272. The Structure of Vincarodine[1,2])

by **Norbert Neuss, Harold E. Boaz** and **John L. Occolowitz**
Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206, USA
Ernest Wenkert and **F. M. Schell**
Department of Chemistry, Indiana University, Bloomington, Indiana 47401, USA
Pierre Potier and **Christiane Kan**
Institut de Chimie des Substances Naturelles, C.N.R.S., 91190 Gif-sur-Yvette, France
M. M. Plat and **M. Plat**
U.E.R. de Chimie Thérapeutique, Université de Paris-Sud, 92290 Chatenay Malabry, France

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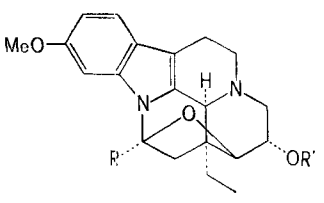
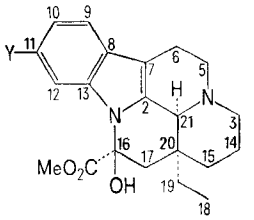
Summary. The structure of vincarodine has been determined to be **1** by an investigation of its IR.-, UV.-, ^{1}H- and ^{13}C-NMR.- and mass spectra. A ^{13}C-NMR. analysis has been performed on the bases vincamine (**2**), epivincine (**9**), 14,15-dehydrovincine (**7**) and its 16-epimer (**8**).

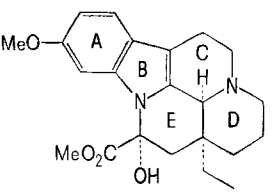
Vincarodine (**1**) [3] is one of the minor alkaloids in the leaves of *Vinca rosea* L. (*Catharanthus roseus* G. DON). While the base, – m.p. 235–238° (dec.), $[\alpha]_D^{26} = -139°$ ($CHCl_3$), pK_a 5.9 (66% DMF), – was believed to be a dimer [3], examination of the spectral properties of recently re-isolated alkaloid and its derivatives indicate it to be a monomeric, $C_{22}H_{26}N_2O_5$ compound related to the alkaloids of the vincamine (**2**) type [4].



1 R = CO_2Me, R' = H
3 R = CO_2Me, R' = Ac
4 R = CH_2OH, R = H
5 R = CH_2OAc, R = Ac



2 Y = H
6 Y = OMe
7 Y = OMe, $\Delta^{14(15)}$



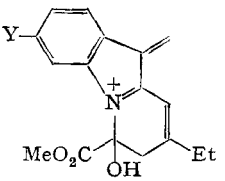
8 $\Delta^{14(15)}$
9

Acetylation of the alkaloid yielded a monoacetate (**3**) and lithium aluminium hydride reduction a diol (**4**) whose acetylation produced a diacetate (**5**). These data, substantiated by elemental analyses, high resolution mass spectra (Tables 1 and 2) and IR., UV. and ^{1}H-NMR. spectra demonstrated the presence of an aromatic methoxy group, a carbomethoxy unit, a secondary hydroxy function and an ether moiety in vincarodine. The close relationship of the latter and vincine [11-methoxy-

[1]) Vinca Alkaloids. XXXI. For part XXX see [1].
[2]) Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances. XX. For part XIX see [2].

vincamine (**6**), an alkaloid first reported in *Vinca minor*] [5], apparent from the similarity of their IR. and UV. spectra, was of particular importance. Their IR. spectra in chloroform solution are superimposable in the 1400–2000 cm^{-1} region [6]. The UV. spectrum of vincarodine shows absorption maxima at 231 nm (ε 31,000), 270 (8220) and 297 (4300) in comparison with vincine's maxima at 229 nm (ε 32,300), 273 (2900) and 296 (5025) [6]. The ^{1}H-NMR. spectrum of vincarodine furnishes corroborative evidence for the nature of the substitution in the indole ring by revealing a 1,2,4-hydrogen substitution pattern at 7.32 ppm (*d*, 8 Hz *ortho* splitting); 6.80 (*d* × *d*, 8 and 2 Hz *ortho* and *meta* splitting) and 6.52 (*d*, 2 Hz *meta* splitting) for the hydrogens at C(9), C(10) and C(12) respectively (*cf.* [7]).

The location of the secondary hydroxy group and the nature of the oxide unit were deduced from analyses of the mass and ^{1}H-NMR. spectra of vincarodine and its derivatives. The high resolution MS. of the alkaloid exhibits several high-intensity peaks one of whose most significant is the fragment of *m/e* 296 (**d**), reminiscent of the characteristic fragment **z** from vincamine (**2**) and vincine (**6**) [8]. Other peaks represent the loss of water and the carbomethoxy and ethyl groups as well as more deep-seated fragmentation (Table 1). One of the most abundant peaks in the MS. of vincarodinol (**4**) is **h**, the alcohol equivalent of fragment **d**, indicating that reduction of the ester function of the alkaloid did not modify a major fragmentation mode of the ring skeleton (Table 2). In conjunction with considerations of the biogenesis of vincarodine (*vide infra*) the mass-spectral findings suggested formula **1** for the natural base.

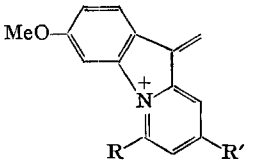


z

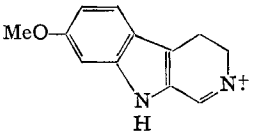
Table 1. *Characteristic Mass-spectral Fragments of Vincarodine* (**1**)

Fragment		Analysis	
		Calc.	Found
1$^+$	$C_{22}H_{26}N_2O_5$	398.1842	398.1818
a	$C_{22}H_{24}N_2O_4$	380.1748	380.1736
b	$C_{20}H_{23}N_2O_3$	339.1716	339.1709
c	$C_{18}H_{19}NO_3$	297.1360	297.1365
d	$C_{18}H_{18}NO_3$	296.1287	296.1287
e	$C_{16}H_{14}NO_3$	268.0971	268.0971
f	$C_{13}H_{15}N_2O$	215.1184	215.1186
g	$C_{12}H_{12}N_2O$	200.0940	200.0950

The 220-MHz-^{1}H-NMR. spectra of vincarodine (**1**) (Fig. 1) and its acetate (**3**) are consistent with the proposed structures in chemical shifts and geminal, vicinal and long-range coupling constants. The equatorial, C(14) oxymethine hydrogen signal at 3.72 ppm in the spectrum of vincarodine shifts to 4.90 ppm in the acetate spectrum. Irradiation of this hydrogen of both substances results in the coalescence of the C(3)



d R = CO_2Me, R′ = Et
e R = CO_2Me, R′ = H
h R = CH_2OH, R′ = Et
i R = H, R′ = Et



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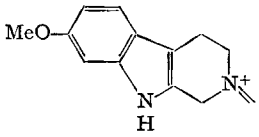
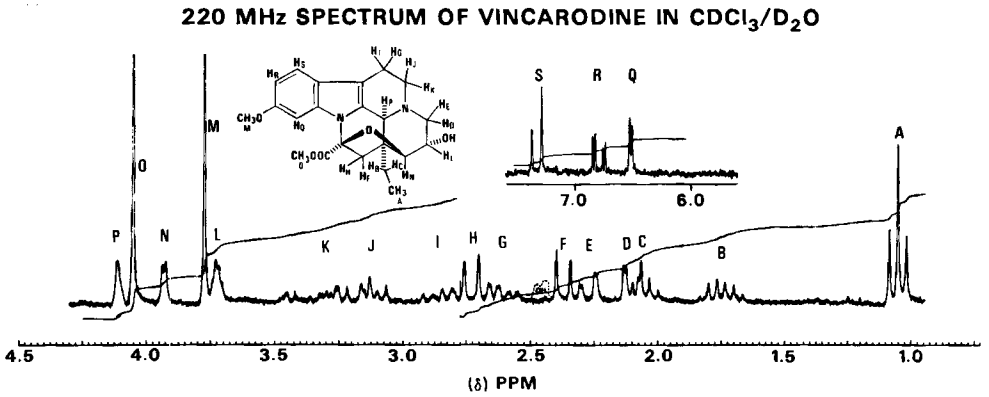


g

Table 2. *Characteristic Mass-spectral Fragments of Vincarodinol* (4)

Fragment		Analysis Calc.	Found
4^+	$C_{21}H_{26}N_2O_4$	370.1906	370.1893
h	$C_{17}H_{18}NO_2$	268.1342	168.1338
i	$C_{16}H_{16}NO$	238.1223	238.1232
f	$C_{13}H_{15}N_2O$	215.1184	215.1155
g	$C_{12}H_{12}N_2O$	200.0940	200.0950

methylene doublet of doublets into a simple doublet and the collapse of the C(15) methine doublet into a singlet. These experiments define the substitution pattern of ring D and thereby the relative stereochemistry of the alkaloid.



For corroboration of the above assignment it was decided to submit the base to ^{13}C-NMR. spectroscopy and simultaneously to gather information on ^{13}C chemical shifts of alkaloids of the more general vincamine (**2**) type, an area for which such data are lacking. Vincamine (**2**), 16-epivincine (**9**), the new natural base 14,15-dehydrovincine (**7**) and its 16-epimer (**8**) [9] were chosen as models and their natural abundance, ^{13}C- and ^{1}H-NMR. decoupled and single-frequency off-resonance decoupled spectra recorded on a *Fourier* transform spectrometer operating at 15.08 MHz [2]. Analysis of the chemical shifts of the three substances as well as of vincarodine (**1**) yielded the data summarized in Table 3.

Table 3. ^{13}C- *Chemical Shifts*[a]

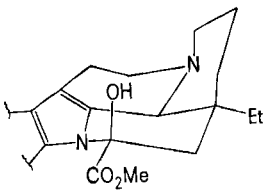
	1	2	9	7	8
C(2)	133.2	131.4	130.5	130.2	131.5
C(3)	45.4[b]	44.5	44.3	43.4	43.4
C(5)	50.1	50.9	50.8	49.3	49.5
C(6)	18.4	16.9	16.4	16.4	16.5
C(7)	110.9	105.9	105.9	106.0	105.9
C(8)	125.3	128.9	123.0	123.4	123.2
C(9)	118.6	118.4	118.3	118.6	118.1
C(10)	109.6	121.5	109.0	109.2	108.9
C(11)	156.3	120.1	155.6	156.0	155.7
C(12)	96.2	110.2	97.3	95.2	97.7
C(13)	137.8	134.1	136.3	134.8	137.6
C(14)	66.3	20.8	20.5	125.3	125.6
C(15)	82.0	25.2	24.0	127.9	126.5
C(16)	90.5	81.9	82.9	82.0	84.0
C(17)	46.1[b]	44.5	46.9	43.1	45.6
C(18)	9.3	7.6	7.3	8.1	8.1
C(19)	25.7	28.8	28.6	34.5	34.9
C(20)	43.9	35.1	36.0	36.6	38.0
C(21)	56.5	59.1	58.6	57.3	56.9
C=O	168.6	174.3	171.1	172.9	171.8
OMe	52.9	54.1	52.9	53.7	52.2
ArOMe	55.3	–	55.6	55.5	55.6

[a]) In ppm downfield from TMS; spectra of deuteriochloroform solutions; $\delta^{TMS} = \delta^{CDCl_3} + 76.9$ ppm.
[b]) These signals may be reversed.

The aromatic shifts are based on those of N_b-methyltetrahydroharmine, reserpine and several of its derivatives [10], while the shifts of the O-methyl and C-methyl groups and the carbonyl function are derived from chemical shift theory [11]. Since C(21) is a unique methine in the four models (**2**, **7**, **8**, **9**), its signal is readily recognizable for these substances as well as for vincarodine (**1**). The presence of oxy and nitrogenous substituents at C(16) differentiates this non-protonated center from the other one at C(20). Furthermore, the low-field δ(C(16)) value distinguishes the vincamine-like alkaloids from their *Aspidosperma* relatives [2] and other indole bases and characterizes vincarodine as a natural product of the former structure type.

The remaining saturated carbons of the models are solely methylenes. The chemical shifts C(3), C(5) and C(6) can be assigned by analogy with the shifts of related carbons in pseudoyohimbine [10]. Both the latter and the vincines are held in *cis*-indoloquinolizidine configurational constraint (*cf.* stereostructure **10** for vincine) which imposes 1,3-diaxial interactions between C(3) and C(6) and gives the latter a diagnostically significant high-field position. Being sandwiched between two heavily substituted carbons and thus feeling strong deshielding β-effects, C(17) is at low field. Since the chemical shift distribution for C(3), C(5), C(6) and C(17) applies also to vincarodine, its oxygen bridge emanating from C(16) must terminate at C(14) or C(15), both of which sites are reflected in the ^{13}C-NMR. spectrum as oxymethine units. The C(14) oxygen terminus is preferred, since not only is the alternate structure of questionable stability for reasons of strain, but it also would be expected to modify

ring C and D carbon shifts from the pattern emerging from the spectra of **2**, **7**, **8**, **9**. While in the resulting stereostructure **11** for vincarodine C(3) is expected to feel the presence of the neighboring oxygen atoms, its mildly deshielded position with respect to C(3) of vincamine (**2**) must be the consequence of almost a cancellation of the shielding γ-effect of the ether linkage and deshielding β-effect of the hydroxy group in conjunction with minor unpredictable effects caused by the mild conformational changes imposed on ring D by the rigidity of the ether bridge.



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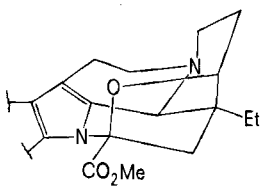

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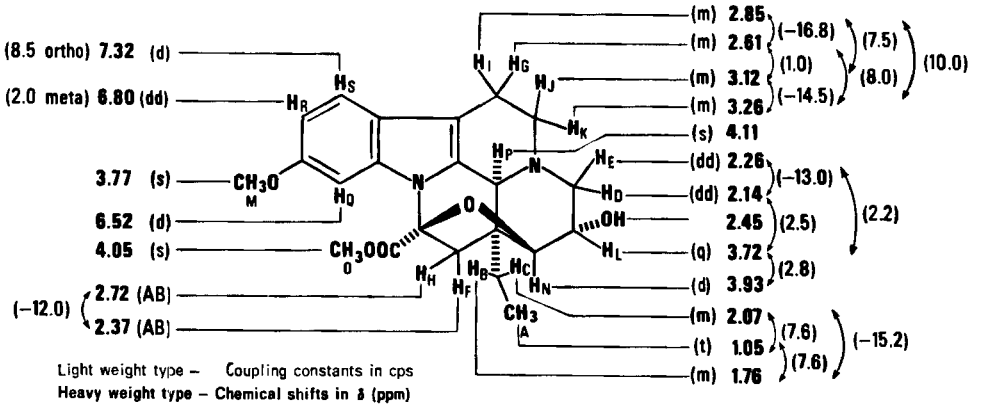
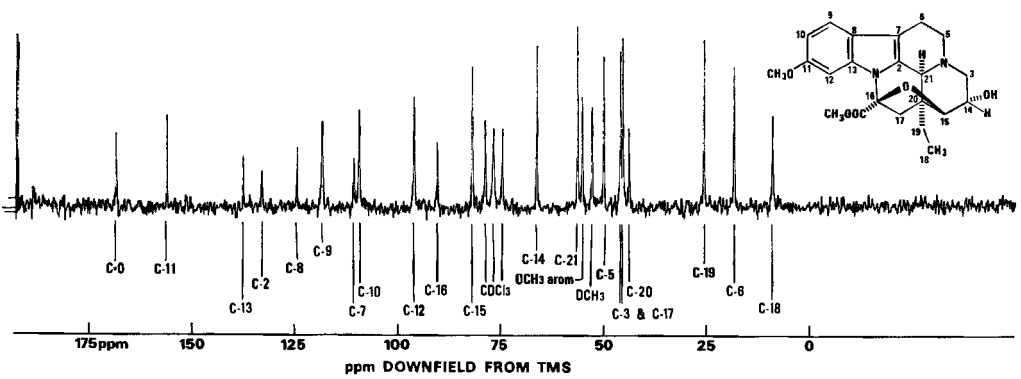


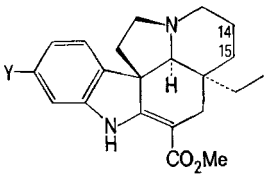
The C(19) shift of the olefinic bases **7** and **8** is derived from this center being the only remaining methylene group. Olefinic carbons 14 and 15 are distinguished from each other by more β-effects deshielding the latter. Shift assignment of C(14), C(15) and C(19) of vincamine (**2**) and epivincine (**9**) follows from substituent parameters analogous to those of vincadifformine (**12**) [2]. Finally, C(19) of vincarodine is the only remaining methylene group of the alkaloid and C(14) and C(15) are differentiable by the latter being exposed to more β-effects (Fig. 3).

The above ^{13}C-NMR. results are in full agreement with the assignment of structure **1** to vincarodine and reveal several points of significance for rapid structure determination of alkaloids of the vincamine (**2**) type. The high-field position of C(6) reflects a *cis*-quinolizideine moiety. A non-protonated carbon shift in the vicinity of 85 ppm shows the C(16) environment characteristic of vincamine-like compounds. The chemical shifts of C(13) and C(17) and, to a less extent, those of C(12) and C(15) reveal the stereochemistry of the carbinol amine unit.

On the assumption of the last stages of the biogenesis of vincine (**2**) involving oxidative transformation of 11-methoxytabersonine (**13**) [12] (*cf.* [13]) the simplest

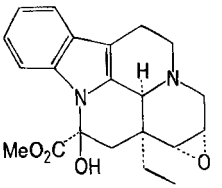
Natural Abundance ^{13}C Proton Decoupled Fourier Transform Spectrum 15.08 MHz, 973 Scans





12 Y = H

13 Y = OMe, $\Delta^{14(15)}$



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explanation for the biogenetic origin of vincarodine (**1**) requires the epoxidation of vincine (**2**) followed by intramolecular displacement within the resultant 14,15-epoxyvincine (**14**).

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Experimental Part

High Resolution Mass Spectra were recorded on a *CEC* 21-110 spectrometer. – *Nuclear Magnetic Resonance Spectra.* The ^1H-NMR. spectra were recorded on *Varian* HA-100 and *HR*-220 spectrometers. The ^{13}C-NMR. spectra of deuteriochloroform solutions in 13 mm o.d. tubes were recorded on a pulsed *Fourier* transform spectrometer operating at 15.08 MHz with a *Varian* DP-60 magnet working at 14 kGauss.

Purification of Vincarodine (**1**). The alkaloid was purified as described by *Svoboda et al.* [3] and recrystallized repeatedly from 1:1 methylene chloride/methanol, m.p. 236–238° (dec.), $[\alpha]_D^{26} = -193°$ ($CDCl_3$).

$C_{22}H_{26}N_2O_5$	Calc.	C 66.32	H 6.58	N 7.03	O 20.08%
	Found	,, 66.42	,, 6.51	,, 6.82	,, 20.30%

Vincarodine Acetate (**3**) was prepared in a conventional manner using 150 mg of the base, pyridine and acetic anhydride at room temperature overnight. After the usual work-up and crystallization of the product from methanol 140 mg of crystalline acetate, m.p. 206–208° (dec.), was obtained.

$C_{22}H_{28}N_2O_6$	Calc.	C 65.44	N 6.41	N 6.36%
	Found	,, 65.19	,, 6.29	,, 6.39%

Vincarodinol (**4**). To a suspension of 500 mg of the base in tetrahydrofuran were added 500 mg of lithium aluminium hydride and the mixture refluxed with stirring for 4 h. After cooling with ice, 3.2 ml of water was added and the mixture filtered from insoluble aluminium salt. After evaporation and crystallization from chloroform/acetone 300 mg of the carbinol, m.p. 190–193° (dec.), was obtained.

$C_{21}H_{26}N_2O_4$	Calc.	C 68.09	H 7.07	N 7.56	O 17.28%
	Found	,, 67.85	,, 7.16	,, 7.43	,, 17.25%

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