Anhydrocannabisativine, a New Alkaloid from *Cannabis sativa* L.

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Received February 7, 1977, from the **Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677,* and the [1]*Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261.* Accepted for publication March 30, 1977.

Abstract □ Ethanol extracts of the leaves and roots of a Mexican variant of *Cannabis sativa* L. (marijuana) afforded, after partitioning and chromatography, the new spermidine alkaloid, anhydrocannabisativine. The structure was determined by spectral analysis and semisynthesis.

Keyphrases □ Anhydrocannabisativine—isolated from ethanol extract of leaves and roots of *Cannabis sativa*, structure determined □ *Cannabis sativa*—ethanol extract of leaves and roots, anhydrocannabisativine isolated, structure determined □ Alkaloids—anhydrocannabisativine isolated from ethanol extract of leaves and roots of *Cannabis sativa*, structure determined

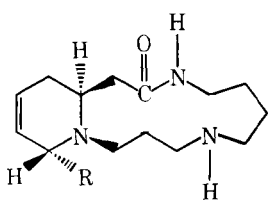
The occurrence of nitrogen-containing substances in *Cannabis sativa* L. was presented recently (1). The most novel compound is the spermidine alkaloid, cannabisativine (I), isolated from the leaves and roots (2–4). The isolation of I represented the first occurrence of the spermidine (pyrido[2,1-*d*][1,5,9]triazacyclotridecine) nucleus in a higher plant. This investigation dealt with the isolation of a related alkaloid, anhydrocannabisativine (II), from the roots and leaves of a Mexican variant of *C. sativa* L.

DISCUSSION

The plant was defatted with hexane and extracted with ethanol, and the extract residue was partitioned between chloroform and 2% citric acid. The acidic layer was made basic with concentrated ammonium hydroxide and extracted with chloroform. This chloroform extract, when chromatographed on a silica gel G column and eluted with 2% ammonium hydroxide in methanol, afforded II. Numerous attempts to crystallize the alkaloid or its salts failed, so spectral data were obtained on the residue.

The IR spectrum showed absorptions at 1715 (C=O), 1661 (amide C=O), and 1615 (C=C) cm^{-1}. The mass spectrum showed a molecular ion at *m/e* 363 for $C_{21}H_{37}N_3O_2$ [18 amu (H_2O) less than I]. A comparison of the mass spectral fragmentations of I and II showed great similarities, except for fragments arising from the C-7 side chain. Peaks at *m/e* 292 ($M - C_5H_{11}$) and 264 ($M - C_5H_{11}CO$) indicated that II was an analog of I with a keto group on C-2 of the side chain.

Direct comparison of the alkaloid with a dehydration product of I confirmed the structure, and the compound was named anhydrocannabisativine (II) [13-(2-oxoheptanyl)-1,4,5,6,7,8,9,10,11,13,16,16a-dodecahydropyrido[2,1-*d*][1,5,9]triazacyclotridecine-2(3*H*)-one].



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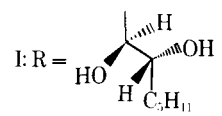

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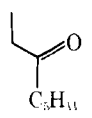

EXPERIMENTAL[1]

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

Extraction—Air-dried ground leaves (5 kg) were first defatted by percolation with hexane (60 liters) and then extracted with ethanol (100 liters) at room temperature. The extract was evaporated *in vacuo* at 40° to leave a dark-green syrup (645 g) (12.9%).

Isolation of II—The ethanol extract was partitioned between chloroform (2 liters) and 2% citric acid (3 × 700 ml). The acidic layer was then made alkaline (pH 9) with concentrated ammonium hydroxide and partitioned with chloroform (3 × 2 liters). The combined chloroform layers were dried over anhydrous sodium sulfate and evaporated *in vacuo* at 40° to yield a dark yellowish-brown residue of the crude alkaloidal fraction (0.7 g) (0.014%). This residue was chromatographed over a 1.8 × 30-cm silica gel G column[3] (40 g).

Elution with 2% concentrated ammonium hydroxide in methanol afforded II (23 mg); $[\alpha]_D^{22}$ + 18.7° (c 0.1, methanol); IR: ν_{max} (film on potassium bromide) 3290, 3020, 2925, 2860, 1715, 1661, 1642, 1615, 1540, 1460, 1365, 1210, 1124, 1100, and 1050 cm^{-1}; NMR: δ 5.8 (2H, m, vinyl) and 9.6 (1H, s, broad, CONH) ppm; mass spectrum: M^+ *m/e* 363 (12%), 348 (1), 292 (4), 264 (22), 250 (68), 208 (55), 198 (60), 192 (25), 171 (20), 84 (60), 80 (40), 70 (100), and 43 (60).

Following this same isolation procedure, additional quantities of II (20 mg) were isolated from air-dried ground roots (66 kg) of this species.

Preparation of II from I—Compound I (5 mg) was dissolved in a saturated aqueous solution of oxalic acid (0.3 ml). The solution was evaporated to dryness, and the reaction flask was flushed with nitrogen and then attached to a drying tube. The reaction mixture was heated (180–185°) for 2 min and left to cool to room temperature. The mixture was dissolved in water (5 ml), made alkaline with concentrated ammonium hydroxide (pH 9), and extracted with chloroform (3 × 5 ml).

The combined layers were dried with anhydrous sodium sulfate and evaporated *in vacuo* at 40° to leave a residue (4 mg). This residue was chromatographed on a 0.9 × 12-cm silica gel G column[3] (10 g). Elution with 2% concentrated ammonium hydroxide in methanol afforded II (2 mg), identical (TLC, $[\alpha]_D$, and mass spectrum) with the natural product.

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ACKNOWLEDGMENTS

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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 E. I. du Pont de Nemours 21-492 spectrometer.

[2] Lot CMEF-72ME-A(2)C-69; voucher specimens were deposited in the herbarium of the School of Pharmacy, University of Mississippi.

[3] Silica gel G–water (1:2) slurried and dried at 100° (3 hr), passed through 100-mesh sieve, and packed in an elution solvent system.