[Chem. Pharm. Bull. 22(9)2113—2117(1974)]

UDC 547.91.057.09 : 615.33.011.5.015.11

Studies on Pentenomycins. IV.[1] Preparation and Antimicrobial Activities of Pentenomycin Derivatives

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(Received March 2, 1974)

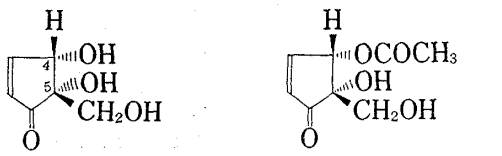
Eleven acyl and alkyliden derivatives of pentenomycin I (I) were synthesized for obtaining an improved antibiotic.

All derivatives synthesized showed same order of or improved antimicrobial activity than the natural antibiotic.

Among these derivatives, 2-bromo-4,5,6-triacetylpentenomycin I (XIV) showed strongest antimicrobial activity.

The new cyclopentenone antibiotics named pentenomycin I (I) and II (II) were isolated from the culture filtrate of *Streptomyces eurythermus* MCRL 0738 in our laboratory.[3]

The chemical structures of I and II were elucidated as (4: S, 5: S)-4,5-dihydroxy-5-hydroxymethyl-cyclopent-2-en-1-one and (4: S, 5: S)-4-acetoxy-5-hydroxy-5-hydroxymethyl-cyclopent-2-en-1-one respectively by chemical and X-ray analysis (Fig. 1).[1,4]



pentenomycin I (I) pentenomycin II (II)

Fig. 1. Structures of Pentenomycins

Both antibiotics showed specific activities against limited strains of Gram negative bacteria as reported previously.[3]

It raised our interest that their antimicrobial activities were almost similar, notwithstanding the hydroxy group at the C-4 position in pentenomycin I was acetylated in pentenomycin II, and further, examination of triacetyl pentenomycin I (III) showed that acylation of three hydroxy groups of I did not decrease antimicrobial activity as shown in Table I.

These observations led us to prepare some acyl derivatives with improved biological activity. Then, five acyl derivatives were prepared by treating I with an appropriate acid anhydride or acid chloride in pyridine solution. The reaction of I with excess butyric anhydride afforded a mixture of di- and tri-acylated derivatives, which were separated by silicagel column chromatography.

However, in the case of isobutyryl, benzoyl and furoyl derivatives only the corresponding diacyl derivatives were obtained. On the preliminary tests on these derivatives, dibenzoyl derivative of I was antibiotically more active than the others. Thus the 4-O-monobenzoyl derivative which seemed to be attractive in the respect of its biological activity, was attempted to synthesize by the route shown in Chart 1. But unfortunately the compound showed rather weak activity than expected.

Chemical structures of these derivatives were proved by nuclear magnetic resonance (NMR), infrared (IR) and ultraviolet (UV) spectra and elementary analysis as shown in Table

1) Part III: T. Date, K. Aoe, K. Kotera and K. Umino, *Chem. Pharm. Bull.* (Tokyo), **22**, 1963 (1974).
2) Location: *Toda-shi, Saitama.*
3) Part I: K. Umino, T. Furumai, N. Matsuzawa, Y. Awataguchi, Y. Ito and T. Okuda, *Journal of Antibiotics*, **26**, 506 (1973).
4) Part II: K. Umino, N. Takeda, Y. Ito and T. Okuda, *Chem. Pharm. Bull.* (Tokyo), **22**, 1233 (1974).

II and III. Especially, NMR spectra of the derivatives in DMSO-d_6 were most useful to confirm the position of an acyl group. As described previously,[4] in the NMR spectrum of II, a doublet at δ 3.52 (2H, J=5.5), a triplet at δ 4.91 (1H, J=5.5) and a singlet at δ 5.52 (1H) were assingned to the underlined proton in $C\underline{H}_2OH$, $CH_2O\underline{H}$ and $-\dot{C}-O\underline{H}$ groups respectively.

Table I. Antimicrobial Activities of Pentenomycin I, II and Pentenomycin I Derivatives
(Serial Agar Dilution Method: M.I.C. mcg/ml)

Compound No.		Test organisms		
		S. aureus 209-P[a]	*B. pertussis* Tohama[b]	*N. gonorrhoeae* Yoshioka[b]
I	pentenomycin I	125	31.2	15.6
II	pentenomycin II	250	31.2	31.2
III	4,5,6-triacetyl pentenomycin I	250	62.5	62.5
IV	4,5,6-tributyryl pentenomycin I	62.5	62.5	62.5
V	4,6-di-*n*-butyryl pentenomycin I	62.5	62.5	31.2
VI	4,6-di-iso-butyryl pentenomycin I	62.5	31.2	15.6
VII	4,6-dibenzoyl pentenomycin I	31.2	7.8	7.8
VIII	4-monobenzoyl pentenomycin I	25	25	25
IX	4,6-difuroyl pentenomycin I	62.5	31.2	31.2
X	4,5-isopropylidene pentenomycin I	250	31.2	31.2
XI	4,5-isobutylidene pentenomycin I	125	15.6	15.6
XII	4,5-(2′-methylisobutylidene) pentenomycin I	125	15.6	15.6
XIII	4,5-benzylidene pentenomycin I	125	15.6	15.6
XIV	2-bromo-4,5,6-triacetyl pentenomycin I	31.2	3.9	3.9

a) Brain Heart Infusion Agar (Difco).
b) Brain Heart Infusion Agar plus 10% horse serum.

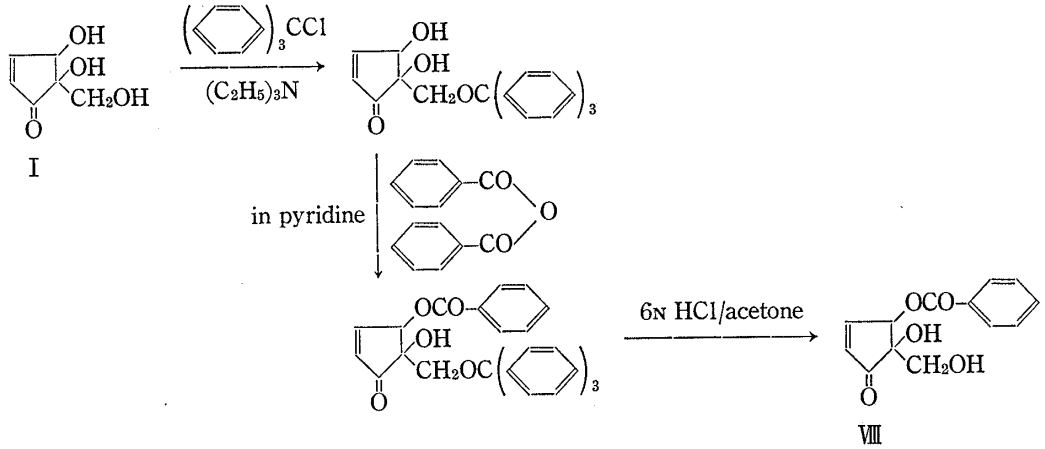
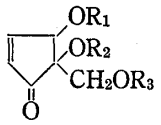


Chart 1. Synthesis of 4-O-Monobenzoyl Pentenomycin I (VIII)

TABLE II. Physical Properties of Pentenomycin I Acyl Derivatives



Compound No.	R_1	R_2	R_3	mp (°C)	Mol formula
III	$COCH_3$	$COCH_3$	$COCH_3$	111—112	$C_{12}H_{14}O_7$
IV	$CO(CH_2)_2CH_3$	$CO(CH_2)_2CH_3$	$CO(CH_2)_2CH_3$	syrup	$C_{18}H_{26}O_7$
V	$CO(CH_2)_2CH_3$	H	$CO(CH_2)_2CH_3$	syrup	$C_{14}H_{20}O_6$
VI	$COCH(CH_3)_2$	H	$COCH(CH_3)_2$	76— 77	$C_{14}H_{20}O_6$
VII	CO-Ph	H	CO-Ph	61— 63	$C_{20}H_{16}O_6$
VIII	CO-Ph	H	H	77— 78	$C_{13}H_{12}O_5$
IX	CO-furyl	H	CO-furyl	103—104	$C_{16}H_{12}O_8$

Compound No.	Analysis (%) Calcd. (Found) C	H	N	UV λ_{max}^{MeOH} nm (ε)	IR $\nu_{C=O}$ cm^{-1}
III	53.33 (53.52)	5.22 (5.40)	41.44 (41.42)	216(8770)	1760, 1740, 1730
IV	61.01 (60.42)	7.40 (7.73)	31.60 (32.19)	215(7190)	1750, 1740
V	59.15 (56.02)	7.09 (7.20)	33.77 (31.60)	214(9030)	1750, 1739, 1725
VI	59.15 (58.97)	7.09 (7.09)	33.77 (33.71)	214(13100)	1735, 1730, 1718
VII	68.17 (67.84)	4.58 (4.63)	27.24 (27.13)	230, 275, 282 (17600, 1590, 1320)	1739, 1722, 1710
VIII	62.89 (62.50)	4.87 (4.72)	32.22 (31.71)	229, 274, 281 (15600, 817, 657)	1758, 1740, 1721
IX	57.83 (56.76)	3.64 (3.60)	38.52 (38.30)	213, 254 (27300, 54000)	1739, 1725, 1704

TABLE III. NMR Spectral Data of Pentenomycin I Derivatives (60 MHz in DMSO-d_6)

Compound No.	Chemical shift[a,b] CH_2OH	CH_2OH	C_5-OH
II	3.52(d)	4.91(t)	5.52(s)
III	4.40(s)	—	—
IV	4.36(s)	—	—
V	4.18(s)	—	6.04(s)
VI	4.14(s)	—	6.06(s)
VII	4.47(s)	—	6.35(s)
VIII	3.53(d)	4.98(t)	5.71(s)
IX	4.84(s)	—	6.29(s)
X	3.89(d)	5.34(t)	—
XI	3.62(d)	5.02(t)	—
XII	3.65(d)	5.10(t)	—
XIII	3.70(d)	5.17(t)	—

a) Chemical shifts are expressed in δ values (ppm from TMS as the internal reference).
b) abbreviations: s=singlet, d=doublet, t=triplet

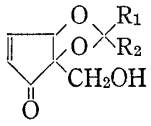
As summarized in Table III, in the NMR spectra of the triacylated derivatives (III, IV), the above described two hydroxy protons disappeared and the methylene protons in the hydroxymethyl group appeared as a singlet. In the NMR spectra of diacylated derivatives (V, VI, VII, IX), only the tertiary hydroxy proton was observed, indicating acyl groups were substituted at the C_4 and C_6 hydroxy groups and the tertiary hydroxy group was intact.

On the other hand, isopropylidene derivative of I, which was prepared for the structural determination, was also found to be biologically active. Then, four alkylidene derivatives were synthesized by reacting I with an appropriate ketone in the presence of catalytic amount of sulfuric acid.

The physico-chemical properties were summarized in Table IV and the structures of these alkylidene derivatives were also determined by checking the hydroxy proton summarized in Table III. There were three structures to be considered for alkylidene derivatives of I but in the NMR spectra of these derivatives the hydroxymethyl group was proved to be free for all alkylidene derivatives.

It was considered that the space distance of C_4–OH and C_5–OH was more favorable than the other possible form of alkylidene derivatives.

TABLE IV. Physical Properties of Pentenomycin I Alkylidene Derivatives



Compound No.	R_1	R_2	mp (°C) or bp (°C/mmHg)[a]	Mole formula	Analysis (%) Calcd. (Found) C	H	O	UV λ_{max}^{MeOH} nm (ε)
X	CH_3	CH_3	65— 66	$C_9H_{12}O_4$	58.69 (58.56)	6.57 (6.49)	34.75 (35.13)	211(4650)
XI	CH_3	CH_2CH_3	140—160/2	$C_{10}H_{14}O_4$	60.59 (59.14)	7.12 (7.46)	32.29 (32.91)	211(3960)
XII	CH_3	$CH<^{CH_3}_{CH_3}$	120—140/2	$C_{11}H_{16}O_4$	62.23 (61.38)	7.60 (7.74)	30.15 (30.32)	212(6300)
XIII	H		105—108	$C_{13}H_{12}O_4$	67.23 (66.92)	5.21 (5.40)	27.57 (27.51)	256, 261, 267 (696, 465, 232)

a) bath temperature

Monobromopentenomycin I triacetate (XIV),[1] a derivative useful for X-ray analysis, showed strongest activity among the derivatives synthesized. As listed in Table I, biological activities of eleven synthetic acyl and alkylidene derivatives of I showed the same order of or improved microbial activity than the natural antibiotics.

Experimental[5]

Isolation of Pentenomycin I (I)——Isolation of I was achieved as already reported.[3]

Acyl Derivatives of Pentenomycin I——a) General Preparation Method: To a pyridine solution of I (1 mmole), excess amount of an acid anhydride (3.5 mmoles) or an acid chloride was added dropwise and kept standing overnight at room temperature. Then, the reaction mixture was evaporated *in vacuo* to remove pyridine and the residue was extracted with EtOAc. The extract was washed twice with 5% sodium carbonate solution and water. The organic layer was separated and concentrated to dryness.

5) All melting points were uncorrected. The IR spectra were recorded in thin film or nujol mulls with a Hitachi EPI-5 spectrophotometer, the NMR spectra were measured with a JNM-NH-60 at 60 MHz using tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. The UV spectra were measured on a Hitachi EPS-3 UV spectrophotometer.

The crude material thus obtained was usually purified by column chromatography on silica gel (Mallinckrodt Silicic Acid CC-7).

The elution was made by $CHCl_3$ for IV, V, VI, VII and IX or by $CHCl_3$: MeOH (100: 2) for VIII. The eluates were monitored on thin-layer chromatography (TLC, Merck Kieselgel GF_{254}) and main fractions containing desired compound were combined and concentrated. The residue thus obtained was recrystallized to give a purified product.

b) 4-O-Benzoyl Derivative (VIII): To a suspended solution of I (898 mg, 6.3 mmoles) in $CHCl_3$ containing trityl chloride (1770 mg, 6.2 mmoles), triethyl amine (1260 mg, 12 mmoles) was added dropwise, then the mixture was stirred under room temperature for one and half hr. After the reaction was completed, the organic layer was washed twice with water, separated, dried and concentrated to dryness to give a syrup (2.5 g). Two spots corresponding to mono- and ditrityl derivatives of I were observed on thin-layer chromatogram.

This crude tritylated product was further subjected to benzoylation by addition of pyridine (5 ml) and benzoic anhydride (1.0 g) without any purification. After the reaction mixture was kept standing overnight at room temperature, the mixture was concentrated *in vacuo* to remove pyridine. The residue was extracted with EtOAc and the organic layer was washed with water. The concentration of EtOAc layer gave syrup of crude benzoyl derivative, which was detritylated in a mixture of 6N HCl (2.6 ml) and acetone (14 ml) at 40°C for one and half hr. The reaction mixture was diluted with water and extracted with EtOAc. The obtained detritylated benzoyl derivative was chromatographed on silica gel (Mallinckrodt CC-7) using $CHCl_3$: MeOH (100: 2) as developing solvent. Fraction 35—38 gave, after recrystallization from EtOH, colorless needles of VIII, mp 77—78° (80 mg).

Alkylidene Derivatives of Pentenomycin I——To a solution of I in an excess ketone, catalytic amount of sulfuric acid was added and then stirred overnight. Then, the solution was neutralized with solid sodium carbonate and the resulting salt was removed by filtration. The filtrate was concentrated *in vacuo*. The obtained crude material was purified by vaccum distillation or recrystallization.

Determination of Antimicrobial Activities——The minimum inhibitory concentration (MIC) of pentenomycin I derivatives were determined by two-fold serial agar dilution method on Brain Heart Infusion Agar (BHI, Difco) with or without 10% horse serum. The test organisms were previously cultivated for 18—24 hours on BHI agar or serum-BHI agar, and one loopful of a suspension containing about 10^5—10^6 viable cells per ml of the test organism was streaked on each assay plate. The plates were incubated at 37° and the antimicrobial readings were made routinely 18 hours later.

Acknowledgement We are indebted to Dr. T. Okuda, Manager of this laboratory, for his interest and advice and to Dr. K. Kotera and his collaborators of the Analytical Center of this company for their elementary and instrumental analysis. Thanks are expressed to Miss Akemi Uno of this laboratory for her technical assistance.