Tumor Inhibitors. I. Aristolochic Acid, the Active Principle of *Aristolochia indica*[1]

S. MORRIS KUPCHAN AND RAYMOND W. DOSKOTCH

Department of Pharmaceutical Chemistry, University of Wisconsin, Madison, Wisconsin

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In the course of a continuing screening program for tumor inhibitors from plant sources, an alcoholic extract of the roots of *Aristolochia indica*[2] was found to have reproducible activity against Adenocarcinoma 755 in mice.[3] We report herein the fractionation of the active extract and the isolation and characterization of the active principle, aristolochic acid.

The dried ground roots of *A. indica* were extracted in a Soxhlet extractor with 95% ethanol. After concentration of the extract under water-pump pressure at a temperature no higher than 40°, a thick brown sirup was obtained. About 120 g. of concentrated extract was obtained per kg. of root. Preliminary studies of the alcoholic extract indicated that partition between chloroform and water resulted in concentration of the activity in the chloroform phase. Upon extraction of the chloroform solution with dilute aqueous base, the active principle was extracted into the alkaline layer. Further fractionation of the alkali-soluble active material was effected by adsorption chromatography on silicic acid (Mallinckrodt):Celite 545 (Johns-Manville), 4:1, whereby the active material was concentrated into a single yellow solid fraction. The latter fraction was readily crystallized from absolute alcohol to afford a high yield of aristolochic acid [1-ethoxy-5,6-methylenedioxy-9-nitro-8-phenanthroic acid (I)].

The flow sheet for the isolation is given in Fig. 1, and the biological data[3] for the fractions obtained in a typical experiment are reported in Table I. The evaluation of the assay results by CCNSC on a statistical basis in sequential testing is such that a material is considered active if it causes reduction of tumor weight to 42% or less when at least seven of the ten animals survive. The data in Table I, consequently, indicate that only subfractions F and G show tumor-inhibitory activity.

Characterization of the active principle as aristolochic acid (I) was effected by comparison of melting point, ultraviolet and infrared spectra with those reported by Pailer, *et al.*[4] Decarboxylation of I

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(2) Gathered in Madras, India in May, 1958. The authors thank Rajaranga and Company, Madras, India, for gathering and forwarding the dried plant materials to us, and Dr. C. B. Sulochana, University Botany Laboratory, Madras, for confirming the identity of the plant.

(3) Assays were performed by the Wisconsin Alumni Research Foundation under contract to the Cancer Chemotherapy National Service Center. The procedures were those described in *Cancer Chemotherapy Reports*, **1**, 42 (1959).

(4) M. Pailer, L. Belohlav, and E. Simonitsch, *Monatsh.*, **87**, 249 (1956).

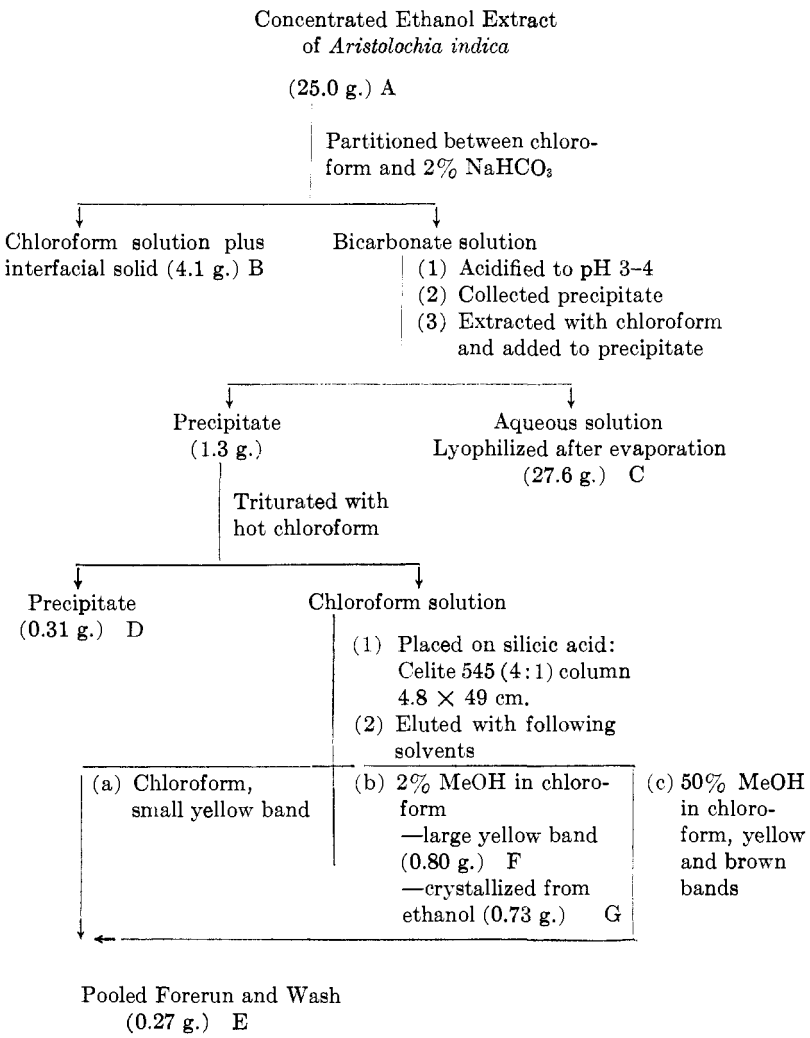
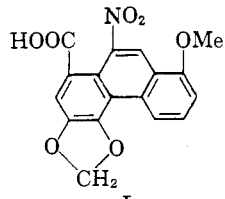
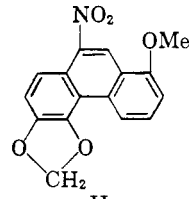


Fig. 1.—Flow sheet for the isolation of the tumor inhibiting principle of *Aristolochia indica*.

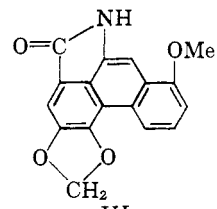
with copper powder in quinoline gave 1-methoxy-5,6-methylenedioxy-9-nitrophenanthrene (II), with physical properties identical to those reported.[4] Reduction of I with zinc in glacial acetic acid yielded 9-amino-1-methoxy-5,6-methylenedioxy-8-phenanthroic lactam (III), identical in regard to melting point and spectral properties with reported values.[4]



I



II



III

It is of considerable interest to note that extracts of various species of *Aristolochia* have been used in the treatment of cancer since antiquity; references to such use occur at least as far back as the Graeco-Roman period.[5]

TABLE I

ACTIVITY OF FRACTIONS FROM *A. indica* IN ADENOCARCINOMA 755

Fraction	Control no.	Dose, mg./kg.	Survivors	Change in weight (g.) (test/control)	Tumor weight (mg.) (test/control)	T/C
A	1290	90	10/10	−2.8/1.3	723/1633	0.44
		60	10/10	−3.3/1.3	687/1633	0.42
		40	9/10	−2.6/1.3	1623/1633	0.99
		20	9/10	−0.1/1.3	1687/1633	1.03
B		90	10/10	−3.6/1.3	1118/1633	0.68
		60	10/10	−2.2/1.3	1221/1633	0.75
		40	9/10	−1.3/1.3	1214/1633	0.74
		20	9/10	−0.8/1.3	1057/1633	0.64
C		120	9/10	3.0/1.3	2203/1633	1.34
		80	10/10	1.7/1.3	2383/1633	1.45
		55	10/10	1.0/1.3	1576/1633	0.96
		37	9/10	0.4/1.3	1798/1633	1.10
D		50	8/10	0.1/1.3	1926/1633	1.17
		33	10/10	0.0/1.3	1974/1633	1.20
		22	10/10	−0.2/1.3	1644/1633	1.00
		11	10/10	1.7/1.3	2184/1633	1.33
E		30	10/10	−1.5/1.3	1458/1633	0.89
		20	10/10	−0.6/1.3	1644/1633	1.00
		15	10/10	−0.5/1.3	1533/1633	0.93
		7.5	10/10	0.7/1.3	1768/1633	1.08
F		10	2/10	1.8/1.3	1275/1633	Toxic
		7	4/10	−2.7/1.3	561/1633	Toxic
		5	8/10	−3.6/1.3	591/1633	0.36
		3	9/10	−0.7/1.3	1219/1633	0.74
G	1278	5	2/10	−2.3/1.8	350/1518	Toxic
		4	7/10	−2.3/1.8	357/1518	0.23
		3	6/10	−2.3/1.8	602/1518	Toxic
		2	7/10	−2.2/1.8	744/1518	0.49

(5) G. Urdang, G. D. Goldat, D. Queller, and G. Sonnedecker, "An Examination of Old Literature (Especially Herbals) for Drugs with Supposed Effects on Cancer," Report to National Institutes of Health, on Contract No. C-2089 (C) with the University of Wisconsin (1956).