Communications to the Editor

STUDIES ON THE IONOPHOROUS ANTIBIOTICS XXVIII[1]. MOYUKAMYCIN, A NEW GLYCOSYLATED POLYETHER ANTIBIOTIC

Sir:

The polyether antibiotics are microbial metabolites produced mainly by *Streptomyces*[2] and effective against coccidia[3]. They have the characteristic physico-chemical property known as ion transport across biological and artificial membranes[4]. Some of them are being widely used as coccidiostats in poultry and as growth promotors in ruminants[3].

In the course of our continuous screening program for polyether antibiotics, a new compound moyukamycin sodium salt (**I**) was found to be produced by *Streptomyces hygroscopicus* TM-581 (FERM-BP 274). This communication describes the isolation, structural elucidation and antimicrobial activity of **I**.

Fermentation was carried out at 30°C for 4 days in two 5-liter jar fermentors each containing 3 liters of a medium consisting of glucose 2%, oatmeal 2%, meat extract 0.3%, NaCl 0.3%, $CaCO_3$ 0.3%, $FeSO_4 \cdot 7H_2O$ 0.04%, and $MnCl_2 \cdot 4H_2O$ 0.04% (pH 7.0). After the mycelia were removed by centrifugation, the supernatant was extracted with benzene. The mycelia were extracted with acetone and the solvent was removed by evaporation *in vacuo* to give a concentrated aqueous solution, which was then extracted with benzene. The combined benzene extract was concentrated *in vacuo* to dryness and the syrupy residue was applied to a Sephadex LH-20 column which was developed with acetone. Fractions active against *Bacillus subtilis* were collected and concentrated *in vacuo* to dryness. The pale yellow powder thus obtained was chromatographed on a silica gel column packed with benzene. The column was washed successively with benzene and benzene - acetone (8: 2) and then the active substance was eluted by benzene - acetone (6: 4). The combined enriched fraction was concentrated *in vacuo* to give moyukamycin free acid which was converted to the sodium salt by washing its ethyl acetate solution with 0.01 N NaOH. The solvent layer was concentrated

Fig. 1. Structure of moyukamycin sodium salt (**I**).

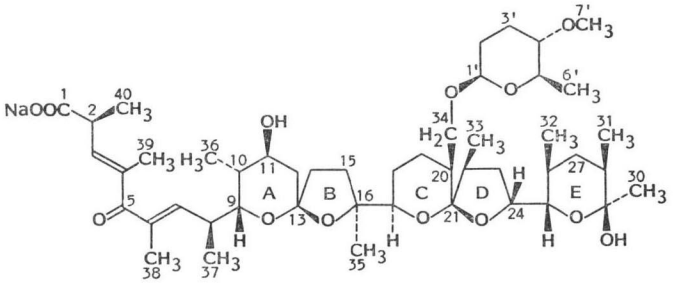
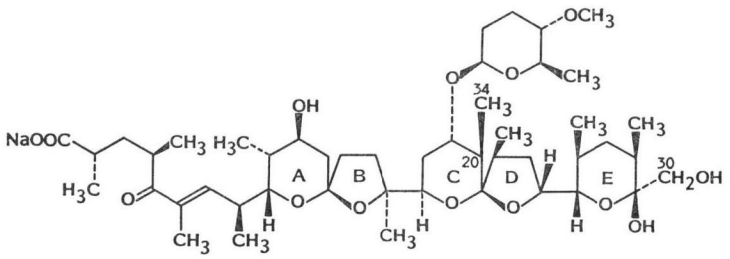


Fig. 2. Structure of dianemycin sodium salt (**II**).



and subjected to Sephadex LH-20 chromatography with EtOH to give a colorless powder of **I**, 99.6 mg, mp 129~133°C.

Moyukamycin sodium salt (**I**) has the following physico-chemical properties; $[\alpha]_D^{26}$ +91.4° (*c* 0.5, $CHCl_3$); UV λ_{max}^{EtOH} 242 nm ($E_{1cm}^{1\%}$ 146.9); IR $\nu_{max}^{CHCl_3}$ 3400 (OH), 1633 (C=C-C=O), and 1570 cm^{-1} ($-COO^-$). These characteristics are similar to those of the polyether antibiotics having an α,β-unsaturated ketone and two spiroketals such as dianemycin[5] (A-130A[6]), A-130B[7], A-130C[7], leuseramycin (TM-531A)[8], TM-531B (4′-*O*-demethyldianemycin)[1], TM-531C (3′-hydroxydianemycin)[1], X-14931A (19-deoxyaglycone of dianemycin)[9], and lenoremycin[10].

The molecular formula of **I** was established to be $C_{47}H_{75}O_{13}Na$ by elemental analysis (Found C 65.04, H 8.54, Na 2.00, Calcd C 64.79, H 8.68, Na 2.64) and mass spectral data [FAB-MS *m/z* 871 $(M+H)^+$]. This formula is reminiscent of a dehydro derivative of leuseramycin. Unlike leuseramycin, however, the 400 MHz ^1H NMR spectrum of **I** suggested the presence of one $-CHCH_2O-$ (δ_H 3.79, dd and 4.22, dd), and two $-CHCH=C(CH_3)-$ (δ_H 5.96, d and 6.01, d; 1.85, s and 1.92, s).

The structure of **I** was determined by comparison of its ^{13}C NMR spectral data with those of dianemycin sodium salt (**II**) (see Table 1) and by the use of the empirical rules for the polyether antibiotics[11]. The ^{13}C NMR data were obtained by measurements of the completely decoupled, INEPT[12,13] and ^{13}C-^1H shift correlation spectra[14].

The presence of an α,β-unsaturated carbonyl system in **I** as found in **II** was suggested by the similar chemical shifts of the ketone (C-5, δ_C 202.2), olefinic carbons (C-6, δ_C 133.8 and C-7, δ_C 144.9) and allylic methyl (C-38, δ_C 12.1). The other olefinic carbon signals at δ_C 136.2 and 147.5 also indicated that they are conjugated to a carbonyl carbon. Taking the UV absorption of **I** into consideration, this double bond must be connected to C-5. The ^{13}C chemical shifts of the allylic methyls (C-38, δ_C 12.1 and C-39, δ_C 13.2) proved the *E* configurations for these two double bonds. The methine signal at δ_C 44.1 was

Table 1. ^{13}C NMR chemical shifts (ppm) of sodium salts of moyukamycin (**I**) and dianemycin (**II**) at 100.6 MHz.

No.	Moyukamycin (**I**)	Dianemycin (**II**)	No.	Moyukamycin (**I**)	Dianemycin (**II**)
1	179.8	183.8	25	73.7	73.2
2	44.1	40.2	26	33.4	32.9
3	147.5	41.5 (CH)	27	37.0	36.5
4	136.2	37.5 (CH_2)	28	40.0	35.9
5	202.2	206.2	29	97.9	98.5
6	133.8	133.6	30	28.7	65.3 (CH_2OH)
7	144.9	144.9	31	17.1	16.7
8	35.2	37.8	32	18.1	17.7
9	69.4	69.6	33	15.9	16.1
10	36.4	35.9	34	69.2	13.1 (CH_3)
11	71.2	70.4	35	27.9	26.6
12	34.6	34.0	36	10.7	10.0
13	107.6	106.9	37	16.8	14.4
14	39.6	39.7	38	12.1	11.2
15	32.3	32.2	39	13.2	16.9
16	86.5	86.6	40	17.3	19.5
17	80.5	75.7	1′	102.5	101.9
18	20.1	25.4	2′	30.0	30.7
19	24.1	79.2 (CH-O-)	3′	27.7	27.7
20	35.6	34.6	4′	79.5	80.1
21	109.7	109.8	5′	75.6	74.6
22	36.1	35.9	6′	18.4	18.4
23	29.7	29.9	7′	57.3	56.8
24	79.4	77.9			

assigned to C-2 adjacent to the terminal carboxylic acid from the empirical rules. This methine was ascertained to be attached to a methyl (δ_H 1.14, δ_C 13.2) and an olefinic proton (H-3, δ_H 6.01) by spin decoupling irradiating at δ_H 3.44 (H-2). The configuration of C-2 was assumed to be identical with the related polyether antibiotics mentioned above due to the similarity between **I** and **II**.

The empirical rules indicated that **I** possesses the same ether rings as **II** does except for the C ring. As shown in Table 1, the ^{13}C chemical shifts of C-9 to C-16 and C-21 to C-29 are in good agreement in both the compounds. In particular, the oxymethine (C-9, δ_C 69.4), ketal (C-13, δ_C 107.6), oxygenated quaternary (C-16, δ_C 86.5) and methyl (C-35, δ_C 27.9) signals in **I** indicated the structures of the A ring including C-8 and the B ring to be identical with those of **II**. Similarly, the characteristic signals for the D ring (C-21, δ_C 109.7 and C-24, δ_C 79.4), E ring except for C-30 carbon (C-29, δ_C 97.9: C-31, δ_C 17.1: and C-32, δ_C 18.1), and 4-*O*-methylamicetose moiety (C-1′, δ_C 102.5: C-4′, δ_C 79.5: C-5′, δ_C 75.6: and C-7′, δ_C 57.3) in **I** suggested the structural identity of these moieties in **I** and **II**. The disappearance of the oxymethylene signal at δ_C 65.3 due to C-30 in **II** indicated the presence of a methyl group at C-30 (δ_C 28.7) in **I**. The remaining carbon resonances due to rings A, B, D and E in **I** were assigned by detailed comparison of ^{13}C NMR spectral data of **I** and **II** (Table 1). The downfield shift of C-28 in **I** is explained in terms of the lack of γ-effect by the hydroxyl function on C-30.

The remaining carbons, *i.e.* two methylenes (δ_C 20.1 and 24.1) and one each of methine (δ_C 35.6), oxymethylene (δ_C 69.2) and oxymethine (δ_C 80.5), constitute the six-membered C ring moiety from C-17 to C-20. The decoupled difference ^1H NMR spectrum irradiating at δ_H 3.79 (1H, dd, J=8.8 and 3.1 Hz, H-34a) and 4.22 (1H, dd, J=8.8 and 7.2 Hz, H-34b) indicated the connectivity of $-CH_2\underset{|}{C}HCH_2O-$, in which the methine was attached to a quaternary carbon (C-21) due to its splitting pattern at δ_H 2.00 (dd, J=4.4 and 2.2 Hz). Consequently, the oxymethine assignable to C-17 was linked to the above partial structure *via* the only remaining methylene. The downfield shift of C-17 in **I** was reasonably attributed to the loss of the γ-effect by the substituent at C-19 of **II**. This substituent effect gave the assignment of C-18 and -19 methylenes δ_C 20.1 and 24.1, respectively. The orientation of the oxymethylene C-34 was determined to be axial (β-orientation) because of the coupling constant of H-20 (J=4.4 and 2.2 Hz) in the decoupled difference spectrum mentioned above.

The sugar moiety was combined to the oxymethylene at C-34 on the C ring, because the NOE experiment irradiating at H-1′ (δ_H 4.45) showed the enhancement of the signals at δ_H 3.79 and 4.22 (H-34a and -34b) in addition to those at δ_H 3.30 (H-5′). Thus, the structure of moyukamycin sodium salt has been established as shown in Fig. 1.

As far as we know, **I** is the first polyether antibiotic to possess a sugar at a branched hydroxymethyl group located in the middle position of polyketide chain.

Although the absolute structure of **I** remains to be clarified, it seems most reasonable to assume that **I** has the same configuration as **II** because of their similar optical rotation values.

Moyukamycin has activity against a wide range of Gram-positive bacteria, while no ac-

Table 2. Antimicrobial activity of moyukamycin sodium salt (**I**).

Test microorganism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	3.13
S. aureus Smith (SA-, PC-, TC-, SM-, KM-, CP- and Mac-R)	6.25
S. aureus TPR-23 (SA-, PC-, TC-, SM-, KM-, CP- and Mac-R)	6.25
S. epidermidis TPR-25	6.25
B. subtilis ATCC 6633	3.13
Bacillus licheniformis	1.56
Micrococcus luteus NIHJ	3.13
Escherichia coli NIHJ C-2	>50
Pseudomonas aeruginosa P-32	>50
Aspergillus niger	>50
Tricophyton asteroides	>50
Candida albicans	>50
Saccharomyces cerevisiae	>50

Abbreviations: SA; sulfonamide, PC; benzylpenicillin, TC; tetracycline, SM; streptomycin, KM; kanamycin, CP; chloramphenicol, Mac; macrolide, R; resistant strain.
Medium: Heart-infusion agar.
Incubation: 24 hours at 37°C.

tivity was observed against Gram-negative bacteria as shown in Table 2. Its anticoccidial activity will be reported elsewhere.

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