Tumor Inhibitors XIX. Desoxypodophyllotoxin, the Cytotoxic Principle of *Libocedrus decurrens*

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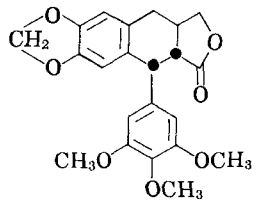
An alcoholic extract of *Libocedrus decurrens* Torr. was found to show significant inhibitory activity when tested *in vitro* against cells derived from human carcinoma of the nasopharynx (KB). Systematic fractionation of the extract led to the isolation and characterization of desoxypodophyllotoxin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

In the course of continuing search for tumor inhibitors from plant sources, an alcoholic extract of *Libocedrus decurrens* Torr.[1] (*Pinaceae*) was found to have significant inhibitory activity against cells derived from human carcinoma of the nasopharynx in tissue culture (KB).[2] Consequently, a systematic study aimed at isolation of the KB-inhibitory principle of *L. decurrens* Torr. was undertaken. The preliminary fractionation of the alcohol extract is summarized in Scheme I. Fraction H material, dissolved in chloroform, was subjected to chromatography on silicic acid, whereupon the total activity was eluted in the first yellow band using chloroform as eluant. Further careful rechromatography on silicic acid, collecting fractions which were analyzed by thin-layer chromatography, led to the isolation of desoxypodophyllotoxin (I) from the fraction eluted with 10% chloroform in benzene. The assay data are reported in Table I. Evaluation of the tissue culture assay results by the CCNSC is such that a material is considered active if the ED_{50} (dose inhibiting growth to 50% of control growth) equals or is less than 1 mcg./ml. The fractions containing desoxypodophyllotoxin were found to show the highest cytotoxicity. The results indicate that desoxypodophyllotoxin is the major cytotoxic principle of the extract of *L. decurrens* Torr.[3]



I

EXPERIMENTAL

Melting points were determined on a Hoover Uni-Melt capillary melting point apparatus. Infrared spectra were determined in chloroform solution on a Beckman IR5A infrared spectrophotometer. The petroleum ether used was Skellysolve B, b.p. 60–68°. Evaporations were carried out at temperatures less than 40°.

Extraction and Preliminary Fractionation of L. decurrens—Coarsely ground leaves and stems of *L. decurrens* (500 Gm.) were twice extracted continuously with 95% ethanol for 5 hr., and the ethanol extract was concentrated under water pump pressure to a thick dark syrup (A, 142 Gm.). A portion of the extract (96 Gm.) was partitioned between water (1.5 L.) and chloroform (2.5 L.), and the two solutions were evaporated under reduced pressure (B, 59 Gm., and D, 27 Gm., respectively) and the insolubles (C, 8.1 Gm.) collected separately. The chloroform solubles, except a small assay sample (50 mg.), were partitioned between petroleum ether (1 L.) and 10% aqueous methanol (750 ml.) and the two solutions were evaporated under reduced pressure (E, 9.0 Gm., and F, 15.0 Gm., respectively). The 10% aqueous methanol solubles were dissolved in methanol (250 ml.) and treated with a saturated methanol solution of neutral lead acetate until no more precipitate formed. The precipitate was removed by centrifuging and washed with methanol before suspending in methanol and bubbling in hydrogen sulfide. The lead sulfide was filtered after regeneration had been completed and the filtrate was evaporated to a gummy residue (G, 1.94 Gm.). The supernatant liquid from lead acetate precipitation was also treated with hydrogen sulfide, and the lead sulfide was filtered off and the solvent evaporated (H, 11.9 Gm.).

Isolation of Desoxypodophyllotoxin—A larger batch of fraction H (151 Gm.) was prepared from the crude extract (1.5 Kg.) and a portion (57.9 Gm.) was dissolved in chloroform and added to a column of silicic acid (2.6 Kg.). The column was eluted with chloroform until the first orange-yellow band was collected (I, 16.445 Gm.) and the remaining material was stripped with methanol (J, 36.0 Gm.).

A portion of the active fraction I (5.6 Gm.), was dissolved in benzene, added to a column of silicic acid (80 Gm.) packed in benzene, and chromatographed using 10% chloroform in benzene as solvent. Fractions were collected and examined by thin-layer chromatography on silica gel using chloroform as solvent and ceric sulfate (3% in 3 *N* sulfuric acid) spray reagent. The fractions richest in desoxypodophyllotoxin were combined, evaporated to dryness (533 mg.), and crystallized from ethanol. The colorless crystalline product (K, 195 mg.) was characterized as desoxypodophyl-

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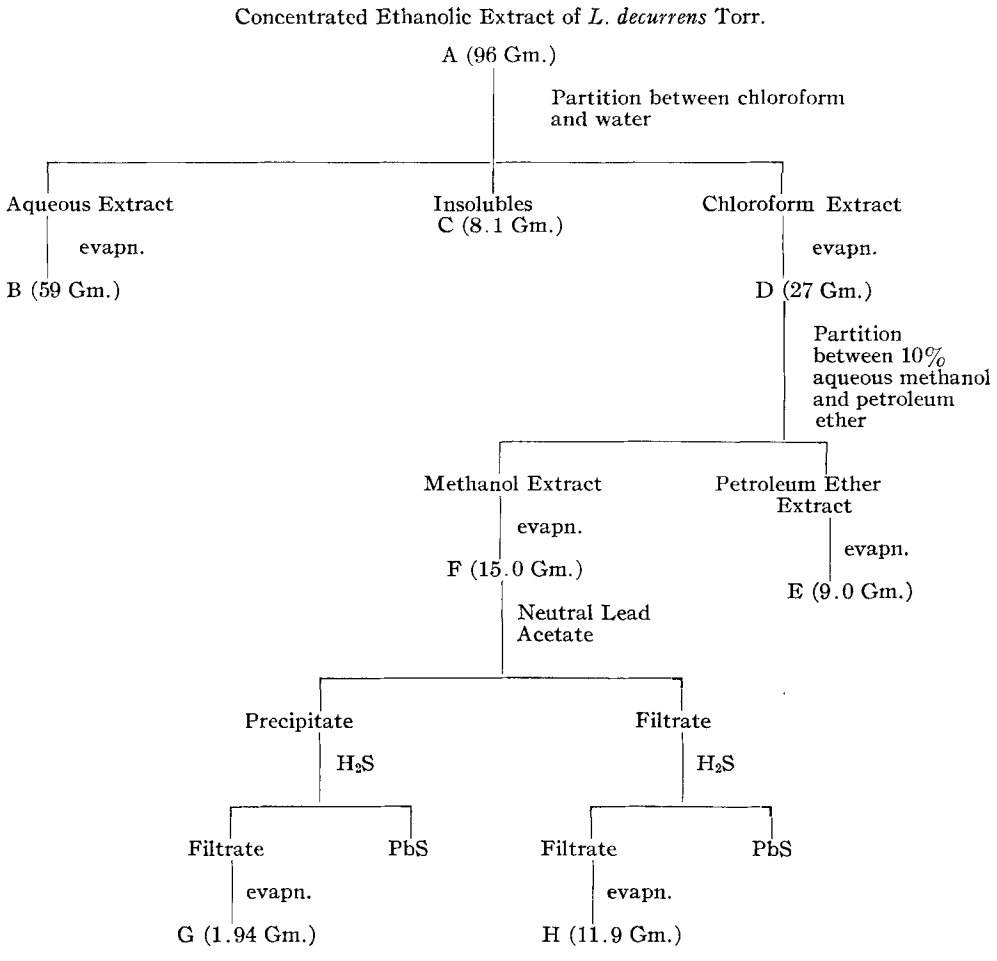
Previous paper: Abbott, B. J., Hartwell, J. L., Iltis, H. H., Kupchan, S. M., Leiter, J., Perdue, R. E., Jr., and Schepartz, S. A., *Cancer Res. (Suppl.)*, **26**, 1131(1966).

[1] Leaves and stems gathered in California in September 1964. The authors acknowledge the receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Md., in accordance with the program developed with the USDA by the Cancer Chemotherapy National Service Center.

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

[3] It has been reported previously (1) that desoxypodophyllotoxin occurs in *Libocedrus chilensis* and is active against sarcoma 37. The same authors, however, found that their sample of *L. decurrens* was inactive against sarcoma 37.



Flow Sheet for Fractionation of Cytotoxic Extract from *L. decurrens* Torr.

Scheme I

TABLE I—CYTOTOXICITY OF FRACTIONS FROM *L. decurrens* TORR.

Fraction	ED_{50}, mcg./ml.
A	0.26
B	>100
C	27
D	0.063
E	3.0
F	0.024
G	2.6
H	0.23
I	<0.001
J	2.5
K	1.6×10^{-9}

lotoxin by mixed melting point, mixed thin-layer chromatography, and infrared spectral comparison with an authentic sample.[4]

Other chromatographic fractions with contained desoxypodophyllotoxin also showed cytotoxic activity. The desoxypodophyllotoxin-free fractions were found to be essentially inactive in the KB assay.

REFERENCE

(1) Fitzgerald, D. B., Hartwell, J. L., and Leiter, J., *J. Natl. Cancer Inst.*, **18**, 83(1957).

[4] The authors thank Dr. J. L. Hartwell, CCNSC, National Cancer Institute, for the authentic sample of desoxypodophyllotoxin.