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Studies of Aminosugars. XVIII. Syntheses of Amino Derivatives of Schardinger α-Dextrin and Raffinose[1]

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In continuing our study of the relationship between structural and biochemical characteristics of aminoglycosides, amino-deoxy derivatives of polysaccharides have been synthesized. The six primary hydroxyl groups of Schardinger α-dextrin and the three primary hydroxyl groups of raffinose have successfully been replaced by amino groups respectively, to give hexa-(6-amino-6-deoxy)-cyclohexaglucan (α-1, 4) (IV) and 1'', 6, 6''-triamino-1'', 6, 6''-trideoxyraffinose (X). Their structures were confirmed by their NMR and IR spectra and by the studies of their chemical reactions. It has been found that hexa-(6-amino-6-deoxy)-cyclohexaglucan (α-1, 4) showed significant antitumor activity.

In a previous paper[2] the syntheses of several amino derivatives of disaccharides were reported.

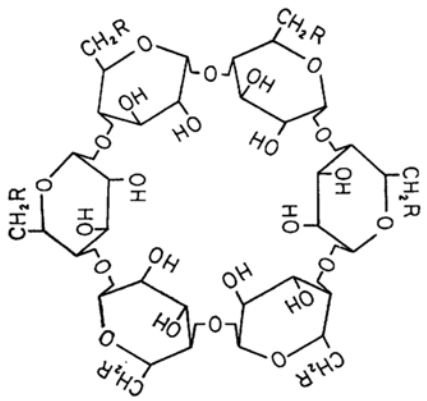
The present paper is concerned with an extension of our study on aminosaccharides and reports the syntheses of amino-deoxy derivatives from the natural polysaccharides with α-anomeric configuration, *i. e.*, Schardinger α-dextrin and raffinose.

Schardinger α-dextrin[3] (I) is a kind of cyclic dextrins and contains eighteen hydroxyl groups, six out of which are primary and the others are secondary. On preferential tosylation of I with *p*-toluenesulfonyl chloride, followed by purification

1) Part XXXIV of "Studies of Antibiotics and Related Substances," by Sumio Umezawa.

2) S. Umezawa, T. Tsuchiya, S. Nakada and K. Tatsuta, This Bulletin, **40**, 395 (1967).

3) D. French, M. L. Levine, J. H. Pazur and E. Norberg, *J. Am. Chem. Soc.*, **71**, 353 (1949).



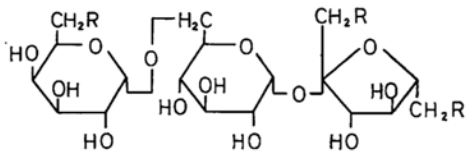
I : R = OH (Schardinger α-dextrin)

II : R = OTs

III : R = N_3

IV : R = NH_2

V : R = NHAc

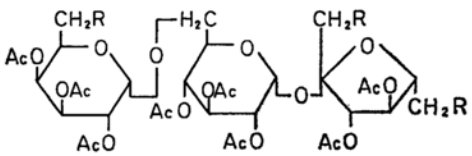


VI : R = OH (Raffinose)

IX : R = N_3

X : R = NH_2

XI : R = NHAc



VII : R = OTs

VIII : R = N_3

by column chromatography, hexa-(6-*O*-tosyl)-cyclohexaglucan (α-1, 4) (II) was obtained in a 12.2% yield. Though the preparation of II was previously reported by W. Lautsch, *et al.*,[4] the product has not been purified by chromatography and seemed to be the mixture of partially tosylated products. In the next step, the replacement of the tosyloxy groups of II with azido groups was carried out by heating II in dimethylformamide with sodium azide, thus affording hexa-(6-azido-6-deoxy)-cyclohexaglucan (α-1, 4) (III) in a 52% yield. The azide compound (III) was finally converted to hexa-(6-amino-6-deoxy)-cyclohexaglucan (α-1, 4) (IV) by catalytic hydrogenation with platinum oxide in aqueous methanol in a yield of 60%. The structure of IV was confirmed by the fact that the hydrolysis of IV with hydrochloric acid gave only 6-amino-6-deoxy-D-glucose on paper chromatogram, and furthermore by the nuclear magnetic resonance spectrum of the hexa-*N*-acetylated derivative (V) of IV. The signal at τ 8.07 (singlet) with a relative intensity of 3 may be ascribed to eighteen *N*-acetyl protons, and the doublet at τ 4.99 with a relative intensity of 1 may be assigned to six anomeric protons.

Raffinose (VI) was preferentially tosylated and acetylated to give 2, 2', 3, 3', 3'', 4, 4', 4''-octa-*O*-acetyl-1'', 6, 6''-tri-*O*-tosylraffinose (VII) in a 40% yield. The three tosyloxy groups of VII were then replaced by azido groups similarly as has been described in the preparation of III to give 2, 2', 3, 3', 3'', 4, 4', 4''-octa-*O*-acetyl-1'', 6, 6''-triazido-1'', 6, 6''-trideoxyraffinose (VIII). Treatment of VIII with sodium methoxide in methanol gave the de-*O*-acetylated product (IX), which was finally hydrogenated with platinum oxide to give 1'', 6, 6''-triamino-1'', 6, 6''-trideoxyraffinose (X). Structural evidences for X were obtained by the determination of NMR spectrum of the tri-*N*-acetylated derivative (XI) of X, and by means of the periodate oxidation of XI, where five moles of periodate were consumed for one mole of XI.

Experimental

Thin-layer Chromatography, Silica-gel Column Chromatography, and Paper Chromatography. Thin-layer chromatography was conducted by the use of silica gel (Daiichi Pure Chemicals Co.); the prepared plate was activated at 110°C and then stored in a desiccator. The spray reagent used was concentrated sulfuric acid. Solvent systems used: benzene-ethanol (4 : 1) (Solvent A); benzene-ethanol (3 : 1) (Solvent B); methanol-chloroform (5 : 2) (Solvent C); benzene-ethyl acetate (8 : 5) (Solvent D), and chloroform-ethanol (1 : 1) (Solvent E). Silica-gel column chromatography was carried out by the use of silica gel (Kanto Chemical Co.) activated at 110°C before use. Paper chromatography was conducted by the descending technique on Toyo filter paper No. 50, and the substances were detected by the use of ninhydrin spray (0.25% in pyridine). Solvent system used: *n*-butanol - pyridine - water - acetic acid (6 : 4 : 3 : 1) (Solvent F).

General Procedure for Nuclear Magnetic Resonance Spectrometry. The NMR spectrum was determined at a frequency of 60 Mcps with a Japan

4) W. Lautsch, R. Wiechert and H. Lehmann, *Kolloid-Zeitschrift*, **135**, 134 (1954).

Electron Optics JNM-C-60 spectrometer in deuterium oxide. Sodium 2, 2-dimethyl-2-silapentane-5-sulfonate was used as an internal reference in the sample. Peak positions are given in τ-values.

Hexa-(6-*O*-tosyl)-cyclohexaglucan (α-1, 4) (II). To a suspension of Schardinger α-dextrin anhydride [cyclohexaglucan (α-1, 4), I, 2.43 g, 2.50 mmol] in dry pyridine (35 m*l*) cooled at -15°C, *p*-toluenesulfonyl chloride (4.40 g, 23.1 mmol) was added; the mixture was then stirred at -15°C for 1 hr and allowed to stand overnight at 0°C, at 5°C for 5 hr and at room temperature for 3 days. After the addition of a small volume of water, the solution was left for 30 min. The resulting solution was poured into a large volume of ice and water. The precipitate was collected by a centrifuge and washed with water; yield 2.7 g (S, 10.12%). Thin-layer chromatography with Solvent A proved the product to be composed of six components, with R_f-values of 0.66, 0.53, 0.39, 0.23, 0.09 and 0.00. This product was dissolved in a solvent mixture of benzene-ethanol (4 : 1) and chromatographed on a silica-gel column (49×540 mm) with the same solvent. After about 600 m*l* of eluate, fractions (15 g each) were tested by thin-layer chromatography. Each substance having the corresponding R_f value was recrystallized from methanol, and their yields and S-contents (%) were summarized as follows:

R_f value	Fraction No.	Yield mg	S-content %
0.66	5—16	965	11.49
0.53	18—26	204	11.15
0.39	27—34	88	10.51
0.23	33—45	572	10.36
0.09	45—64	250	9.57

As the substance at the origin seemed to be less tosylated than the substance having an R_f 0.09, it was not further investigated. The substance having an R_f 0.23 had mp 184—188°C (decomp.), $[\alpha]_D^{17}$ +101° (*c* 0.72, chloroform) [lit.,[4] mp 174°C, $[\alpha]_D^{20}$ +95° (*c* 1, chloroform)]; IR spectrum (KBr disk): 3440 (νOH), 2930 (νCH), 1600 (phenyl), 1175 (sulfonate) cm^{-1}.

Found: C, 49.64; H, 5.49; S, 10.36%; mol wt (Vapor pressure osmometer), 1970. Calcd for $(C_{13}H_{16}O_7S)_6$: C, 49.36; H, 5.10; S, 10.14%; mol wt 1898.

On the basis of these data, it seemed reasonable to assume that the product (R_f 0.23) was regarded as II, which contained six tosyloxy groups; Yield 572 mg (12.2%).

Hexa-(6-azido-6-deoxy)-cyclohexaglucan (α-1, 4) (III). To a solution of II (1.14 g) in dry *N*, *N*-dimethylformamide (11 m*l*) sodium azide (1.6 g) was added; the suspension was then vigorously agitated at 135°C for 6.5 hr. The resulting dark solution was filtered, and the filtrate was evaporated and coevaporated with toluene to give the residue, which showed essentially a single spot (R_f 0.20) on thin-layer chromatography with Solvent B. The residue was dissolved in a solvent mixture of benzene-ethanol (3 : 1) and chromatographed on a silica-gel column (49×325 mm) with the same solvent. After about 400 m*l* of eluate, fractions (15 g each) were tested by thin-layer chromatography. The substance having an R_f 0.20 was eluted in the fractions of tube Nos. 4—18; these fractions were combined and evaporated to yield 350 mg (52%) of III as a slightly yellow solid, which was used for the next step without further purification; IR spectrum (KBr disk): 3400 (νOH), 2920 (νOH), 2105 (azide) cm^{-1}, no sulfonate band.

Hexa-(6-amino-6-deoxy)-cyclohexaglucan (α-1, 4) (IV). A sample (350 mg) of III was dissolved in 75% aqueous methanol (24 m*l*) and hydrogenated with platinum oxide (160 mg) under 3 atm of hydrogen-pressure at 30—40°C for 4 hr. After removal of the catalyst, the filtrate was treated with active charcoal and evaporated to give a base of IV (260 mg). The aqueous solution of the base was neutralized with hydrochloric acid to pH 2 and evaporated to dryness. Recrystallization of the residue from aqueous ethanol gave crystalline hexahydrochloride of IV; yield, 240 mg (60%); mp 214°C (decomp.), $[\alpha]_D^{17}$ +103° (*c* 0.67, water). On paper chromatography with Solvent F, hexahydrochloride of IV showed a single spot with an R_f 0.01. IR spectrum (KBr disk): 3400—3300 (νOH, NH), 2910 (νCH), 1600 (δ_{as} NH_3^+), 1500 (δ_s NH_3^+) cm^{-1}.

Found: C, 36.91; H, 6.48; N, 6.71%. Calcd for $(C_6H_{11}O_4N\cdot HCl)_6$: C, 36.47; H, 6.12; N, 7.09%.

A small quantity of IV was heated with 3 N hydrochloric acid for 3 hr in a boiling water bath, and the hydrolyzate was paper chromatographed with Solvent F. Detections by ninhydrin and ammoniacal silver nitrate colorations showed only a spot corresponding to 6-amino-6-deoxy-D-glucose*1 as monosaccharide, respectively.

Hexa-(6-acetamido-6-deoxy)-cyclohexaglucan (α-1, 4) (V). To a stirred suspension of IV base (80 mg) in dry methanol (2 m*l*) acetic anhydride (0.3 m*l*) was added; the mixture was stirred for 1 hr and allowed to stand overnight at room temperature. After evaporation, the aqueous solution of the residue was treated with active charcoal and evaporated to give a solid, which was recrystallized from water by adding ethanol; yield, 70 mg (69%); mp 135—137°C (decomp.), $[\alpha]_D^{19}$ +92° (*c* 0.79, water).

On thin-layer chromatography with Solvent C, the product V showed a single spot with an R_f value of 0.21. NMR spectrum data: τ 4.99 (doublet, J= 2.9 cps, 6 protons, anomeric hydrogen), 8.07 (singlet, 18 protons, *NAc*); IR spectrum (KBr disk): 3380—3300 (νOH, NH), 2920 (νCH), 1645 (amide I), 1550 (amide II) cm^{-1}.

Found: C, 46.97; H, 6.31; N, 6.52%. Calcd for $(C_8H_{13}O_5N)_6$: C, 47.29; H, 6.45; N, 6.89%.

2, 2′, 3, 3′, 3″, 4, 4′, 4″-Octa-*O*-acetyl-1″, 6, 6″-tri-*O*-tosylraffinose (VII). To a solution of raffinose anhydride (VI, 1.01 g, 2.00 mmol) in dry pyridine (30 m*l*), Drierite (anhydrous calcium sulfate, 1.2 g) was added; the mixture was then allowed to stand for 30 min. After the mixture had been cooled to -15°C, *p*-toluenesulfonyl chloride (1.34 g, 7.06 mmol) was added in one portion, and the mixture was kept at -15°C for 1.5 hr, at 0°C for 1 day and then at 5°C for 2 days. Acetic anhydride (4.5 m*l*) was added to the resultant cold solution, and the solution was again

*1 The specimen of 6-amino-6-deoxy-D-glucose was obtained from the hydrolyzate of kanamycin.

set aside overnight at room temperature. After the addition of a small volume of water, the solution was left for 30 min. The reaction mixture was then poured into a large volume of ice and water. The precipitate which separated was collected by a centrifuge and washed with water, yielding 2.1 g of a crude product. Thin-layer chromatography with Solvent D proved the product to be composed of two components, with R_f values of 0.48 (main) and 0.36 (minor). This product was dissolved in a solvent mixture of benzene - ethyl acetate (8 : 5) and chromatographed on a silica-gel column (47×220 mm) with the same solvent. After about 110 m*l* of eluate, fractions (15 g each) were tested by thin-layer chromatography. The substance having an R_f 0.48 appeared in the fractions of tube Nos. 24—34; these fractions were combined and evaporated under reduced pressure, and the residue was recrystallized from ethanol affording VII; yield, 1.04 g (40%), mp 90—93°C, $[\alpha]_D^{17}$ +95° (*c* 0.70, chloroform); IR spectrum (KBr disk): 2950 (νCH), 1755 (ester), 1600 (phenyl), 1370 (OAc), 1175 (sulfonate) cm^{-1}.

Found: C, 51.05; H, 5.31; S, 7.20%. Calcd for $C_{55}H_{66}O_{30}S_3$: C, 50.69; H, 5.10; S, 7.38%.

2, 2′, 3, 3′, 3″, 4, 4′, 4″-Octa-*O*-acetyl-1″, 6, 6″-triazido-1″, 6, 6″-trideoxyraffinose (VIII). A suspension of sodium azide (4.0 g) in dry *N, N*-dimethylformamide (55 m*l*)was stirred with VII (5.5 g) at 130°C for 7 hr. The solvent was then removed from the dark solution by distillation and codistillation with toluene under reduced pressure. As a part of the acetyl groups of VII had been found to be removed by the above procedure, the dark residue obtained was acetylated with acetic anhydride (40 m*l*) and dry pyridine (110 m*l*) for 2 days. After filtration, the filtrate was evaporated to a syrup, which, on thin-layer chromatography with Solvent D, showed two spots with R_f values of 0.72 (main) and 0.60 (minor). The syrup was then dissolved in a solvent mixture of benzene - ethyl acetate (8 : 5) and chromatographed on a silica-gel column (54×630 mm) with the same solvent. After about 830 m*l* of eluate, fractions (15 g each) were tested by thin-layer chromatography. The substance having an R_f 0.72 was eluted in the fractions of tube Nos. 46—60; these fractions were combined and evaporated to give a solid, which was recrystallized from aqueous ethanol affording VIII; yield, 1.48 g (38%); mp 180—184°C (decomp.), $[\alpha]_D^{17}$ +131° (*c* 0.63, chloroform); IR spectrum (KBr disk): 2930 (νCH), 2105 (azide), 1755 (ester), 1375 (OAc) cm^{-1}.

Found: C, 44.98; H, 5.13; N, 13.32%. Calcd for $C_{34}H_{45}O_{21}N_9$: C, 44.59; H, 4.95; N, 13.77%.

1″, 6, 6″-Triazido-1″, 6, 6″-trideoxyraffinose (IX) A suspension of VIII (380 mg) in dry methanol (7.6 m*l*) was treated with 0.75 N methanolic sodium methoxide (0.6 m*l*) at room temperature for 5 days. After neutralization to pH 7 with Amberlite IRC 50 (H form) (previously washed with methanol), the solution was evaporated to give the residue, which showed two spots with R_f values of 0.71 (minor) and 0.61 (main) on thin-layer chromatography with Solvent E. The residue was then dissolved in the same solvent system and chromatographed on a silica-gel column (27×230 mm) with the same solvent. After about 40 m*l* eluate, fractions (3 g each) were tested by thin-layer chromatography. The substance having an R_f 0.61 was eluted in the fractions of tube Nos. 29—54; these fractions were combined and evaporated to give a solid of IX; yield, 132 mg (55%); mp 80—83°C (decomp.), $[\alpha]_D^{17}$ +117° (*c* 0.71, water); IR spectrum (KBr disk): 3370 (νOH), 2930 (νCH), 2110 (azide) cm^{-1}.

Found: C, 37.20; H, 5.27%. Calcd for $C_{18}H_{29}O_{13}N_9$: C, 37.31; H, 5.04%.

1″, 6, 6″-Triamino-1″, 6, 6″-trideoxyraffinose (X). A solution of IX (107 mg) in 50% aqueous ethanol (10 m*l*) was hydrogenated with platinum oxide (40 mg) under 3 atm of hydrogen-pressure at 35—40°C for 4 hr. After removal of the catalyst, the filtrate was evaporated and the aqueous solution of the residue was passed through a column (6×110 mm) of Dowex 1X2 (OH form). The ninhydrin-positive eluate, which was evaporated to give a base of X, was neutralized with hydrochloric acid to pH 2 and evaporated to dryness. Recrystallization of the residue from water-acetone gave crystalline trihydrochloride of X; yield, 76.8 mg (68%); mp 139—141°C (decomp.), $[\alpha]_D^{17}$ +114° (*c* 0.68, water). On paper chromatography with Solvent F, trihydrochloride of X showed a single spot with an R_f 0.02. IR spectrum (KBr disk): 3370—3260 (νOH, NH), 2900 (νCH), 1610 (δ_{as} NH_3^+), 1505 ($\delta_s NH_3^+$) cm^{-1}.

Found: C, 35.55; H, 6.57; N, 6.89%. Calcd for $C_{18}H_{35}O_{13}N_9 \cdot 3HCl$: C, 35.39; H, 6.27; N, 6.88%.

1″, 6, 6″ - Triacetamido - 1″, 6, 6″ - trideoxyraffinose (XI). To a suspension of X base (82 mg) in dry methanol (0.85 m*l*) acetic anhydride (0.25 m*l*) was added; the mixture was treated as has been described in the synthesis of V. The product was recrystallized from water by adding acetone; yield, 66 mg (64%); mp 142—144°C (decomp.), $[\alpha]_D^{17}$ +123° (*c* 0.65, water). On thin-layer chromatography with Solvent C, the product XI showed a single spot with an R_f value of 0.44. NMR spectrum data: τ ~8.0 (three singlets, 9 protons, NAc-1″, 6, 6″), 4.57 (doublet, 1 proton, H-1′, J=3 cps), 5.07 (doublet, 1 proton, H-1, J=2.5 cps); IR spectrum (KBr disk): 3400—3300 (νOH, NH), 2930 (νCH,) 1650 (amide I), 1555 (amide II) cm^{-1}.

Found: C, 45.48; H, 6.86; N, 6.70%. Calcd for $C_{24}H_{41}O_{16}N_3$: C, 45.93; H, 6.58; N, 6.70%.

The Periodate Oxidation of XI. An accurately-weighed sample (32.5 mg) of XI was dissolved in a mixture of a 0.1 N sodium acetate buffer solution (pH 4.7, 10m*l*) and a 0.1 M sodium metaperiodate solution (5.26 m*l*); the oxidation was then carried out at 0°C in the dark. Iodometric titrations with sodium arsenite[5] showed that the periodate consumption in moles per mole of XI was (time in hr, moles of oxidant consumed per mole of XI): 0.5, 3.16; 3.0, 4.17; 6.0, 4.37; 24.0, 4.55; 48.0, 4.99; 96.0, 5.00.

Preliminary Bioassay. It is noteworthy that the daily intraperitoneal injection of 100 mg/kg of the amino-deoxy derivative (IV) of Schardinger α-dextrin inhibited the ascites increase and prolonged the survival period of mice bearing ascites-type of Ehrlich carcinoma by intraperitoneal route. The compound IV also inhibited the growth of *Bacillus subtilis* PCI 219 completely at a dilution of 1 : 500, but showed no inhibition against *M. pyogenes var. aureus* 209p, *E. coli* and *Mycobacterium tuberculosis* 607 in a dilution of 1 : 1000. Compound

5) J. M. Bobbitt, "Advances in Carbohydrate Chemistry," Vol. 11, Academic Press, New York (1956), pp. 1—14.

X did not show any activity against the above-mentioned test organisms and carcinoma.

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