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ISOLATION, STRUCTURAL ELUCIDATION, AND SYNTHESIS OF TABERNAMINE, A NEW CYTOTOXIC BIS-INDOLE ALKALOID FROM *TABERNAMONTANA JOHNSTONII*[1]

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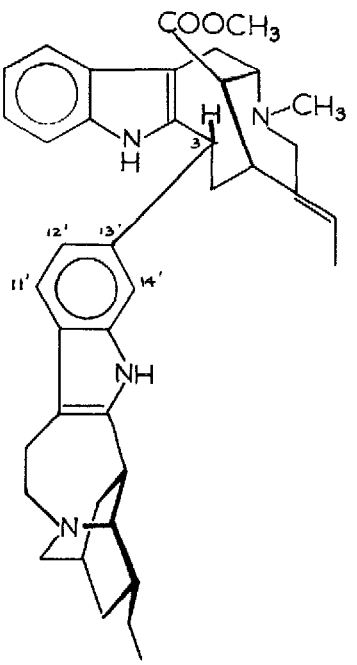
In the course of a continuing search for tumor inhibitors of plant origin, ethanol extracts of the stem bark of *Tabernaemontana johnstonii* Pichon. (Apocynaceae), collected in Kenya, were shown to possess significant inhibitory activity against P388 lymphocytic leukemia (PS) (*in vivo* and *in vitro*) and against human carcinoma of the nasopharynx (KB).[2] Systematic fractionation of the ethanol extract indicated that activity was concentrated successively in the basic alkaloidal portion of this extract and in the fraction eluted with benzene from a column of neutral alumina (Woelm, act. III). Further chromatography on silica gel PF-254 (E. Merck) with elution by methanol-chloroform yielded an active fraction which was rechromatographed on silica gel PF-254 and eluted with ethanol-ethyl acetate. Final purification of the active fraction by preparative thin-layer chromatography and high-pressure liquid chromatography yielded tabernamine (1) as a colorless amorphous powder. The material had an ED_{50} of 1.9 μg/ml in the PS cell culture system.

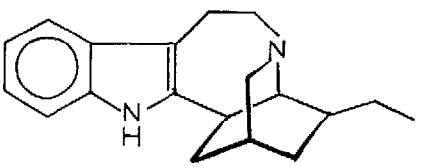
Tabernamine was homogeneous on TLC in two solvent systems and also on HPLC (Partisil-10 with elution by 10% hexane in ethyl acetate[3]). It had $[\alpha]_D^{22}$ -51° (c = 0.18, CH_3OH); mass spectrum m/e 630(4), 616(M^+,40), 585(20), 182(94), 180(60), 136(84), 122(100); λ_{max}^{EtOH} (log ε) 235(4.53), 287(4.02), and 295(4.00) nm. Its IR spectrum showed ν_{max} 1720 cm^{-1}, and its NMR spectrum (CD_3OD) showed peaks at 0.90 (3H, triplet), 1.68 (3H, doublet), 2.50 (3H, singlet), 2.56 (3H, singlet), 5.38 (1H, m), 6.88 (1H, doublet, J = 8 Hz), 7.04 (4H, complex), 7.28 (1H, doublet, J = 8 Hz), and 7.54 (1H, complex).

The mass spectrum of tabernamine suggests that it belongs to the class of bis-indole alkaloids of which voacamine is the best known example.[4] In particular, the peaks at m/e 122, 180 and 182 are due to characteristic ions formed from the vobasine portion of such an alkaloid while peaks at m/e 122 and 136 result from the formation of characteristic iboga fragment ions.[5] The molecular ion peak at m/e 616 is consistent with a voacamine-type alkaloid lacking a carbomethoxy group and a methoxy group. The low intensity of the peak at m/e 630(M+14), due to intermolecular methyl transfer, is consistent with the absence of the carbomethoxy group in the iboga portion of the molecule.[6]

The NMR spectrum of tabernamine offers further evidence for the structure deduced above. In particular, signals due to the methyl portion of an ethyl group, to an N-methyl and a high field O-methyl group, and to the methyl and methine portions of a $=CH-CH_3$ group are all readily detectable. The high field signal (2.50 or 2.56 ppm) associated with the carbomethoxy group is consistent with this group's presence on a vobasine moiety where it is shielded by the diamagnetic anisotropy of the indole nucleus.[7]



1



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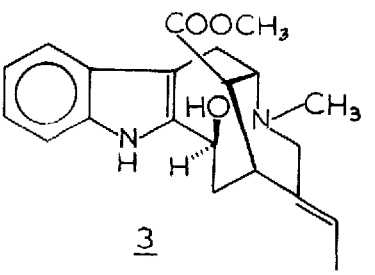

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Acid cleavage of tabernamine[4] yielded ibogamine (2), identified by TLC and HPLC and comparison of its mass spectrum with that of an authentic sample. Confirmation of the nature of the units linked together in tabernamine was obtained by partial synthesis from the monomeric units.[4] Condensation of vobasinol (3),[7] prepared from perivine,[8] with ibogamine yielded an alkaloid which was identical (TLC, HPLC, NMR, IR, optical rotation) with that isolated from *T. johnstonii*.

The remaining points to be considered concern the position and stereochemistry of the linkage between the vobasine and ibogamine moieties. Electrophilic substitution on the benzene ring of indoles occurs most readily at the 6-position,[9] and hence the vobasine unit in 1 is most probably linked at the 13' position of the ibogamine moiety. Confirmation of this conclusion was reached on the basis of the NMR spectrum of tabernamine, which shows a doublet at 7.28 ppm. Since the 4 proton of indole absorbs downfield from the remaining aromatic protons,[10] this doublet may be assigned to the proton in the 11' position in 1, thus indicating the linkage to be at the 13' position. The stereochemistry at C-3 of the vobasine moiety is proposed on the basis that attack of the ibogamine molecule on the vobasinol-derived iminium salt intermediate would occur from the much less crowded α-side of the molecule.[4]

Tabernamine is the first bis-indole alkaloid of the voacamine type to be isolated that lacks a methoxy group in the iboga ring, although a recent paper describes the isolation of capuvosine derived from a cleavamine-type indole alkaloid which lacks a ring methoxy group.[11] Since the conditions required for the synthesis of tabernamine were rather vigorous, we do not believe the compound to be an artefact of the relatively mild isolation procedure although this possibility cannot be totally excluded.

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