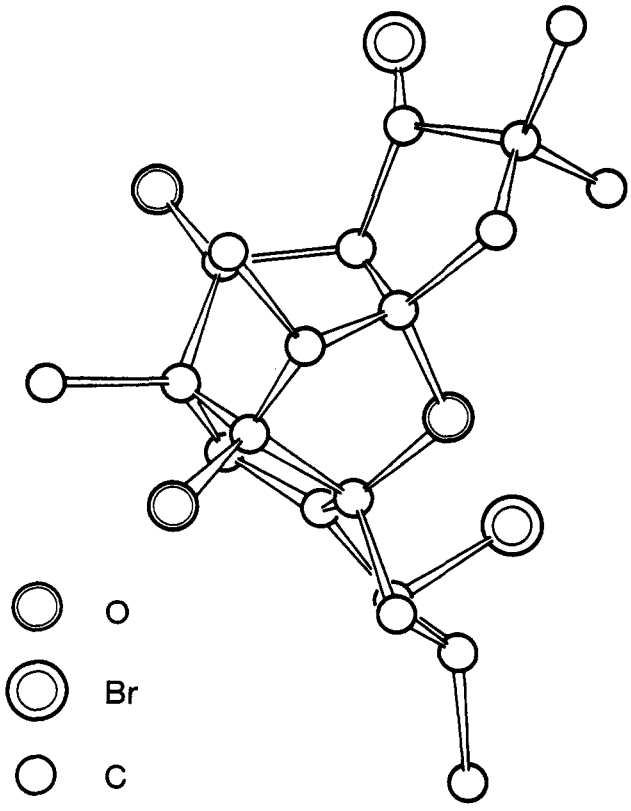


Figure 1. Model showing the absolute configuration of jatrophone dihydrobromide.

phone and the dihydrobromide adduct made the latter derivative an attractive target for X-ray crystallographic analysis.

Crystals of the dihydrobromide have orthorhombic symmetry with space group $P2_12_12_1$ and $a = 21.974$ (3), $b = 12.486$ (3), $c = 7.127$ (2) Å. On the basis of four formula units of $C_{20}H_{26}Br_2O_3$ in the unit cell, the calculated density is 1.610 g/cm³, in reasonable agreement with the observed value of 1.61 (1) g/cm³.

Intensity data were collected by diffractometry using monochromatic Mo Kα radiation, scintillation counting, and pulse height analysis.

The locations of the two bromine atoms in the unit cell were derived from a three-dimensional Patterson synthesis, and the carbon and oxygen atoms were found from two successive three-dimensional electron-density syntheses using the heavy atom method of phase determination. The approximate atomic parameters were refined by the block-diagonal least-squares method with individual isotropic thermal parameters to give $R = 0.104$, and with individual anisotropic thermal parameters to yield $R = 0.047$ and $R' = ([\Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2])^{1/2} = 0.057$ for the 903 independent significant reflections measured. The molecular structure illustrated by Figure 1 is in the correct absolute configuration with respect to a right-handed coordinate system, as judged by independent least-squares refinement of the two possible enantiomeric structures taking into account the anomalous dispersion terms for the bromine atoms ($\Delta f' = -0.21$, $\Delta f'' = 2.68$). For the alternate configuration $R = 0.060$ and $R' = 0.074$.

From the spectral properties of jatrophone and its derivatives and the reversible interrelation with **3** it may be concluded that jatrophone has structure **1**. The ready formation of the dihydrobromide **3** is envisioned as a result of two novel 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.

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,[4,9] investigations are in progress to determine the significance of various structural features in relation to the biological activity of jatrophone.

(9) S. M. Kupchan, D. C. Fessler, M. A. Eakin, and T. J. Giacobbe, *Science*, 168, 376 (1970).

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Isomer Distribution in the Aromatic Nitramine Rearrangement. Solvent Viscosity Effects within the Solvent Cage

Sir:

The composition of the product resulting from the acid-catalyzed nitramine rearrangement depends on the nature of the reaction medium. Thus, the yield of nitrated product from N-nitroaniline increases from 60 to 95% and the *ortho–para* isomer ratio changes from 3.5 to 19.0 as the concentration of the catalyzing acid is increased.[1] Similar results were obtained in the rearrangements of N-nitro-1-naphthylamine and its N-methyl derivative.[2] This behavior was attributed to the differential effect of the acid medium's basic strength on proton loss from the *ortho* and *para* positions. However, the data are poorly correlated by reference to either the water activity or the acidity function of the reaction solvent.

Interestingly, the published isomer ratios yield a smooth curve when plotted against the viscosity of the medium—results obtained in sulfuric, phosphoric, and perchloric acid falling along the same correlation line. The notion that the solvent viscosity determines the isomer distribution and the nitrated product yield in the nitramine rearrangement was pursued by rearranging N-nitro-N-methylaniline in a series of methanol–glycerol mixtures of different compositions and viscosities. This solvent pair was chosen because of the similar polar character of the components (Z (methanol) = 83.6 and Z (glycerol) = 85.3), but widely different viscosities (η (methanol) = 0.55 cP and η (glycerol) = 954 cP). Reactions were run both in the presence and absence of hydroquinone as scavenger. The results obtained are plotted in Figure 1. As the solvent viscosity was changed from 0.60 to 80.5 cP the total yield of nitrated product increased (from 34 to 90% in the presence of hydroquinone and from 76 to 100% in its absence) and the *ortho–para* isomer ratio was enhanced (from 1.30 to 18.5 in the presence of scavenger and from 1.23 to 13.7 in its absence).

(1) D. V. Banthorpe, E. D. Hughes, and D. L. H. Williams, *J. Chem. Soc.*, 5349 (1964).
(2) D. V. Banthorpe and J. A. Thomas, *ibid.*, 7149 (1965).