AMICOUMACIN-A, A NEW ANTIBIOTIC WITH STRONG ANTIINFLAMMATORY AND ANTIULCER ACTIVITY*

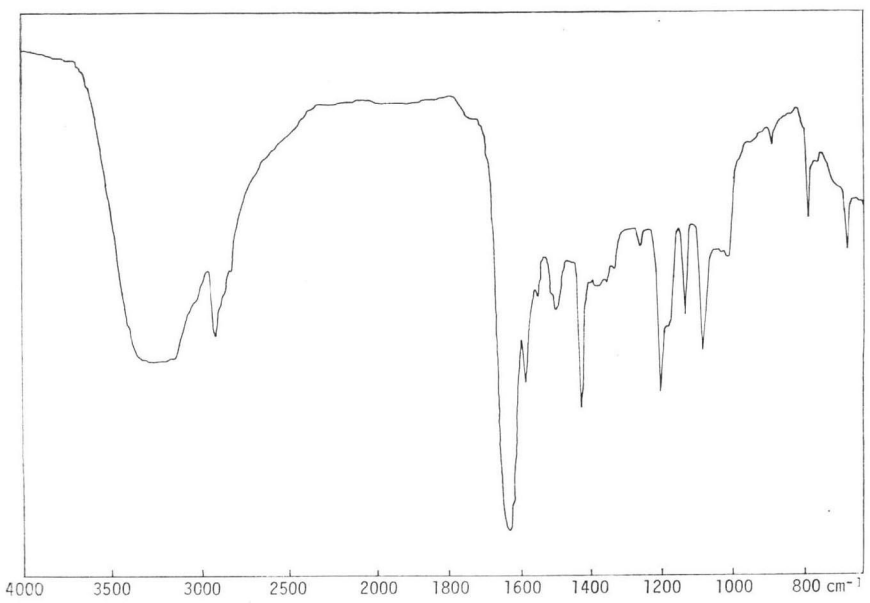
Sir:

In our screening program for new antibiotics produced by bacteria, a strain of *Bacillus pumilus* was found to produce a new antibiotic named amicoumacin-A (**I**). **I** was a major component of a mixture of antibiotics produced by this strain. It exhibited antibacterial activity, and strongly suppressed inflammatory and ulcer activity. The present communication describes the isolation, structure determination and bioactivity of amicoumacin-A.

Bacillus pumilus BN-103 was incubated for 40 hours at 28°C in a reciprocal shaker in forty 500 ml flasks each containing 100 ml of medium with the following composition: 3.0% glucose, 1.5% soybean meal, 0.3% meat extract, 0.5% peptone, 0.3% NaCl and 0.25% $CaCO_3$ (pH 7.0). The fermentation broth was subjected to successive column chromatography over Amberlite IRC-50 (H^+; 400 ml, eluted with a 1: 1 mixture of 0.2 N HCl and acetone; 2.0 liters) and activated carbon (100 ml, eluted with the same solvent as above; 500 ml) to yield a preparation of **I** (350 mg) of 75% purity (estimated by HPLC: Nucleosil $5C_{18}$; ϕ4.6×150 mm; RT=8.1 minutes at 1.0 ml/minute; eluted with a 70: 30 mixture of 0.025 M KH_2PO_4 and acetonitrile). Further purification of an aliquot of this preparation (100 mg) was accomplished by chromatography over Sephadex G-10 (200 ml, eluted with 0.1 M NaCl) followed by desalting by Diaion HP-20 (eluted with 50% aqueous acetone). This resulted in an analytical amorphous **I** hydrochloride (48 mg): m.p. 132~135°C (dec.); λ_{max}^{MeOH} nm (ε): 208 (27300), 247 (6400), 315 (4380); $[\alpha]_D^{25}$ −97.2° (*c* 1.0, MeOH); positive to ninhydrin reaction. The IR and PMR spectra are shown in Figs. 1 and 2. The CMR spectrum in D_2O of **I** showed twenty signals at 21.7 (q), 23.6 (q), 25.2 (d), 30.0 (t), 32.3 (t), 39.1 (t), 50.2 (d), 51.2 (d), 71.2 (d), 73.2 (d), 82.1 (d), 108.6 (s), 116.2 (d), 119.8 (d), 137.6 (d), 140.5 (s), 160.8 (s), 170.5 (s), 173.8 (s) and 175.1 (s) ppm respectively. Elemental analysis coupled with CMR and FD-MS (*m/z* 424 $(M+1)^+$) correspond to the molecular formula $C_{20}H_{29}N_3O_7$ for **I**.

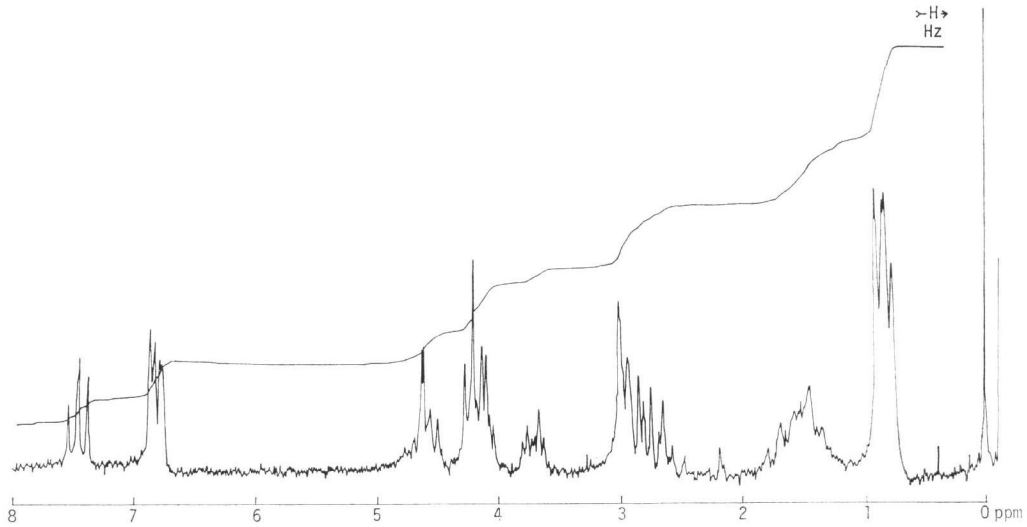
On acid hydrolysis of **I**, a crystalline chromophore ($C_{14}H_{19}NO_3 \cdot HCl$; m.p. 235~239°C; $[\alpha]_D^{23}$ −58.5° (*c* 0.5, MeOH)) was isolated and identified with **II** by its physico-chemical properties[1]. The

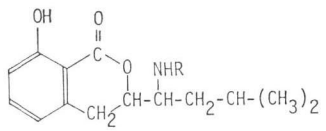
Fig. 1. Infrared spectrum of amicoumacin-A hydrochloride in KBr.

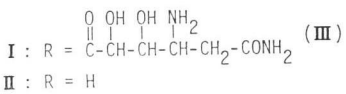


* A part of this work was presented at the 5th Symposium on Microbial Science, Hiroshima, 1980.

Fig. 2. 100 MHz PMR spectrum of amicoumacin-A hydrochloride in D_2O.







structure of **II** agreed with the chromophoric moiety of baciphelacin[2].

The non-chromophoric fragment could not be isolated following acid degradation, but a comparison of data on **I** and **II** suggested that an amino acid moiety (**III**) was attached to the chromophore (**II**) by an amide bond (IR; 1530 and 1660 cm^{-1}).

Upon treatment of **I** with acetic anhydride in methanol in presence of Dowex 1×2 (CO_3^{--}), a mono-N-acetyl derivative (m.p. 127~129°C; $[\alpha]_D^{23}$ −79.8° (*c* 0.5, MeOH)) was obtained. Acetylation of **I** with acetic anhydride in pyridine resulted in a tetra-N,O-acetyl derivative ($[\alpha]_D^{23}$ −79.0° (*c* 0.5, MeOH)). These results revealed the presence of two hydroxy groups and one amino group in **III**. Detailed NMR study of the N-DNP-tri-O-acetyl derivative (m.p. 143°C; $[\alpha]_D^{23}$ −10.8° (*c* 0.5, MeOH)) indicated a partial structure (–A–C(=O)–C(H)(OH)–C(H)(OH)–C(H)(NH$_2$)–CH$_2$–C(=O)–B–) in **III**.

Table 1. Antibacterial activity (MIC) of amicoumacin-A hydrochloride.

Test organisms	Amicoumacin-A (**I**) (mcg/ml)*
Staphylococcus aureus Rosenbach 209P JC-1	0.39
Staphylococcus aureus Smith (I)	0.39
Staphylococcus aureus No. 26	0.39
Staphylococcus aureus 606	0.39
Staphylococcus aureus N-0018	0.78
Staphylococcus epidermidis ATCC 14990	0.20
Staphylococcus epidermidis 109	0.39
Staphylococcus faecalis ATCC 8043	1.56
Bacillus anthracis No. 119	3.13
Escherichia coli NIHJ JC-2	100
Escherichia coli W 3630 RGN 823	12.5
Escherichia coli JR 66/W 677	12.5
Citrobacter freundii GN 346	100
Salmonella enteritidis No. 11	1.56
Shigella sonnei EW 33 Type I	6.25
Proteus vulgaris OX-19	50
Pseudomonas aeruginosa MB-3829	100
Pseudomonas cepacia M-0527	100

* The MICs were determined on heart infusion agar with inoculum size of 10^6 cell/ml.

The carbonyl group, A or B in the above partial structure, should attach to the chromophore (**II**) to form amicoumacin-A. The extra NH_2 group

Table 2. Effects of amicoumacin-A on carrageenin induced paw edema in rats.*

Compound	Dose (mg/kg)	Swelling (%)	Inhibition (%)
Control		72.2	—
Amicoumacin-A	10 (p.o.)	66.7	7.6
Amicoumacin-A	25 (p.o.)	61.0	15.5
Amicoumacin-A	50 (p.o.)	49.4	31.6
Phenylbutazone	50 (p.o.)	50.9	29.5

* Five male Wister strain rats were in each group. All drugs were suspended in 0.5% gum acacia and administered intragastrically 1 hour before injection of 1.0% carrageenin solution. Paw edema was measured 3 hours after injection.

Table 3. Effects of amicoumacin-A on gastric ulceration induced by stress (23±1°C, 6 hours) in rats.*

Compound	Dose (mg/kg)	Ulcer index*** Mean± S.D.	Preventive ratio (%)
Normal saline		4.25±0.95	—
Amicoumacin-A	10 (p.o.)	2.80±1.30	34.1
Amicoumacin-A	25 (p.o.)	1.20±0.44	71.8
Sulpiride**	30 (p.o.)	2.80±1.09	34.1

* Five male Sprague Dawley strain rats were used at each add dosage.

** N-(1-Ethyl-2-pyrrolidinylmethyl)-2-methoxy-5-sulfamoyl-benzamide.

*** 1: 1~12 (mm^2), 2: 13~25 (mm^2), 3: 26~37 (mm^2), 4: 38~50 (mm^2), 5: >50 (mm^2).

was considered most probably to be present as a carboxylic amide, since **I** gave only mono-N-acetyl derivative. Attachment of the carbonyl A to the chromophoric moiety (**II**) was supported by the periodate oxidation of **I**, which yielded a product giving rise to an anticipated M^+ at m/z 305. From these results, structure **I** was proposed for amicoumacin-A.

The MIC values of amicoumacin-A are listed in Table 1. **I** is active only against Gram-positive bacteria. It exhibits antiinflammatory activity against carrageenin induced paw edema (Table 2). At a dose of 50 mg/kg p.o., amicoumacin-A has suppression of edema comparable to that of phenylbutazone. Antiulcer activity of amicoumacin-A, using the stress induced ulcer procedure[3] is greater than that of sulpiride (Table 3).

The acute LD_{50} value of amicoumacin-A in mice is 132 mg/kg by the p.o. route.

Details of taxonomic studies of strain BN-103, of the determination of the structure and, biological evaluation of amicoumacin-A will be described in separate papers.

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(Received February 5, 1981)

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