Antitumor Agents from *Bursera microphylla* (*Burseraceae*) I. Isolation and Characterization of Deoxypodophyllotoxin

By E. BIANCHI, M. E. CALDWELL, and J. R. COLE

Deoxypodophyllotoxin has been isolated and characterized as one of several antitumor agents from *Bursera microphylla* (*Burseraceae*). β-Sitosterol has also been shown to be present in this plant.

As a result of a routine screen of Southwestern plants for potential antitumor activity, the chloroform extract of *Bursera microphylla* A. Gray (*Burseraceae*)[1] demonstrated activity toward the human epidermoid carcinoma of the nasopharynx test system (cell culture) of the CCNSC (9KB).

Bursera microphylla is a tree usually less than 30 ft. tall and can be found in Southwestern Arizona, Baja California, Sonora, and southward into Central Mexico. The material used in this research was collected in March and June from rocky dry slopes in the Gila mountains near Ligurta in Yuma County, Arizona. *Bursera microphylla*, as well as other *Bursera* species, have been the subject of previous investigations: various substances such as phellandrene and phellandral (1), α-hydroxylupene and α-amyrin (2), and others (3) have been reported.

The above materials did not show any tumor inhibition activity (4). The plant was then subjected to a systematic fractionation in order to characterize the active principle. The fractionation procedure involved the utilization of solvent extraction followed by adsorption chromatography on alumina, thick-layer chromatography on silica gel, and paper chromatography.

Four substances (I, II, III, and IV) were separated. Substance IV was identified as β-sitosterol on the basis of mixed melting point, superimposable infrared spectra of an authentic specimen, and a comparison of R_f values with a known specimen utilizing thin-layer chromatography. The solvent systems employed were dichloromethane–benzene–ethyl acetate (12:24:3) and ethyl ether–dichloromethane (1:5). Substances I, II, and III showed a strong tumor inhibition against the 9KB system. Substance III was identified as deoxypodophyllotoxin. This is somewhat unique as there are apparently no reports in the literature of this compound being isolated from this plant or any closely related genus and species. In addition, III was treated with a mild alkali and was converted into deoxypicropodophyllin (the "picro" or "B" series). Substance II was subjected to paper and thin-layer chromatography and showed a single spot but all attempts at crystallization failed. The isolation, purification, and characterization of this compound will be reported in a forthcoming publication. Substance I was not present in sufficient quantities to warrant further chemical investigation at this time but will also be investigated at a later date.

The preliminary chloroform extract exhibited activity of less than 1.0×10^{-2} mcg./ml. Deoxypodophyllotoxin showed activity in this system of 2.6×10^{-3} mcg./ml. Activity is defined as $ED_{50} \leqq 10$ mcg./ml. for plant extracts. Results are expressed as the dose that inhibits growth to 50% of control growth 3 days after drug addition (5).

EXPERIMENTAL

Extraction—The stems (12 Kg.) of *Bursera microphylla* were dried in the air, ground, and extracted exhaustively in a Soxhlet with chloroform. After removal of the solvent, the residue (470 Gm.) was treated several times with petroleum ether (b.p. 40–60°). The material obtained from the solution was inactive and, therefore, discarded. The insoluble part separated from the liquid by centrifugation was extracted with ethyl ether. The insoluble portion was inactive and discarded. The organic layer was stirred (not shaken in order to prevent emulsion) three times with a 10% aqueous solution of sodium carbonate. The ethereal extract, after removal of the alkali aqueous solution yielded the active material (Scheme I).

Isolation and Identification of Deoxypodophyllotoxin—The active material was chromatographed through a column of alumina (grade III). The second fraction obtained by elution with benzene yielded material showing improved activity. The fractions obtained by elution with chloroform, ethyl ether, acetone, and methanol were inactive and were discarded. The active fraction was then subjected to thin-layer chromatography. The matrix used was Silica Gel G, Merck. The solvent system employed was dichloromethane–benzene–ethyl acetate (12:24:3). A double run was employed. There appeared to be four predominant materials. In order to obtain each of these in a pure state, the mixture was applied on preparative chromatoplates which were run under the same general conditions. This procedure afforded more precise separation of the four substances designated as I, II, III, and IV. Substance I was neglected because the amount of material obtained was insufficient for further study at this time. Substance IV was identified as β-sitosterol by the procedures indicated above. The yield of β-sitosterol from the dry plant was approximately 0.021%. Since the resolution of II and III was not satisfactory because of the closeness of their R_f values when the above indicated solvent system was used, they were eluted together, applied on preparative chromatoplates (1 mm. thick), and

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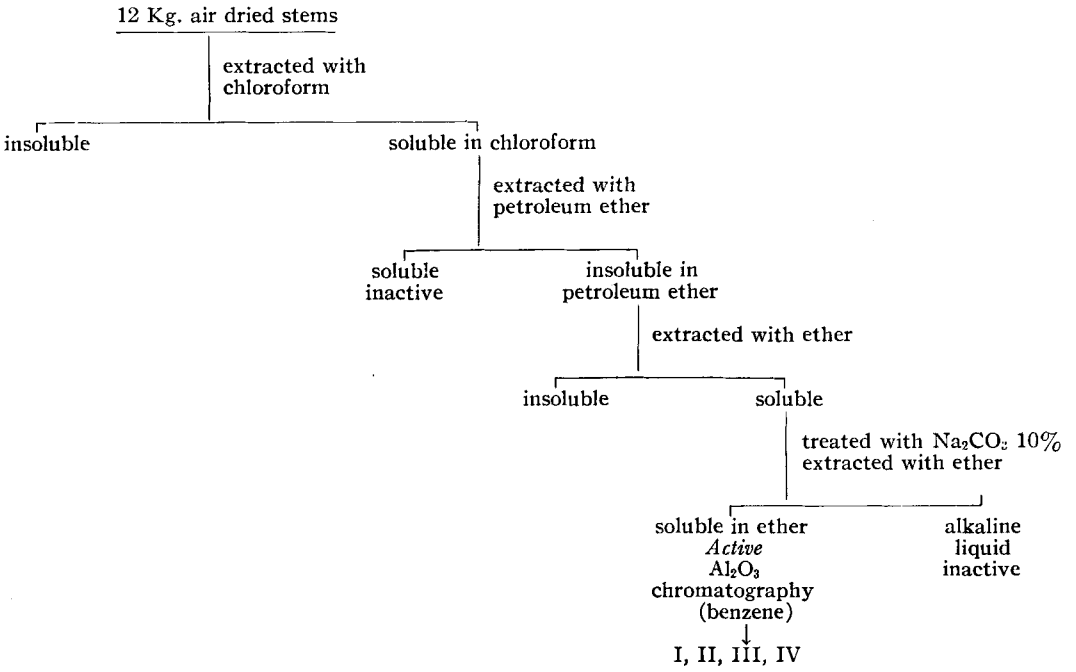
[1] Identification confirmed by Robert Barr, College of Pharmacy, and Dr. Charles Mason, Botany Department University of Arizona, Tucson. A reference specimen was also deposited.

Schematic Presentation of Extraction
B. microphylla



Scheme I

run with a dichloromethane–ethyl acetate (5:1) system. This procedure gave good resolution for the separation of III. It was eluted with acetone and recrystallized from methanol, m.p. 162–163°; $[\alpha]_D$ −112° (chloroform). These data are in agreement with that reported by the literature for deoxypodophyllotoxin. Mass spectrometry indicated a parent ion of *m/e* 398 compatible with this compound. In addition, no depression in a mixed melting point and a superimposable infrared spectrum with an authentic sample further proved the identification of III as deoxypodophyllotoxin.[2] The yield of deoxypodophyllotoxin from the dry plant was approximately 0.09%.

Conversion of Deoxypodophyllotoxin into Deoxypicropodophyllin—To a solution of deoxypodophyllotoxin in methanol, a few drops of an aqueous methanolic solution of potassium bicarbonate was added and the mixture was allowed to stand overnight. The mixture was treated as indicated above and yielded deoxypicropodophyllin. The same result was obtained using sodium acetate as the basic catalyst for this conversion (6). The compound showed an inversion of the sign of the optical rotation, $[\alpha]_D$ +30° in chloroform. Its mass spectrum pattern again indicated a parent ion *m/e* 398 indicating that only a geometrical rearrangement took place during the alkali treatment.

CONCLUSIONS

Bursera microphylla has been the subject of a phytochemical investigation in order to determine the chemical agents responsible for the apparent antitumor activity in the 9KB system of the CCNSC. Deoxypodophyllotoxin has been isolated and characterized as being one of these agents. Two other agents have been isolated and will be the subject of future reports. β-Sitosterol has also been shown to be present in this plant.

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Keyphrases

Antitumor agents—*Bursera microphylla*
Deoxypodophyllotoxin—isolation, characterization
Chromatography, adsorption, thick layer, paper—separation
IR spectrophotometry—identity
TLC—identity
Mass spectroscopy—identity
Tumor inhibition, ED_{50}—deoxypodophyllotoxin