## Notes

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# Rearrangement of α to β-Aspartyl Peptide with Anhydrous Hydrogen Fluoride

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On the way of peptide synthesis β-esterified aspartyl peptide in aspartylglycyl and aspartylseryl sequences rearranges α to β-peptide form by base or acid treatment for deprotection of functional groups. The intermediate of this rearrangement is proved to be a succinimide derivative.[2] Sakakibara, *et al.* found that anhydrous hydrogen fluoride with anisole is a very excellent deprotecting reagent and removes almost all protecting groups for peptide synthesis, and hydrogen fluoride is strongly acidic in anhydrous state.[3] It is useful to examine whether this excellent reagent makes aspartyl rearrangement.

N-Benzyloxycarbonyl-β-benzylaspartylglycine benzyl ester (**1**), N-benzyloxycarbonyl-β-benzylaspartylhistidine methyl ester (**2**), N-benzyloxycarbonyl-β-benzylaspartyl-O-benzylserine methyl ester (**3**), and N-benzyloxycarbonyl-β-benzylaspartylalanine benzyl ester (**4**) were chosen for this examination. Each peptide was treated with anhydrous hydrogen fluoride in the presence of anisole for 1 hr at various temperature. After removal of hydrogen fluoride and anisole, the peptides were treated with aqueous 1% ammonium hydrogen carbonate at 40° for 24 hr.[2] Under this condition α-aspartyl peptide does not rearrange, the succinimide intermediate is hydrolized completely to α and β peptide form and ester to acid. The products were applied on paperchromatography for separation and identification with authentic samples. The product ratio was estimated gravimetrically.

As shown in Table, **1**, **2**, and **3** rearranged at room temperature, but **1** did not at −25°. The other hand, three fourths of **2** rearranged even at −25°. Peptide (**4**) did not rearrange at room temperature.

Aspartyl β-peptide form of aspartylglycine and aspartylhistidine was confirmed by nuclear magnetic resonance (NMR) spectroscopy. The absorption of α-proton of α-peptide appears little lower field than one of α-carboxyl anion of amino acid.[4] As shown in Table, α-aspartylhistidine showed aspartyl α-proton absorption overlapped on water's, but aspartyl α-proton of β-aspartylhistidine overlapped on histidine α-one. Aspartyl α-absorption of β-aspartylglycine overlapped on glycine one, but one of α-aspartylglycine appeared 0.2 ppm lower field than that of β-form. Since the amount of aspartylserines was too small, NMR spectra of α and β-aspartylserine were taken by the accumulation method and amino acid analysis was used. NMR spectra of those two compounds also showed the same kind of fact as the above mentioned.

The reaction mechanism may follow one which was suggested by Ondetti, *et al.*[2] In the case of aspartylglycine sequence, glycine has only a steric advantage for the rearrangement, but in the case of aspartylhistidine sequence histidine imidazole might be a very excellent catalyst, so this sequence rearranges even at −25°.

1) Location: *1-1, Katahira-2-chome, Sendai, 980, Japan.*
2) M.A. Ondetti, A. Deer, J.T. Sheehan, J. Plušcěc, and O. Kocy, *Biochemistry*, **7**, 4069 (1968) and papers cited in the above paper.
3) S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Japan*, **38**, 1412 (1965); J. Lenard and A.B. Robinson, *J. Am. Chem. Soc.*, **89**, 181 (1967).
4) F. Conti, C. Pietroneso, and P. Viglino, *Org. Mag. Resonance*, **2**, 131 (1970); J.J.M. Rowe, J. Minton, and K.L. Rowe, *Chem. Rev.*, **70**, 1 (1970).

## Experimental

N-Benzyloxycarbonyl-β-benzylaspartic acid and amino acid esters, which were used for this paper, were prepared according to the procedure of Schroeder, *et al.*[5] NMR spectra were taken by JEOL C-60 HL and PS-100 high resolution instruments and EC-5 electronic computer.

**Protected Aspartyl Dipeptides**——To a solution of 500 mg (1.4 mmole) of Cbz-Asp(−β-OBz)-OH in 10 ml of tetrahydrofuran–acetonitrile (1:1), 350 mg (1.7 mmole) of dicyclohexylcarbodiimide was added at 0°. After 30 min standing, 1.4 mmole of an amino acid ester salt (glycine benzyl ester *p*-toluenesulfonate, 470 mg; alanine benzyl ester *p*-toluenesulfonate, 490 mg; O-Bz-serine methyl ester hydrochloride, 340 mg;) and 140 mg (1.4 mmole) of triethylamine in 10 ml of tetrahydrofuran–acetonitrile (1:1) were added to the above solution and the mixture was stood for overnight at 4°. In the case of histidine methyl ester dihydrochloride, 340 mg of this salt (1.4 mmole) suspended in chloroform was treated with dry ammonia gas at 0° for 10 min. After removal of precipitated salt, the solvent was evaporated and the resulted clear oil was used for the coupling reaction. After overnight reaction, the precipitate of urea derivative was removed. After evaporation of the solvent, the residue was dissolved in ethyl acetate. A small amount of acetic acid was added to decompose excess dicyclohexylcarbodiimide. The precipitate was filtered off and the solvent was evaporated again. The resulting oil was applied on silica gel chromatography. Elution solvents were as follows, benzene–ethyl acetate (10:1) for peptide (**1** and **4**), ethyl acetate–ethanol (10:1) for **2**. **1**, **2**, and **4** were recrystallized from benzene–hexane and chloroform–hexane. **3** was obtained directly by recrystallization from benzene–hexane. **1**; yield 420 mg, mp 89—91°. *Anal.* Calcd. for $C_{28}H_{28}O_7N_2$: N, 5.55. Found: N, 5.68. **2**; yield 540 mg, mp 103—105°. *Anal.* Calcd. for $C_{26}H_{28}O_7N_4$: N, 11.02. Found: N, 10.93. **3**; yield 470 mg, mp 98—99.5°. *Anal.* Calcd. for $C_{29}H_{30}O_8N_2$: N, 5.24. Found: N, 5.37. **4**; yield 510 mg, mp 74—75.5°. *Anal.* Calcd. for $C_{29}H_{30}O_7N_2$: N, 5.40. Found; N, 5.40.

**Catalytic Hydrogenolysis of Protected Dipeptides**——Protected peptides (0.6—1 mmole) were deprotected by hydrogenolysis over 30—50 mg of 10% palladium on carbon at room temperature in the following solvents, ethanol–water (1:1) for **1** and **3**, acetic acid for **2**, and ethanol–water–acetic acid (5:5:1) for **4**. After filtration of the catalyst, the solvent was evaporated to dryness. The resulting products were recrystallized from ethanol–water. Aspartylglycine; mp 164—165°. *Anal.* Calcd. for $C_6H_{10}O_5N_2$: N, 14.73. Found: N, 14.74. Aspartylalanine; mp 197—199°. *Anal.* Calcd. for $C_7H_{12}O_5N_2$: N, 13.72. Found: N, 13.98. Aspartylhistidine methyl ester diacetic acid salt; mp 91—93°. *Anal.* Calcd. for $C_{15}H_{21}O_9N_1$: N, 13.86. Found: N, 13.78. Aspartylserine methyl ester; mp 159—161°. *Anal.* Calcd. for $C_8H_{14}O_6N_2$: N, 11.96. Found: N, 12.21.

Table I. Product Ratio, *Rf* Value of Paperchromatography and NMR δ Value in $D_2O$ from Internal DSS

| Reaction temperature | Asp-Gly | | Asp-His | | Asp-Ser | | Asp-Ala |
|---|---|---|---|---|---|---|---|
| | α | β | α | β | α | β | α |
| Room temp. | 69% | 31% | 39% | 61% | 70% | 30% | 100% |
| 0° | 79% | 21% | 26% | 74% | | | |
| −25° | 100% | | 26% | 74% | | | |
| *Rf* value in BuOH:AcOH:$H_2O$ 2:1:1 | 0.293 | 0.217 | 0.234 | 0.312 | 0.369 | 0.213 | 0.479 |
| NMR δ in $D_2O$ from DSS | 2.89 Asp-β (d, 2H) | 3.00 Asp-β (d, 2H) | 2.98 Asp-β (d, 2H) | 2.30 Asp-β (m, 2H) | 2.82 Asp-β (m, 2H) | 2.93 Asp-β (m, 2H) | 1.37 Ala-β (d, 3H) |
| | 3.98 Gly (s, 2H) | 3.98 Gly (s, 2H) | 3.30 His-β (d, 2H) | 3.32 His-β (d, 2H) | 3.89 Ser-β (m, 2H) | 3.89 Ser-β (m, 2H) | 2.86 Asp-β (t, 2H) |
| | 4.33 Asp-α (t, 1H) | 4.13 Asp-α (m, 1H) | 4.30 His-α (t, 1H) | 4.30 α-H (m, 2H) | 4.15 Asp-α (m, 1H) | 4.08 Asp-α (m, 1H) | 4.25 Ala-α (q, 1H) |
| | | | 7.30 His-im (s, 1H) | 7.30 His-im (s, 1H) | 4.32 Ser-α (m, 1H) | 4.34 Ser-α (m, 1H) | 4.29 Asp-α (t, 1H) |
| | | | 8.60 His-im (s, 1H) | 8.45 His-im (s, 1H) | | | |

5) E. Schroeder and K. Luebke, "The Peptides," Vol. 1, Academic Press, New York, 1965.

**Hydrogen Fluoride Treatment of Protected Dipeptides**——Protected dipeptide (50 mg) and excess anisole (110 mg) were placed in a Toho Kasei Hydrogen fluoride apparatus and 5 ml of anhydrous hydrogen fluoride was introduced. After 1 hr stirring at various temperature (see Table), hydrogen fluoride was completely distilled off *in vacuo*. The residue was dissolved in water and extracted with ethyl acetate. The aqueous layer was lyophilized. The white residue was dissolved in aqueous 1% ammonium hydrogen carbonate and stood at 40° for 24 hr. After lyophilization of the reaction mixture, the product was applied on paper chromatography (Toyo No. 50 and in butanol: acetic acid: water=2:1:1). Amino acid analysis of α and β-aspartylserine. α, $Asp_{1.09}$, $Ser_{1.00}$: β, $Asp_{1.05}$, $Ser_{1.00}$ (6N HCl at 110° for 24 hr).

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# Purines. X.[1] A Convenient Method for Synthesis of 2',3'-O-Isopropylideneadenosine 1-Oxide

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The preparation of 2',3'-O-isopropylideneadenosine 1-oxide (III) from 2',3'-O-isopropylideneadenosine (II)[3] by the 1-N-oxidation was first reported in 1958 by Brown and coworkers.[4] In a continuing study of the chemistry of 1-alkoxy-9-alkyladenine salts[1,5] we had occasion to examine an alternative synthesis of this N-oxide (III), which constitutes the major portion of this paper.

The starting material selected for the present synthesis was adenosine 1-oxide (IV),[4,6] and it was prepared from adenosine (I) in 65% yield according to the procedure reported by Stevens *et al.*[4] except that the excess of hydrogen peroxide and peracetic acid were removed by passing the reaction mixture in the cold (2—5°) through a column packed with Amberlite CG-120 ($H^+$) and the column was eluted with dilute aqueous ammonium hydroxide. Since we have noticed that the original procedure often requires stirring a few days long with palladium-on-charcoal for destroying the peroxides, this modification will prove of great use.

In order to prepare isopropylidene derivative III, condensation of IV with acetone in the presence of *p*-toluenesulfonic acid was then attempted under conditions patterned after those[3] employed for the synthesis of II from I. However, the *p*-toluenesulfonate of IV that formed was only sparingly soluble in acetone, and the desired reaction seemed to occur only very slowly. Accordingly, we next tried to use perchloric acid as a catalyst in a mixture of acetone and 2,2-dimethoxypropane because of its simplicity, ready availability, and the ease with which it could convert certain nucleosides into the corresponding isopropylidene derivatives.[8] Treatment of IV with acetone containing 2,2-dimethoxypropane and 70%

1) Part IX: T. Fujii, T. Itaya, and S. Moro, *Chem. Pharm. Bull.* (Tokyo), **20**, 1818 (1972).
2) Location: *13-1 Takara-machi, Kanazawa 920, Japan.*
3) A. Hampton, *J. Am. Chem. Soc.*, **83**, 3640 (1961), and references cited.
4) M.A. Stevens, D.I. Magrath, H.W. Smith, and G.B. Brown, *J. Am. Chem. Soc.*, **80**, 2755 (1958).
5) T. Fujii, T. Itaya, and S. Moro, *Chem. Pharm. Bull.* (Tokyo), **20**, 958 (1972), and earlier papers cited therein.
6) For a monohydrate of N-oxide IV, see footnote 8 in ref. 7.
7) T. Fujii, C.C. Wu, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **19**, 1368 (1971).
8) *a*) J.A. Zderic, J.G. Moffatt, D. Kau, K. Gerzon, and W.E. Fitzgibbon, *J. Med. Chem.*, **8**, 275 (1965); *b*) T. Ueda, Y. Iida, K. Ikeda, and Y. Mizuno, *Chem. Pharm. Bull.* (Tokyo), **16**, 1788 (1968).