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JUSTICIDIN B, A BIOACTIVE TRACE LIGNAN FROM THE SEEDS OF *SESBANIA DRUMMONDII*

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In an early report, the seeds of *Sesbania drummondii* (Rydb.) Cory (Fabaceae) showed in vivo 3PS (P-388) murine antileukemic and in vitro 9KB carcinoma cytotoxic activities, and these activities were not explained by a series of commonly isolated phytochemicals (1). The subsequent isolation of the novel compounds, sesbanine and drummondol, from the active extracts still failed to explain the in vivo antitumor activity (2,3). Persistent efforts, with extracts of nearly 500 kg of seeds, finally yielded sesbanimide as an exceptionally potent, novel, antitumor component (4), and this compound is undergoing further evaluation. More recently, drummondone A and B, two abscisic acid derivatives, have been isolated and characterized (5).

A re-examination of unfractionated extracts resulting from the previous large-scale isolation of sesbanimide detected potent bioactivity in the form of brine shrimp (*Artemia salina*) lethality (LC_{50} 51 μg/ml) (6) in a pool of column fractions eluting prior to those which yielded sesbanimide. The brine shrimp assay was then used to guide the fractionation of this pool through three successive chromatography columns and a final purification by preparative tlc to yield another trace bioactive component new to this plant and to this plant family. Spectral (uv, ir, eims, cims, hrms, and ^{1}H nmr) and physical (mp, mmp, and co-tlc) data subsequently identified this component as justicidin B (brine shrimp LC_{50} 1.1 μg/ml), a known phenylnaphthalene lignan (7). This compound has piscidal activity comparable to rotenone (8-10), cytotoxicities (9PS ED_{50} 3.3 μg/ml, 9KB ED_{50} 7.3×10^{-2} and 1.2×10^{-2} μg/ml) but no 3PS in vivo activity (in doses up to 200 mg/kg) (11,12).[1] The effectiveness of the brine shrimp lethality bioassay in detecting small amounts of such bioactive plant components is emphasized; the yield of justicidin B represents only 0.0000009% of the dried weight of the seeds.

[1]G.M. Cragg, National Cancer Institute, personal communication, May 2, 1986.

EXPERIMENTAL

PLANT MATERIAL.—Seeds (including a few pods) of *S. drummondii* were harvested from the wild in Texas (PR-55305) under the auspices of Dr. James A. Duke, USDA, Beltsville, Maryland, where voucher specimens are maintained.

EXTRACTION.—The ground seeds (546 kg) were extracted with MeOH, and the concentrate was diluted with H_2O and partitioned with hexane followed by CH_2Cl_2. A portion (1.9 kg) of the CH_2Cl_2 solids (11.05 kg total) was chromatographed through 62 kg of Si gel using a gradient (2.5-100%) of MeOH in CH_2Cl_2. Fifty fractions (18.5 liters each) were collected and combined into fifteen pools; the pool of fractions 26-29 was used to isolate sesbanimide. The large-scale extraction and preliminary separation was carried out by Polysciences, Inc., Warrington, Pennsylvania.

ISOLATION OF JUSTICIDIN B.—Fractions 30 and 31 contained sesbanimide and were active in the brine shrimp assay (LC_{50} 171 and 152 μg/ml, respectively) (6). A less-polar pool of fractions 5-7, which was free of sesbanimide, was the most active in the brine shrimp assay (LC_{50} 51 μg/ml); additional unexplained bioactivity (LC_{50} 209 μg/ml) resided in a more-polar pool of fractions 44-50.

A portion (6.1 g) of the residue (15.2 g total) of the active pool of fractions 5-7 was chromatographed through three successive Si gel columns, pooling similar fractions after tlc analysis, and separating the brine shrimp-active fractions on the succeeding columns. The bioactive fraction (LC_{50} 3.1 μg/ml) from the third column crystallized (13 mg), but tlc indicated the co-occurrence of two approximately equal components in the crystals. Preparative tlc (Si gel, hexane-EtOAc, 1:1) was used to resolve the two components; the lesser component (4.8 mg, 0.0000009% yield) was active (LC_{50} 1.1 μg/ml, 95% confidence limits 1.6-0.7 μg/ml), while the major component (7.7 mg) was inactive (LC_{50}>100 μg/ml) in the shrimp assay. Identification of the active component as justicidin B was based on uv, ir, eims, cims, hrms, and ^{1}H-nmr spectral data (7-12) followed by direct comparison (mp, mmp, co-tlc in five solvent systems) with an authentic sample (12).

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