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VINCARUBINE, A NOVEL BISINDOLE ALKALOID FROM VINCA MINOR L.

Bohumil PROKSA, Dušan UHRÍN, Eduard GROSSMANN and Zdeno VOTICKÝ

Institute of Chemistry, Centre for Chemical Research, Slovak
Academy of Sciences, 84238 Bratislava, Czechoslovakia

The structure of a novel bisindole alkaloid vincarubine (1) was adduced from the spectral data. Vincarubine is the first dimeric alkaloid isolated from the leaves of lesser periwinkle.

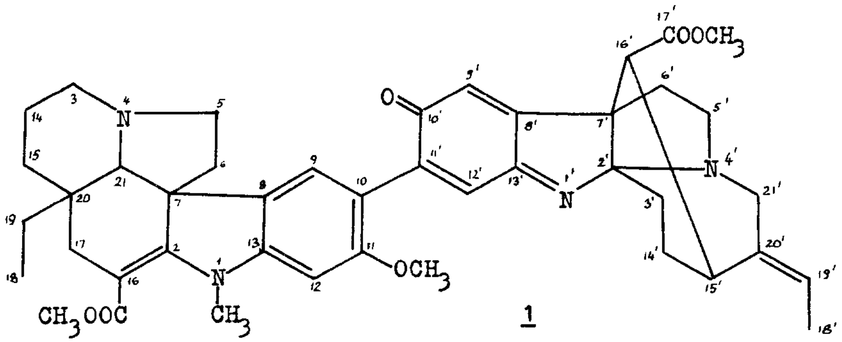
Vincarubine, the dark-red indole alkaloid was separated by counter-current distribution followed by column chromatography of the mixture of tertiary strong bases fraction from the leaves of lesser periwinkle (Vinca minor L.). This cryptocrystalline base, $C_{43}H_{50}N_4O_6$ (718.9), m.p. 170°C (decomp.), $[\alpha]_D^{23}$ -550 ± 10°(ethanol) displayed absorption bands in the UV region at λ_{max}, nm (log ε, m^2. mol^{-1}) 273 (3.21), 340 (3.29) and 480 (2.40). Its IR spectrum (KBr, $\tilde{\nu}$, cm^{-1}) indicated the presence of an ester group (1730), an α,β-unsaturated carbonyl grouping (1680) and a double bond (1605). The ^1H NMR spectrum showed signals attributable to four isolated protons of an aromatic system, three methoxyl, one $N-CH_3$, one ethylidene, and one ethyl groupings. Upon $NaBH_4$ reduction in methanol vincarubine (1) formed a yellow compound, which, in turn, underwent a spontaneous oxidation in air to afford the starting material. Therefore, 1 was reduced with sodium dithionate and the product was directly acetylated to the stable 2, $C_{45}H_{54}N_4O_7$ (762.9). The UV spectrum of 2 revealed absorption bands at λ_{max}, nm (log ε, m^2. mol^{-1}) 259 (3.11) and 347 (3.46). Addition of methanolic HCl to the sample was associated with both hypsochromic shift to 343 nm and an appearance of a shoulder at 320 nm. This phenomenon is reported[1,2] as diagnostic of the vincorane type of alkaloid having a C_6H_5-N-C-N grouping. This assignment was also backed by the shift of the ^{13}C NMR signal of the carbon between two nitrogen atoms from δ 104.0 ppm for 1 to 94.7 ppm for 2, the latter value being close to that published for vincorine[3] and (-)-1-norvincorine (3). A contrast of the ^{13}C NMR spectra of 1 and 2 with those of flexicorine and dihydroflexicorine[5] showed the vincorine moiety of both types of alkaloids to be closely related. The absence of $N_{(1')}-CH_3$ in the structures of 1 and 2 caused, as follows also from the analysis of the spectra of vincorine and (-)-1-norvincorine[4] that carbons $C_{(3')}$ resonate at δ 27.8 and 26.4 ppm, respectively and the respective carbons $C_{(6')}$ at δ 41.0 and 41.1 ppm. Assignment of all signals of 1 was confirmed by analysis of homo- and heterocorrelated 2D spectra. As a result, the signals ascribed to $C_{(3')}$ and $C_{(6')}$ for flexicorine[5] should be interchanged.

TABLE 1. ^{13}C NMR chemical shifts (δ, ppm) of compounds 1 – 4

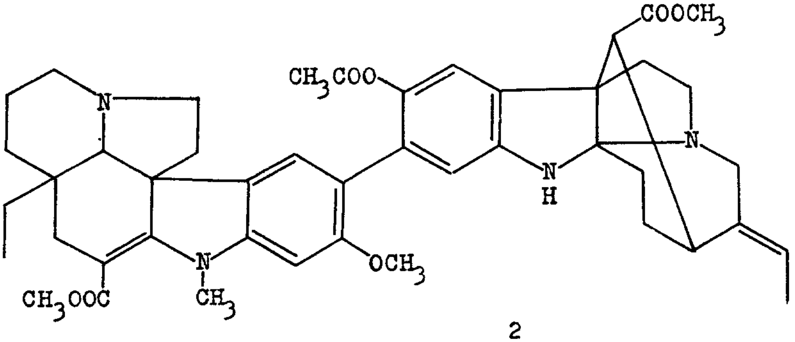
Carbon	1[a]	2[a]	4[b]	Carbon	1[a]	2[a]	3[c]
2	166.6	167.3	167.8	2′	104.0	94.7	94.4
3	50.3	50.4	51.7	3′	27.8	26.4[+]	26.6
5	51.8	51.7	50.7	5′	53.8	54.5	56.0
6	47.0	47.2	45.3	6′	41.0	41.1	41.3
7	56.0	56.2	55.5	7′	56.8	57.7	57.8
8	130.6	130.3	138.0	8′	158.0	136.4	139.4
9	122.8	123.2	120.5	9′	123.4	118.8	112.2[+]
10	116.7	118.9	120.5	10′	187.0	140.8	153.1
11	157.7	156.8	127.4	11′	144.7	130.9	111.9[+]
12	93.5	93.3	109.3	12′	131.1	111.7	109.3
13	148.9	147.3	143.4	13′	164.5	145.4	141.8
14	22.0	22.2	22.2	14′	26.7	26.5[+]	26.6
15	32.6	32.6	32.9	15′	35.7	35.1	35.3
16	94.2	93.5	92.8	16′	50.3	50.6	50.6
17	30.2	30.0	25.6	17′	172.6	173.3	173.5
18	7.1	7.2	7.3	18′	13.8	13.6	13.5
19	29.4	29.3	29.3	19′	123.1	123.3	122.0
20	37.5	37.6	38.2	20′	139.2	138.2	138.2
21	75.0	75.2	72.7	21′	59.2	57.7	58.1
$N-CH_3$	35.9	36.1	-	OCH_3	-	-	54.6
OCH_3	56.1	56.1	-	$COO\underline{C}H_3$	51.9	51.7	51.7
$\underline{C}OOCH_3$	168.0	168.0	169.2	$\underline{C}H_3COO$	-	20.9	-
$COO\underline{C}H_3$	50.9	50.9	50.9	$CH_3\underline{C}OO$	-	169.7	-

[a] in $CDCl_3$ at 303 K, internal standard tetramethylsilane; [b] ref.[10]; [c] ref.[4]; [+] may be interchanged.

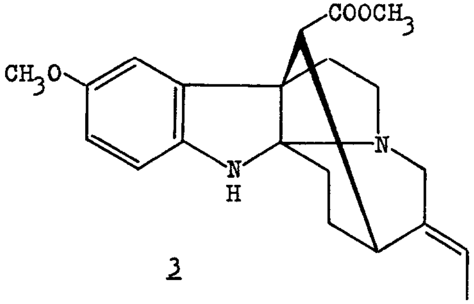
The mass spectrum of 1 disclosed peaks associated with M^+ at *m/z* 718 (42%) and further ones corresponding to M + 2 (100%), M + 14 and M + 2 + 14 ions. The M + 2 ion formed by a thermal *in situ* hydrogenation of the quinonimine moiety of the molecule was observed in the spectrum of flexicorine, as well. The M + 14 peak reflected the thermal transmethylation of bisindole alkaloids having methoxycarbonyl and amine substitutions as *e.g.* voacamine[6] or vinblastine.[7] Other prominent peaks appeared at *m/z* M + 2 - 15 (3%),



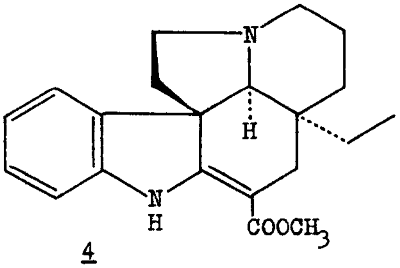
1



2



3



4

M - 15 (4%), M + 2 - 28 (4%), M + 2 - $COOCH_3$ (8%), M - $COOCH_3$ (12%), 258 (2%), 124 (42%), 107 (45%) and 92 (63%). The dihydroacetate 2 revealed peaks at m/z 776 (M + 14, 2%), 762 (M^+, 21%), 720 (M - C_2H_2O, 2%), 381 (the vincorane moiety of the molecule) and 124 (100%). The 1 : 4 intensity ratio of the peak at m/z 762 to that at 124 indicated the vincadifformine type of alkaloids[8]. The IR absorption band at 1680 cm^{-1} of compound 2 remained unchanged, this being in favour of an α,β-unsaturated carbonyl group in this part of the alkaloid vincarubine. This band is diagnostic of anilino-acrylate alkaloids, as evidenced by the ^1H- and ^{13}C NMR spectra analysis. The selective transfer of polarization[9] through $H_{(9)}$ at δ 7.0 ppm afforded signals at 157.7 ($C_{(11)}$), 148.9 ($C_{(13)}$) and 144.7 ppm. The latter is due to $C_{(11')}$ of the vincorane moiety, and therefore, an opposite arrangement of $C_{(8')}$ and $C_{(11')}$ signals was suggested in contrast to[5]. The remaining signals of the alicyclic carbons are in line, as far as the quantity and multiplicity are concerned, with those[10] of vincadifformine (4); some differences in signal positions might be due to different substitution at $N_{(1)}$. The band at 2776 cm^{-1} in the IR spectra of 1 and 2 comparable with that at 2760 cm^{-1} of compound 4 favourizes the trans orientation[11] of the $H_{(21)}$ with respect to the lone electron pair at $N_{(4)}$.

The presented data entitle us to ascribe structure 1 to vincarubine, which is the first bisindole alkaloid isolated from the leaves of *Vinca minor L.*

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