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NOTES

SYNTHESES OF ALPHA AMIDE AND PEPTIDE DERIVATIVES OF DL-ASPARTIC ACID

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In previous work we have prepared α and β amide and peptide derivatives of aspartic acid starting with N-benzyl-aspartic acid as the anhydride hydrochloride (1) or as the mixed anhydride with chloroformic acid obtained by reaction with phosgene (2, 3, 4). Exclusive preparation of the β derivatives in good yield was obtained starting with maleic anhydride (5). In the previous methods, α amides and peptides were obtained sometimes exclusively, and sometimes in admixture with β derivatives (2, 3, 4). The use of active amines or aminoacid esters led to the formation of α derivatives, while with weak aromatic amines β derivatives were obtained (2, 4, 6). From weak aromatic amines, α derivatives were obtained only on using large excess of amine (6).

In the present work the use of β-benzyl N-benzyl-DL-aspartate (I) as starting material for the exclusive formation of α-aspartyl amides and peptides is described. In this compound the β-carboxyl group is masked with a benzyl group, which can be removed easily by hydrogenolysis. The use of other ester groups such as the methyl group [the corresponding N-benzyl derivative has been prepared (7)] did not seem feasible, as these can be removed only by hydrolysis, and it has already been shown that such hydrolysis leads to the formation of imide derivatives as cyclic intermediates, which afterwards may open to yield either the α or β derivatives (8, 9).

β-Benzyl N-benzyl-DL-aspartate (I) was prepared starting from benzyl maleate as follows:

$$\begin{array}{l} CHCOOCH_2C_6H_5 \\ \| \\ CHCOOH \end{array} + C_6H_5CH_2NH_2 \rightarrow \begin{array}{l} CH_2COOCH_2C_6H_5 \\ | \\ CHCOOH \\ | \\ NHCH_2C_6H_5 \end{array}$$

I

One equivalent of benzylamine was used, and it led exclusively to opening of the double bond without attacking the ester group, showing that addition to the double bond is preferable to amidation (7). DL-Aspartic acid was obtained on hydrogenolysis of (I). Reaction of (I) with phosgene in dioxane gave an active intermediate from which α-aspartyl amide and peptide derivatives were obtained on reaction with two equivalents of amine or amino-acid ester in dioxane. The product usually was difficult to crystallize, so that the dioxane solution was evaporated after filtration from the amine hydrochloride that precipitated, and the product was hydrogenolyzed with alcohol in the presence of palladium chloride (30% on carbon). In the case of cyclohexylamine, the intermediate product N^2-benzyl-N-cyclohexyl-α-asparagine β-benzyl ester was isolated. By this method the yields in many cases were much higher than those obtained previously (2, 4).

The α amides and peptides gave positive biuret reactions and purple spots on paper chromatograms as was found previously (2).

Structure of the Active Intermediate (II)

To ascertain the nature of the active intermediate (II), the known determinations (10) of acid chlorides, anhydrides, and amino acids by anhydrous titrations with sodium

methoxide and Triton B (trimethylbenzylammonium hydroxide) were used, as shown in the equations below:

$$RCOCl + NaOCH_3 \rightarrow RCOOCH_3 + NaCl \text{ (no back-titration with } HClO_4)$$

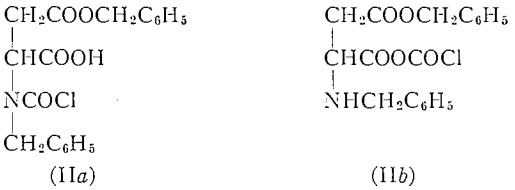
$$RCOCl + 2\,(CH_3)_3NC_7H_7OH \rightarrow RCOON(CH_3)_3C_7H_7 + (CH_3)_3NC_7H_7Cl + H_2O$$

$$\underset{\underset{NH_2}{|}}{RCHCOOH} + NaOCH_3 \rightarrow \underset{\underset{NH_2}{|}}{RCHCOONa} + CH_3OH \xrightarrow[\text{back-titration}]{2HClO_4} \underset{\underset{NH_2.HClO_4}{|}}{RCHCOOH} + NaClO_4$$

It may be noted that while 1 equivalent of sodium methoxide is required for the titration of a chloride or anhydride, 2 equivalents of Triton B are required. However, we found that a solution of carbon dioxide in dioxane required 1 equivalent of Triton B for titration, using thymol blue as indicator, the same as with sodium methoxide.

The intermediate (II) was prepared directly in dioxane solution (excess phosgene expelled) and aliquot portions analyzed. Volhard titration for chlorine showed the presence of approximately 1 equivalent of chlorine. Anhydrous titration with sodium methoxide using thymol blue as indicator required the use of 2 equivalents of reagent, while titration with Triton B required the use of 3 equivalents of reagent (10). Back-titration of the sodium methoxide titrated aliquot with perchloric acid in dioxane required the use of 2 equivalents of reagent, one to titrate the sodium carboxylate formed, and the other the amino group. Addition of *p*-toluidine to the dioxane solution of II led to the generation of 1 mole equivalent of CO_2. Also, the dioxane solution of the intermediate did not titrate directly with perchloric acid in dioxane with thymol blue as indicator, presenting evidence that the amino group was blocked.

All these results are consistent with the formation of an N-chloroformyl derivative (II*a*), but not a mixed anhydride with chloroformic acid (II*b*), which has a free amino group, or a simple anhydride hydrochloride between two molecules of the benzyl ester derivative.



(II*a*)

(II*b*)

Such an N-chloroformyl derivative was formed in the reaction of phosgene with N-phenyl-β-alanine or N-*p*-tolyl-β-aminobutyric acid (11). It can cyclize (11) on reaction with amines to an N-carboxyanhydride, which decomposes with carbon dioxide evolution on reaction with additional amine.

We tried to obtain more evidence from examination of the infrared spectra of the active intermediate (II). A mixed anhydride structure, such as (II*b*), must have double absorption peaks at about 5.4 μ and 5.6 μ which are not given by the N-chloroformyl structure (II*a*). The dioxane solution was evaporated to dryness *in vacuo* at 30°, and the residue showed a double absorption peak in the infrared at 5.4 μ and 5.65 μ, which is consistent with either a mixed anhydride or an N-carboxyanhydride structure. However, during the evaporation, the compound must have undergone considerable transformation as its chlorine content was reduced by some 50%. The dry compound also evolved fumes of hydrogen chloride on standing. After several fractional crystallizations of the residue from dioxane – petroleum ether or chloroform–ether, the N-carboxyanhydride derivative of (I) was isolated. This was identical with that obtained on addition of an equivalent of

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TABLE I

α-DL-Aspartyl amides and peptides

N-Alkyl (or aryl)-DL-α-asparagine	M.p.,* °C	Yield, %	R_f	Formula	Carbon, %		Hydrogen, %		Nitrogen, %		Nitrogen, % (Van Slyke)	
					Calc.	Found	Calc.	Found	Calc.	Found	Calc.	Found
Phenyl (6)	252	60	0.79	$C_{10}H_{12}N_2O_3$	57.7	57.6	5.8	5.9	13.4	13.0	6.7	6.5
α-Naphthyl	227	95		$C_{14}H_{14}N_2O_3$					10.8	10.6	5.4	5.2
p-Tolyl (1)	235	60	0.79	$C_{11}H_{14}N_2O_3$	59.5	59.5	6.3	6.2	12.6	12.7	6.3	6.3
Isobutyl	242	80	0.87	$C_8H_{16}N_2O_3$	51.1	51.1	8.5	8.6	14.9	14.5	7.4	7.3
n-Butyl (4)	226	70	0.89	$C_8H_{16}N_2O_3$	51.1	51.1	8.5	8.5	14.9	14.7	7.4	7.4
n-Hexyl (4)	223	65	0.83	$C_{10}H_{20}N_2O_3$	55.5	55.1	9.3	9.3	13.0	13.1	6.5	6.6
Carbethoxymethyl (2)	175	60	0.89	$C_8H_{14}N_2O_5$	44.0	43.7	6.4	6.2	12.8	12.7	6.4	6.5
2-Carbethoxyethyl (3)	188	50	0.93	$C_9H_{16}N_2O_5$	46.5	46.5	6.9	6.9	12.0	12.3	6.0	6.3

*Substances were recrystallized from ethanol or water.

triethylamine to the dioxane solution of the active intermediate, filtration of the precipitated triethylamine hydrochloride, evaporation of the dioxane, and crystallization of the N-carboxyanhydride from chloroform–ether. The N-carboxyanhydride showed absorptions at 5.4 μ and 5.65 μ besides the absorption at 5.8 μ (ester group).

EXPERIMENTAL

Melting points were determined in a Fisher–Johns apparatus, and the ascending method of paper partition chromatography (80% phenol) was used. A procedure for one typical example of each reaction step is given and the remainder are summarized in Table I. Benzyl maleate (12) m.p. 51–52°, was prepared in 92% yield by heating equimolar quantities of maleic anhydride and benzyl alcohol at 125°.

*β-Benzyl N-benzyl-*DL*-aspartate (I).*—Benzyl maleate (51.5 g, 0.25 mole) was dissolved in dry dioxane (400 ml) and cooled. Benzylamine (26.8 g, 0.25 mole) was added in one portion and the reaction mixture was stirred at 125° for 3 hours. The product started to precipitate after a few minutes. It was filtered and washed with acetone, yield 60 g (76%), m.p. 195° on recrystallization from water or better from 50% ethanol. Anal. Calc. for $C_{18}H_{19}NO_4$: C, 69.0; H, 6.1; N, 4.5. Found: C, 68.9; H, 5.9; N, 4.7.

*Reaction of β-Benzyl N-Benzyl-*DL*-aspartate with Phosgene.*—Dry, finely powdered β-benzyl N-benzyl-DL-aspartate (15.7 g, 0.05 mole) was suspended in dry dioxane (400 ml) in a three-necked flask equipped with a gas leading tube, a reflux condenser connected to a calcium chloride tube, and a mechanical stirrer. Dry phosgene was bubbled in with stirring for 90 minutes and the temperature was kept constant at 50°. After 10 minutes the solution cleared. Excess phosgene, but not the solvent, was removed *in vacuo* at 20° (4–5 hours). The solution was used as such for the preparation of the amides and peptides.

*N²-Benzyl-N-cyclohexyl-α-*DL*-asparagine β-Benzyl Ester.*—To an ice-cold solution of the reactive intermediate (II) prepared from (I) (4.1 g, 0.013 mole) in dioxane (100 ml) was added cyclohexylamine (2.7 g, 0.028 mole). The reaction mixture was shaken in the cold for 30 minutes and left overnight at room temperature. The precipitate consisting of cyclohexylamine hydrochloride was filtered off, and the filtrate evaporated *in vacuo*. On cooling the residue crystallized. It was dissolved in a small volume of ethanol, water was added to incipient cloudiness, and the substance was left to recrystallize; yield 4.85 g. (95%), m.p. 80°. Anal. Calc. for $C_{24}H_{30}N_2O_3$: C, 73.1; H, 7.6; N, 7.1. Found: C, 73.4; H, 7.9; N, 7.1.

*N-Cyclohexyl-*DL*-α-asparagine.*—N²-Benzyl-N-cyclohexyl-DL-α-asparagine (1 g) was dissolved in ethanol (100 ml) and 0.2 g of 30% palladium chloride on charcoal (2) was added. The hydrogenolysis was carried out in a Parr low-pressure hydrogenation apparatus for 10 hours at 50°. The product precipitated in the ethanol solution and was extracted from the catalyst by boiling water. The aqueous solution was evaporated, and the product which crystallized was washed with ethanol and filtered; yield 0.55 g. (quantitative), m.p. 247° on recrystallization from water, R_f 0.92. Anal. Calc. for $C_{10}H_{18}N_2O_3$: C, 56.1; H, 8.4; N, 13.1; N (Van Slyke), 6.6. Found: C, 56.0; H, 8.5; N, 13.2; N (Van Slyke), 6.6.

*N-Carboxyanhydride of β-Benzyl N-Benzyl-*DL*-aspartate.*—To a solution of the reactive intermediate (III) in dioxane prepared as above from 3.1 g (I), an equivalent amount of dry triethylamine (sufficient to react with the chlorine) was added dropwise in the cold with stirring. The reaction mixture was stirred for 2 hours and the precipitate of triethylamine hydrochloride was filtered. The dioxane filtrate was evaporated to dryness at 40°, and the N-carboxyanhydride crystallized on standing overnight in the cold under petroleum ether, yield 2.8 g (82%). It was recrystallized from chloroform – petroleum ether or chloroform–ether, m.p. 92°. Anal. Calc. for $C_{19}H_{17}NO_5$: C, 67.2; H, 5.0; N, 4.1; mol. wt. 339. Found: C, 66.9; H, 5.2; N, 4.3; mol. wt. 335, determined by anhydrous titration with 0.1 *N* sodium methoxide in dioxane using thymol blue as indicator (13).

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