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Isolation of Cannabisativine, an Alkaloid, from *Cannabis sativa* L. Root

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Abstract □ An ethanol extract of the root of a Mexican variant of *Cannabis sativa* L. (marijuana) afforded, after partitioning and chromatography, the new spermidine alkaloid cannabisativine.

Keyphrases □ *Cannabis sativa*—cannabisativine isolated from ethanol extract of roots □ Alkaloids—cannabisativine isolated from ethanol extract of roots of *Cannabis sativa* □ Cannabisativine—isolated from roots of *Cannabis sativa*

The occurrence of several low molecular weight, nitrogen-containing substances in *Cannabis sativa* L. has been reported including choline (1–4), trigonelline (2, 3), muscarine (5), piperidine (6), *N*-(*p*-hydroxy-β-phenylethyl)-*p*-hydroxy-*trans*-cinnamamide (7), neurine (8), L-proline (8), and L-(+)-isoleucine betaine (9). Small amounts of four alkaloids were isolated (10), but the high-resolution and mass spectra were inconclusive.

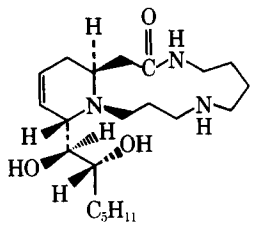
Recently, the presence of a new spermidine alkaloid, cannabisativine (I) [13-(1,2-dihydroxyheptanyl)-1,4,5,6,7,8,9,10,11,13,16,16a-dodecahydropyrido[2,1-*d*][1,5,9]triazacyclotridecin-2(3*H*)-one], was found in the roots of this species (11). This report details the procedure used in the isolation of this substance.

EXPERIMENTAL[1]

Plant Material—Roots of a Mexican variant of *C. sativa* L. were used[2].

Extraction—Air-dried ground roots of *C. sativa* (11.72 kg) were extracted by percolation with methanol (150 liters). The extract was evaporated *in vacuo* at 40° to leave a dark-brown syrup (323 g, 2.8%).

Isolation of Cannabisativine—The methanol extract (323 g) was partitioned between water (4 liters) and chloroform (4 liters) to give fractions of 266.0 g (2.3%) and 47.7 g (0.4%), respectively. The chloroform fraction was then partitioned between petroleum ether (bp 60–90°) (2 liters) and methanol–water (9:1) (2 liters) to yield fractions of 12.7 g (0.1%) and 33.4 g (0.3%), respectively.



I

The aqueous methanol fraction was chromatographed on silicic acid[3] (450 g, 5 × 49 cm) packed in petroleum ether (bp 60–90°). Elution with 8% methanol–chloroform afforded a residue (3.0 g), which was dissolved in chloroform (25 ml) and partitioned with 1% hydrochloric acid (3 × 25 ml). The combined acid layers (75 ml) were basified to pH 8 with concentrated ammonium hydroxide and partitioned with chloroform (3 × 75 ml). The combined chloroform layers were dried over anhydrous sodium sulfate and evaporated, *in vacuo*, at 40° to yield a white residue (48 mg).

Crystallization from acetone afforded cannabisativine (29 mg), mp 167–168°; $[\alpha]_D^{25}$ + 55.1° (c 0.53, $CHCl_3$); UV: no maximum above 210 nm; IR: ν_{max} (KBr) 3300, 3020, 2960, 2920, 2850, 1628, 1580, 1470, 1250, 1130, 1045, and 707 cm^{-1}; NMR: δ 5.90 (2H, s, vinyl) and 9.6 (1H, s, broad, CONH); mass spectrum (M^+): *m/e* 381 (1%), 363 (1), 352 (1), 310 (2), 280 (3), 250 (64), 208 (100), 198 (6), 171 (12), 129 (5), 114 (5), 112 (6), 96 (4), 94 (6), 84 (8), 80 (6), 72 (7), 70 (8), and 55 (6). The structure of this compound was recently determined to be I (11).

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[1] Melting points were determined on a Thomas-Hoover Uni-Melt melting-point apparatus and are corrected. IR spectra were run in potassium bromide using a Perkin-Elmer 257 spectrophotometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. UV spectra were run on a Perkin-Elmer 202 spectrophotometer. NMR spectra were obtained in deuterated chloroform on a Hitachi Perkin-Elmer R-24 spectrometer, with tetramethylsilane as the internal standard. Mass spectra were recorded on a LKB-9000 spectrometer.

[2] Lot Me-A(2)-C-69 grown in 1971; voucher specimens were deposited in the herbarium of the School of Pharmacy, University of Mississippi.

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Synthesis and Antibacterial and Antifungal Activities of Alkyl and Polyhalophenyl Esters of Benzo[*b*]-3-methyl-2-furancarbamic Acid

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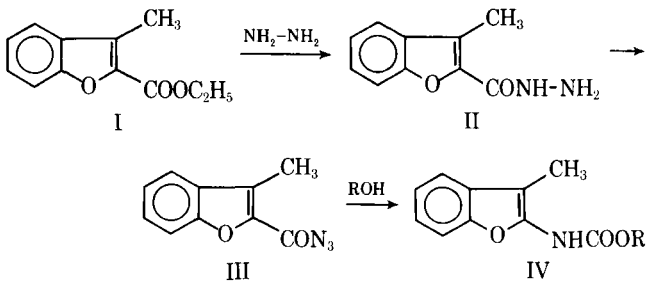
Abstract □ Several alkyl and polyhalophenyl esters of benzo[*b*]-3-methyl-2-furancarbamic acid were prepared and tested for antifungal activity against *Candida albicans, Penicillium notatum*, and *Aspergillus niger*. The pentachlorophenyl ester was the most active substance and the only compound to show antibacterial activity against *Staphylococcus aureus*.

Keyphrases □ Carbamic acid esters—synthesized, screened for antifungal and antibacterial activities □ Furancarbamic acid esters—synthesized, screened for antifungal and antibacterial activities □ Antifungal activity—alkyl and polyhalophenyl esters of furancarbamic acid synthesized and screened □ Antibacterial activity—alkyl and polyhalophenyl esters of furancarbamic acid synthesized and screened

In continuing studies on the chemistry and antibacterial and antifungal activities of carbamic acid esters (1–3), alkyl and polyhalophenyl esters of benzo[*b*]-3-methyl-2-furancarbamic acid were synthesized from benzo[*b*]-3-methyl-2-furancarboxazide (III) and the appropriate alcohol or phenol (Scheme I). The physical data of the compounds prepared are summarized in Table I. All compounds listed in Table I were tested against *Candida albicans* (28012), *Penicillium notatum* (S-13), and *Aspergillus niger* (23171) *in vitro* using Sabouraud dextrose agar medium[1].

Each compound was dissolved in acetone to a concentration of 1 mg/ml. These solutions were diluted with hot culture medium to the desired concentrations and autoclaved at 120° for 2 hr. Five replicates of each concentration were prepared.

The antifungal activity of all compounds tested, except IV*n*, was insignificant at a concentration of 5 μg/ml. All compounds were active against *P. notatum* and *A. niger* but inactive against *C. albicans* at a concentration of 10 μg/ml. However, they were active against *C. albicans* at a concentration of 30 μg/ml. Griseofulvin was used as a control (Table II).



Scheme I

All compounds also were tested against *Bacillus subtilis* (NCTC 3610), *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumoniae* (ATCC 10031), and *Sarcina lutea* (ATCC 9341). Nitrofurazone was used as a control. Standard paper disks, 6 mm in diameter, were immersed in solution and placed on an inoculated assay medium surface[2].

The antibacterial activity of all compounds that dissolved in acetone at the 0.5% concentration was insignificant. However, IV*n* at the same concentration showed 12-mm inhibition zones against *S. aureus* and had no activity against other strains.

EXPERIMENTAL[3]

Benzo[*b*]-3-methyl-2-furancarboxyhydrazide (II)—To a stirring solution of 25 g (0.5 mole) of hydrazine hydrate in 150 ml of ethanol was added dropwise a solution of 20.4 g (0.1 mole) of ethyl benzo[*b*]-3-methyl-2-furancarboxylate (I) (5) in 50 ml of ethanol.

[1] These microorganisms were obtained from the Department of Parasitology, Public Health Institute, Tehran, Iran.

[2] Antibiotic assay medium, British Pharmacopoeia, 1968.

[3] Melting points were taken on a Kofler hot-stage microscope and are uncorrected. IR spectra were recorded using a Leitz model III spectrograph. NMR spectra were recorded on a Varian A60A instrument.