solvent gave a reddish glass. Recrystallization from chloroform petroleum ether gave 4,5-dihydroxy-2,4′-bis (2-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydro-1-isoquinolylmethyl) biphenyl ether (I) as a pale brown powder (16 mg.), m.p. 112~116° (sinters at 102°). *Anal.* Calcd. for $C_{36}H_{40}O_7N_2$: C, 70.57; H, 6.58; N, 4.57. Found: C, 70.64; H, 6.67; N, 4.65. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610 (OH) and 2817 ($N-CH_3$). UV λ_{max}^{EtOH} mμ (log ε): 286 (4.07).*5 Rf (paper chromatography) (synthetic) 0.497, (natural) 0.489 [BuOH-AcOH-H_2O (5:1:4) as solvent; the spots were detected by their fluorescence under UV light (short wave 2536 Å, cycle 50)]. NMR (τ) spectrum ($CDCl_3$ as solvent and tetramethylsilane as internal reference were used) showed 5.41~5.70 (OH) (4H), 6.21 (broad singlet) (6H) (OCH_3) and 7.60 (broad doublet) (6H) (NCH_3).

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Summary

The diamide (Ⅸ) was prepared from the 2,4′-oxy-4,5-bisbenzyloxydibenzoic acid (Ⅶ) and 3-methoxy-4-benzyloxyphenethylamine by Arndt-Eistert reaction. Cyclization of Ⅸ gave the dihydroisoquinoline (Ⅹ); reduction of the dimethiodide (Ⅺ) with sodium borohydride, followed by hydrolysis with concentrated hydrochloric acid-ethanol (1:1), gave the stereoisomeric mixture of magnolamine (I).

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*5 Natural magnolamine, UV λ_{max}^{EtOH} mμ (log ε): 286 (4.09).

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15. Shigeru Takanashi, Yoshinobu Hirasaka, Minoru Kawada, and Morizo Ishidate*2: Synthesis of Amino Sugar Containing Disaccharides. The Synthesis of Hyalobiuronic Acid and Chondrosine.*3

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Biochemical interests have been taken in aminosugar containing polysaccharides. Hyaluronic acid and chondroitin sulfuric acid A or C are typical of these polysaccharides. The former is composed of equivalent quantities of D-glucuronic acid and N-acetyl-D-glucosamine, and the latter consists of D-glucuronic acid and sulfonated N-acetyl-D-galactosamine. The unit disaccharide of hyaluronic acid is hyalobiuronic acid and that of chondroitin sulfuric abid A or C is chondrosine.

In a preliminary communication, there has been outlined the synthesis of these disaccharides.[1]

Hyalobiuronic acid was isolated form hydrolysate of human umbilical cord hyaluronate[2] and its structure was shown to be 2-amino-2-deoxy-3-O-β-D-glucopyranuronosyl-D-glucose by Weissmann, *et al.*[3] Chondrosine was obtained in crystalline form[4] by Davidson *et al.*[5] and characterized as 2-amino-2-deoxy-3-O-β-D-glucopyranuro-

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*2 *Ibid.* (石館守三).

*3 Part of this work was presented at the 35th Annual Meeting of the Japanese Biochemical Sociey, Tokyo, 1962.

1) S. Takanashi, Y. Hirasaka, M. Kawada, M. Ishidate: J. Am. Chem. Soc., **84**, 3029 (1962).
2) B. Weissmann, M. M. Rapport, A. Linker, K. Meyer: J. Biol. Chem., **205**, 205 (1953).
3) B. Weissmann, K. Meyer: J. Am. Chem. Soc., **76**, 1753 (1954).
4) E. A. Davidson, K. Meyer: *Ibid.*, **76**, 5686 (1954).
5) *Idem*: *Ibid.*, **77**, 4796 (1954).

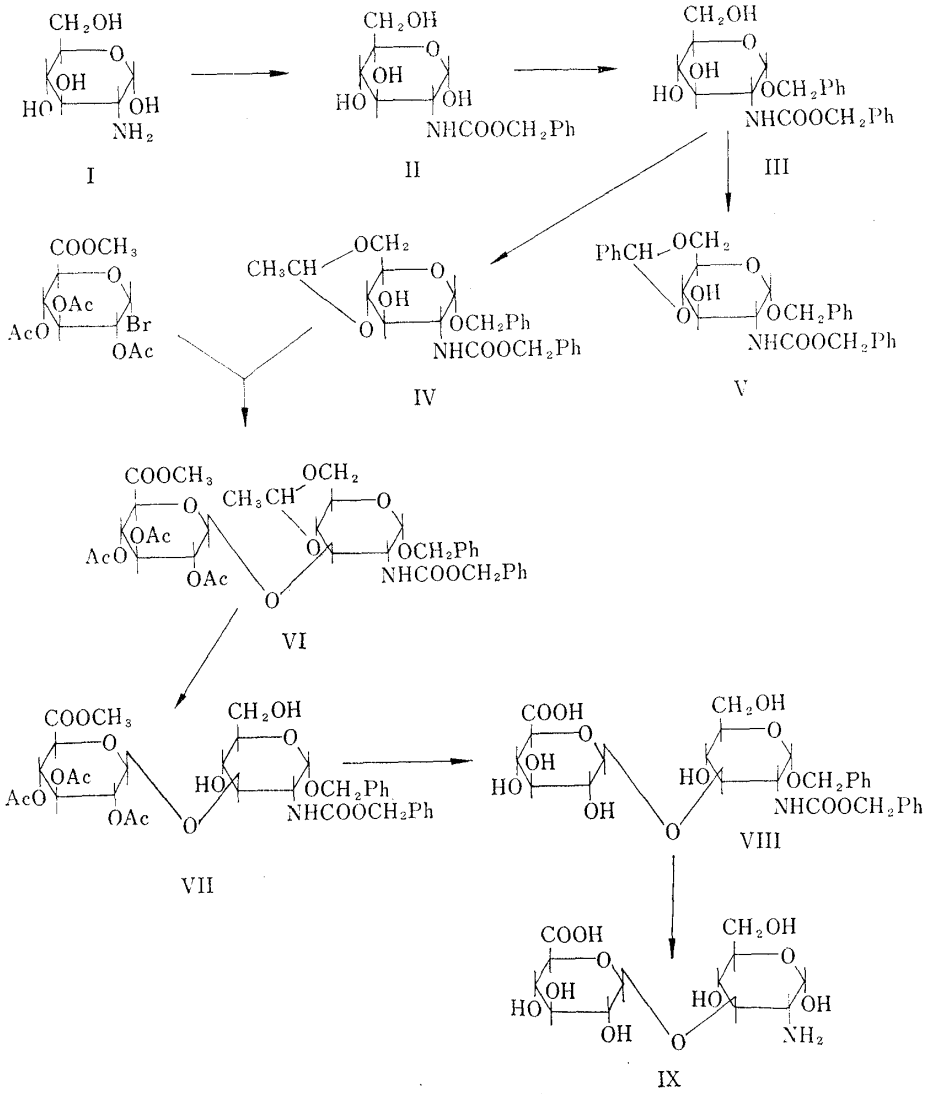

Chart 1. Each galactosamine derivative corresponds to I, II, ... and IX was numbered as X, XI, ... and XVIII

nosyl-D-galactose. The chemical synthesis of the heptaacetyl-methyl ester of hyalobiuronic acid has been accomplished independently by Jeanloz, *et al.*[6,7]

The authors synthesized these disaccharides for the purpose of confirming the structure of them synthetically and providing for some biochemical studies. The synthetic process is shown in Chart 1.

The synthesis of hyalobiuronic acid was made by condensing methyl (tri-O-acetyl-α-D-glucopyranosyl bromide) uronate[8] by the Koenigs-Knorr reaction with a glucosamine derivative which had the amino group and all the hydroxyl groups except C_3 substituted.

2-Benzyloxycarbonylamino-2-deoxy-D-glucopyranose (II) was prepared according to the method of Chargaff, *et al.*[9] and then converted to benzyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (III) by the method of Heyns, *et al.*[10]

6) R. W. Jeanloz, H. M. Flowers: J. Am. Chem. Soc., **84**, 3030 (1962).
7) H. M. Flowers, R. W. Jeanloz: Biochemistry, **3**, 123 (1964).
8) G. N. Bollenback, J. W. Long, D. G. Benjamin, J. A. Lindquist: J. Am. Chem. Soc., **77**, 3310 (1955).
9) E. Chargaff, M. Bovarnick: J. Biol. Chem., **118**, 421 (1937).
10) K. Heyns, H. Paulsen: Ber., 88, 188 (1955).

In order to block the hydroxyl groups in C_4 and C_6 of Ⅲ, two kinds of acetal derivatives, an ethylidene and a benzylidene compound, were prepared. The procedure of Akiya, *et al.*[11] was applied to the preparations of benzyl 2-benzyloxycarbenylamino 2-deoxy-4,6-O-ethylidene-α-D-glucopyranoside (Ⅵ) and benzyl 2-benzyloxycarbonylamino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (Ⅴ).

An attempt to condense methyl (tri-O-acetyl-α-D-glucopyranosyl bromide)uronate with Ⅳ in the presence of silver oxide, iodine and calcium sulfate in chloroform was successful but Ⅴ failed to enter into the reaction probably because of some steric effect of benzylidene group described by Curtis, *et al.*[12]

The product of the condensation reaction was chromatographed on Florisil*4 column and benzyl 2-benzyloxycarbonylamino-2-deoxy-4,6-O-ethylidene-3-O-[(methyl tri-O-acetyl-β-D-glucopyranosyl)uronate]-α-D-glucopyranoside (Ⅵ) was isolated as white needles from the first fraction eluted with a mixture of chloroform and petroleum ether (4:1). This compound possesses infrared absorption bands at 1750 and 1690 cm^{-1} due to acetyl and N-benzyloxycarbonyl group.

The removal of ethylidene group in Ⅵ was carried out by heating Ⅵ in 50% acetic acid on a boiling water bath, and resulting benzyl 2-benzyloxycarbonylamino-2-deoxy-3-O-[(methyl tri-O-acetyl-β-D-glucopyranosyl)uronate]-α-D-glucopyranoside (Ⅶ) was treated with 0.2*N* sodium hydroxide at room temperature to hydrolyse the acetyl and methyl group, and benzyl 2-benzyloxycarbonylamino-2-deoxy-3-O-β-D-glucopyranuronosyl-α-D-glucopyranoside (Ⅷ) was precipitated by acidifying with hydrochloric acid.

The N-benzyloxycarbonyl and benzyl glucosidic linkages in Ⅷ were split simultaneously by catalytic hydrogenation over 10% palladium-carbon in water and resulting 2-amino-2-deoxy-3-O-β-D-glucopyranuronosyl-D-glucopyranose (Ⅸ) was recrystallized by dissolving in 0.2*N* hydrochloric acid followed by neutralizing to pH 5 with sodium bicarbonate.

The mixture of Ⅸ and authentic hyalobiuronic acid obtained by hydrolysis of umbilical cord hyaluronate*5 gave a single spot on a paper chromatogram, and infrared absorption spectrum (Fig. 1) and specific optical rotation of Ⅸ coincided with authentic material.

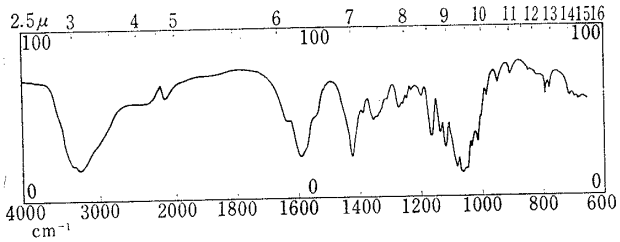

Fig. 1. Infrared Absorption Spectrum of Ⅸ (in KBr)

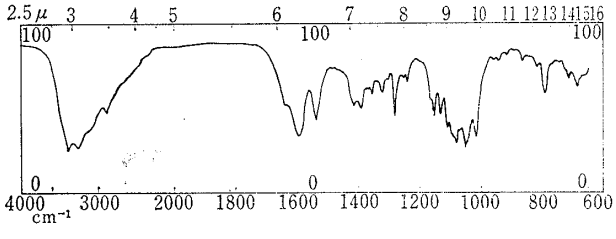

Fig. 2. Infrared Absorption Spectrum of XVⅢ (in KBr)

Quite similarly, benzyl 2-benzyloxycarbonylamino-2-deoxy-α-D-galactopyranoside[13] (Ⅹ) was converted to benzyl 2-benzyloxycarbonylamino-2-deoxy-4,6-O-ethylidene-α-D-galactopyranoside (Ⅺ) and benzyl 2-benzyloxycarbonylamino-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (Ⅻ).

*4 Synthetic magnesia-silica gel. A product of Floridin Co.
*5 Kindly supplied by Dr. K. Konno and Dr. T. Tetsuka, Department of Biochemistry, Faculty of Medicine, University of Tokyo.

11) S. Akiya, T. Osawa : Yakugaku Zasshi, **76**, 1276 (1956).
12) E. J. C. Curtis, J. K. N. Jones : Can. J. Chem., **38**, 1305 (1960).
13) K. Heyns, M. Beck : Ber., **90**, 2443 (1957).

In this case also, the condensation of methyl (tri-O-acetyl-α-D-glucopyranosyl bromide)uronate with the ethylidene derivative by the Koenigs-Knorr reaction was effected, but not with the benzylidene derivative. The reaction product was isolated by Florisil column chromatography and then the protecting groups were removed. 2-Amino-2-deoxy-3-O-β-D-glucopyranuronosyl-D-galactose was recrystallized from aqueous ethanol and was identified with authentic chondrosine obtained form hydrolysate of chondroitin sulfate by paper chromatography and infrared absorption spectra (Fig. 2).

Experimental

Benzyl 2-Benzyloxycarbonylamino-2-deoxy-4,6-O-ethylidene-α-D-glucopyranoside(IV)——Finely powdered III (28.2 g.) was suspended in freshly distilled paraldehyde (300 ml.), and concentrated H_2SO_4 (0.5 ml.) was added to it. The mixture was stirred for 5 hr. at room temperature, then $CHCl_3$ (300 ml.) was added to obtain clear solution, which was neutralized by adding K_2CO_3 and filtered. The filtrate was concentrated and the residue was washed with ligroin, recrystallized from benzene as long needles of m.p. 165° (20 g., yield 67%). $[\alpha]_D^{20}$ +95.4°(c=1.95, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{27}O_7N$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.61; H, 6.26; N, 3.11.

Benzyl 2-Benzyloxycarbonylamino-2-deoxy-4,6-O-ethylidene-α-D-galactopyranoside (XIII)——A mixture of XII (4.9 g.), paraldehyde (50 ml.) and concentrated H_2SO_4 (5 drops) was treated as above and XIII was recrystallized from CCl_4 (3.3 g., yield 67%). m.p. 183°. $[\alpha]_D^{20}$ +135.8°(c=1.62, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{27}O_7N$: C, 64.32; H, 6.34; N, 3.26. Found: C, 64.58; H, 6.27; N, 3.06.

Benzyl 2-Benzyloxycarbonylamino-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (V)——A suspension of III (8.1 g.) and anhydrous $ZnCl_2$ (8 g.) in benzaldehyde was stirred for 7 hr. at room temperature. To the resulting mush H_2O and ligroin were added. The mixture was stirred and filtered. The filtered mass was washed with H_2O and ligroin, recrystallized from AcOEt as white needles which possessed two molecules of H_2O (8.4 g., 80%). m.p. 218°. $[\alpha]_D^{20}$ +102.2°(c=2.23, dioxane). *Anal.* Calcd. for $C_{28}H_{29}O_7N \cdot 2H_2O$: C, 63.74; H, 6.31; N, 2.66. Found: C, 63.27; H, 6.28; N, 2.85. Water of crystallization in V was lost on heating *in vacuo* over P_2O_5 at 80° for 24 hr. *Anal.* Calcd. for $C_{28}H_{29}O_7N$: C, 68.42; H, 5.95; N, 2.85. Found: C, 68.47; H, 5.76; N, 2.71.

Benzyl 2-Benzyloxycarbonylamino-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside (XIV)——Benzaldehyde (20 ml.), XII (4 g.) and $ZnCl_2$ (4 g.) were treated as above and resulting XIV was recrystallized from MeOH (4 g., yield 82%). m.p. 199°. $[\alpha]_D^{20}$ +130.0°(c=0.90, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{29}O_7N$: C, 68.42; H, 5.95; N, 2.85. Found: C, 68.61; H, 5.87; N, 2.73.

Benzyl 2-Benzyloxycarbonylamino-2-deoxy-4,6-O-ethylidene-3-O-[(methyl tri-O-acetyl-β-D-glucopyranosyl)uronate]-α-D-glucopyranoside (VI)——To a solution of IV (17.2 g.) in alcohol-free anhydrous $CHCl_3$ (160 ml.), alkaline-free $CaSO_4 \cdot \frac{1}{2}H_2O$*6 (48 g.) and freshly prepared Ag_2O (23.2 g.) were added. In another flask were put methyl (tri-O-acetyl-α-D-glucopyranosyl bromide)uronate (17.2 g.), $CaSO_4 \cdot \frac{1}{2}H_2O$ (3 g.) and $CHCl_3$ (15 ml.). Both the mixtures were stirred respectively under protecting from light for 1 hr. at room temperature, then combined together and iodine (1.3 g.) was added. Stirring was continued in the dark until no ionizable bromine was detected (3 days) after which the solids were removed by filtration and washed with $CHCl_3$. The combined solutions were evaporated to a syrup, which was dissolved in benzene. The benzene solution was passed through a column containing Florisil (240 g., 3×90 cm.). The reaction products absorbed on the column were eluted with a mixture of $CHCl_3$ and petr. ether (4:1). The eluates which showed positive reaction to qualitative tests for uronic acid and nitrogen were collected and evaporated. The residue was recrystallized from MeOH (7.5 g., yield 25%). m.p. 189°. $[\alpha]_D^{20}$ +46.7° (c=1.65, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{43}O_{16}N$: C, 57.98; H, 5.81; N, 1.88. Found: C, 58.07; H, 5.80; N, 1.65. Further elution with AcOEt afforded a material which was recrystallized form AcOEt (decomp. 230°) and had no infrared absorption band at 1690 cm^{-1} due to N-benzyloxycarbonyl group.

Condensation Reaction of V——A mixture of anhydrous V (9.8 g.), methyl (tri-O-acetyl-α-D-glucopyranosyl bromide)uronate (12 g.), $CaSO_4 \cdot \frac{1}{2}H_2O$ (60 g.), Ag_2O (15 g.), iodine (2 g.) and $CHCl_3$ (400 ml.) was treated as above. No condensation product was obtained and V (6.1 g.) was recovered.

Benzyl 2-Benzyloxycarbonylamino-2-deoxy-4,6-O-ethylidene-3-O-[(methyl tri-O-acetyl-β-D-glucopyranosyl)uronate]-α-D-galactopyranoside (XV)——A mixture of XIII (4.3 g.), methyl (tri-O-acetyl-α-D-glucopyranosyl bromide)uronate (4.3 g.), $CaSO_4 \cdot \frac{1}{2}H_2O$ (15 g.), Ag_2O (5.8 g.), iodine (0.5 g.) and $CHCl_3$ (55 ml.) was treated as in the case of VI and resulting XV was recrystallized from CCl_4 (1.3 g., yield 17%). m.p. 162°. $[\alpha]_D^{20}$ +86.8° (c=1.52, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{43}O_{16}N$: C, 57.98; H, 5.81; N, 1.88. Found: C, 58.30; H, 5.83; N, 2.03. In this case, about 25% of the starting material (XIII, 1.1 g.) was

*6 Prepared by washing $CaSO_4 \cdot 2H_2O$ with diluted H_2SO_4 and then H_2O thoroughly and drying at 120~130° for 8 hr.

recovered.

Benzyl 2-Benzyloxycarbonylamino-2-deoxy-3-O-β-D-glucopyranuronosyl-α-D-glucopyranose (VIII)——A suspension of VI (6.0 g.) in 50% AcOH (300 ml.) was heated on a boiling water bath for 3 hr. and resulted clear solution was concentrated to one-tenth in volume, to which H_2O was added and white precipitate VII was collected by filtration. Attempts to crystallize VII from several solvents were unsuccessful. Amorphous VII (5.5 g.) was suspended in 0.2*N* NaOH (320 ml.), stirred at room temperature until a clear solution was obtained, which was allowed to stand overnight and then acidified with 2*N* HCl. White precipitate of VIII was filtered, washed with H_2O and recrystallized from H_2O as needles (3.2 g., yield 69% based on VI). m.p. 190°(decomp.). $[\alpha]_D^{20}$ +48.1°(c=1.83, MeOH). *Anal.* Calcd. for $C_{27}H_{33}O_{13}N$: C, 55.95; H, 5.74; N, 2.42. Found: C, 55.88; H, 5.77; N, 2.45.

Benzyl 2-Benzyloxycarbonylamino-2-deoxy-3-O-β-D-glucopyranuronosyl-α-D-galactopyranoside (XVII)——Heating of XV (3.0 g.) in 50% AcOH (200 ml.) afforded XVI, which was treated with 0.2*N* NaOH (100 ml.) as described above. The solution neutralized with 2*N* HCl was concentrated *in vacuo*. The residue was dissolved in EtOH and insoluble material was removed by filtration. The evaporation of the filtrate gave a crystalline residue which was recrystallized from H_2O (1.6 g., yield 66% based on XV). m.p. 210°(decomp.). $[\alpha]_D^{20}$ +75.2°(c=1.80, MeOH). *Anal.* Calcd. for $C_{27}H_{33}O_{13}N\cdot H_2O$: C, 54.27; H, 5.90; N, 2.34. Found: C, 54.20; H, 5.96; N, 2.15.

2-Amino-2-deoxy-3-O-β-D-glucopyranuronosyl-D-glucopyranose (IX)——A suspension of finely ground VIII (2.0 g.) and 10% Pd-C (1.0 g.) in H_2O (200 ml.) was stirred vigorously in the stream of H_2 at room temperature for 16 hr. The catalyst was removed by centrifugation and filtration, washed with hot 0.1% NaCl solution. The combined solutions were concentrated to form fine crystals and allowed to stand overnight at 5°. The crystals were recrystallized by dissolving in 0.2*N* HCl followed by neutralizing to pH 5 with $NaHCO_3$. This compound slowly crystallized as prisms with one molecule of H_2O which was lost on heating *in vacuo* over P_2O_5 at 80° for 8 hr. and darkened at about 190°, having no characteristic melting or decomposing point (0.9 g., yield 73%). $[\alpha]_D^{20}$ +34.3°~+30.1°(5 hr.; c=1.08, 0.1*N* HCl). *Anal.* Calcd. for $C_{12}H_{21}O_{11}N\cdot H_2O$: C, 38.61; H, 6.21; N, 3.75. Found: C, 38.81; H, 6.09; N, 3.75. After drying: Calcd. for $C_{12}H_{21}O_{11}N$: C, 40.56; H, 5.96; N, 3.94. Found: C, 39.89; H, 5.95; N, 3.92.

2-Amino-2-deoxy-3-O-β-D-glucopyranuronosyl-D-galactopyranose (XVIII)——Catalytic hydrogenation of XVII (1.6 g.) was carried out over 10% Pd-C (0.8 g.) in H_2O (40 ml.) as described above. The filtered solution was evaporated *in vacuo* to dryness and a crystalline residue was dissolved in hot water, to which equal volume of EtOH was added. Fine needles were formed on standing. This compound was obtained as a crystalline hydrate which darkened gradually from 185° without melting (0.6 g., yieid 60%). $[\alpha]_D^{20}$ +38°(c=1.00, H_2O). *Anal.* Calcd. for $C_{12}H_{21}O_{11}N\cdot H_2O$: C, 38.61; H, 6.21; N, 3.75. Found: C, 38.80; H, 6.12; N, 3.64. After heating *in vacuo* over P_2O_5 at 80° for 8 hr.: Calcd. for $C_{12}H_{21}O_{11}N$: C, 40.56; H, 5.96; N, 3.94. Found: C, 40.12; H, 6.01; N, 3.98.

Paper Chromatography——Paper chromatography was carried out by ascending method at 20° for 16~18 hr. using Toyo filter paper No. 50. The mixture of IX and authentic hyalobiuronic acid obtained by hydrolysis of umbilical cord hyaluronate, gave a single pink spot of Rf 0.16 on a paper chromatogram developed with butanol-AcOH-H_2O (38:12:50) and sprayed with ninhydrine. Co-chromatography of XVIII with authentic chondrosine was done by the same method as described above, and a single spot of Rf 0.14 was detected.

The authors express their gratitude to Dr. Nitta, a department head of this laboratories, for his encouragements during the work. The authors are also indebted to the members of the Analytical Section of the laboratories for taking IR spectra and the elementary analysis.

Summary

Hyalobiuronic acid, the unit disaccharide of hyaluronic acid, and chondrosine, that of chondroitin sulfuric acid A or C, were synthesized by condensing corresponding aminosugar derivatives with acetobromo derivative of glucuronic acid by the Koenigs-Knorr reaction.

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