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# 16(S)-HYDROXY-16,22-DIHYDROAPPARICINE, A NEW ALKALOID FROM THE LEAVES OF *TABERNAEMONTANA DICHOTOMA*

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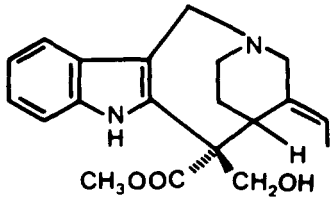
ABSTRACT.—From an extract of *Tabernaemontana dichotoma* leaves, two minor alkaloids were isolated. One was identified as voaphylline hydroxyindolenine, and the other one is a new alkaloid, for which the structure 16(S)-hydroxy-16,22-dihydroapparicine is proposed on the basis of spectral data.

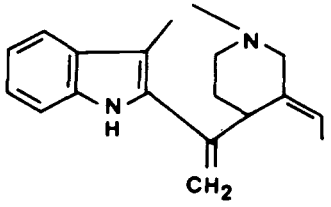
In previous publications, we have reported on the major alkaloids present in the leaves of *Tabernaemontana dichotoma* Roxb. (1,2). During our further studies of the biologically active alkaloids from this plant, we isolated some minor compounds from extracts of the leaves. The identification and structure elucidation of two of these are reported here.

One of the isolated minor alkaloids showed a molecular ion at *m/z* 282 in the ms. Major fragments were observed at *m/z* 172, 158, 130, and 110. Also, loss of $H_2O$ was observed (*m/z* 264). The uv was that of an unsubstituted indole. In the pmr, two AB doublets of geminal protons were observed at 4.73, 3.95 ($J=17.5$ Hz) and 3.66, 3.58 ppm ($J=17$ Hz). Similar AB doublets are observed for the vallesamine (**1**) (3) and apparicine (**2**) (1) type of alkaloids. In fact, the pmr spectrum shows a great deal of similarity to that of vallesamine (3). The presence of a three-proton singlet, superimposed on the three-proton doublet of H-18, points to the presence of a methyl-group on a quaternary carbon.

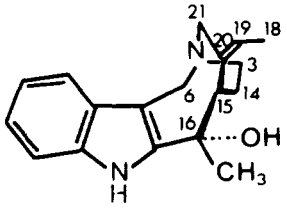
All these data suggested structure **3**, 16-hydroxy-16,22-dihydroapparicine, and the cmr of the alkaloid was in excellent agreement (see Table 1). The cmr shifts observed are similar to those previously reported for vallesamine (3), with the exception of C-15 and C-16. These, however, correspond to shifts reported for the 16-hydroxy-



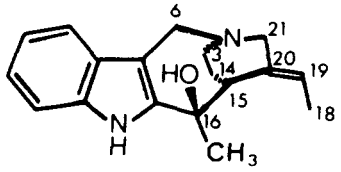

**1** Vallesamine




**2** (−)-Apparicine




conformation 1




conformation 2

**3** 16-hydroxy-16,22-dihydroapparicine (according to nOe experiments, conformation 1 represents the structure of this alkaloid)

16,17-dihydrouleines (4). This leaves only the stereochemistry at C-16 to be determined. As there are two conformations possible, the number of possibilities is four. Of these, two seem less likely because of steric hindrance (conformation 1 with α-16-methyl and conformation 2 with β-16-methyl). In both remaining conformations, the 16-methyl group is in the plane of the indole nucleus. The large and small coupling constants observed for H-15 are in agreement with both of these conformations. The resolutions of the signals of H-3 and H-14 protons are too small to allow a determination of the coupling constants for these protons and, thus, of their positioning.

TABLE 1. Cmr Chemical Shifts for 16-Hydroxy-16,22-dihydroapparicine (**3**) (in $CDCl_3$)

| Carbon | Chemical shifts (in ppm) |
|---|---|
| 2 | 138.1 |
| 3 | 48.4 |
| 6 | 50.4 |
| 7 | 107.3 |
| 8 | 129.9 |
| 9 | 118.5 |
| 10 | 119.2 |
| 11 | 122.3 |
| 12 | 110.3 |
| 13 | 135.2 |
| 14 | 23.4 |
| 15 | 43.2 |
| 16 | 74.5 |
| 18 | 13.8 |
| 19 | 124.9 |
| 20 | 134.5 |
| 21 | 53.2 |
| 22 | 30.2 |

In order to establish the definite stereochemistry, some nOe experiments were performed to determine the position of the two C-6 protons. Irradiation of one of the C-6 protons (4.00 ppm) gave an nOe for H-3, H-21, and H-9. Due to overlapping by the signal of the solvent ($Me_2CO$), nOes for H-14 could not be observed. In $CDCl_3$ solution, upon irradiation of the C-14 protons, nOes were observed for H-3, H-15, and one of the H-6 protons (3.95 ppm). From these nOe experiments, conformation 1 can be concluded as the most likely. In conformation 2, neither of the two H-6 protons is expected to cause an nOe on H-3 protons; whereas, an nOe for H-21 can be expected by irradiation of H-6β, but that proton should not cause an nOe for H-9 in this conformation. On the other hand, in conformation 1, H-6β is in a position that could lead to an nOe for one of the H-21 protons. Irradiation of the multiplet of the H-21 protons showed nOes for H-6β (4.75 ppm), H-19, and H-3. Irradiation at 1.75 ppm (H-18 and H-22) causes nOes for H-15, H-19, and the NH. All of these experiments are in accordance with conformation 1, and it is thus concluded that 16-hydroxy-16,22-dihydroapparicine has a 16S configuration.

A further minor alkaloid was identified as voaphylline hydroxyindolenine by its spectral data (uv, ms, pmr) and tlc-comparison with an authentic sample (5).

## EXPERIMENTAL

PLANT MATERIAL.—The collection, identification, and extraction procedures of the leaves of *T. dichotoma* were previously described (1).

ISOLATION.—The alkaloids were isolated from fractions from the previously described column chromatographic separation (1) by means of repeated preparative tlc on Merck silica gel 60 $GF_{254}$, using solvent systems: (a) Cyclohexane-$CHCl_3$-diethylamine (6:3:1), (b) $Et_2O$-absolute EtOH-diethylamine (18:0.8:0.6), (c) $CHCl_3$-MeOH (95:5), (d) Diisopropylether-absolute EtOH saturated with 25% $NH_3$ (19:1)[1], and (e) Toluene-absolute EtOH saturated with 25% $NH_3$ (95:5)[1]. Voaphylline hydroxyindolenine was purified by sequentially using systems A, B, and E, and 16-hydroxy-16,22-dihydroapparicine by the systems A, C, and D. The alkaloids were eluted from the plates by extraction with $CHCl_3$-MeOH (1:1).

The alkaloids were detected by uv light of 254 and 366 nm wavelength and spraying with 0.2 M $FeCl_3$ in 35% perchloric acid and 1% ceric sulfate in 10% $H_2SO_4$ followed by heating.

SPECTRAL METHODS.—The uv spectra were recorded on a Shimadzu MPS 5000 instrument; ir spectra were obtained with Jasco IRA-1 spectrometer; eims were recorded on a LKB 9000 instrument with direct inlet; pmr spectra were run on a Bruker WM 300 apparatus. Because of the poor solubility of the new alkaloid in $CDCl_3$, the nOe experiments were performed in degassed deuterioacetone solution. The cmr noise-decoupled spectrum was recorded on a JEOL 100 in $CDCl_3$.

VOAPHYLLINE HYDROXYINDOLENINE.—Spectral data of this alkaloid were identical to those previously reported (5). Rf values in systems A and E were 0.38 and 0.26; a purple color with ceric sulfate and an orange color with $FeCl_3$ spray reagent was observed.

16-HYDROXY-16,22-DIHYDROAPPARICINE.—$[\alpha]^{20}D = +129°$ (c=0.1 in EtOH); uv (EtOH) λ max (log ε) 220 (4.43), 284 (3.79), 292 (sh) nm; ir (KBr-disc): 3400, 2950, 1460, 1310, 1100, 750 $cm^{-1}$; eims (70 eV) *m/z* (rel. int. %): 282 ($M^+$, 43), 264 (6), 239 (8), 222 (6), 208 (7), 173 (26), 172 (100), 158 (20), 154 (7), 152 (9), 144 (8), 131 (7), 130 (46), 123 (12), 111 (36), 110 (44), 109 (10), 108 (14), 103 (10); cmr, see Table 1, and pmr, see Table 2.

TABLE 2. Pmr of 16-Hydroxy-16,22-dihydroapparicine (**3**) (300 MHz in $CDCl_3$)

| C-H | δ (ppm) | *J* (Hz) |
|---|---|---|
| NH | 9.10 bs | 1H |
| H-3a<br>H-3b | 2.89-2.95m | 2H |
| H-6α | 3.95 d | 1H $J_{6\alpha,6\beta}=17.5$ |
| H-6β | 4.73 d | 1H $J_{6\beta,6\alpha}=17.5$ |
| H-9 | 7.46 bd | 1H $J_{9,10}=8.0$ |
| H-10 | 7.18 ddd | 1H $J_{10,9}=8.0, J_{10,11}=7.5, J_{10,12}=1.0$ |
| H-11 | 7.08 ddd | 1H $J_{11,12}=8.0, J_{11,10}=7.5, J_{11,9}=1.0$ |
| H-12 | 7.33 bd | 1H $J_{12,11}=8.0$ |
| H-14a<br>H-14b | 2.01-2.22 m | 2H |
| H-15 | 3.32 dd | 1H $J_{15,14b}=12.0, J_{15,14a}=3.5$ |
| H-18 | 1.75 ddd | 3H $J_{18,19}=6.9, J_{18,21b}=2.5, J_{18,21a}=1$ |
| H-19 | 5.69 q | 1H $J_{19,18}=6.9$ |
| H-21a | 3.66 bd | 1H $J_{21a,21b}=17.0$ |
| H-21b | 3.58 bd | 1H $J_{21b,21a}=17.0$ |
| H-22 | 1.73 s | 3H |

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[1]Prior to development the plates were placed in an atmosphere of $NH_3$ for 20 min.

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