Anticancer Evaluation of *Amphipterygium adstringens*

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The antineoplastic activity of numerous *Amphipterygium adstringens* fractions has been studied on dba mice bearing RC mammary adenocarcinoma. Methods of anticancer evaluation are fully described. Several tumor inhibitors, as well as activators, were found in the various drug fractions. The methanol extract of the whole drug exhibited the most pronounced inhibitory activity. The tumor-damaging capacity of the drug has been shown to be mainly associated with the saponin-containing fractions.

THE POSSIBILITIES of discovering natural products with anticancer activity have been the motivation for establishing and maintaining many screening programs in cancer laboratories throughout the country. Although research in this area is mainly concerned with negative data, it is hoped that reports of this nature will spare others the time and effort of investigating sources which have proved to be of no value, and secondly, will focus attention on substances that are worthy of further exploration. Little progress has been reported in the field of cancer chemotherapy and promising leads are still as scarce today as they were several years ago (1, 2).

Several laboratories with cancer screening programs have reported the testing of plant extracts for possible adverse tumor effects. Belkin and associates have published a series of papers on the tumor-damaging action of numerous plant preparations (3–5). Faust and coworkers have reported in some detail the antineoplastic activity of *Citrullus colocynthis* on Sarcoma-37 (6). More recently, McKenna, *et al.* (7), examined numerous essential oils as potential antitumor agents. Testing and screening materials for anticarcinogenic activity have been routine in the Clayton Foundation Biochemical Institute cancer research laboratories for a number of years (8–12).

This study was initiated after preliminary screening tests showed that *Amphipterygium adstringens* Schlecht, fam. *Julianiaceae* possessed a certain degree of anticancer activity in both mice and embryonated eggs (9). Since *Amphipterygium adstringens* was readily available in ufficient quantity, a simultaneous chemical investigation was conducted on the drug in an effort to ascertain the nature of the component responsible for the antineoplastic activity (13).

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EXPERIMENTAL

Fractionation of Drug.—The drug fractions used in this study were essentially those isolated during the course of the phytochemical investigation (13). The various drug fractions that were isolated and prepared for this study are schematically outlined in Fig. 1.

Preparation of Drug Fractions.—The drug fractions were stored in the refrigerator and processed for testing as soon as possible to avoid possible loss of biological activity. Before the drug fractions were tested biologically, they were sterilized by one of two methods. Water-soluble extracts were sterilized by filtering through a Selas No. 2 porcelain filter. Other extracts were sterilized by placing the material in a sterile Erlenmeyer flask containing a small quantity of 70% alcohol. The flask was placed in a desiccator under aseptic conditions and the alcohol evaporated by use of a vacuum pump. Acetone-soluble extracts were tested by the latter method with the exception that acetone was used in place of 70% alcohol. The extracts were then checked for sterility according to procedures described in previous reports (9). Sterile extracts were tested for toxicity by trials in mice. Subdermal injections of various concentrations were made and the effect produced on weight and visceral organs was noted. It was important to select a dosage range that would give distinguishable results between possible toxic reactions and tumor damage.

Type of Neoplasms.—The fractions were tested for antineoplastic activity against RC mammary adenocarcinoma in dba mice. This mammary tumor has been employed for screening purposes in other cancer research laboratories (14). Transplants of RC mammary adenocarcinoma in the dba strain have been maintained in the Clayton Foundation Biochemical Institute cancer laboratories since the time of their spontaneous development in 1941. The preliminary screening tests were conducted on C_3H mouse mammary carcinoma cultivated in eggs (15), since the time and effort involved is much less than that required by the animal transplant method.

Methods of Anticancer Evaluation.—The method described for the anticancer evaluation studies is somewhat similar to that reported earlier by

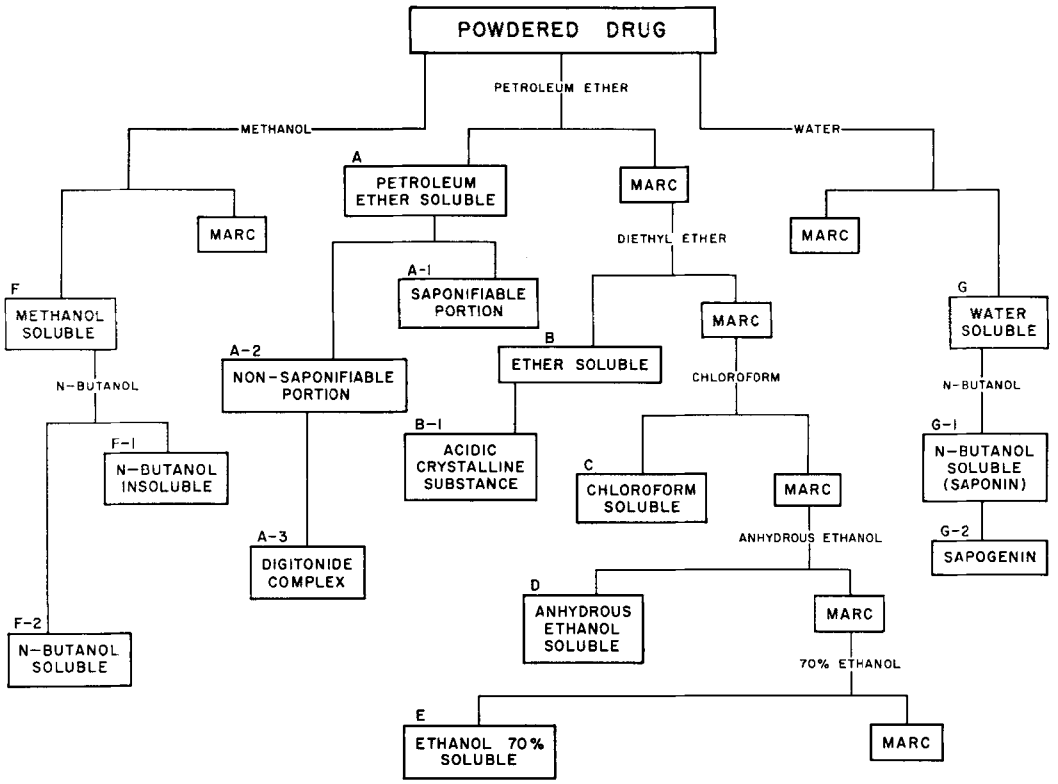

Fig. 1.—Fractionation of *Amphipterygium adstringens*.

McKenna and co-workers (9, 11). The dba mice used in this study were kept in stock at least 10 days before use and were maintained on regular laboratory chow and water, *ad libitum*. Transplants of the RC mammary adenocarcinoma were made by subdermal injections of sterile tumor suspensions. Fresh homogenized tumor tissue (0.02 ml.) dispersed in 0.2 ml. of 0.85% saline solution was injected in the right inguinal area. The mice were then divided randomly into equal groups. One group was kept as control, and received in the left inguinal area an injection of 0.2 ml. of sterile water for injection. The remaining mice, designated as the experimental group, received daily injections of the test fraction for 7 days. The test fractions were likewise administered in the left inguinal region, in the form of either aqueous solutions or suspensions, 72 hours after tumor implantation. All of the test fractions were used in concentrations of 750 mcg./ml. Only during the preliminary experiments were saturated solutions near maximum tolerated doses employed. All the animals were sacrificed on

Table I.—Antitumor Activity of Some *Amphipterygium adstringens* Extracts in dba Mice Bearing RC Mammary Adenocarcinoma

Type of Extract	Dosage Form	No. of Mice Used	Av. Body Weight at Autopsy, Gm.	Av. Tumor Weight at Autopsy Gm.	Percentage Body Growth at Autopsy[a]	Percentage Tumor Growh at Autopsy[a]
A	Suspension	12	22.80	0.47	102	157
A-1	Suspension	10	25.10	0.77	97	70
A-2	Suspension	12	27.10	0.95	105	86
A-3	Solution	8	23.10	0.51	104	68
B	Suspension	12	26.50	0.78	103	70
B-1	Solution	12	19.80	0.38	89	127
C	Suspension	12	22.80	0.64	102	213
D	Solution	12	24.10	0.40	109	53
E	Solution	12	22.30	0.30	97	60
F	Solution	16	25.70	0.51	100	46
F-1	Solution	12	20.30	0.40	91	133
F-2	Solution	8	23.60	0.47	102	50
G	Solution	10	24.40	0.42	95	52
G-1	Solution	10	22.30	0.38	92	51
G-2	Solution	10	26.00	1.10	101	100

[a] Control equals 100%.

the eleventh day of tumor growth and the weights of the animals and tumors recorded. The effectiveness of the drug fractions was evaluated on the basis of the difference in the average weight of the tumor in the experimental group as compared with the tumor growth in the control animals.

RESULTS AND DISCUSSION

The experimental results are given in Table I. It will be noted from the data shown that the methanol extract (F) of the whole drug exhibited the greatest amount of inhibition. This particular extract (F) induced a 54% decrease in tumor growth as compared with controls. Comparable inhibitory activity was exhibited by the *n*-butanol fraction (F-2) of the methanol extract (F). The F-2 fraction evinced a 50% decrease in tumor growth as compared with controls. In general, the most significant inhibitory activities were demonstrated by fractions D, E, F, F-2, G, and G-1. In addition to these inhibitory fractions, growth activators were found among the various drug fractions. Tumor growth was stimulated primarily by the petroleum ether (A) and chloroform (C) extracts of the drug. The petroleum ether extract (A) increased tumor growth by 57% as compared with controls. The chloroform extract (C) accelerated and increased tumor growth by as much as 113%. Body weight was not affected by the various fractions, with the possible exception of the acidic crystalline substance (B-1) isolated from the ether extract (13). The B-1 treated animals experienced an average body weight loss of 4.21 Gm. as compared with the average body weight of all other treated animals.

None of the experimental animals showed any toxic drug symptoms. However, upon autopsy, it was noted that some of the experimental animals did have irritation at the site of drug release and that, in a few cases, the drug was not thoroughly dispersed and absorbed. These manifestations were mainly associated with those fractions (A, A-1, A-2, B) which were administered in the form of suspensions.

Since the inhibitory activities of the saponin-containing fractions (D, E, F, F-2, G) were comparable to that of the isolated saponin (G-1), it is suggested that the anticarcinogenic action of *Amphipterygium adstringens* is primarily attributed to the presence of the steroidal saponin. Although this generalization is well supported by the fact that the saponin-exhausted fraction (F-1) exhibited a loss of inhibitory activity, it is rather difficult to justify completely the inhibitory activity on this observation since saponins are well known for their characteristic hemolytic activity, and yet no deleterious effects were observed. It should be noted also that the aglycon fraction (G-2) possessed no biological activity. Compared with the saponin (G-1), the aglycon fraction (G-2) lost inhibitory activity by 49%. These data indicate that the carbohydrate residues may play an important role in the biological action of the steroidal saponin. It may well be that the carbohydrate residues significantly affect absorption and distribution of the agent, as in the case of the cardioactive glycosides.

SUMMARY

Over 150 dba mice bearing RC mammary adenocarcinoma were used to study the antineoplastic effect of *Amphipterygium adstringens*, Schlecht. Numerous drug fractions were prepared and evaluated for anticancer activity. The results of this investigation are summarized.

1. The methanol extract (F) of the whole drug exhibited the most pronounced inhibitory activity. A 54% decrease in tumor growth was induced by the crude extract as compared with controls

2. Tumor growth was stimulated by the petroleum ether (A) and chloroform (C) extracts of the drug.

3. The saponin-containing fractions (D, E, F, F-2, G) exhibited inhibitory activity comparable to the isolated saponin (G-1).

4. The saponin-exhausted fraction (F-1) showed complete loss of inhibitory activity.

5. In contrast to the saponin (G-1), the aglycon (G-2) evinced no biological activity. Compared with the saponin (G-1), the aglycon (G-2) lost inhibitory activity by 49%.

6. Body weight was affected only by the acidic crystalline substance (B-1) isolated from the ether extract. An average body weight loss of 4.21 Gm., as compared with other treated animals, was noted.

7. The experimental results indicate that the anticarcinogenic activity of *Amphipterygium adstringens* may be due to the presence of the steroidal saponin (G-1).

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