Studies on Antibiotics BN-227 and BN-227-F, New Antibiotics

II. Chemical Structure of Antibiotics BN-227 and BN-227-F

Jiro Itoh, Shoichi Amano, Yasuaki Ogawa, Yoshio Kodama, Norio Ezaki and Yujiro Yamada

Central Research Laboratories, Meiji Seika Kaisha, Ltd., Morooka-cho, Kohoku-ku, Yokohama, Japan

(Received for publication December 17, 1979)

The structure of a new antibiotic BN-227 was determined as I by physico-chemical methods. The X-ray analysis also suggested the structure of I. The structure of antibiotic BN-227-F was determined as II. The antibiotic BN-227-F was prepared by addition of a solution of ferric chloride to antibiotic BN-227.

The production, isolation and characterization of antibiotic BN-227 and BN-227-F have been previously described.

This paper reports the structure determination of these antibiotics.

Structure of Antibiotic BN-227

The molecular formula of antibiotic BN-227 was established as C_{7}H_{9}NO_{3} from the results of elemental analysis and mass spectrometry (m/e 155, M^{+}) as reported in the previous paper. The IR spectrum showed the presence of hydroxy group (3470 cm⁻¹) and carbonyl group (1650 cm⁻¹).

Fig. 1 shows the mass spectrum of antibiotic BN-227 (I). Important ions were observed at m/e 155 (M^{+}), 140 (M^{+}—CH₃) and 124 (M^{+}—OCH₃). The NMR spectrum of I is shown in Fig. 2. A singlet at 9.70 ppm was assigned to a hydroxy proton, two doublets at 7.20 ppm and 6.53 ppm were assigned to olefinic protons. Singlets at 3.73 ppm and 2.43 ppm were assigned to methoxy and methyl protons.

The antibiotic BN-227 formed a typical 3:1 complex with Fe^{3+} ion as afterwards described. The results suggest that the antibiotic BN-227 is a hydroxamic acid. Many of natural products which contain a hydroxamic acid have been found mainly from microbial sources. Thus, the structure of antibiotic BN-227 was deduced as I and confirmed by the chemical modification studies summarized in Chart 1.
Catalytic reduction\(^{5,6}\) of 1 with platinum oxide gave, after absorption of 2 moles of hydrogen, tetrahydro derivative 111. The NMR spectrum of tetrahydro derivative (III) revealed the presence of two methylene protons at 2.46 ppm and 1.88 ppm and two multiplets (1H x 2) at 4.02 ppm and 3.62 ppm (Fig. 3).

Fig. 4 shows the mass spectrum of the tetrahydro derivative (III). Ions were observed at \( m/e \) 58, 72, 110, 128, 144 and 159; they are 4 mass units higher than the corresponding peaks of antibiotic BN-227.

Reduction\(^{5,6}\) of the tetrahydro derivative (III) with Zn powder in acetic acid afforded a new derivative IV. The NMR spectrum of IV is shown in Fig. 5.
Fig. 5. NMR spectrum of IV (100 MHz in CDCl₃).

Fig. 6. Mass spectrum of IV.

Fig. 7. Molecular profile of antibiotic BN-227

Table 1. Chemical shifts of antibiotic BN-227 (I) (CDCl₃).

<table>
<thead>
<tr>
<th>Chemical shift (ppm)</th>
<th>Proton integration</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.43</td>
<td>3H</td>
<td>CH₃ at C6</td>
</tr>
<tr>
<td>3.73</td>
<td>3H</td>
<td>CH₂O at C5</td>
</tr>
<tr>
<td>6.53</td>
<td>1H</td>
<td>H at C3</td>
</tr>
<tr>
<td>7.20</td>
<td>1H</td>
<td>H at C4</td>
</tr>
<tr>
<td>9.70</td>
<td>1H</td>
<td>OH</td>
</tr>
</tbody>
</table>

Table 2. Chemical shifts of tetrahydro derivative (III) (CDCl₃).

<table>
<thead>
<tr>
<th>Chemical shift (ppm)</th>
<th>Proton integration</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.32</td>
<td>3H</td>
<td>CH₃ at C6</td>
</tr>
<tr>
<td>1.88</td>
<td>2H</td>
<td>H at C4</td>
</tr>
<tr>
<td>2.46</td>
<td>2H</td>
<td>H at C3</td>
</tr>
<tr>
<td>3.40</td>
<td>3H</td>
<td>CH₂O at C5</td>
</tr>
<tr>
<td>3.62</td>
<td>1H</td>
<td>H at C5</td>
</tr>
<tr>
<td>4.02</td>
<td>1H</td>
<td>H at C6</td>
</tr>
<tr>
<td>7.98</td>
<td>1H</td>
<td>OH</td>
</tr>
</tbody>
</table>

Fig. 6 shows the mass spectrum of derivative (IV), with ions at m/e 128 and 143; they are 16 mass unit lower than the corresponding ions in the tetrahydro derivative (III).

Compound IV was produced by the loss of an oxygen atom from the tetrahydro derivative (III) and no longer gave a deep red color with
ferric chloride.

In the NMR spectrum of tetrahydro derivative (III), on irradiation at 1.32 ppm a multiplet at 4.02 ppm collapsed into a doublet. On irradiation of a multiplet at 4.02 ppm, a doublet of methyl proton at 1.32 ppm collapsed to a singlet.

An examination of the NMR spectrum of the tetrahydro derivative of 1-hydroxy-2-pyridone, showed that the proton at C5 is at higher field than the proton at C6. Therefore a methyl group is attached to C6. The results of NMR spectrum of antibiotic BN-227 (I) and tetrahydro derivative (III) are summarized in Table 1 and Table 2.

**X-ray Analysis of Antibiotic BN-227**

To confirm the above structure, we carried out an X-ray analysis. The crystal of antibiotic BN-227 was triclinic, space group P1, with four molecules in a unit cell of dimensions, a=15.453Å, b=7.787Å, c=6.929Å, cos α=−0.2078, cos β=−0.1620, cos γ=−0.0201 and z=4. Total reflection intensities of 1859 were measured with a Philips PW-1100 four-circle diffractometer.

The structure was solved by the direct method using the MULTAN7, from which E-map of the most probable set of phases easily revealed the molecular profile. Final refinement of the structure was carried out using the weight \( W = \frac{C + |F_o| + d |F_o|^2}{2} \), where \( c = 30.0 \) and \( d = 0.003 \), and gave an R value of 6.1%. The results shown in Fig. 7, coincide with the one expected.

**Structure of Antibiotic BN-227-F**

The molecular formula of antibiotic BN-227-F was established as C\(_{21}\)H\(_{24}\)N\(_3\)O\(_9\)Fe from the results of elemental analysis, mass spectrometry (m/e 518, M\(^+\)) and atomic absorption analysis as reported in the previous paper\(^1\). The IR spectrum was similar to that of antibiotic BN-227. The NMR spectrum could not be measured, because antibiotic BN-227-F contains iron.

The structure of antibiotic BN-227 (I) and the molecular formula of antibiotic BN-227-F suggested that antibiotic BN-227-F is a chelate compound of three hydroxamates and one ferric ion\(^8,9\). In order

---

Fig. 8. IR spectra of native BN-227-F (-----) and synthetic BN-227-F (------).
to confirm the structure of antibiotic BN-227-F, an attempt was made to convert antibiotic BN-227 into antibiotic BN-227-F by the addition of a ferric chloride. Addition of ferric chloride to the antibiotic BN-227 afforded dark reddish prisms, whose melting point, IR spectrum, mass spectrum and Rf value on TLC were identical with those of native antibiotic BN-227-F. Fig. 8 shows IR spectra of native antibiotic BN-227-F and synthetic BN-227-F.

On the basis of the evidence already described, the structure of antibiotic BN-227-F should be formulated as II.

Experimental

General methods

Melting points were determined with a Yamato MT-1 melting apparatus, and are uncorrected. Specific rotations were determined in a 10 mm cell with a Perkin-Elmer Model 141 polarimeter. IR spectra were obtained with a Hitachi 215 Infrared Spectrophotometer. UV spectra were recorded with a Hitachi Model 200-20 Spectrophotometer. NMR spectra were observed with Varian T-60 or XL-100-12 NMR Spectrometer with TMS as the internal standard. Mass spectra were determined with a JEOL JMS-01SG Spectrometer.

Antibiotic BN-227 (I)

Crystals from ethyl acetate were used in the structural studies: m. p. 115°C, [α]25° 0 (c 1, MeOH).

Anal. Calcd. for C7H9N03: C 54.18, H 5.86, N 9.02.

Found: C 53.79, H 5.84, N 8.73.

Masses of fragment ions: m/e 155 (M+), 140 (M+ CH3), 124 (M+ OCH3).

Preparation of the tetrahydro derivative (III) from I

A solution of I (200 mg) in ethanol (10 ml) was hydrogenated over PtO2 (100 mg) for 2 hours. The reaction mixture was filtered, and the filtrate evaporated to dryness. The residue dissolved in methanol (1.0 ml) and chromatographed on a Sephadex LH-20 column (300 ml). The fractions containing III were collected and evaporated to give a colorless syrup (163 mg).

Anal. Calcd. for C7H13NO3 (M. W., 159): C 52.81, H 8.25, N 8.79.

Found: C 53.06, H 8.48, N 8.92.

Fragment ions: m/e 159 (M+), 144 (M+ CH3), 128 (M+ OCH3).

Preparation of IV from III

A solution of III (163 mg) in acetic acid (6 ml) was reduced with zinc powder (400 mg) for 3 days at room temperature. The reaction mixture was filtered, the filtrate evaporated to dryness. The residue dissolved in methanol (1 ml) and chromatographed on a Sephadex LH-20 column (300 ml). The fractions containing IV were collected and evaporated to give a colorless syrup (132 mg).


Found: C 58.71, H 9.17, N 9.78.

Fragment ions: m/e 143 (M+), 128 (M+ CH3), 112 (M+ OCH3).

Antibiotic BN-227-F (II)

Crystals from chloroform (m. p. 156°C) were used for the structural studies.


Fragment ions: m/e 518 (M+), 489, 364, 335.
Preparation of BN-227-F (II) from BN-227 (I)

To a solution of 100 mg of I in methanol (5 ml), ferric chloride (300 mg) in water (2 ml) was added
and kept at room temperature for 2 hours. The dark red precipitate was separated, washed with ethyl
acetate and dried. It was dissolved in chloroform and crystallized upon standing (80 mg): m. p. 156°C.
Anal. Calcd. for C_{21}H_{24}N_{3}O_{9}Fe (M. W., 518): C 48.50, H 4.65, N 8.21, Fe 10.20.

Acknowledgements

The authors express their gratitude to Miss Miki for the mass spectra, Miss Ohmori for elemental analysis.

References

and BN-227-F, new antibiotics. I. Taxonomy, isolation and characterization. J. Antibiotics 32: 1089~
1095, 1979
3) Middleton, A. J.; D. S. Cole & K. D. Macdonald: A hydroxamic acid from Aspergillus nidulans with
antibiotic activity against Proteus species. J. Antibiotics 31: 1110~1115, 1978
Mikroorganismen. Archiv Mikrobiol. 45: 119~135, 1963
5) Shaw, E.: Analogs of aspergilllic acid. I. The tautomeric of the the hydroxyipyridine-N-oxides. J.
Amer. Chem. Soc. 71: 67~70, 1949
Heterocycl. Chem. 12: 389~390, 1975
8) Shirahata, K.; T. Deguchi, T. Hayashi, I. Matsubara & T. Suzuki: The structures of fluopsins C and
9) Enery, T. & J. B. Neilands: Contribution to the structure of the ferrichrome compounds: Characteriza-
tion of the acyl moieties of the hydroxamate functions. J. Amer. Chem. Soc. 82: 3658~3662, 1960