Antitumor Agents from *Jatropha macrorhiza* (Euphorbiaceae) I: Isolation and Characterization of Jatropham

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Abstract □ A new lactam has been isolated from *Jatropha macrorhiza* (Euphorbiaceae) and has shown tumor-inhibitory properties against the P-388 lymphocytic leukemia test system. The compound was identified by means of NMR, UV, IR, rotary dispersion, mass spectrometry, and classical chemical procedures. It has the structure 5-hydroxy-4-methyl-3-pyrrolin-2-one and has been named jatropham.

Keyphrases □ *Jatropha macrorhiza* (Euphorbiaceae)—isolation and characterization of jatropham (5-hydroxy-4-methyl-3-pyrrolin-2-one), antitumor activity □ 5-Hydroxy-4-methyl-3-pyrrolin-2-one (jatropham)—isolation from *Jatropha macrorhiza*, characterization, antitumor activity □ Antitumor agents—isolation and characterization of jatropham (5-hydroxy-4-methyl-3-pyrrolin-2-one) from *Jatropha macrorhiza*

As a result of the continuing search for plants having tumor-inhibiting constituents, it was found that the chloroform extract of the leaves, stems, flowers, and fruits of *Jatropha macrorhiza* Benth. (Euphorbiaceae)[1] showed inhibitory activity toward the P-388 lymphocytic leukemia test system (3PS)[2]. The plant was collected in Cochise County, Ariz., in June 1968.

DISCUSSION

Solvent extractions of the chloroform extract with hexane followed by acetonitrile yielded a crystalline compound from the acetonitrile solution. This compound demonstrated activity of 125% test/control (T/C) at 45 mg./kg. in the 3PS test system. Activity in the 3PS system is defined as an increase in the survival of treated animals over that of controls resulting in a T/C $\geq$ 125% (1).

The compound was soluble in water, alcohol, and acetone but insoluble in less polar organic solvents. Elemental analysis and molecular weight (mass spectrum) indicated that the molecular formula was $C_5H_7NO_2$. The IR spectrum showed broad absorption at 3400–3450 (OH and NH) as well as 1725 (carbonyl) and 1640 cm.$^{-1}$ (double bond). The NMR spectrum showed a methyl group (δ 1.7, 3H, singlet), and the remaining four protons appeared individually at δ 4.9 (doublet), 5.4 (doublet), 6.5 (singlet), and 7.6 (singlet). When deuterium oxide was added to the solution, the doublet at δ 4.9 and the singlet at δ 7.6 disappeared and the doublet at δ 5.4 was reduced to a singlet. Apparently, in deuteroacetone, used as the solvent here, the hydrogen of the hydroxyl group does not exchange rapidly and couples with the adjacent proton. The UV spectrum showed strong end absorption and a shoulder at 230 nm.

The spectra are consistent with several pyrrolin-2-one structures with a secondary alcohol and a methyl group on the ring. In particular, they are similar to the spectra reported for a series of alkyl 5-hydroxy-3-pyrrolin-2-one derivatives (2). The location of the methyl group with respect to the carbonyl was demonstrated by reduction of the compound over platinum oxide to the known 4-methyl-2-pyrrolidone (II, Scheme I). The positions of the hydroxyl group and double bond were established by oxidation of the jatropha lactam (which was given the trivial name jatropham) with chromic acid in acetone to citraconic acid imide (III). The reactions show the structure of jatropham to be 5-hydroxy-4-methyl-3-pyrrolin-2-one (I).

Jatropham is optically active, levorotatory in the visible region, thus indicating that there is no ring tautomerism with the open-chain aldehyde-amide. This fact is consistent with the work of Scheffold and Dubs (2) who did not find evidence for ring opening with their 5-hydroxy-3-pyrrolin-2-ones, although corresponding lactones were in equilibrium with open-chain acids. The optical rotatory dispersion (ORD) curve has one peak (at 260 nm.) and two troughs (300 and 215 nm.) in the 600–200-nm. region. The higher inversion, molecular amplitude $\alpha = -22$, is centered near 270 nm. where there is no peak in the UV spectrum. The second inversion, molecular amplitude $\alpha = +208$, is centered near 235 nm. where there is a shoulder in the UV spectrum.

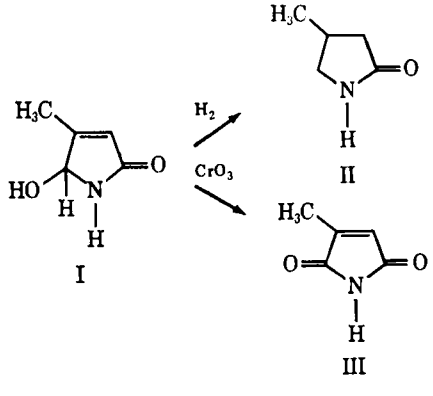
EXPERIMENTAL[3]

Isolation Procedure—Fresh-frozen leaves, stems, flowers, and fruits were placed in a lyophilizer and dried for 24 hr. The dried material (2 kg.) was ground in a Wiley mill and macerated with 12 l. of chloroform for 24 hr. The material was filtered and air dried, yielding an extract weighing 70 g. The chloroform extract was repeatedly extracted with hexane (technical) in 2-l. portions until the hexane was clear. The residue was then extracted with acetonitrile (technical) in 1-l. portions until the acetonitrile portion was clear. Evaporation of the acetonitrile solution yielded a crystalline solid material (1.3 g.).

A sample recrystallized from acetonitrile for analysis had m.p. 131–132°; $[\alpha]_{549}^{25}$ −62°; ORD data (c, 1.6 × 10^{-2}, water), $[\phi]_{589}$ −67°, $[\phi]_{300}$ −560°, $[\phi]_{260}$ +1700°, and $[\phi]_{215}$ −19,100°; IR (acetonitrile): 3550 (s), 1725 (s), 1600 (m), 1050 (s), and 870 (m) cm.$^{-1}$; UV (methanol): strong end absorption with a shoulder at 230 nm.; log ϵ 3.06.

Anal.—Calc. for $C_5H_7NO_2$: C, 53.09; H, 6.24; N, 12.38; mol. wt. 113. Found: C, 52.63; H, 6.21; N, 12.56; *m/e* 113.

Hydrogenation of Jatropham—A sample of lactam weighing 0.910 g. (8.0 mmoles) was dissolved in 70 ml. of ethanol, 0.110 g. of plat-



Scheme I

[1] Identification was confirmed by Robert J. Barr, College of Pharmacy, and Dr. Charles T. Mason, Botany Department, University of Arizona, Tucson. A reference specimen was deposited in the University of Arizona Herbarium.

[2] Drug Evaluation Branch, Drug Research and Development, Chemotherapy, National Cancer Institute.

[3] Carbon, hydrogen, and nitrogen analyses were performed by Huffman Laboratories, Inc., Wheatridge, Colo., and Chemalytics, Inc., Tempe, Ariz. Mass, NMR, UV, and IR spectra were determined using a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), a Varian T-60 spectrometer, a Beckman DB-G spectrophotometer, and a Beckman IR-33, respectively. ORD measurement was obtained from a Cary 60, and melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

inum oxide was added, and hydrogen was admitted to the system at atmospheric pressure. The solution absorbed 400 ml. (13.6 mmoles) of hydrogen in 30 min., after which no further uptake was observed. The reaction mixture was filtered to remove platinum and evaporated to dryness, leaving 0.851 g. of residual oil.

The crude product was separated by chromatography on 20 g. of silica gel into two main fractions, which were eluted from the column with acetone. The first of these weighed 0.451 g. and crystallized as long needles. Recrystallization from hexane gave material melting at 53–56° [lit. (4) m.p. 54–56°] and having the properties expected of 4-methyl-2-pyrrolidinone; mass spectrometry: M^+, *m/e* 99; ν_{max}^{CHF}: 3460 (m), 3240 (s), 3020 (s), and 1700 (s) cm.$^{-1}$; NMR (60 MHz.): δ 1.3 (3H, doublet), 1.8 (1H, multiplet), 2.4 (2H, unresolved), 3.4 (2H, two doublets), and 7.4 (1H, broad).

Anal.—Calc. for C_5H_9NO: C, 60.61; H, 9.09; N, 14.14; mol. wt. 99. Found: C, 60.79; H, 8.84; N, 14.33; *m/e* 99.

The second fraction, 0.364 g., did not crystallize. The IR spectrum differed from that described for the first fraction in having strong, broad absorption near 3300 cm.$^{-1}$. The NMR spectrum had a peak at δ 5.3 (1H) that was removed when deuterium oxide was added to the solution. This material, presumably a mixture of 5-hydroxy-4-methyl-2-pyrrolidinones, was not investigated further.

Oxidation of Jatropham—A solution of 0.815 g. (7.2 mmoles) of lactam in 40 ml. of acetone was cooled to 5°, and a solution of chromic acid (5) was added slowly with occasional addition of portions of anhydrous magnesium sulfate until the red-orange color of the oxidizing agent persisted. The mixture was filtered, the insoluble portion was washed thoroughly with acetone, and the solution was evaporated to dryness. The residue weighed 0.62 g. (78%). The material was recrystallized from carbon tetrachloride, giving a sample melting at 99–102°. This sample was further purified by sublimation, yielding long needles, m.p. 103–105°. The purified material was identical in IR spectrum (chloroform solution), TLC behavior, and mixed melting point (103–105°) with a sample of citraconic acid imide, m.p. 104–105°, synthesized by heating citraconic anhydride with ammonium hydroxide (6).

Anal.—Calc. for $C_5H_6NO_2$: C, 54.05; H, 4.50; N, 12.61; mol. wt. 111. Found: C, 54.35; H, 4.54; N; 12.32; *m/e* 111.

REFERENCES

(1) "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems," Cancer Chemotherapy Reports No. 25, Cancer Chemotherapy National Service Center, U. S. Department of Health, Education, and Welfare, Washington, D. C., Dec. 1962.

(2) R. Scheffold and P. Dubs, *Helv. Chim. Acta*, **50**, 798(1967).

(3) B. Litman and J. Schellman, *J. Phys. Chem.*, **69**, 978(1965).

(4) C. P. Gutsche and I. Y. C. Tao, *J. Org. Chem.*, **32**, 1778(1967).

(5) J. Meinwald, J. Crandall, and W. L. Hymans, *Org. Syn.*, **45**, 77(1965).

(6) J. Gottlieb, *Ann.*, **77**, 274(1851).

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COMMUNICATIONS

Dissolution Rates of Cholesterol Monohydrate Crystals and Human Cholesterol Gallstones in Bile Acid–Lecithin Solutions: Enhancing Effect of Added Alkyl Quaternary Ammonium Salts

Keyphrases □ Cholesterol monohydrate crystals and human cholesterol gallstones—dissolution in bile acid–lecithin solutions, effect of alkyl quaternary ammonium salts □ Cholate–lecithin dissolution media for cholesterol dissolution—effect of alkyl quaternary ammonium salts on interfacial barriers □ Benzalkonium chloride—effect on cholesterol dissolution in cholate–lecithin solutions □ Quaternary ammonium compounds—effect on cholesterol dissolution in cholate–lecithin solutions □ Bile acid–lecithin solutions—cholesterol dissolution, effect of alkyl quaternary ammonium salts □ Gallstone dissolution in bile acid–lecithin solutions—effect of quaternary ammonium salts

Sir:

Recent experiments on cholesterol dissolution in micellar bile salt solutions showed that added lecithin decreases the dissolution rates even though it increases the solubility of cholesterol monohydrate crystals in the solvent media (1, 2). From these data, effective crystal–solution interfacial barriers for the dissolution process were deduced, assuming a model based upon the interfacial barrier in series with a Nernst diffusion layer. Table I shows: (*a*) the dissolution rates of a compressed cholesterol monohydrate pellet (J/A), and (*b*) the solubilities (C_s) and diffusivities (D) of cholesterol molecules in various media determined by reported methods (2). The total transfer resistance (R) was calculated as the sum of diffusional resistance (h/D) and interfacial resistance ($1/P$). For example, in 2% cholate–1% lecithin solution, the interfacial resistance for cholesterol dissolution is nearly 20 times the diffusional resistance based upon the benzoic acid dissolution experiment. These findings are important in view of the recent clinical studies showing that *in vivo* diminution of cholesterol gallstones may be controlled by dissolution kinetics. This communication reports findings with benzalkonium chloride and other quaternary ammonium compounds in counteracting or eliminating the interfacial barriers present in the cholate–lecithin media for cholesterol dissolution.

As shown in Table I, in 2% cholate–1% lecithin and in 5% cholate–2.5% lecithin, added benzalkonium chloride significantly reduced the large R values to