Protection of Amide-Nitrogen for Peptide Synthesis. A Novel Synthesis of Peptides Containing C-Terminal Glutamine

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It is known that troublesome side reactions[1] occur in the synthesis of asparagine- or glutaminepeptides due to the instability of the β- or γ-amide groups. Therefore, a suitable protection of amide nitrogen is desirable, in which the protecting group should be easily removable after formation of the peptide bonds. Frankel et al.[2] reported that removal of the benzyl group from N^{β}-benzyl-asparagine was very difficult because of the unexpected stability of the group.

In the present work, glycyl-L-glutamine and L-alanyl-L-glutamine ware prepared using xanthydrol[3] (I) as a protective agent by the following procedures. N^{α}-Cbzo*-N^{γ}-xanthyl-L-glutamine (II) was prepared from N^{α}-cbzo-L-glutamine (III) in glacial acetic acid containing an equimolar amount of I. Yield 80%; m. p. 182~183°C, $[\alpha]_D^{17}-5.7°$ (*c* 5.7, dimethylformamide). Found: C, 66.36; H, 5.55; N, 6.13. Calcd. for $C_{26}H_{24}O_6N_2 \cdot 1/2H_2O$: C, 66.52; H, 5.37; N, 5.97%.

When II was treated with hydrogen bromide in glacial acetic acid, L-glutamine (IV) was recovered in 55% yield. IV had the same optical activity with the glutamine which was obtained directly from the starting material III, m. p. 187°C, $[\alpha]_D^{32.5}+6.4°$ (*c* 2.8, H_2O). It is interesting that N^{γ}-xanthyl-L-glutamine (V) was not obtained from IV and I in acetic acid. The substance V was first obtained by selective hydrogenation of II in ethanol using palladium-charcoal as catalyst; m. p. 222°C (dicomp.). Found: C, 65.71; H, 5.60; N, 8.97. Calcd. for $C_{18}H_{18}O_4N_2$: C, 66.24; H, 5.56; N, 8.58%.

Synthesis of Glycyl-L-glutamine. — N^{α}-Cbzo-N^{γ}-xanthyl-L-glutamine methylester (VI) was prepared from II in dioxane by treatment with diazomethane. Recrystallization of VI from dioxane gave fine colorless needles (80~85%); m. p. 235~235.5°C. Found: C, 68.21; H, 5.58; N, 5.94. Calcd. for $C_{27}H_{26}O_6N_2$: C, 68.34; H, 5.52; N, 5.90%. The cbzo-group was removed by catalytic hydrogenation, and N^{γ}-xanthyl-L-glutamine methylester acetate (VII) was obtained as colorless needles (67%); m. p. 148~148.5°C. Fuond: C, 62.72; H, 6.07; N, 6.90. Calcd. for $C_{21}H_{24}O_6N_2$: C, 62.99; H, 6.04; N, 7.00%. Substance VII was coupled with cbzo-glycine *p*-nitrophenylester (VIII) in anhydrous chloroform[4,5], and cbzo-glycyl-N^{γ}-xanthyl-L-glutamine methylester (IX) was obtained as fine colorless needles after recrystallization from dioxane (70%); m. p. 184°C. Found: C, 65.49; H, 5.64; N, 7.89. Calcd. for $C_{29}H_{29}O_7N_3$: C, 65.52; H, 5.50; N, 7.91%. The product IX was carefully saponified in dioxane using *N*-sodium hydroxide, and cbzo-glycyl-N^{γ}-xanthyl-L-glutamine (X) was obtained. It was recrystallized from methanol-toluene (4 : 1) and water (77%) : m. p. 198°C. Found: C, 65.17; H, 5.39; N, 8.04; eq. wt.: Na, 516. Calcd. for $C_{28}H_{27}O_7N_3$: C, 64.98; H, 5.26; N, 8.12%; mol. wt. 517.5. Treatment of X with hydrogen bromide, foffowed by purification by Amberlite IR-4B, gave glycyl-L-glutamine monohydrate (XI) in 70% yield; m. p. 206°C (decomp.). Found: C, 38.02; H, 7.02; N, 19.12. Calcd. for $C_7H_{13}O_4N_3 \cdot H_2O$: C, 38.00; H, 6.84; N, 19.00%. $[\alpha]_D^{15}=-1.8°$ (*c* 3.8, water). Thierfelder[6] reported; m. p. 199~200°C, $[\alpha]_D^{15}-2.47°$ (*c* 4.20, water).

Synthesis of L-Alanyl-L-glutamine. — Cbzo-L-alanyl-N^{γ}-xanthyl-L-glutamine methyl ester (XII) was prepared by coupling cbzo-L-alanine *p*-nitrophenyl ester with VII in the same manner, and was recrystallized from ethanol (90%); m. p. 208°C (decomp.). Found: C, 65.17; H, 5.69; N, 7.79. Calcd. for $C_{30}H_{31}O_7N_3$: C, 66.04; H, 5.73; N, 7.70%. L-Alanyl-L-glutamine monohydrate was obtained from XII by the similar procedure to that of XI, m. p. 214~215°C (decomp.). Found: C, 40.42; H, 7.52; N, 17.82. Calcd. for $C_8H_{15}O_4N_3 \cdot H_2O$: C, 40.84; H, 7.28; N, 17.86%. $[\alpha]_D^{14}=+11.4°$ (*c* 3.70, water).

Detailed results will be published elsewhere.

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