NEW ANTIBIOTICS, ENAMINOMYCINS A, B AND C
III. THE STRUCTURES OF ENAMINOMYCINS A, B AND C

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The structures of enaminomycins A and B were determined by their physico-chemical properties and X-ray crystallographic analyses to be 4-amino-2,5-dioxo-7-oxa-bicyclo[4,1,0]-hept-3-ene-3-carboxylic acid and 2-oxo-4-amino-5-hydroxy-5-acetonyl-7-oxa-bicyclo[4,1,0]-hept-3-ene-3-carboxylic acid, respectively. The structure of enaminomycin C was also determined by the analysis of NMR spectrum and other physico-chemical properties to be 2-oxo-4-amino-5-hydroxy-7-oxa-bicyclo[4,1,0]hept-3-ene-3-carboxylic acid.

As reported in the previous papers,[1,2] new antibiotics, enaminomycins A, B and C, were isolated from the culture broth of *Streptomyces baarnensis* No. 13120 and are presumed to be new members of the epoxy quinone family. They are active against Gram-positive and Gram-negative bacteria as well as against some species of fungi. Enaminomycin A is also active against L1210 mouse leukemia cells *in vitro*.

The present paper deals with the structural elucidation of enaminomycins A, B and C.

Structural Elucidation of Enaminomycin A

Enaminomycin A (**I**), $C_7H_5NO_5$ (M^+ 183), is an acidic, lipophilic, white amorphous powder; m.p. 105°C (dec.), $[\alpha]_D^{20}-20.9°$ (*c* 0.83, MeOH), UV λmax. ($E_{1cm}^{1\%}$), 245(285) and 293 nm (530) in MeOH. The IR spectrum (KBr pellet) of enaminomycin A showed absorption at 3500~2500, 1730 and 1710 cm^{-1} corresponding to carboxyl and carbonyl groups. In the NMR spectrum in acetone-d_6, two prominent signals at δ 4.10 (J=4.5 Hz) and 4.15 (J=4.5 Hz) for vicinally coupled protons suggested an epoxide residue in enaminomycin A.

Reaction of enaminomycin A with diazomethane gave a crystalline substance **II**, $C_9H_9NO_5$ (M^+ 211), whose UV spectrum showed a close resemblance to that of **I**. The IR spectrum of compound **II** indicated ester carbonyl absorption band at 1600 cm^{-1}. The NMR spectrum of compound **II** at 100 MHz in $CDCl_3$ showed two signals at δ 3.32 (J=4.2~4.4 Hz) and 3.69 (J=4.2~4.4 Hz) corresponding to the epoxide protons and similar to those of enaminomycin A. One methoxy at 3.80 for methyl ester was also present, and one AB type methylene at δ 3.09 (J=4.8~5.0 Hz) and 3.20 (J=4.8~5.0 Hz) was formed upon diazomethane treatment. It was strongly suggested by comparison of the molecular formulae of enaminomycin A and compound **II** that the extra methylene group derived from diazomethane was introduced into enaminomycin A to form **II**. This was further confirmed by the NMR spectrum of the reaction product of **I** with phenyldiazomethane, compound **III**, $C_{21}H_{17}NO_5$ (M^+ 363). The NMR spectrum of the benzyl ester derivative (**III**) revealed the presence of two phenyl

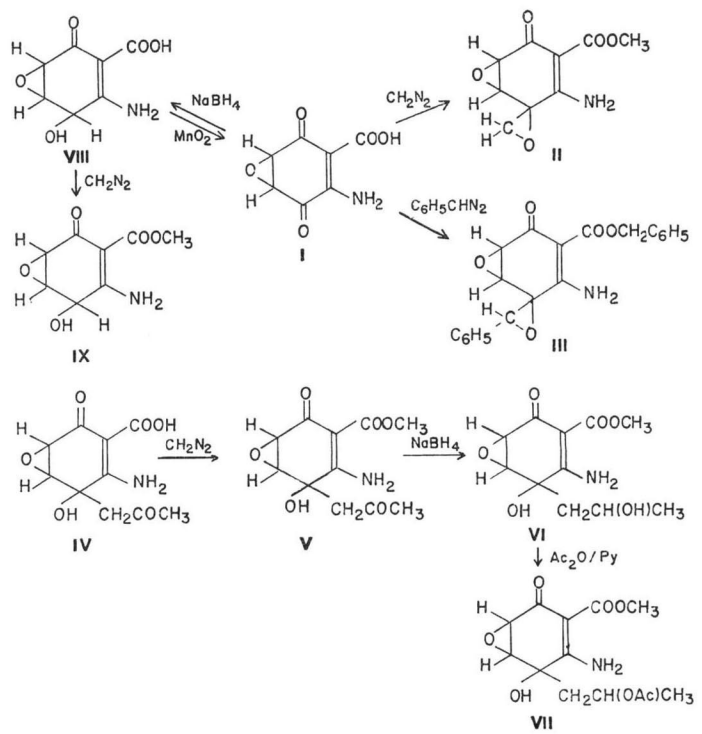
groups in compound **III**. The structure of **II** was finally determined by X-ray crystallographic analysis as shown in Scheme 1. From the data described above, the spiro epoxide in compound **II** was established to be derived by the reaction of enaminomycin A with diazomethane and the structure of enaminomycin A was thus determined to be 4-amino-2,5-dioxo-7-oxa-bicyclo[4,1,0]hept-3-ene-3-carboxylic acid (**I**).

Structural Elucidation of Enaminomycin B

Enaminomycin B (**IV**), $C_{10}H_{11}NO_6$ (M^+ 241), is an acidic, lipophilic, colorless crystalline substance; m.p. 160°C (dec.), $[\alpha]_D^{20}+60.1°$ (*c* 0.99, MeOH), UV λmax. ($E_{1cm}^{1\%}$), 244 (315) and 293 nm (580) in MeOH. The IR spectrum showed absorption at 3500~2500, 1720, 1670 and 1650 cm^{-1}. The NMR spectrum in acetone-d_6 exhibited a methyl singlet at δ 2.20, a methylene singlet at δ 3.15 and a hydroxy singlet at δ 5.22, in addition to two epoxide protons at δ 3.60 (J=4.5 Hz) and δ 4.10 (J=4.5 Hz). These physical and chemical data suggested that enaminomycin B possessed a ring structure closely related to enaminomycin A.

Reduction of enaminomycin B methyl ester (**V**), $C_{11}H_{13}NO_6$ (M^+ 255) gave dihydroenaminomycin B methyl ester (**VI**). Two singlets due to methyl and methylene protons in compound **V** were converted to two doublets which appeared at δ 1.20 and δ 2.00, each with a 6.0 Hz coupling constant. Irradiation of a proton multiplet centered at δ 4.28 made the two doublets collapse to singlets. In NMR spectrum of monoacetyl derivative of compound **VI** (**VII**), the proton multiplet shifted from δ 4.28 to δ 5.15 with the same coupling system. The presence of a secondary alcohol moiety in compounds **VI** was thus established. From these experiments, the existence of one hydroxy and one acetonyl moieties in the structure of enaminomycin B, in addition to epoxy, carboxyl and primary amine groups found in enaminomycin A, was confirmed. These data described above, together with comparative studies on the physicochemical characteristics of enaminomycins, proved the structure of enaminomycin B is 2-oxo-4-amino-5-hydroxy-5-acetonyl-7-oxa-bicyclo[4,1,0] hept-3-ene-3-carboxylic acid (**IV**); this was further supported by X-ray analysis. The study of X-ray analysis of enaminomycin B will be pre-

Scheme 1.



sented in detail elsewhere.

Structural Elucidation of Enaminomycin C

The structure of enaminomycin C was established mainly by the analysis of the NMR data and by comparison with enaminomycins A and B. Enaminomycin C (**VIII**), $C_7H_7NO_5$ (M^+ 185) is also an acidic, colorless, crystalline substance; m.p. 173°C (dec.), $[\alpha]_D^{20}+31.4°$ (*c* 0.14, MeOH), UV λmax. ($E_{1cm}^{1\%}$), 245 (436) and 290 nm (930) in MeOH. The IR spectrum showed absorption at 3500~2500, 1680 and 1605 cm^{-1}. The NMR spectrum in DMSO-d_6 exhibited two protons at δ 3.63 (J=4.5 Hz) and δ 3.82 (J=4.5 Hz) corresponding to epoxide protons, a proton giving a broad double doublet at δ 4.81 (J=8.5, 2.0 Hz) and an exchangeable proton giving doublet at δ 6.56 (J=8.5 Hz). As it will be described below, one of the epoxide protons coupled to a proton (δ 4.81) on the carbon linked to hydroxy group. Treatment of enaminomycin C with MnO_2 in methanol gave a biologically active substance, which showed the same Rf value as enaminomycin A on silica gel TLC. Treatment of this resulting compound with diazomethane yielded a crystalline substance, which was identical with compound **II** in all its physico-chemical properties. It was thus established that oxidation of enaminomycin C with MnO_2 gave enaminomycin A. On the other hand, the reduction of enaminomycin A with sodium borohydride gave a dihydroenaminomycin A whose characteristics were identical with those of enaminomycin C. This mutual transformation suggests that one of the two carbonyl groups in enaminomycin A is reduced to a secondary alcohol in enaminomycin C. To establish which of the two carbonyl groups is reduced, we conducted NMR analysis of the enaminomycin C methyl ester (**IX**), $C_8H_9NO_5$ (M^+ 199), prepared by treatment of enaminomycin C with diazomethane. The signals of compound **IX** at 100 MHz (pyridine-d_5) in NMR spectrum corresponded to the methoxy of the methyl ester at δ 3.71, the two epoxide protons at δ 3.75 (J=4.0, 1.0 Hz) and δ 4.00 (J=4.0, 2.0~2.2 Hz), a proton giving broad double doublet at δ 5.27 (J=2.0~2.2, 1.0 Hz), a hydroxy proton giving singlet at δ 8.60 or δ 4.86, and two amino protons at δ 4.86 or δ 8.60 and δ 9.90. A signal at δ 5.27, which was assigned to a proton on the carbon-bearing hydroxy group, was coupled to one of the epoxide protons at δ 4.00 with a coupling constant of 2.0~2.2 Hz and to another proton at δ 3.75 with a coupling constant of 1.0 Hz. By irradiation at δ 4.00, the signal assigned to one of the amino protons at δ 9.90 was simplified and increased in the magnitude of its peak height. This evidence supported that the structure of enaminomycin C is 2-oxo-4-amino-5-hydroxy-7-oxa-bicyclo[4,1,0]hept-3-ene-3-carboxylic acid (**VIII**).

All reactions mentioned above are summarized in Scheme 1.

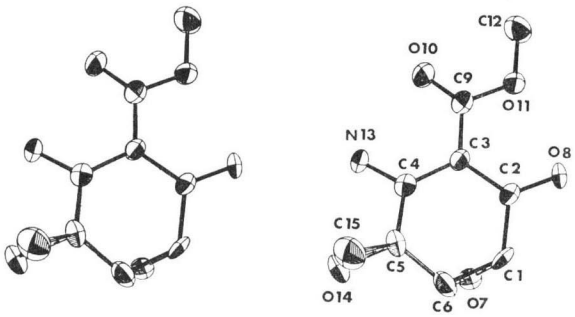
X-Ray Analysis of Methylated Enaminomycin A

The crystals of compound **II** for X-ray analysis were obtained from an ethyl acetate-acetone solution. The crystal data of this compound are: a=9.328(4), b=13.092(4), c=7.50(4)Å, space group $P2_12_12_1$, $D_{obs.}=1.52$, $D_{calc.}=1.53$ g/cm^3, and Z=4. The intensities were measured for 50° on a Rigaku four-circle diffractometer with LiF monochromated Mo Kα radiation, and the 632 independent reflections with $F \geq 3\sigma(F)$ were used in the structure refinement.

The structure was solved with the MULTAN program[3] using 208 E's$\geq$1.30 and refined by block-diagonal least squares; the H atoms were discerned in a difference synthesis. The refinement converged with anisotropic temperature factors for the non-hydrogen atoms, and isotropic factors for H. The

atomic scattering factors were taken from International Tables for X-ray Crystallography.[4] Unit weight was given to all reflections. Final R value was 0.058. The final atomic coordinates, the interatomic distances and the bond angles with their estimated standard deviations are listed in Tables 1, 2 and 3, respectively. The structure of the molecule and the atom-numbering scheme are shown in Fig. 1.

Fig. 1. Molecular geometry and atom-numbering scheme of methylated enaminomycin A.



The C(2)-O(8) bond is significantly longer than the C(9)-O(10) bond, due to the less-double bond character. In the C(2)-C(3) and C(3)-C(4) bond length, the former is shorter than the normal C-C single bond (1.54 Å), while the latter is longer than the double bond (1.34 Å), and both lengths are close to each other. These facts in-

Table 1. Positional parameters of methylated enaminomycin A with e.s.d.'s in parentheses.

(a) Nonhydrogen atoms. The parameters are multiplied by 10^4.

	x	y	z
C (1)	4283 (8)	1431 (7)	4627 (10)
C (2)	2689 (8)	1371 (6)	4446 (9)
C (3)	1848 (7)	1162 (6)	6017 (10)
C (4)	2503 (8)	1300 (5)	7695 (10)
C (5)	3902 (8)	1812 (6)	7844 (9)
C (6)	4905 (9)	1657 (6)	6359 (10)
O (7)	4961 (6)	646 (4)	5675 (7)
O (8)	2212 (6)	1479 (5)	2939 (6)
C (9)	366 (8)	819 (6)	5999 (10)
O (10)	−351 (6)	667 (6)	7281 (8)
O (11)	−141 (5)	710 (5)	4348 (7)
C (12)	−1596 (10)	356 (8)	4257 (13)
N (13)	1910 (6)	1061 (5)	9221 (8)
O (14)	4550 (6)	1792 (5)	9598 (7)
C (15)	4019 (10)	2721 (7)	8973 (13)

b) Hydrogen atoms. The parameters are multiplied by 10^3.

	x	y	z
H (1)	485 (7)	162 (5)	360 (9)
H (6)	572 (7)	211 (5)	639 (8)
H (12A)	−242 (10)	92 (7)	458 (13)
H (12B)	−174 (10)	−29 (8)	500 (14)
H (12C)	−176 (8)	25 (6)	315 (10)
H (13A)	204 (7)	129 (5)	1017 (9)
H (13B)	92 (6)	88 (4)	910 (8)
H (15A)	313 (9)	299 (6)	900 (12)
H (15B)	455 (6)	321 (4)	856 (7)

Table 2. Bond lengths of methylated enaminomycin A.

		Bond length (Å)
C (1)	–C (2)	1.496 (11)
C (1)	–C (6)	1.454 (11)
C (1)	–O (7)	1.440 (10)
C (2)	–C (3)	1.442 (10)
C (2)	–O (8)	1.223 (8)
C (3)	–C (4)	1.411 (10)
C (3)	–C (9)	1.454 (10)
C (4)	–C (5)	1.471 (11)
C (4)	–N (13)	1.310 (9)
C (5)	–C (6)	1.469 (11)
C (5)	–O (14)	1.449 (9)
C (5)	–C (15)	1.465 (12)
C (6)	–O (7)	1.421 (10)
C (9)	–O (10)	1.189 (10)
C (9)	–O (11)	1.334 (9)
O (11)	–C (12)	1.436 (11)
O (14)	–C (15)	1.395 (11)
C (1)	–H (1)	0.96 (7)
C (6)	–H (6)	0.97 (6)
C (12)	–H (12A)	1.10 (9)
C (12)	–H (12B)	1.03 (10)
C (12)	–H (12C)	0.86 (7)
N (13)	–H (13A)	0.79 (7)
N (13)	–H (13B)	0.96 (6)
C (15)	–H (15A)	0.90 (8)
C (15)	–H (15B)	0.86 (5)

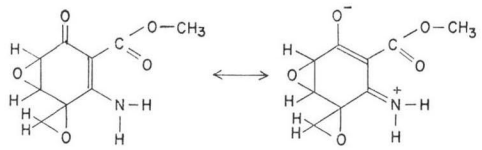
dicate that the π-electrons may be delocalized considerably. The C(4)-N(13) bond of 1.310 Å is significantly shorter than the reported $C_{sp}2$-N single bonds: the aminobenzoic acid derivatives (1.349[5], 1.367[6], 1.378[7] and 1.381 Å[7]) and the complexes of aniline and phenol derivatives (1.437[8] and 1.483 Å[9]). The C-NH_2 bond in 9-substituted adenine acquires, through the protonation at the N-1 position, more double bond character, and is shortened to 1.316 Å[10], which is typical of this compound. Accordingly, the resonance may occur in the molecule involving a dipolar ion structure as shown in Fig. 2.

The atoms C(1), C(3), C(4), C(6) are roughly in a plane and C(2), C(5) are both displaced by 0.21 and 0.35 Å on the same side of this plane. Thus the six-membered ring adopts a boat conformation.

Stereochemistry of Enaminomycins A, B and C

To solve the stereochemical problem, it was necessary to select one of two theoretically postulated isomers for enaminomycin A and one of four isomers for each of enaminomycins B and C (Scheme 2). The problems were in some respects analogous to that encountered in the

Fig. 2. The resonance structure of methylated enaminomycin A.



Scheme 2.

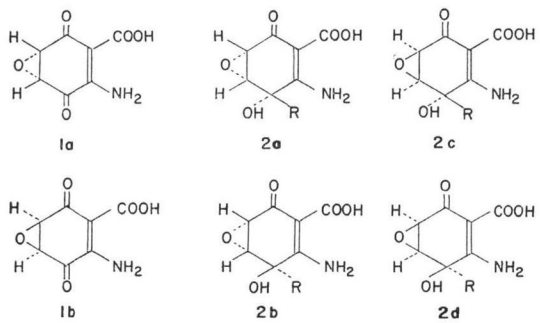


Table 3. Bond angles of methylated enaminomycin A.

			Bond angle(°)
C (2)	–C (1)	–C (6)	119.2 (8)
C (2)	–C (1)	–O (7)	116.6 (7)
C (6)	–C (1)	–O (7)	58.8 (3)
C (1)	–C (2)	–O (8)	116.0 (8)
C (1)	–C (2)	–C (3)	118.5 (8)
C (3)	–C (2)	–O (8)	125.5 (10)
C (2)	–C (3)	–C (4)	118.0 (7)
C (2)	–C (3)	–C (9)	124.6 (10)
C (4)	–C (3)	–C (9)	117.4 (9)
C (3)	–C (4)	–C (5)	120.7 (9)
C (3)	–C (4)	–N (13)	124.5 (9)
C (5)	–C (4)	–N (13)	114.6 (8)
C (4)	–C (5)	–C (6)	116.4 (7)
C (4)	–C (5)	–O (14)	115.6 (7)
C (4)	–C (5)	–C (15)	118.7 (8)
C (6)	–C (5)	–O (14)	114.9 (7)
C (6)	–C (5)	–C (15)	120.2 (7)
O (14)	–C (5)	–C (15)	57.2 (3)
C (1)	–C (6)	–C (5)	116.9 (8)
C (5)	–C (6)	–O (7)	115.2 (7)
C (1)	–C (6)	–O (7)	60.1 (3)
C (1)	–O (7)	–C (6)	61.1 (2)
C (3)	–C (9)	–O (10)	125.4 (14)
C (3)	–C (9)	–O (11)	112.3 (8)
O (10)	–C (9)	–O (11)	122.3 (11)
C (9)	–O (11)	–C (12)	114.5 (6)
C (5)	–O (14)	–C (15)	62.0 (2)
C (5)	–C (15)	–O (14)	60.8 (2)

Table 4. Circular dichroism of enaminomycins A, A-Me, B and C in MeOH.

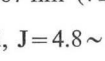
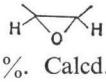
Enaminomycin	Peak (nm)	[ϕ]
A	301	−17200
	271	+19100
A-Me	307	−89900
	280	+94400
B	296	−80400
	269	+94400
C	294	−78000
	268	+64200

determination of the constitution of epoxydon,[11] terremutin[12] and panepoxydon.[13] As reported for epoxydon, terremutin and panepoxydon, an "inverted octant rule[14]" was applied to predict the absolute configurations of enaminomycins. Since the COTTON effect was negative in enaminomycins A, A-Me, B and C (Table 4), enaminomycins A, B and C were respectively assigned the configurations shown in **1a** and in **2a** or **2b**, in similarity to the absolute configuration of terremutin. Moreover, X-ray analysis revealed that the hydroxy group at C_5 of enaminomycin B possesses the same direction as epoxide. Thus the absolute configuration of enaminomycin B was determined as **2a**. From the NMR spectrum of enaminomycin C the spin-spin coupling ($J=2.0\sim2.2$ Hz) was observed between C_4 and C_5 protons, but this was not sufficient to predict the configuration at C_5 of enaminomycin C. In enaminomycin A, the reaction with diazomethane proceeded from less hindered site to yield compound **II**, which was confirmed by X-ray analysis (Fig. 1). It was also assumed that in the reduction of enaminomycin A sodium borohydride attacked from the same site as in the case of diazomethane. The configuration of enaminomycin C was thus determined as **2a**.

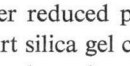
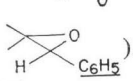
Experimental

Melting points were taken in a Yamato micro-melting point apparatus and were uncorrected. UV spectra were run on a Hitachi 124 recording spectrophotometer, and IR spectra on a Hitachi infrared spectrophotometer. Optical rotations were determined in Perkin-Elmer 241 polarimeter. NMR spectra were run on a Varian model NMR or Hitachi R-24 and chemical shifts were measured to an internal standard, TMS, and recorded as δ values.

Compound **II**.

Ethereal diazomethane was added to an ethyl acetate solution containing 100 mg of enaminomycin A and the mixed solution was allowed to stand for 15 minutes at room temperature with occasional stirring. After removal of the solvent under reduced pressure, the residue was purified on a preparative silica gel thin-layer plate (Merck Co., Ltd., F_{254} 2.0-mm thick) using ethyl acetate as the developing solvent. Compound **II** was eluted from TLC plate with ethyl acetate and obtained as colorless needles (30 mg); m.p. 183°C (dec.); *m/e* 211; UV λ_{max}^{MeOH} ($E_{1cm}^{1\%}$), 248 (377), 287 nm (716); IR ν_{max}^{KBr}, 3340, 3020, 3000, 1663 and 1600 cm^{-1}; NMR $\delta_{ppm}^{CDCl_3}$, 3.09 and 3.20 (2H, d, $J=4.8\sim5.0$ Hz, ), 3.32 and 3.69 (2H, d, $J=4.2\sim4.4$ Hz, ), 3.80 (3H, s, -COO<u>CH</u>$_3$), 6.10 and 9.90 (-NH_2); Found: C, 51.26; H, 4.45; N, 6.46%. Calcd. for $C_9H_9NO_5$: C, 51.19; H, 4.30; N, 6.63%.

Compound **III**.

Sufficient amount of ethereal phenyldiazomethane was added to an ethyl acetate solution containing 40 mg of enaminomycin A, and the mixed solution was allowed to stand overnight at 4°C with occasional stirring. After removal of the solvent under reduced pressure, the residue was dissolved in a small amount of benzene and passed through a short silica gel column (Merck Co., Ltd., Kieselgel 40) packed with benzene. After removal of the solvent under reduced pressure, the residue was purified by preparative silica gel thin-layer chromatography, developed and eluted with ethyl acetate. Compound **III** was obtained as colorless needles (18.5 mg); m.p. 60°C; *m/e* 366; IR $\nu_{max}^{CCl_4}$, 3460, 3280, 3040, 2940, 1670, 1600 and 1500 cm^{-1}; UV λ_{max}^{MeOH} ($E_{1cm}^{1\%}$), 247 (200), 293 nm (330); NMR $\delta_{ppm}^{CDCl_3}$, 3.50 and 3.75 (2H, d, $J=5$ Hz, ), 4.55 (1H, s, ), 5.25 (2H, s, -COO<u>CH</u>$_2$C$_6$H$_5$), 7.40 (10H, -CH$_2$<u>C$_6$H$_5$</u>, ). High resolution mass spectrometry, Found: 363.1119, Calcd. for $C_{21}H_{17}NO_5$: 363.1106.

Compound **V**.

Sufficient amount of ethereal diazomethane was added to an ethyl acetate solution containing

150 mg of enaminomycin B and the mixture allowed to stand for 30 minutes at room temperature with occasional stirring. After removal of the solvent under reduced pressure, the residue was dissolved in a small volume of ethyl acetate and applied to the silica gel column (2×10 cm, Merck Co., Ltd., Kieselgel 40) packed and eluted with a solvent system of benzene - ethyl acetate (2 : 1). Fractions of 5.4 ml were collected. Fractions 110 through 150 were collected and, after removal of the solvent *in vacuo*, the resultant crystalline residue was recrystallized from ethyl acetate to give colorless needles (75.7 mg); m.p. 135°C; IR ν_{max}^{KBr}, 3400, 3270, 3130, 1720, 1667 and 1600 cm^{-1}; NMR $\delta_{ppm}^{CDCl_3}$, 2.23 (3H, s, $-CH_2-COCH_3$), 2.28 and 2.89 (2H, d, J=18 Hz, $-CH_2COCH_3$), 3.53 and 3.88 (2H, d, J=4.5 Hz, ), 3.79 (3H, s, $-COOCH_3$), 5.22 (1H, -OH), 6.90 and 9.65 ($-NH_2$); UV λ_{max}^{MeOH} ($E_{1cm}^{1\%}$), 248 (390), 287 nm (830); Found: C, 51.96; H, 5.32; N, 5.52%. Calcd. for $C_{11}H_{13}NO_6$: C, 51.76; H, 5.13; N, 5.49%.

Compound **VI**.

A 196-mg quantity of compound **V** was dissolved in 2 ml MeOH, 18 mg of sodium borohydride added and the mixture was allowed to stand overnight in an ice-water bath. After addition of 5 ml of distilled water, the reaction mixture was acidified to pH 3.0 with acetic acid, and extracted with ethyl acetate. The extract was dried on anhydrous sodium sulfate and concentrated to give 127.7 mg of **VI** as a colorless oil. UV λ_{max}^{MeOH} ($E_{1cm}^{1\%}$), 251 (325), 286 nm (630); NMR $\delta_{ppm}^{CDCl_3}$, 1.20 [3H, d, J=6 Hz, $-CH_2CH(OH)-CH_3$], 2.0 [2H, d, J=6 Hz, $-CH_2CH(OH)CH_3$], 3.55 and 3.90 (2H, d, J=4.5 Hz, ), 4.28 [1H, multiplet, $-CH_2CH(OH)CH_3$], 7.40 and 9.80 (2H, $-NH_2$); High resolution mass spectrometry, Found: 257.0907, Calcd. for $C_{11}H_{15}NO_6$: 257.0899.

Compound **VII**.

A 127.7-mg quantity of compound **VI** was dissolved in 0.5 ml pyridine and 0.4 ml of acetic anhydride, and allowed to stand in an ice-water bath overnight. After addition of 5 ml of distilled water, the reaction mixture was acidified to pH 3.0 with acetic acid, and extracted with ethyl acetate. The extract was concentrated and further purified on a preparative silica gel thin-layer plate using ethyl acetate as a solvent; the band corresponding to **VII** was eluted with the same solvent. Evaporation of the solvent gave 66.5 mg of **VII** as a colorless oil; *m/e* 299; IR $\nu_{max}^{CHCl_3}$, 3550, 3430, 3280, 3000, 2950, 1730, 1720, 1650 and 1595 cm^{-1}; UV λ_{max}^{MeOH} ($E_{1cm}^{1\%}$), 253 (210), 287 nm (397.5); NMR $\delta_{ppm}^{CDCl_3}$, 1.18 [3H, d, J=6 Hz, $-CH_2CH(OAc)CH_3$], 1.90 (3H, s, -OAc), 1.96 [2H, d, J=6 Hz, $-CH_2CH(OAc)CH_3$], 3.55 and 3.68 (2H, d, J=4.5 Hz, ), 5.15 [1H, multiplet, $-CH_2CH(OAc)CH_3$], 5.43 (1H, -OH), 7.38 and 9.77 (2H, $-NH_2$); High resolution mass spectrometry, Found: 299.1009, Calcd. for $C_{13}H_{17}NO_7$: 299.1005.

Compound **IX**.

A sufficient amount of ethereal diazomethane was added to a methanolic solution containing 40 mg of enaminomycin C, and the reaction mixture allowed to stand for 15 minutes at room temperature. After removal of the solvent under reduced pressure, the residue was purified on a preparative silica gel thin-layer plate (Merck Co., Ltd., F_{254}, 2-mm thick), using ethyl acetate for development and elution of compound **IX**. Finally, **IX** was obtained as colorless needles (27 mg); m.p. 137°C (dec.); *m/e* 199; UV λ_{max}^{MeOH} ($E_{1cm}^{1\%}$), 250 (480), 286 nm (1030); IR ν_{max}^{KBr}, 3360, 3200, 2950, 1710, 1675, 1605 and 1510 cm^{-1}; NMR $\delta_{ppm}^{pyridine\text{-}d_5}$, 3.71 (3H, s, $-COOCH_3$), 3.75 (1H, d, J=4.0, 1.0 Hz, ), 4.00 (1H, dd, J=4.0, 2~2.2 Hz, ), 5.27 (1H, ), 4.86, 8.60 and 9.90 ($-NH_2$ and -OH); Found: C, 47.88; H, 4.74; N, 6.83%. Calcd. for $C_8H_9NO_5$: C, 48.24; H, 4.56; N, 7.03%.

Reduction of enaminomycin A.

A 3.1-mg quantity of sodium borohydride was added to an ethyl acetate solution containing 15 mg of enaminomycin A, and the solution allowed to stand for 30 minutes in an ice-water bath with stirring. After the reaction was stopped by adding 20% phosphoric acid, the solvent was removed under

reduced pressure. The residue was purified on Sephadex LH-20 column (1 × 16 cm), using a solvent system of ethyl acetate - methanol (95 : 5). Five-ml fractions were collected. Fractions 12 through 15 were collected and, after removal of the solvent under vacuum, the resultant crystalline residue was recrystallized from methanol as colorless needles (5.0 mg). The results of UV spectrum, NMR spectrum and mass spectrometry of this compound were consistent with those of enaminomycin C.

Oxidation of enaminomycin C.

One gram of manganese oxide was added to a methanol solution containing 60 mg of enaminomycin C, and the reaction mixture was allowed to stand for 150 minutes at room temperature with stirring. One ml of ethereal diazomethane was added to the filtrate of this reaction mixture, and the mixture allowed to stand for 15 minutes at room temperature. After removal of the solvent under vacuum, the residue was purified on preparative silica gel thin-layer plate (Merck Co., Ltd., F_{254}, 0.25-mm thick) using ethyl acetate for development and elution. Finally, 50 mg of the reaction product were obtained as colorless needles; all physical properties, such as NMR spectrum, UV spectrum and mass spectrometry, were identical with those of compound **II**.

Discussion

Structural elucidation of enaminomycins A, B and C revealed that all three antibiotics are new members of the epoxy quinone family. Enaminomycin C was found to be the reduced form of enaminomycin A and chemical interconversion between A and C by oxidation and reduction was successfully achieved.

Other microbial metabolites of the epoxy quinone family were reported before: terreic acid,[15] terremutin,[12] phyllostine,[16] epoxydon[11] (=phyllosinol[17]), senépoxyde,[18] crotepoxide,[19] panepoxydion,[13] panepoxydon[13] and G7063-2.[20] These compounds are fungal metabolites, except the last one which is produced by a streptomycete.

Stereochemistry of the epoxide part of enaminomycins determined by CD spectra was consistent with that of terremutin but opposite to epoxydon.

Terreic acid and G7063-2 were reported to be active against Gram-positive and Gram-negative bacteria, and epoxydon, crotepoxide, panepoxydion and panepoxydon were described to have antitumor activity; none was reported to be active against both bacteria and tumors. It is of interest that both epoxydon and enaminomycin A are active against tumor cells despite the fact that they are different in the configuration of the epoxide ring attached to the quinone nucleus; this suggests that the configuration of the epoxide ring in relation to the quinone nucleus may be irrelevant to the antitumor activity of the epoxide quinone family.

After completion of the present work, we noticed the report on I-581,[21] an antagonist of chloramphenicol, whose structure (without stereochemistry) was described to be identical with enaminomycin A, but a final identification must await comparative studies of these two substances.

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