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NOTES

Cytotoxic and Tumor Inhibitory Agent from *Polygala macradenia* Gray (Polygalaceae): 4′-Demethyldeoxypodophyllotoxin

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Abstract □ The chloroform fraction of *Polygala macradenia* exhibited activity against the P-388 lymphocytic leukemia and human epidermoid carcinoma of the nasopharynx test systems. The constituent responsible for this activity was a lignan, 4′-demethyldeoxypodophyllotoxin ($C_{21}H_{20}O_7$). The identity was proven by elemental analysis; PMR, IR, mass spectrometric, and melting-point determinations; and preparation of a derivative.

Keyphrases □ *Polygala macradenia*—chloroform fraction of extract of aerial parts, lignan isolated, evaluated for cytotoxic and tumor inhibitory activity □ 4′-Demethyldeoxypodophyllotoxin—isolated from chloroform fraction of extract of aerial parts of *Polygala macradenia*, evaluated for cytotoxic and tumor inhibitory activity □ Cytotoxic activity—lignan isolated from chloroform fraction of extract of aerial parts of *Polygala macradenia* evaluated □ Tumor inhibitory activity—lignan isolated from chloroform fraction of extract of aerial parts of *Polygala macradenia* evaluated

In the continuing search for plants having tumor inhibitory constituents, it was found that the chloroform fraction of *Polygala macradenia* Gray (Polygalaceae)[1], obtained by partitioning the ethanol extract of the stems, leaves, flowers, and fruit between chloroform and water, showed inhibitory activity against the P-388 lymphocytic leukemia (PS) test system and the human epidermoid carcinoma of the nasopharynx (KB) test system[2].

DISCUSSION

The lignan 4′-demethyldeoxypodophyllotoxin (I), previously isolated from *Polygala paenea* L. (Polygalaceae) (1), was isolated from the chloroform fraction by column chromatography, treatment with activated charcoal, preparative TLC, and recrystallization. The structure of the lignan was confirmed by elemental analysis; PMR, IR, mass spectrometric, and melting-point determinations; and conversion to the known lignan, deoxypodophyllotoxin (II).

Compound I demonstrated an ED_{50} of 0.0012 μg/ml in the KB test system. Activity in the KB system is defined as $ED_{50} \leq 20$ μg/ml (2). In the PS test system, the lignan demonstrated activity of 132% test/control (T/C) at 2.1 mg/kg. Activity in the PS system is defined as an increase in the survival of treated animals over that of control animals resulting in a T/C ≥ 125% (3).

EXPERIMENTAL[3]

Isolation Procedure—The stems, leaves, flowers, and fruit of *P. macradenia* (16 kg) were ground and extracted exhaustively in a Lloyd-type extractor with ethanol. The air-dried residue was partitioned between chloroform (10 liters) and water (10 liters). A 200-g sample of the air-dried chloroform phase was then subjected to alumina III (2 kg) column (7 × 65 cm) chromatography. The solvents used, in increasing order of polarity, were hexane, hexane–benzene (1:1), benzene, chloroform, and methanol; elution was continued with each solvent until the eluent was colorless. The benzene fraction (1.2 g) was subjected to silica gel 60 (75 g) column (3.5 × 24 cm) chromatography, eluting with benzene–ether (4:1). Fractions 16–38 (25 ml each), consisting of one major component according to TLC, exhibited activity.

Isolation of I—The combined active fractions were treated with activated charcoal and purified by preparative TLC with a chloroform–

[1] The plant was collected in Texas in August 1974. Identification was confirmed by Dr. Robert E. Perdue, Jr., Medicinal Plant Resources Laboratory, U.S. Department of Agriculture, Bethesda, Md. A reference specimen is maintained by the U.S. Department of Agriculture.

[2] Of the Drug Evaluation Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, Md.

[3] Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. PMR, IR, and mass spectra were determined using a Varian T-60 spectrophotometer, a Beckman IR-33, and a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), respectively. The melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

ethyl acetate (9:1) solvent system, and the residue was crystallized from methanol to yield colorless plates, mp 246–248° [lit. (1) mp 244–248°]. The IR and PMR spectra were identical to the previously reported spectra (1). Mass spectrometry indicated a parent peak at *m/e* 384.

Anal.—Calc. for $C_{21}H_{20}O_7$: C, 65.61; H, 5.24; mol. wt., 384. Found: C, 65.52; H, 5.08; *m/e* 384.

Compound II—Methylation of I (25 mg) with ethereal diazomethane in dry dichloromethane for 48 hr at room temperature yielded II, which crystallized from methanol as colorless prisms, mp 168° [lit. (1) mp 167–169°]. The IR spectrum was identical to the previously reported spectrum (1).

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Topical Mosquito Repellents X: 2-Oxazolidones

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Abstract □ A number of 2-oxazolidones were evaluated for their effectiveness as topical mosquito repellents. Although some compounds approached diethyltoluamide in potency, none was superior.

Keyphrases □ 2-Oxazolidones, various—synthesized, evaluated as topical mosquito repellents □ Repellents, mosquito—various 2-oxazolidones synthesized and evaluated □ Structure–activity relationships—various 2-oxazolidones evaluated as topical mosquito repellents

Several derivatives of 2-oxazolidone have been prepared and evaluated for effectiveness as topical mosquito repellents. The repellency of all of the compounds against female *Aedes aegypti* (yellow fever) mosquitoes was determined by topical application on human subjects as previously described (1).

Since the majority of effective repellents are either amides or esters, it is surprising that carbamates have not been more extensively investigated. The two literature reports (2, 3) of repellent carbamates indicate that simple alkyl derivatives of the type $R_1OC(=O)N(R_2)R_3$ have provided some degree of protection against *A. aegypti* mosquitoes.

The fact that little data were available in a structural area of reported repellent activity, combined with the intriguing similarity between the two most common classes of repellents (esters and amides) and carbamates, encouraged further studies. 2-Oxazolidones were chosen because of practical considerations. Physical properties for a number of 2-oxazolidones were available (proper volatility is an important consideration for a topically applied insect repellent), as were some well-documented synthetic methods for their preparation.

With few exceptions, all compounds tested were prepared *via* published procedures. Table I gives their structures, boiling points, and topical repellency data. Diethyltoluamide was used as the standard repellent in all tests.

EXPERIMENTAL[1]

Preparation of 3-Methoxymethyl-2-oxazolidone (XIX)—Thionyl chloride (9.5 g, 0.08 mole) was placed in a 20-ml flask and stirred at room temperature. 3-Hydroxymethyl-2-oxazolidone (3.2 g, 0.03 mole) was added dropwise over 30 min, and the resulting solution was stirred overnight.

Excess thionyl chloride was removed *in vacuo*, and the residue was distilled. The desired product (bp 100–103°/0.25 mm) was obtained as a colorless oil, 2.9 g; IR (film): 2970, 1740, 1480, 1260, 1031, and 766 cm^{-1}; NMR ($CDCl_3$): δ 5.31 (s, 2H), 4.45 (t, 2H), and 3.74 (t, 2H).

The 3-chloromethyl-2-oxazolidone was not stable at room temperature (loss of hydrogen chloride), but it could be stored for short periods at −20°.

A solution of sodium methoxide in methanol was prepared by the addition of sodium (0.345 g, 0.015 mole) to a stirred solution of dry methanol under nitrogen. 3-Chloromethyl-2-oxazolidone (2.0 g, 0.015 mole) was then added dropwise over 30 min. After stirring for 1 hr at room temperature, TLC indicated no remaining starting material.

The crude reaction mixture was filtered, and methanol was removed under reduced pressure. Distillation (bp 76°/0.20 mm) afforded XIX as a colorless oil, 1.2 g; IR (film): 2930, 1740, 1421, 1259, and 1080 cm^{-1}; NMR ($CDCl_3$): δ 4.70 (s, 2H), 4.45 (t, 2H), 3.71 (t, 2H), and 3.38 (s, 3H).

Anal.—Calc. for $C_5H_9NO_3$: C, 45.79; H, 6.92; N, 10.68. Found: C, 45.82; H, 6.81; N, 10.95.

Preparation of 3-Acetoxymethyl-2-oxazolidone (XX)—Acetic anhydride (2.04 g, 0.02 mole) was added dropwise to a stirred solution of 3-hydroxymethyl-2-oxazolidone (2.0 g, 0.017 mole) in dry pyridine (5 ml). The colorless solution was then stirred overnight at room temperature.

The crude reaction mixture was concentrated *in vacuo;* the residue was dissolved in methylene chloride, washed with saturated sodium chloride, dried, and reconcentrated *in vacuo*. Distillation (100–104°/0.1 mm) afforded XX as a colorless oil, 1.9 g; IR (film): 2950, 1760, 1740, 1420, 1210, 1020, and 940 cm^{-1}; NMR ($CDCl_3$): δ 5.37 (s, 2H), 4.40 (t, 2H), 3.76 (t, 2H), and 2.07 (s, 3H).

[1] Melting points were determined on a capillary melting-point apparatus and are uncorrected. Boiling points were determined using a short path distillation apparatus and also are uncorrected. IR and NMR spectra were taken of all compounds and were consistent with the assigned structures. Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Stanford University, Stanford, Calif.