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Studies on Pentenomycins. II.[1] The Structures of Pentenomycin I and II, New Antibiotics

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The structures of pentenomycins I and II, new antibiotics produced by *Streptomyces eurythermus* MCRL 0738, were proposed to be 4,5-dihydroxy-5-hydroxymethyl-cyclopent-2-en-1-one and 5-hydroxy-5-hydroxymethyl-4-acetoxy-cyclopent-2-en-1-one respectively by chemical and spectroscopic methods.

The new antibiotics, pentenomycins I and II, which were moderately active against Gram-positive and Gram-negative bacteria were isolated from the culture filtrate of *Streptomyces eurythermus* MCRL 0738.

The isolation and properties of the antibiotics were reported in the preceeding paper.[1] The present paper deals with the structure determination of the antibiotics by chemical and spectroscopic methods. The determination of the absolute configuration of the antibiotics by X-ray analysis will be reported in the succeeding paper.[3]

As previously reported,[1] pentenomycin I was obtained as a hygroscopic white amorphous powder: $C_6H_8O_4 \cdot 1/2H_2O$ (I) and was well characterized as its crystalline triacetate: mp 111—112° (II).

The elemental analysis and molecular ion peak in mass spectrum of II accorded to the molecular formula $C_{12}H_{14}O_7$ for II (Fig. 1).

The nuclear magnetic resonance (NMR) spectrum showed the presence of nine proton signals due to the three acetoxy groups, while the spectrum of I showed none of them as listed in Table I.

Since the IR spectrum of II (Fig. 2) did not indicate the presence of another hydroxyl group, it was concluded that I contained three hydroxyl groups in the molecule. As shown

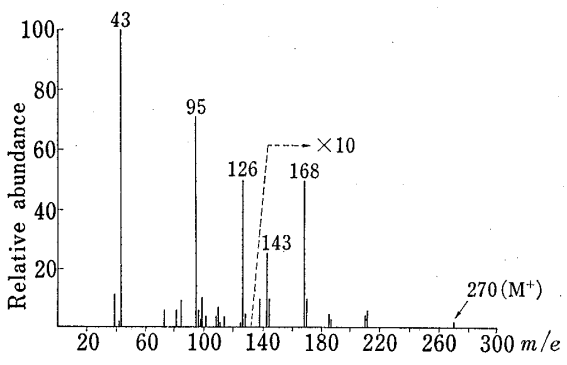


Fig. 1. Mass Spectrum of Pentenomycin I Triacetate (II)

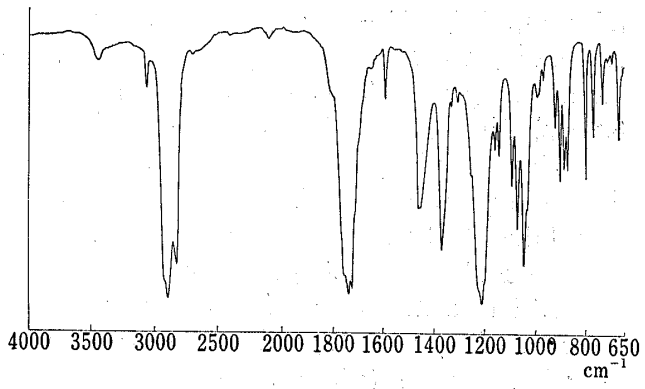


Fig. 2. IR Spectrum of Pentenomycin I Triacetate (II) (in Nujol)

1) Part I: K. Umino, T. Furumai, N. Matsuzawa, Y. Awataguchi, Y. Ito, and T. Okuda, *J. Antibiotics*, **26**, 506 (1973).
2) Location: *Todashi, Saitama.*
3) Part III: T. Date, K. Aoe, K. Kotera, and K. Umino, *Chem. Pharm. Bull.* (Tokyo), to be published.

Table I. NMR Spectral Data (60 MHz)

Compound No.	Solvent	Chemical shift (δ)				J (Hz)				Miscellaneous
		H_2	H_3	H_4	CH_2OH	$J_{2,3}$	$J_{3,4}$	$J_{H_2,OH}$	$J_{2,4}$	
I	D_2O	6.39	7.81	4.79	3.67	6.0	3.0	—	1.5	
	DMSO-d_6	6.58	7.94	4.88	3.81[d]	6.0	3.0	5.5	—	
II	$CDCl_3$	6.60	7.48	5.90	4.40	6.0	3.0	—	1.5	$-COCH_3$: 2.03 (3H, s), 2.07 (3H, s), 2.08 (3H, s)
III	$CDCl_3$	—	—	5.49[t]	4.31	—	—	—	—	$-COCH_3$: 2.02 (9H, s), $-(H_2)_2$ and $-(H_3)_2$: 2.2–3.0 (4H,m)
IV	$CDCl_3$	6.34[d]	7.78	5.29[d]	3.88	6.0	3.0	—	—	$\times^{CH_3}_{CH_3}$: 1.34(3H,s), 1.45(3H,s)
	DMSO-d_6	6.61[d]	7.63	5.20[d]	4.36[d]	6.0	3.0	5.5	—	$\times^{CH_3}_{CH_3}$: 1.45(3H,s),1.58(3H,s), $-CH_2OH$: 5.34 (1H, t, J=5.5)
V	$CDCl_3$	6.31[d]	7.63	5.20[d]	4.36	6.0	3.0	—	—	$\times^{CH_3}_{CH_3}$: 1.01(3H,s),1.33(3H,s), $-COCH_3$: 2.01 (3H, s)
VI	D_2O	6.58	7.86	5.82	3.77	6.0	3.0	—	1.5	$-COCH_3$: 2.14 (3H, s)
	DMSO-d_6	6.45	7.72	5.78	3.52[d]	6.0	3.0	5.5	1.5	$-COCH_3$: 2.01 (3H, s), $+OH$: 5.52 (1H, s) $-CH_2OH$: 4.91 (1H, t, J=5.5)
VII	$CDCl_3$	—	7.60	5.77[d]	4.40	—	3.0	—	—	$-COCH_3$: 2.06(3H,s),2.10(3H, s), 2.11 (3H, s)

Signal multiplicities were represented by s (singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Otherwise suffixed, H_2, H_3, H_4 and CH_2OH were appeared as dd, d, and s, respectively.

in Table I, the NMR spectrum of I suggested the presence of one olefinic group (δ 7.81, 1H, dd and δ 6.39, 1H, dd) and one methine group (δ 4.79, 1H, dd) and one methylene group (δ 3.67, 2H, s). When measured in DMSO-d_6, the methylene signal appeared as a doublet at δ 3.81 (J=5.5 Hz) and this was changed to a singlet by the addition of D_2O, indicating the one of the three hydroxyl groups in I should constitute hydroxymethyl group. The presence of olefinic group in I was also supported by the infrared (IR) spectrum absorption band at 1590 cm^{-1} and by the following hydrogenation experiment of II. Catalytic hydrogenation of II (Pd-C in MeOH) afforded dihydropentenomycin I triacetate (III): $C_{12}H_{16}O_7$ *m/e* 272 (M^+), mp 60°, $[\alpha]_D^{21}$ −152° (*c*=0.34, EtOH). In the NMR spectrum of III, the signals corresponding to the olefinic protons in I have disappeared and the signals arising from two methylene groups (δ 2.2—3.0, 4H, m) appeared. Judging from the coupling constant (J= 6.2 Hz), the configuration of the olefinic protons in I and II was assumed to be *cis*.

This olefinic group in II was further extended to the –CH=CH–CHOAc system by spin decoupling in the NMR measurement of II (100 MHz, Fig. 3). The irradiation of the signal due to the olefinic proton at δ 7.45 simultaneously collapsed the methine proton signal (double doublet at δ 5.83) to a doublet and the double doublet signal of another olefinic proton at δ 6.51 to a doublet having a small long range coupling ($J_{2,4}$=1.5 Hz). Furthermore, the presence of α,β-unsaturated ketone function could be assumed by ultraviolet (UV) spectrum of I: λ_{max}^{MeOH} 216 nm (ε 3750).

Subtracting the above described $C_4H_4O_2$ (–CH–C=C–C–) unit from the molecular formula of I ($C_6H_8O_4$), only $C_2H_4O_2$ (–C–CH_2OH) unit was left. Combination of the both units easily suggests that the structure of I is shown by A.

The UV absorption maximum (216 nm) was quite in agreement with a calculated value

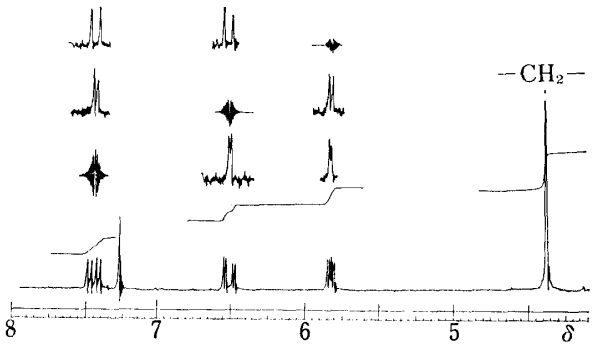
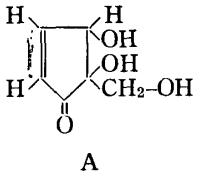


Fig. 3. NMR Spectra of II, 100 MHz in $CDCl_3$

Top three spectra showed the results of spin decoupling experiments.



A

(217 nm) for 2-cyclopentenone.[4] Also, the IR absorption at 1709 cm^{-1} supported cyclopentenone structure[4] and vinyl protons in NMR spectrum corresponded to the known substituted five membered ring systems.[5,6]

The *cis* diol system in A was confirmed by the formation of an isopropylidene derivative. Treatment of I with acetone in the presence of acid catalyst followed by vacuum distillation gave an isopropylidene derivative (IV): $C_9H_{12}O_4$, mp 65—66°. The NMR spectrum of (IV) in DMSO-d_6 showed a doublet signal at δ 4.36 (2H, J=5.5 Hz) due to the methylene protons of a hydroxymethyl group and a triplet signal at δ 5.34 (1H, J=5.5 Hz) arising from the proton of a hydroxyl group. On addition of D_2O, the former changed to a singlet and the latter disappeared, indicating the hydroxymethyl group was free in IV. This was supported by the fact that IV gave colorless syrupy monoacetate (V) $C_{11}H_{14}O_5$ and methylene protons due to the hydroxymethyl were observed in lower field than that of IV in the NMR spectrum. As summarized in Table I, the NMR spectra of IV and V were well in accordance with their structures.

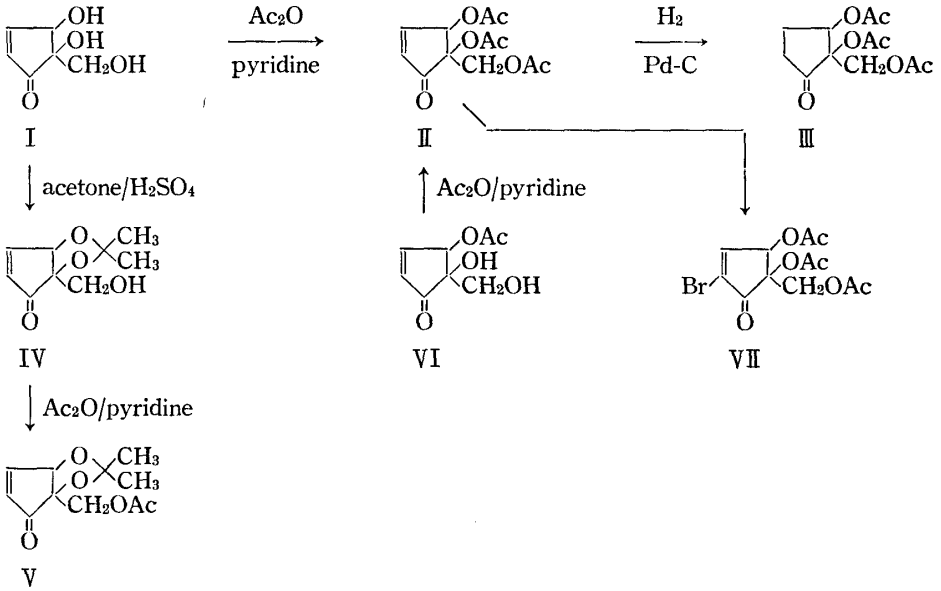


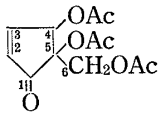
Chart 1. Reaction Schema

4) J.R. Dyer, "Application of Absorption Spectroscopy of Organic Compounds," Prentice-Hall Inc., U.S.A., 1965.
5) G. Stork, G.L. Nelson, F. Roussac, and O. Gringore, *J. Am. Chem. Soc.*, **93**, 3091 (1971).
6) R.A. Coburn and L. Long, Jr, *J. Org. Chem.*, **31**, 4312 (1966).

The reaction schema utilized for the structural determination were outlined in Chart 1. Consequently, the structure of pentenomycin I was proposed to be 4,5-dihydroxy-5-hydroxymethyl-cyclopent-2-en-1-one (A).

Carbon-13 NMR spectrum of pentenomycin I triacetate (II) were also well in accordance with the proposed structure as summarized in Table II.

TABLE II. Carbon-13 Chemical Shifts of Pentenomycin I Triacetate (II)[a]



II

Assignment	δ (ppm)	Assignment	δ (ppm)
C-1	199.6	C-4	72.2[c]
C-2	135.8[b]	C-5	77.2
C-3	154.2[b]	C-6	64.3

a) The spectra were taken at 22.6 MHz on Brucker HFX-10 Fourier transform spectrometer in $CDCl_3$: Chemical shifts are expressed as parts per million down field from TMS.
b) Signal assignments were accomplished by selective decoupling technique.
c) The signal was overlapped to $CDCl_3$, but the chemical shift was acertained by another spectrum taken in CD_3COCD_3.

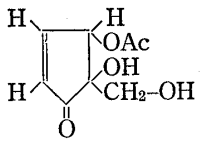
Mass spectrum of II (Fig. 1) gave the following fragment ion peaks which were also well accorded to the triacetate of the proposed structure A. *m/e*: 270 (M^+), 210 (M^+–CH_3COOH), 185 (M^+–(CH_3CO+CH_2CO)), 168 (210–CH_2CO), 143 (185–CH_2CO), 126 (168–CH_2CO), 95 (143–CH_4O_2), 84 (126–C_2H_2O).

On the other hand, pentenomycin II (VI), a minor antibiotic was isolated as a colorless syrup: $C_8H_{10}O_5 \cdot 1/2H_2O$.

The NMR spectrum of VI in D_2O showed the presence of one acetyl group (δ 2.14, 3H, s), one methylene group (δ 3.77, 2H, s), one methine group (δ 5.82, 1H, dd) and one olefinic group (δ 7.86, 1H, dd and δ 6.58, 1H, dd). Comparison of the NMR spectrum of I and VI revealed that the spectrum of VI was essentially identical with that of I except that the former had one additional acetoxy signal and showed lower shift of signals due to H_4. This would be interpreted by thinking that one of the three hydroxy groups of pentenomycin I was substituted with an acetoxyl group in VI. The molecular formula of V $C_8H_{10}O_5 \cdot 1/2H_2O$ and the fact that VI afforded a crystalline peracetate $C_{12}H_{10}O_7$ which was identical with a triacetyl pentenomycin I (II) were not contradictory to this assumption.

As described above, H_4 of VI resonated at a lower field (Δ=1.03 ppm) than that of I, therefore the hydroxyl group on C_4 seemed to be substituted with an acetyl group. The NMR spectrum of pentenomycin II in DMSO-d_6 was most useful to confirm this position.

In addition to the signals arising from H_2, H_3, H_4 and acetoxy groups, a doublet at δ 3.52 (2H, J=5.5, $C\underline{H}_2OH$), a triplet at δ 4.91 (1H, J=5.5, $CH_2O\underline{H}$) and a singlet at δ 5.52 (1H, –C–OH) were obserbed in the spectrum of VI. On addition of D_2O, the above doublet was collapsed to a singlet and simultaneously the above triplet and singlet were disappeared, indicating the hydroxymethyl and the tertiary hydroxyl groups were free in VI. On the basis of these NMR data, it was reasonably concluded that the structure of pentenomycin II was to be 5-hydroxy-5-hydroxymethyl-4-acetoxy-cyclopent-2-en-1-one (B).



B

On treatment of II with bromine in acetic acid afforded monobromopentenomycin I triacetate (VII) $C_{12}H_{13}O_7Br$. The structure of VII was easily determined to be 4,5-diacetoxy-5-acetoxymethyl-2-bromo-cyclopent-2-en-1-one by the interpretation of NMR as shown

in Table I. The compound (VII) gave a promising crystals for X-ray analysis, from which the absolute configurations of pentenomycins I and II were decided as 4,5-dihydroxy-5-hydroxymethyl-(4: S, 5: S)-cyclopent-2-en-1-one and 4-acetoxy-5-hydroxy-5-hydroxymethyl-(4: S, 5: S)-cyclopent-2-en-1-one respectively. The details of the X-ray analysis will be reported in the succeeding paper.[3]

Only a few biologically active substances having a cyclopentenone skeleton have been found in nature: terrein,[7] prostaglandins,[8] crystosporiopsin,[9] methylenomycins.[10] Finding of pentenomycins will add a new member to this group substances.

Experimental[11]

Pentenomycin I (I)——Isolation of pentenomycin I (I) was achieved as already reported[1] and was obtained as an amorphous powder.

Anal. Calcd. for $C_6H_8O_4 \cdot 1/2H_2O$: C, 47.07; H, 5.92. Found: C, 47.99; H, 5.98. $[\alpha]_D^{21}$ −32° (c=0.24, EtOH). UV λ_{max}^{MeOH} (ε): 216 nm (3750). IR ν_{max}^{Nujol} cm^{-1}: 1709 (C=O), 1590 (C=C), 1333, 1240, 1189, 1135, 1103, 1058, 1036, 990, 909, 871, 838, 785.

Pentenomycin I Triacetate (II)——To a solution of I (0.103 g, 0.78 mmole) in pyridine (2 ml) was added acetic anhydride (1.0 g) at 10°, and the mixture was kept standing at room temperature for 24 hr. Then, the mixture was concentrated *in vacuo* to remove pyridine. The concentrate was diluted with water and extracted with ethylacetate.

The organic layer was separated and dried over anhydrous Na_2SO_4. On removal of the solvent brown oil was obtained, which was crystallized from isopropyl ether to give colorless crystals (178 mg, 84% yield). Recrystallization from ethyl ether afforded crystals of pentenomycin I triacetate (II), mp 111—112°. $[\alpha]_D^{21}$ −24° (c=0.36, EtOH). *Anal.* Calcd. for $C_{12}H_{14}O_7$: C, 53.37; H, 5.18; O, 41.48. Found: C, 53.52; H, 5.40; O, 41.42. m/e 270 (M^+). UV λ_{max}^{MeOH} nm (ε): 216 (8770). IR spectrum was shown in Fig. 3. II was also derived by acetylation of pentenomycin II as described after.

Dihydropentenomycin I Triacetate (III)——A solution of II (142 mg, 0.53 mmole) in MeOH (2 ml) was catalytically hydrogenated in the presence of 10% palladium on charcoal (7 mg) at an atomospheric pressure. After 25 min, II absorbed 10.2 ml (0.46 mmole) of hydrogen. The catalyst was then removed by filtration and the filtrate was evaporated to yield crude crystals of III (120 mg), which was recrystallized from ethyl ether to give 87 mg of III as colorless crystals: mp 60°. $[\alpha]_D^{21}$ −152° (c=0.34, EtOH). *Anal.* Calcd. for $C_{12}H_{16}O_7$: C, 52.94; H, 5.88. Found: C, 53.08; H, 6.11. m/e: 272 (M^+). IR ν_{max}^{Nujol} cm^{-1}: 1733 (C=O), 1461, 1221, 1162, 1097, 1060, 1018, 970, 910, 895, 869, 823, 751.

Isopropylidene Derivative of Pentenomycin I (IV)——Pentenomycin I (200 mg) was dissolved in acetone (2 ml) containing catalytic amount of sulfuric acid and the solution was stirred at room temperature. After 16 hrs the solution was neutralized by addition of Na_2CO_3. Resulting salts were removed by filtration and the filtrate was evaporated to yield a pale yellow oil (178 mg). On fractional distillation of the oil, pure IV was distilled at bath temp. 120—140° under the pressure of 1 mmHg. The pure distillate was crystallized to colorless crystals of IV by standing in an ice box: mp 65—66°. *Anal.* Calcd. for $C_9H_{12}O_4$: C, 58.69; H, 6.57; O, 34.75. Found: C, 58.56; H, 6.49; O, 35.13. m/e 184 (M^+).

UV λ_{max}^{MeOH} nm (ε): 211 (4650). IR ν_{max}^{Nujol} cm^{-1}: 3420 (OH), 3270, 3190, 1720 (C=O), 1584 (C=C), 1459, 1368, 1341, 1320, 1279, 1239, 1218, 1168, 1120, 1080, 1065, 1037, 990, 962, 902, 859, 793, 722, 703, 670.

Monoacetate of Isopropylidene Derivative (V)——To a solution of IV (250 mg) in pyridine (3 ml) was added acetic anhydride (1.3 g) and the reaction mixture was kept standing at room temperature overnight. Then the reaction mixture was diluted with H_2O and extracted with ethyl acetate. The organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated to yield a pale brown oil. On fractional distillation, pure syrupy V was distilled at bath temp. 110—130° under 1 mmHg. *Anal.* Calcd. for $C_{11}H_{14}O_5$: C, 58.40; H, 6.24; O, 35.36. Found: C, 58.21; H, 5.98; O, 35.70. IR ν_{max}^{film} cm^{-1}: 2980, 2920, 1746 (C=O), 1722 (C=O), 1598 (C=C), 1452, 1372, 1343, 1220, 1170, 1123, 1087, 1067, 1040, 997, 898, 858, 803.

7) D.H.R. Barton and E. Miller, *J. Chem. Soc.*, **1955**, 1028.
8) A.J. Weinheimer and R.L. Spraggins, *Tetrahedron Letters*, **59**, 5185 (1969).
9) G.M. Strunz, A.S. Court, J. Komlossy, and M.A. Stillwell, *Can. J. Chem.*, **47**, 2087 (1969).
10) Presented by T. Haishi, N. Kitahara, Y. Takiguchi, A. Terahara, S. Sugawara, T. Hatake, S. Tamura, and M. Arai at the Annual Meeting of Agricultual Chemical Society of Japan, Sendai, April 1, 1972.
11) All melting points were uncorrected. The IR spectra were recorded in thin film or nujol mulls with a Hitachi EPIS-5 spectrophotometer. The NMR spectra were measured with a JEOL PS-100 at 100 MHz and JNM-NH·60 at 60 MHz using tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. The mass spectra were determined with a Hitachi RMS-4 spectrometer.

Pentenomycin II (VI)——Pentenomycin II (VI) was obtained as a colorless syrup by the isolation method described previously.[1] $[\alpha]_D^{28}$ −55° (c=1.45, MeOH). *Anal.* Calcd. for $C_8H_{10}O_5 \cdot 1/2H_2O$: C, 49.22; H, 5.68. Found: C, 49.71; H, 5.69. UV $\lambda_{max}^{H_2O}$ nm (ε): 216 (5040). IR ν_{max}^{film} cm^{-1}: 3425 (OH), 2940, 1711 (C=O), 1593 (C=C), 1376, 1238, 1149, 1040, 920, 821.

Pentenomycin II Diacetate——To a solution of VI (49.8 mg) in pyridine (1 ml) was added acetic anhydride (0.2 g) and the mixture was kept standing at room temperature overnight. The solution was evaporated and the residue was extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated. The resulting crude product was purified by recrystallization from ethyl ether to give colorless crystals of pentenomycin II acetate (22.4 mg, 62% yield).

This compounds was identical with II in all respects of physicochemical and spectra data.

Monobromopentenomycin I Triacetate (VII)——A solution of bromine (210 mg, 1.33 mmole) in carbon tetrachloride (2.1 ml) was added dropwise to a stirred solution of II (340 mg, 1.26 mmole) in acetic acid (6.8 ml) and the mixture was stirred at room temperature for 2 hr. Then the solution was poured into water and extracted with carbon tetrachloride. The organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated to give a light brown oil. The oil was crystallized from isopropyl ether. Recrystallization from ethyl ether gave colorless needles (305 mg, yield 69%) of monobromopentenomycin I triacetate (VII), mp 107—108°. *Anal.* Calcd. for $C_{12}H_{13}O_7Br$: C, 41.29; H, 3.72; O, 32.09; Br, 22.89. Found: C, 40.95; H, 3.72; O, 31.17; Br, 23.96. *m/e*: 348, (M^+−1) 350 (M^++1). $[\alpha]_D^{18}$ +59° (c=0.35, $CHCl_3$).

UV λ_{max}^{MeOH}nm (ε): 243 (8007). IR ν_{max}^{Nujol} cm^{-1}: 1745, 1592, 1462, 1371, 1312, 1278, 1250, 1221, 1162, 1124, 1093, 1058, 1038, 935, 916, 882, 854, 840, 766, 712.

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