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CANNABISATIVINE, A NEW ALKALOID FROM *CANNABIS SATIVA* L. ROOT

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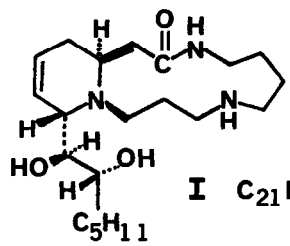
An ethanol extract[1] of the root of *Cannabis sativa* L. afforded, after partitioning and repeated chromatography, a new spermidine (pyrido[2,1-d][1,5,9]triazacyclotridecine) alkaloid, cannabisativine (I) with the chemical name of 13-(1,2-dihydroxyheptanyl)-1,4,5,6,7,8,9,10,11,13,16,16a-dodecahydropyrido[2,1-d][1,5,9]triazacyclotridecin-2(3H)-one. Crystallization of I from acetone gave white plates with mp 167-168° and $[\alpha]_D^{25}$ + 55.1° (c 0.53, $CHCl_3$)



I $C_{21}H_{39}N_3O_3$

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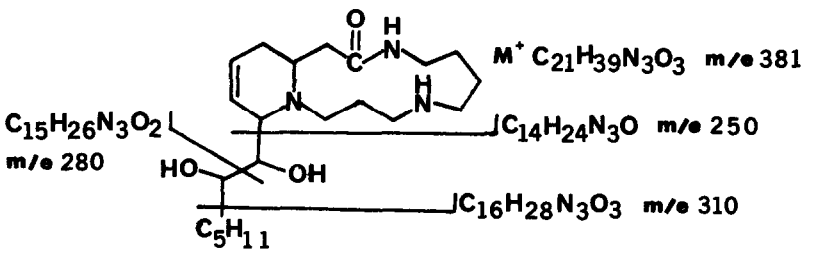
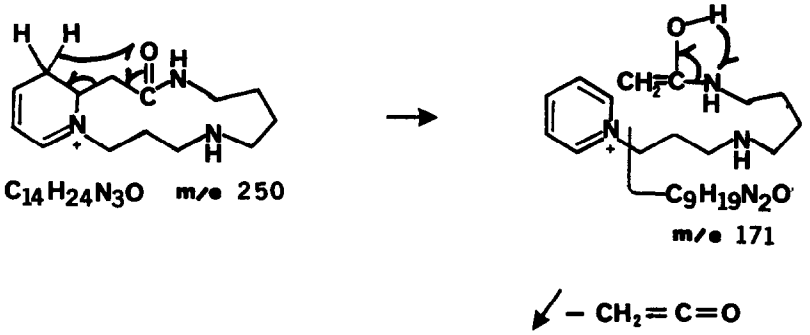
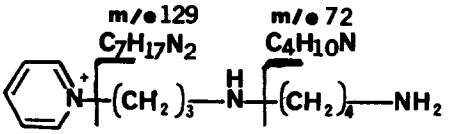
Single crystal data on I was collected on an automated Siemens diffractometer with Ni filtered CuKα radiation. The cell constants were a = 23.70, b = 13.43, c = 7.26 Å and the space group, as determined by systematic absences, was $P2_12_12_1$ with four molecules in the unit cell. The measured density by flotation was 1.097 which agreed with the calculated 1.095 g/cc. A total of 1682 independent reflections were measured out of which 1486 were recorded as observed. The structure was solved using the direct method program Phase DT[2]. An E map using 258 phases gave positions for 22 out of the 27 nonhydrogen atoms Subsequent Fourier maps revealed the positions of the other five atoms (excluding hydrogens). The last atoms to appear were associated with the hydrophobic C_5H_{11} side chain. All 27 atoms were then refined as carbon atoms using isotropic temperature factors and after two cycles of full matrix least squares a conventional R factor of 16.2% was obtained. This calculation clearly indicated which of the six atoms were O or N via the temperature factors for these six atoms which were much lower than those of the carbons. At this point it was also easy to interpret the mass spectrum which clearly showed the presence of the two adjacent hydroxyl functions in agreement with the X-ray structure. Isotropic refinement with the correctly placed oxygens and nitrogens produced a R factor of 12.9% after two cycles. The last calculation revealed very high temperature factors for the C_5H_{11} side chain indicating much thermal motion in this portion of the molecule. After three cycles of anisotropic refinement (R = 9 7%), several difference maps produced tentative positions for 30 hydrogen atoms which included all protons except those residing on the C_4H_9 terminal end of the side chain. The final R with these hydrogens was 8 8% Further refinement is in progress

The 100 Hz NMR spectrum clearly showed the presence of 2 vinyl protons, curiously as a singlet, at 5.9δ and the amide proton as a broad singlet at 9 6δ. The remainder of the spectrum was quite complex This NMR did negate the possibility of an extra double bond on the side chain whose precise atomic distances for atoms could not be obtained from X-ray data due to the high thermal motion of the side chain The IR spectrum also revealed the amide absorption at 1628 cm^{-1} The high resolution mass spectrum could easily be interpreted as shown below including the following of the fragmentation pathway via strong metastable peaks.

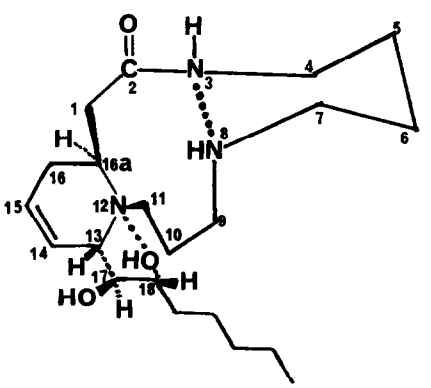






To our knowledge this is the first report of the isolation of a non-quaternary alkaloid from the roots of *Cannabis sativa* L. Although two other alkaloids, palustrine[3-5] and palustridine[6], with the pyrido[1,2-d][1,5,9]triazacyclotridecine nucleus have been previously isolated from members of the genus *Equisetum* (Equisetaceae-Horsetail) this is the first reported occurrence of this system in a higher plant

The X-ray structure shows some interesting interactions. There appears to be a hydrogen bond between N_3 and N_8 which constricts the thirteen membered ring into the approximate conformation shown below. There also appears to be a strong hydrogen bond between the OH at C_{18} and



N_{12} The relative configuration of this nucleus is different than that reported previously for palustrine[5]. In this case C_{17} and C_1 are *trans* to each other and C_{11} off of N_{12} is in a *cis*-conformation with respect to C_1. The alcohols at C_{17} and C_{18} are erythro. The structures represented here are, needless to say, relative and not absolute.

Finally, thin-layer chromatography indicates the presence of this substance in the leaves of *Cannabis sativa* L.

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