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ALKALOIDS FROM CATHARANTHUS ROSEUS

^{1}H NMR STUDY OF 20'S-DEOXYVINBLASTINE AND ITS N-2' BORANE COMPLEX

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ABSTRACT

The ^{1}H NMR spectra of 20' S-deoxyvinblastine and its N-2' borane complex have been unraveled. 2D δ-J-resolved NMR allowed to measure the isolated peaks in the overlapping patterns of the amine borane complex. Both compounds can now easily be discriminated on basis of ^{1}H NMR data.

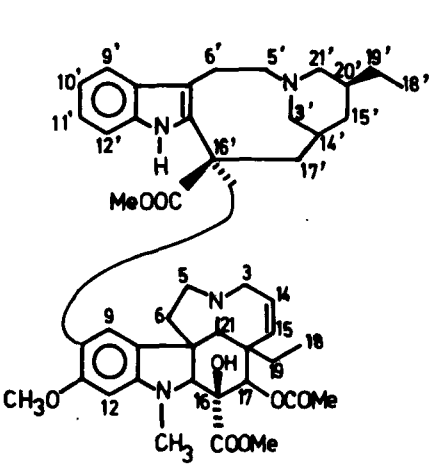
INTRODUCTION

^{1}H NMR is an established way to identify the bisalkaloids isolated from Catharanthus roseus[1-4]. So far only ^{1}H NMR spectra of the natural bisalkaloids as isolated from the plant were studied in our laboratory[2-4]. In order to synthesize the antitumor dimeric indole alkaloids of the vinblastine group[5], the vindoline moiety may be attached to the catharanthine moiety by the Potier-Polonowsky reaction[6]. By this method we have prepared 15',20'-anhydrovinblastine. The same compound was also obtained from vinblastine isolated from the plant[10]. Potier[6] and Kutney[7] observed that 20'-deoxyvinblastine is obtainable by hydrogenation of 15',20'-anhydrovinblastine in the presence of Adam's catalyst, the double bond in the vindoline fragment remaining unaffected.

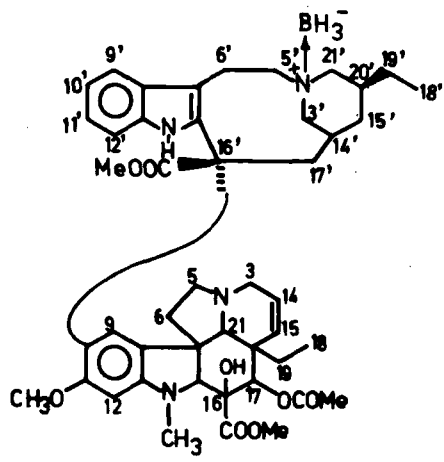
In our hands, following the experimental conditions of the formation of 15',20'-anhydrovinblastine, we obtained after hydrogenation two different dimeric products.

The ^{1}H NMR spectrum of these two products were readily run, and certain chemical shifts may immediately be diagnostic for discrimination of possible isomers. Some ^{1}H NMR data have indeed already been published separately by Potier[6] and Kutney[7]. These data are not in agreement with each other, nor with the present data. Therefore a closer examination was necessary. Thus it was found that one of the two products was 20' S-deoxyvinblastine; it was also found that the

second dimer is not an isomer at C-16' or at C-20', but the N-borane complex of 20' S-deoxyvinblastine. IR was diagnostic (2380 cm^{-1})[8]. Moreover the "natural" configuration of both dimers was verified by CD measurements. During the ^1H NMR measurements (e.g. double irradiation experiments) we observed a continuous change of the spectrum of the N-borane complex. The data provided here are from the compound stabilized in solution. We assume that changement at N-2' is responsible for a transformation with the non-stabilized $CDCl_3$ in the NMR tube providing a quaternary $R_3\overset{+}{N}H$ complex whose spectrum was in fact measured.



20'S-deoxyvinblastine



N-2' borane complex of 20'S-deoxyvinblastine

RESULTS AND DISCUSSION

The ^1H NMR data of 20' S-deoxyvinblastine (1) and its N-borane complex (2) in $CDCl_3$ solution are gathered in Table 1. As the ^1H NMR data of vinblastine are well established[2], the patterns of the vindoline moiety could immediately be assigned in the present compounds. Indeed, for this fragment no important differences with the corresponding chemical shifts in vinblastine are expected (except perhaps H-9). Important differences with those in vinblastine are expected in the catharanthine or rather velbamine part. These patterns were identified by extensive nmdr experiments. In order to measure the coupling constants in hidden patterns, a 2D δ-J-resolved NMR spectrum[9] was helpful for (2). Because of the multiple overlaps in the regions δ 0.80-0.90, δ 1.20-2.20 and most of all in the region δ 3.10-3.50, it is almost impossible to measure the patterns of the ^1H NMR spectrum of the borane complex (2) by a first-order analysis. Therefore we have : 1) tried to identify the position of the patterns

by nmdr and 2) we have isolated the overlapping patterns by a 2D δ-J-resolved NMR experiment, as obtained by the performance of a two-pulse spin echo experiment of the type $90°\text{-}t_{1/2}\text{-}180°\text{-}t_{1/2}$-scan[7]. For 20'S-deoxyvinblastine there were so many close overlaps in the regions at ∿ δ 2.80 and ∿ δ 3.20, that 2D δ-J-resolved NMR seemed to be not helpful.

I. 20'S-deoxyvinblastine (1)

Two methods to obtain the 20'S-deoxyvinblastine (1) were worked out. First the 20'S-deoxyvinblastine (1) was synthesized from catharanthine and vindoline following Potier[6].

The same 20'S-deoxyvinblastine (1) was also prepared from vinblastine, following a patent description[10], implicating that in this case we start with a product having the natural configuration. Other physical data point to the fact that after hydrogenation we have the 20'S isomer. The ^{1}H NMR of 20'S-deoxyvinblastine (1) was run and analyzed. We have gathered the data also in Table 1.

Table I : ^{1}H NMR data of 20' S-deoxy VLB (1), N'-2-borane complex of 20' S-deoxy-VLB (2) and VLB[2]

Chemical shifts	(1)	(2)	VLB		(1)	(2)	VLB
H-3A	3.37	3.39	3.37	H-3A'	3.18	3.59	3.39
H-3B	2.83	2.85	2.86	H-3B'	2.80	3.27	2.41
H-14	5.89	5.88	5.85	H-14'	1.12	1.47	0.85
H-15	5.26	5.27	5.30	H-15A'	2.06	2.10	1.48
H-5A	3.30	3.31	3.30	H-15B'	0.71	0.83	1.44
H-5B	2.44	2.49	2.47	H-17A'	3.23	3.21	4.00
H-6A	2.18	2.17	2.18	H-17B'	2.30	2.35	2.32
H-6B	1.80	1.75	1.85	H-20'	1.71	1.96	(-)
H-2	3.73	3.82	3.74	H-21A'	2.81	3.22	2.81
H-17	5.47	5.45	5.45	H-21B'	2.81	2.79	2.81
H-21	2.74	2.77	2.68	H-5A'	3.65	3.73	3.70
CH_2-19	1.78 1.23	1.79 1.36	1.80 1.32	H-5B'	3.15	3.10	3.12
				H-6A'	3.30	3.49	3.30
CH_3-18	0.90	0.91	0.88	H-6B'	2.90	3.10	3.12
H-9,-12	6.58; 6.12	6.47; 6.10	6.55; 6.10	CH_2-19'	1.34 1.27	1.42 1.20	1.34 1.37
OH	-	9.60	9.75	CH_3-18'	0.83	0.81	0.82
CH_3-N	2.75	2.82	2.81	H-9',-10'	7.43; ∿7.15	7.43; ∿7.15	7.52; ∿7.10
CH_3-OØ	3.58	3.62	3.62				
CH_3-OOC	3.80	3.85	3.80	-11',-12'	∿7.15; ∿7.15	∿7.15; ∿7.15	∿7.10; ∿7.10
CH_3-COO-	2.12	2.13	2.12				
				NH	7.95	8.00	8.50
				$COOCH_3$	3.80	3.85	3.80

Coupling constants

	(1)	(2)		(1)	(2)*
^3J(3A,14)	4.8	4.8	^3J(3A',14')		∿1
^3J(3B,14)	∿1.0	∿1.0	^3J(3B',14')		∿2
^3J(14,15)	10.0	10.0	^3J(3A',3B')		14.2
^2J(3A,3B)	14.2	14.0	^3J(14',15A')	∿2.0	∿3
^3J(5A,6A)	9.0	9.0	^3J(14',15B')	∿1.0	∿1.0
^3J(5A,6B)	4.0	4.0	^2J(15A',15B')	∿14.0	∿14.0
^2J(5A,5B)	11.0	11.0	^3J(15A',20')	∿7.0	∿7.0
^3J(5B,6A)	6.4	6.4	^3J(15B',20')	∿11.0	∿11.0
^3J(5B,6B)	10.0	10.0	^3J(20',21A')		∿2.0
^2J(6A,6B)	13.6	14.0	^3J(20',21B')		∿11.4
			^2J(21A',21B')		∿11.4
			^3J(14',17A')		12.0
			^3J(14',17B')	∿2.0	∿2.0
			^2J(17A',17B')	∿14.0	∿14.0
			^3J(18A',20')		∿6.4
			^3J(18B',20')		∿8.0
			^2J(18A',18B')		∿13.0
			^3J(5A',6A')	10.8	
			^3J(5A',6B')	∿1.0	
			^2J(5A',5B')	14.0	
			^3J(5B',6A')	∿1.0	
			^3J(5B',6B')	5.2	
			^2J(6A',6B')	14.8	

* The system H-5',-6' in 2 is degenerated but completely alike to that in VLB.

The broad triplet at δ 1.12 is ascribed to H-14'. Irradiation at δ 3.23 (ascribed to H-17A') and at δ 2.06 (ascribed to H-15A') reduces this pattern each time to a broad doublet. Thus ^3J(14',17A') and ^3J(14',15') can be extracted. Irradiation of this pattern shows 1) a sharpening at the triplet structure of δ 0.71 (ascribed to H-15B') 2) a sharpening of the doublet-like structure at δ 2.30 (ascribed to H-17B', this assignment is further verified by the fact that by irradiation at δ 3.23 ascribed to H-17A', this pattern becomes a broad singlet); 3) the pattern at δ 2.06 (ascribed to H-15A') is reduced from an octet to a quartet; 4) important other changes are observed in the region δ 3.20.

Very different compared to N-2' borane complex of 20'S-deoxyvinblastine (2) is the fact that the system for the H-5',-6' protons is degenerated. These protons show now patterns that are very similar to the corresponding protons in vinblastine in benzene-solution[2]. This is verified by following experiments : 1) irradiation at δ 2.90 causes changes at δ 3.15, and δ 3.65; 2) irradiation

at δ 3.15 causes a change at δ 3.30 and δ 2.90; 3) irradiation at δ 3.30 causes a change at δ 3.15 and δ 3.65; 4) irradiation at δ 3.65 causes a collapse at δ 2.90 and δ 3.30. Although the patterns are very close to those of vinblastine, the coupling constants could not be extracted with certainty.

It is very difficult to differentiate between the H-3' and H-21' protons. The couplings between H-3' and H-14' are expected to be very small as well as those between H-21' and H-20'. One pattern at δ 3.18 can be as well H-3A' or H-21A'. Irradiation at δ 1.71 (H-20') shows a change in the pattern at δ 2.81, but not at δ 3.18, which is an indication that H-3A' may be found at δ 3.18. Irradiation at δ 3.18 shows indeed a change at δ 2.81. Irradiation at δ 1.12 (H-14') shows changes at δ 3.18, which cannot be explained by changes of H-17A' only in this region. Therefore we assume that the pattern at δ 3.18 may be ascribed to H-3A'. Exact coupling constants could not be extracted. Even 2D δ-J-NMR allowed no further refinements in the present case.

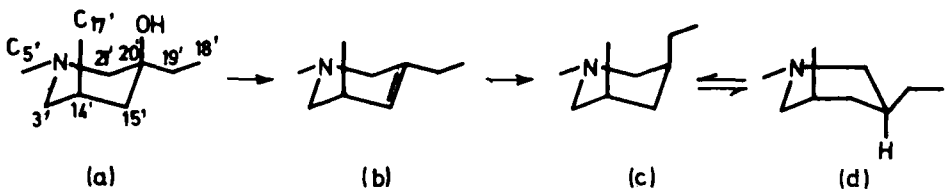


Fig. 2

For vinblastine we have accepted the chair form as shown in fig. 2(a), and this conformation is preferred probably by the occurrence of the hydrogen bridge between OH-20' and N-4' or between OH-20' and the ester group on C-16'[2].

For preparing the 20'S-deoxy derivative (1), first a precursor is obtained having a double bond between C-15' and C-20'. The hydrogenation of the double bond of this compound leads to the 20'S-deoxy derivative (1). As the reduction occurs along the less hindered side, the ethyl group on C-20' must be in a β-position. If a chair form is accepted, the ethyl group should here be syn-axial with the C(14')-C(17') bond as well as with the lobe of the free electrons on N-4'. Therefore it is quite acceptable that the piperidine ring accepts a twist-boat conformation.

Inspection of the vicinal coupling constants may point in this direction, although with a strained chair ambivalence is possible. Indeed the coupling constants between H-14' and H-3' as well as with H-15' remain very small. For the coupling constants between H-20' and H-21' or H-15' each time a large coupling is found, namely $^{3}J(15B',20')$ and $^{3}J(21B',20')$, and a small coupling constant. Such values can be easily rationalised if a twist-boat form is

accepted. The different conformations of the piperidine ring in the catharanthine moiety of vinblastine and 20'S-deoxyvinblastine are the reason why their corresponding chemical shifts cannot be compared.

II. N-2' borane complex of 20'S-deoxyvinblastine (2)

The N-2' borane complex of 20'S-deoxyvinblastine was synthesized from catharanthine and vindoline (modification of the Potier procedure). Under the methyl peaks CH_3-18 and CH_3-18', a triplet is found assigned to H-15B', from which we measure $^2J(15A',15B') \sim 14.0$ Hz and $^3J(15B',20') \sim 11.0$ Hz. Indeed, H-14' appears as a broad triplet and as will be scrutinized further, its larger coupling constants originate from H-17A' and H-15B', as is proven by irradiating these patterns. That means that $^3J(14',15'B)$ must be very small.

Because of their symmetry, it could be derived that the patterns at δ 1.20 and δ 1.42, as well as those at δ 1.79 and δ 1.36 belong respectively to the 19'- and 19-methylene group, as is verified by nmdr experiments on the methyl groups. The pattern of H-6B was extracted from the multiplet at δ 1.75, and allowed to calculate $^3J(6A,6B) = 4.0$ Hz, $^3J(5B,6B) = 10.0$ Hz and $^2J(6A,6B) = 14.0$ Hz, indeed the values found for this proton in the spectrum of vinblastine. From the multiplet at δ 2.15 a broad pattern was extracted, ascribed to H-15A', as well as the multiplet for H-5B, allowing to calculate $^3J(5A,6A) = 9.0$ Hz, $^3J(5B,6A) = 6.4$ Hz and $^2J(6A,6B) = 14.0$ Hz. These values were confirmed by inspection of the pattern of H-5B (isolated at δ 2.49).

In the complex pattern at δ 3.10-3.30 we first encounter the presence of two collapsing broad doublets, namely the degenerated A_2 part of the A_2XY spin system formed by H-5',-6', as well as H-21A'. The triplet at δ 3.23 is assigned to H-17A' from which we can extract $^3J(14',17A') = \sim 12$ Hz and $^2J(17A',17B') = \sim 14.0$ Hz.

At about δ 3.30 we find the expected pattern of H-5A and a broad doublet ascribed to H-3B'. At δ 3.49 we fond a quasi-triplet as usually found for H-6A' in vinblastine and the doublet for H-3A'. With 2D δ-J-resolved NMR it was possible to see and measure separate patterns as close as δ 3.391, δ 3.314 and δ 3.200.

Although it was not possible to extract all the coupling constants of 1, we have enough data to accept that the conformations of the azanonene ring system may have the same conformation. In such a case chemical shift differences may be diagnostic in order to discover the origins of the changes in both molecu-

les[11]. As the differences are most outspoken in H-5',-6',-21',-3',-14' and 17', one of the possible sources for the differences was thought to be an epimerization at C-16'. C.D.-experiments showed that this was excluded. With such a set of parameters, we know 1) all the protons and 2) their mutual dispositions. Therefore the only possible origin for the differences - which also explains the changes occurring in the NMR tube - is a complexation of N-2'.

Some ^{1}H NMR data of the epimers of vinblastine at C-16' and C-20' have been published separately by Potier[6] and Kutney[7]. The chemical shifts of Me-18 and Me-18' as well as H-9 and H-12 are expected to be diagnostic for these isomers. For the isomers with a natural configuration on C-16' the methyl groups are found at δ 0.85, where for the isomers with an unnatural configuration they are found at δ 0.64/0.66 and δ 0.39/0.91. The former is the case for the present compounds. Although the position of H-9 seems to be diagnostic for Kutney, as he finds it at δ 6.94/6.96 for the 16'R isomers, and at δ 6.38/6.61 for the 16'S isomers, Potier finds H-9 at δ 6.58 for the 16'R,20'S isomer.
For the two isomers with the natural configuration (16'S) Potier only provides the data for the 16'S,20'S epimer, while no discrepancy can be made between the isomers at C-20' with the data of Kutney. For the 20'S isomer Potier finds H-9 at 6.49, Kutney finds it at δ 6.61 and we find it at δ 6.58. So for the data as provided here, the chemical shifts of H-9 and H-12 are close to those of Kutney, and those of the methyl groups are close to the values provided by Potier. As the data afforded by the borane complex are in fair agreement with data provided by Kutney, this means that it is dangerous to discriminate between both compounds on basis of the most striking ^{1}H NMR data provided by Kutney and Potier, but a more general view is necessary.

EXPERIMENTAL

I. Synthesis of 20'S-deoxyvinblastine (1)

a) 15'20'-anhydrovinblastine

Method A[6]

Trifluoroacetic anhydride (0.41 ml, 2.9 mmole) was added at -20°C to a solution of catharanthine N_b-oxyde (450 mg, 1.27 mmole) and vindoline (500 mg, 1.09 mmoles) in CH_2Cl_2 (4 ml) under argon. After two hours at -20°C, the CH_2Cl_2 and the excess of anhydride were evaporated under reduced pressure at 10°C.
The residue was dissolved in methanol (15 ml) and $NaBH_4$ was added at 0°C (to

pH $\simeq$ 7).

After 30 minutes, the reaction mixture was poured into brine, made alkaline and extracted by CH_2Cl_2.

After usual work-up and crystallization (methanol) of the product mixture, 15' 20'-anhydrovinblastine (260 mg, 30 %) was obtained.

IR ($CHCl_3$) : 2985, 1741, 1610, 1500 cm^{-1}.

Mass (chemical ionization with isobutane as reactant gas) : 793 (M^+).

UV (CH_3OH) : 217, 263, 289 nm.

Method B[9]

Thionyl chloride (1.2 ml, 16 mmoles) was added at -10°C to a stirred solution of the sulphate of natural vinblastine (600 mg, 0.66 mole) in dry dimethylformamide (8 ml). The resulting mixture was stirred for 2 hours at room temperature. The mixture was then poured into a saturated aqueous solution of NaCl (200 ml), made alkaline with NH_4OH, extracted four times with benzene, washed with water.

After usual work-up, 15' 20'-anhydrovinblastine is isolated (284 mg, 54 %) identical with the compound obtained by the first method.

b) 20' S-deoxyvinblastine

The hydrogenation of a solution of 15' 20'-anhydrovinblastine (300 mg, 0.37 mmoles) in ethanol (45 ml) with PtO_2 (60 mg) at atmospheric pressure was stopped after 5 hours.

Usual work-up lead to a residue purified by preparative TLC. (AcOEt-EtOH, 1-1), yielding 20' S-deoxyvinblastine (153 mg, 52 %).

mp : 214°C (CH_3OH).

IR : ($CHCl_3$) : 2995, 1741, 1616 cm^{-1}.

$|\alpha|_D$: +67.5° (c = 0.21) ($CHCl_3$).

UV (CH_3OH) : 218, 264, 288 mm; CD : 257 (+), 302 (+) nm.

Mass (chemical ionization with isobutane as reactant gas) : 795 (M^+), 810 (M^+ + 15).

II. Synthesis of N-2' borane complex of 20' S-deoxyvinblastine (2)

a) 15' 20'-anhydrovinblastine

Trifluoroacetic anhydride (0.88 ml, 6.23 mmole) was added at -70°C to a stirred solution of catharanthine N_b-oxyde (900 mg, 2.55 mmole) and vindoline (1 g, 2.19 mmole) in CH_2Cl_2 (6 ml) under argon.

After 1 hour at -78°C, the CH_2Cl_2 and the excess of anhydride were evaporated under reduced pressure at 10°C.

The residue was dissolved in dry methanol (30 ml) and excess $NaBH_4$ was added at 0°C (pH > 9).

After 30 minutes, the reaction mixture was poured into a saturated aqueous solution of NaCl (50 ml), extracted three times with CH_2Cl_2 and washed with water. After drying with sodium sulphate and filtration the CH_2Cl_2 extract was evaporated under reduced pressure. After purification of the product mixture by crystallization (ethanol) a 50 % yield of 15' 20'-anhydrovinblastine (875 mg) is obtained.

mp : 216°C (ethanol).

IR ($CHCl_3$) : 2990, 2350, 1740, 1611, 1500, 1459, 1245, 1040 cm^{-1}.

Mass (chemical ionization with isobutane as reactant gas) 793 (M^+).

UV (EtOH) : 219 (4.33), 261 (3.74), 288 (3.60) nm.

b) 20' S-deoxyvinblastine (borane complex)

Hydrogenation of 250 mg of 15' 20'-anhydrovinblastine in CH_3OH (PtO_2, 50 mg) lead after usual work-up and purification by preparative TLC 175 mg of the borane complex of 20' S-deoxyvinblastine (70 %).

$[\alpha]_D$ = +50°C (c = 0.179) ($CHCl_3$).

mp : 224°C.

UV (CH_3OH) : 216, 265, 289 nm; CD : 257 (+), 302 (+) nm.

IR ($CHCl_3$) : 2990, 2380, 1740, 1614 cm^{-1}.

Mass (chemical ionization with isobutane as reactant gas) : 795 (M^+), 810 (M^+ + 15).

The ^1H NMR spectra were run on a Bruker WH 360 spectrometer, at 18°C for 2 % solutions (F.T.-mode, pulse width 2 µsec, quadrature detection, resolution : 0.208 Hz/Point).

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