[CONTRIBUTION FROM THE WEIZMANN INSTITUTE OF SCIENCE]

THE USE OF HYDROGEN BROMIDE IN ACETIC ACID FOR THE REMOVAL OF CARBOBENZOXY GROUPS AND BENZYL ESTERS OF PEPTIDE DERIVATIVES[1]

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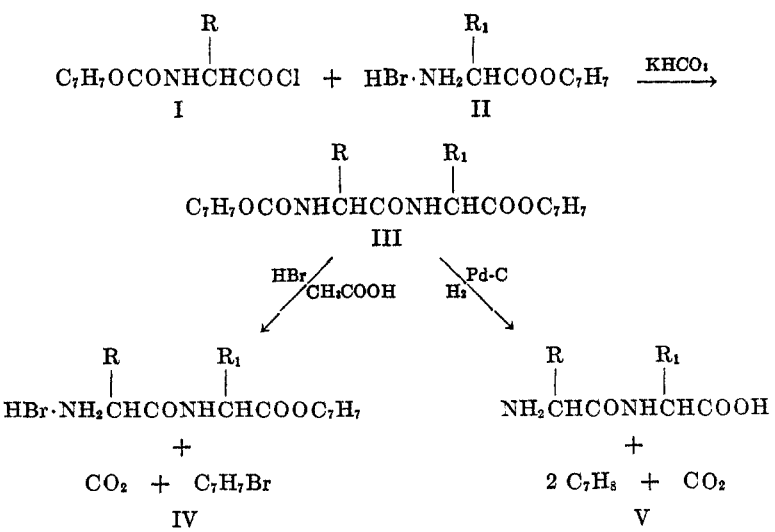
The introduction, in 1932, by Bergmann and Zervas (1) of carbobenzoxy chloride as a reagent for the protection of amino groups in peptide synthesis enabled the preparation of a wide variety of peptides (2). The carbobenzoxy groups are generally removed in the last step of the synthesis by catalytic hydrogenation. Since in the case of sulfur-containing peptides, catalytic hydrogenation cannot be carried out satisfactorily, the catalysts being inactivated, alternative procedures for the removal of the blocking group have been investigated, such as the use of phosphonium iodide as a non-hydrolytic cleaving agent (3) and the removal of carbobenzoxy and benzyl groups in general by treatment with metallic sodium in liquid ammonia (4).

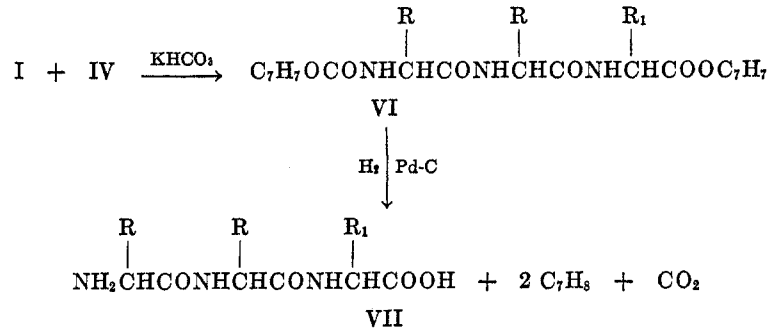
In an investigation of the action of dry hydrogen bromide, or hydrogen chloride on benzyl carbamates (5), it was observed that hydrogen bromide in glacial acetic acid causes smooth non-hydrolytic cleavage of carbobenzoxy groups attached to nitrogen.[2] In the case of benzyl esters of N-carbobenzoxy-α-amino acids, the carbobenzoxy groups are *preferentially* removed and benzyl ester hydrobromides of α-amino acids are obtained.

These observations support the following modification of peptide syntheses:



[1] Presented before the Chemical Division of the Israeli Chemical Society, December 1952.

[2] The same observation was independently reported by Anderson, *et al.* (12).



(a) Carbobenzoxy-α-amino acid chlorides (I) are condensed with α-amino acid benzyl ester hydrobromides (II) in a mixture of ethyl acetate and water in the presence of potassium bicarbonate, giving benzyl esters of carbobenzoxy-dipeptides (III) in over 70 % yield. The use of amino acid benzyl esters is already known to save one step in the peptide synthesis (2), since the benzyl group can be removed together with the carbobenzoxy groups by catalytic hydrogenation, thus obviating the necessity for alkali treatment of the esters (III). It is advisable to wash the ethereal solutions of the acid chlorides with ice-water before condensation reaction (6, 7). The use of aqueous potassium bicarbonate as a condensing agent makes it unnecessary both to isolate the acid chloride from its ethereal solution and to prepare the free benzyl ester in an anhydrous solvent.

(b) The carbobenzoxy groups in the intermediate carbobenzoxypeptide benzyl esters (III) are *preferentially* cleaved by short treatment with hydrogen bromide in glacial acetic acid leading to hydrobromides of dipeptide benzyl esters (IV). Thus, the hydrobromides of glycylglycine benzyl ester and glycyl-L-phenylalanine benzyl ester were prepared in 94 % and 89 % yield, respectively.

(c) Renewed condensation of the dipeptide esters (IV) with a second molecule of carbobenzoxy-α-amino acid chloride (I) gives carbobenzoxytripeptide benzyl esters (VI) which are converted by catalytic hydrogenation directly to the free tripeptides (VII). Four tripeptides of glycine and L-phenylalanine have been prepared in this way. By coupling carbobenzoxy-L-phenylalanyl chloride with (diglycyl)glycine benzyl ester hydrobromide one tetrapeptide, L-phenylalanyl-(diglycyl)glycine, was obtained in good yield (Table I).

In the case of carbobenzoxyglycylglycine benzyl ester and carbobenzoxy-(diglycyl)glycine benzyl ester it was observed that both the carbobenzoxy and benzyl groups are removed simultaneously when a current of dry hydrogen bromide is passed through their acetic acid solutions for one hour at 60°.

EXPERIMENTAL[3, 4]

L-Phenylalanine benzyl ester hydrobromide. This compound was prepared in analogy to the racemic compound (5). Attempts to crystallize the intermediate carbobenzoxy-L-phenylalanine benzyl ester were unsuccessful; the crude product was treated directly with the re-

[3] All m.p.'s are uncorrected.

[4] The specific rotations of the intermediates are not given since the solubility and rotations of these compounds are low.

TABLE I

PEPTIDES

PEPTIDE	YIELD, %	M.P., °C (dec)	$[\alpha]_D^{25}$ (in water)	FORMULA	ANALYSES					
					Calc'd			Found		
					C	H	N	C	H	N
Glycylglycine	83	222[a]		$C_4H_8N_2O_3$	36.4	6.0	21.2	36.6	5.8	21.0
Glycyl-L-phenylalanine	93	264–268[b]	+41.8 (*c*, 2.5)[b]	$C_{11}H_{14}N_2O_3$	59.5	6.3	12.6	59.3	6.5	12.6
L-Phenylalanylglycine monohydrate	87	270–271	+93.5 (*c*, 1.85)[c]	$C_{11}H_{16}N_2O_4$	55.0	6.7	11.7	55.3	6.8	11.9
(Diglycyl)glycine	87	244–246[e]		$C_6H_{11}N_3O_4$	38.1	5.8	22.2	38.4	6.0	22.0
(Diglycyl)-L-phenylalanine	84	228–230	+35.6 (*c*, 1.0)	$C_{13}H_{17}N_3O_4$	55.9	6.1	15.0	55.6	5.9	15.2
L-Phenylalanylglycylglycine	71	218–220	+79.4 (*c*, 1.75)	$C_{13}H_{17}N_3O_4$	55.9	6.1	15.0	55.6	6.3	15.1
L-Phenylalanylglycyl-L-phenylalanine monohydrate	80	266–268	+45.5 (*c*, 1.66)[e]	$C_{20}H_{25}N_3O_5$	62.0	6.4	10.9	61.8	6.5	11.1
L-Phenylalanyl(diglycyl)glycine	80	235–236	+65.3 (*c*, 2.02)	$C_{15}H_{20}N_4O_5$	53.6	6.0	16.6	53.7	6.1	17.0

[a] Ref. (8) gives m.p. 215–220°. [b] Ref. (9) gives m.p. 267°, $[\alpha]_D^{20}$ 42.0°. [c] Ref. (9, 10) gives $[\alpha]_D^{20}$ +54.2° and $[\alpha]_D^{28}$ +84.4°. [d] Ref. (11) gives m.p. 246°. [e] Specific rotation measured in 1 *N* HCl.

TABLE II

CARBOBENZOXYPEPTIDE BENZYL ESTERS

BENZYL ESTER[a]	YIELD, %	M.P.,[b] °C.	FORMULA	ANALYSES					
				Calc'd			Found		
				C	H	N	C	H	N
Cbzglycylglycine	73	110	$C_{19}H_{20}N_2O_5$	64.0	5.6	7.9	64.2	5.4	8.1
Cbzglycyl-L-phenylalanine	86	74	$C_{26}H_{26}N_2O_5$	69.9	5.8	6.3	69.8	5.8	6.2
Cbz-L-phenylalanylglycine	72	138	$C_{26}H_{26}N_2O_5$	69.9	5.8	6.3	69.9	6.0	6.5
Cbz(diglycyl)glycine[c]	89	162	$C_{21}H_{23}N_3O_6$	61.0	5.5	10.2	60.8	5.7	10.4
Cbz(diglycyl)-L-phenylalanine	79	146	$C_{28}H_{29}N_3O_6$	66.8	5.7	8.1	66.4	5.6	8.3
Cbz-L-phenylalanylglycylglycine	74	109	$C_{28}H_{29}N_3O_6$	66.8	5.7	8.1	66.6	5.5	8.4
Cbz-L-phenylalanylglycyl-L-phenylalanine	83	150–151	$C_{35}H_{35}N_3O_6$	70.8	5.9	7.1	70.6	5.9	7.3
Cbz-L-phenylalanyl(diglycyl)glycine	86	114–115	$C_{30}H_{32}N_4O_7$	64.3	5.7	10.0	64.2	5.5	10.0

[a] Cbz = Carbobenzoxy. [b] The esters were crystallized from ethyl acetate-petroleum ether. [c] Crystallized from alcohol.

agent (hydrogen bromide in glacial acetic acid) and the hydrobromide formed was triturated with dry ether, filtered, and washed with ether. It was recrystallized from hot water and melted at 209°. Yield, 75%, based on carbobenzoxy-L-phenylalanine; $[\alpha]_D^{25}$ 5.4° in alcohol (*c*, 2.3).

Anal. Calc'd for $C_{16}H_{18}BrNO_2$: N, 4.2; Br, 23.8.

Found: N, 4.4; Br, 23.8.

Carbobenzoxyglycyl-L-phenylalanine benzyl ester. To 10.5 g. (0.05 mole) of carbobenzoxyglycine, suspended in 150 ml. of dry ether, there was added with stirring and cooling (ice-salt) 12 g. (0.058 mole) of phosphorus pentachloride. After 45 minutes, the ethereal solution was decanted from the excess phosphorus chloride into a separatory-funnel containing crushed ice. The water layer was separated and the ethereal solution of the acid chloride used immediately for the coupling reaction.

It was added, within five minutes, with stirring and cooling (ice-water) together with 100 ml. of a saturated solution of potassium bicarbonate to 10 g. (0.03 mole) of finely powdered L-phenylalanine benzyl ester hydrobromide, suspended in 100 ml. of ethyl acetate. The reaction mixture was stirred for another 30 minutes, and the organic layer was separated and washed with aqueous potassium bicarbonate (50 ml. of a 5% solution), water, and dilute hydrochloric acid (50 ml. of 2 *N* solution). After drying over sodium sulfate, the solvent was removed under reduced pressure and the residual oil was recrystallized from ethyl acetate-petroleum ether (Table II).

Glycyl-L-phenylalanine. Carbobenzoxyglycyl-L-phenylalanine benzyl ester (3 g.) in methanol (100 ml.) was catalytically hydrogenated, under 4 atm. pressure and in the presence of 5% palladized charcoal (0.3 g.) and acetic acid (1 ml.). After three hours, water was added to dissolve the peptide which had separated and the solution was filtered from the catalyst. After concentration *in vacuo* to a small volume (5 ml.) absolute alcohol was added and the peptide was filtered, washed with alcohol and anhydrous ether, and dried *in vacuo* over sulfuric acid (48 hours) (Table I).

Glycyl-L-phenylalanine benzyl ester hydrobromide. A 33% solution (8 g.) of dry hydrogen bromide in glacial acetic acid, was added to carbobenzoxyglycyl-L-phenylalanine benzyl ester (3 g.). The mixture, protected with a calcium chloride tube, was allowed to stand at room temperature, with occasional shaking, until the evolution of carbon dioxide ceased (20 minutes). Dry ether (150 ml.) was then added and the reaction flask was kept in the refrigerator (4 hours). The solid hydrobromide which separated was filtered, washed with dry ether, and dried *in vacuo* over sulfuric acid and sodium hydroxide. The product melted at 193° after recrystallization from alcohol and ether; Yield, 2.3 g. (89%).

Anal. Calc'd for $C_{18}H_{21}BrN_2O_3$: N, 7.1; Br, 20.3.

Found: N, 7.3; Br, 20.5.

Glycylglycine benzyl ester hydrobromide. Carbobenzoxyglycylglycine benzyl ester (10 g.) was treated with hydrogen bromide in glacial acetic acid (20 g. of a 33% solution), under exclusion of humidity. After the evolution of carbon dioxide had ceased (20 minutes), absolute ether (100 ml.) was added to precipitate the hydrobromide formed. The reaction flask was kept in the refrigerator overnight and the solid was filtered, washed with dry ether, and dried over sulfuric acid and potassium hydroxide, m.p. 144°. The yield was 8 g. (94%).

Anal. Calc'd for $C_{11}H_{15}BrN_2O_3$: N, 9.2; Br, 26.4.

Found: N, 9.3; Br, 26.6.

Glycylglycine. Carbobenzoxyglycylglycine benzyl ester (5 g.) was suspended in glacial acetic acid (25 ml.) and a stream of dry hydrogen bromide was bubbled through the hot solution (60°) for one hour. During the reaction the starting material dissolved, and after 15 minutes the dipeptide hydrobromide began to separate.

Dry ether (100 ml.) was then added and the reaction flask kept in the refrigerator (4 hours). The supernatant liquid was decanted and the solid was washed with small portions of ether and dried *in vacuo*. In order to obtain the free peptide, the hydrobromide was dissolved in the minimum amount of water, excess pyridine was added, and the peptide was

precipitated with absolute alcohol. It was filtered, washed with absolute alcohol and dry ether, and dried *in vacuo* over sulfuric acid (48 hours) (Table I).

(Diglycyl)glycine benzyl ester hydrobromide. Carbobenzoxy(diglycyl)glycine benzyl ester (9 g.) was treated with hydrogen bromide in glacial acetic acid (20 g. of a 33% solution) as described above. The hydrobromide so obtained was recrystallized from absolute alcohol and dry ether and melted at 174–175°. The yield was 7.0 g. (89%).

Anal. Calc'd for $C_{13}H_{18}BrN_3O_4$: N, 11.6; Br, 22.2.
Found: N, 11.6; Br, 22.5.

SUMMARY

The preparation of the peptides of glycine and L-phenylalanine by a modified "carbobenzoxy" method is described. Hydrogen bromide in glacial acetic acid is used as a non-hydrolytic cleaving agent for the removal of the carbobenzoxy groups in the intermediates benzyl esters.

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