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# ABSOLUTE CONFIGURATION OF AMINOGLYCOSIDE ANTIBIOTICS P-2563 (P) AND (A)

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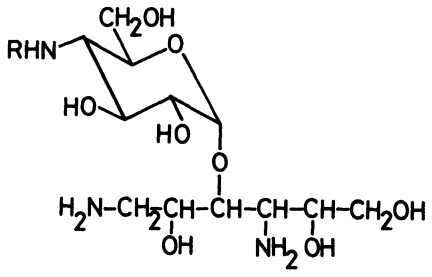
The absolute configuration of the aminopolyol moiety (XI) of the aminoglycoside antibiotics P-2563 (P) (I) and (A) (II) was established to be 2S, 3S, 4R and 5S from the evidence that XI gave methyl 3,6-diacetamido-3,6-dideoxy-α-L-gulopyranoside through the oxidation of the hydroxymethyl group at C-6. From this fact, together with the plane structures of I and II[1] and $^{13}$C-NMR studies, the absolute configuration of I and II were elucidated to be 3-O-(4-deoxy-4-propionamido-α-D-glucopyranosyl)-(2S, 3S, 4R, 5S)-1,4-diamino-1,4-dideoxyhexitol and 3-O-(4-acetamido-4-deoxy-α-D-glucopyranosyl)-(2S, 3S, 4R, 5S)-1,4-diamino-1,4-dideoxy-hexitol, respectively.

In the previous paper,[1] the plane structures I* and II were proposed for P-2563 (P) and (A).



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

In this paper, the stereochemistry of 1,4-diamino-1,4-dideoxyhexitol (XI), I, II and related compounds are described.

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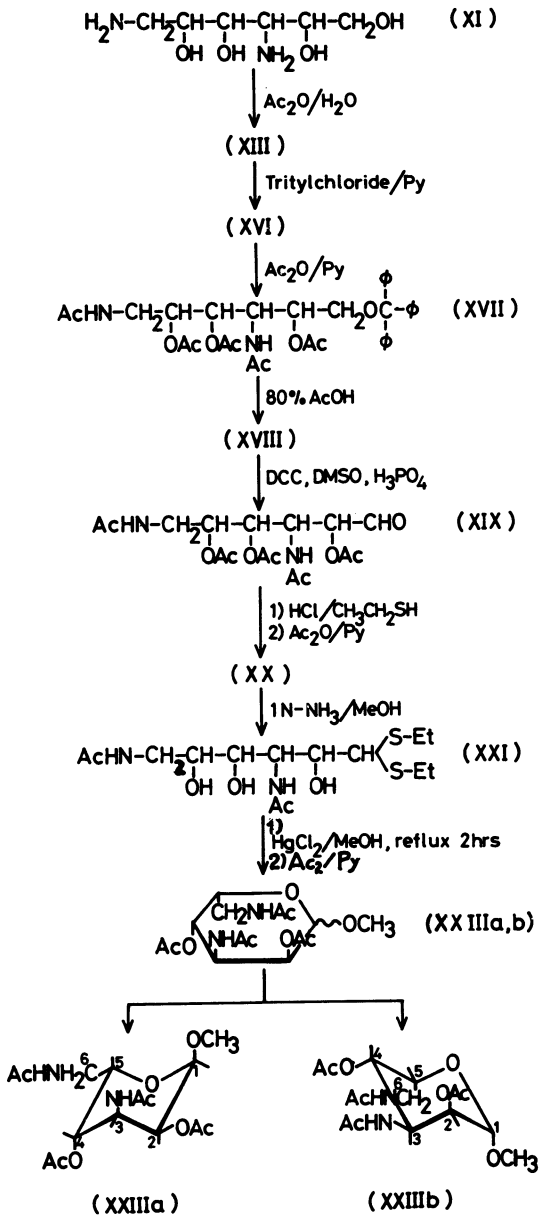
* The compound numbers in this paper are as same as those described in the previous paper.[1]

The oxidation of the hydroxymethyl group of XI at C-6 afforded methyl glycosides (XXIIIa and b) as shown in Scheme 1.




Scheme 1

N-Acetylation, tritylation and O-acetylation of XI afforded 2,3,5-tri-O-acetyl-6-O-trityl-1,4-diacetamido-1,4-dideoxyhexitol (XVII), prisms, mp 105-106°C (decomp), $C_{35}H_{40}N_2O_9$, $[\alpha]_D$ +17.4° (c=0.5, $CHCl_3$). Detritylation of XVII with 80% AcOH gave 2,3,5-tri-O-acetyl-1,4-diacetamido-1,4-dideoxyhexitol (XVIII), prisms, mp 175-180°C (decomp), $C_{16}H_{26}N_2O_9$, $[\alpha]_D^{24}$ +41.8° (c=1.0, $CHCl_3$). Oxidation of XVIII with DCC, anhydrous crystalline orthophosphoric acid and pyridine in DMSO yielded 2,4,5-tri-O-acetyl-3,6-diacetamido-3,6-dideoxyhexose (XIX), prisms, mp 131-135°C (decomp), $C_{16}H_{24}N_2O_9$, $[\alpha]_D^{24}$ +23.7° (c=1.0, $CHCl_3$). Thioacetallization with $CH_3CH_2SH$ and reacetylation of XIX gave 2,4,5-tri-O-acetyl-3,6-diacetamido-3,6-dideoxyhexose diethyl dithioacetal (XX), prisms, mp 183-185°C (decomp), $C_{20}H_{34}N_2O_8S_2$, [m/e 495 ($M^+$+1)], $[\alpha]_D^{24}$ +26.6° (c=0.5, $CHCl_3$). Treatment of XX with 1N-$NH_3$ in MeOH afforded O-deacetyl-derivative (XXI), $C_{14}H_{28}N_2O_5S_2$. Methanolysis of XXI with $HgCl_2$ in MeOH, followed by acetylation and separation gave methyl glycoside tetra-acetate (XXIIIa), $C_{15}H_{24}N_2O_8$, [m/e 360 ($M^+$), 361 ($M^+$+1), 329 ($M^+-OCH_3$)], $[\alpha]_D^{27}$ -25.1° (c=1.0, $CHCl_3$) and its anomer (XXIIIb), $C_{15}H_{24}N_2O_8$, [m/e 360 ($M^+$), 361 ($M^+$+1), 329 ($M^+-OCH_3$)], $[\alpha]_D^{27}$ +59.5° (c=1.0, $CHCl_3$).

The chemical shifts and coupling constants in PMR spectra of XXIIIa and XXIIIb are shown in Table 1.

It was found from these data that (1) a relatively broad long-range coupling of about 1.5Hz between H-1 and H-3, which is ascribed to a "W" letter arrangement of bonds[2] is present, (2) the chemical shift of methoxy proton is 3.48 ppm, indicating that the methoxy group is axial,[3] (3) coupling-constants of ring-protons are relatively small. These findings and Hudson's rule[4] indicated the presence of only six structures [i.e. β-D-glucopyranoside (1C), β-D-galactopyranoside (1C), β-D-mannopyranoside (1C), β-D-talopyranoside (1C), α-L-idopyranoside (1C) and α-L-gulopyranoside (1C)] for XXIIIa, among the probable sixty four structures.

Table I. PMR-Spectra of XXIIIa and b (100MHz in $CDCl_3$ + $D_2O$)

| | | H 2 | H 4 | H 1 | H 3 | H 5 | H 6a | H 6b | Ac | $OCH_3$ |
|---|---|---|---|---|---|---|---|---|---|---|
| XXIIIa | δ (ppm) | 5.16 | 5.00 | 4.89 | 4.40 | 4.05 | 3.54 | 2.97 | 2.18<br>2.10<br>2.04<br>2.00 | 3.48 |
| | J (Hz) | $J_{1-2}$ 3.7<br>$J_{2-3}$ 5.0 | $J_{4-3}$ 4.0<br>$J_{4-5}$ 1.5 | $J_{1-2}$ 3.7<br>$J_{1-3}$ 1.5 | $J_{3-2}$ 5.0<br>$J_{3-4}$ 4.0<br>$J_{3-1}$ 1.5 | $J_{5-4}$ 1.5<br>$J_{5-6a}$ 6.0<br>$J_{5-6b}$ 8.0 | $J_{6a-5}$ 6.0<br>$J_{6a-6b}$ 14.0 | $J_{6b-5}$ 8.0<br>$J_{6b-6a}$ 14.0 | | |
| XXIIIb | δ (ppm) | 4.80 | 4.99 | 4.90 | 5.14 | 3.51 | 3.58 | 3.16 | 2.18<br>2.16<br>2.00 | 3.36 |
| | J (Hz) | $J_{2-1}$ 2.8<br>$J_{2-3}$ 5.0 | $J_{4-3}$ 8.0<br>$J_{4-5}$ 3.0 | $J_{1-2}$ 2.8 | $J_{3-2}$ 5.0<br>$J_{3-4}$ 8.0 | $J_{5-4}$ 3.0<br>$J_{5-6a}$ 5.2<br>$J_{5-6b}$ 8.0 | $J_{6a-5}$ 5.2<br>$J_{6a-6b}$ 15.0 | $J_{6b-5}$ 8.0<br>$J_{6b-6a}$ 15.0 | | |

On the other hand, XXIIIb was assumed to be β-L-guloside (C1) from (1) the chemical shift (3.36 ppm) of methoxy group, indicative of axial configuration,[3] and (2) small coupling-constants of ring-protons, except $J_{3,4}$=8Hz. The downfield shift of H-3 due to 1,3 diaxial deshielding effect[5] supports that XXIIIb is β-L-guloside (C1) which is more stable than 1C form from the energetic point of view.[6]

Separately, the authors synthesized 3,6-diacetamido-3,6-dideoxy-D-gulose from D-glucose by the procedures reported by H. Weidmann.[7]

The methyl glycosidation and O-acetylation of this compound afforded methyl 3,6-diacetamido-2,4-O-diacetyl-3,6-dideoxy-α-D-gulopyranoside (XXIVa), $C_{15}H_{24}N_2O_8$, [m/e 360 ($M^+$), 361 ($M^+$+1), 329 ($M^+-OCH_3$)], $[\alpha]_D^{21}$ +21°, (c=1.0, $CHCl_3$). The physicochemical properties of XXIVa were identical with those of XXIIIa except their specific rotations.

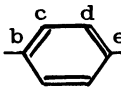
Thus, the structure of XXIIIa was confirmed to be the mirror image of XXIVa.

From these facts, the absolute configuration of XI was concluded to be 2S, 3S, 4R, 5S-1,4-diamino-1,4-dideoxyhexitol.

Next, the confirmation of the configuration of I by the $^{13}C$-NMR-spectrum was carried out. The assignment and chemical shift*1) of XI and its mono-N-alkyl derivative, i.e. 1-N-(p-methoxybenzyl)-1,4-diamino-1,4-dideoxyhexitol (XXVI), prisms, mp 102-104°C, $C_{14}H_{24}N_2O_5 \cdot \frac{1}{2}H_2O$, $[\alpha]_D^{24}$ -9.4° (c=1.0, $H_2O$), obtained by methanolysis of mono-N-(p-methoxybenzyl) derivative of I, are shown in Table II.

Table II. $^{13}C$-NMR-Spectra of XI and XXVI

| | XI | XXVI | XI-XXVI |
|---|---|---|---|
| C-1 | 44.1 (t) | 52.8 or 51.4 (t) | -8.7 or -7.3 |
| C-4 | 55.1 (d) | 55.3 (d) | -0.2 |
| C-6 | 63.8 (t) | 63.9 (t) | -0.1 |
| C-3 | 70.8 (d) | 71.4 (d) | -0.6 |
| C-5 | 73.2 (d) | 73.3 (d) | -0.1 |
| C-2 | 75.1 (d) | 72.7 (d) | +2.4 |

In the spectrum of XI, three of six peaks at 44.1 (t) ppm, 63.8 (t) ppm and 55.1 (d) ppm were directly assigned to C-1, C-6, and C-4 by the chemical shifts and off-resonance experiment. Each peak at 70.8 (d) ppm, 73.2 (d) ppm or 75.1 (d) ppm was remained as C-2, 3 or 5. The six peaks of p-methoxybenzyl group in XXVI [  ] were assigned as follows: C-a, 52.8 or 51.4 (t); C-b, 128.1 (s); C-c, 131.0 (d); C-d, 115.1 (d); C-e, 159.6 (q) ppm. A downfield shift [52.8 or 51.4 (t) ppm] of C-1 from 44.1 (t) ppm in XI and an upfield shift [72.7 (d) ppm] of an O-methine carbon from 75.1 (d) ppm were observed, while other peaks remained relatively constant. It has been reported by G. C. Levy et al.[8] that N-alkyl substituents cause a downfield shift at the carbon of attachment and a small upfield shift (2-4 ppm) at the carbon of α-position (C-2) from C-N-alkyl and that influence of substituents is primarily over a short range. From these facts, remaining three peaks were assigned as shown in Table II.

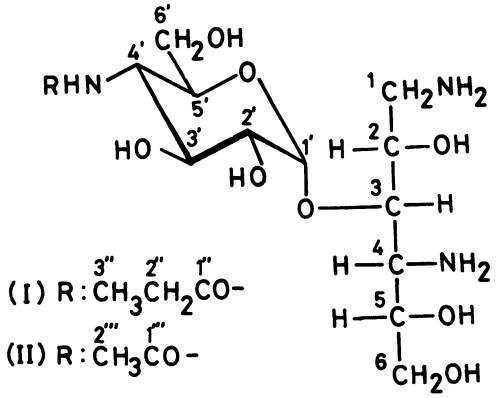
$^{13}$C-NMR chemical shifts of I is shown in Table III. By comparing these data with those of propionic acid[9] (III), methyl 4-acetamido-4-deoxy-α-D-glucopyranoside (XXVa), prisms, mp 183-185°C (decomp), $C_9H_{17}NO_6$, $[\alpha]_D^{24}$ +158° (c=1.0, $H_2O$), and 1,4-diamino-1,4-dideoxyhexitol (XI), all of the chemical shifts in I were assigned as shown in Table III.

Table III. $^{13}$C-NMR-Spectra of I, III, XXVa and XI

| | I | III | XXVa | XI |
|---|---|---|---|---|
| C-1" | 179.1(s) | 180.4(s) | | |
| 2" | 30.1(t) | 27.8(t) | | |
| 3" | 10.4(q) | 9.0(q) | | |
| 1' | 101.1(d) | | 100.1(d) | |
| 2' | 72.9(d) | | 72.5(d) | |
| 3' | 72.4(d) | | 71.5(d) | |
| 5' | 71.1(d) | | 71.5(d) | |
| 6' | 61.6(t) | | 61.7(t) | |
| 4' | 52.3(d) | | 52.4(d) | |
| 3 | 80.9(d) | | | 70.8(d) |
| 2 | 74.5(d) | | | 75.1(d) |
| 5 | 73.1(d) | | | 73.2(d) |
| 6 | 63.8(t) | | | 63.8(t) |
| 4 | 54.5(d) | | | 55.1(d) |
| 1 | 43.9(t) | | | 44.1(t) |
| $CH_3O$- | | | 55.9(q) | |
| $\underline{C}H_3CO$- | | | 23.0(q) | |
| $CH_3\underline{C}O$- | | | 175.1(s) | |

The configuration of the glycosidic linkage between aminosugar and aminopolyol was concluded to be α from the fact that chemical shift of C-1' (101.1 ppm) was more consistent with that (100.1 ppm) of XXVa than that (104.1 ppm) of methyl 4-acetamido-4-deoxy-β-D-glucopyranoside (XXVb), prisms, mp 300-302°C (decomp), $C_9H_{17}NO_6$, $[\alpha]_D^{24}$ +2° (c=1.0, $H_2O$).

Considering that the absolute configuration of aglycone is held by methanolysis with HCl in MeOH, the absolute configuration of I and II, which are drawn according to Fischer's rule, are proposed for P-2563 (P) and (A), respectively.




This stereo structure has been supported by X-ray analysis, the details of which will be reported elsewhere.

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*1) The $^{13}C$-NMR-spectra were obtained at 25.2MHz on VARIAN XL-100 12. The samples were examined as 10% aqueous solution containing approximately 2% (v/v) of 1,4-dioxane to serve as an internal reference. The $^{13}C$-shifts obtained were converted to the TMS scale.

Authors' comments after the contribution.

The authors' independent work has revealed that the structures of P-2563 (P) and (A) are identical with those of Sorbistin $A_1$ and B, respectively. M. Konishi, S. Kamata, T. Tsuno, K. Numata, H. Tsukiura, T. Naito, and H. Kawaguchi, J. Antibiotics XXIX, No. 11, 1152 (Nov. 1976); also presented at the 203rd Scientific Meeting of Japan Antibiotics Research Association Nov. 26, 1976. (Dec. 20, 1976)

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