SYNTHESIS OF DIPEPTIDES FROM β-(8-THEOBROMINYL)-α-ALANINE AND GLYCINE

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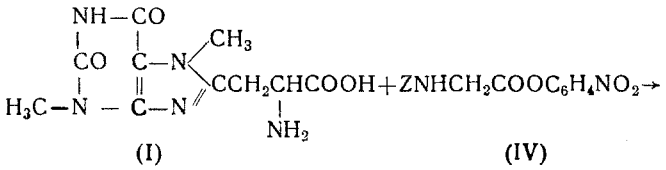
Continuing our investigation of amino acid derivatives of purine substituted at carbon C-8 by the β-carbon atom of α-alanine, we carried out the synthesis of dipeptides from the β-(8-xanthinyl)-α-alanines described previously [1, 2].

In the present communication we give the results of the synthesis of two dipeptides obtained by condensing derivatives of β-(8-theobrominyl)-α-alanine (I) [1] with derivatives of glycine. These dipeptides differ from one another in the fact that in glycyl-β-(8-theobrominyl)-α-alanine (II) the amino group and the carboxy group of the β-(8-theobrominyl)-α-alanine (I) remain free and in β-(8-theobrominyl)-α-alanylglycine (III) the carboxy group of the theobrominylalanine (I) takes part in the formation of the peptide bond.

In all cases, the blockage of the amino group was effected by carbobenzoxylation [3]. The protection of the carboxy groups for both amino acids, I and glycine, was effected by their conversion into the triethylammonium salts [4]; in the case of glycine, esterification with ethanol, tert-butanol, and benzyl alcohol was also used.



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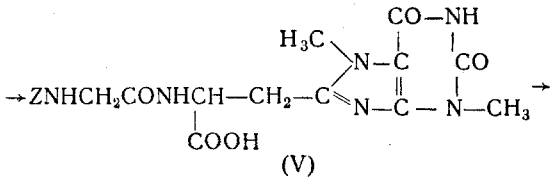

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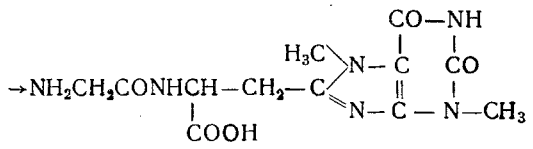

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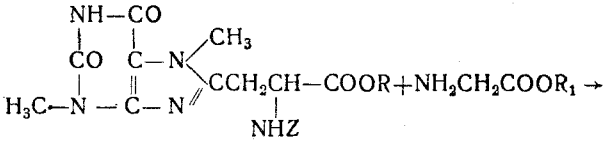
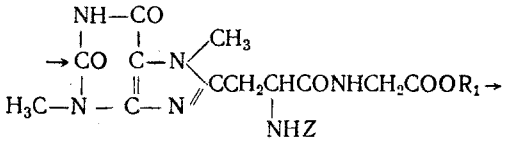
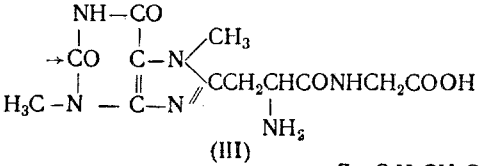


R=H (VI); CH_2CN (VII); p-$C_6H_4NO_2$ (VIII).



R_1=H (IX); C_2H_5 (X) $(CH_3)_3C$ (XI); $C_6H_5CH_2$ (XII).



(III)

Z=$C_6H_5CH_2OCO$—carbobenzoxy group.

The following methods for the creation of a peptide bond were tested: the mixed anhydride method [5], the activated esters method (cyanomethyl [6] and p-nitrophenyl [7] esters), and the dicyclohexylcarbodiimide method [8]. The

best results in the syntheses of dipeptides II and III were obtained by the reaction of esters of the carbobenzoxyamino acids IV and VIII with the triethylammonium salts of the amino acids. In addition, the dipeptide III was obtained from carbobenzoxy-β-(8-theobrominyl)-α-alanine (VI) and glycine esters by the mixed anhydride method with ethyl chlorocarbonate. It was impossible to use the same condensation to prepare the dipeptide II because of the low solubility of esters of β-(8-theobrominyl)-α-alanine (I). An attempt to condense the cyanomethyl ester of carbobenzoxy-β-(8-theobrominyl)-α-alanine (VII) with glycine ethyl ester was unsuccessful because of the low solubility of VII.

The carbobenzoxy groups in the dipeptide derivatives obtained could be split off either by catalytic hydrogenation or by the action of hydrobromic acid in glacial acetic acid. The selection of the method of eliminating the carbobenzoxy group was determined by the nature of the second protective grouping. If this was a benzyl group, it was most convenient to use catalytic hydrogenation. If, however, the carboxy group was esterified with tert-butanol, the best results were obtained by the use of hydrobromic acid in glacial acetic acid. Just as in catalytic hydrogenation, both protective groups were removed simultaneously. In working with triethylammonium salts, catalytic and chemical reduction are equivalent.

Experimental

Carbobenzoxyglycyl-β-(8-theobrominyl)-α-alanine (V)

With heating, 2.7 g of I [1] was dissolved in 110 ml of water containing 2.5 ml of triethylamine, and a hot solution of 3.3 g of the p-nitrophenyl ester of carbobenzoxyglycine (IV) [9] in 135 ml of ethanol was added, after which the mixture was stirred at 50° C for 18 hr. It was then evaporated to half its original volume and extracted with ethyl acetate. The aqueous solution was acidified with concentrated hydrochloric acid to Congo Red and left in a refrigerator. The precipitate that deposited, 2.65 g (57.7%), proved to be V with mp 139–140° C (from ethanol).

Found, %: C 52.20; H 4.93; N 18.02. Calculated for $C_{20}H_{22}O_7N_6$, %: C 52.39; H 4.83; N 18.31.

Glycyl-β-(8-theobrominyl)-α-alanine hydrobromide (II-HBr)

A suspension of 2.8 g of V in 4 ml of glacial acetic acid was treated with 28 ml of a 33% solution of hydrogen bromide in glacial acetic acid. After 30 min, the solid matter had dissolved, and the reaction mixture was poured into 175 ml of absolute ether. This gave a precipitate [2 g (80%)] of II-HBr with mp 216–218° C (from ethanol).

Found, %: C 35.54; H 4.50; N 20.90; Br 19.88. Calculated for $C_{12}H_{17}O_5N_5Br$, %: C 35.5; H 4.23; N 20.74; Br 19.71.

Glycyl-β-(8-theobrominyl)-α-alanine (II)

With cooling, triethylamine was added to a solution of 2 g of II-HBr in 100 ml of 70% ethanol to give pH 5. The solution was left in the cold. A precipitate of II [1.5 g (90%)] deposited, mp 352–355° C (from water).

Found, %: C 44.19; H 5.06; N 25.81. Calculated for $C_{12}H_{16}O_5N_6$, %: C 44.44; H 4.97; N 25.91.

Cyanomethyl ester of carbobenzoxy-β-(8-theobrominyl)-α-alanine (VII)

One gram of VI [10] was treated successively with 0.5 ml of triethylamine and 1 ml of chloroacetonitrile. The reaction mixture was heated at 70° C for 30 min with stirring and was left to stand at 20° C for 2 hr. The viscous mass was crystallized from ethanol. This gave 0.9 g (82.6%) of VII with mp 185–186° C.

Found, %: C 54.81; H 4.36; N 19.09. Calculated for $C_{20}H_{20}O_6N_6$, %: C 54.67; H 4.55; N 19.13.

p-Nitrophenyl ester of carbobenzoxy-β-(8-theobrominyl)-α-alanine (VIII)

With stirring, 0.75 g of p-nitrophenol was added to a suspension of 2 g of VI in 20 ml of ethyl acetate, the mixture was cooled to 0° C, and 1 g of dicyclohexylcarbodiimide in 5 ml of ethyl acetate was added. Stirring was continued at 0° C for another hour and at room temperature for 4.5 hr, after which the mixture was left to stand for 18 hr. The precipitate of dicyclohexylurea that had deposited was separated off and the filtrate was evaporated in vacuum. The residue was crystallized from ethanol. This gave 1.5 g (57.7%) of VIII with mp 182–183° C.

Found, %: C 54.89; H 4.32; N 15.81. Calculated for $C_{24}H_{22}O_8N_6$, %: C 55.16; H 4.24; N 16.09.

Carbobenzoxy-β-(8-theobrominyl)-α-alanylglycine (IX)

A mixture of 0.65 g of (VIII) and 0.1 g of glycine in 55 ml of 50% ethanol was treated with 0.35 ml of triethylamine and the mixture was heated at 55–60° C for 14 hr. The ethanol was evaporated off in vacuum and the aqueous layer was extracted once with 10 ml of ethyl acetate and acidified to Congo Red. The substance that deposited was left in a refrigerator and filtered off. The precipitate was crystallized from ethanol. This gave 0.3 g (52%) of IX with mp 125–129° C.

Found, %: C 51.99; H 4.93; N 17.82. Calculated for $C_{20}H_{22}O_7N_6$, %: C 52.39; H 4.83; N 18.31.

Ethyl ester of carbobenzoxy-β-(8-theobrominyl)-α-alanylglycine (X)

A solution of 2 g of VI in 200 ml of tetrahydrofuran at +5° C was treated with 0.9 ml of triethylamine and 0.65 ml of ethyl chlorocarbonate, and the mixture was left for 10 min. Then 1 g of freshly distilled glycine ethyl ester was added and the mixture was left at 0-5° C for 30 min and at room temperature for 18 hr. After this, 20 ml of water was added and it was evaporated in vacuum until a precipitate was formed, which was dissolved in water and extracted with ethyl acetate (3 × 50 ml). The extract was dried with magnesium sulfate and evaporated to half bulk. The precipitate was filtered off and boiled with 70% ethanol. This gave 0.6 g (24%) of X, mp 177-179° C.

Found, %: C 54.44; H 5.49; N 16.97. Calculated for $C_{22}H_{26}O_7N_2$, %: C 54.31; H 5.39; N 17.28.

Tert-butyl ester of carbobenzoxy-β-(8-theobrominyl)-α-alanylglycine (XI)

A solution of 5 g of VI in 200 ml of dioxane and 1.8 ml of triethylamine at +12° C was treated with 1.3 ml of ethyl chlorocarbonate and the mixture was left for 10 min, after which 2.3 g of glycine tert-butyl ester was added and it was left at 20° C for 20 hr. Then 50 ml of water was added to the reaction mixture, the precipitate of unchanged VI was filtered off, and the filtrate was evaporated in vacuum to 30 ml and was extracted with ethyl acetate and 2 N hydrochloric acid, with water, with 5% sodium bicarbonate solution, and with water again. The organic fraction was dried with sodium sulfate and evaporated. The oily residue crystallized on standing. The yield of XI was 4 g (63%), mp 166-168° C (from benzene).

Found, %: C 56.06; H 6.20; N 16.23. Calculated for $C_{24}H_{30}O_7N_6$, %: C 56.02; H 5.89; N 16.34.

Benzyl ester of carbobenzoxy-β-(8-theobrominyl)-α-alanylglycine (XII)

A mixture of 1.6 g of VI and 100 ml of tetrahydrofuran containing 1.1 ml of triethylamine was heated until the solid matter had dissolved almost completely and then 0.8 ml of ethyl chlorocarbonate was added at +10° C. The mixture was kept at +12° C for 10 min and was then treated with 1.6 g of freshly prepared glycine benzyl ester. The reaction mixture was left at 20° C for 2 hr and was then treated with 35 ml of water and evaporated in vacuum. The residue was extracted with methylene chloride and washed with 5% Na_2CO_3 solution, water, 2 N hydrochloric acid, and water again. The methylene chloride was dried with sodium sulfate and evaporated. The residue was triturated in absolute ether. This gave 1.7 g (78%) of XII, mp 155-156° C (from ethanol).

Found, %: C 59.13; H 5.54; N 24.63. Calculated for $C_{27}H_{28}O_7N_6$, %: C 59.11; H 5.14; N 15.32.

β-(8-Theobrominyl)-α-alanylglycine (III)

With boiling, 1.1 g of XII was dissolved in 60 ml of methanol and hydrogenated in the presence of 0.1 ml of glacial acetic acid with 0.8 g of 10% palladium on carbon at 25° C. The catalyst was filtered off, the solution repeatedly boiled with water until the ninhydrin reaction was negative, and the aqueous solution was evaporated. The precipitate formed was filtered off and crystallized from water. This gave 0.4 g (65%) of III with mp 349-352° C.

Found, %: C 42.94; H 5. 54; N 24.63. Calculated for $C_{12}H_{16}O_5N_6$, %: C 42.11; H 5.30; N 24.55.

β-(8-Theobrominyl)-α-alanylglycine (III)

A suspension of 1 g of XI in 2 ml of glacial acetic acid was treated with 10 ml of hydrobromic acid in glacial acetic acid. The substance gradually dissolved, and after 30 min the solution was treated with 40 ml of absolute ether, which was then poured off from the precipitate. The residue was dissolved in water and extracted with ether. The aqueous solution was neutralized with concentrated ammonia to pH 7, ethanol was added (4 vol), and the mixture was left in a refrigerator. The precipitate of III that desposited was filtered off. This gave 0.35 g (53%) of a substance with mp 347-350° C.

Summary

The dipeptides glycyl-β-(8-theobrominyl)-α-alanine and β-(8-theobrominyl)-α-alanylglycine have been synthesized.

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