Stereochemistry of (−)-Virantmycin

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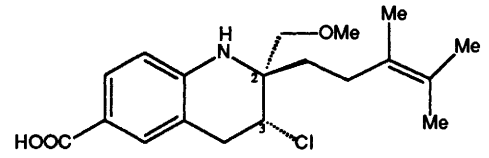
The stereochemistry of the antiviral metabolite (−)-virantmycin has been determined by NOE difference spectroscopy to be 2*R*,3*R*. This reverses an earlier stereochemical proposal for C-2. NOEs from a slowly exchanging hydroxy proton in a synthetic intermediate provided the key stereochemical information.

The unusual metabolite (−)-virantmycin (**1**) isolated by Japanese workers,[1] from *Streptomyces nitrosporeus* has been found to possess potent antiviral activity. Its gross structure was elucidated[2] mainly by NMR studies but the relative and absolute stereochemistry at the two chiral centres (C-2 and C-3), have remained unknown. No derivatives suitable for *X*-ray examination have yet been found. We report here the relative stereochemical assignment of these two centres as determined using NOE difference experiments.



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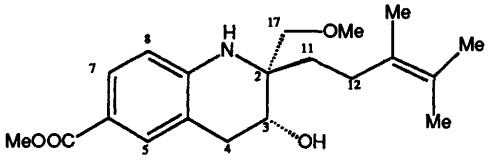

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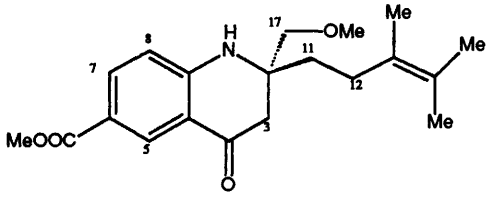

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The total synthesis of racemic virantmycin from *p*-aminobenzoic acid has been achieved in Cambridge[3] but did not resolve the stereochemical ambiguity. Very recently a chiral synthesis of the enantiomer, (+)-virantmycin, has been achieved in Sapporo,[4] leading to a suggestion for the relative and absolute stereochemistry of the metabolite. An intermediate from the Cambridge synthesis, the racemic amino alcohol ±-(**2**), has formed the basis of this present study because of its more plentiful supply. This material was shown[3] to be a single diastereoisomer and, on treatment with thionyl chloride, followed by hydrolysis with lithium hydroxide, gave, by a double inversion process involving an intermediate aziridine (*i.e.* an overall retention), a racemic chloro acid identical in all chromatographic and spectroscopic respects with natural virantmycin.

Another intermediate from the total racemic synthesis, the bicyclic ketone (**3**), was also examined in preliminary NOE difference experiments to establish the existence of NOEs at both the 3_{ax} and 3_{eq} positions.

Details of the NOE difference experiments performed with these two compounds are given below, along with observations and conclusions drawn from the spectra obtained.

Results

The assigned chemical shifts and couplings constants for both (**2**) and (**3**) are given in Tables 1 and 3. These assignments were straightforward, or result from observed NOEs. The stereochemically significant NOEs observed for (**2**) and (**3**) are shown in Tables 2 and 4.

The experiments with the bicyclic ketone (**3**) focussed on the doublet signals observed for 3_{eq}-H and 3_{ax}-H. These are very strongly coupled, forming an AB quartet. At this stage we did not attempt to determine which doublet was which. It later became clear in the light of NOEs observed in compound (**2**) that the more shielded doublet results from 3_{ax}-H and the less shielded doublet from 3_{eq}-H. However, the primary aim of the NOE experiment with compound (**3**) was to determine if NOEs existed between 11-H, 12-H, and 17-H and the protons at C-3. This was indeed the case, strong positive enhancements being observed both to and from 3_{ax}-H and 3_{eq}-H: separate irradiation of 11-H and 12-H primarily gave enhancements to the less shielded doublet side of the $3_{ax}/3_{eq}$ multiplet, and irradiation of 17-H (double doublet) essentially enhanced the more shielded doublet. Interestingly, the $3_{ax}/3_{eq}$ multiplet showed the effect of strong coupling in these NOE experiments, essentially appearing as three peaks when 11-H, 12-H, or 17-H were irradiated. Close inspection showed the multiplet to be composed of the less shielded doublet and one peak of the other doublet when 11-H and 12-H were irradiated, and the more shielded doublet and one peak of the other doublet when 17-H was irradiated. This effect is rarely observed but it is a well understood consequence of strong coupling.[5] It was difficult to be selective when irradiating the $3_{ax}/3_{eq}$ multiplet but it appeared that the more shielded doublet gave a large NOE to 11-H and 12-H.

Inspection of the ^{1}H spectrum of (**2**) suggested, by virtue of its measured coupling constants, that 3-H was equatorial. The NOE experiments summarised in Figure 1 confirmed this and also mapped the stereochemistry around the other chiral centre at C-2. Here, as with (**3**), the key enhancements were expected to and from 3-H and 3-OH. The observation that 3-H was equatorial was confirmed by essentially equal enhancements both to and from both 4-H resonances. Consistent with our

Table 1. ^{1}H Chemical shifts and couplings for compound (3) in $CDCl_3$ solution.

Proton(s)	Chemical shift (ppm)	Multiplicity	Coupling (*J*/Hz)
15, 18, 19	1.58	br s	
11	1.68	m	
12	2.00	t	8.4
3_{ax}	2.56	d	16.2
3_{eq}	2.67	d	16.2
OMe	3.33	s	
17	3.34	d	9.3
17′	3.39	d	9.3
CO_2Me	3.83	s	
NH	4.96	br s	
8	6.61	d	8.9
7	7.89	dd	8.9, 2.1
5	8.44	d	2.1

Table 2. Nuclear Overhauser enhancements in compound (3).[a]

Proton(s) irradiated	Proton(s) observed						
	11	12	3_{ax}	3_{eq}	17	17′	8
11		s	m	s	w	w	
12	s		m	s	m	m	
3_{ax}	w	w			m	m	
3_{eq}	m	m			w	w	
17[b]	w		s	m			
17′[b]	w		s	m			
NH	w		w		w	w	s

[a] Enhancements are positive and are subjectively listed as s = strong; m = medium; w = weak. [b] Irradiated together.

experience gained from (3), strong enhancements were given from 3-H to the 12-H protons and the non-equivalent 17-H protons. Subjectively, the enhancement was larger to 12-H but we did not feel this to be sound evidence of the stereochemistry at C-2. However, when 3-OH was irradiated, strong enhancement was given to both 17-H resonances and essentially none to 12-H. Importantly, only one of the 4-H resonances was enhanced when 3-OH was irradiated, indicating this to be 4_{eq}-H. These observations on the 'bottom' face of the molecule are supported by those on the 'top' face, particularly the mutual NOEs between 12-H and the other 4-H which must, therefore, be axial as shown in Figure 1. This assignment of the H_4 protons is confirmed by the larger NOE given to the aromatic 5-H proton when 4_{eq}-H is irradiated (Figure 2).

These results enable us to define the relative stereochemistry of (±)-(2) as shown in Figure 1, with C-2 having the alkene side chain axial and the methoxymethyl group equatorial. This arrangement can be related directly to the stereochemistry of natural virantmycin, which is conclusively determined to be as shown in (1).

Discussion

The above stereochemistry is different from that proposed in the recent chiral synthesis achieved by Morimoto, Oda, Shirahama, Matsumoto, and Omura.[4] They agree that the 3-OH is axial but have the positions of the alkenyl side-chain and the methoxymethyl group reversed. Their proposal is based on NOE evidence to and from the 4-H resonances: like us they have determined that the alkenyl side-chain is on the same side as the 4-H signal at 3.08 ppm; and that the methoxymethyl group is on the same side as the 4-H signal at 2.82 ppm. However, they

Table 3. ^{1}H Chemical shifts and couplings for compound (2) in $CDCl_3$ solution.

Proton(s)	Chemical shift (ppm)	Multiplicity	Couplings (*J*/Hz)
11	1.54	m	
15, 18, 19	1.59	br s	
11′	1.78	ddd	13.8, 10.6, 6.4
12	2.05	m	
OH	2.53	d	8.8
4_{eq}	2.82	dd	16.6, 5.8
4_{ax}	3.08	dd	16.6, 4.3
OMe	3.38	s	
17	3.46	d	9.6
17′	3.64	d	9.6
CO_2Me	3.82	s	
3	3.95	m	
NH	4.35	br s	
8	6.47	d	8.5
7	7.67	dd	8.5, 1.9
5	7.70	d	1.9

Table 4. Nuclear Overhauser enhancements in compound (2).[a]

Proton(s) irradiated	Proton(s) observed										
	11	12	OH	4_{eq}	4_{ax}	17	17′	3	NH	8	5
11		s			w	w	w	w	w		
12	s				w	m	m	s	w		
OH				m		s	m	s	ST	s	
4_{eq}					s			m			m
4_{ax}	w	w		s				m			w
17			m				s		m		
17′	w	w	w	w		s		w	w		
3	w	s	s	s	s	w	m				
NH			ST			w				s	

[a] Abbreviations as for Table 3; ST = saturation transfer by chemical exchange.

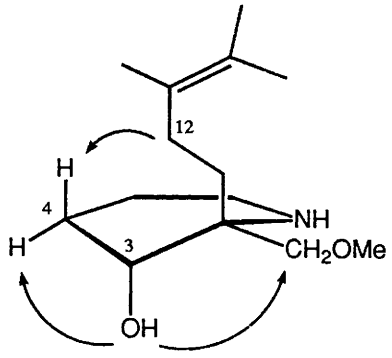

Figure 1. Conformation and some NOEs in (±)-(2).

assign the 3.08 ppm signal as the equatorial proton and the 2.82 ppm signal as the axial; we reverse the assignment. If we ignore any NOE information from the hydroxy proton, the relative configuration of C-2 and C-3 depend on the assignment of the 4-H protons. It would appear the basis for the assignment made by Morimoto *et al.* is the small chemical-shift difference between the 4-H protons. The hydroxy group and the alkenyl side-chain will undoubtedly have large influences on the chemical shift of these two protons, making any assignment based solely on chemical shift very tenuous. Our assignment of the 4-H protons is based on two independent strands of NOE evidence. The observation of an NOE from 3-OH to 4-H at 2.82 ppm but not

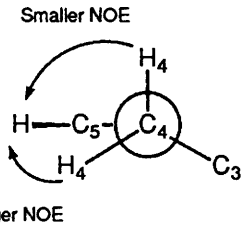

Figure 2. NOEs from C-4 protons to 5-H.

to 4-H at 3.08 ppm unambiguously assigns the former as 4_{eq}-H. In addition, the NOE from this resonance to the aromatic 5-H is significantly larger than the NOE from the resonance at 3.08 ppm. This is independent confirmation that our assignment is correct since the equatorial proton will be closer than the axial proton to 5-H (Figure 2). With the 4-H assignments established, there can be only one interpretation of the remaining NOE evidence and the same conclusion is drawn from the Japanese evidence if their 4-H assignments are reversed.

The chiral synthesis employed by the Japanese workers to generate the asymmetry at C-2 utilised a stereoselective Sharpless epoxidation of an allylic alcohol. From all precedents this reaction should, under the conditions used, produce the final relative stereochemistry we have deduced.[6] We feel this also lends support to our conclusions, as a reversal of the expected stereochemistry from the Sharpless epoxidation is unlikely. We certainly accept the Sapporo group's determination of the absolute stereochemistry at C-3 as 3*R*, and therefore conclude that the absolute configuration of natural (−)-virantmycin is 2*R*,3*R*.

Many chemists feel that hydroxy resonances contain little useful structural information. However, from the point of view of structure determination in general, these results provide further evidence that slowly-exchanging hydroxy groups are strategically valuable 'entry points'.[7] There are many published examples of the use of NOEs and couplings from such hydroxy groups;[8] in some cases the exchange is inhibited by intramolecular hydrogen-bonding, while in others it has to be slowed by careful sample preparation.[9] In either case, slow hydroxy exchange is worth encouraging and exploiting.

Experimental

A Bruker WM 300 instrument operating at 300 MHz was used to obtain the NOE difference spectra. These were acquired with 16 K data points over 2 450 Hz using a standard pulse sequence: an irradiation time of 4 s with no relaxation delay was used. 1 Hz exponential line broadening was applied to Fourier transformation. Spectra were referenced to the solvent, $CDCl_3$ (7.24 ppm).

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