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# ANTIMICROBIAL ALKALOIDS FROM *BOEHMERIA CYLINDRICA*

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**Key Word Index**—*Boehmeria clyindrica*; Urticaceae; alkaloids; cryptopluerine; julandine; 3,4-dimethoxy-ω-(2′-piperidyl)acetophenone; antimicrobial activity.

**Abstract**—Investigation of extracts of *Boehmeria cylindrica* resulted in identification of cryptopleurine and 3,4-dimethoxy-ω-(2′-piperidyl)acetophenone, known alkaloids, as agents responsible for intense activity against *Candida albicans*. Julandine, the secophenanthroquinolizidine alkaloid related to cryptopleurine, was also active but not definitely identified in the extracts examined.

In connection with our screening program for antimicrobial agents from higher plants, we wish to report the surprisingly intense antimicrobial activity of alkaloidal constituents of *Boehmeria cylindrica* (L.) Sw (Urticaceae), of Kansas origin. The crude alcoholic extracts of this plant were active *in vitro* in an agar-dilution assay at less than 1000 μg/ml, so bioassay directed fractionation studies were initiated following our usual scheme (cf. [1]). The anticandidal activity was found to reside in the crude alkaloidal fraction (MIC = 6 μg/ml), but the relatively small amount of plant material available precluded detailed fractionation. Accordingly, we turned to the more abundant *B. cylindrica* var. *drummondiana* Wedd., whose spectrum and potency paralleled those of the original species.

Interestingly, autobiographical techniques involving TLC strips of *B. cylindrica* alkaloids laid on seeded lawns of *C. albicans* in agar plates demonstrated two well-separated zones of inhibition in areas of the strips where UV light, various spray reagents and iodine vapors failed to reveal the presence of definite components. These findings showed that too little of the active constituents were present for detection in the usual fashion. Due to the generosity of Dr. J. A. Lamberton, we were able to make a side-by-side comparison, using TLC bioautographs, with the following known *Boehmeria* alkaloids: the cytotoxic agent cryptopleurine (**1**), synthetic [2] julandine (**2**), and 3,4-dimethoxy-ω-(2′-piperidyl)acetophenone (**3**). The TLC spots for **2** and **3** were rather close to one another and paralleled the inhibition zone of lower $R_f$. Compound **1** paralleled the inhibition zone of higher $R_f$. Prep. TLC finally resulted in the isolation of small amounts of incompletely pure **1** and **3**, accounting for about 0.00002% of the weight of the dried plant material, while **2** was not found in isolable amounts. The isolated compounds were identical to the authentic samples of **1** and **3** both by TLC and by HPLC spiking experiments.

Agar dilution assays of **1**, **2**, and **3** revealed minimum inhibitory concentration values against *C. albicans* of 0.1, 12.5 and 3.12 μg/ml, respectively. Plant-derived antimicrobial agents, in our experience,

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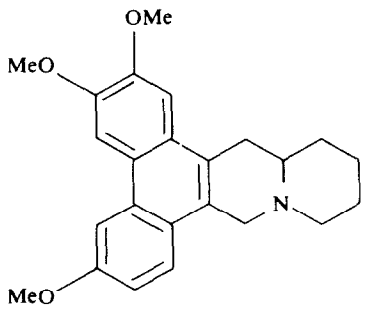


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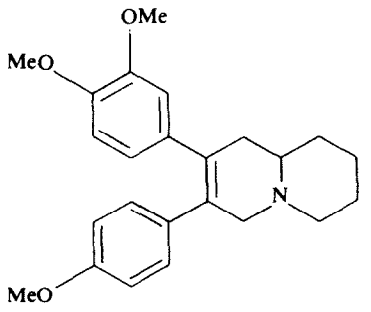


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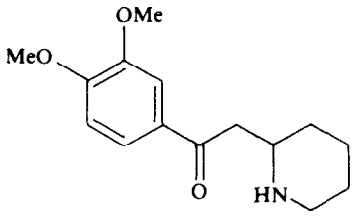


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are rarely as potent as **1** and **3**. Our results demonstrate the power of bioassay-directed fractionation and the impracticality of large-scale isolation of **1–3** from these species. The content of these alkaloids in Kansas plants is below the level of reasonable phytochemical detection methods and they would not have been detected were it not for their exceptional antimicrobial potency *in vitro*.

Cryptopleurine (**1**) was previously isolated from *Cryptocarya pleurosperma* [3] and *C. laevigata* [4] (Lauraceae), and from *B. caudata* [4], *B. platyphylla* [5] and *B. cylindrica* [6]. Its anti-yeast activity was noted as early as 1950 [7] and confirmed against *Saccharomyces cerevisiae* in 1968 [8]. Anti-fungal activity has been noted [9] and a number of analogues have been prepared and tested [10]. Cryptopleurine has also been shown to be vesicant and irritant and to have significant mammalian toxicity [3], to be toxic to oyster eggs [7], to be antiviral [11] and to be cytotoxic in cancer cell test systems [4, 6, 12]. Julandine (**2**) has been isolated from *B. platyphylla* [5], while the piperidylacetophenone (**3**) has been isolated both from *B. platyphylla* [5] and *B. cylindrica* [6]. Neither compound has been reported previously to have biological activity.

The cytotoxicity of cryptopleurine has been attributed to inhibition of protein biosynthesis at the ribosome level [8, 14, 15], and *S. cerevisiae* cell-free systems have been used commonly as a model for such studies. It is interesting to note in the context of this work that **2** was recently found to be inactive as an inhibitor of poly(U)-mediated peptide synthesis in this model [15].

## EXPERIMENTAL

*Plant material. Boehmeria cylindrica* (L.) Sw. was gathered near Lakeview, KS, and near the Kansas River outside of Lecompton, KS. *B. cylindrica* var. *drummondiana* was gathered below the spillway of Kingman State Fishing Lake outside Kingman, KS, with bulk collection on 5 Oct. 1979 (before first frost). Voucher specimens are on deposit at the University of Kansas Herbarium, and were identified by Mr. Ralph Brooks of the Kansas Biological Survey. Whole plants, still bearing seeds, were air-dried and ground with a mill.

*Extraction.* The finely ground plant (18.1 kg) was extracted in 500–700 g batches with 95% EtOH using large, modified Soxhlet extractors. The EtOH solution was evaporated under reduced pressure to yield 1160 g crude extract. The extract was treated with 5% HCl in a blender and filtered; the filter cake was washed with 5% HCl, and the combined filtrates were washed with $CHCl_3$ ($CHCl_3$ discarded). The aq. solution was adjusted to pH 9.8 with $NH_4OH$, and was extracted with $CHCl_3$. The combined $CHCl_3$ extracts were backwashed with aq. $NH_3$, pH 10, dried with $Na_2SO_4$, evaporated, and vacuum-dried to yield 1.33 g crude alkaloids. The antimicrobial activity of the plant resided in this fraction.

*Antimicrobial assay.* MIC determinations were performed by use of serial agar dilutions as described previously [16], using *Candida albicans* ATCC 10231 grown on trypticase–soy agar. TLC bioautography was performed by laying a developed alkaloid analytical TLC plate (*ca* 1-cm-wide bands) face down over an immature culture of *C. albicans*; after allowing the culture to grow, inhibition zones around active TLC bands could be noted. In system A (see below), the broad upper inhibition zone had a $R_f$ of 0.72, while the very broad zone had a $R_f$ of about 0.25.

*Chromatography.* HPLC (Perkin–Elmer Series 2/2 with 254-nm detector) analysis and spiking experiments were performed on a 4.6 mm i.d. × 25 cm aminoalkyl bonded-phase column using 2% or 10% MeOH in $CH_2Cl_2$, with a flow rate of 2.0 ml/min. Retention times for **1** and **2** were 9.75 min and 3.38 min, respectively, using 10% MeOH; **3** required 2.70 min with 2% MeOH. Analytical TLC was performed on commerical plates precoated with E. Merck Si gel 60 F-254, using the following solvent systems: (a) $NH_3$-

saturated 1:9 MeOH–$CHCl_3$; (b) 2:8:1 *i*-PrOH–$CHCl_3$–TEA; (c) 9:2 $CHCl_3$–TEA; (d) 5:5:1 $CHCl_3$–$C_6H_6$–TEA. $R_f$s of **1**, **2**, and **3** in system A were 0.77, 0.28, and 0.21, respectively. Prep. TLC was performed on 1.0-mm-thick commerical plates precoated with E. Merck $Al_2O_3$ GF-254, developing with ammoniated $CHCl_3$; $R_f$s of **1** and **3** were 0.64 and 0.13, respectively.

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