Desoxypodophyllotoxin, the Cytotoxic Principle of *Callitris columellaris* F. Muell

Y. AYNEHCHI

Abstract □ An alcoholic extract of *Callitris columellaris* F. Muell was found to show significant inhibitory activity when tested *in vitro* against cells derived from human carcinoma of the nasopharynx (KB). Systematic fractionation of the extract led to the isolation and characterization of desoxypodophyllotoxin as the active principle.

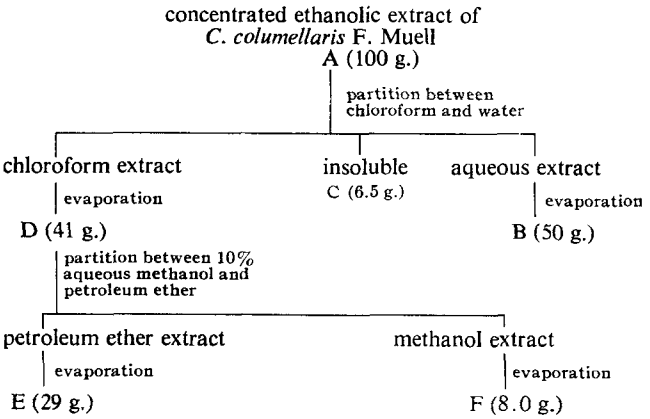
Keyphrases □ Desoxypodophyllotoxin—isolation from *Callitris columellaris*, cytotoxicity evaluation □ *Callitris columellaris*—isolation of cytotoxic principle, desoxypodophyllotoxin, evaluation □ TLC—separation, identification

It was reported earlier (1) that leaves of several junipers possess tumor-necrotizing activity for a number of transplant mouse tumors. In several species the active material was podophyllotoxin (2); in one species, a previously undescribed but related lignin, silicicolin (3), now called desoxypodophyllotoxin (4), was the active material. Further investigation of the leaves of four additional genera (*Libocedurs*, *Podocarpus*, *Chamacyparis*, and *Callitris*) revealed the presence of tumor-necrotizing substances in these plants.

Previous examination of *Callitris columellaris* indicated the presence of *l*-citronellic acid, eudesmol or cryptomeridol, guaiol, and azulene (5). The identification of these compounds was confirmed by GLC and IR and NMR spectroscopy (6). Furthermore, it has been shown that the extract from *C. columellaris* has fungistatic activity. The antifungal property is mainly due to the presence of *l*-citronellic acid, which is an antifungal agent toward *Conifora olivocea* and cryptomeridol, which are antifungal agents toward *C. sanguineus* (7).

In the course of a continuing search for tumor inhibitors from plant sources, an alcoholic extract of *C. columellaris* F. Muell[1] (Pinaceae) was found to have significant inhibitory activity against cells derived from human carcinoma of the nasopharynx in tissue culture (KB). Consequently, a systematic study aimed at isolation of the KB inhibitory principle of *C. columellaris* was undertaken.

The preliminary fractionation of the alcohol extract is summarized in Scheme I. Fraction F material dissolved in chloroform was subjected to chromatography on magnesia–silica gel,[2] whereupon the total activity



Scheme I—Flow Sheet for Fractionation of Cytotoxic Extract from C. columellaris

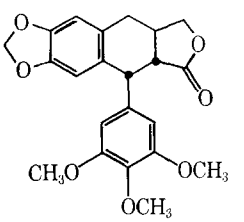
was eluted by using chloroform and 2% methanol in chloroform as eluent.

Further careful chromatography on a silica gel column, collecting fractions which are analyzed by TLC, led to the isolation of desoxypodophyllotoxin (I) from Fraction G.

Results of the tissue culture assays are given in Table I. Material was considered active if the ED_{50} (dose inhibiting to 50% of control growth) was equal or less than 1 mcg./ml. The fractions containing desoxypodophyllotoxin were found to show the highest cytotoxicity. The results indicate that desoxypodophyllotoxin is the major principle of the extract of *C. columellaris* F. Muell.

EXPERIMENTAL

Melting points were obtained on a Hoover Uni-Melt capillary melting-point apparatus. IR spectra were obtained in chloroform solution on a Beckman IR5-A spectrophometer. TLC was carried out on silica gel G and H plates (E. Merck), and the chromatograms were sprayed with a $Ce(SO_4)_3$–H_2SO_4 solution followed by heating until brown spots appeared. Petroleum ether was used,[3] b.p. 60–68°. Evaporation was carried out at less than 40°.



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[1] Leaves and stems gathered in Australia in June 1965 by the U. S. Department of Agriculture (USDA). The author acknowledges the receipt of the dried plant material from Dr. Robert E. Perdue, Jr., USDA, Beltsville, Md., in accordance with the program developed with USDA by Cancer Chemotherapy National Service Center. A voucher specimen has been kept by USDA, Beltsville, Md.

[2] Florisil, Floridin Co., Pittsburgh, Pa.

[3] Skellysolve B; Skelly Oil Co., Kansas City, Mo.

Table I—Cytotoxicity of Fractions from *C. columellaris*[a]

Fractions	ED_{50}, mcg./ml.
A	0.40
B	64.0
C	100.0
D	0.57
E	32.0
F	3×10^{-2}
G	2.4×10^{-2}
Desoxypodophyllotoxin	5.8×10^{-7}

[a] Assays were performed under the auspices of the Cancer Chemotherapy National Service Center. The procedures were those described in *Cancer Chemother. Rep.*, **25**, 1(1962).

Extraction and Preliminary Fractionation of *C. columellaris*— Coarsely ground leaves and stems of *C. columellaris* (5 kg.) were twice extracted continuously with 95% ethanol for 10 hr., and the ethanol extract was concentrated under water pump pressure to a thick dark syrup (A, 1690 g.). A portion of the crude extract (100 g.) was partitioned between water (1.0 l.) and chloroform (2.0 l.), and the two solutions were evaporated under reduced pressure (B, 59.0 g.; and D, 41 g.). The insolubles (C, 6.50 g.) were collected separately.

The chloroform-soluble portions were partitioned between petroleum ether (2.0 l.) and 10% aqueous methanol (1750 ml.), and the two solutions were evaporated under reduced pressure (E, 29.10 g.; and F, 8.0 g.). The 10% aqueous methanol-soluble portions were dissolved in chloroform and chromatographed on a column (60 × 4 cm.) containing 800.0 g. magnesia–silica gel (100–200 mesh, F-101); then they were eluted with 2.0 l. chloroform followed with 1.0 l. 2% methanol in chloroform. The eluents (chloroform and 2% MeOH in chloroform solutions) were combined and evaporated under reduced pressure (G, 4.10 g.).

Isolation of Desoxypodophyllotoxin—A large batch of Fraction G (55.0 g.) was prepared from crude extract (5.0 kg.), dissolved in benzene, added to a column (75 × 6 cm.) of silica gel (1200 g.), and chromatographed, using 20% chloroform in benzene as the solvent. Fractions (30 ml.) were collected and examined by TLC on silica gel G and H, using 1% MeOH in chloroform as eluent and ceric sulfate (3% in 3 *N* sulfuric acid) spray reagent. The fractions richest in desoxypodophyllotoxin were combined, evaporated to dryness (4.20 g.), and crystallized from ethanol. The colorless crystalline product (3.10 g.) was characterized as desoxypodophyllotoxin by mixed melting point, m.p. 166–168°, mixed TLC, and IR spectral comparison with the authentic sample.

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3-Substituted-2-thiohydrothymines as Potential Antitumor Agents

A. C. GLASSER* and JOHN TRIPLETT

Abstract □ A series of 3-substituted-2-thiohydrothymines was synthesized through the cyclization of the corresponding 1-(2-methyl)-carboxyethyl-3-substituted-2-thioureas which were formed from the appropriate isothiocyanate and β-aminoisobutyric acid. The cyclized products were screened for antitumor activity against lymphoid leukemia L-1210. Results of the screening indicated the compounds have little significant activity in the test system employed.

Keyphrases □ 2-Thiohydrothymines, 3-substituted—synthesis, antitumor testing □ Antitumor agents, potential—2-thiohydrothymines, synthesized, screened □ IR spectrophotometry—structure, identity

Most chemical agents that prevent cancerous growth do so by affecting nucleotide or nucleic acid metabolism. This is both a conclusion of screening (empirical) and a vindication of the search for such agents on purely theoretical reasons (1). Uncontrolled growth is a hereditary property of the neoplastic cell, and this heritable nature of cancer is one reason for considering interference with nucleic acid metabolism in the search for new agents (2).

An earlier report from these laboratories on the synthesis and antituberculous and antitumor activities of a series of 3-substituted-2-thiohydrouracils showed a degree of antitumor activity slightly above the level of inhibition needed for further experimental testing according to the Cancer Chemotherapy National Service Center (CCNSC) Protocol for tumor weight inhibition (3). Compounds in the thiohydrouracil series, such as 3-*p*-ethoxyphenyl- and 3-*p*-(*n*-butoxyphenyl)-, showed test/control (T/C) % values of 62 and 63, respectively, against Sarcoma 180 as tested by CCNSC.

On further consideration of the general class of compounds, it was felt that modifying the structures to correspond to that of thymine, which has a methyl