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ISOLATION AND SYNTHESIS OF VOBPARICINE, A NOVEL TYPE DIMERIC INDOLE ALKALOID

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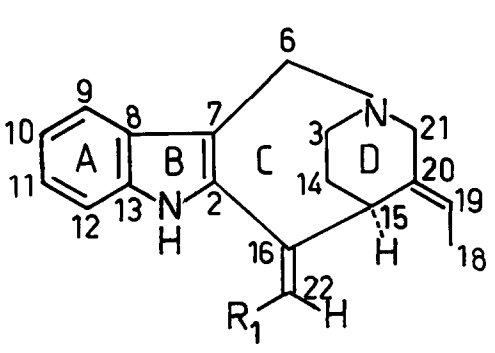
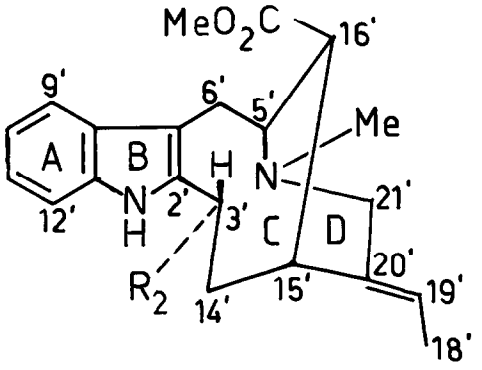
Summary: From the rootbark of *Tabernaemontana chippii* a novel antimicrobially active dimeric indole alkaloid was isolated which was assigned structure 1 on the basis of spectroscopic evidence and its synthesis.

From 250 g rootbark of *Tabernaemontana chippii* (Stapf) Pichon (Apocynaceae) approximately 4 mg of a new alkaloid has been isolated which was named vobparicine. With ceric sulphate it gave a pink-gold colour. The colour with ferric chloride perchloric acid was greyish purple upon heating. In the ^{1}H 500 MHz ^{1}H-NMR the most characteristic signals were 2 indolic NH, 8 aromatic protons, a doublet (1 H) at 5.96 ppm, 2 quartets (1 H each) at 5.41 and 5.39 ppm, 2 doublets (1 H each) at 4.49 and 4.39 ppm, 2 singlets (3 H each) at 2.61 and 2.47 ppm and 2 doublets (3 H each) at 1.68 and 1.67 ppm. From thesedata it could be concluded that it was a dimeric indole alkaloid with 2 unsubstituted aromatic rings, indicating a different attachment between the 2 halves than is usual for dimeric *T.* alkaloids[2]. The shifts for the 2 methyl singlets suggested the presence of a vobasine half, while the 2 doublets (J=17.5 Hz) at 4.49 and 4.39 ppm suggested the presence of an apparicine-like half. This was confirmed by comparing the spectrum with reference 300 MHz spectra of vobasinol, conodurine (vobasinyl--half) and apparicine[3].

The only 3 major differences between the spectrum of vobparicine and a combination of the spectra of apparicine and vobasinyl were the presence of a doublet (J=10.1 Hz) at 5.96 ppm, the absence of 2 singlets in the 5.3 ppm region for the 2 apparicine H_{22} and the appearance of an extra coupling of 10.1 Hz in the signal for the vobasinyl $H_{3'}$. These facts suggested structure 1 or its C_{22} epimer for vobparicine. The assignment of certain signals in the ^{1}H-NMR (table 1) was confirmed by some homonuclear decouping experiments, which most importantly, showed the doublet at 5.96 ppm to be coupled (J=10.1 Hz) with the assumed signal for the vobasinyl $H_{3'}$ at 4.53 ppm.

Additional evidence for the correctness of structure 1 or its E-isomer was gained from the UV spectrum, the ^{13}C-NMR and the MS. The UV spectrum (λ_{max}: 222, 290 (sh), 296 (sh) and 304 nm) is approximately equal to a summation of the UV spectra of vobasinol and apparicine. The ^{13}C-NMR spectrum of vobparicine (table 2) is also similar to a summation of the ^{13}C-NMR spectra of apparicine[4] and the vobasinyl-part of voacamine[5] except for the upfield shift of C_{16} and the downfield shift of C_{22} due to the newly formed bond between the 2 monomers. The high resolution MS confirmed the molec.formula (M^+ at m/z 600.3464, $C_{39}H_{44}N_4O_2$) and also characteristic fragments for the vobasinyl-part at m/z 337,336, 277, 194, 182, 180 and 122 and the apparicine part (at m/z 263,249, 233, 220, 208, 167 and 107) were observed. A differentation between 1 (Z-configuration) and its E-epimer was made by means of a careful study of Dreiding models and some NOE experiments. The rather large coupling of 10.1 Hz between H_{22} and $H_{3'}$ suggested either a large angle (150° - 180°) or a small angle (0° - 30°) between these 2 protons. Thus 2 configurations (E or Z) were possible of which each could occur in 2 different conformations (either large or small angle). From the similar coupling constants of the apparicine and vobasinyl-halves in vobparicine as compared with apparicine and vobasinyl it was assumed that the D-ring of the apparicine half and the C-and D-rings of the vobasinyl--half possessed the same conformation as in their monomeric counterparts. The small chemical shift difference between $H_{6\alpha}$ and $H_{6\beta}$ indicated that the flexible 8-membered C-ring of the apparicine half still possessed the same conformation in vobparicine as in apparicine[6]. The Dreiding models showed the small angle conformations in both the E-and the Z-isomer to be impossible due to severe stereochemical hindrance between many protons. The E-isomer with





1 VOBPARICINE $R_1 + R_2$ = BOND

2 APPARICINE R_1 = H

3 VOBASINYL R_2 = BOND

4 VOBASINOL R_2 = OH

the large angle between H_{22} and $H_{3'}$, was considered to be unlikely due to some stereochemical hindrance between $H_{14\alpha}$ and H_{15} and $H_{3'}$. In the large angle Z-isomer there was relatively little hindrance between the different protons. Only the $H_{1'}$ and H_{22} and H_1 and $H_{3'}$ were somewhat proximate. Thus for the E-isomer on irradiation of $H_{3'}$, a NOE-effect for $H_{14\alpha}$ would be expected and on irradiation of H_{22} a NOE for $H_{1'}$. For the Z-isomer irradiation of $H_{3'}$ should give no NOE for the $H_{14\alpha}$, but a NOE for $H_{1'}$ instead. Irradiation of H_{22} was expected to give a NOE for $H_{1'}$. In the actual experiments irradiation of H_{22} gave a strong NOE for the lowfield NH. Irradiation of $H_{3'}$ gave a small NOE for the high field NH and no NOE for $H_{14\alpha}$. These data could only fit the proposed structure 1 for vobparicine (Z-isomer). The assignment of the 2 NH signals, not previously possible, was also determined.

Biosynthetic origin. It was speculated that vobparicine is formed in a similar way to the dimeric alkaloids of the voacamine type, namely by an attack of the electrophilic C_3 of vobasinol on an electron rich center, such as in apparicine C_{22}. To prove this hypothesis a semisynthesis of vobparicine from vobasinol and apparicine was attempted. When vobasinol was refluxed for 2 hrs with excess apparicine in 1.5 % methanolic HCl under nitrogen, no vobasinol could be detected on TLC afterwards. Instead a product of a polarity between apparicine and vobasinol was formed. After separation from the excess apparicine and purification, the product was in all respects, - coTLC, chromogenic reactions, UV and CD (table 3) - identical with vobparicine giving extra proof for structure 1. An explanation of the fact that vobparicine has not been isolated previously from species containing both apparicine and vobasine/vobasinol could be its low abundance in comparison with the dimeric alkaloids of the voacamine type, which are seemingly more easily formed. In the species investigated here the ratio between the yield of all the alkaloids of the dimeric voacamine type and vobparicine was at least 100 : 1[3].

Although the possibility that vobparicine might be an artefact could not be excluded with 100 % certainty, vobparicine was considered to be a genuine alkaloid because the drastic circumstances used in its synthesis were not achieved during the isolation procedure and also because the alkaloids of the voacamine type, which are probably formed in a similar fashion, are not considered to be artefacts[7]. It seems likely that vobparicine will turn out to be a fairly common minor alkaloid in the genus *T.* as in 9 of the 67 chemically investigated species apparicine and vobasine co-occur.

In a preliminary screening for activity against gram-positive bacteria vobparicine showed strong activity.

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References:

1 - Part 9 in the series "Pharmacognostical studies on *Tabernaemontana* species". For part 8 see reference 2.

2 - T.A. van Beek, R. Verpoorte, A. Baerheim Svendsen, A.J.M. Leeuwenberg and N.G. Bisset, J. Ethnopharmacology (1984) in press.

3 - T.A. van Beek, unpublished results.

4 - M. Damak, Thesis, Université de Paris-Sud, p. 145 (1977).

5 - R.M. Braga, H.F. Leitao Filho and F. De A.M. Reis, Ciência e Cultura, suplemento, 142 (1980).

6 - F. Heatley, L. Akhter and R.T. Brown, J. Chem. Soc., Perkin 2, 919 (1980).

7 - G. Büchi, R.E. Manning and S.A. Monti, J. Am. Chem. Soc., 86, 4631 (1964).

Table 1: ^{1}H-NMR data vobparicine ($CDCl_3$, 500 MHz, TMS = 0 ppm)

H-nr	δ	J (Hz)	H-nr	δ	J (Hz)
1	7.56	bs	1'	7.78	bs
3 α	3.19	m, Σ J ~ 30	3'	4.53	12.1 (14α'), 10.1 (22), 3.2 (14β')
3 β	3.65	m, Σ J ~ 26	5'	4.00	10.4 (6α'), 8.2 (6β'), 3.1 (16')
6 α	4.49	17.5 (6β)	6 α'	3.38	14.7 (6β'), 10.4 (5')
6 β	4.39	17.5 (6α)	6 β'	3.21	14.7 (6α'), 8.2 (6β')
9	7.37	8 (10)	9'	7.53	7.5 (10')
10	7.05	8 (9), 7.5 (11)	10'	7.09	7.5 (9'), 7.5 (11')
11	7.14	8 (12), 7.5 (10)	11'	7.12	7.5 (10'), 7.5 (12')
12	7.21	8 (11)	12'	7.20	7.5 (11')
14 α	2.20	~ 13.5 (14β), ~ 7.5 (3α), ~ 4.3 (3β), ~ 2.2 (15)	14α'	2.64	15.1 (14β'), 12.6 (15'), 12.1 (3')
			14β'	1.89	15.1 (14α'), 6.8 (15'), 3.2 (3')
14 β	2.37	m, Σ J ~ 37	15'	3.74	12.6 (14α'), 6.8 (14β'), 3.1 (16')
15	4.45	~ 5 (14β), ~ 2.2 (14α)	16'	2.74	3.1 (5'), 3.1 (15')
18	1.68	6.8 (19), 1.9 (21β)	18'	1.67	6.8 (19'), 1.9 (21')
19	5.39	6.8 (18)	19'	5.41	6.8 (18')
21α	3.45	15.2 (21β)	21α'	2.99	13.8 (21β')
			21β'	3.77	13.8 (21α')
21β	3.97	15.2 (21α), 1.9 (18)	CO_2Me'	2.47	s
22	5.96	10.1 (3')	NMe'	2.61	s

Table 2: Experimental ^{13}C-NMR data of vobparicine ($^{12}CDCl_3$, 75.2 MHz, $^{13}CDCl_3$ = 77.0 ppm) and literature ^{13}C-NMR data of apparicine[4] and 3'-vobasinyl-half of voacamine[5]

C-nr	apparicine	vobparicine	C-nr	3'-vobasinyl	vobparicine
2	135.7	133.7	2'	135.5	135.1
3	45.3	46.6	3'	37.4	33.9
6	54.3	54.1	5'	59.9	59.7
7	111.1	110.4	6'	19.8	19.4
8	129.0	128.4	7'	109.6	109.5
9	118.6*	118.1	8'	129.4	129.9
10	119.3*	119.6	9'	117.2	117.7
11	123.0	123.0	10'	119.8*	119.8
12	110.2	110.4	11'	121.3	122.2
13	137.8	136.3	12'	110.1	110.0
14	29.6	28.7	13'	136.2	135.8
15	41.3	37.8	14'	36.6	33.7
16	142.6	133.7	15'	33.3	33.4
18	12.5	13.0	16'	46.0	46.0
19	120.3	119.8	18'	12.3	12.3
20	131.3	132.1	19'	118.7*	119.6
21	54.3	54.1	20'	137.7	136.9
22	112.2	123.0	21'	52.3	52.2
			NMe'	41.9	42.3
			$CO_2\underline{Me}$'	49.8	50.1
			$\underline{CO}_2$Me'	170.7	not observed

Note: The assignments of vobparicine signals, which differ less than 3 ppm from each other, may be interchanged.

Table 3: CD data vobparicine (MeOH)

(λ, $\Delta\epsilon$): 347, 0.0; 327, -5.2; 320, -4.6; 310, -6.0; 300, 0.0; 297, +1.4; 294, 0.0; 291, -2.0; 282, 0.0; 246, +28.1; 238, 0.0; 227, -79.1; 215, 0.0; 205, +37.3.

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