## Communications to the Editor

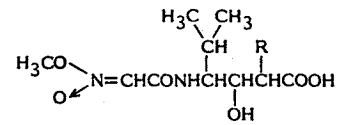
# NITRACIDOMYCINS A AND B, NEW ENTEROMYCIN-GROUP ANTIBIOTICS

Sir:

In the course of our screening program for new antibiotics with spheroplast formation, nitracidomycins A (**1a**) and B (**1b**)[1] were obtained from the culture filtrate of a streptomycete. They were characterized and shown to have a nitronic acid and an 4-amino-3-hydroxy acid moiety in their structures; they both had moderate activities against Gram-positive bacteria and some species of Gram-negative bacteria, such as *Bacteroides fragilis*. In this report, the production, isolation, physicochemical properties, structures and biological properties of **1a** and **1b** are reported.

A streptomycete which produced **1a** and **1b** was isolated from the soil sample collected at Ogose, Saitama Prefecture, Japan, and identified as *Streptomyces viridochromogenes* SANK 60784 (FERM P-7582). Fermentation of *S. viridochromogenes* SANK 60784 was carried out in a 600-liter fermentor, containing 300 liters of medium composed of glucose 3.0%, pressed yeast 1.0%, soybean meal 3.0%, $CaCO_3$ 0.4%, $MgSO_4 \cdot 7H_2O$ 0.2%, and CB-442 0.01% (pH 7.0 before sterilization), at 28°C for 73 hours with agitation of 220 rpm and aeration of 300 liters/minute.

The culture filtarate (280 liters) was adjusted to pH 2.0 and the antibiotics were extracted with 300 liters of EtOAc. The active principles in the organic layer were extracted further with 290 liters of diluted alkaline solution (pH 10). The



**1a** R = $CH_3$
**1b** R = $CH_2CH_3$

Fig. 1. IR spectrum of nitracidomycin A (KBr).

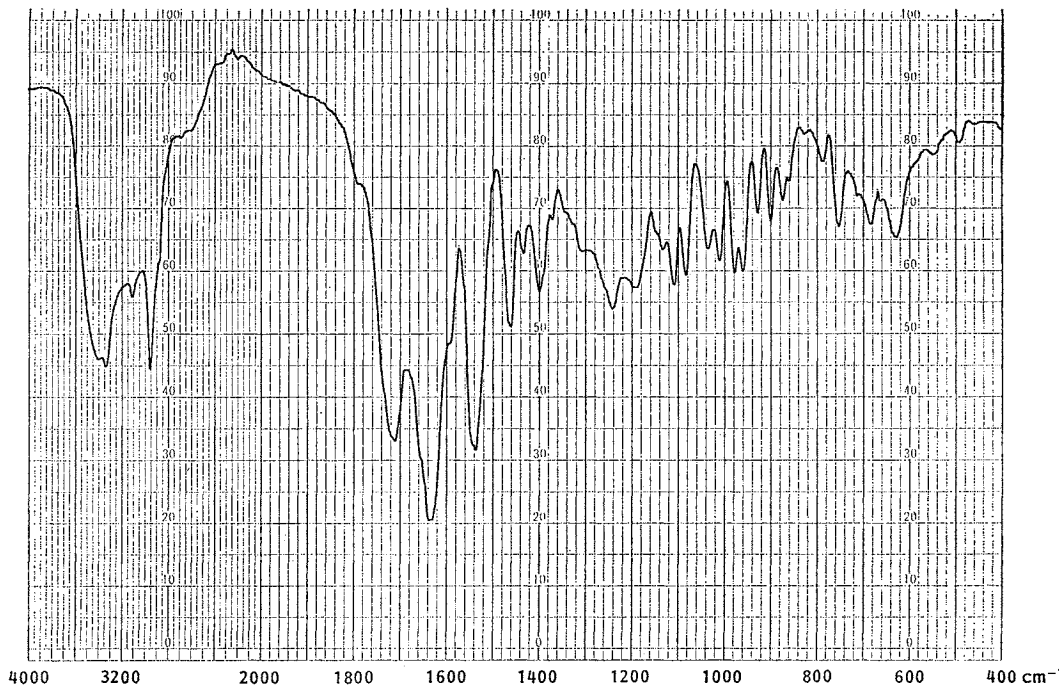


Fig. 2. $^{1}H$ NMR spectrum of nitracidomycin A in $CDCl_3$ (270 MHz).

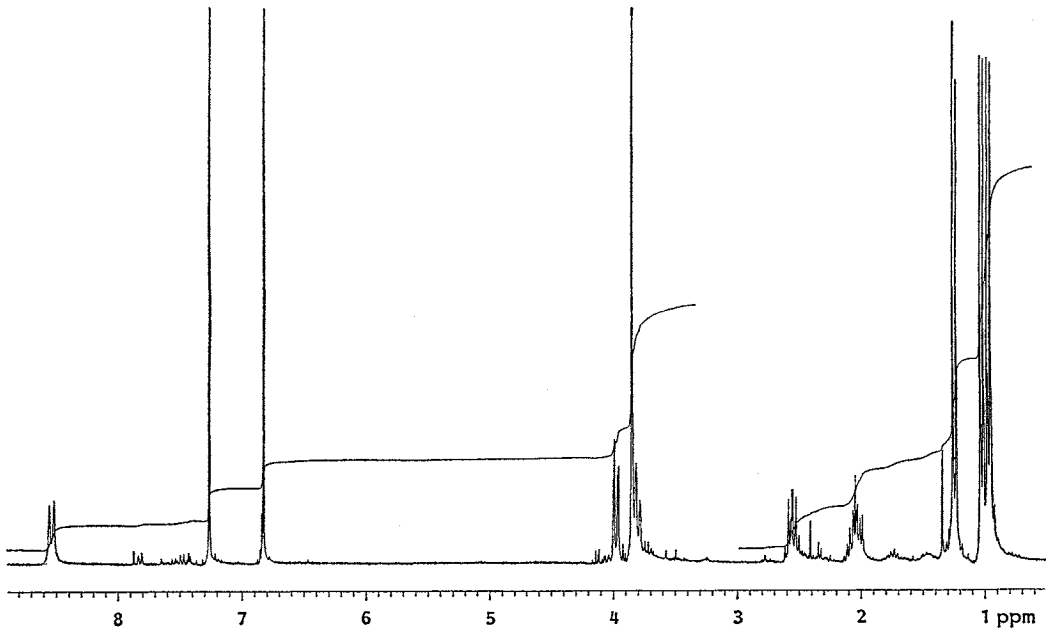
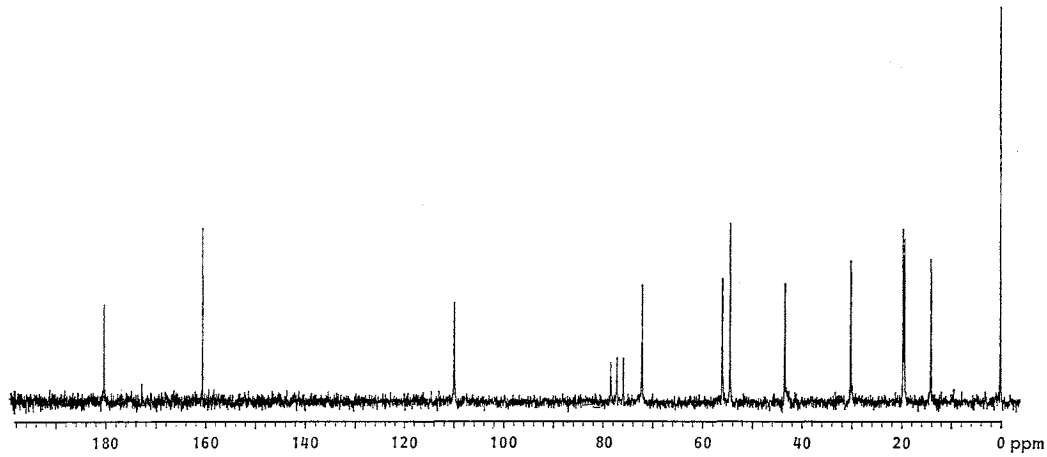
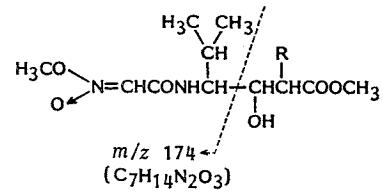



Fig. 3. $^{13}C$ NMR spectrum of nitracidomycin A in $CDCl_3$.

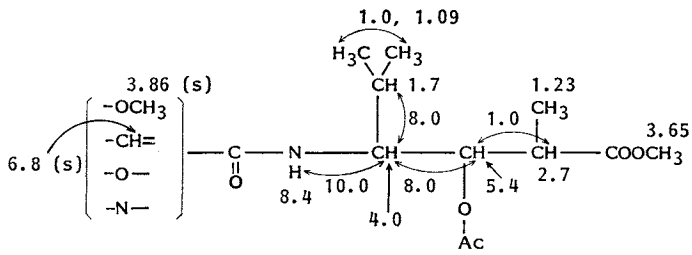



aqueous layer obtained in this way was adjusted to pH 2.0, and then re-extracted with the same volume of EtOAc. The final extract was dried over $Na_2SO_4$ and concentrated under reduced pressure. The residue dissolved in 200 ml of a mixture of EtOAc and $CHCl_3$ (1 : 1) was subjected to chromatography on a column of Sephadex LH-20 (2 liters) with the same solvent mixture. Fractions 1 and 2 were further purified by rechromatography on Sephadex LH-20 using a solvent system composed of $CHCl_3$ - EtOAc -




**2** R = $CH_3$

**5** R = $CH_2CH_3$

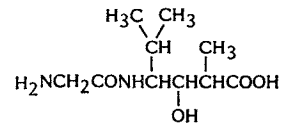



**3**

*n*-hexane (2:1:1) to yield 200 mg of nitracidomycin B (**1b**) and 2.5 g of nitracidomycin A (**1a**), respectively.

Nitracidomycin A (**1a**) showed UV max at 251 nm (ε 5,100 in MeOH) and $[\alpha]_D^{25}$ −31.9° (*c* 2.0, $CHCl_3$). IR and $^1H$ and $^{13}C$ NMR spectra of **1a** are shown in Figs. 1, 2 and 3. Reaction of **1a** with diazomethane gave **2**. **2** was deduced a monomethyl ester of **1a** from the analysis of $^1H$ NMR spectrum. Elemental analysis of **2** gave C 49.91, H 7.50, N 9.53% (C 49.65, H 7.64, N 9.65, calcd for $C_{13}H_{22}N_2O_6$). The high resolution mass spectrum (HR-MS) of **2** showed the molecular ion peak at *m/z* 291.1538 ($C_{12}H_{23}N_2O_6$, $M+H^+$) and base ion peak at *m/z* 174.0965 ($C_7H_{14}N_2O_3$). Acetylation of **2** with acetic anhydride in pyridine gave a monoacetate (**3**); $C_{14}H_{25}N_2O_7$, *m/z* 333 ($M+H^+$). The detailed analyses of $^1H$ and $^{13}C$ NMR spectra of **2** and **3** in $CDCl_3$ indicated the assigned partial structure of **3**.

The UV absorption maximum at 251 nm in the structure of nitracidomycin A suggested a nitronic acid moiety. Further evidence for a nitronic acid moiety was obtained as follows: Hydrogenation of **1a** with platinum oxide in MeOH gave a peptide (**4**). Hydrolysis of **4** with 12 N HCl - AcOH (1:1) gave glycine and a new amino acid, 4-amino-3-hydroxy-2,5-dimethylhexanoic acid (AHDMHA) by amino acid analysis. This new hydroxy amino acid, AHDMHA, $C_8H_{17}NO_3$, was also obtained by hydrolysis of **1a** under the same condition as mentioned above, followed by column chromatography on Sephadex G-10 developed with BuOH - AcOH - $H_2O$ (4:1:5, upper layer). The $^1H$ NMR spectrum of AHDMHA displayed three doublet methyl protons at δ 0.80 ($CH_3$), 0.86 ($CH_3$) and 1.01 ($CH_3$) and four methine protons at 1.90 (δ), 2.35 (α), 2.93 (γ) and 3.63 (β), respectively.




**4**

These assignments strongly supported the partial structure described above and the structure of nitracidomycin A as **1a**.

Nitracidomycin B (**1b**) also gave monomethyl ester (**5**), $C_{13}H_{25}N_2O_6$, *m/z* 305 ($M+H^+$), by treatment with diazomethane. In the $^1H$ NMR spectrum of **5**, a newly appeared ethyl signal was detected at 0.87 (t, $CH_3$), and 1.7 (m, $CH_2$), but doublet at 1.20 ppm of α-methyl signal derived from the structure **2** was disappeared. This result indicated that nitracidomycin B has an ethyl group instead of an α-methyl group in nitracidomycin A. Further structural evidence was obtained from the mass spectrometry. In the mass spectrum of **5**, the base ion peak at *m/z* 174 appeared as same as **2**. This fragment ion supported the presence of the same moiety in **2** and **5**.

Therefore, the structure of nitracidomycin B was determined as **1b**. The structural characteristics of nitracidomycins A and B include the *O*-methyl nitronic acid moiety reported as a constituent of enteromycin[2], enteromycin carboxamide[3], U-22956[2], YN-0165J-A[4], seligocidin[5] and thermycetin[6], and the presence of 4-amino-3-hydroxy acid moiety such as 4-amino-3-hydroxy-6-methylheptanoic acid in pepstatin[7], and 4-amino-3-hydroxy-2-methylpentanoic acid in bleomycin[8]. The configurations of **1a** and **1b** are now under investigation.

Antimicrobial activity of nitracidomycin A was determined by conventional agar-dilution method as shown in Table 1. It is moderately

Table 1. Antimicrobial activity of nitracidomycin A.

| Test organism | Medium | MIC ($\mu$g/ml) |
|---|---|---|
| *Staphylococcus aureus* FDA 209P JC-1 | A | 50 |
| *S. aureus* SANK 71075 | A | 50 |
| *S. epidermidis* SANK 71575 | A | 50 |
| *Bacillus subtilis* PCI 219 | A | 50 |
| *Enterococcus faecalis* SANK 71478 | A | 25 |
| *Micrococcus luteus* PCI 1001 | A | 50 |
| *Escherichia coli* NIHJ JC-2 | A | >200 |
| *Pseudomonas aeruginosa* SANK 73575 | A | >200 |
| *Klebsiella pneumoniae* SANK 74975 | A | >200 |
| *Serratia marcescens* SANK 73060 | A | >200 |
| *Bacteroides fragilis* SANK 71176 | B | 50 |
| *B. fragilis* SANK 70478 | B | 100 |
| *B. fragilis* SANK 70678 | B | 200 |
| *B. fragilis* SANK 70878 | B | 200 |

Medium A: Mueller-Hinton agar (Difco).
Medium B: GAM agar (Nissui).
Inoculum size: $10^6$ cells/ml.

active against Gram-positive and Gram-negative bacteria, especially against *Enterococcus faecalis* and *B. fragilis*. Antimicrobial activity of nitracidomycin B was very similar to that of nitracidomycin A determined by conventional paper-disc agar diffusion assay.

Nitracidomycins A and B were also able to induce spheroplast formation of Gram-negative bacteria such as *Proteus mirabilis* SANK 71873 at a concentration equivalent to the MIC.

The acute toxicity ($LD_{50}$) of nitracidomycin A in mice was 150 mg/kg by intravenous administration.

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