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55. Masuo Akagi, Setsuzo Tejima, and Masanobu Haga : Biochemical Studies on Thiosugars. II.*1 Synthesis of N-Acetyl-1-thio-D-glucosamine and its Derivatives.

(*Faculty of Pharmacy, School of Medicine, Hokkaido University**2)

D-Glucosamine, one of natural amino sugars, is widely distributed in animals and plants, and it has a considerable significance to animals as a mucopolysaccharides.

Concerning sulfur-containing derivatives of glucosamine, none has been found in nature with the exception of a sulfate, but in expectation of some biochemical activities of sulfur-containing amino sugars, the present work was undertaken to synthesize N-acetyl-1-thio-D-glucosamine and its derivatives.

For the synthesis of 1-thio-sugar, several procedures*1 are available, but in the present work, hydrolysis of glycosyl xanthate, which was synthesized from glycosyl halide and potassium ethylxanthate, was employed.

As regards glycosyl halides of glucosamine, various synthetic methods for numerous derivatives have been reported,[1] but most of them required tedious procedures and gave a poor yield.

The method by Leaback and Walker[2] was improved and acetochloroglucosamine (2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl chloride) (I) was synthesized directly from glucosamine hydrochloride in 60% yield.

D-Glucosamine hydrochloride and anhydrous sodium acetate were suspended in acetic anhydride and heated on a steam bath with continuous stirring for 2 hours.

Glucosamine was acetylated and resulting sodium chloride precipitated. To this mixture, dry hydrogen chloride was saturated at below 10°. After standing at room temperature for 24 hours, chloroform was added and the mixture was washed thoroughly with

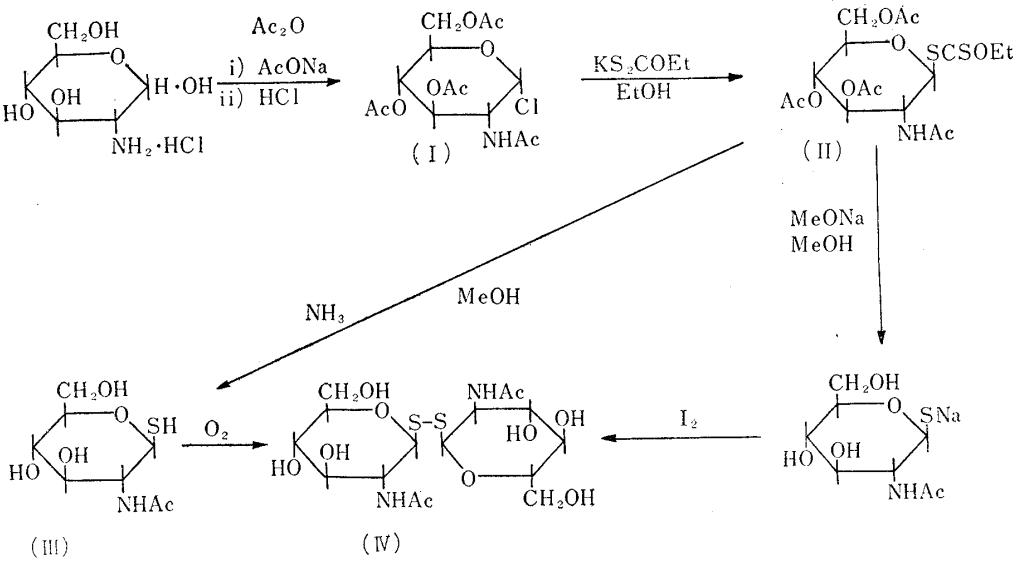

Chart 1.

*1 Part I : This Bulletin, 8, 1114 (1960).

*2 Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (赤木満洲雄, 手島節三, 羽賀正信).

1) A. B. Foster, D. Horton : "Advances in Carbohydrate Chemistry," **14**, 214 (1959). Academic Press Inc., New York.

2) D. H. Leaback, P. G. Walker : J. Chem. Soc., **1957**, 4754.

ice-water. After usual treatment, (I) was obtained as crystals of m.p. 127~128°.

For the preparation of acetoxanthogenoglucosamine (2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranosyl ethylxanthate) (II), potassium ethylxanthate and (I) were heated in dehydrated ethanol and (II), m.p. 142~143°, was obtained in 50% yield.

By treatment of (II) with cold methanolic ammonia, deacetylation and hydrolysis of xanthate occurred and N-acetyl-1-thio-D-glucosamine (III) was obtained as highly hydroscopic powder which was easily oxidized to crystalline disulfide (IV), m.p. 212~213°.

Deacetylation of (II) with calculated amount of sodium methoxide gave a sodium salt of (III) as hygroscopic crystals of m.p. 120~122°.

The sodium salt of (III) was oxidized by iodine to disulfide (IV) quantitatively so that the disulfide (IV) is bis(2-acetamido-2-deoxy-β-D-glucopyranosyl) disulfide. In analogy with (IV), the sulfide was synthesized. Refluxing of (I) and potasium sulfide in dehydrated ethanol gave acetylated sulfide (V), m.p. 314~316°, which was deacetylated by methanolic ammonia to N-acetylated sulfide (VI), m.p. 219~220°. In another way, (VI) was synthesized from (I) and the sodium salt of (III) by refluxing in dehydrated ethanol and deacetylation and, therefore, this sulfide (VI) is bis(2-acetamido-2-deoxy-β-D-glucopyranosyl) sulfide (isotrehalose type).

(III) and its sodium salt reduced the Fehling solution and gave color reaction with sodium nitroferricyanide but (IV) and (VI) did not reduce the Fehling solution and did not give color reaction for thiol.

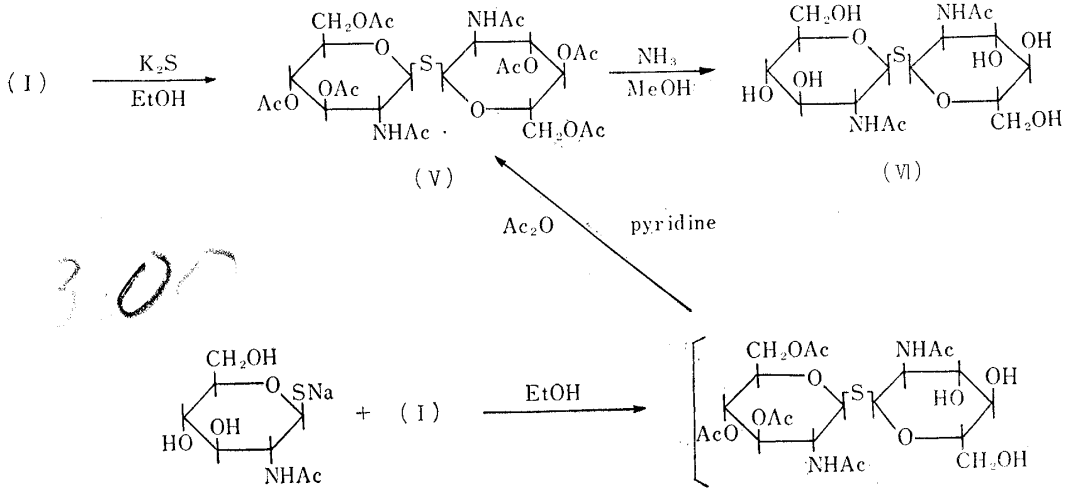

Chart 2.

Experimental

2-Acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl Chloride (I)——30.0 g. of glucosamine hydrochloride and 15.0 g. of anhyd. AcONa were suspended in 300 cc. of Ac_2O and heated on a steam bath with continuous stirring for 2 hr. To this mixture, without removal of NaCl, dry HCl was passed below 10° to be saturated. The mixture was kept aside at room temperature for 24 hr., protected from moisture, $CHCl_3$ (400 cc.) was added to it, and the mixture was poured into 1 L. of ice-water. The separated $CHCl_3$ layer was extracted with cold saturated $NaHCO_3$ solution and twice with cold H_2O. The $CHCl_3$ layer was dried over Na_2SO_4 and evaporated in a reduced pressure to a syrup which crystallized from Et_2O. Recrystallization from $CHCl_3$ and Et_2O gave colorless crystals, m.p. 127~128°. Yield, 32 g. (60%). $[\alpha]_D^{16}$ +108° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{14}H_{20}O_8NCl$: C, 45.42; H, 5.51; N, 3.83; Cl, 9.42. Found : C, 45.61; H, 5.41; N, 3.88; Cl, 9.54.

2-Acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranosyl Ethylxanthate (II)——A solution of K-ethylxanthate dissolved in 150 cc. of dehyd. EtOH was heated on a steam bath to boiling. To this solution, 18.0 g. of (I) was added in small portions with stirring for 20 min. After cool, the mixture was poured into 400 cc. of H_2O and allowed to stand in a refrigerator. The precipitated crystals were

collected, washed with H_2O and recrystallized from hydr. EtOH to white needles, m.p. 142~143°. Yield, 11.5 g. (50%). $[\alpha]_D^{15}$ +36.1° (c=1.2, $CHCl_3$). *Anal.* Calcd. for $C_{17}H_{25}O_9NS_2$: C, 45.01; H, 5.54; N, 3.10; S, 14.18. Found : C, 45.08; H, 5.64; N, 3.31; S, 14.01.

N-Acetyl-1-thio-D-glucosamine (III)——A solution of (II) (5.0 g.) in 30 cc. of MeOH saturated with NH_3 at 0° was allowed to stand overnight in a refrigerator. The solution was evaporated to syrup in a reduced pressure. The residue was washed with a small amount of cold EtOH and Et_2O. Yield, 1.8 g. (70%).

This material was highly hygroscopic and had no distinct melting point. $[\alpha]_D^{16}$ −220° (c=1.0, H_2O). *Anal.* Calcd. for $C_8H_{15}O_5NS$: C, 40.49; H, 6.37; N, 5.82; S, 13.51. Found : C, 40.54; H, 6.42; N, 5.76; S. 13.54.

(III) reduced (blackend) the Fehling solution at room temperature and gave color reaction with Na-nitroferricyanide. On dissolving in hot EtOH, (III) was easily oxidized to disulfide (IV).

Sodium Salt of (III)——To a solution of 5.0 g. of (II) dissolved in 10 cc. of $CHCl_3$ and cooled to 0°, MeONa solution (Na, 0.25 g. in 10 cc. of MeOH) was added and the mixture was allowed to stand at 0° for 6 hr. The resulting precipitate was collected and washed with cold EtOH, m.p. 120~122°. $[\alpha]_D^{20}$ −25.5° (c=0.8, H_2O). *Anal.* Calcd. for $C_8H_{14}O_5NSNa$: C, 37.06; H, 5.44; N, 5.40; S, 12.36. Found : C, 37.12; H, 5.54; N, 5.32; S, 12.33. This material was slightly hygroscopic and reduced (blackened) the Fehling solution at room temperature and gave color reaction as thiol.

Bis(2-acetamido-2-deoxy-β-D-glucopyranosyl) Disulfide (IV)——1) This material was obtained by dissolving (III) in hot EtOH and after 24 hr., (IV) appeared as fine crystals, m.p. 213°.

2) I_2-KI solution was added to the solution of sodium salt of (III) (2.5 g.) in 10 cc. of H_2O until the color of iodine remained a little. The solution was concentrated to dryness in a reduced pressure and the residue was extracted with 10 cc. of MeOH which was allowed to stand in a refrigerator, m.p. 212~213°. Yield, 1.8 g. $[\alpha]_D^{16}$ −223° (c=0.9, H_2O). *Anal.* Calcd. for $C_{16}H_{28}O_{10}N_2S_2$: C, 41.01; H, 5.17; N, 6.00; S, 13.69. Found : C, 40.91; H, 5.08; N, 6.11; S, 13.76.

Bis(2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranosyl) Sulfide (V)——1) From (I) and K_2S: 10.0 g. of (I) was added to the hot solution of K_2S, prepared from 1.1 g. of K and H_2S in 30 cc. EtOH, and heated on a steam bath with continuous stirring. After 5 min., (V) appeared as needle crystals. The mixture was poured into 100 cc. of H_2O. Yield, 3.4 g. (36%). After recrystallization from $CHCl_3$-EtOH, (V) melted at 314~316° (decomp.).

2) From (I) and Sodium Salt of (III). A solution of 3.7 g. of (I) and 2.6 g. of sodium salt of (III) dissolved in 20 cc. of dehyd. EtOH was refluxed on a steam bath for 25 min., the solution was evaporated in a reduced pressure to a syrup, and the residue was acetylated with pyridine and Ac_2O as usual. Yield, 1.8 g. (50%). $[\alpha]_D^{16}$ −146.4° (c=0.8, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{40}O_{16}N_2S$: C, 48.83; H, 5.81; N, 4.07; S, 4.06. Found : C, 48.51; H, 5.88; N, 4.14; S, 4.09.

Bis(2-acetamido-2-deoxy-β-D-glucopyranosyl) Sulfide (VI)——A solution of 1.0 g. of (V) dissolved in 10 cc. of MeOH saturated with NH_3 at 0° was allowed to stand overnight at room temperature. The solution was concentrated under a reduced pressure below 40° and the syrupy residue was crystallized from H_2O-EtOH. Recrystallization form H_2O-EtOH gave colorless powder, m.p. 219~220°. $[\alpha]_D^{16}$ −36.7° (c=1.0, H_2O). *Anal.* Calcd. for $C_{16}H_{28}O_{10}N_2S$: C, 43.62; H, 6.46; N, 6.36; S, 7.28. Found: C, 42.66; H, 6.62; N, 6.22; S, 7.20.

The chemical properties of (VI) closely resembled (IV).

The authors wish to express their gratitude to Chugai Pharmaceutical Co., for the supply of glucosamine hydrochloride.

Summary

A convenient synthesis for 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl chloride was described. Several derivatives of 1-thio-D-glucosamine were synthesized through xanthate.

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