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CHEMISTRY OF AMINOGLYCOSIDE ANTIBIOTICS P-2563 (P) AND (A)

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Two antibiotics* active against Gram-positive and -negative bacteria, P-2563 (P) (I) and (A) (II) were isolated from a cultured broth of Pseudomonas fluorescence P-2563[1]. It was found that I and II have acylaminoglycoside having the same new aminopolyol in their molecule. From their chemical degradation studies and physico-chemical properties structures of I and II were proposed to be 3-O-(4-deoxy-4-propionamido-α-D-glucopyranosyl)-1,4-diamino-1,4-dideoxyhexitol and 3-O-(4-acetamido-4-deoxy-α-D-glucopyranosyl)-1,4-diamino-1,4-dideoxyhexitol, respectively.

Two antibiotics P-2563 (P) (I) and P-2563 (A) (II) were separated by column chromatography of Amberlite IRC-50 (NH_4) from the cultured filtrate of Pseudomonas fluorescence P-2563. I is colorless prisms, monohydrochloride, dihydrate, mp 98-108°C (decomp): $C_{15}H_{31}N_3O_9 \cdot HCl \cdot 2H_2O$, $[\alpha]_D^{23}$ +60.3° (c=1.0, H_2O), pKa' 9.6 and 7.2, UV $\lambda_{max}^{H_2O}$ end absorption, IR ν_{max}^{KBr} 3370, 2910, 1640, 1550, 1150, 1085, 1030, and 940 cm^{-1}, NMR*1) $\delta_{ppm}^{D_2O}$ 1.34 (3H, t, J=7Hz, $-CH_2\underline{CH_3}$), 2.54 (2H, q, J=7Hz, $-CO\underline{CH_2}CH_3$), 5.40 (1H, d, 3.5Hz, anomeric proton), 3.0-4.5 (14H, N,O-methine, N,O-methylene). II is obtained as a base, colorless powder, mp 148-150°C (decomp): $C_{14}H_{29}N_3O_9$, $[\alpha]_D^{23}$ +76.1° (c=1.0, H_2O), pKa' 9.5 and 7.2, UV $\lambda_{max}^{H_2O}$ end absorption, IR ν_{max}^{KBr} 3370, 2950, 1660, 1570, 1390, 1320, 1150, 1080, 1035, and 560 cm^{-1}, NMR*1) $\delta_{ppm}^{D_2O}$ 2.25 (3H, s, $-COC\underline{H_3}$), 5.36 (1H, d, 4.0Hz, anomeric proton), 3 - 4.5 (14H, N,O-methine, N,O-methylene). Both I and II are positive to ninhydrin, Molisch and Ehrlich reagents and do not reduce Fehling's reagent. Both I and II show similar antibacterial activity[1].

N-Acetylation of I with Ac_2O in H_2O gave the diacetate (III), colorless prisms, mp 156-158°C (decomp) and acetylation of III with Ac_2O in pyridine gave

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* The aminoglycoside antibiotics which have appeared in Japanese patent application [TOKKAISHO-51-91389 Bristol BANYU KENKYUSHO K.K.] include ones the same as authors' antibiotics P-2563 (P) and (A) on the physico-chemical properties. The structures of Bu-2183 A and B have been reported, but the details of the determination have not been described. The authors had given the same plane structures to authors' compounds in Japanese patent application [TOKKAISHO-51-123886]. The authors wish to describe the chemical studies of the antibiotics carried out independently; the stereochemistry will be reported later.

the octa-acetate (IV), colorless prisms, mp 199-201°C (decomp), $C_{31}H_{47}N_3O_{17}$, [m/e 733 (M^+)], $[\alpha]_D^{27}$ +65.5° (c=1.0, $CHCl_3$). From these facts, it was assumed two primary amino groups and six hydroxyl groups were present in I. The presence of two primary amino groups was also supported by next results. First, when I was treated with p-methoxybenzaldehyde and the resultant Shiff's base was reduced with $NaBH_4$ and the product was acetylated with Ac_2O in pyridine, the di-N-(p-methoxybenzyl) octa-acetate (V), $C_{47}H_{63}N_3O_{19}$ was obtained. Second, when aqueous solution of I was treated with benzylchloride, and the resultant product was treated with Ac_2O in pyridine, the tetra-N-benzyl-hexa-acetate (VI), colorless prisms, mp 80°C, $C_{55}H_{67}N_3O_{15}$ was obtained.

An alkaline hydrolysis of I yielded propionic acid (VII) and the depropionyl derivative (VIII), colorless powder, mp 130-140°C (decomp), $C_{12}H_{27}N_3O_8$, $[\alpha]_D^{23}$ +82.4° (c=1.0, H_2O), pKa' 8.7 (2 mole) and 6.5 (1 mole). Acetylation of VIII with Ac_2O in pyridine afforded the nona-acetate (IX), colorless prisms, mp 189-191° (decomp), $C_{30}H_{45}N_3O_{17}$, [m/e 719 (M^+)]. $[\alpha]_D^{27}$ +69.0° (c=1.0, $CHCl_3$). Comparison of NMR-spectrum of IX with IV revealed that the propionyl group in IV had been replaced by acetyl group in IX.

Methanolysis of I and VIII with MeOH-HCl for 12 hrs under refluxing afforded the same methyl glycosides of amino sugar (Xa,b), $C_7H_{15}NO_5$ [m/e 193 (M^+), 162 (M^+-OCH_3)] and ninhydrin positive substance (XI). After acetylation of Xa,b with Ac_2O in pyridine, the acetates were chromatographed on silica gel to give XIIa, colorless prisms, mp 138-140°C, $C_{15}H_{23}NO_9$, [m/e 361 (M^+), 330 (M^+-OCH_3)], $[\alpha]_D^{23}$ +156° (c=1.0, $CHCl_3$), NMR*1) $\delta_{ppm}^{CDCl_3}$ 4.94 (1H, d, J=4.0 Hz, anomeric proton) and XIIb, colorless prisms, mp 202°C, $C_{15}H_{23}NO_9$, [m/e 361 (M^+), 330 (M^+-OCH_3)], $[\alpha]_D^{20}$ +0.5° (c=1.0, $CHCl_3$), NMR*1) $\delta_{ppm}^{CDCl_3}$ 4.38 (1H, d, J=7.4Hz, anomeric proton). The structures of XIIa and XIIb were elucidated by the NMR spin-decoupling studies to be methyl 4-amino-4-deoxy-α-D-glucopyranoside tetra-acetate and its anomer, respectively. The physico-chemical data of XIIa were identical with those of methyl 4-amino-4-deoxy-α-D-glucopyranoside tetra-acetate reported by E. J. Reist[2] and H. Agahigian[3].

Table 1. NMR spectrum of 1,4-diamino-1,4-dideoxyhexitol hexa-acetate (XIV) (100MHz in $CDCl_3$)

	H	H	H	H	H	H	H	H	OAc	NH	NH
	3	2	5	4	6a	6b	1a	1b		on C-4	on C-1
δ (PPm)	5.24	4.99	4.91	4.60	4.30	4.05	3.66	3.38	2.12 1.94	6.30	6.17
	q	oct.	oct.	hex.	q	q	oct.	oct.	s	d	q
XIV	1 H	1 H	1 H	1 H	1 H	1 H	1 H	1 H	3H x 6	1 H	1 H
J (Hz)	2.2 8.5	8.5 5.7 4	2.7 5.7 9.7	9.7 2.2	12.5 2.7	12.5 5.7	5.7 14.8	4 14.8		9.7	6 5
	AcO-C-	AcO-C-	AcO-C-	AcN-C-	AcO-C-	AcO-C-	AcN-C-	AcN-C-	O-Ac	AcN-C-	AcN-C-

XI gave colorless prisms from water, mp 108-110°C, $C_6H_{16}N_2O_4$, $[\alpha]_D^{20}$ -1.85° (c=1.0, H_2O), pKa' 8.6, IR ν_{max}^{KBr} 3100-3400, 1550-1600, 1000-1100 cm^{-1}, NMR $\delta_{ppm}^{D_2O}$ 2.8-4.1 (8H, N,O-methine, N,O-methylene). XI is positive to ninhydrin and $KMnO_4$ but negative to Molisch and Fehling's reagents. N-Acetylation of XI with Ac_2O in H_2O gave the di-N-acetate (XIII), mp 81-82°C, $C_{10}H_{20}N_2O_6 \cdot H_2O$, $[\alpha]_D^{25}$ +14.5° (c=1.0, H_2O) and acetylation of XIII with Ac_2O in pyridine gave the hexa-acetate (XIV), mp 115-116°C, $C_{18}H_{28}N_2O_{10}$, [m/e 432 (M^+), 433 (M^++1)], $[\alpha]_D^{23}$ +55° (c=1.0, $CHCl_3$). By the NMR spin-decoupling studies of XIV, all the protons in XIV were unambiguously assigned as shown in Table 1, and the structure of XIV was elucidated to be 1,4-diamino-1,4-dideoxyhexitol hexa-acetate.

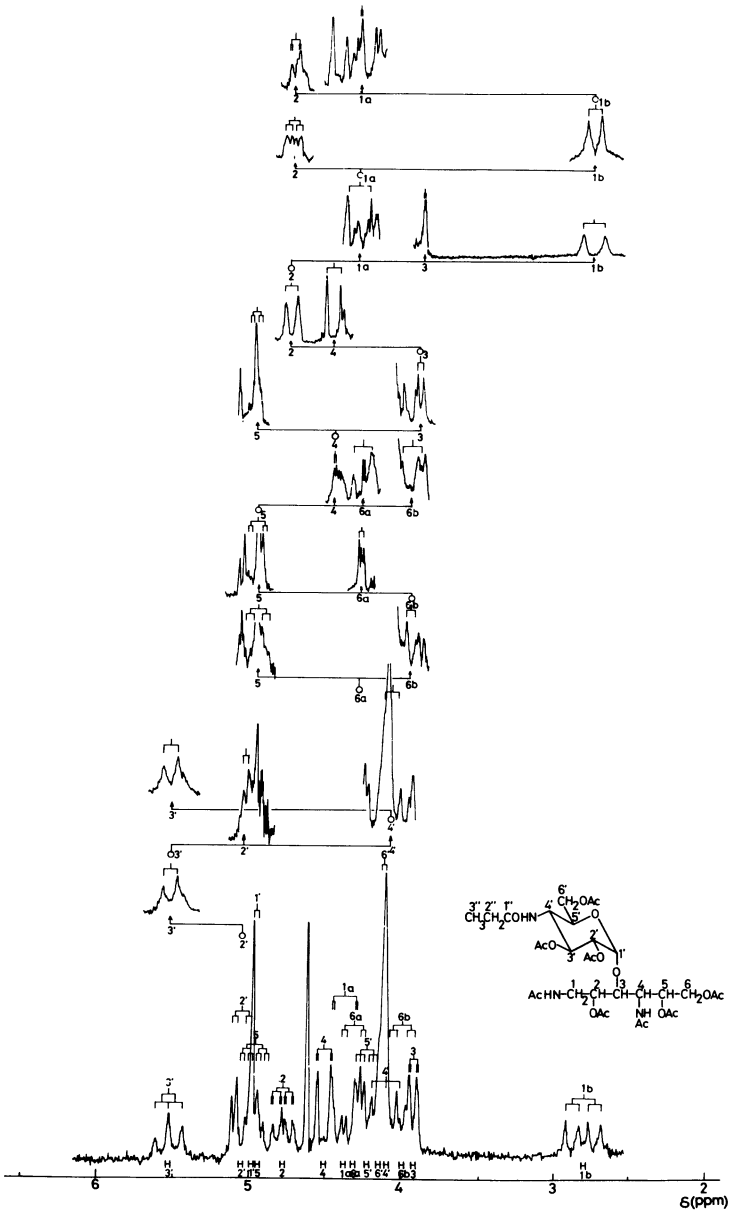


Fig. 1. NMR spectrum and spin-decoupling of IV ($CDCl_3$ + D_2O, 100MHz)

From above results, it was clarified that I is composed of propionyl, 4-amino-4-deoxy-D-glucose and 1,4-diamino-1,4-dideoxyhexitol moieties.

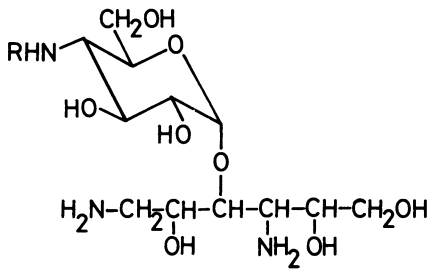
Propionyl group was assumed to be combined with amino group of 4-amino-D-glucose as amido linkage from the following facts; first, the pKa' values of I are 9.6 (1 mole) and 7.2 (1 mole), and those of the depropionyl derivative (VIII) are 8.7 (2 mole) and 6.5 (1 mole); second, the amide group is observed at 1640 cm^{-1} in the IR spectrum of I, third, methanolysis of V gave amino sugar derivative (Xa,b) and di-N-(p-methoxybenzyl)-1,4-diamino-1,4-dideoxyhexitol (XV), mp 134-135°C, $C_{22}H_{32}N_2O_6 \cdot 2HCl \cdot 1\frac{1}{2}H_2O$, [m/e 420 ($M^+$)].

Methanolysis of I gave the methylglycoside (Xa,b) and the aminopolyol, diaminohexanetetraol (XI). By the NMR spin-decoupling studies of IV, all the protons in IV were unambiguously assigned as shown in Fig. 1. The signal due to C_3-H of XIV showed a downfield shift on comparing with that of IV. From these evidence it is assumed that aminosugar combined with C_3-OH of the aminopolyol as glycoside linkage.

The configuration of the glycosidic linkage of I was assigned to be α by the coupling constant of the anomeric proton (J=3.5Hz) and the evidence of $[M]_D$ of VIII, XI, and Xa.

The structure of II was elucidated to be 3-O-(4-acetamido-4-deoxy-α-D-glucopyranosyl)-1,4-diamino-1,4-dideoxyhexitol by the fact that the nona-acetate of II was identical with that obtained from VIII in IR and NMR spectra, optical rotation and mixed melting point.

In conclusion, the structures I and II are proposed for P-2563 (P) (I) and (A) (II), respectively.



(I) R: CH_3CH_2CO-, (II) R: CH_3CO-

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*1) Measured at 100MHz.

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