Notes

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Akira Yamamoto and Hisao Tsukamoto: Preparation of α-Benzyl N-Benzyloxycarbonyl-DL-aspartate and its Resolution by 2-Acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucosylamine.*1

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In the previous paper,*1 the preparation of L-β-aspartyl derivative (X) of 2-acetamido-2-deoxy-β-D-glucosylamine has been described.

Since during the course of the preparation of X some difficulties were experienced in purification of N-benzyloxycarbonyl-L-aspartic acid α-benzyl ester (II),[1~4] an intermediate for the synthesis of X, more convenient methods for the preparation of II, have been reinvestigated. When II is prepared *via* N-benzyloxycarbonyl-L-aspartic anhydride (III)[4~6] by treating it with benzyl alcohol in a sealed tube, it is reasonable that both α- and β-ester can be formed. Marks, *et al.*[3] succeeded in separating the mixture of the isomers by fractional extraction of the mixture from an organic solvent with aqueous sodium carbonate.

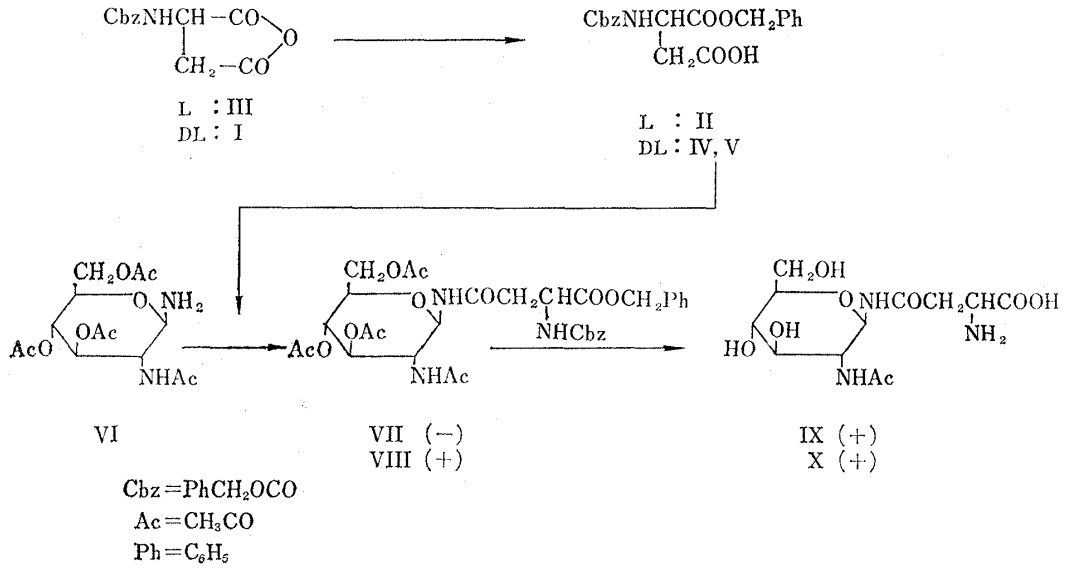

Chart 1.

We have attempted to develop an alternative method for preparation, in which N-benzyloxycarbonylaspartic acid α-benzyl ester (IV) was prepared *via* its dicyclohexylamine salt, which was obtained by the treatment of N-benzyloxycarbonyl-L-aspartic anhydride in the presence of dicyclohexylamine and benzyl alcohol in absolute ether, according to

*1 This Bulletin, **13**, 1041 (1965).

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1) M. Bergmann, L. Zervas, L. Salzmann: Ber., **66**, 1288 (1933).
2) P. M. Bryant, R. H. Moore, P. J. Pimlott, G. T. Young: J. Chem. Soc., **1959**, 3868.
3) G. S. Marks, A. Neuberger: *Ibid.*, **1961**, 4872.
4) G. L. Miller, O. K. Behrens, V. du Vigneaud: J. Biol. Chem., **140**, 411 (1941).
5) M. Bergmann, L. Zervas: Ber., **65**, 1192 (1932).
6) W. J. LeQuesne, G. T. Young: J. Chem. Soc., **1952**, 24.

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a similar reaction to that by which N-benzyloxycarbonyl-L-glutamic acid α-benzyl ester[7] can be prepared. Unexpectedly, this procedure did not give a product which melt at 85° (m.p. of pure II),*3 but the product (IV) which melted sharply at 104.5°, after being recrystallized thoroughly from toluene.

The material was observed to have no rotation and an analytical value agreed with II, but its infrared spectrum was quite different from II or pure N-benzyloxycarbonyl-L-aspartic acid β-benzyl ester, m.p. 107°. A plausible explanation, therefore, would appear to involve the formation of a racemate, although it is known[9] that N-benzoyl-L-aspartic anhydride is completely racemised with *N* NaOH at room temperature and N-acetyl derivative partly under the same condition, but N-benzyloxycarbonyl derivative is hardly racemised in general.

In order to clarify the fact of racemisation, N-benzyloxycarbonyl-DL-aspartic acid α-benzyl ester (V) was prepared from DL-aspartic acid, applying the method of Marks, *et al.*[8] As the results, V was demonstrated to be identical with IV in infrared spectrum, melting point, mixed melting point and elemental analysis. Thus, it seems possible to conclude that the racemisation occurred on the way of the treatment by dicyclohexylamine from N-benzyloxycarbonyl-L-aspartic anhydride in the presence of benzyl alcohol.

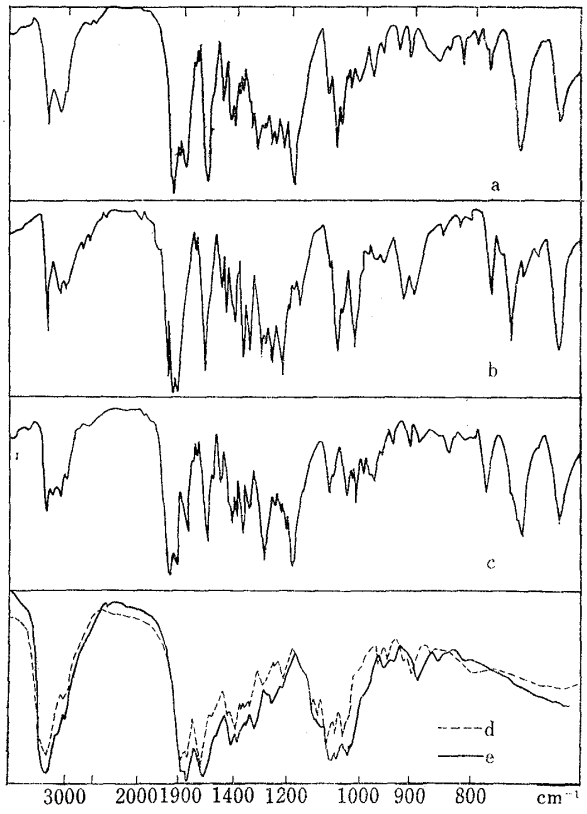

Fig. 1. Infrared Absorption Spectra (KBr)

a : N-Cbz-L-aspartic acid α-benzyl ester
b : N-Cbz-DL-aspartic acid α-benzyl ester
c : N-Cbz-L-aspartic acid β-benzyl ester
d : N-(L-β-Aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine
e : N-(D-β-Aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine

Next step involved the resolution of IV by 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucosylamine (VI), in which IV was condensed with VI in tetrahydrofuran in the presence of dicyclohexylcarbodiimide, according to the method in the previous papers.*1 The condensation product was separated by recrystallization from EtOH into two expected products, which were diastereomeric, *i.e.*, m.p. 196~197° (VII), $[\alpha]_D$ −13.8 and m.p. 216~217° (VIII), $[\alpha]_D$ +10.2. The latter was identified with the authentic specimen, 1-(α-benzyl N-benzyloxycarbonyl-L-β-aspartamido)-2-acetamido-1,2-dideoxy-3,4,6-tri-O-acetyl-β-D-glucose.

Finally, VII and VIII were freed from the protecting groups according to the method described in the previous reports.*1 Since the structure of the resulting product of VIII was established as N-(L-β-aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine (X) by comparison of its properties with those of the authentic specimen, it is possible to deduce that the other diastereomeric product (IX), which has the similar analytical value as X, may be the corresponding D-β-aspartamido derivative.

*3 These were kindly offered by Prof. Izumiya, Faculty of Sciences, Kyushu University.

7) E. Klieger, H. Gibian : Ann., **655**, 195 (1962).
8) N. Izumiya, S. Uchio, T. Yamashita : Nippon Kagaku Zasshi, **79**, 420 (1959).
9) C. C. Barker : J. Chem. Soc., **1953**, 453.

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The possibility must also be supported by the following facts: The first, in paper chromatography in butanol-acetic acid-water=12:3:5 and paper electrophoresis in 5*N* AcOH, Ⅸ and Ⅹ were revealed upon spraying with ninhydrin reagent as a single spot having the same mobilities. Ninhydrin color detected on paper for Ⅸ was specific blue in close agreement with those obtained with β-aspartylpeptides and glycopeptide (Ⅹ), which is easily distingishable from the coloration of α-aspartylglycopeptide, the other amino acids and glucosamine as discussed in the previous paper.*1 These findings suggest that Ⅸ is an N-glucosaminide, involving the amide group of asparagine, but not isoasparagine. The second, the resemblance between rotation of both compounds, $[\alpha]_D$ +26.3, and $[\alpha]_D$ +10.2 suggests that both compounds have β-configuration at C-1 in carbohydrate moiety as discussed in the previous papers.*1 All these observations support that Ⅸ must have a structure of N-(D-β-aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine.

Experimental

N-Benzyloxycarbonyl-DL-aspartic Acid——This was prepared from DL-aspartic acid according to the method of Bergmann, *et al.*[5] Needles from AcOEt-petr. ether, m.p. 114~115°, yield 81.5%.

N-Benzyloxycarbonyl-DL-aspartic Anhydride——This was prepared according to the method of Bergmann, *et al.*[1] Plates, from abs. AcOEt-abs. ether, m.p. 130°, yield 86%.

N-Benzyloxycarbonyl-DL-aspartic Acid α-Benzyl Ester (Ⅴ)——This was prepared from N-benzyloxycarbonyl-DL-aspartic anhydride according to the method of Marks.[3] Needles from toluene, m.p. 104.5°, yield 76%. *Anal.* Calcd. for $C_{19}H_{19}O_6N$: C, 63.86; H, 5.32; N, 3.92. Found: C, 63.96; H, 5.46; N, 4.20.

N-Benzyloxycarbony-L-aspartic Anhydride——The method of LeQuesne, Young was used.[6] Needles from abs. benzene, m.p. 109~112°, yield 52%.

N-Benzyloxycarbonyl-DL-aspartic Acid α-Benzyl Ester (Ⅳ)——The method of Klieger, *et al.*[7] was applied. To a suspension of 3 g. of N-benzyloxycarbonyl-L-aspartic anhydride and 1.8 ml. of benzyl alcohol in 20 ml. of abs. ether was added dropwise under stirring a solution of 2.38 g. of dicyclohexylamine in 23 ml. of abs. ether for 40 min. and the stirring was continued for further 6 hr. The mixture was evaporated to syrup under reduced pressure, and solidified by addition of petr. ether. Recrystallization from EtOH-petr. ether gave 2 g. of dicyclohexylamine salt of Ⅳ, plates, m.p. 121°.

To a solution of 9 ml. of AcOEt containing 0.9 ml. of 5*N* HCl was added 1.2 g. of dicyclohexylamine salt of Ⅳ and the mixture was stirred for one hr. Dicyclohexylamine HCl salt separated was filtered off and washed with AcOEt. The filtrate and washings were combined, washed with water, dried with Na_2SO_4 and evaporated to dryness under reduced pressure. The crystalline residue was recrystallized from AcOEt-petr. ether, benzene and toluene, successively, to give 0.5 g. of colorless needles, m.p. 104.5°, no rotation. IR spectra of Ⅳ and Ⅴ were completely superimpossible. *Anal.* Calcd. for $C_{19}H_{19}O_6N$: C, 63.86; H, 5.32; N, 3.92. Found: C, 64.07; H, 5.39; N, 4.10.

1-(α-Benzyl N-Benzyloxycarbonyl-D-β- and -L-β-aspartamido)-2-acetamido-1,2-dideoxy-3,4,6-tri-O-acetyl-β-D-glucose——To a solution of 2.0 g. of Ⅳ and 1.94 g. of 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucosylamine in 108 ml. of dry tetrahydrofuran was added 1.3 g. of dicyclohexylcarbodiimide. The solution was stirred for 2 hr. at room temperature and allowed to stand overnight. A few drops of AcOH was added to the reaction mixture and stirred for a further short time. Dicyclohexylurea separated was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was extracted with a small amount of $CHCl_3$ and filtered from insoluble materials. The extract was washed with cold diluted HCl and water, then with aqueous $NaHCO_3$ and water. The solution was dried over Na_2SO_4 and was evaporated to dryness under reduced pressure.

The residue, 2.9 g., was separated by fractional crystallization from EtOH into two products, which were diastereomeric, 0.63 g. of needles (Ⅷ) and 0.92 g. of small needles (Ⅶ), more soluble than the former, respectively.

The former, m.p. 216~217°, $[\alpha]_D^{35}$ +10.2° (c=0.98, $CHCl_3$), was identified with authentic specimen, 1-(α-benzyl N-benzyloxycarbonyl-L-β-aspartamido)-2-acetamido-1,2-dideoxy-3,4,6-tri-O-acetyl-β-D-glucose in the points of melting point, mixed melting point and IR spectra. *Anal.* Calcd. for $C_{33}H_{39}O_{13}N_3$: C, 57.81; H, 5.73; N, 6.13. Found: C, 58.07; H, 5.59; N, 6.25.

The latter (Ⅶ), m.p. 194~196°, $[\alpha]_D^{35}$ −13.8° (c=1.44, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{39}O_{13}N_3$: C, 57.81; H, 5.73; N, 6.13. Found: C, 57.72; H, 5.63; N, 6.15.

N-(L-β-Aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine (Ⅹ)——Ⅷ was freed from the substitutes by the method described in the previous paper.*1 Needles from warm aquenus EtOH, m.p. 264~266° (decomp.), $[\alpha]_D^{36}$ +26.4° (c=0.68, H_2O). *Anal.* Calcd. for $C_{12}H_{21}O_8N_3$: C, 42.98; H, 6.31; N, 12.53. Found:

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C, 42.51; H, 6.60; N, 12.39.

N-(D-β-Aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine (IX)——A solution of 0.9 g. of VIII in 114 ml. of MeOH was catalytically hydrogenated with 1.68 g. of 8% Pd-C at atmospheric pressure. Evolution of CO_2 stopped after 3.5 hr. and it was continued for further 1.5 hr. The solution was filtered from the catalyst, which was washed with MeOH until the filtrate had a negative ninhydrin test. The filtrate and washings were combined and evaporated under reduced pressure. The residue was dried over P_2O_5 overnight. To a 4% solution of the residue in abs. MeOH was added an equal amount of 2% solution of $Mg(OCH_3)_2$[*1,10] in abs. MeOH at 0°. The reaction was continued until ferric-hydroxamate test became only negligible. After being kept at 0° for 1.5 hr., 15 ml. of water and 7 ml. of Dowex 50 (H^+) resin were added to the reaction mixture and vigorously stirred for ten min. The mixture (neutralized solution and resin) was poured into the column (0.9×6.2 cm.) of the same resin. The column was thoroughly washed with water and then, followed by the displacement elution with 0.15*N* NH_3. Finally, the ninhydrin-positive fractions collected were passed through Amberlite IRC-50 column (H^+) (0.9×9.3 cm.) to remove NH_3. The effluent was combined and evaporated to dryness under reduced pressure to give crystalline materials. Recrystallization from aqueous EtOH gave 0.32 g. of colorless plates, m.p. 240~245° (decomp.), but turned brown at 222°, $[\alpha]_D^{26}$ +10.2 (c=1.47, H_2O). *Anal.* Calcd. for $C_{12}H_{21}O_8N_3 \cdot 2H_2O$: C, 39.11; H, 6.43; N, 11.32. Found: C, 39.11; H, 6.87; N, 11.55.

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Summary

N-Benzyloxycarbonyl-DL-aspartic acid α-benzyl ester was prepared from N-benzyloxycarbonyl-L-aspartic anhydride on racemisation in the presence of dicyclohexylamine and condensed with 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucosylamine to give diastereomeric two glycopeptide acetates, 1-(α-benzyl N-benzyloxycarbonyl-L-β-aspartamido)-2-acetamido-1,2-dideoxy-3,4,6-tri-O-acetyl-β-D-glucose and 1-(α-benzyl N-benzyloxycarbonyl-D-β-aspartamido)-2-acetamido-1,2-dideoxy-3,4,6-tri-O-acetyl-β-D-glucose.

Both compounds were subjected to hydrogenolysis and de-O-acetylation to give the resulting two glycopeptide, N-(L-β-aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine and N-(D-β-aspartyl)-2-acetamido-2-deoxy-β-D-glucosylamine.

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10) D. R. Whitaker, M. E. Tate, C. T. Bishop: Can. J. Chem., **40**, 1885 (1962).

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