Chemical Modification of Validamycin A

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The chemical modification of validamycin A was examined. One of the synthetic validamycin analogs, *N*[(1*S*)-(1,4,6/5)-4,5,6-trihydroxy-3-hydroxymethyl-2-cyclohexenyl][*O*-β-2-amino-2-deoxy-D-glucopyranosyl-(1→3)-(1*S*)-(1,2,4/3,5)-2,3,4-trihydroxy-5-hydroxymethyl-cyclohexyl] amine monohydrochloride had almost the same activity against the sheath blight of rice plants as that of validamycin A. Partial synthesis of validamycin A was also examined.

Validamycin A¹⁾ has been found as a main component of validamycin complex²⁾ effective against the sheath blight of rice plants. Validamycin A is about twenty times more active than its D isomer only due to the difference in anomeric configuration of the glucosidic linkage. In addition, validoxylamine A lacking D-glucose still has one-tenth of the activity of validamycin A.³⁾ Thus, the chemical modifications of the sugar moiety as well as the glycosidic linkage of validamycin might be interesting in elucidating the structure-activity relationship of validamycins.

We here report the synthesis of validamycin analogs *via* a validoxylamine A derivative prepared from natural validamycin A, and

their inhibitory effects against the sheath blight of rice plants. Partial synthesis of validamycin A is also described.

RESULTS AND DISCUSSION

Validamycin A (**Iβ**) was treated with benzyl chloride and sodium hydroxide in dry dimethyl sulfoxide to give a fully benzylated derivative of validamycin A (**IIβ**) in a good yield. The D-glucose portion of **IIβ** was hydrolyzed in a mixture of acetic acid and aq. hydrochloric acid. The *N*[(1*S*)-1,4,6/5)-4,5,6-tribenzyloxy-3-benzyloxymethyl-2-cyclohexenyl][(1*S*)-(1,2,4/3,5)-2,4-dibenzyloxy-5-benzyloxymethyl-3-hydroxy-cyclohexyl]amine (**IIIb**, a validoxylamine A derivative) obtained was coupled with 1,3,4,6-tetra-*O*-acetyl-2-(2,4-dinitrophenyl)amino-2-deoxy-α-D-glucopyranosyl bromide (**IV**)⁴⁾ to synthesize validamycin analogs in which D-glucose is replaced by D-glucosamine. For the partial synthesis of validamycin A, **IIIb** was glycosylated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride (**V**).⁵⁾

The coupling of **IIIb** with **IV** was conducted by a modified Konigs-Knorr reaction using silver carbonate and silver perchlorate as catalysts in three different solutions shown in Table II. When the reaction was conducted in benzene-dioxane (9:1), α-glycoside (**VIIα**) was obtained as a major product.

TABLE I. SYNTHETIC VALIDAMYCIN
DERIVATIVES

Compound ^a	R ₁ ^b	R ₂ ^b	R ₃ ^b
Iβ (Validamycin A)	H	H	OH
IIβ	Bn	Bn	OBn
VIβ	Bn	Ac	OAc
VIIα , VIIβ	Bn	Ac	NHDNP
VIIIα , VIIIβ	Bn	H	NHDNP
IXα , IXβ	Bn	H	NHAc
Xα , Xβ	Ac	Ac	NHAc
XIα , XIβ	H	H	NHAc
XIIα , XIIβ	H	H	NH ₂ ·HCl

^a α or β indicates configuration of the glycosidic linkage.

^b Bn=CH₂Ph; Ac=COCH₃; DNP=2,4-dinitrophenyl.

The formation of β -glycoside (**VII β**), however, became predominant with an increase in polarity of solvents used, and in nitromethane only **VII β** was isolated. If benzene-nitromethane (1:1) is used, both isomers are simultaneously obtainable and they are readily separable by column chromatography on silica gel.

Compound (**VII α**) or (**VII β**) was treated with methanolic sodium methoxide to remove the *O*-acetyl group and then with 2 N sodium hydroxide in acetone to remove the *N*-dinitrophenyl group. The resulting free amino group was acetylated with acetic anhydride in methanol to give **IX α** or **IX β** , respectively. To prepare unprotected validamycin analogs, seven benzyl groups in **IX α** or **IX β** must be removed without giving any damage to the double bond on the valienamine⁶⁾ moiety, since the biological activity of validamycin A is lost by hydrogenation. This problem was solved by use of sodium metal in liquid ammonia as follows. Compound (**IX α**) or (**IX β**) in tetrahydrofuran and sodium metal were alternately added to liquid ammonia with stirring to afford a completely debenzylated material which was

subsequently acetylated for purification. The pure **X α** or **X β** was converted, *via O*-deacetylation, into validamycin analog **XI α** or **XI β** having validamycin D or A configuration, respectively, which was further transformed into compound **XII α** or **XII β** by treatment with hydrazine hydrate.

In examination of the partial synthesis of validamycin A, the condensation of **IIIb** with glucosyl chloride (**V**) was conducted in nitromethane-benzene (1:1). The resulting glucoside (**VI β**), however, could not be converted into unprotected validamycin A, since the part of D-glucose was subject to elimination even on a silica gel column. Such a phenomenon was also observed in the courses of isolation and purification of validamycin A producing a lot of undesirable validoxylamine A lacking D-glucose as a major byproduct. On the other hand, the synthetic validamycin derivatives in which D-glucose is replaced by D-glucosamine were very stable against such chemical treatments.

TABLE III. INHIBITORY EFFECT OF VALIDAMYCIN ANALOGS ON THE EXPANSION OF THE SHEATH BLIGHT OF RICE PLANTS

Compound (30 ppm)	Expanding rate (%) ^a
Iβ (Validamycin A)	0
XIα	80
XIβ	41
XIIα	36
XIIβ	4
Untreated	100

^a [Average length of lesion per stem treated with drugs/Average length of lesion per stem untreated] $\times 100\%$ for 10 days.

TABLE II. SOLVENT EFFECT ON GLYCOSYLATION OF **IIIb** BY **IV**.

Solvent	Glycosylation Yield (%) ^a	
	VIIα	VIIβ
Benzene: Dioxane (9:1)	62	13
Benzene: Nitromethane (1:1)	31	28
Nitromethane	trace	46

^a % isolated.

The effects of the synthetic validamycin analogs on the sheath blight of rice plants were tested^{1a)} (Table III). The analogs in validamycin A series (**XI β** and **XII β**) were always more effective than those in validamycin D series (**XI α** and **XII α**) indicating that the β -glycosidic linkage is essential for higher activities. Among these analogs, **XII β** was most active and inhibited the expansion of lesion on rice plants as effectively as validamycin A itself.

EXPERIMENTAL

Melting points are uncorrected. Specific rotations were determined with a Yanagimoto OR-50 polarimeter, and IR spectra were recorded with a Jasco IR-A spectrophotometer. NMR spectra were recorded at 90 MHz with a Hitachi R-22 spectrometer, and the data were confirmed by use of decoupling techniques. Preparative chromatography was performed on 300-mesh silica gel (Wako Co) with solvent systems specified. Solvents used for glycosylation were dried over sodium (benzene, dioxane) or Drierite (nitromethane), and distilled. Evaporations were conducted *in vacuo*.

N[(1*S*)-(1, 4, 6/5)-4, 5, 6-tribenzyloxy-3-benzyloxymethyl-2-cyclohexenyl] [O- β -2, 3, 4, 6-tetra-O-benzyl-D-glucopyranosyl-(1 $\rightarrow$ 3)]-(1*S*)-(1, 2, 4/3, 5)-2, 4-dibenzyloxy-5-benzyloxymethyl-3-hydroxy-cyclohexyl] amine (**II β**). To a solution of validamycin A (**I β**) (10 g) in dimethyl sulfoxide (500 ml) was added powdered sodium hydroxide (90 g), and the mixture was stirred for 2 hr at room temperature. Benzyl chloride (150 ml) was gradually added to this mixture and stirring was continued for 45 hr at 70°C. Benzyl chloride (50 ml) was further added and the reaction was continued for another 3 hr at 85°C. The suspension was filtered with filter aid and washed with benzene. The filtrate and washings were combined and evaporated to a syrup, which was then dissolved in benzene (400 ml) and successively washed with water, 2M hydrochloric acid, and water, dried and evaporated. The residual syrup underwent distillation to remove most of the dibenzyl ether, and chromatographed on a column of silicic acid (250 g) with benzene and benzene-methanol (300:1). The latter eluate yielded the title compound (**II β**) as a syrup (18.1 g, 62%), IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 3320 (NH), 3100~2800 (CH), 2000~1700, 1600~1400, 1020~1150, 740 and 690 (OCH₂Ph); NMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 5.87 (1H, olefinic) and 7.1~7.4 (55H, Phenyl). *Anal.* Found: C, 78.15; H, 6.80; N, 0.93. Calcd. for C₉₇H₁₀₁NO₁₈: C, 78.25; H, 6.84; N, 0.94%.

N[(1*S*)-(1, 4, 6/5)-4, 5, 6-tribenzyloxy-3-benzyloxymethyl-2-cyclohexenyl] [(1*S*)-(1, 2, 4/3, 5)-2, 4-dibenzyloxy-5-benzyloxymethyl-3-hydroxycyclohexyl] amine (**IIIb**). A solution of **II β** (13 g) in acetic acid (26 ml) and 6N hydrochloric acid (13 ml) was stirred for 20 hr at 50~55°C. The mixture was concentrated by evaporation at 40~50°C to give a syrup which was neutralized with Amberlite IRA-410 ion-exchange resin in acetone (100 ml). The suspension was filtered and the filtrate was evaporated to a syrup from which both of **IIIa** and **IIIb** are obtainable by column chromatography. In routine work, this syrup was successively treated with catalytic amount of sodium methoxide in benzene-methanol (1:1) solution. After neutralized with Amberlite IRC-50, the mixture was filtered and the filtrate was evaporated to a syrup which was chromatographed on a column of silicic acid. The eluate from benzene-methanol (300:1) yielded the title compound (**IIIb**) as a syrup (2.91 g, 57%), IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 3600-3000 (NH, OH), 3100~2800 (CH), 2000~1700, 1600~1400, 1150~1020, 740 and 690 (OCH₂Ph); NMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 5.88 (1H, olefinic) and 7.1-7.4 (35 H, Phenyl). *Anal.* Found: C, 78.25; H, 7.22; N, 1.29. Calcd. for C₈₃H₈₇NO₈: C, 78.31; H, 6.99; N, 1.45%. (**IIIa**), IR $\nu_{\text{max}}^{\text{film}}$: 3320 (NH), 3100~2800 (CH), 1740 and 1240 (OAc), 2000~1700, 1600~1400, 1150~1020, 740 and 690 (OCH₂Ph); NMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 1.88 (3 H, OAc), 5.9 (1 H, olefinic), and 7.1~7.4 (35 H, Phenyl). *Anal.* Found: C, 77.21; H, 7.16; N, 1.10. Calcd. for C₈₆H₈₉NO₉: C, 77.43; H, 6.90; N, 1.39%.

N[(1*S*)-(1, 4, 6/5)-4, 5, 6-tribenzyloxy-3-benzyloxymethyl-2-cyclohexenyl] [O- β -2, 3, 4, 6-tetra-O-acetyl-D-glucopyranosyl-(1 $\rightarrow$ 3)]-(1*S*)-(1, 2, 4/3, 5)-2, 4-dibenzyloxy-5-benzyloxymethyl-3-hydroxy-cyclohexyl] amine (**VI β**). A mixture of **IIIb** (4.56 g), Drierite (11 g), silver carbonate (8 g), benzene (40 ml) and nitromethane (40 ml) was stirred overnight at 30°C. To this mixture was added the chloride (**V**) (8 g) and silver perchlorate (110 mg), and the mixture was stirred overnight at 40~45°C. The suspension was filtered and the filtrate was evaporated to a syrup which was chromatographed on a column of silicic acid. The eluate from chloroform-methanol (200:1) yielded the title compound **VI β** as a syrup (2.02 g, 32%), $[\alpha]_D^{25} + 52.5^\circ$ ($c=1.6$, CHCl₃); IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 3320 (NH), 3100~2800 (CH), 1750, 1240 and 1220 (OAc), 1150~1020, 740 and 690(OCH₂Ph); NMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 1.8, 2.0, 2.20 and 2.04 (12H, OAc), 5.72 (1H, anomeric), 5.89 (1H, olefinic), and 7.1~7.4 (35H, Phenyl). *Anal.* Found: C, 71.05; H, 6.60; N, 1.22. Calcd. for C₇₇H₈₅NO₁₇: C, 71.33; H, 6.61; N, 1.08%.

N[(1*S*)-(1,4,6/5)-4,5,6-tribenzyloxy-3-benzyloxymethyl-2-cyclohexenyl] [O- α , β -3,4,6-tri-O-acetyl-2-(2,4-dinitro-

phenyl) amino-2-deoxy-D-glucopyranosyl-(1→3)-(1S)-(1,2,4/3,5)-2,4-dibenzyloxy-5-benzoyloxymethyl-3-hydroxy-cyclohexyl] amine (**VIIa** and **VIIβ**).

Procedure a): A mixture of **IIIb** (5.94 g), Drierite (14 g), silver carbonate (9.4 g), benzene (45 ml) and nitromethane (45 ml) was stirred overnight at 30°C. To this suspension was added the bromide **IV** (10 g) and silver perchlorate (93 mg), and the mixture was stirred for 5 hr at 30°C and filtered. The filtrate was evaporated to a syrup which was chromatographed on a column of silicic acid. The title compounds **VIIa** (2.31 g, 31%) and **VIIβ** (2.05 g, 28%) were obtained from the chloroform-methanol (400:1) and (300:1) eluates, respectively.

Procedure b): The coupling reaction using **IIIb** (5.25 g), Drierite (13 g), silver carbonate (9 g), the bromide **IV** (8 g) and silver perchlorate (90 mg) was conducted in the mixture of benzene (72 ml) and dioxane (8 ml) in the same manner as that used in procedure a) to give **VIIa** (4.12 g, 62%) and **VIIβ** (0.87 g, 13%).

Procedure c): The same coupling reaction as described above was performed in nitromethane (80 ml) using **IIIb** (6.98 g), drierite (15 g), silver carbonate (10 g), the bromide **IV** (10 g) and silver perchlorate (100 mg) to afford only **VIIβ** (4.08 g, 46%).

Compound (**VIIa**) (yellow amorphous): mp 57~79°C, $[\alpha]_D^{25} + 29^\circ$ ($c=1$, CHCl_3); IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3320 (NH), 3100-2800 (CH), 1740 and 1220 (OAc), 1620~1500 (NO_2), 1150~1020, 740 and 690 (OCH_2Ph); NMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 1.72-2.0 and 2.02 (9 H, OAc), 5.9 (2 H, anomeric and olefinic), 6.8~7.4 (35 H, Phenyl), and 8.05, 8.92 and 9.03 (3 H, dinitrophenyl). *Anal.* Found: C, 68.38; H, 6.42; N, 3.71. Calcd. for $\text{C}_{81}\text{H}_{88}\text{N}_4\text{O}_{19}$: C, 68.53; H, 6.11; N, 3.95%.

Compound (**VIIβ**) (yellow amorphous): mp 52~54°C, $[\alpha]_D^{25} - 23^\circ$ ($c=1$, CHCl_3); IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3320 (NH), 1740 and 1240 (OAc), 1620~1500 (NO_2), 1150~1030, 740 and 690 (OCH_2Ph); NMR $\delta_{\text{MeSi}}^{\text{CDCl}_3}$: 1.83, 2.0 and 2.02 (9 H, OAc), 5.81 (1 H, olefinic), 6.8~7.5 (35 H, Phenyl), 7.88, 8.28 and 8.78 (3 H, dinitrophenyl). *Anal.* Found: C, 68.34; H, 6.29; N, 3.68. Calcd. for $\text{C}_{81}\text{H}_{88}\text{N}_4\text{O}_{19}$: C, 68.53; H, 6.11; N, 3.95%.

N [(1S)-(1,4,6/5)-4,5,6-tribenzyloxy-3-benzoyloxymethyl-2-cyclohexenyl] [O- α , β -2-(2,4-dinitrophenyl) amino-2-deoxy-D-glucopyranosyl-(1→3)-(1S)-(1,2,4/3,5)-2,4-dibenzyloxy-5-benzoyloxymethyl-3-hydroxy-cyclohexyl] amine (**VIIIa** and **VIIIβ**). A mixture of **VIIa** (2.31 g) or **VIIβ** (2.05 g), benzene (15 ml), methanol (35 ml) and a catalytic amount of sodium methoxide was stirred for 3 hr at room temperature, and then neutralized with Amberlite IRC-50 ion-exchange resin. The suspension was filtered and the filtrate was evaporated to give **VIIIa** (2.28 g, 98%) or **VIIIβ** (1.58 g, 85%), respectively.

Compound (**VIIIa**) (yellow amorphous): mp 60~62°C; $[\alpha]_D^{25} + 10^\circ$ ($c=0.5$, CHCl_3); IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 3600~3200 (NH, OH), 3100~2800 (CH), 1620~1500 (NO_2), 2000~1700, 1150~1020, 740 and 690 (OCH_2Ph).

Compound (**VIIIβ**) (yellow amorphous): mp 67~69°C; $[\alpha]_D^{25} - 22^\circ$ ($c=0.5$, CHCl_3); IR data is the same as that of **VIIIa**.

N [(1S)-(1,4,6/5)-4,5,6-tribenzyloxy-3-benzoyloxymethyl-2-cyclohexenyl] [O- α , β -2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-(1S)-(1,2,4/3,5)-2,4-dibenzyloxy-5-benzoyloxymethyl-3-hydroxy-cyclohexyl] amine (**IXa** and **IXβ**). A mixture of **VIIIa** (2.9 g) or **VIIIβ** (2.0 g), acetone (20 ml) and 2N sodium hydroxide (20 ml) was stirred for 2 hr at 50°C. The acetone was removed by evaporation and the residue was extracted with chloroform. The extract was successively washed with water, dried and evaporated to a syrup which was then acetylated with acetic anhydride (5 ml) in methanol (50 ml). The mixture was evaporated and the residue was chromatographed on a column of silicic acid. The eluate from chloroform-methanol (80:1) yielded **IXa** (1.96 g, 74%) or **IXβ** (1.44 g, 78%), respectively.

Compound (**IXa**) (amorphous): mp 52~54°C; $[\alpha]_D^{25} + 67^\circ$ ($c=1$, CHCl_3). Compound (**IXβ**) (amorphous): mp 51~53°C; $[\alpha]_D^{25} + 25.5^\circ$ ($c=1$, CHCl_3); IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} for **IXa** and **IXβ**: 3600~3200 (NH, OH), 3100~2800 (CH), 200~1800, 1150~1020, 740 and 690 (OCH_2Ph), 1640 and 1560 (amide). *Anal.* Found (**IXa**): C, 72.88; H, 6.93; N, 2.55. (**IXβ**): C, 72.81; H, 6.65; N, 2.15. Calcd. for $\text{C}_{71}\text{H}_{80}\text{N}_2\text{O}_{13}$: C, 72.92; H, 6.89; N, 2.40%.

N [(1S)-(1,4,6/5)-4,5,6-triacetoxy-3-acetoxymethyl-2-cyclohexenyl] [O- α , β -2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-D-glucopyranosyl-(1→3)-(1S)-(1,2,4/3,5)-2,4-diacetoxy-5-acetoxymethyl-3-hydroxy-cyclohexyl] amine (**Xa** and **Xβ**). To liquid ammonia (150 ml) were alternately added with stirring **IXa** (5.51 g) or **IXβ** (6.20 g) in tetrahydrofuran (25 ml) and sodium metal (6 g to **IXa**, 7 g to **IXβ**) in portions over 20~30 min at -70°C. After reaction, ammonium chloride (20 g to **IXa**, 23 g to **IXβ**) was carefully added and the ammonia was evaporated at room temperature. Water was added to the residue and organic materials were extracted with benzene. The water layer was evaporated and the insoluble materials in methanol were filtered off. The filtrate was evaporated and the resulting residue was acetylated with acetic anhydride (25 ml) and pyridine (50 ml). Methanol was added under cooling to decompose the excess acetic anhydride and the mixture was evaporated. The residue was dissolved in chloroform and successively washed with ice-cold 1 N hydrochloric acid, water, 10% sodium bicarbonate and water. The chloroform layer was dried and evaporated to a syrup which was then chromatographed on a column of silicic acid.

The eluate from chloroform-methanol (50:1) yielded **Xa** (3.1 g, 68%) or **Xβ** (2.9 g, 57%) as a syrup, respectively. **Xa**, $[\alpha]_D^{25} + 68^\circ$ ($c=0.5$, CHCl_3); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1740 and 1230 (OAc), 1670 and 1520 (amide). **Xβ**, $[\alpha]_D^{25} + 34^\circ$ ($c=0.5$, CHCl_3); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1740 and 1230 (OAc), 1660 and 1540 (amide). No phenyl proton was observed in NMR spectra of **Xa** as well as **Xβ**. Anal. Found **Xa**: C, 52.88; H, 6.05; N, 2.81. (**Xβ**): C, 52.90; H, 5.76; N, 3.25. Calcd. for $\text{C}_{42}\text{H}_{55}\text{N}_2\text{O}_{23}$: C, 52.61; H, 6.10; N, 2.92%.

N [(1*S*)-(1,4,6/5)-4,5,6-trihydroxy-3-hydroxymethyl-2-cyclohexenyl] [*O*-α, β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-(1*S*)-(1,2,4/3,5)-2,3,4-trihydroxy-5-hydroxymethyl-cyclohexyl]amine (**XIa** and **XIβ**). Compound (**Xa**, 3.1 g) or (**Xβ**, 2.9 g) was treated with methanolic sodium methoxide and the solution was neutralized with Amberlite IRC-50 ion-exchange resin. The suspension was filtered and the filtrate was evaporated to afford **XIa** (1.48 g, 84%) or **XIβ** (1.36 g, 82%) as an amorphous solid, respectively. **XIa**, mp 147°C (dec.); $[\alpha]_D^{25} + 113^\circ$ ($c=1$, H_2O); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3600~3000 (OH, NH), 1640 and 1550 (amide), 1100~1000 (COH); NMR $\delta_{\text{Me}_4\text{Si}(\text{ext.})}^{\text{D}_2\text{O}}$: 2.1 (3H, NAc), 5.35 (1H, d., $J=3.5$ Hz, anomeric) and 6.02 (1H, olefinic). **XIβ**, mp $124\sim 125^\circ$ (dec.); $[\alpha]_D^{25} + 40^\circ$ ($c=1$, H_2O); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3600~3000 (OH, NH), 1640 and 1560 (amide), 1100~1000 (COH); NMR $\delta_{\text{Me}_4\text{Si}(\text{ext.})}^{\text{D}_2\text{O}}$: 2.1 (3H, NAc), 4.6 (1H, d., $J=8$ Hz, anomeric) and 6.0 (1H, olefinic).

N [(1*S*)-(1,4,6/5)-4,5,6-trihydroxy-3-hydroxymethyl-2-cyclohexenyl][*O*-α,β-2-amino-2-deoxy-D-glucopyranosyl-(1→3)-(1*S*)-(1,2,4/3,5)-2,3,4-trihydroxy-5-hydroxymethyl-cyclohexyl]amine monohydrochloride (**XIIa**

and **XIIβ**). A solution of **XIa** (1.38 g) or **XIβ** (1.24 g) in commercial hydrazine hydrate (10 ml) was heated for 24 hr under reflux. After reaction, hydrazine hydrate was removed by evaporation, and to the residue was added the calculated amount of 0.1 N hydrochloric acid equivalent to the amine and charcoal (3 g). After standing for 30 min at 40°C , the mixture was filtered and the filtrate underwent freeze-drying. The title compound **XIIa** (1.02 g) or **XIIβ** (1.12 g) was obtained as an amorphous solid, respectively.

XIIa, mp $166\sim 167^\circ\text{C}$ (dec.); $[\alpha]_D^{25} + 59^\circ$ ($c=0.6$, H_2O); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3600~3000 (OH, NH), 1650~1550 (NH_2) and 1100~1000 (COH); NMR $\delta_{\text{Me}_4\text{Si}(\text{ext.})}^{\text{D}_2\text{O}}$: 5.55 (1H, d., $J=3.5$ Hz, anomeric) and 5.9 (1H, olefinic). **XIIβ**, mp $148\sim 149^\circ\text{C}$ (dec.); $[\alpha]_D^{25} + 42^\circ$ ($c=1$, H_2O); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3600~3000 (OH, NH_2), 1650~1550 (NH_2) and 1100~1000 (COH); NMR $\delta_{\text{Me}_4\text{Si}(\text{ext.})}^{\text{D}_2\text{O}}$: 6.15 (1H, olefinic).

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