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CLEAVAGE OF N-CARBOBENZOXY GROUPS BY DRY HYDROGEN BROMIDE AND HYDROGEN CHLORIDE

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In search of a method for the removal of carbobenzoxy groups from the nitrogen atoms in thyroxine-containing peptides, Harington (1) observed that hydrogen iodide (or, better, phosphonium iodide) in glacial acetic acid achieves the desired reaction, giving the amine hydriodide, benzyl iodide, and carbon dioxide.

$$RNHCOOC_7H_7 + HI \rightarrow RNH_2 \cdot HI + C_7H_7I + CO_2$$

This reaction represents a non-hydrolytic cleavage and not, as claimed by several investigators (1, 2), a reduction process. Indeed hydrogen *chloride* (in anhydrous ethanol) eliminates the carbobenzoxy group in (N-carbobenzoxy-tyrosyl)tyrosine or carbobenzoxyglycine (3) and (in glacial acetic acid) cleaves ethyl carbamate to ammonium chloride, ethyl chloride, and carbon dioxide (4).

The general application of this reaction especially to N-carbobenzoxy compounds has been investigated. It was found that benzyl carbamates, N-carbobenzoxy-α-amino acids, N-carbobenzoxy-α-amino acid benzyl esters, and also poly-carbobenzoxy-L-lysine are cleaved in the expected manner by a solution of hydrogen bromide in glacial acetic acid. The reaction, which is carried out at room temperature within 15 minutes to one hour, gives excellent yields of the corresponding hydrobromides (Tables I and II). Ethyl carbamate reacts with hydrogen bromide in glacial acetic acid at 75° to give ammonium bromide, carbon dioxide, and ethyl bromide.

In two cases the reaction was carried out using dry hydrogen chloride, and it was found that both benzyl carbamate and as complex a substance as poly-carbobenzoxy-L-lysine are cleaved in glacial acetic acid at 75° to the corresponding amine hydrochloride in over 90% yield.

Anhydrous dioxane and anhydrous ethanol can be used as solvents in this reaction. Ethanol has, however, the disadvantage that the amine hydrobromide formed cannot be precipitated directly from the alcohol by the addition of ether, as the latter does not mix with an alcoholic solution of hydrogen bromide. The use of dioxane or acetic acid has the additional advantage that esterification is avoided.

In the case of N,N′-dicarbobenzoxy-L-lysine, the optical activity was preserved in the lysine dihydrobromide formed, also the lysine recovered from poly-carbobenzoxy-L-lysine by successive application of the above method and hydrolysis with hydrochloric acid, has been shown by bioassay to possess quantitatively the original L-configuration.

The method described has proven particularly useful in its application to the benzyl esters of N-carbobenzoxyamino acids. Harington and Mead (5) have

TABLE I

REACTION OF HYDROGEN BROMIDE WITH BENZYL CARBAMATES AND CARBOBENZOXY-α-AMINO ACIDS

STARTING MATERIAL	HYDROBROMIDE	YIELD, %	ANALYSIS				BENZYL BROMIDE Yield, %
			Nitrogen		Bromine		
			Calc'd	Found	Calc'd	Found	
$NH_2COOCH_2C_6H_5$	NH_4Br	92	14.4	14.1	81.6	81.1	90
$CH_3NHCOOCH_2C_6H_5$	$CH_3NH_2 \cdot HBr$	96	12.5	12.7	71.4	71.8	92
$(CH_3)_2NCOOCH_2C_6H_5$	$(CH_3)_2NH \cdot HBr$	94	11.1	11.4	63.5	64.0	92
$C_6H_5NHCOOCH_2C_6H_5$	$C_6H_5NH_2 \cdot HBr$	92	8.0	8.2	45.9	46.2	91
$C_6H_5N(CH_3)COOCH_2C_6H_5$	$C_6H_5NH(CH_3) \cdot HBr$	91	7.4	7.4	42.5	42.0	96
$C_6H_5CH_2NHCOOCH_2C_6H_5$	$C_6H_5CH_2NH \cdot HBr$	85	7.4	7.6	42.5	42.2	91
$(C_6H_5)_2NCOOCH_2C_6H_5$	$(C_6H_5)_2NH \cdot HBr$	88	5.6	5.7	32.0	32.4	89
N-Carbobenzoxyglycine	Glycine·HBr	96	9.0	8.8	51.3	50.5	
N-Carbobenzoxy-DL-alanine	DL-Alanine·HBr	92	8.3	8.0	47.2	46.6	
N-Carbobenzoxy DL-phenylalanine	DL-Phenylalanine·HBr	90	5.7	5.7	32.5	32.9	
N,N′-Dicarbobenzoxy-L-lysine	L-Lysine·2HBr[a]	94	9.1	9.0	51.9	51.5	
N-Carbobenzoxy-S-benzyl-L-cysteine	S-Benzyl-L-cysteine·HBr[b]	88	4.8	5.1	27.4	27.8	

[a] The rotation of the lysine was determined in 6 N hydrochloric acid; $[\alpha]_D^{25}$ 25.8°. Ref. (19) gives $[\alpha]_D^{25}$ 25.72°. [b] The compound gave a negative test for free SH-groups. The free acid melts at 218°. Ref. (20) gives m.p. 216–218°.

TABLE II

AMINO ACID BENZYL ESTER HYDROBROMIDES

BENZYL ESTER HYDROBROMIDE OF	YIELD, %	M.P., °C	FORMULA	ANALYSIS			
				Nitrogen		Bromide	
				Calc'd	Found	Calc'd	Found
Glycine	92	147	$C_9H_{12}BrNO_2$	5.7	5.9	32.5	32.6
DL-Alanine	91	107	$C_{10}H_{14}BrNO_2$	5.4	5.8	30.8	30.6
β-Alanine[a]	93	57	$C_{10}H_{14}BrNO_2$	5.4	5.5	30.8	31.7
DL-Valine	91	109	$C_{12}H_{18}BrNO_2$	4.9	5.2	27.8	28.1
DL-Phenylalanine[b]	93	200	$C_{16}H_{18}BrNO_2$	4.2	4.1	23.8	24.2
S-Benzyl-L-cysteine[b, c]	92	127	$C_{17}H_{20}BrNO_2S$	3.7	4.0	20.9	21.2
L-Aspartic acid dibenzyl ester[d]	92	117	$C_{18}H_{20}BrNO_4$	3.6	4.0	20.3	21.0
L-Aspartic acid-β-benzyl ester[e]	80	154	$C_{11}H_{14}BrNO_4$	4.6	4.6	26.4	26.9

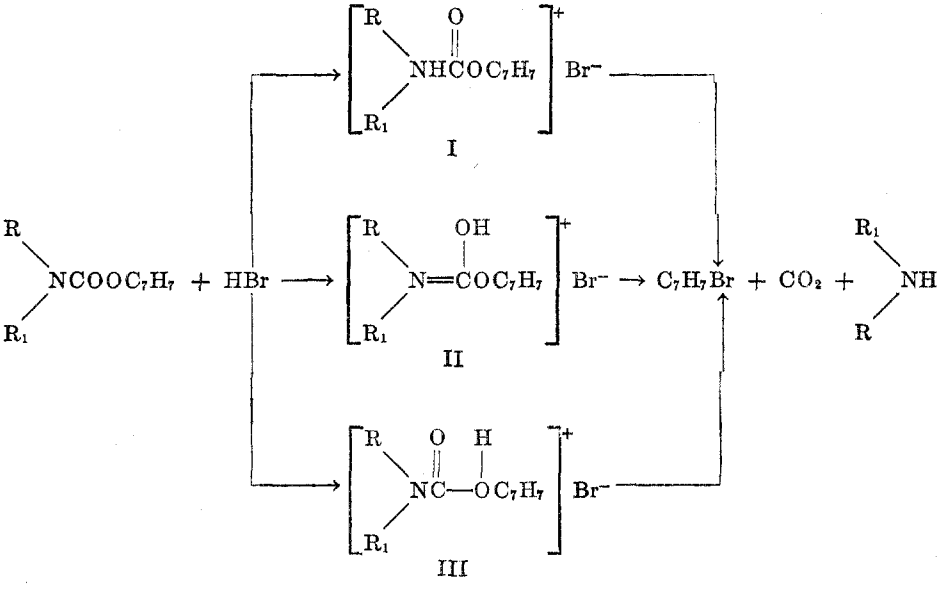
[a] This hydrobromide is somewhat hygroscopic. [b] The hydrobromide was recrystallized from water. [c] $[\alpha]_D^{23}$ −21.7° (*c*, 1.7) in alcohol (96%). *Anal.* Calc'd for $C_{17}H_{20}BrNO_2S$: S, 8.4. Found: S, 8.4. [d] $[\alpha]_D^{23}$ −3.9° (*c*, 9.6) in water. [e] $[\alpha]_D^{23}$ +11.4° (*c*, 7.7) in water.

already observed that cysteine benzyl ester hydriodide can be obtained from N,N′-dicarbobenzoxycystine dibenzyl ester by *preferential* cleavage of the N-

carbobenzoxy groups with phosphonium iodide (which is accompanied, in this case, by reduction of the disulfide bond). Hydrogen bromide in glacial acetic acid acts more smoothly than phosphonium iodide (within 15 minutes at room temperature); the method appears generally applicable and leads to the benzyl ester hydrobromides, which are easily isolated as non-hygroscopic salts (Table II).

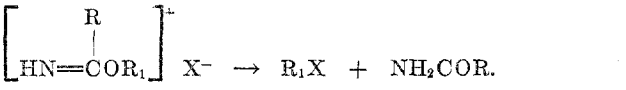
Benzyl esters are also cleaved by hydrogen bromide in glacial acetic acid, but under more stringent conditions than N-carbobenzoxy groups. Benzyl hippurate, prepared by benzoylation of glycine benzyl ester, gave hippuric acid on treatment with hydrogen bromide in glacial acetic acid for 12 hours. N-Benzoyl-DL-phenylalanine benzyl ester gives under the same conditions, N-benzoyl-DL-phenylalanine.

The reaction reported here recalls the interaction of urethans with acetyl bromide (6); a similar mechanism might be operative in both cases. The first step then would be the addition of a proton to a nucleophilic center in the urethan system, followed by the attack of the bromide ion on the benzyl group. According to the following scheme three pathways of the reaction are possible:



When N-methyl carbamate is treated with anhydrous hydrogen bromide a crystalline mass forms immediately upon addition of the reagent; this mass disappears as the reaction progresses, and carbon dioxide is evolved. Towards the end of the reaction, methylamine hydrobromide begins to separate.

Structure II resembles that of the hydrohalides of iminoethers which are known to decompose, on heating, to alkyl halides and amides (7, 8):



Likewise, structure III recalls that of the oxonium salts of ethers which have been suggested as intermediates in the hydrogen halides fission of ethers to alcohols and alkyl halides (9, 10);

$$\left[\begin{array}{c} H \\ | \\ ROR_1 \end{array}\right]^+ X^- \rightarrow R_1X + ROH$$

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TABLE III

BENZYL ESTERS OF N-CARBOBENZOXYAMINO ACIDS

BENZYL ESTERS	YIELD, %	M.P., °C.	FORMULA	ANALYSIS					
				N		C		H	
				Calc'd	Found	Calc'd	Found	Calc'd	Found
N-Carbobenzoxyglycine	93	72	$C_{17}H_{17}NO_4$	4.7	4.4	68.2	68.2	5.7	6.0
N-Carbobenzoxy-DL-alanine	90	59	$C_{18}H_{19}NO_4$	4.5	4.8	69.0	68.7	6.1	6.0
N-Carbobenzoxy-β-alanine[a]	90	22	$C_{18}H_{19}NO_4$	4.5	4.6	69.0	69.0	6.1	5.8
N-Carbobenzoxy-DL-valine	88	45	$C_{20}H_{23}NO_4$	4.1	4.1	70.3	71.0	6.8	7.0
N-Carbobenzoxy-DL-phenylalanine	85	84	$C_{24}H_{23}NO_4$	3.6	3.7	74.0	74.4	5.9	6.3
N-Carbobenzoxy-S-benzyl-L-cysteine[b]	92	66	$C_{25}H_{25}NO_4S$	3.2	3.5	68.9	69.0	5.8	6.2

[a] B.p. 218° (0.3 mm.). [b] $[\alpha]_D^{23}$ −42.1° (*c*, 9.5) in glacial acetic acid. *Anal.* Calc'd for $C_{25}H_{25}NO_4S$: S, 7.4. Found: S, 7.1.

EXPERIMENTAL[1]

Preparation of benzyl carbamates. Benzyl carbamate was synthesized from benzyl chlorocarbonate and ammonia (11); benzyl-N-methyl- and benzyl N, N-dimethyl-carbamate were prepared analogously from methyl- and dimethyl-amine (6). Benzyl N-phenylcarbamate was prepared by trans-esterification of isopropyl N-phenylcarbamate with benzyl alcohol (6), and benzyl N,N-diphenylcarbamate was obtained from diphenylcarbamyl chloride and benzyl alcohol (12).

Benzyl N-phenyl-N-methylcarbamate. Equimolar amounts of methylaniline and benzyl chlorocarbonate were condensed by the Schotten-Baumann method. The product was extracted with ether, and the ethereal solution was washed with dilute hydrochloric acid, dried over sodium sulfate, and concentrated. The oily residue was distilled *in vacuo*, b.p. 171°/1.5 mm.; n_D^{25} 1.5655; d_4^{25} 1.128; yield, 83%.

Anal. Calc'd for $C_{15}H_{15}NO_2$: C, 74.7; H, 6.2; N, 5.8.

Found: C, 74.9; H, 6.4; N, 6.0.

Benzyl N-benzylcarbamate was prepared analogously, using benzylamine. Obtained from petroleum ether, it had m.p. 64°; yield, 87%.

[1] All m.p. s are uncorrected.

Anal. Calc'd for $C_{15}H_{15}NO_2$: C, 74.7; H, 6.2; N, 5.8.
Found: C, 75.1; H, 6.3; N, 5.8.

N-Carbobenzoxy-α-aminoacids. N-Carbobenzoxy-glycine was prepared according to *Organic Syntheses* (13); N-carbobenzoxy-DL-alanine, N-carbobenzoxy-DL-phenylalanine, and N,N'-dicarbobenzoxy-L-lysine according to Bergmann and Zervas (14, 15); N-carbobenzoxy-S-benzylcysteine according to Harington and Mead (5); and poly-carbobenzoxy L-lysine according to Katchalski, Grossfeld, and Frankel (16).

N-Carbobenzoxyamino acid benzyl esters.[2] *General procedure.* A solution of the N-carbobenzoxy amino acid (0.05 mole) in benzene (75 ml.) was refluxed with benzyl alcohol (8 g.) and *p*-toluenesulfonic acid (0.75 g.), the water being removed azeotropically. Refluxing was continued until the theoretical quantity of water had distilled off (1–3 hours). The benzene solution was washed with two portions (25 ml. each) of aqueous potassium bicarbonate solution (5%), dried over sodium sulfate, and concentrated. The residue was recrystallized from petroleum ether. The results are summarized in Table III.

Reaction of benzyl carbamates with dry hydrogen bromide in glacial acetic acid. General procedure. A saturated solution (50 g.) of dry hydrogen bromide in glacial acetic acid (36%) was added to the carbamate (0.05 mole) in a reaction flask protected with a calcium chloride tube. Immediately after the addition of the reagent, carbon dioxide began to develop; in some cases the reaction was markedly exothermic. The mixture was allowed to stand at room temperature, with occasional shaking, for one hour (in most cases, the reaction was completed after one-half hour), and 150-cc. of dry ether was added to precipitate the amine hydrobromide formed. The supernatant liquid was decanted and the solid triturated with ether, filtered, and washed with ether. Finally, the amine hydrobromide was dried *in vacuo* over sulfuric acid and sodium hydroxide. For identification, it was converted into the N-benzoyl derivative or the corresponding thiourea.

In order to isolate the benzyl bromide formed the combined ethereal solutions were concentrated, water was added, and the oil which separated was extracted with ether and distilled *in vacuo*. The results are summarized in Table I.

Reaction of carbobenzoxyamino acids with dry hydrogen bromide in glacial acetic acid. The procedure described above was applied using 10 g. of carbobenzoxyamino acid and 50 g. of a 36% solution of dry hydrogen bromide in glacial acetic acid. The amino acid hydrobromide which formed was repeatedly digested with dry ether, centrifuged, and isolated by decantation. It was finally dried *in vacuo* over sulfuric acid and sodium hydroxide (Table I).

Amino acid benzyl ester hydrobromides. The above general procedure was applied using 0.01 mole of N-carbobenzoxyamino acid benzyl ester and 10 g. of the same reagent. After the evolution of carbon dioxide had ceased (15 minutes), 100 ml. of dry ether was added to precipitate the benzyl ester hydrobromide formed. The ethereal solution was placed in the refrigerator for a few hours and the hydrobromide was filtered off, washed with several portions of anhydrous ether, and dried as above.

Reaction of poly-carbobenzoxy-L-lysine with dry hydrogen bromide in glacial acetic acid. Finely powdered poly-carbobenzoxy-L-lysine (1 g.) (average degree of polymerization $n = 30$) was treated, as described above, with 20 g. of a 36% solution of hydrogen bromide in acetic acid. The poly-L-lysine hydrobromide thus obtained was purified by dissolving it in the minimum of water, filtering, and precipitating with anhydrous alcohol and dry ether. After centrifuging, the supernatant was decanted and the solid white product was dried *in vacuo* over sulfuric acid and sodium hydroxide; yield, 93%.

Anal. Calc'd for $C_6H_{13}BrN_2O$: N (amino), 6.7; Br, 38.3.
Found: N (amino), 7.0; Br, 38.6.

Reaction of benzyl carbamate with dry hydrogen chloride in glacial acetic acid. Through a hot (75°) solution of 7.5 g. of benzyl carbamate in 50 ml. of glacial acetic acid, dry hydrogen

[2] N-Carbobenzoxy-L-aspartic acid dibenzyl ester and N-carbobenzoxy-L-aspartic acid β-benzyl ester were prepared according to Berger and Katchalski (21).

chloride was bubbled for two hours. The ammonium chloride was precipitated in the usual way with dry ether, filtered, and washed with ether (yield 93%); for identification it was converted into *benzamide*, m.p. 128°.

Reaction of poly-carbobenzoxy-L-lysine with dry hydrogen chloride in glacial acetic acid. The above procedure was applied, using 1 g. of polymer dissolved in 50 ml. glacial acetic acid. The poly-L-lysine hydrochloride which separated was isolated in analogy with the poly-L-lysine hydrobromide (see above). Yield of dry product, 94%.

Anal. Calc'd for $C_6H_{13}ClN_2O$: N (amino), 8.5; Cl, 21.6.

Found: N (amino), 8.2; Cl, 21.9.

Reaction of ethyl carbamate with dry hydrogen bromide in glacial acetic acid. Ethyl carbamate (9 g.) was dissolved in 50 ml. of glacial acetic acid and gaseous hydrogen bromide was bubbled for 1½ hours through the hot solution (75°). Ammonium bromide was isolated in the usual way, yield, 92%. Only a very small amount of ethyl bromide could be isolated, most of it being entrained with the stream of hydrogen bromide.

Benzyl hippurate. To an ice-cold solution of glycine benzyl ester hydrobromide (5 g.) in 1 *N* sodium hydroxide (20 ml.), there was added benzoyl chloride (3 g.) and 4 *N* sodium hydroxide (5.5 ml.) in three portions, with shaking. The reaction mixture was extracted with ether, and the ethereal solution was washed with aqueous sodium bicarbonate solution and dilute hydrochloric acid, dried over sodium sulfate, and concentrated. The solid residue was crystallized from benzene-petroleum ether; m.p. 88° [Ref. (17) gives m.p. 85–86°]. Yield, 74%.

Anal. Calc'd for $C_{16}H_{15}NO_3$: N, 5.2. Found: N, 4.8.

Hippuric acid. To benzyl hippurate (4 g.), there was added hydrogen bromide in glacial acetic acid (10 g. of a 36% solution) and the mixture was left overnight at room temperature. Then water (25 ml.) was added and the product was extracted with ethyl acetate. The hippuric acid was isolated by transfer into aqueous bicarbonate, re-extraction (after acidification) into ethyl acetate and evaporation of the solvent; m.p. 189°; yield, 73%. No depression of the m.p. was observed on admixture of an authentic sample of hippuric acid (189°).

N-Benzoyl-DL-phenylalanine benzyl ester, prepared from DL-phenylalanine benzyl ester hydrobromide (6.7 g.) and benzoyl chloride (3 g.), was recrystallized from petroleum ether, m.p. 111°; yield, 70%.

Anal. Calc'd for $C_{23}H_{21}NO_3$: C, 76.9; H, 5.8; N, 3.9.

Found: C, 77.3; H, 5.8; N, 4.0.

N-Benzoyl-DL-phenylalanine was prepared from the benzyl ester (1 g.) and the hydrogen bromide reagent (4 g.). After three hours at room temperature, benzoyl-DL-phenylalanine was isolated, as described above (ether instead of ethyl acetate). The residue was recrystallized from alcohol-water, m.p. 187° (18). Yield, 66%. A mixture melting point with an authentic sample of N-benzoyl-DL-phenylalanine showed no depression.

SUMMARY

Benzyl carbamates, carbobenzoxy-α-amino acids, and poly-carbobenzoxy-L-lysine are cleaved at room temperature by hydrogen bromide in glacial acetic acid to the corresponding amine hydrobromides, benzyl bromide, and carbon dioxide. Dry hydrogen chloride reacts analogously at 75°.

A general method for the preparation of amino acid benzyl esters is proposed. N-Carbobenzoxyamino acids are azeotropically esterified with benzyl alcohol and the carbobenzoxy groups are removed selectively by a short treatment of the products with hydrogen bromide in glacial acetic acid.

The reaction mechanism is discussed.

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