Antitumor Agents from *Jatropha macrorhiza* (Euphorbiaceae) III: Acetylaleuritolic Acid

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Abstract □ The triterpene acetylaleuritolic acid was isolated from *Jatropha macrorhiza* (Euphorbiaceae) and showed tumor-inhibitory properties toward the P-388 lymphocytic leukemia test system. The compound was identified by means of IR, PMR, and mass spectrometry and by its transformation into the known methyl acetyloleanolate.

Keyphrases □ Acetylaleuritolic acid—isolated from ethanol extract of roots of *Jatropha macrorhiza*, antitumor activity evaluated □ *Jatropha macrorhiza*—ethanol extract of roots, acetylaleuritolic acid isolated and evaluated for antitumor activity □ Antitumor activity—evaluated in acetylaleuritolic acid isolated from ethanol extract of roots of *Jatropha macrorhiza*

In the continuing search for plants having tumor-inhibitory constituents[1], the ethanol extract of the roots of *Jatropha macrorhiza* Benth. (Euphorbiaceae)[2] was found to possess inhibitory activity toward the P-388 lymphocytic leukemia test system[3].

DISCUSSION

One constituent of the ethanol extract of *J. macrorhiza* Benth. roots is the triterpene acetylaleuritolic acid (I), $C_{32}H_{50}O_4$ (1). The IR spectrum of I indicated that this compound contained carboxylic acid (1695 cm^{-1}) and acetate (1730 and 1255 cm^{-1}) functional groups. The mass spectrum of I exhibited a fragmentation pattern consistent with that reported (1) for Δ^{14}-taraxerene derivatives and identical to the data for aleuritolic acetate (2). The physical data (2) for aleuritolic acid and its derivatives were also in agreement with those observed for I and its derivatives (II and III).

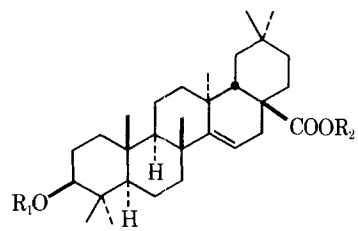
Because an authentic sample of I was unobtainable, its methyl ester (III) was converted *via* acid isomerization (2, 3) into IV, identical in all respects (TLC, IR and mass spectra, and mixed melting point) to an authentic specimen[4] of methyl acetyloleanolate (4).

Compound I demonstrated activities of 158 and 128% test/control (T/C) at dose levels of 1.0 and 1.4 mg/kg, respectively, in the P-388 system. Activity in the P-388 test system is defined as an increase in the survival of treated animals over that of controls resulting in a T/C ≥ 125% (5).

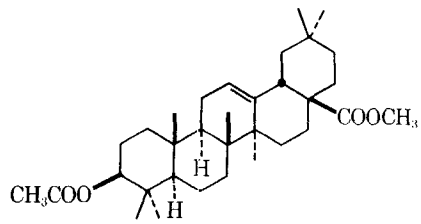
EXPERIMENTAL[5]

Extraction Procedure—The fresh ground roots (90.8 kg) of *J. macrorhiza* were extracted exhaustively in a Lloyd-type extractor with ethanol. The solvent from the ethanol extract was removed in air, and the residue was partitioned between chloroform and water (1:1). After the layers separated, the chloroform was removed in air, resulting in 130.3 g of residue.

Isolation of I—The residue from the chloroform phase (100 g) was stirred vigorously with ether (1.4 liters) and filtered. This process provided 7 g of ether-insoluble material and, after removal of the solvent *in vacuo*, 92 g of ether-soluble residue. The ether-soluble fraction (50 g) was then chromatographed over 850 g (55 × 840 mm) of silica gel 60[6]. Elution of the column was begun with benzene and continued with increasing amounts of chloroform in benzene. Elution with 80% chloroform produced a semicrystalline fraction (2 g), which was recrystallized from hexane, resulting in 375 mg of I as colorless needles, mp 298–300° (sublimes above 140°) [lit. (1) mp 278–281°], $[\alpha]_D^{25}$ −49° (c 0.2, $CHCl_3$). The IR [($CHCl_3$): 1730, 1695, and 1255 cm^{-1}], PMR [($CDCl_3$): δ 0.86 (s, 3H), 0.95 (s, 15H), 1.25 (s, 3H), 2.02 (s, 3H), 4.45 (m, 1H), and 5.51 (m, 1H) ppm], and mass [*m/e* 498 (M^+), 483, 438, 423, 377, 344, 329, 269, 248, 234 (base), 219, 203, 189, 135, and 119] spectra were in accord with Structure I.



I: $R_1 = CH_3CO$, $R_2 = H$
II: $R_1 = R_2 = H$
III: $R_1 = CH_3CO$, $R_2 = CH_3$



IV

Anal.—Calc. for $C_{32}H_{50}O_4 \cdot \frac{1}{2}H_2O$: C, 75.69; H, 10.12. Found: C, 75.31; H, 10.11.

The methyl ester (III) of I melted at 239–241° [lit. (1) mp 241–243°], and the desacetyl compound (aleuritolic acid, II) melted at 303–306° dec. (sublimes above 205°, turning into tiny needles) [lit. (1) mp 300–302° dec.].

Isomerization of Methyl Acetylaleuritolate (III) and Preparation of Methyl Acetyloleanolate (IV)—Compound III, 25 mg, in acetic acid (5 ml) was heated with concentrated hydrochloric acid (0.2 ml) on a steam bath for 30 min. The crystalline solid (22 mg) obtained after workup was recrystallized from ether–hexane, giving pure IV, mp 219–220°, $[\alpha]_D^{25}$ +58° (c 1.0, $CHCl_3$), identical (TLC, IR and mass spectra, and mixed melting point) with an authentic sample[4].

Anal.—Calc. for $C_{33}H_{52}O_4 \cdot \frac{1}{2}H_2O$: C, 75.96; H, 10.23. Found: C, 76.23; H, 10.36.

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(5) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep. Part 3*, **3**, 9 (1972).

[1] For Part I, see *J. Pharm. Sci.*, **62**, 1206 (1973). For Part II, see *J. Org. Chem.*, **41**, 1855 (1976).

[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] Tested by the Drug Evaluation Branch, Drug Research and Development, Chemotherapy, National Cancer Institute.

[4] An authentic specimen of oleanolic acid was provided by Drug Research and Development, Chemotherapy, National Cancer Institute.

[5] Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. Mass, PMR, and IR spectra were recorded using a Hewlett-Packard model 5930 spectrometer, a Varian T-60 spectrometer, and a Beckman IR-33, respectively. Optical rotations were obtained on a Rudolph model 70 polarimeter, and melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

[6] E. Merck.

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Anthelmintic Dihydroquinoxalino[2,3-*b*]quinoxalines

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Abstract □ A series of dihydroquinoxalino[2,3-*b*]quinoxalines was synthesized and tested for anthelmintic activity in a model assay. The most promising compound, 5,12-diacetyl-5,12-dihydroquinoxalino[2,3-*b*]quinoxaline, was orally effective in sheep at a dose of 200 mg/kg against a broad range of helminths.

Keyphrases □ Quinoxalines, substituted—synthesized, anthelmintic activity evaluated □ Dihydroquinoxalino[2,3-*b*]quinoxalines, substituted—synthesized, anthelmintic activity evaluated □ Anthelmintic activity—evaluated in series of substituted dihydroquinoxalino[2,3-*b*]quinoxalines □ Structure–activity relationships—series of substituted dihydroquinoxalino[2,3-*b*]quinoxalines evaluated for anthelmintic activity

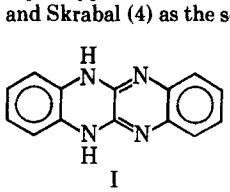
In the continuing search for anthelmintic agents of novel structure, dihydroquinoxalino[2,3-*b*]quinoxaline showed erratic but definite anthelmintic activity in a preliminary assay. Therefore, several analogs were synthesized and tested for anthelmintic efficacy.

RESULTS AND DISCUSSION

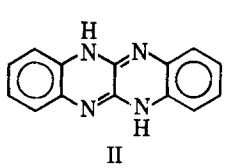
Fluoflavin was first prepared by Hinsberg and Pollak (1), who formulated the structure as 5,12-dihydroquinoxalino[2,3-*b*]quinoxaline (I). Since then, there has been considerable speculation as to whether the compound is more accurately represented by the tautomeric 5,11-dihydro structure, II. A spectroscopic study (2) indicated that the 5,12-dihydro structure, I, is the most probable, and it is used for fluoflavin derivatives in this report. Derivatives having substituents in the benzo rings also may have ambiguous structures; no attempt was made to establish their exact structures.

The new compounds prepared are listed in Tables I and II together with some previously described compounds. Synthetic procedures, generalized where possible, are described under *Experimental*. All derivatives were synthesized by reacting appropriately substituted 1,2-phenylenediamines with 2,3-dichloroquinoxalines over sodium carbonate in refluxing dimethylformamide (3) (Scheme I). Acylation was generally accomplished by long heating in a large excess of the anhydride. Alternatively, conventional acylation with the acyl halide in pyridine was possible.

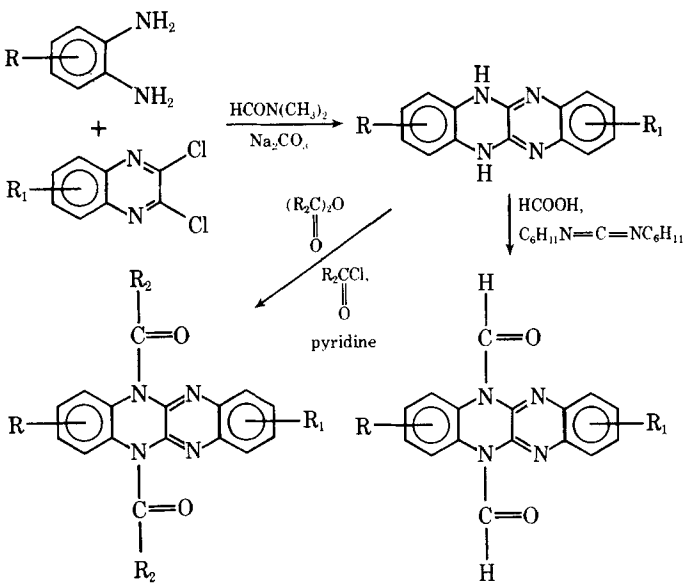
The diformyl derivative could only be prepared with 100% formic acid and *N,N'*-dicyclohexylcarbodiimide. Reduction of the nitro derivative (XXVIII) with hydrogen over palladium-on-carbon gave the amine (XXIX), which was acylated with methyl chloroformate to the carbamate (XXX). Oxidation of I with hydrogen peroxide in trifluoroacetic acid gave quinoxalino[2,3-*b*]quinoxaline 5,11-dioxide (XLIII), previously described by Kuhn and Skrabal (4) as the sole product isolated.



I



II



Scheme I

Biological results (the lowest oral doses demonstrating activity) are shown in Tables I and II. Compounds were tested for anthelmintic activity against *Trichostrongylus* in a standard laboratory animal model assay (5). The most potent compounds in the series, VIII–X, were lower acyl derivatives of the unsubstituted I.

The nonacylated compounds were all extremely insoluble; since I showed erratic but definite activity at 400 mg/kg, the main contribution of acylation was to achieve better absorption and distribution of the intrinsically active ring system. Acylation with groups larger than propionyl diminished activity, as did any of the ring substitutions tried. The aromatic analog (XLII) was inactive, but its bis(*N*-oxide) derivative (XLIII) was surprisingly among the most active compounds in the series. 5,12-Diacetyl-5,12-dihydroquinoxalino[2,3-*b*]quinoxaline (IX) was selected as the most promising and evaluated for anthelmintic efficacy in sheep. At an oral dose of 200 mg/kg, it was effective against *Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus axei, Trichostrongylus colubriformis,* and *Cooperia* sp.

EXPERIMENTAL[1]

Method A: 2-Methoxy-5,12-dihydroquinoxalino[2,3-*b*]quinoxaline (XXIII)—A suspension of 20.0 g (0.144 mole) of 4-methoxy-1,2-phenylenediamine, 28.8 g (0.144 mole) of 2,3-dichloroquinoxaline, and 15.3 g (0.144 mole) of sodium carbonate in 100 ml of dimethylformamide was refluxed for 5 hr and then cooled to room temperature. The product was filtered off and crystallized from acetic acid, 23.1 g (60.3%), mp >340°.

[1] Melting points were taken on a Thomas-Hoover Unimelt apparatus and are uncorrected.