THE STRUCTURE OF NOCAMYCIN, A NEW ANTITUMOR ANTIBIOTIC

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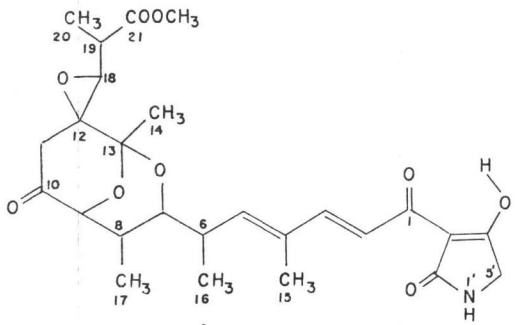
The structure of nocamycin, a new antitumor antibiotic, has been elucidated with the aid of mass- and PMR-spectroscopic investigation of the antibiotic and its various chemical transformation products. Nocamycin is structurally related to tirandamycins.

Recently the isolation and physico-chemical characteristics of nocamycin have been reported[1,2].

In this paper we report on studies dealing with the structural elucidation of nocamycin, which has resulted in the assignment of structure **1** to this new antibiotic.



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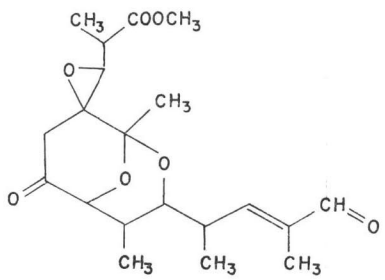

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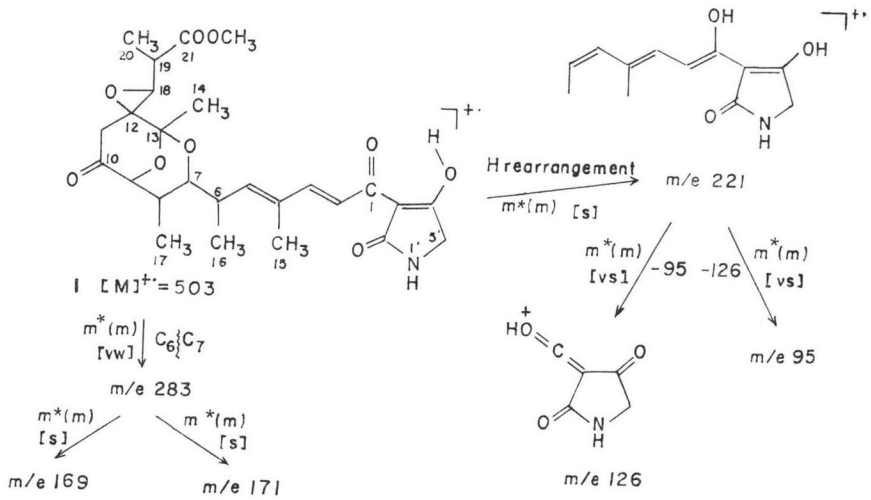
The mass spectrum of nocamycin showed a molecular ion at 503.21607 (calcd. for $C_{26}H_{33}NO_9$ 503.21553). The characteristic fragment ions of nocamycin are depicted in Scheme 1; the respective mass spectral data are given in Table 1.

Ion *m/e* 221 which gives rise to the base peak in the 70 and 12.5 eV mass spectra of **1** is formed by the cleavage of the C-6–C-7 bond, preceded by a hydrogen rearrangement to the charged portion. The same ion has also been observed in the mass spectra of tirandamycins A and B[3]. Further fragmentation of this odd-electron ion results in the formation of ion *m/e* 126 representing the tetramic acid moiety as well as in that of ion *m/e* 95 representing its hydrocarbon part. Ion *m/e* 126 has also been reported in the case of tirandamycin A[4]. Direct cleavage of the C-6–C-7 bond gives rise to ion *m/e* 283 in the case of **1**. The analogous fragmentation process has been reported in the case of tirandamycins A and B[3,4] and of methyl streptolate[5] but not observed in the spectra of streptolydigin[5]. The further fragmentation of ion *m/e* 283 results in two abundant fragment ions at *m/e* 171 and 169, respectively. Both of these ions contain the 1-carbomethoxyethyl side chain.

Scheme 1.



The symbol m*(m) at the arrows indicates that the respective metastable transitions have been measured by using accelerating voltage scans. The intensity of the observed metastable peaks are denoted by abbreviations vw (very weak), s (strong) and vs (very strong).

Table 1. Characteristic mass spectral data of compounds **1** and **2.**

Compound	*m/e*	Relative intensity[a] (in %)		Mass decimals		Elemental compositions	$\lvert\Delta\rvert$[c] (ppm)
		A	B	measured[b]	calculated		
1	503	1.2	3	0.21607	0.21553	$C_{26}H_{33}NO_9$	1.1
	283	32	25	0.11652	0.11817	$C_{14}H_{19}O_6$	5.8
	221	100	100	0.10433	0.10519	$C_{12}H_{15}NO_3$	3.8
	171	12	1.5	0.06486	0.06574	$C_8H_{11}O_4$	5.1
	169	48	9	0.08600	0.08647	$C_9H_{13}O_3$	2.7
	126	18	0	0.02005	0.01912	$C_5H_4NO_3$	7.4
	95	6	0.2	0.08682	0.08608	C_7H_{11}	7.8
2	380	16		0.18594	0.18351	$C_{20}H_{28}O_7$	6.4
	283	100					
	171	11		0.06613	0.06574	$C_8H_{11}O_4$	2.3
	169	33		0.08600	0.08647	$C_9H_{13}O_3$	2.7
	98	21		0.07237	0.07317	$C_6H_{10}O$	8.1

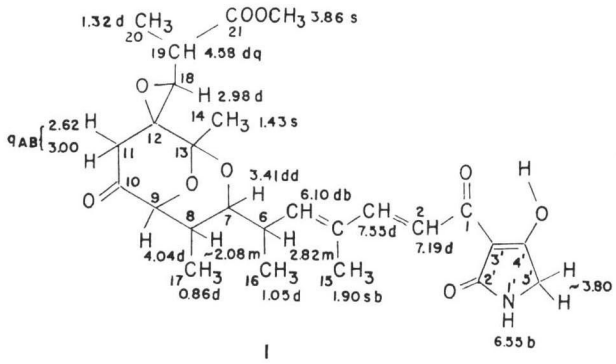
a) Mass spectra were taken on a Varian MAT SM-1 instrument under the following operating conditions: resolution, 1250; accelerating voltage, 8 kV; electron energy, 70 eV (A) vs 12.5 eV (B); electron current, 300 μA; source temperature, 250°C (A) vs 150°C (B); evaporation temperatures, **1**: 175°C (A) vs 180°C (B), **2**: 100°C.

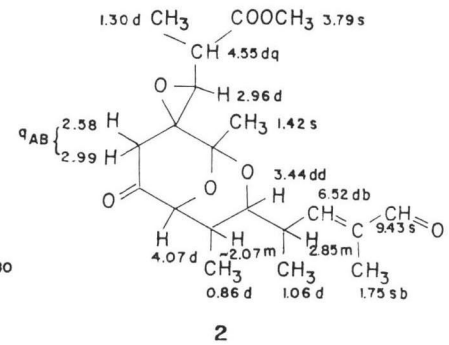
b) High resolution mass measurements were performed at a resolution of 10,000 (10% valley), using PFK as the reference standard.

c) Accuracy of mass measurements: $|\Delta| = \frac{|m_{measured} - m_{calculated}|}{m}$

The mass spectrum of the compound formed by the hydrolysis of nocamycin with 0.1 N NaOH[2] showed a molecular ion at 489. In accordance with the fragmentation mechanism depicted in Scheme 1 ions *m/e* 221, 126 and 95 remain at the same *m/e* values in this spectrum while ions *m/e* 283,

Fig. 1. a) Assignments of the 250 MHz ^{1}H-NMR data of compound **1**. Spectrum was taken in $CDCl_3$ on a Cameca instrument.
b) Assignments of the 100 MHz ^{1}H-NMR data of compound **2**. Spectrum was taken in $CDCl_3$ on a Varian XL-100 instrument.
Chemical shifts are given in δ (ppm) using TMS as the internal standard.
Abbreviations of multiplicities: s=singlet, d=doublet, q=quartet, m=multiplet, b=broad.





171 and 169 are shifted to *m/e* 269, 157 and 155, respectively, indicating the transformation of the carbomethoxyl into a carboxyl group upon hydrolysis of nocamycin.

Compound **2** (m.p. 97~98°C; yield: 20% of theor.) was isolated from products formed upon oxidation of **1** with $KMnO_4$ in acetone - chloroform. In accordance with its structure, the UV spectrum of **2** (in 95% ethanol) showed a single maximum at λ_{max} 226.5 nm (ε_{max} 16,600). The mass spectrum of **2** showed a molecular ion at 380.18594 (calcd. for $C_{20}H_{28}O_7$ 380.18351). When compared with the mass spectral behavior of **1**, the formation of ion *m/e* 283 becomes more favored in the case of **2** and gives rise to the base peak. Ions *m/e* 171 and 169 are also prominent. The fragmentation pathway leading to ion *m/e* 221 in the case of **1** results in the formation of ion *m/e* 98 in the spectrum of **2**. The mass spectral data of the above discussed ions of compound **2** are given in Table 1.

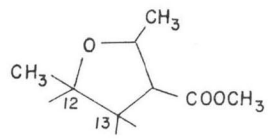
The 250 MHz ^{1}H-NMR spectrum of nocamycin has been published[2]. The assignment of the signals of the ^{1}H-NMR spectrum of **1** is shown in Fig. 1. The assignment has greatly been facilitated by the similarity of parts of the NMR spectrum of **1** to those of streptolic acid[6] and tirandamycin A[7] whose NMR spectra have been discussed in detail[4]. The similarity of the structural parts of nocamycin and tirandamycin A (and tirandamycic acid) is reflected in similarity of the chemical shift and coupling constant data of the protons on carbons 2 to 9 and 15 to 17 of **1** and of the analogous protons of tirandamycin A (and tirandamycic acid)[4]. The signals of the protons on C-5′ of **1** and tirandamycin A[4] also appear at similar chemical shifts although in the case of **1** this signal coincides with the singlet of the protons of the $COOCH_3$ group. The coupling constant $J_{2,3}=16$ Hz indicates a *trans* double bond between C-2 and C-3. The identical spectral pattern discussed above has been confirmed in spin decoupling experiments, irradiating at the resonance frequencies of the protons on carbons 5 to 9 of **1**, *i.e.*, at $\delta\delta$ 6.10, 2.82, 3.42, 2.08, and 4.04, respectively. As regards signals of the remaining protons in the NMR spectrum of **1**, the proton at C-18 appears as a doublet at δ 2.98 one line of which coincides with one of the lines of the AB quartet ($J_{AB}=17.2$ Hz) of the C-11 protons. This indicates one vicinal proton at C-19, whose pentet-like signal appears at δ 4.58. The doublet of the C-20 methyl protons appears at δ 1.32, the singlet of the C-14 methyl protons at δ 1.43.

Data of the 100 MHz ^{1}H-NMR spectrum of compound **2** given in Fig. 1, further confirm the structure assigned to nocamycin. The signals of the tetramic acid moiety and of the olefinic protons at C-2 and C-3 are, of course, absent and the singlet of an aldehyde proton appears at δ 9.43. Due to the presence of the new aldehyde group the signals of the C-5 olefinic and the C-15 methyl protons are shifted downfield and upfield, respectively. The other signals of **2** have similar chemical shifts and coupling parameters to the respective moiety of nocamycin. Nevertheless, the coupling characteristics of the proton at C-19 are better seen in this spectrum, namely, this signal is split into a doublet with $J_{18,19}=8.2$ Hz, both lines of which are further split into a quartet with $J_{19,20}=6.0$ Hz. This observation together with the δ 4.55 value of the chemical shift of this signal further confirm the structure of the C-19–C-21 side chain of nocamycin.

The absolute stereochemical assignment of tirandamycin A and streptolydigin have been reported[8]. Based upon the high similarity of NMR data it can be assumed that the stereochemistry of nocamycin is the same as that of tirandamycin A as regards portions C-1 to C-10 and C-13 to C-17.

Note added in proof:

After this paper had been submitted for publication a Dutch patent (No. 7807570) was published which describes a compounds (Bu 2313B) whose physico-chemical data seem to be similar to those of nocamycin. Although not proven, a structure is proposed in that patent for Bu 2313B which differs from that of nocamycin, suggested here in positions C-12–C-14 and C-18–C-21. The structural part proposed there may also fit to our data presented here.



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