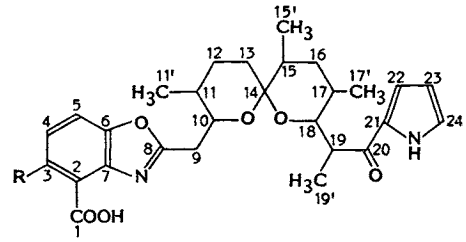
A NOVEL POLYETHER ANTIBIOTIC, AC7230 (3-HYDROXYCEZOMYCIN OR ITS STEREOISOMER)

Sir:

In the course of a screening program for new antibiotics from microorganisms, a novel polyether antibiotic AC7230 has been isolated from a culture broth. The producing organism was obtained from a soil sample collected at Nagano Prefecture, Japan. It was identified as *Dactylosporangium* sp. AC7230 (FERM P-8502). Fermentation was carried out on a rotary shaker (250 rpm, 5 cm-gyration) at 28°C for 3 days. The medium consisted of sucrose 2.0%, soybean flour 1.0%, cotton seed flour 0.5%, dry yeast 0.25%, NaBr 0.1% and $CaCO_3$ 0.1% (pH 6.5).

The fermented broth (10 liters) was centrifuged and the antibiotic was extracted from the harvested mycelial cake with acetone (2 liters). The extract was concentrated to remove acetone. The antibiotic was extracted with ethyl acetate (1 liter) from the concentrate. The ethyl acetate extract was concentrated to an oil, dissolved in chloroform washed sequentially with equal volumes of 0.5 N HCl, saturated sodium carbonate and water, and then was dried over sodium sulfate. After filtration and concentration, the resulting oil was applied to a Sephadex LH-20 column (420 ml) and developed with a mixture of chloroform - methanol (1: 1). The bioactive fractions against *Bacillus subtilis* ATCC 6633 that gave a single spot on thin-layer chromatogram were concentrated to yield the crystalline sodium salt of the antibiotic (210 mg). The free acid of the antibiotic was obtained by extraction with chloroform at pH 1 from an aqueous solution of the sodium salt.

Fig. 1. Structure of AC7230.



AC7230	R = OH
Cezomycin	R = H
A23187	R = $NHCH_3$

The antibiotic thus obtained behaved as an acidic substance and was stable at acidic or alkaline pH. It was soluble in chloroform, dimethyl sulfoxide and insoluble in water and hexane. The color reactions of the antibiotic revealed that it was positive for $FeCl_3$, but negative for Molisch and ninhydrin reactions. Physico-chemical properties are summarized in Table 1. The ^1H NMR spectrum (100 MHz, in $CDCl_3$) of the antibiotic (free acid) showed the presence of five aromatic protons. Two of these occur as an isolated AB spectrum (δ_A 7.57, δ_B 7.07, J_{AB}=9.0 Hz) characteristic of a 1,2,3,4-tetrasubstituted benzene derivative. The remaining three aromatic protons (δ 7.32, 7.02, 6.28) appear as multiplets with somewhat smaller coupling constants, reminiscent of five membered hetero-aromatics. The ^1H NMR spectrum also showed the resonances of four secondary methyl groups (δ 0.99, 0.85, 0.80, 0.74), phenolic hydroxyl group (δ 12.9) and carboxylic acid group (δ 14.0). The physico-chemical properties, such as UV, IR and ^1H NMR spectra were very

Table 1. Physico-chemical properties of AC7230 (Na salt).

Appearance	White needle crystals
Anal Calcd:	C 63.15, H 6.25, N 5.26, Na 4.32.
Found:	C 63.31, H 6.37, N 5.24, Na 4.63.
Formula	$C_{28}H_{33}N_2O_7 \cdot Na$
MW (FAB-MS, MH^+)	533
MP (°C)	>300
Optical rotation	$[\alpha]_D^{26}$ +328° (*c* 0.6, $CHCl_3$)
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	203 (599), 251 (sh, 277), 258 (304), 267 (sh, 225), 306 (442)
IR (KBr) cm^{-1}	3160 (phenolic OH), 1640 (C=C−C=O), 1610 (COO^-)
TLC (Silica gel f) Rf	0.31 ($CHCl_3$ - MeOH, 10: 1),
	0.63 ($CHCl_3$ - MeOH - AcOH, 10: 0.2: 0.1),
	0.38 ($CHCl_3$ - acetone, 10: 0.5)

FAB-MS: Fast atom bombardment mass spectra.

Table 2. ^{13}C NMR chemical shifts (δ) of AC7230, cezomycin and A23187.

Carbons	AC7230 (free acid)	Cezomycin[1] (free acid)	A23187[1] (Calcimycin) (free acid)
1	169.8 (s)	167.8 (s)	168.1 (s)
2	101.8 (s)	120.4 (s)	98.2 (s)
3	159.8 (s)	126.8 (d)	150.8 (s)
4	117.6 (d)	125.0 (d)	108.4 (d)
5	117.1 (d)	117.1 (d)	116.7 (d)
6	143.3 (s)	150.6 (s)	141.7 (s)
7	139.8 (s)	140.8 (s)	140.8 (s)
8	168.5 (s)	165.4 (s)	166.1 (s)
9	32.8 (t)	32.7 (t)	32.4 (t)
10	68.7 (d)	68.8 (d)	68.4 (d)
11	29.3 (d)	29.3 (d)	28.8 (d)
12	25.7 (t)	25.8 (t)	25.7 (t)
13	25.4 (t)	25.5 (t)	25.4 (t)
14	98.5 (s)	98.4 (s)	98.5 (s)
15	32.4 (d)	32.4 (d)	32.3 (d)
16	35.2 (t)	35.3 (t)	35.2 (t)
17	28.4 (d)	28.4 (d)	28.3 (d)
18	73.0 (d)	73.1 (d)	72.9 (d)
19	42.6 (d)	42.6 (d)	42.5 (d)
20	194.2 (s)	194.4 (s)	193.7 (s)
21	133.1 (s)	133.3 (s)	133.0 (s)
22	114.9 (d)	115.2 (d)	116.3 (d)
23	110.4 (d)	110.3 (d)	110.1 (d)
24	124.8 (d)	124.8 (d)	124.3 (d)
11′	11.4 (q)	11.4 (q)	11.3 (q)
15′	16.3 (q)	16.2 (q)	16.1 (q)
17′	11.0 (q)	10.9 (q)	10.7 (q)
19′	13.1 (q)	12.9 (q)	13.0 (q)
NCH_3			30.0 (q)

δ: ppm from TMS in $CDCl_3$ using $CDCl_3$ (δ 77.09 ppm) as the internal reference.

Single frequency off-resonance (SFOR) multiplicities are indicated in parenthesis.

similar to those of cezomycin (3-demethylamino-A23187)[1], A23187[1~3] and X-14885A.[4] But, mass spectrum differentiated the antibiotic (Na salt: MH^+ 533, free acid: MH^+ 511) obtained here from cezomycin (free acid: MH^+ 495), A23187 (free acid: MH^+ 524) or X-14885A (Na salt: MH^+ 519).

The ^{13}C NMR chemical shifts of the antibiotic were assigned by comparing with those of cezomycin and A23187 as shown in Table 2. The replacement of the proton at C-3 by OH has little influence on the *meta*-position carbons 5 and 7, but markedly shifts the peaks due to the other aromatic carbons.

DAVID and KERGOMARD reported the same phenomenon in cezomycin and A23187.

Table 3. Antimicrobial spectrum of AC7230.

Test microorganism	MIC (μg/ml)
Staphylococcus aureus ATCC 6538P	0.4
S. epidermidis sp-al-1	≦0.2
Streptococcus pyogenes N.Y. 5	≦0.2
S. faecalis 1501	≦0.2
S. agalactiae 1020	0.8
Micrococcus luteus ATCC 9341	≦0.2
Bacillus subtilis ATCC 6633	≦0.2
Corynebacterium diphtheriae P.W. 8	≦0.2
Escherichia coli NIHJ-JC2	>100
Klebsiella pneumoniae ATCC 10031	>100
Shigella flexneri type 3a	>100
Proteus vulgaris OX 19	>100
Pseudomonas aeruginosa IAM 1095	>100

The chemical shifts of the other part except benzoxazole moiety were found constant for the three antibiotics listed in Table 2.

Based on the data presented, we propose the structure shown in Fig. 1 for this antibiotic which is therefore the 3-hydroxycezomycin or its stereoisomer. The absolute configuration of antibiotic AC7230 will be reported elsewhere.

AC7230 is a new polyether antibiotic belonging to the class of these natural acid ionophores known as pyrrolethers, which includes cezomycin, A23187, X-14885A and X-14547A.[5] The antibiotic reported here, AC7230 was isolated from *Dactylosporangium* sp., but the others pyrrolethers (class 3) antibiotics[6] were produced by *Streptomyces* sp.

AC7230 has exhibited antimicrobial activity against Gram-positive bacteria as indicated in Table 3. The acute toxicity of AC7230 in mice expressed as LD_{50} is about 50 mg/kg when the antibiotic is administered intraperitoneally.

SATOSHI YAGINUMA
MASASHI AWATA
NAOKI MUTO
KENJI KINOSHITA
KIMIO MIZUNO

Medicinal Research Laboratories,
Toyo Jozo Co., Ltd.,
Ohito, Shizuoka 410-23, Japan

(Received August 20, 1986)

References

1) DAVID, L. & A. KERGOMARD: Production by controlled biosynthesis of a novel ionophore

antibiotic, cezomycin (demethylamino A23187). J. Antibiotics 35: 1409~1411, 1982

2) GALE, R. M.; C. E. HIGGENS & M. M. HOEHN (Eli Lilly): Antibiotic A23187 and process for preparation thereof. U.S. 3,923,823, Dec. 2, 1975
3) CHANEY, M. O.; P. V. DEMARCO, N. D. JONES & J. L. OCCOLOWITZ: The structure of A23187, a divalent cation ionophore. J. Am. Chem. Soc. 96: 1932~1933, 1974
4) WESTLEY, J. W.; C.-M. LIU, J. F. BLOUNT, L. H. SELLO, N. TROUPE & P. A. MILLER: Isolation and characterization of a novel polyether antibiotic of the pyrrolether class, antibiotic X-14885A. J. Antibiotics 36: 1275~1278, 1983
5) WESTLEY, J. W.; R. H. EVANS, Jr., C.-M. LIU, T. HERMANN & J. E. BLOUNT: Structure of antibiotic X-14547A, a carboxylic acid ionophore produced by *Streptomyces antibioticus*, NRRL 8167. J. Am. Chem. Soc. 100: 6784~6786, 1978
6) WESTLEY, J. W.: Introduction to "Polyether Antibiotics: Naturally Occurring Acid Ionophores" Vol. 1, Biology. *Ed.* J. W. WESTLEY, Marcel Dekker, Inc., New York, 1982