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174. Mitsuya Tanaka and Yoshiko Toshimitsu : Syntheses of N-Aminoacyl-D-glucosamine Derivatives and Periodate Oxidation Thereof.

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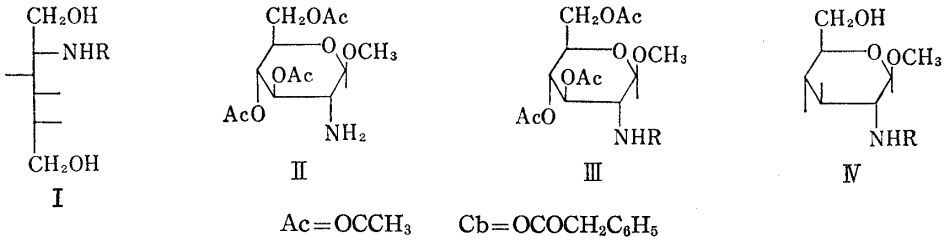
Glucosamine is a prevailing component of carbohydrate moiety of glycoproteins, and so there have been many reports on the syntheses of N-aminoacyl-[*2,1~4] and N-peptidyl-D-glucosamines[5,6] as model compounds of glycoproteins. In this connection, the present paper deals with the syntheses and periodate oxidation experiments of several N-benzyloxycarbonylaminoacyl derivatives of methyl β-glucosaminide and glucosaminol.

N-(N-Benzyloxycarbonylglycyl)- and N-(N-benzyloxycarbonyl-DL-alanyl)-D-glucosaminol (Ia and Ib) were prepared by reduction of corresponding glucosamine derivatives with sodium borohydride.

By the carbodiimide coupling of methyl 3,4,6-tri-O-acetyl-β-D-glucosaminide (II) with N-benzyloxycarbonylglycine, N-benzyloxycarbonyl-DL-alanine, hippuric acid, β- and α-benzyl N-benzyloxycarbonyl-L-aspartate, corresponding methyl N-(acylaminoacyl)-3,4,6-tri-O-acetyl-β-D-glucosaminides (IIIa~e) were prepared. Subsequent treatment with sodium methoxide furnished N-(acylaminoacyl)-D-glucosaminides (IVa~e) (Chart 1).



Ac=$OCCH_3$ Cb=$OCOCH_2C_6H_5$

Ia, IIIa, IVa : R=$OCCH_2NHCb$
Ib, IIIb, IVb : R=$OCCH(CH_3)NHCb$
IIIc, IVc : R=$OCCH_2NHOCC_6H_5$
IIId : R=$OCCH(NHCb)CH_2COOCH_2C_6H_5$
IIIe : R=$OCCH_2CH(NHCb)COOCH_2C_6H_5$
IVd=IVe : R=$OCCH_2CH(NHCb)COOH$
IIIf, IVf : R=$OCCH(NHCb)CH_2COOH$

Chart 1.

Since Liefländer[1] obtained N-(N-benzyloxycarbonyl-β-aspartyl)-D-glucosamine from N-(β-benzyl-N-benzyloxycarbonyl-α-aspartyl)-tri-O-acetyl-D-glucosamine by saponification accompanied by α→β conversion, the occurrence of the conversion during saponification of methyl N-(β-benzyl-N-benzyloxycarbonyl-α-L-aspartyl)-tri-O-acetyl-β-D-glucosaminide (IIId) was anticipated. Saponification of IIId offered a compound (IVd) which was shown to be identical with β(?)-aspartyl derivative (IVe) similarly obtained from methyl N-(α-benzyl-N-benzyloxycarbonyl-β-L-aspartyl)-tri-O-acetyl-

*1 Okubo, Narashino-shi, Chiba-ken (田中光也, 利光淑子).
*2 For a review of the literature on this subject, readers are referred to 1).

1) M. Liefländer : Z. physiol. Chem., Hoppe-Seyler's, **329**, 1 (1962).
2) M. Liefländer, K. Thomas : *Ibid.*, **331**, 154 (1963).
3) G. S. Marks, A. Neuberger : J. Chem. Soc., **1961**, 4872.
4) A. Bertho, I. Schmidt, E. Strecker : Ann., **651**, 185 (1962).
5) O. Wacker, M. Liefländer : Z. physiol. Chem., Hoppe-Seyler's, **335**, 255 (1964).
6) S. M. Amir, J. S. Brimacombe, M. Stacey : Nature, **203**, 401 (1964).

β-D-glucosaminide (IIIe), while, on removal of the benzyloxycarbonyl and benzyl group by hydrogenolysis, IIId yielded N-α-aspartyl-tri-O-acetyl-compound (Vd), which was distinguishable from the β-isomer obtained by hydrogenolysis of IIIe. Paper chromatography showed that α-aspartyltriacetyl compound (Vd) ran faster than the β-isomer and stained reddish purple with ninhydrin on filter paper while the β-isomer stained blue purple.

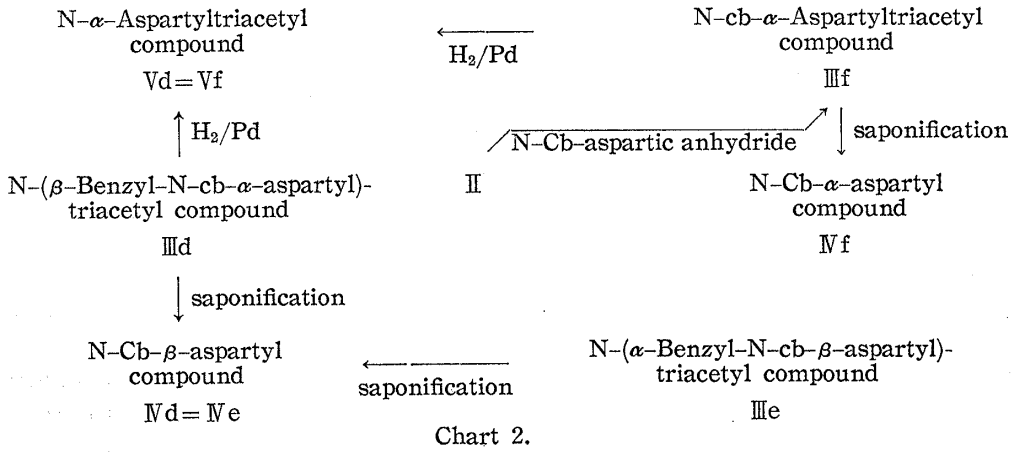


Chart 2.

Methyl N-(N-benzyloxycarbonyl-α-L-aspartyl)-β-glucosaminide (IVf) was synthesized by deacetylation of methyl N-(N-benzyloxycarbonyl-α-L-aspartyl)-tri-O-acetyl-β-D-glucosaminide (IIIf). The reaction of N-benzyloxycarbonyl-L-aspartic anhydride with II in the presence of pyridine in chloroform gave IIIf, the structure of which was confirmed by converting IIIf to an N-aspartyl-tri-O-acetylglucosaminide (Vf) identical with the above α-aspartyltriacetyl compound (Vd). Deacetylation of IIIf yielded an N-benzyloxycarbonyl-aspartylglucosaminide (IVf) which differed from the isomeric N-benzyloxycarbonyl-β(?)-aspartyl compound (IVd, IVe). On hydrogenolysis IVf gave a product, presumably α-aspartylglucosaminide which differed from the similar products obtained from IVd and IVe, presumably β-aspartylglucosaminide : paper electrophoresis at pH 2.4 showed that the "α-isomer," which would be expected to be slightly weaker acid than the "β-isomer," migrated faster than the "β-isomer" toward cathode, and the "α-isomer" stained red with ninhydrin while the "β-isomer" gave a blue color characteristic for β-aspartyl peptides, as in the case of the N-aspartyltriacetyl isomers above mentioned. These observations indicate that the compound (IVf) is the α-aspartyl-isomer, and IVd (identical with IVe) is the β-aspartyl-isomer.

The periodate oxidation of N-(N-benzyloxycarbonylglycyl)-D-glucosaminol (Ia) in unbuffered solution was normal : 3 moles of sodium metaperiodate were consumed rapidly with liberation of 1 mole of formaldehyde and 2 moles of formic acid. Paper chromatography and paper electrophoresis showed that reduction of the oxidation product with sodium borohydride to diol, hydrogenolysis to remove the benzyloxycarbonyl group, and subsequent acid hydrolysis offered glycine and serinol as expected.

Methyl N-acylglucosaminides (IVa~f), which have one pair of hydroxyl groups attached to the adjacent carbon atoms, took up slowly 1.03~1.14 moles of oxidant with formation of acid (0.03~0.037 mole) and considerable amounts of formaldehyde (0.14~0.34 mole). It was expected that, periodate oxidation, reduction with sodium borohydride, mild acid hydrolysis (with 0.5*N* sulfuric acid at room temperature) which probably hydrolyzes the acetal but does not affect the peptide bond, reduction of the resultant aldehyde with sodium borohydride and final removal of benzyloxycarbonyl group by hydrogenolysis, would convert N-(N-benzyloxycarbonyl-α-aspartyl)-glucosaminide and the β-isomer (IVf, IVd) to α- and β-aspartylserinol respectively without

serious contamination. Paper chromatography and paper electrophoresis of these products obtained by the above treatments, however, revealed unknown components besides the major components presumably α- and β-aspartylserinol. Moreover, on acid hydrolysis of both products, the formation of an appreciable amount of glucosamine and traces of contaminants was shown chromatographically together with aspartic acid and serinol. Thus, some difficulty was encountered in the conversion.

Experimental*3

Paper Chromatography and Paper Electrophoresis——Ascending paper chromatograms were run on Toyo Roshi No. 51 paper with BuOH saturated with water (Rf_1), BuOH saturated with 1% aqueous NH_3 (Rf_2), BuOH–AcOH–water (4:1:2) (Rf_3) and BuOH–pyridine–water (6:4:3) (Rf_4). N-Acylglucosaminides were detected with hypochlorite–KI–tolidine[7] and compounds containing a free amino-group with ninhydrin. Paper electrophoresis was performed in buffers : *N* AcOH (pH 2.4), pyridine–AcOH–water (10:2:488) (pH 5.8), the filter paper (20 cm. in length) being used with a potential difference of 500 volts for ca. 30 min. : the migration rates (cm./hr., toward the cathode) refer to this condition.

N-(N-Benzyloxycarbonylglycyl)-D-glucosaminol (Ia)——N-(N-Benzyloxycarbonylglycyl)-D-glucosamine[1] (2.62 g.) was dissolved in hot water (200 ml.), and after cooling $NaBH_4$ (0.42 g.) was added. After standing for 5.5 hr. at room temperature, the solution was acidified with AcOH and treated with Amberlite IR 120 (H^+ form). Evaporation of the solution and repeated distillation with MeOH under reduced pressure to remove boric acid, gave a crystalline mass. Two recrystallizations from EtOH gave 1.75 g. (77%) of the product, m.p. 140.7~142.5°, $[\alpha]_D^{23.5}$ −9.0° (c=1.0, water). *Anal.* Calcd. for $C_{16}H_{24}O_{16}N_2$: C, 51.60; H, 6.50; N, 7.52. Found : C, 51.70; H, 6.65; N, 7.62. Rf_1 0.50; Rf_2 0.48.

N-(N-Benzyloxycarbonyl-DL-alanyl)-D-glucosaminol (Ib)——a) N-(N-Benzyloxycarbonyl-DL-alanyl)-1,3,4,6-tetra-O-acetyl-D-glucosamine : Dicyclohexylcarbodiimide (3.2 g.) in CH_2Cl_2 (50 ml.) was added to a solution of N-benzyloxycarbonyl-DL-alanine (2.96 g.) and 1,3,4,6-tetra-O-acetylglucosamine[8] (4.6 g.) in CH_2Cl_2 (80 ml.). After 18 hr. at 10°, the mixture was acidified with dilute AcOH and kept for 1 hr. Dicyclohexylurea, which had separated, was removed by filtration and the filtrate was washed with 2*N* HCl, water, 5% $NaHCO_3$ and water successively, and dried. After evaporation of the solution, the residue was crystallized from EtOH to give the product, 5.75 g., 77.6%, m.p. 140~140.5°. On further purification from EtOH the melting point rose to 145.5~146.5°. $[\alpha]_D^{19}$ +10.5° (c=2.0, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{32}O_{12}N_2$: C, 54.34; H, 5.84; N, 5.07. Found : C, 54.22; H, 6.08; N, 5.11.

b) N-(N-Benzyloxycarbonyl)-DL-alanyl)-D-glucosamine : To a stirred solution of the tetraacetate (5.52 g., 10 mmoles) in $CHCl_3$ (40 ml.) was added 1.7*N* NaOMe (23.5 ml., 40 mmoles) dropwise at −5°. After 20 min. at −5° and further 20 min. at room temperature, the solution was neutralized with *N* HCl and evaporated to a small volume under reduced pressure. The crystalline precipitate that occurred during the evaporation was collected and washed with water, EtOH and $CHCl_3$ successively. The product (3.8 g.) had m.p. 198.5~200°. On further recrystallizations from MeOH the melting point rose to 223~223.5°. $[\alpha]_D^{18}$ +55.5° (c=2.0, dimethylformamide). *Anal.* Calcd. for $C_{17}H_{24}O_8N_2$: C, 53.12; H, 6.29 N, 7.29. Found : C, 52.97; H, 6.28; N, 7.43.

c) Ib : To a solution of N-(N-benzyloxycarbonyl-DL-alanyl)-glucosamine (787 mg., 2.05 mmoles) in 50% MeOH was added 160 mg. of $NaBH_4$. After standing for 42 hr. at 10°, the reaction mixture was treated as Ia. Recrystallization from 50% MeOH gave the product, 0.75 g. (95%). On further purification it melted at 144.5~146.5°. *Anal.* Calcd. for $C_{17}H_{26}O_8N_2$: C, 52.84; H, 6.78; N, 7.25. Found : C, 52.29; H, 6.98; N, 6.85. Rf_1 0.60; Rf_2 0.58. $[\alpha]_D^{24.5}$ −19.9° (c=1.0, MeOH).

Methyl N-(N-Benzyloxycarbonylglycyl)-3,4,6-tri-O-acetyl-β-D-glucosaminide (IIIa)——Dicyclohexylcarbodiimide (3.2 g.) in $CHCl_3$ (10 ml.) was added to a solution of methyl 3,4,6-tri-O-acetyl-β-D-glucosaminide (II) (4.5 g., 14 mmoles) and N-benzyloxycarbonylglycine (2.95 g., 14 mmoles) in CH_2Cl_2 (60 ml.). The reaction and the treatment, when carried out as described in Ib-a), gave a syrup. On treatment with hot EtOAc, the syrup crystallized readily to give the product, m.p. 145~146°, 6.2 g. (87%). $[\alpha]_D^{20}$ +1.6° (c=2.0, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{30}O_{11}N_2$: C, 54.08; H, 5.92; N, 5.49. Found : C, 54.13; H, 6.18; N, 5.61.

Methyl N-(N-Benzyloxycarbonylglycyl)-β-D-glucosaminide (IVa)——*N* NaOMe (36.6 ml.) was added to a solution of IIIa (6.2 g., 12.2 mmoles) in $CHCl_3$–MeOH (1:1 v/v) under cooling with ice. The mixture was allowed to stand for 20 min. at 0° and for further 20 min. at room temperature. The solution was neutralized with *N* HCl (36.6 ml.) and evaporated to dryness, and the residue was extracted several times

*3 All melting points are uncorrected.

7) C. G. Greig, D. H. Leaback : Nature, **188**, 310 (1960).

8) M. Bergmann, L. Zervas : Ber., **64**, 975 (1931).

with hot EtOH (0.7 L. in total). On cooling the combined extract separated long needles, 3.0 g. (64%), m.p. 205~207°. $[\alpha]_D^{31}$ −23.0° (c=0.98, water). An additional product (0.6 g.) could be obtained from the EtOH filtrate. *Anal.* Calcd. for $C_{17}H_{24}O_8N_2$: C, 53.12; H, 6.29; N, 7.29. Found : C, 52.95; H, 5.96; N, 7.29. Rf_1 0.64; Rf_2 0.60.

Methyl N-(N-Benzyloxycarbonyl-DL-alanyl)-3,4,6-tri-O-acetyl-β-D-glucosaminide (IIIb)——Dicyclohexylcarbodiimide (1.25 g., 6.0 mmoles) was added to a solution of II (1.60 g., 5 mmoles) and N-benzyloxycarbonyl-DL-alanine (1.10 g., 5 mmoles) in CH_2Cl_2 (40 ml.). As worked out as in Ib-a), 2.9 g. of crude product were obtained. Recrystallization from EtOH gave the product, m.p. 147~155° (sintered at 143.5°). $[\alpha]_D^{17}$ −0.3° (c=1.3, $CHCl_3$). *Anal.* Calcd. for $C_{24}H_{32}O_{11}N_2$: C, 54.95; H, 6.15. Found : C, 54.79; H, 6.26.

Methyl N-(N-Benzyloxycarbonyl-DL-alanyl)-β-D-glucosaminide (IVb)——Deacetylation was carried out as described in IVa. The EtOH extract of the product was evaporated to dryness and the residue was crystallized from water. IIIb (1.6 g.) was deacetylated to IVb in 62% yield (0.46 g.), needles. On repeated recrystallizations from water, the melting point rose to 198~198.3°. $[\alpha]_D^{26}$ −37.9° (c=1.0, water). *Anal.* Calcd. for $C_{18}H_{26}O_8N_2$: C, 54.26; H, 6.58; N, 7.03. Found : C, 54.57; H, 6.60; N, 6.59. Rf_1 0.68; Rf_2 0.68.

Methyl N-Hippuryl-3,4,6-tri-O-acetyl-β-D-glucosaminide (IIIc)——Hippuric acid (1.79 g., 10 mmoles) and II (3.2 g., 10 mmoles) in tetrahydrofuran (90 ml.) were warmed until they went into solution and dicyclohexylcarbodiimide (2.3 g.) was added after cooling. After 24 hr. at room temperature, the mixture was acidified with AcOH and dicyclohexylurea, which had separated, was filtered off and the solvent was removed. On treatment with EtOH, the oily residue crystallized to give the crude product, m.p. 160°, 2.45 g. (51%). Repeated recrystallizations from EtOH gave pure IIIc, m.p. 178~179° (sintered at 160°). $[\alpha]_D^{31}$ −8.4° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{28}O_{10}N_2$: C, 54.99; H, 5.87; N, 5.83. Found : C, 54.54; H, 6.09; N, 5.92.

Methyl N-Hippuryl-β-D-glucosaminide (IVc)——*N* NaOMe (2.2 ml.) was added to a suspension of IIIc (2.10 g., 4.4mmoles) in absolute MeOH (10 ml.). The mixture was shaken for 15 min. and the resultant homogeneous solution was allowed to stand over night in an ice box. The crystalline product separated was washed with MeOH and recrystallized from water to afford the product, m.p. 233° (decomp.), 1.3 g., (83%). $[\alpha]_D^{29}$ −25.7° (c=1.01, water). The product gave combustion values that coincide with those of the hydrated compound. *Anal.* Calcd. for $C_{16}H_{22}O_7N_2 \cdot 1\frac{1}{2}H_2O$: C, 50.39; H, 6.61; N, 7.35. Found : C, 50.48; H, 6.26; N, 7.19. Rf_1 0.51; Rf_2 0.51.

Methyl N-(β-Benzyl-N-benzyloxycarbonyl-α-L-aspartyl)-3,4,6-tri-O-acetyl-β-D-glucosaminide (IIId)——The experiment was carried out as described in IIIc. The reaction of β-benzyl N-benzyloxycarbonyl-L-aspartate (1.6 g., 4.5 mmoles), II (1.43 g., 4.5 mmoles) and dicyclohexylcarbodiimide (1.1 g., 5.3 mmoles) gave 3.5 g. of the crude product. Two recrystallizations from EtOH gave 2.2 g. of the product in 73% yield, m.p. 141° (sintered at 136°). $[\alpha]_D^{25}$ +5.07° (c=2.03, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{38}O_{13}N_2$: C, 58.35; H, 5.81; N, 4.25. Found : C, 58.32; H, 5.84; N, 4.43.

Methyl N-(α-Benzyl-N-benzyloxycarbonyl-β-L-aspartyl)-3,4,6-tri-O-acetyl-β-D-glucosaminide (IIIe)——Dicyclohexylcarbodiimide (1.25 g., 5.5 mmoles) in CH_2Cl_2 (20 ml.) was added to a solution of II (1.7 g., 5.34 mmoles) and α-benzyl N-benzyloxycarbonyl-L-aspartate[3] (1.79 g., 5 mmoles) in CH_2Cl_2 (20 ml.). After 48 hr. at room temperature, the treatment was carried out as described in Ib-a) to give the crude product, 3.5 g., m.p. 152~162°. After recrystallization from EtOAc, the product (1.9 g., 58%) had m.p. 160~165°. $[\alpha]_D^{14}$ +3.4° (c=2.08, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{38}O_{13}N_2$: C, 58.35; H, 5.81; N, 4.25. Found : C, 58.27; H, 6.10; N, 4.51.

Methyl N-(N-Benzyloxycarbonyl-β-L-aspartyl)-β-D-glucosaminide (IVd and IVe)——*N* NaOMe (4.00 ml., 4.0 mmoles) was added to a solution of IIId (0.658 g., 1.0 mmoles) in $CHCl_3$ (10 ml.) under cooling with ice. After 20 min. in the cold and further 30 min. at room temperature, water was added to the solution and the mixture was freed from cations by Amberlite IR 120 (H^+ form). The aqueous layer was evaporated under reduced pressure and the residue was crystallized from EtOH to give IVd, 0.16 g., in 36% yield, m.p. 161° (decomp.). Three recrystallizations from EtOH gave the pure product, m.p. 176~177° (decomp.). $[\alpha]_D^{30}$ −24.4° (c=1.09, water). *Anal.* Calcd. for $C_{19}H_{26}O_{10}N_2$: C, 51.58; H, 5.92; N, 6.33. Found : C, 51.28; H, 5.80; N, 6.34. Rf_1 0.21; Rf_2 0.16; Rf_3 0.78.

In the same manner, IIIe was treated with NaOMe to give the product (IVe) in 20% yield. After two recrystallizations the product had m.p. 175° (decomp.), mixed melting point with IVd, 175~176° (decomp.), $[\alpha]_D^{30}$ −29.4° (c=1.0, water). Rf_1~Rf_3 : identical with those of IVd. In addition, after hydrogenolysis of IVd and IVe in MeOH-H_2O (1:1) with Pd black as the catalyst, paper chromatography and paper electrophoresis of both products (aspartylglucosaminide) revealed an identical component (Rf_3 0.15; migration rate at pH 2.4, 2.4; blue color with ninhydrin) which differed from the isomeric α-aspartyl compound described in IVf.

Methyl N-(N-Benzyloxycarbonyl-α-L-aspartyl)-3,4,6-tri-O-acetyl-β-D-glucosaminide (IIIf)——A mixture of II (3.19 g., 10 mmoles), N-benzyloxycarbonyl-L-aspartic anhydride (2.49 g., 10 mmoles) and pyridine

(4.0 ml., ca. 50 mmoles) in $CHCl_3$ (30 ml.) was allowed to stand at room temperature for a week. The mixture was then washed successively with 2*N* HCl and water and dried over Na_2SO_4 for a time. Evaporation of the solution under reduced pressure and trituration of the residue with EtOH gave the crystalline product, 3.9 g., 69% yield, m.p. 187~194°. Recrystallization from EtOH gave prisms, m.p. 196~197°. $[\alpha]_D^{25}$ +2.8° (c=0.8, $CHCl_3$), slightly soluble in $CHCl_3$. *Anal.* Calcd. for $C_{25}H_{32}O_{13}N_2$: C, 52.81; H, 5.63; N, 4.93. Found : C, 52.54; H, 5.77; N, 4.95.

Methyl N-(N-Benzyloxycarbonyl-α-L-aspartyl)-β-D-glucosaminide (IVf)——IIIf (0.568 g., 1 mmole) in $CHCl_3$ (10 ml.) was deacetylated by adding *N* NaOMe (4.00 ml., 4.0 mmoles), according to the procedures described in IVd. The aqueous layer freed from cations was concentrated under reduced pressure to separate the crystalline product which was washed successively with water and EtOH. On heating the product (0.285 g., 65%) started to color at 194° and melted at 197°, a mixed melting point with IIIf (m.p. 196~197°) was 175~176°, $[\alpha]_D^{27}$ −32.2° (c=1.0, water). *Anal.* Calcd. for $C_{19}H_{26}O_{10}N_2$: C, 51.58; H, 5.92; N, 6.33. Found : C, 52.09; H, 6.05; N, 6.68. Rf_1 0.29; Rf_2 0.16; Rf_3 0.82.

On hydrogenolysis, IVf gave N-α-aspartylglucosaminide : Rf_3 0.22; migration rate (pH 2.4) 5.0; red color with ninhydrin.

Methyl N-α-L-Aspartyl-3,4,6-tri-O-acetyl-β-D-glucosaminide (Vd and Vf)——1) Vd from IIId. IIId (0.658 g., 1.0 mmole) in AcOH (10 ml.) was stirred with Pd black (60 mg.) in the stream of H_2 gas, until the generation of CO_2 gas ceased. After removal of the catalyst the solution was evaporated under reduced pressure to give a syrup (0.55 g.). Treatment with hot EtOH furnished needles, 0.33 g. (76%), m.p. 208° (decomp.), $[\alpha]_D^{25}$ −0.3° (c=1.0, water). *Anal.* Calcd. for $C_{17}H_{26}O_{11}N_2$: C, 47.00; H, 6.03; N, 6.45. Found; C, 46.77; H, 6.03; N, 6.06.

2) Vf from IIIf. As worked out as described above, IIIf (284 mg.) afford VIf, 160 mg. (68%), m.p. 208° (decomp.), mixed melting point with Vd, 208° (decomp.).

The N-α-aspartyl-tri-O-acetyl compounds (Vd and Vf) had the identical Rf value (Rf_3 0.66) and stained reddish purple with ninhydrin, while the hydrogenolysis product of IIIe, β-isomer of Vd, had Rf_3 0.56 and stained blue purple. Paper electrophoresis gave similar results : migration rate at pH 2.4 were for α-isomer, 3.3 and for β-isomer, 1.5 respectively.

Periodate Oxidation——To an aqueous solution of the compound was added 0.05*M* $NaIO_4$, and the unbuffered oxidation was carried out at 10° in the dark. The consumption of periodate was followed according to Fleury and Lange.[9] The acid produced was determined by titration with 0.01*N* NaOH after destruction of excess of periodate with ethylene glycol, and the liberated formaldehyde was determined by the dimedone method.[10] Treatment of N-(N-benzyloxycarbonylglycyl)-glucosaminol and N-(acylaminoacyl)-derivatives of methyl β-glucosaminide with sodium metaperiodate gave the following results.

Ia in 0.025*M* $NaIO_4$: oxidant consumption, 3.07 (40 min.), 2.99 (24 hr.) moles; production of acid, 1.85 moles; liberation of HCHO, 1.00 mole (24 hr.).

IVa in 0.01*M* $NaIO_4$: oxidant consumption, 0.75 (24 hr.), 0.98 (48 hr.), 1.12 (72 hr.) moles; acid produced, 0.037 mole (72 hr.); HCHO liberated, 0.24 mole (72 hr.).

IVb in 0.024*M* $NaIO_4$: oxidant consumption, 0.15 (40 min.), 1.05 (24 hr.), 1.10 (48 hr.), 1.10 (72 hr.) moles; acid produced, 0.036 mole (72 hr.); HCHO liberated, 0.143 mole (72 hr.).

IVc in 0.02*M* $NaIO_4$: corresponding figures were : oxidant, 0.31, 1.09, 1.16, 1.14 : acid, 0.032; HCHO, 0.27.

IVd in 0.01*M* $NaIO_4$: oxidant consumption, 0.14 (40 min.), 0.74 (24 hr.), 0.88 (48 hr.), 1.03 (72 hr.), 1.12 (96 hr.) moles; HCHO, 0.34 mole (72 hr.).

IVf in 0.01*M* $NaIO_4$: oxidant consumption, 0.92 (24 hr.), 1.14 (48 hr.), 1.16 (72 hr.) moles; HCHO, 0.170 (50 hr.) mole.

Investigation of Periodate Oxidation Products of IVd, IVf, and Ia——The following general procedures were adopted.

1) The periodate oxidation mixture was treated with $Pb(OAc)_2$ to precipitate iodate and periodate ions, and H_2S gas was bubbled to the filtrate. After removal of PbS and evaporation of the solution under reduced pressure, the residue in a small amount of water was treated with $NaBH_4$ overnight at 10°. The solution was acidified with dil. AcOH and treated with Amberite IR 120 (H^+ form) and evaporated repeatedly with MeOH.

2) The mild acid hydrolysis of the residue was carried out in 0.5*N* H_2SO_4 for 24 hr. at room temperature and neutralized with $BaCO_3$. The filtrate was treated with $NaBH_4$ as described above.

3) The products in dil. AcOH were hydrogenolysed by stirring with Pd black in the stream of H_2 gas for 6 hr. After removal of the catalyst, the solution was evaporated under reduced pressure.

4) The residue, which were expected to be N-acylserinol, was hydrolyzed with 2*N* HCl at 100° for 6 hr. and the hydrolysate was dried over KOH in vacuum.

9) P. Fleury, J. Lange : J. pharm. chim., **17**, 107, 196 (1933).
10) R. E. Reeves : J. Am. Chem. Soc., **63**, 1476 (1941).

Ⅳd and Ⅳf (0.08 mmole) were oxidized for three days as described above. The oxidation mixtures were treated according to the procedure 1)~3) to give a syrup correspondent to β- and α-aspartylserinol. Paper chromatography and detection with ninhydrin revealed following components, indicating the products were not homogeneous: Ⅳd; Rf_3 0.21 blue, presumably β-aspartylserinol, 0.35 orange, 0.72 orange; Ⅳf; Rf_3 0.25 reddish purple, presumably α-aspartylserinol, 0.38 orange, 0.73 orange. Electrophoresis at pH 2.4 also showed the presence of several components: Ⅳd; migration rate 0.2 yellow. 2.6 blue, 4.1, 5.3 purple. Ⅳf; migration rate 0.4 yellow, 2.4, 3.9 purple, 5.1 reddish purple. After acid hydrolysis 4) of the products from Ⅳd and Ⅳf, chromatography showed the presence of serinol (Rf_4 0.27), aspartic acid (Rf_4 0.02), a smaller amount of glucosamine (Rf_4 0.22) and negligible amounts of components with Rf_4 0.17, 0.13, and 0.10.

When the periodate oxidation mixture of Ia was treated as in 1), 3), and 4), chromatography revealed two components with Rf_3 values identical with those of glycine and serinol. Paper electrophoresis at pH 5.8 showed similar results: migration rates were serinol, 5.4; glucosamine, 3.6; aspartic acid, −3.6; glycine, 0.0.

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Summary

Methyl N-(benzyloxycarbonylaminoacyl)-β-D-glucosaminides and N-(benzyloxycarbonylaminoacyl)-D-glucosaminols were prepared and the periodate oxidation of some of these compounds was studied. Saponification of N-(β-benzyl-N-benzyloxycarbonyl-α-L-aspartyl)-tri-O-acetyl-D-glucosaminide (Ⅲd) caused a transpeptidation to give the β-aspartyl derivative (Ⅳd) and the α-isomer (Ⅳf) was prepared by deacetylation of N-(N-benzyloxycarbonyl-α-aspartyl)-tri-O-acetyl-D-glucosaminide (Ⅲf), which was obtained by the reaction of N-benzyloxycarbonyl-L-aspartic anhydride with methyl-tri-O-acetyl-β-D-glucosaminide (Ⅱ).

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