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 reported1,2). 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.

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 B3). 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 A4). 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 B3,4) and of methyl streptolate5) but not observed in the spectra of streptolydigin5). 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*| Mass decimals | Elemental compositions | \(|A|^{(c)}\) (ppm) |
|----------|-----|-------------------|---------------|-----------------------|-----------------|
|          |     | (in %)            | measured\(^{b)}\) | calculated            |                 |
| 1        | 503 | 1.2              | 3             | 0.21607               | 0.21553         | 1.1            |
|          | 283 | 32               | 25            | 0.11652               | 0.11817         | 5.8            |
|          | 221 | 100              | 100           | 0.10433               | 0.10519         | 3.8            |
|          | 171 | 12               | 1.5           | 0.06486               | 0.06574         | 5.1            |
|          | 169 | 48               | 9             | 0.08600               | 0.08647         | 2.7            |
|          | 126 | 18               | 0             | 0.02005               | 0.01912         | 7.4            |
|          | 95  | 6                | 0.2           | 0.08682               | 0.08608         | 7.8            |
| 2        | 380 | 16               |               | 0.18594               | 0.18351         | 6.4            |
|          | 283 | 100              |               |                       |                 |                |
|          | 171 | 11               |               | 0.06613               | 0.06574         | 2.3            |
|          | 169 | 33               |               | 0.08600               | 0.08647         | 2.7            |
|          | 98  | 21               |               | 0.07237               | 0.07317         | 8.1            |

* 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: \(|A| = \frac{|m_{\text{measured}} - m_{\text{calculated}}|}{m}\)

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 $\delta$ (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 $\lambda_{\text{max}}$ 226.5 nm ($e_{\text{max}}$ 16,600). The mass spectrum of 2 showed a molecular ion at 380.18594 (calcd. for C$_{20}$H$_{23}$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$^{15}$. 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$^{16}$ and tirandamycin A$^{17}$ whose NMR spectra have been discussed in detail$^{18}$. 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 $\delta$ 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 $\delta$ 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 $\delta$ 4.58. The doublet of the C-20 methyl protons appears at $\delta$ 1.32, the singlet of the C-14 methyl protons at $\delta$ 1.43.
Data of the 100 MHz ¹H-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 J₁₈,₁₉ = 8.2 Hz, both lines of which are further split into a quartet with J₁₉,₂₀ = 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. 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 compound (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.

References