GLYSPERIN, A NEW ANTIBIOTIC COMPLEX OF BACTERIAL ORIGIN

II. STRUCTURES OF GLYSPERINS A, B AND C

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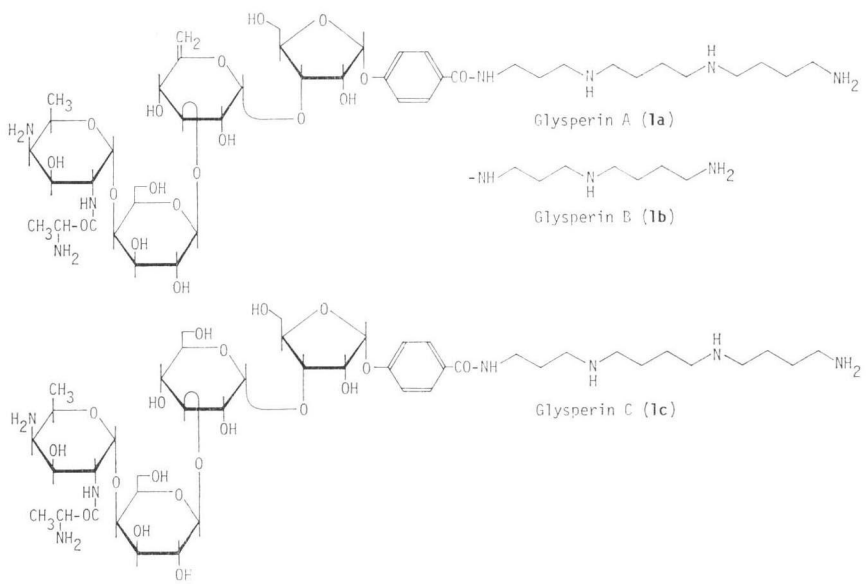
Structures of glysperins A, B and C were determined on the basis of chemical degradation studies in conjunction with spectroscopic analyses. Glysperin A consisted of L-alanine, *p*-hydroxybenzoic acid, a C_{11}-alkyl tetramine and four sugar moieties, three of which were identified as D-ribose, D-galactose and 2,4-diamino-2,4,6-trideoxy-D-galactose. The fourth sugar was a novel exoenose, 6-deoxy-D-*xylo*-hex-5-enose. Structural difference between glysperins A and B resided solely in the terminal polyamine moiety which was spermidine in glysperin B. Glysperin C contained D-glucose in place of the exoenohexose moiety of glysperin A. Glysperins A, B and C are, in some respects, structurally related to the glycocinnamoyl-spermidine antibiotics, LL-BM 123 β, γ_1 and γ_2.

Glysperin* is a complex of basic water-soluble antibiotics produced by strains of *Bacillus cereus*. In the preceding paper, characteristics of the producing strain, fermentation and isolation methods, as well as the chemical and biological properties of the antibiotics were described[1]. This paper presents evidence to assign the following structures to glysperins A (**Ia**), B (**Ib**) and C (**Ic**).



General Structural Characteristics

Glysperin components showed positive responses to ninhydrin, anthrone and ELSON-MORGAN reagents but were negative to SAKAGUCHI and ferric chloride reactions. The molecular formulae of

* This antibiotic was formally called Bu-2349.

$C_{44}H_{75}N_7O_{18}$, $C_{40}H_{66}N_6O_{18}$ and $C_{44}H_{77}N_7O_{19}$ were assigned to **Ia**, **Ib** and **Ic**, respectively, based on microanalyses and ^{13}C-NMR (CMR) spectra. The three components showed similar UV spectra, exhibiting a single maximum at 247 nm which did not shift in acidic or alkaline solution. The IR spectra indicated the presence of multi-hydroxyl (3,400 and 1,040 cm^{-1}) and amide groups (1,630 and 1,560 cm^{-1}). The proton NMR (PMR) spectrum of **Ia** showed, in common with that of **Ib** and **Ic**, two doublet methyl groups (δ in ppm, 1.28 and 1.49), multiple methylene protons (1.6~2.4) and 4 aromatic protons (AB-quartet, 7.12 and 7.71). The PMR spectra of **Ia** and **Ib** indicated 6 protons in the anomeric and/or olefinic region (4.8~6.0 ppm), while the PMR of **Ic** contained 4 protons in that field. The CMR spectra also reflected this difference: **Ia** and **Ib** exhibited two sp^2 carbons (155.8, s and 98.0, t) in addition to the 6 sp^2 carbons also present in **Ic**. On the other hand, the CMR of **Ic** showed two sp^3 carbons (at around 60~80 ppm) not present in the spectra of **Ia** and **Ib**.

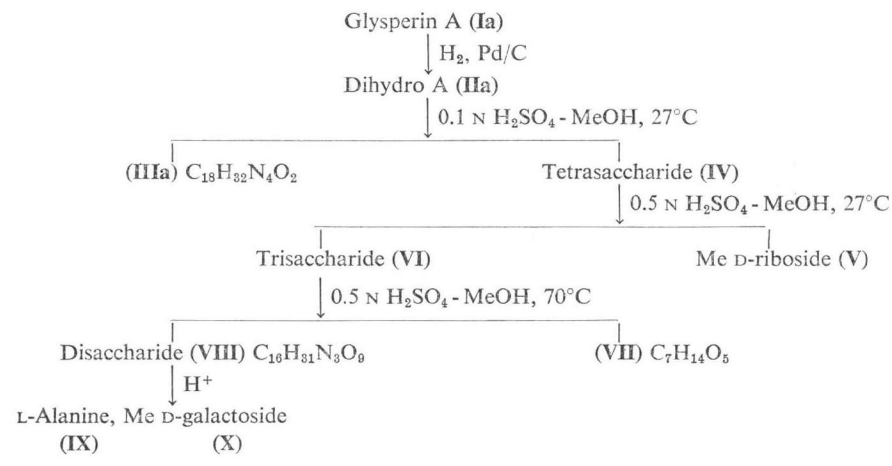
Catalytic Hydrogenation of Ia and Ib

The catalytic hydrogenation of **Ia** in ethanol afforded dihydroglysperin A (**IIa**, $C_{44}H_{77}N_7O_{18}$). The UV and IR spectra of **IIa** were very similar to those of **Ia**. The PMR spectrum of **IIa** differed from **Ia** in the presence of an additional methyl group (δ 1.33, d) and the absence of two protons in the olefinic region (δ 4.8~6.0). This suggested that a $CH_2=C\langle$ group in **Ia** had been reduced to a CH_3–$CH\langle$ group in **IIa**. Dihydroglysperin B (**IIb**) was obtained similarly from **Ib**. Further structural studies were carried out mainly on **IIa** and **IIb** because the degradation products obtained from their acid hydrolysis were more stable than those from **Ia** and **Ib**.

Acid Hydrolysis of IIa and IIb (Chart 1)

Upon mild acid hydrolysis (0.1 N H_2SO_4 in MeOH, 27°C, 16 hours), **IIa** was cleaved into two fragments, a UV-absorbing basic compound (**IIIa**) and a tetrasaccharide (**IV**). Further methanolysis of **IV** in 0.5 N H_2SO_4 at 27°C afforded methyl glycosides of a pentose (**V**) and a trisaccharide fragment (**VI**). The pentose was identified as methyl riboside by TLC and GLC (as a trimethylsilyl derivative). The D-configuration was assigned from the optical rotational value of its crystalline tosylhydrazone. Further hydrolysis of **VI** in 0.5 N H_2SO_4 at 70°C afforded another neutral sugar (**VII**) and a disaccharide (**VIII**)

Chart 1.



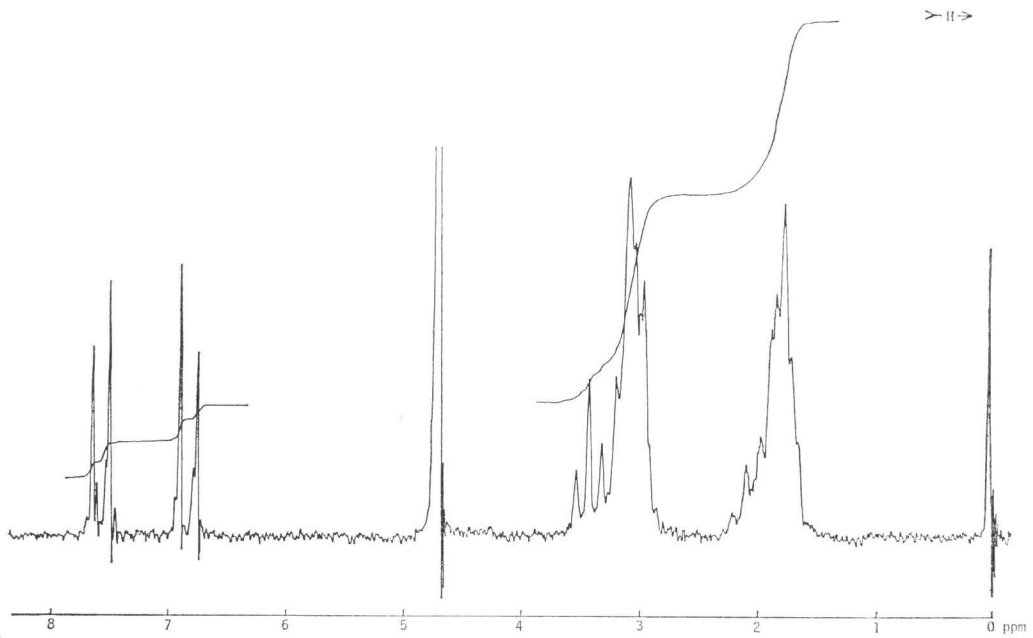
which was presumed to contain a basic sugar moiety. The disaccharide resisted further methanolysis but liberated L-alanine (**IX**) on vigorous aqueous acid hydrolysis. N-Acetylation of **VIII** followed by heating with methanolic hydrogen chloride afforded methyl D-galactoside (**X**) which was identified by TLC, GLC and specific rotation. The presumed basic sugar moiety was not isolated in the above acid hydrolysis studies.

On treatment with 0.1 N H_2SO_4 in methanol, **IIb** was hydrolyzed to yield a new UV-absorbing fragment (**IIIb**) and tetrasaccharide **IV**, the latter being identical with that obtained from **IIa**. Thus, the structural difference between **Ia** and **Ib** was found to reside only in the chromophoric aglycone moiety.

Structures of IIIa and IIIb

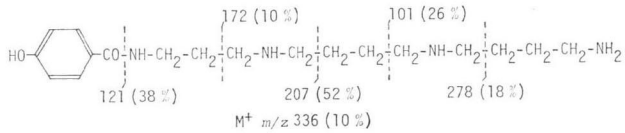
The basic aglycone **IIIa** was obtained as a crystalline hydrochloride ($C_{18}H_{32}N_4O_2 \cdot 3HCl$) and gave a positive reaction with ferric chloride. The UV spectrum of **IIIa** exhibited a maximum at 252 nm in water and in 0.1 N HCl, and the absorption underwent a bathochromic shift to 289 nm in 0.1 N NaOH. As shown in Fig. 1, the PMR spectrum of **IIIa** displayed 10 higher-field and 12 lower-field methylene protons along with four aromatic (AB quartet) protons. Acid hydrolysis of **IIIa** in 6 N HCl yielded a UV-absorbing acidic compound (**XI**, $C_7H_6O_3$) and a strongly basic amine (**XIIa**, $C_{11}H_{28}N_4$). From its UV ($\lambda_{max}^{H_2O}$ 252 nm) and IR spectra, **XI** was proved to be *p*-hydroxybenzoic acid. The tetrahydrochloride of **XIIa** was isolated as fine needles and showed physicochemical properties similar to those of spermidine or spermine. The PMR spectrum of **XIIa** indicated an asymmetric structure and the presence of six C–$\underline{CH_2}$–N and five C–$\underline{CH_2}$–C groups. The mass spectrum (MS) of **XIIa** included the molecular ion at *m/z* 216 and prominent fragment ions (*m/z* 172, 158, 101, 87) produced by β-cleavages. These data suggested **XIIa** to be 1,13-diamino-4,9-diazatridecane, a novel compound, and the structure was proven by the following synthesis: utilizing the method of KUTTAN[2], two molecules of 4-amino-

Fig. 1. PMR Spectrum of **IIIa** (60 MHz, D_2O).



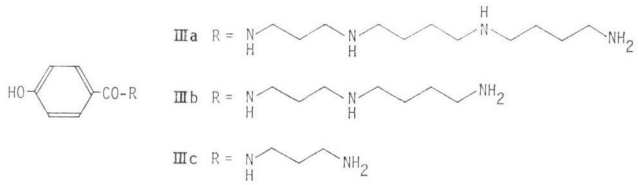
butyric acid were coupled to obtain 4-(4′-aminobutyramido)butyramide. Reaction of the amide with N-hydroxysuccinimide ester of 3-benzyloxycarbonylamidopropionic acid, followed by deblocking, afforded 4-[4′-(3′′-aminopropionamido)butyramido]butyramide. Reduction of the triamide with borane in THF gave 1,13-diamino-4,9-diazatridecane. The synthetic compound was identical with **XIIa** in all respects. The MS of **IIIa** showed, in addition to the M^+ ion at *m/z* 336, abundant ion peaks at *m/z* 278 ($M^+ - NH_2(CH_2)_3$) and *m/z* 207 ($M^+ - NH_2(CH_2)_4NH(CH_2)_3$), indicating that the terminal propylamine group of **XIIa** was acylated with **XI** (Fig. 2).

Fig. 2. Mass spectrum of **IIIa**.



The physico-chemical properties of **IIIb** ($C_{14}H_{23}N_3O_2$), the aglycone of **Ib**, were similar to those of **IIIa**. Upon acid hydrolysis, **IIIb** yielded **XI** and spermidine (**XIIb**). The MS and CMR of **IIIb** indicated that **XI** was also linked to the aminopropyl site of **XIIb**.

The structures of **XIIa** and **XIIb** assigned above were supported by microbiological transformation using spermidine oxidase[3]. Upon incubation with the enzyme preparation of *Serratia marcescens*, **IIIa** was converted into two ninhydrin-positive, UV-absorbing fragments. One was identified as **IIIb** and the other (**IIIc**, *m/z* 194 in MS) afforded **XI** and 1,3-diaminopropane on acid hydrolysis. **IIIb** was converted to **IIIc** by similar enzymatic reaction. Consequently, the structures of **IIIa**, **IIIb** and **IIIc** are as shown below:

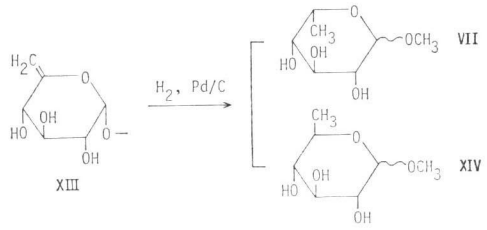


Structure of VII

The neutral sugar fragment, **VII** ($C_7H_{14}O_5$), was separated by silica gel chromatography into two anomers, **VIIa** and **VIIb**. Both anomers exhibited one C–CH_3 group in their PMR spectrum and an $(M+1)^+$ ion peak at *m/z* 179, indicating that they were methyl glycosides of 6-deoxyhexose. The magnitude of coupling constants observed for the anomeric protons (J_{1-2}=2.0 Hz for **VIIa** and 4.6 Hz for **VIIb**) and for the C_5 proton (J_{4-5}=3.3 Hz for **VIIa** and 4.0 Hz for **VIIb**) suggested an *ido* or a *talo* pyranoside configuration for **VII**. Eventually, **VIIa** was identified as methyl 6-deoxy-β-L-idopyranoside[4] by direct comparison with an authentic sample, and hence **VIIb** was the α-anomer.

Since **VII** was not formed when **Ia** was hydrolyzed, **VII** arises by catalytic hydrogenation of the CH_2=C< group of **Ia**. Thus the antecedent structure for **VII** in **Ia** was concluded to be 6-deoxy-D-*xylo*-hex-5-enose (**XIII**). It has been reported[4] that catalytic reduction of 6-deoxy-α-D-*xylo*-hex-5-enopyranoside gave 6-deoxy-L-idose predominantly along with a small amount of 6-deoxy-D-glucose, while reduction of the β-anomer of the enopyranoside afforded 6-deoxy-D-glucose as a major product[5].

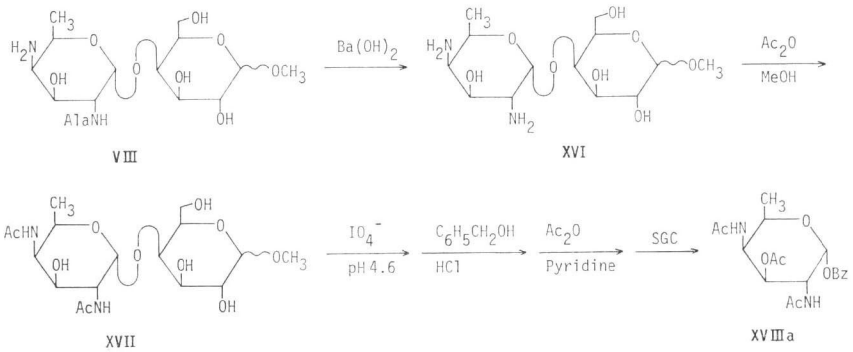
In fact, the acid hydrolyzate of **VI** was found to contain a small amount of another sugar which was identified as an anomeric mixture of methyl 6-deoxy-D-glucopyranoside (**XIV**). The structures of **VII**, **XIII** and **XIV** are:



Structures of VIII

As described earlier the disaccharide fragment, **VIII** ($C_{16}H_{31}N_3O_9$), contains L-alanine (**IX**) and D-galactose (**X**). The presence of a diaminohexose moiety (**XV**) in **VIII** was presumed from the analytical and spectral data. Heating **VIII** with saturated $Ba(OH)_2$ afforded the dealanyl derivative, **XVI** (Chart 2). This was converted to its N-acetyl derivative, **XVII**, which was treated in acetate buffer (pH 4.6) with excess periodate. The oxidation product was heated with benzyl alcohol containing 2% hydrogen chloride and then acetylated in pyridine to obtain benzyl N,O-acetyl-glycoside (**XVIII**). Chromatographic purification of **XVIII** gave a homogeneous anomer (**XVIIIa**, $(M+1)^+$: m/z 379, M_D: +488) which was identified as benzyl 2,4-diacetamido-3-O-acetyl-2,4,6-trideoxy-α-D-galactopyranoside[6].

Chart 2.

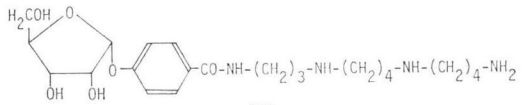


Therefore, **XV** was determined to be 2,4-diamino-2,4,6-trideoxy-D-galactose. The MS of **XVI** exhibited an intense fragment ion at m/z 145, suggesting that **XV** was linked glycosidically to **X** in **XVI**. Since **XVII** consumed 1 molar equivalent of periodate, the site of glycosidic linkage should be either the C-2 or C-4 hydroxyl group of **X**. It was concluded to be C-4 because no axial O-acetyl group was observed in the PMR spectrum of the N,O-peracetyl derivative of **XVI** and because the C-2 proton showed a downfield shift on acetylation. The α-glycosidic configuration of **XV** was assigned from the PMR (anomeric proton: δ 4.89, d, J=3.5 Hz) and the CMR (anomeric carbon: δ 99.0) spectra of **XVI**, and from the modified HUDSON's rule[7] (calculated M_D for **XV**: +559). The alanine-containing disaccharide fragment (**VIII**) showed an amide carbonyl absorption in the IR spectrum. The PMR spectrum of **VIII** indi-

cated a lower-field shift of the methine proton at C-2 than observed in **XVI**. Thus the site of acylation with L-alanine was determined to be the C-2 amino group of **XV**.

Structures of Ia and Ib

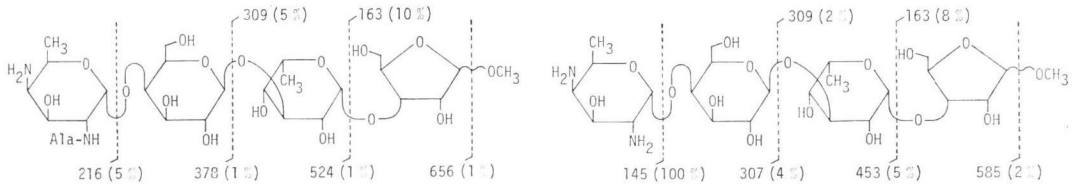
Treatment of **Ia** with acidic water (pH 4.0) or 50% acetic acid at 27°C gave rise to another UV-absorbing fragment (**XIX**) which afforded **IIIa** and **V** upon acid methanolysis. An α-furanoside structure was assigned to **V** based on the PMR spectrum (anomeric proton at δ 5.78, J=4.0 Hz), specific rotation (M_D: +445) and periodate oxidation (1 mole consumed) of **XIX**.



XIX

The sequence of four sugar moieties in **IV** was deduced to be **XV**→**X**→**VII**→**V** by the MS analysis of tetrasaccharide **IV** and its dealanyl derivative as shown in Fig. 3.

Fig. 3. Mass spectra of **IV** and its dealanyl derivative.

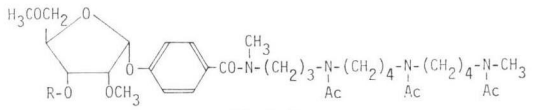


IV
$(M+1)^+$: m/z 688 (1.4%)

Dealanyl-**IV**
$(M+1)^+$: m/z 617 (21%)

The anomeric proton of **X** was observed at δ 4.62 (J=7.0 Hz) in the PMR spectrum of **VI** and the anomeric carbon was located at relatively low field (δ 104.6) in the CMR spectrum, indicating a β-glycosidic configuration for **X** in the trisaccharide fragment. The N-diacetyl derivative of **VI** consumed only one mole of periodate, hence **X** should be linked to the C-3 hydroxyl group of **VII** which exists as a pyranoside form in the trisaccharide.

Treatment of the N-pentaacetyl derivative of **Ia** with excess methyl iodide yielded the N-acetyl-N,O-permethyl derivative which was hydrolyzed in 50% acetic acid to give a UV-absorbing compound (**XX**, M^+: m/z 650). Acetylation of **XX** in pyridine afforded its mono-O-acetate (**XXI**). The acetyl group was located at the C-3 hydroxyl of **V** by comparative PMR analysis of **XX** and **XXI** and by a spin-decoupling experiment. Consequently **XX** and **XXI** have the structures shown below. The site of linkage of **XIX** with the trisaccharide fragment was determined to be the C-3 hydroxyl group of the ribose moiety.



XX R = H
XXI R = Ac

Thus, structures **Ia** and **Ib** shown previously were assigned to glysperins A and B, respectively.

Structure of Ic

Upon hydrolysis in methanolic 0.1 N H_2SO_4, **Ic** afforded **IIIa** and a new tetrasaccharide fragment (**IVc**). Further degradation of **IVc** by refluxing with 0.5 N H_2SO_4 in methanol gave **V**, **VIII** and another sugar **XXII**. **XXII** was identified as methyl D-glucoside by its TLC, GLC and optical rotational values. An α-pyranoside configuration was indicated by the PMR (anomeric proton: δ 5.02, J=3.5 Hz) and CMR (anomeric carbon: δ 99.3) spectra of **IVc**. Thus the structure of **Ic**, as previously shown, had D-glucose in place of the unsaturated sugar moiety of **Ia**.

Discussion

Glysperins A, B and C are a new class of antibiotic with a glycobenzoyl-polyamine structure and several unusual constituents. The occurrence of 2,4-diamino-2,4,6-trideoxy-D-galactose was recently reported in the polysaccharide of *Streptococcus pneumoniae*[8] and *Shigella sonnei*[9]. Glysperins A and B contained 6-deoxy-D-*xylo*-hex-5-enose, which is the first reported instance of its occurrence in nature although its synthesis has been reported[4]. The polyamine component of glysperins A and C, 1,13-diamino-4,9-diazatridecane, is a hitherto undescribed compound.

Glysperins are, in some respects, structurally related to the glycocinnamoylspermidine antibiotics, LL-BM 123 β and γ[10], which are produced by *Nocardia* species[11]. However, the two groups of antibiotics differ in both the chromophore and sugar components. Glysperins do not contain ureido and guanidino groups which are present in the LL-BM 123 antibiotics.

Experimental

UV spectra were determined on a Shimadzu UV-200 spectrophotometer and IR spectra on a JASCO IRA-1 spectrometer. Optical rotational values were measured by use of a JASCO DIP-140 automatic polarimeter. PMR spectra were recorded on a JEOL C-60-HL, a Hitachi 90 MHz or Varian HR-220 apparatus using tetramethylsilane (TMS) as internal or external (in case of D_2O) references. CMR spectra were determined on a Varian FT-80A apparatus operated in FOURIER transform system. Mass spectra were measured on a Hitachi RMU-6MG mass spectrometer modified with an in-beam/direct inlet ion source. Thin-layer chromatography (TLC) was performed on silica gel plates (Kieselgel 60 F_{254}) using the solvent systems shown below.

System No.	Solvent system
S-102	MeOH - 10% $AcONH_4$ (1: 1)
S-114	MeOAc - *n*-PrOH - 28% NH_4OH (45: 105: 60)
S-117	$CHCl_3$ - MeOH - 28% NH_4OH (1: 3: 2)
SD-108	$CHCl_3$ - MeOH (93: 7)
SD-109	$CHCl_3$ - *n*-PrOH - 28% NH_4OH (45: 105: 25)
SD-110	$CHCl_3$ - EtOH (9: 1)

Hydrogenation of glysperin A (**Ia**)—Isolation of **IIa**

A solution of **Ia** (1.3 g) in 100 ml of 50% aqueous EtOH was hydrogenated over PtO_2 (560 mg) at 41 p.s.i. in a Parr apparatus for 18 hours. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residual solid was loaded on a column of Amberlite CG-50 (NH_4^+, ϕ 1.8×40 cm) which was developed successively with water, 0.5 N NH_4OH and 1.0 N NH_4OH. The ninhydrin-positive fractions eluted with 1.0 N NH_4OH were pooled, concentrated *in vacuo* and lyophilized to afford 798 mg of **IIa** as a white amorphous powder, m.p. 143~149°C (dec.). TLC: Rf 0.21 (S-102) and 0.32 (S-117). $[\alpha]_D^{25.5}$ +135° (*c* 0.5, H_2O), $\lambda_{max}^{H_2O}$ 247 nm (ε 14,000). PMR $\delta_{TMS}^{D_2O+DCl}$ in ppm: 1.26 (3H, d, J=6.5 Hz), 1.33 (3H, d, J=6.5 Hz), 1.50 (3H, d, J=7.0 Hz), 5.83 (1H, d, J=4.0 Hz), 7.20 (2H, d, J=9.0 Hz), 7.77 (2H, d, J=9.0 Hz), *etc.* *Anal.* Calcd. for $C_{44}H_{77}N_7O_{18}\cdot\frac{3}{2}H_2CO_3$: C, 50.36; H, 7.47; N, 9.04. Found: C, 50.11; H, 7.62; N, 9.03.

Acid hydrolysis of **IIa**—Isolation of **IIIa** and **IV**

A solution of **IIa** (550 mg) in 50 ml of 0.1 N H_2SO_4-MeOH was stirred at 27°C for 16 hours. The resulting solution was neutralized with Amberlite IR-45 (OH^-) and then evaporated *in vacuo* to dryness. The residue was chromatographed on a column of Amberlite CG-50 (NH_4^+, ϕ 1.8×45 cm) which was developed with water, 0.05 N NH_4OH and 1.0 N NH_4OH, successively. The elution was followed by ninhydrin and anthrone tests. Concentration of anthrone-positive fractions eluted by water gave 240 mg of white solid which contained a small amount of **V** and, for the most part, inorganic salts. Anthrone and ninhydrin-positive fractions from the 0.05 N NH_4OH eluate afforded crude **IV** (324 mg). The ninhydrin-positive eluate with 1.0 N NH_4OH gave **IIIa** (165 mg) after evaporation *in vacuo*. Crude **IV** (320 mg) was purified by silica-gel chromatography (ϕ 1.6×50 cm) using the solvent system, $CHCl_3$ - *n*-PrOH - 28% NH_4OH (5: 8: 2). Elution was monitored by the DAP test[12] (diphenylamine - aniline - acetone - 80% H_3PO_4) and appropriate fractions were evaporated *in vacuo*. A small amount of trisaccharide **VI** (58 mg) was eluted first, followed by the major tetrasaccharide **IV** (252 mg).

IIIa: Crystallized as colorless needles from aqueous EtOH containing dil. HCl, m.p. 255.5~256°C. TLC: Rf 0.34 (S-102) and 0.15 (S-114). $\lambda_{max}^{H_2O \text{ or } 0.1N\ HCl}$ 252 nm (ε 13,900) and $\lambda_{max}^{0.1N\ NaOH}$ 289 nm (ε 20,400). PMR $\delta_{TMS}^{D_2O}$ in ppm: 1.5~2.4 (10H, m), 2.7~3.6 (12H, m), 6.85 (2H, d, J=9.0 Hz) and 7.55 (2H, d, J=9.0 Hz). MS: *m/z* 336 $(M)^+$, 278, 249, 221, 207, 186, 178, 141, 121, 101, *etc.* *Anal.* Calcd. for $C_{18}H_{32}N_4O_2 \cdot 3HCl$: C, 48.49; H, 7.91; N, 12.57; Cl, 23.86. Found: C, 48.48; H, 7.96; N, 12.46; Cl, 23.97.

IV: White amorphous powder. TLC: Rf 0.23 (S-114) and 0.13 (SD-109). $[\alpha]_D^{24}$+94° (*c* 0.5, H_2O). PMR $\delta_{TMS}^{D_2O}$ in ppm: 1.24 (3H, d, J=6.5 Hz), 1.33 (3H, d, J=6.5 Hz), 1.44 (3H, d, J=7.0 Hz), 3.29 (1H, m), 3.40 (3H, s), 4.59 (1H, d, J=7.0 Hz) and 4.8~5.0 (3H, m). MS: *m/z* 688 $(M+1)^+$, 524, 378, 216, *etc.*

Acid hydrolysis of **IIb**—Isolation of **IIIb**

Hydrogenation of **Ib** (45 mg) in the same manner as above afforded 35 mg of **IIb**. TLC: Rf 0.29 (S-102) and 0.45 (S-117). $\lambda_{max}^{H_2O}$ 247 nm (ε 14,500).

IIb (31 mg) was hydrolyzed with 0.1 N H_2SO_4 - MeOH and the hydrolyzate was chromatographed by the procedure described above to isolate **IIIb** (7 mg) and **IV** (10 mg).

IIIb: White powder. TLC: Rf 0.58 (S-102) and 0.28 (S-114). $\lambda_{max}^{H_2O \text{ or } 0.1N\ HCl}$ 252 nm (ε 12,400) and $\lambda_{max}^{0.1N\ NaOH}$ 289 nm (ε 18,500). PMR $\delta_{TMS}^{D_2O+DCl}$ in ppm: 1.4~2.2 (6H, m), 2.6~3.6 (8H, m), 6.64 (2H, d, J=9.0 Hz) and 7.55 (2H, d, J=9.0 Hz). MS: *m/z* 265 $(M)^+$.

Methanolysis of **IV**—Isolation of **VI** and **V**

Saccharide **IV** (550 mg) in 50 ml of 0.5 N H_2SO_4 - MeOH was allowed to stand at 27°C for 16 hours. The reaction mixture was neutralized with Amberlite IR-45 (OH^-) and evaporated to leave an amorphous solid which was chromatographed on a column of Amberlite CG-50 (NH_4^+, ϕ 1.2×30 cm). Development of the column with water gave a mixture of **V** and inorganic salts (292 mg); subsequent development with 0.05 N NH_4OH yielded trisaccharide **VI** (303 mg). **VI**: Amorphous solid. TLC: Rf 0.32+0.39 (S-114) and 0.17+0.22 (SD-109). MS: *m/z* 556 $(M+1)^+$, 555 $(M)^+$, 538, 524, 512, 378, 216, *etc.*

The powder containing **V** (260 mg) was chromatographed on a column of Sephadex LH-20 (ϕ 2.0×75 cm) which was developed with 50% aqueous MeOH. The DAP-positive fractions were concentrated *in vacuo* and lyophilized to afford 136 mg of **V** as an oily solid. **V**: TLC: Rf 0.45 (S-114). $[\alpha]_D^{25}$−88° (*c* 0.3, H_2O). GLC of the TMS-derivative (OV-17, 3%, temperature programing 5°C/min. from 150°C): Rt 3.7 (major) and 3.9 (minor).

V (100 mg) was hydrolyzed with 0.2 N HCl (20 ml) to yield 82 mg of free sugar. Part of the sugar (55 mg) and *p*-toluenesulfonylhydrazide (160 mg) in 10 ml of methanol was heated under reflux for 30 minutes. The yellow precipitate deposited upon cooling was crystallized from a mixture of MeOH and EtOH to give 55 mg of tosylhydrazone. M.p. 116°C. $[\alpha]_D^{27.5}$+14.5° (*c* 0.49, pyridine). The IR spectrum was identical with that of D-ribose tosylhydrazone.

Methanolysis of **VI**—Isolation of **VIIa**, **VIIb**, **VIII** and **XIV**

A solution of **VI** (440 mg) in 40 ml of 0.5 N H_2SO_4 - MeOH was heated under reflux for one hour. After being neutralized with Amberlite IR-45 (OH^-), the solution was evaporated to give an amorphous solid which was applied to a column of Amberlite CG-50 (NH_4^+, ϕ 2.0×30 cm). Development of the column with water afforded neutral sugar **VII** (anomeric mixture, 84 mg); **VIII** (285 mg) was then eluted with 0.05 N NH_4OH. Sugar **VII** (80 mg) was chromatographed on silica-gel (ϕ 1.8×46 cm) using $CHCl_3$ - MeOH (98: 2) as eluant. The course of elution was monitored by a DAP test and TLC (SD-108); appropriate fractions were combined and concentrated to give **VIIb** (18 mg), **VIIa** (20 mg) and a trace amount of **XIV** (2 mg).

VIIa: Hygroscopic white solid. TLC: Rf 0.57 (S-114) and 0.10 (SD-108). $[\alpha]_D^{24}$ +44° (*c* 0.19, H_2O). MS: *m/z* 179 $(M+1)^+$, 147, 129, 116, 100, 74, 60, *etc.* PMR $\delta_{TMS}^{D_2O}$ in ppm: 1.31 (3H, d, J=6.7 Hz), 3.51 (3H, s), 3.4~3.8 (2H, m), 3.99 (1H, t, J=5.0 Hz), 4.10 (1H, d-q, J=6.7 & 3.3 Hz) and 4.71 (1H, d, J=2.0 Hz). Identified as methyl 6-deoxy-β-L-idopyranoside by direct comparison with an authentic specimen.

VIIb: TLC: Rf 0.57 (S-114) and 0.15 (SD-108). $[\alpha]_D^{23}$ −87° (*c* 0.4, H_2O). PMR $\delta_{TMS}^{D_2O}$ in ppm: 1.26 (3H, d, J=6.7 Hz), 3.44 (3H, s), 3.3~3.9 (3H, m), 4.21 (1H, d-q, J=6.7 & 4.0 Hz) and 4.58 (1H, d, J=4.6 Hz).

XIV: TLC: Rf 0.42 (S-114) and 0.06 (SD-108). Identified as methyl 6-deoxy-D-glucoside by TLC and GLC (TMS derivative) comparison with an authentic sample.

VIII: White amorphous powder. TLC: Rf 0.36 (S-114). pKa′: 7.3 and 8.3 (titration equivalent; 410). IR $\nu_{C=O}^{KBr}$ 1680 and 1570 cm^{-1}. MS: *m/z* 410 $(M+1)^+$, 366, 305, 276, 249, 216, *etc.* PMR $\delta_{TMS}^{D_2O}$ in ppm: 1.23 (3H, d, J=6.5 Hz), 1.38 (3H, d, J=7.0 Hz), 3.13 (1H, m), 3.41 (3H, s), 4.86 (1H, d, J=1.5 Hz) and 4.90 (1H, d, J=3.5 Hz).

Di-N-acetyl-**VIII**: Prepared by acetylation of **VIII** in MeOH. M.p. 167~170°C. MS: *m/z* 494 $(M+1)^+$, 407, 348, 300, *etc.* *Anal.* Calcd. for $C_{20}H_{35}N_3O_{11}\cdot H_2O$: C, 46.96; H, 7.29; N, 8.21. Found: C, 46.87; H, 7.29; N, 8.11.

Acid hydrolysis of **VIII**—Isolation of **IX**

A solution of **VIII** (340 mg) in 6 N HCl (17 ml) was heated at 110°C for 16 hours in a sealed tube. The hydrolyzate was chromatographed on Dowex 50W×4 (H^+, ϕ 1.0×32 cm) which was developed with an increasing concentration of HCl. Ninhydrin-positive fractions eluted with 0.1 N HCl were concentrated to afford 87 mg of **IX** hydrochloride.

IX HCl: Crystalline solid. M.p. 206~207°C (dec.). TLC: Rf 0.81 (S-102) and 0.26 (S-114). $[\alpha]_D^{26}$ +6° (*c* 2.0, 5 N HCl). Identified as L-alanine by IR spectrum.

Acid methanolysis of N-diacetyl **VIII**—Isolation of **X**

N-Diacetyl-**VIII** (130 mg) was hydrolyzed by refluxing with 1.8 N methanolic hydrogen chloride. The hydrolyzate was concentrated and passed through a column of Amberlite CG-50 (NH_4^+) to remove basic products. The fractions containing neutral sugar were concentrated *in vacuo* and the residue was fractionated on a column of Sephadex LH-20 (ϕ 2.0×66 cm) with 50% aqueous MeOH. The DAP-positive eluate was concentrated to give 27 mg of syrupy **X**.

X: TLC: Rf 0.30 (S-114). $[\alpha]_D^{21.5}$ +95° (*c* 0.2, H_2O). GLC of TMS derivative (OV-17, 3%, temperature programing at 5°C/min. from 150°C). Rt 6.32 (minor), 7.00 (major) and 7.38 (minor). Identified as methyl D-galactoside.

Hydrolysis of **IIIa** and **IIIb**—Isolation of **XI**, **XIIa** and **XIIb**

A solution of **IIIa** hydrochloride (450 mg) in 50 ml of 6 N HCl was heated at 120°C for 16 hours in a sealed tube. The reaction mixture was cooled and extracted with two 50-ml portions of ethyl ether. The extracts were combined, evaporated and lyophilized to give 71 mg of **XI**. The aqueous layer was decolorized with active carbon, concentrated to 5 ml and then diluted with EtOH to deposit colorless needles of **XIIa** hydrochloride (322 mg).

XI: $\lambda_{max}^{H_2O}$ 252 nm (ε 12,600) and $\lambda_{max}^{0.1N\ NaOH}$ 280 nm (ε 15,100). *Anal.* Calcd. for $C_7H_6O_3$: C, 60.81; H, 4.38. Found: C, 60.61; H, 4.26. Identified as *p*-hydroxybenzoic acid by IR spectrum.

XIIa: Fine needles, m.p. >300°C. TLC: Rf 0.08 (S-102). MS: *m/z* 216 $(M)^+$, 186, 172, 158, 84, 72, *etc.* PMR $\delta_{TMS}^{D_2O}$ in ppm: 1.5~2.5 (10H, m) and 2.6~3.5 (12H, m). *Anal.* Calcd. for $C_{11}H_{28}N_4 \cdot 4HCl$: C, 36.48; H, 8.90; N, 15.47; Cl, 39.15. Found: C, 36.43; H, 9.14; N, 15.37; Cl, 38.94.

IIIb (10 mg) was hydrolyzed with 6 N HCl at 120°C for 16 hours and the hydrolyzate worked up as above to yield **XI** (2 mg) and **XIIb** hydrochloride (3 mg). **XIIb**: TLC: Rf 0.11 (S-102). Identified as spermidine hydrochloride by IR spectrum and TLC.

Preparation of 1,13-diamino-4,9-diazatridecane

4-Benzyloxycarbonyl(Cbz)-aminobutyric acid (7.12 g) and N-hydroxysuccinimide (NOS, 3.45 g) were condensed in the presence of dicyclohexylcarbodiimide (6.19 g) in 120 ml of ethyl acetate at 5°C to yield 4-Cbz-aminobutyryl-NOS (12.12 g).

An ethanol solution of the active ester (6.0 g) was poured into 15 N NH_4OH at −3°C to deposit 2.89 g of 4-Cbz-aminobutyramide as colorless needles, m.p. 132~135°C. Hydrogenolysis of the amide (2.48 g) in the presence 10% palladium on charcoal in 75% aqueous EtOH gave a quantitative yield of deblocked amide which was coupled with 6 g of 4-Cbz-aminobutyryl-NOS to afford 4-(4′-Cbz-aminobutyramido)butyramide, 2.14 g. M.p. 172~173°C. This compound was hydrogenated with 10% palladium on charcoal to yield free diamide. The amide (1.03 g) was treated with 3-Cbz-aminopropionyl-NOS (1.76 g) in 50 ml of dimethylformamide at 25°C. The reaction solution was concentrated to 10 ml *in vacuo* and diluted with 40 ml of 0.5 N NaOH to precipitate 4-[4′-(3″-Cbz-aminopropionamido)-butyramido]butyramide. Yield 821 mg. M.p. 206~207°C. *Anal.* Calcd. for $C_{19}H_{28}N_4O_5$: C, 58.15; H, 7.19; N, 14.28. Found: C, 57.76; H, 7.18; N, 14.17. Deblocking of the triamide (704 mg) by hydrogenation in 80% aqueous MeOH gave aminotriamide quantitatively. This was refluxed with 40 ml of borane in tetrahydrofuran (1 mole/liter solution) for 26 hours. The amine produced was chromatographed on a column of Dowex 50W×4 (H^+, ϕ 1.0×18 cm) with increasing concentration of HCl. Appropriate fractions were concentrated to afford 159 mg of 1,13-diamino-4,9-diazatridecane. Fine needles from aqueous EtOH. M.p. >300°C. TLC: Rf 0.08 (S-102). *Anal.* Calcd for $C_{11}H_{28}N_4 \cdot 4HCl$: C, 36.48; H, 8.90; N, 15.47; Cl, 39.15. Found: C, 36.51; H, 8.98; N, 15.24; Cl, 38.81. Identified as **XIIa**.

Enzymatic degradation of **IIIa**

A mixture of **IIIa** hydrochloride (20 mg) and an enzyme preparation from *Serratia marcescens* IAM-1223 (40 mg) in 20 ml of M/15 phosphate buffer (pH 7.0) was incubated at 37°C for 4 days. After removal of insolubles by filtration, the filtrate was applied to a column of Amberlite CG-50 (NH_4^+, ϕ 1.0×25 cm) which was eluted successively with water (300 ml), N/20 NH_4OH (200 ml), N/2 NH_4OH (200 ml) and N NH_4OH (200 ml). UV-absorbing fractions eluted first with N/20 NH_4OH were concentrated to afford 2 mg of **IIIc**. A subsequent UV-absorbing fraction eluted with N/2 NH_4OH gave 11.5 mg of white powder which was identified as **IIIb** obtained from **Ib**. **IIIc**: TLC: Rf 0.68 (S-102) and 0.41 (S-114). $\lambda_{max}^{H_2O \text{ or } 0.1N\ HCl}$ 252 nm (ε 7,800) and $\lambda_{max}^{0.1N\ NaOH}$ 289 nm (ε 10,500). MS: *m/z* 194 $(M)^+$, 178, 164, 150, 121, 73, *etc.*

Alkaline hydrolysis of **VIII**—Isolation of **XVI**

VIII (383 mg) was refluxed with 38 ml of saturated barium hydroxide solution for 16 hours. The solution was adjusted to pH 7.0 with an addition of solid carbon dioxide and the mixture filtered. The filtrate was concentrated to a sticky residue which was chromatographed on a column of Amberlite CG-50 (NH_4^+, ϕ 1.8×60 cm). Elution with water afforded **IX** (82 mg); subsequent elution with 0.1 N NH_4OH gave 240 mg of **XVI**.

XVI: White amorphous powder. TLC: Rf 0.36 (S-114). pKa′: 6.6 and 8.5 in water (titration equivalent; 340). MS: *m/z* 339 $(M+1)^+$, 307, 276, 265, 235, 145, *etc.* PMR $\delta_{TMS}^{D_2O}$ in ppm: 1.29 (3H, d, J=6.5 Hz), 2.94 (1H, d-d, J=10.5 & 3.5 Hz), 3.19 (1H, d-d, J=2.0 & 4.0 Hz), 3.42 (3H, s), 4.84 (1H, d, J=1.5 Hz) and 4.89 (1H, d, J=3.5 Hz).

XVII (di-N-acetyl **XVI**): Obtained by acetylation of **XVI** in anhydrous MeOH. TLC: Rf 0.41 (S-114). MS: *m/z* 423 $(M+1)^+$, 405, 391, 373, 306, 277, 229, 211, 156, 99, *etc.* PMR $\delta_{TMS}^{D_2O}$ in ppm: 1.10 (3H, d, J=6.5 Hz), 2.08 (3H, s), 2.11 (3H, s), 3.41 (3H, s), and 4.81 (2H, m). *Anal.* Calcd for $C_{17}H_{30}N_2O_{10} \cdot 2H_2O$: C, 45.02; H, 7.56; N, 6.18. Found: C, 45.28; H, 7.34; N, 5.87.

Periodate oxidation of **XVII**—Isolation of **XVIIIa**

A solution of **XVII** (420 mg) and $NaIO_4$ (340 mg, 1.6 equivalent) in 100 ml of 0.1 M sodium acetate buffer (pH 4.6) was stirred for 24 hours at 20~25°C. After excess periodate was decomposed with ethylene glycol (30 mg), the solution was acidified to pH 3.0 and allowed to stand for 2 hours at 25°C. The mixture was filtered, neutralized with Amberlite IR-45 (OH^-) and evaporated to leave a sticky solid which was applied on a column of Diaion HP-20 (ϕ 1.0×45 cm). After inorganic materials were washed out with water, the fractions containing the sugar fragments were eluted with 50% MeOH. Concentration of the fractions gave 315 mg of amorphous solid. The sugar was heated at 72~75°C for 2 hours with 7.8 ml of benzyl alcohol containing 2% hydrogen chloride. Addition of Et_2O to the mixture gave 277 mg of benzyl glycoside as a white powder.

A solution of glycoside (80 mg) in acetic anhydride (1 ml) and pyridine (2 ml) was kept at 20~25°C for 72 hours. Evaporation of the mixture afforded a syrupy residue which was taken up in $CHCl_3$ (50 ml). The solution was washed with water, dried and concentrated to yield 84 mg of white powder. This was chromatographed on silica gel in a 1.0×50 cm column using $CHCl_3$ with increasing proportion of EtOH as eluants. The major sugar fragment (30 mg) eluted with $CHCl_3$ - EtOH (99: 1) was further purified by preparative TLC developed with $CHCl_3$ - EtOH (9: 1). The appropriate sugar zone (Rf 0.34~0.42) was extracted with $CHCl_3$ and the extract concentrated *in vacuo* to afford 11 mg of **XVIIIa**. TLC: Rf 0.40 (SD-110). $[\alpha]_D^{25.5}$ +129° (*c* 0.14, $CHCl_3$). MS: *m/z* 379 $(M+1)^+$, 361, 335, 319, 270, 227, 199, *etc.* PMR $\delta_{TMS}^{CDCl_3}$ in ppm: 1.12 (3H, d, *J*=6.5 Hz), 1.92 (3H, s), 1.99 (3H, s), 2.08 (3H, s), 4.0~4.5 (3H, m), 4.5~4.7 (2H, m), 4.88 (1H, d, *J*=3.5 Hz), 5.14 (1H, d-d, *J*=4.0 & 12.0 Hz), 5.77 (1H, NH) and 6.19 (1H, NH). Identified as benzyl 2,4-diacetamido-3-O-acetyl-2,4,6-trideoxy-α-D-galactopyranoside.

Mild hydrolysis of **Ia**—Isolation of **XIX**

Ia (1.10 g) in 110 ml of 50% aqueous acetic acid was incubated at 27°C for 15 hours. The resulting mixture was evaporated to leave a sticky solid which was chromatographed on a column of Amberlite XT-2 (ϕ 2.0×64 cm) developed with water. Saccharide fragments were eluted first and the fractions containing **XIX** were evaporated *in vacuo* to yield a syrupy residue (540 mg). A portion of the residue (110 mg) was purified by silica gel chromatography (ϕ 1.0×23 cm) developed with $CHCl_3$ - MeOH - 28% NH_4OH (1: 2: 1). The recovered **XIX** was further fractionated on a column of Sephadex LH-20 (ϕ 1.0×25 cm) using 50% aqueous methanol. Concentration of UV-absorbing fractions afforded 63 mg of homogeneous **XIX** as colorless syrup. TLC: Rf 0.15 (S-114). $[\alpha]_D^{25}$ +95° (*c* 0.5, H_2O). $\lambda_{max}^{H_2O,\ 0.1N\ HCl,\ 0.1N\ NaOH}$ 248 nm (ε 14,500). MS: *m/z* 469 $(M+1)^+$, 410, 339, 278, 249, 133, *etc.* PMR $\delta_{TMS}^{D_2O+DCl}$ in ppm: 1.3~2.2(10H, m), 2.6~3.6 (12H, m), 3.70 (2H, d,), 4.0~4.5 (3H, m), 5.78 (1H, d, *J*=4.0 Hz), 7.13 (2H, d, *J*=9.0 Hz) and 7.71 (2H, d, *J*=9.0 Hz).

Isolation of **XX** and **XXI**

A mixture of **Ia** (1.02 g), acetic anhydride (0.57 ml) and anhydrous methanol (50 ml) was stirred for 3 hours. The solution was evaporated to yield 950 mg of N-pentaacetyl-**Ia**. To a dimethylformamide solution (30 ml) of the acetate (610 mg), 1.32 g of sodium hydride was added and the mixture was stirred at 48~52°C for one hour under a nitrogen atomosphere. Methyl iodine (24 g) was added dropwise to the solution at 0°C and the stirring was continued overnight at room temperature. The insolubles were removed by filtration and the solution was evaporated to dryness under reduced pressure. The residue was taken up in $CHCl_3$, washed with water and concentrated to obtain 660 mg of N-acetyl-N,O-permethyl-**Ia** as a sticky solid. TLC: Rf 0.78 (S-114). This solid (370 mg) was hydrolyzed with 50% aqueous acetic acid (37 ml) at 27°C. The hydrolyzate was chromatographed on a column of silica gel (ϕ 2.0×40 cm) developed with $CHCl_3$ - MeOH (95: 5). The UV-absorbing fractions were combined and concentrated to yield **XX** as a syrup (124 mg).

XX: TLC: Rf 0.51 (S-114). λ_{max}^{MeOH} 245 nm (ε 7,500). MS: *m/z* 650 $(M)^+$, 161, *etc.* PMR $\delta_{TMS}^{CDCl_3}$ in ppm: 3.89 (1H, d-d, *J*=4.0 & 6.0 Hz), 4.1~4.4 (2H, m), 5.73 (1H, d, *J*=4.0 Hz).

XX (60 mg) was acetylated with acetic anhydride (3 ml) and pyridine (6 ml) at room temperature to afford 59 mg of **XXI** as an oil. **XXI**: TLC: Rf 0.67 (S-114). λ_{max}^{MeOH} 245 nm (ε 8,900). PMR $\delta_{TMS}^{CDCl_3}$ in ppm: 3.98 (1H, d-d, *J*=4.0 & 6.2 Hz), 4.27 (1H, q, *J*=2.8 Hz), 5.30 (1H, d-d, *J*=2.8 & 6.2 Hz)

and 5.77 (1H, d, J=4.0 Hz). MS: m/z 692 $(M)^+$, 203.

Acid methanolysis of **Ic**—Isolation of **IVc** and **XXII**

Ic (820 mg) was hydrolyzed with 0.1 N H_2SO_4 - MeOH at 27°C for 16 hours and the hydrolyzate was fractionated by a procedure similar to that used for the methanolyzate of **Ia**. Tetrasaccharide **IVc** (356 mg) was obtained after chromatographic purification, together with 202 mg of chromophore fragment **IIIa**.

IVc: TLC: Rf 0.22 (S-114) and 0.03 (SD-109). $[\alpha]_D^{26.5}$ +135° (c 0.5, H_2O). PMR $\delta_{TMS}^{D_2O}$ in ppm: 1.23 (3H, d, J=6.5 Hz), 1.34 (3H, d, J=7.0 Hz), 3.14 (1H, m), 3.40 (3H, s) and 5.02 (1H, d, J=3.5 Hz). *Anal.* Calcd. for $C_{27}H_{49}N_3O_{18}\cdot H_2O$: C, 44.93; H, 7.12; N, 5.82. Found: C, 44.90; H, 7.12; N, 5.67.

A solution of **IVc** (240 mg) in 24 ml of 0.5 N H_2SO_4 - MeOH was heated under reflux for 30 minutes. The reaction mixture was neutralized with Amberlite IR-45 (OH^-) and evaporated to dryness *in vacuo*. The residue was charged on a column of Amberlite CG-50 (NH_4^+, ϕ 2.0×30 cm) which was developed with water followed by 0.1 N NH_4OH. A mixture of neutral sugars (116 mg) was recovered from the eluate with water and disaccharide **VIII** (142 mg) from the eluate with 0.1 N NH_4OH. The neutral sugars were separated on a silica gel column (ϕ 1.0×30 cm) developed with MeOAc - *n*-PrOH - 28% NH_4OH (45: 105: 60) to yield **XXII** (42 mg) and **V** (35 mg).

XXII: TLC: Rf 0.33 (S-114). GLC of TMS derivative (OV-17, 3%, temperature programing of 5°C/min. from 150°C): Rt 8.6. Identity with methyl D-glucoside was confirmed by direct comparison.

Periodate consumption

Periodate oxidation was carried out by the conventional procedure for the following *N*-acetyl derivatives.

Compound	Periodate consumption (mole)		
	1 hour	3 hours	7 hours
Penta-N-acetyl-**Ia**	0.35	0.47	1.17
Penta-N-acetyl-**IIa**	0.35	0.46	1.12
Di-N-acetyl-**IV**	0.65	0.81	0.96
Di-N-acetyl-**VI**	0.60	0.75	0.98
Di-N-acetyl-**VIII**	0.05	0.33	0.86
XVII	0.25	0.80	0.96
Tri-N-acetyl-**XIX**	0.07	0.49	1.03

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References

1) KAWAGUCHI, H.; M. KONISHI, T. TSUNO, T. MIYAKI, K. TOMITA, K. MATSUMOTO, K. FUJISAWA & H. TSUKIURA: Glysperin, a new antibiotic complex of bacterial origin. I. Production, isolation and properties. J. Antibiotics 34: 381~389, 1981
2) KUTTAN, R.; A. N. RADHAKRISHNAN, T. SPANDE & B. WITKOP: *sym*-Homospermidine, a naturally occurring polyamine. Biochemistry 10: 361~365, 1971
3) BACHRACH, U.: Spermidine oxidase from *Serratia marcescens*. J. Biol. Chem. 237: 3443~3448, 1962
4) IKEDA, D.; T. TSUCHIYA & S. UMEZAWA: Studies on aminosugars. XXVIII. Synthesis of β-L-idopyranosides through the corresponding 5-enopyranosides. Bull. Chem. Soc. Japan 44: 2529~2537, 1971
5) HOUGH, L.; R. KHAN & B. A. OTTER: *in* "Deoxy sugars", Advances in Chemistry. Series No. 74, pp. 120, Am. Chem. Soc., Washington, 1968

6) LIAV, A.; I. JACOBSON, M. SHEINBLATT & N. SHARON: Synthesis of 2,4-diacetamido-2,4,6-trideoxy-D-galactose. Carbohyd. Res. 66: 95~101, 1978
7) CHARLSON, A. J.; P. A. J. GORIN & A. S. PERLIN: Determination of the configuration of glycosidic linkages in oligosaccharides. Methods in Carbohydrate Chemistry. I. pp. 419~426, Academic Press, New York, 1963
8) LINDBERG, B.; B. LINDQVIST, J. LONNGREN & D. A. POWELL: Structural studies of the capsular polysaccharide from *Streptococcus pneumoniae* type 1. Carbohyd. Res. 78: 111~117, 1980
9) KENNE, L.; B. LINDBERG, K. PETERSSON, E. KATZENELLENBOGEN & E. ROMANOWSKA: Structural studies of the O-specific side-chains of the *Shigella sonnei* phase I lipopolysaccharide. Carbohyd. Res. 78: 119~126, 1980
10) ELLESTAD, G. A.; D. B. COSULICH, R. W. BROSCHARD, J. H. MARTIN, M. P. KUNSTMANN, G. O. MORTON, J. E. LANCASTER, W. FULMOR & F. M. LOVELL: Glycocinnamoylspermidines, a new class of antibiotics. 3. The structures of LL-BM 123 β, γ_1 and γ_2. J. Am. Chem. Soc. 100: 2515~2524, 1978
11) TRESNER, H. D.; J. H. KORSHALLA, A. A. FANTINI, J. D. KORSHALLA, J. P. KIRBY, J. J. GOODMAN, R. A. KELE, A. J. SHAY & D. B. BORDERS: Glycocinnamoylspermidines, a new class of antibiotics. I. Description and fermentation of the organism producing the LL-BM 123 antibiotics. J. Antibiotics 31: 394~397, 1978
12) HANSEN, S. A.: Thin-layer chromatographic method for the identification of mono-, di- and trisaccharides. J. Chromatog. 107: 224~226, 1975