ALKALOIDS OF *BOEHMERIA CYLINDRICA* (FAMILY URTICACEAE): IDENTIFICATION OF A CYTOTOXIC AGENT, HIGHLY ACTIVE AGAINST EAGLE'S 9KB CARCINOMA OF THE NASOPHARYNX IN CELL CULTURE, AS CRYTOPLEURINE*

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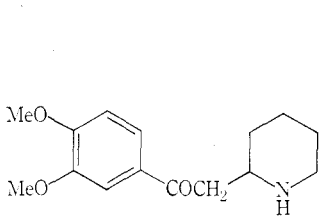
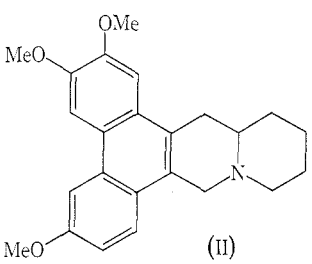
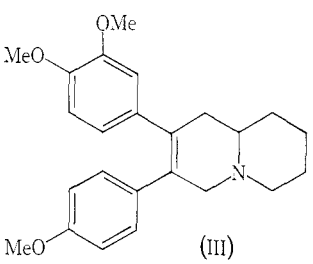
Extracts of the North American species *Boehmeria cylindrica* (L.) Sw. (family Urticaceae) show cytotoxic and antimicrobial activity, and it has been of interest to compare the alkaloids of this species with those of the Australian species *Boehmeria platyphylla* Don. The alkaloids of *B. platyphylla* consist of a major base, 3,4-dimethoxy-ω-(2′-piperidyl)acetophenone (I), and two minor alkaloids, partially racemic cryptopleurine (II) and the secophenanthroquinolizidine base (III).[1,2]



(I)



(II)



(III)

The structure assigned to the latter alkaloid from spectroscopic evidence[2] has recently been confirmed by synthesis (personal communication from Professor P. L. Pauson). Two separate extracts of *B. cylindrica* have been examined and a marked difference has been found in the composition of the alkaloids obtained from them. From only one extract has the alkaloid cryptopleurine been isolated, and like cryptopleurine from *B. platyphylla* it has been obtained as a partial racemate ($[\alpha]_D$ −50° in chloroform). The alkaloid (I) has also been isolated from the extract containing cryptopleurine. The presence of cryptopleurine in this species is thought to be significant, as it probably accounts for the cytotoxic activity of the plant extracts.

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[1] Hart, N. K., Johns, S. R., and Lamberton, J. A., *Aust. J. Chem.*, 1968, **21**, 1397.

[2] Hart, N. K., Johns, S. R., and Lamberton, J. A., *Aust. J. Chem.*, 1968, **21**, 2579.

Although cryptopleurine has not been obtained from the alkaloids of the other batch of *B. cylindrica*, the alkaloid (I) is present as well as an incompletely characterized base that also appears to be related to cryptopleurine. It seems possible that the presence of cryptopleurine may depend upon the stage of development of the plant. Acetamide has been isolated as a major constituent of the crude alkaloid fraction from the second collection of *B. cylindrica*. It is suggested that acetamide could arise as an artefact when the plant material is treated with ammonia, possibly from reaction with a high concentration of acetyl coenzymes.

The incompletely characterized base has been obtained only as a colourless gum, $[\alpha]_D$ −80° in $CHCl_3$, and no crystalline derivatives have been prepared. It appears to be pure, however, from examination by thin-layer chromatography, and although sufficient material has not been available for detailed study, the spectroscopic properties of the base indicate that it is probably related to cryptopleurine and the seco-base (III). The mass spectrum shows a molecular-ion peak at *m/e* 381, consistent with the formula $C_{23}H_{27}NO_4$, and the n.m.r. spectrum shows signals from two methoxy groups at δ 3·55 and δ 3·76, and signals from seven aromatic protons (646–688 c/s). The presence of two hydroxy groups, of which one is probably phenolic and the other alcoholic, is indicated by the formation of an acetylation product that has signals from two *O*-acetyl groups at δ 1·62 and δ 2·09, as well as signals from the two methoxy groups at δ 3·46 and δ 3·68. The acetyl derivative has i.r. absorption bands at ν 1740 cm^{-1} and ν 1760 cm^{-1} (CCl_4 solution.)

Cryptopleurine is inactive against the sarcoma 180, adenocarcinoma 755, Lewis Lung carcinoma, L-1210 leukemia, PS leukemia, and the Walker 256 intramuscular sarcoma, but it shows a highly specific and extremely cytotoxic action against Eagle's 9KB carcinoma of the nasopharynx in cell culture, exhibiting an ED_{50} of $7{\cdot}8\times10^{-4}$ and $2{\cdot}6\times10^{-5}$ μg/ml. Aqueous ethanol (1 : 1) extracts of leaf, stem, and root samples of *B. cylindrica* were cytotoxic, having an ED_{50} of 3·6, 2·5, and 10 μg/ml respectively against Eagle's 9KB carcinoma of the nasopharynx in cell culture. In this system, an $ED_{50} \leqslant 15{\cdot}0$ μg/ml is considered to be significantly cytotoxic for crude plant extracts and an $ED_{50} \leqslant 1{\cdot}0$ μg/ml is cytotoxic for pure compounds.

Experimental

All n.m.r. spectra were measured in $CDCl_3$ solution at 100 Mc/s and chemical shifts are relative to tetramethylsilane (δ 0·00).

Both batches of *B. cylindrica* were collected in the Pittsburgh area, the first on September 1, 1967, and the second which afforded cryptopleurine on October 9, 1968, after a prolonged period of rain.

(i) Whole fruiting plants of *B. cylindrica* (1·0 kg) (first batch) were air-dried, milled, treated with aqueous ammonia (28%) until damp, and again air-dried. This material was then extracted with chloroform in a Soxhlet apparatus, and the chloroform extract was concentrated and shaken repeatedly with 5% aqueous hydrochloric acid. The combined acid extracts were made alkaline by adding 28% ammonia solution, and the crude alkaloid fraction extracted with chloroform. The chloroform extract was dried (Na_2SO_4), filtered, and taken to dryness, to yield 130 mg of residue. Comparison by thin-layer chromatography (Kieselgel G plates; solvent, $CHCl_3$–MeOH (9 : 1)) with the alkaloids of *B. platyphylla* indicated the presence of alkaloid (I), but of negligible traces, if any, of cryptopleurine or the seco-base (III). In a thin-layer chromatogram of the *B. cylindrica* alkaloids there was a spot (R_F 0·55) between the spots from reference cryptopleurine (R_F 0·63) and alkaloid (I) (R_F 0·40). Because of the small amount of material available, preparative thin-layer chromatography was used to separate the alkaloids.

(1) The alkaloid of R_F 0·55 was obtained as a colourless gum (23 mg); $[\alpha]_D$ −80° (*c*, 0·2 in $CHCl_3$); λ_{max} (EtOH) 232 mμ (log ε 4·01), 280 (3·75), which showed no traces of other constituents when examined by t.l.c. The mass spectrum showed peaks at *m/e* 381 (M^+, 14% of base peak), 365 (8), 298 (36), 283 (10), 267 (28), 251 (7), 239 (7), 168 (9), 151 (9), 150 (7), 138 (14), 124 (43), and 84 (100%, base peak). The n.m.r. spectrum showed three-proton singlets at δ 3·55 and δ 3·76 (OCH_3 groups), a seven-proton multiplet at 646–688 c/s (aromatic protons), and a one-proton multiplet at δ 4·35 (CHOH). Acetylation with acetic anhydride–pyridine at room temperature gave a colourless gum, ν_{max} (CCl_4) 1740 and 1760 cm^{-1}.

(2) 3,4-Dimethoxy-ω-(2′-piperidyl)acetophenone (I) was also isolated by preparative thin-layer chromatography. Crystallization from acetone gave colourless needles, m.p. 81–82°, identical in i.r. spectrum with (I) isolated from *B. platyphylla*, and undepressed in m.p. on mixing with authentic (I).

(ii) The second batch of *B. cylindrica*, which consisted of 5·0 kg of whole fruiting plants, was processed in a slightly different manner. The crude chloroform solubles (93·5 g) extracted from the plant extract after basification with ammonia were dissolved in chloroform and repeatedly extracted with aqueous sulphuric acid (2N). The aqueous acid extracts were basified (NH_3), and extraction with chloroform gave the crude alkaloid fraction (760 mg). After storage in a refrigerator it was observed that the crude alkaloid fraction had partly crystallized and that a crystalline sublimate had formed on the walls of the flask. By gentle sublimation under high vacuum 37 mg of this crystalline material was collected. Spectroscopic comparison (i.r., n.m.r.) showed that it was identical with authentic acetamide.

The benzene-soluble portion (680 mg) from the crude alkaloids was chromatographed on a column of neutral alumina. Several fractions eluted by benzene appeared from t.l.c. examination to contain cryptopleurine, while fractions eluted by benzene–chloroform (9 : 1) contained alkaloid (I). Fractions eluted by chloroform contained acetamide and became partly crystalline on standing.

Cryptopleurine was isolated from the appropriate chromatographic fractions by preparative thin-layer chromatography. Crystallization from acetone gave faintly yellowish needles, m.p. 195–197°, $[\alpha]_D$ −50° (*c*, 0·5 in $CHCl_3$). There was no depression of m.p. on mixing with authentic cryptopleurine, and the i.r. spectrum was identical with that of cryptopleurine. It is estimated that the total cryptopleurine content of the alkaloid fraction is about 25 mg.

A small amount of 3,4-dimethoxy-ω-(2′-piperidyl)acetophenone (I) was also isolated by preparative thin-layer chromatography in sufficient quantity to confirm its identity by measurement of its i.r. spectrum.

Acknowledgments

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