CHEMISTRY OF BLEOMYCIN. XXIII NATURAL ABUNDANCE ^{15}N-NMR SPECTROSCOPIC EVIDENCE FOR THE STRUCTURE OF BLEOMYCIN

Sir:

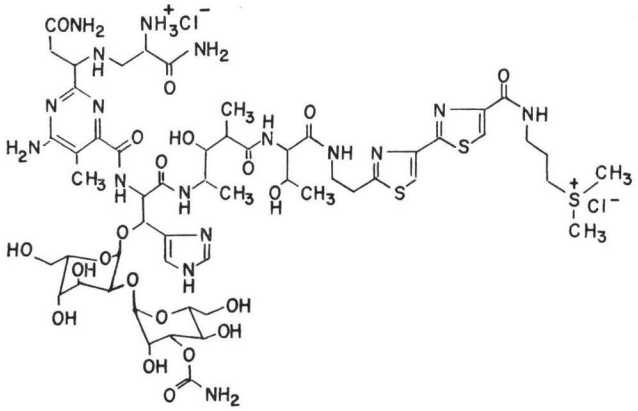
The revised structure[1] of bleomycin (BLM), which was recently proposed by chemical reinvestigation initiated by X-ray crystallographic study of a biosynthetic intermediate[2], contains one more nitrogen atom than the previous structure[3]. The new structure is formally the ammonolysis product at the β-lactam of the previous one. Therefore, the direct observation of the nitrogen atoms in BLM by natural abundance ^{15}N-NMR spectroscopy should give strong evidence for the new structure.

The natural abundance ^{15}N-NMR spectra of metal-free BLM A2 chloride hydrochloride (Fig. 1) were recorded at 36.48 MHz and 26°C with a Bruker WH-360 Fourier transform NMR spectrometer using 1 g of the substance in 5 ml of CH_3OH - CD_3OD (4: 1) and 15 mm diameter sample tube. Two spectra were taken under the following conditions:

A: ^{1}H-decoupled but with the nuclear OVERHAUSER effect(NOE) suppressed by inverse-gated decoupling; pulse width 40 μsec. (*ca.* 60° flip angle), aquisition time 0.54 sec., pulse delay 4.0 sec., spectral width 15 KHz, 23,000 scans (Fig. 2A and 3A).

B: ^{1}H-coupled. pulse width 20 μsec., aquisition time 0.64 sec., repetition rate 1.0 sec., 328,000 scans (Fig. 2B and 3B).

Fig. 1. The structure of metal-free bleomycin A2 chloride hydrochloride.



^{15}N-NMR chemical shifts for BLM A2 and tentative assignments on the basis of chemical shift values in the literature[4~6] and signal multiplicities are shown in Table 1. The imidazole nitrogens showed an unresolved broad peak in the ^{1}H-coupled spectrum where no partial NOE effect is possible. This signal was suppressed by a partial negative NOE in the ^{1}H inverse-gated decoupled spectrum (signal nulling). The two highest field singlet signals, which are not ^{1}H-coupled due to fast proton exchange, should be the primary and secondary amine functions. In an attempted off-resonance continuous decoupling experiment, only 3 nitrogens showed negative signals: −259.9 and −339.8 ppm, weak negative NOE; −341.2 ppm, strong negative NOE. It is typical for molecules of this molecular weight class that at 36 MHz a partial NOE causes signal-nulling for backbone NH's. Internal mobility will then cause a large negative NOE for those mobile nitrogens. Therefore, the peak at −341.2 ppm is suspected to be assigned to the protonated primary amine, −339.9 ppm to the secondary amine, and −259.9 ppm to the most mobile secondary amide, which should be the C-terminal amide function inferred from a ^{13}C spin-lattice relaxation time study of BLM[7]. A more detailed assignment for other secondary amides has not yet been pursued, but it will be possible

Table 1. ^{15}N-NMR chemical shifts for bleomycin A2 and tentative assignments.

Chemical shift[a]	Assignment
−64.9 (s), −73.9 (s)[b]	thiazole
−134.0 (s), −134.2 (s)	pyrimidine
−202.5 (broad) (×2)	imidazole
−244.5 (d), −259.5 (d) −259.9 (d)[c], −260.8 (d) −261.9 (d)	sec. amide
−264.9(t), −271.0 (t)	prim. amide
−292.9 (t)	pyrimidine-NH_2
−302.0 (t)	O-carbamoyl
−339.8 (s)	sec. amine
−341.2 (s)	prim. amine

[a] ppm relative to external NO_3^-.
[b] Multiplicities in parentheses.
[c] The C-terminal amide connected to the bithiazole carboxylic acid.

Fig. 2. ^{15}N-NMR spectra of bleomycin A2.

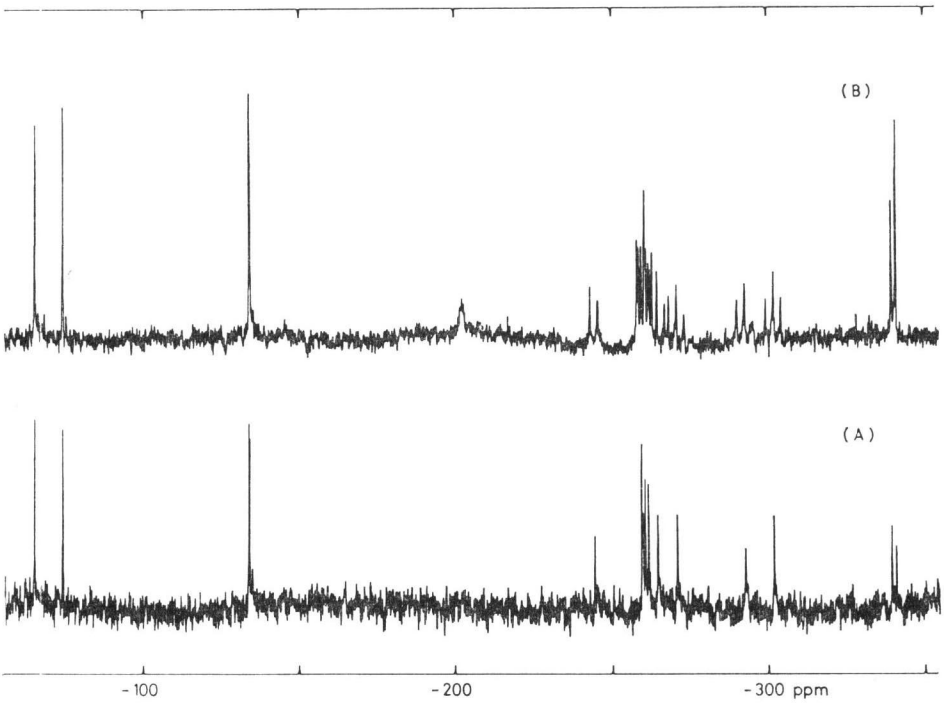
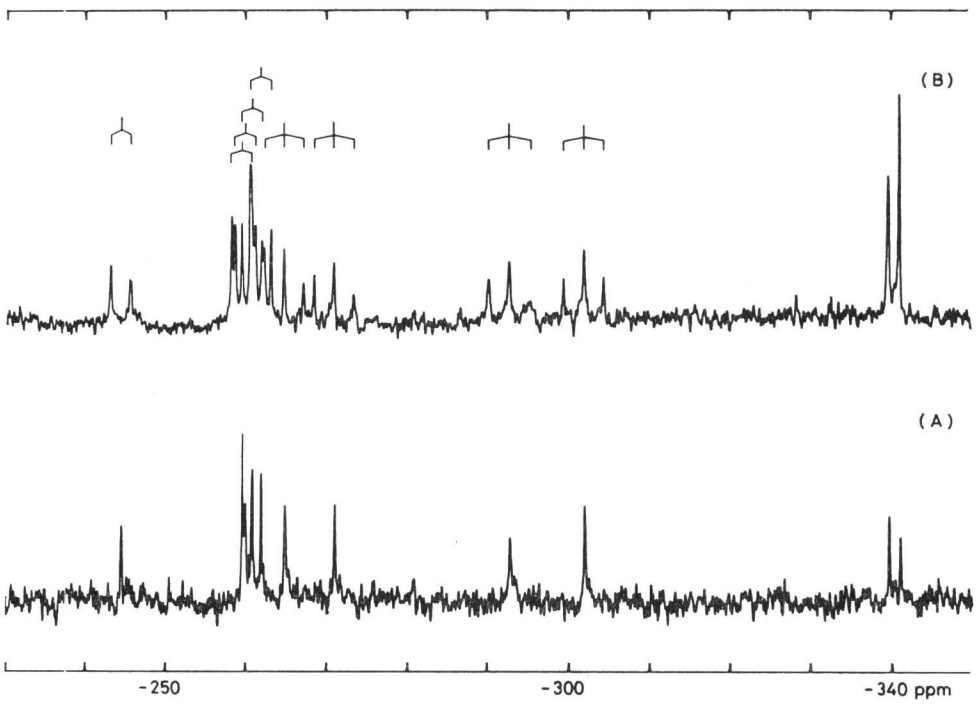


Fig. 3. A part of ^{15}N-NMR spectra of bleomycin A2.



by selective low power ^{1}H-decoupling experiments.

The most important information obtained by this ^{15}N-NMR spectroscopic study for the structure of BLM is: the total number of the nitrogen atoms in BLM A2 is seventeen and the number of the primary amide nitrogens is two. This is the strongest and direct evidence for the validity of the revised structure of BLM. It indicates that natural abundance ^{15}N-NMR spectroscopy of complex molecules such as bleomycin only recently feasible by instrumental improvement is an extremely important tool for the structure elucidation of complex molecules of biological interests.

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