[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, WEIZMANN INSTITUTE OF SCIENCE]

Carbobenzoxy Derivatives of Histidine, Imidazole and Benzimidazole[1]

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1,N-Dicarbobenzoxyhistidine (L and DL) (I) were obtained from benzyl chloroformate and histidine (L and DL, respectively). Compound I was decarbobenzoxylated by amines or alcohols yielding the corresponding N-carbobenzoxy derivatives II. 1,N-Dicarbobenzoxy-L-histidine methyl ester hydrochloride (III) was obtained from I either by direct esterification or *via* the chloride hydrochloride IV. 1-Carbobenzoxyimidazole hydrochloride (V) and 1-carbobenzoxybenzimidazole hydrochloride (VI) were prepared. The 1-carbobenzoxy groups of V and VI were found to be similar in reactivity to those of I and III. 1,3-Dicarbobenzoxy-2-hydroxybenzimidazoline (VII) and the corresponding 2-methoxy and 2-benzyloxy derivatives (VIII and IX) were synthesized, and their reactivity toward sodium methoxide and hydrogen chloride was investigated. Non-aqueous titrations were applied to the investigation of the structure and the reactivity of these compounds (see Table I).

The synthesis of various N-acyl derivatives of histidine has been described.[2] The N-benzoyl,[3,4] N-*p*-nitrobenzoyl[5] and N-carbobenzoxy[6] derivatives were prepared from the amino acid and the respective acyl chlorides in dilute alkali; N-acetylhistidine[7] was synthesized from histidine and acetic anhydride in glacial acetic acid. Although the imino group of imidazole can be acylated, 1,N-di-(2-naphthylsulfonyl)-histidine[8] is the only 1-acyl substituted histidine known. The failure to prepare histidines substituted in the 1-position by acetyl or benzoyl is probably due to the susceptibility of the imidazole acyl bond to alkaline hydrolysis.[2] 1,N-Diacylhistidine ester derivatives, however, are readily obtained from histidine ester and an acyl chloride under anhydrous conditions.[4,9,10] Action of excess acyl chloride on histidine ester in aqueous sodium carbonate causes opening of the imidazole ring which undergoes the well known Bamberger reaction.[11]

Because of the importance of the carbobenzoxy derivatives of amino acids in peptide synthesis,[12] and since little was known concerning the synthesis and properties of carbobenzoxy derivatives of histidine, an investigation of the reaction between L- and DL-histidine and benzyl chloroformate was undertaken.

Dicarbobenzoxy-L-histidine (I) was obtained by coupling L-histidine with two moles of benzyl chloroformate in aqueous solution at 0°, at *p*H 9 to 10, followed by acidification to *p*H 2.4 whereupon it precipitates. Excess mineral acid must be avoided since the product is amphoteric. The crude dicarbobenzoxy derivative is unstable, decomposing at room temperature within several hours to give N-carbobenzoxy-L-histidine (II). Two considerably more stable forms of I, Ia and Ib, were obtained on crystallization from methanol and ethanol, respectively, and were found to contain one molecule of alcohol.

Direct esterification of I with methanolic hydrogen chloride or treatment of I with diazomethane and subsequent acidification with hydrogen chloride led to the methyl ester hydrochloride III. The hydrochloric acid of III was removed by diazomethane to yield the free ester IIIa. Treatment of I with thionyl chloride yielded the corresponding acid chloride hydrochloride IV, which gave III on treatment with methanol. Unlike other N-carbobenzoxyamino acid chlorides, IV failed to cyclize to the respective N-carboxy-α-amino acid anhydride.[13]

Dicarbobenzoxy-L-histidine (I) is readily decarbobenzoxylated by nucleophilic reagents such as sodium methoxide and amines, yielding N-carbobenzoxy-L-histidine (II). With ammonia and aniline, the corresponding benzylurethans formed were also isolated. N-Carbobenzoxy-L-histidine (II) is an amphoteric substance owing to the presence of the acidic α-carboxyl group and the basic imidazole group in the molecule. Its solubility in water, sparing solubility in ethanol and insolubility in acetone, ethyl acetate and benzene suggest a zwitterionic structure.

1,N-Dicarbobenzoxy and N-carbobenzoxy-DL-histidine were prepared analogously to the respective L-histidine derivatives. The melting point and solubility of N-carbobenzoxy-DL-histidine differed considerably from that of N-carbobenzoxy-L-histidine. A comparison of the properties of both forms with those reported by Bergmann and Zervas for N-carbobenzoxy-L-histidine[6] (see Experimental) suggested that the substance isolated by these authors was in fact the racemic form.

For the purpose of comparing the properties of the 1,N-dicarbobenzoxy-histidine with those of other carbobenzoxyimidazole compounds, carbobenzoxyimidazole hydrochloride (V) and carbobenzoxybenzimidazole hydrochloride (VI) were prepared by coupling imidazole and benzimidazole, respectively, with carbobenzoxychloride either in alkaline aqueous medium or under non-aqueous conditions. Both V and VI are decarbobenzoxylated analogously to 1,N-dicarbobenzoxyhistidine by nucleophilic reagents such as amines and sodium methoxide. On heating they split off benzyl chloride and carbon dioxide similarly to 1,N-dicarbobenzoxyhistidine methyl ester hydrochloride (III).

(1) A preliminary report on this work was presented at the 21st Meeting of the Israel Chemical Society; A. Patchornik, *Bull. Res. Counc. of Israel*, **6A**, 10 (1957).

(2) See K. Hofmann, "Imidazole and Its Derivatives," Interscience Publishers, Inc., New York, N. Y., 1953.

(3) H. Pauly, *Ber.*, **43**, 2243 (1910).

(4) O. Gerngross, *Z. physiol. Chem.*, **108**, 50 (1919).

(5) H. Pauly, *ibid.*, **64**, 75 (1910).

(6) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(7) M. Bergmann and L. Zervas, *Biochem. Z.*, **203**, 280 (1928).

(8) H. Pauly, *Z. physiol. Chem.*, **42**, 508 (1904).

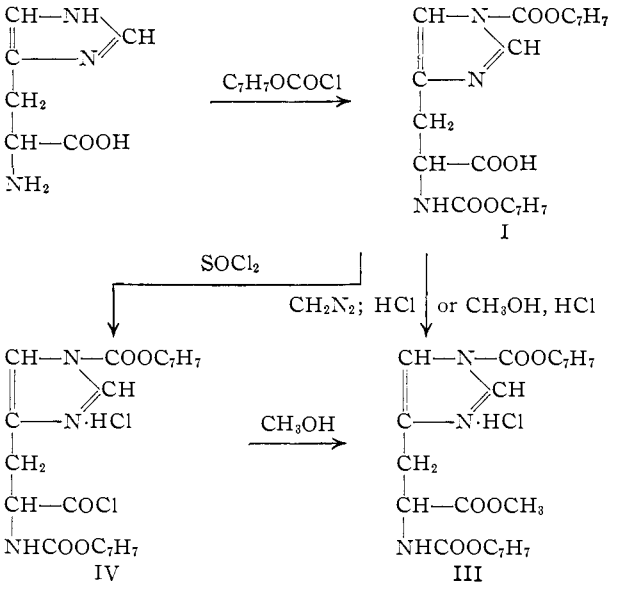
(9) M. Bergmann and L. Zervas, *ibid.*, **175**, 145 (1928).

(10) Th. Wieland, *Ann.*, **580**, 159 (1953).

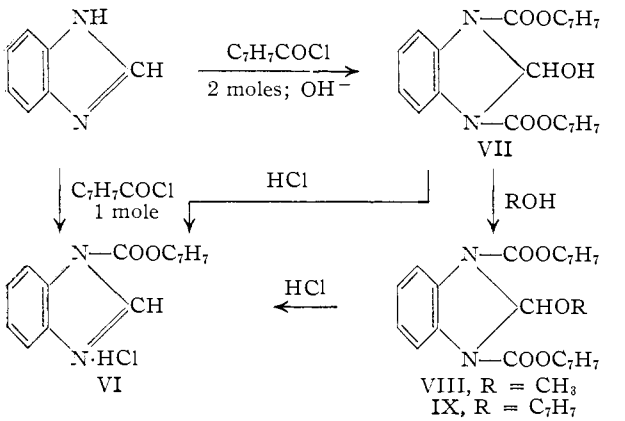
(11) A. Kossel and E. Edlbacher, *Z. physiol. Chem.*, **93**, 396 (1915); E. Bamberger, *Ann.*, **273**, 267 (1893).

(12) H. D. Springall and H. D. Law, *Quart. Revs.*, **10**, 230 (1956).

(13) E. Katchalski, *Advances in Protein Chem.*, **6**, 123 (1951).

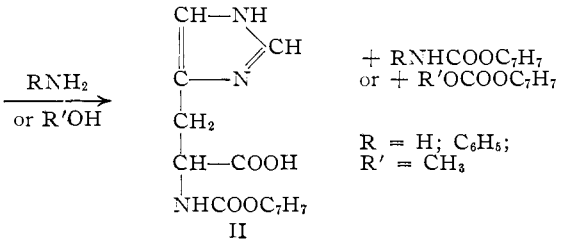


In the case of benzimidazole, 1,3-dicarbobenzoxy derivatives could be prepared. When benzimidazole was coupled with two moles of benzyl chloroformate in the presence of aqueous alkali, 1,3-dicarbobenzoxy-2-hydroxybenzimidazoline (VII)

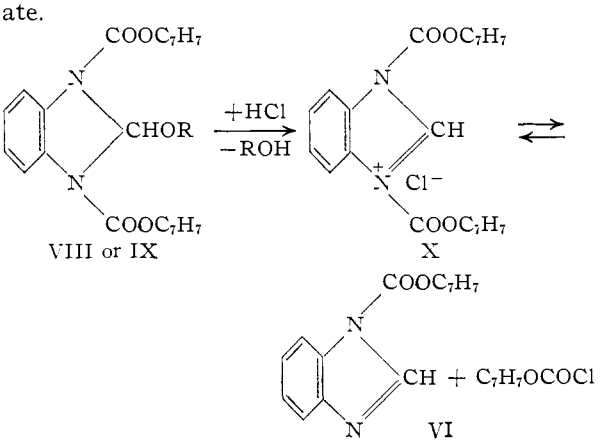


was formed. The pseudobasic hydroxy group of VII is very reactive yielding with alcohols the corresponding 1,3-dicarbobenzoxy-2-alkoxybenzimidazolines. The methoxy and benzyloxy derivatives, to which formulas VIII and IX are ascribed, resemble the corresponding dibenzoyl compounds described by Gerngross.[14] Unlike 1-carbobenzoxyimidazole derivatives, VIII and IX are not decarbobenzoxylated by sodium methoxide in alcohol at room temperature. Anhydrous hydrogen chloride on the other hand causes rapid decarbobenzoxylation with the formation of 1-carbobenzoxybenzimidazole and benzyl chloroformate. The difference in behavior toward nucleophilic reagents between the carbobenzoxy group attached to imidazole and those combined with benzimidazoline is not surprising considering that the pyrrole nitrogen of the former is markedly acidic in character.[2] The sensitivity of the 1,3-dicarbobenzoxy-2-hydroxybenzimidazoline (VII) and its alkoxy derivatives VIII and IX toward hydrogen chloride may be explained

(14) O. Gerngross, *Ber.*, **46**, 1913 (1913).



by an assumed cleavage of the C(2)–O bond by the halogen acid leading to the formation of 1,3-dicarbobenzoxybenzimidazolium chloride (X). Similarly to the labile complexes of tertiary amines with acid chlorides, X decomposes readily into 1-carbobenzoxybenzimidazole and benzyl chloroformate.



For the elucidation of the structure and reactivity of the various imidazole derivatives described above non-aqueous titrations[15] proved of considerable value. The titrimetric properties of imidazole, benzimidazole and histidine and of some of their acyl derivatives are summarized in Table I. The data given demonstrate that the free imidazole groups are considerably more basic than their respective acyl derivatives. Thus while a free imidazole group requires one mole of $HClO_4$ using thymol blue (TB) as indicator (yellow to red) (see no. 1 and 3), its acyl derivatives do not consume $HClO_4$ under these conditions (see no. 7). This behavior explains also the observation that the hydrochlorides of the acyl imidazole derivatives impart a red color to TB (see no. 9). In the presence of crystal violet (CV) in glacial acetic acid, which requires a higher acidity than TB for its color change (violet to green), both the free and the acylated imidazole groups are titratable with one mole of $HClO_4$ (no. 7).

The base-catalyzed alcoholysis of the acylimidazole group discussed previously, is evident from the titrimetric data of these compounds with $HClO_4$ after previous exposure to catalytic quantities of methoxide in the presence of excess methanol (see no. 8, 10 and 12). Thus while benzoylbenzimidazole does not absorb $HClO_4$ when TB is used as indicator, one mole of $HClO_4$ is consumed after exposure of the substance to methanol in the presence of sodium methoxide (no. 9). For III, V, VI and benzoylbenzimidazole HCl (no. 10) one equivalent

(15) J. S. Fritz, "Acid Base Titrations in Nonaqueous Solvents," The G. Fredrick Smith Chemical Co., Columbus, Ohio, 1952.

TABLE I

NON-AQUEOUS TITRATIONS OF IMIDAZOLE DERIVATIVES[a]

No.	Compound	Titrant	Moles per mole compound	Indicator	Color change[b]
1.	Imidazole or benzimidazole	$HClO_4$	1	TB	Y → R
		$HClO_4$	1	CV	V → G
		NaOMe	tr[c]	TB	Y → B
2.	Imidazole·HCl or benzimidazole·HCl	$HClO_4$	tr[c]	TB	Y → R
		$HClO_4$	tr[c]	CV	V → G
		NaOMe	1	TB	Y → B
3.	Histidine, free base	$HClO_4$	2	TB	Y → R
		$HClO_4$	2	CV	Y → G
		NaOMe	1	TB	Y → B
4.	Histidine·HCl	$HClO_4$	1	TB	Y → R
		$HClO_4$	1	CV	V → G
		NaOMe	2	TB	Y → B
5.	N-Carbobenzoxyhistidine (II)	$HClO_4$	1	TB	Y → R
		$HClO_4$	1	CV	V → G
		NaOMe	1	TB	Y → B
6.	II + 1 mole NaOMe	$HClO_4$	2	TB	B → Y → R
7.	1-Benzoylbenzimidazole[d] or 1,N-dicarbobenzoxyhistidine methyl ester (IIIa)	$HClO_4$	tr[c]	TB	Y → R
		$HClO_4$	1	CV	V → G
		NaOMe	tr[c]	TB	Y → B
8.	1-Benzoylbenzimidazole[d] or IIIa + trace NaOMe (MeOH)	$HClO_4$	1	TB	B → Y → R
9.	1,N-Dicarbobenzoxyhistidine methyl ester·HCl (III), 1-carbobenzoxybenzimidazole·HCl (VI), 1-carbobenzoxyimidazole·HCl (V) or 1-benzoylbenzimidazole·HCl	NaOMe	1	TB	R → Y → B
10.	III, V, VI or 1-benzoylbenzimidazole·HCl, + 1 mole NaOMe (MeOH)	$HClO_4$	1	TB	B → Y → R
11.	1,N-Dicarbobenzoxyhistidine (Ia)	$HClO_4$	tr[c]	TB	Y → R
		$HClO_4$	1	CV	V → G
		NaOMe	1	TB	Y → B
12.	Ia + 1 mole NaOMe (MeOH)	$HClO_4$	2	TB	B → Y → R
13.	1,3-Dicarbobenzoxy-2-methoxybenzimidazoline (VIII) or 1,3-dicarbobenzoxy-2-benzyloxybenzimidazoline (IX)	$HClO_4$	tr[c]	TB	Y → R
		$HClO_4$	tr[c]	CV	V → G
		NaOMe	tr[c]	TB	Y → B
14.	VIII, IX, + trace NaOMe (MeOH)	$HClO_4$	tr[c]	TB	Y → R

[a] Samples of 0.1 to 0.2 mmole of the compound were dissolved in 10 ml. of solvent and titrated with 0.1 *N* standard solutions from a 2-ml. microburet. Thymol blue (TB) was used as indicator and dioxane as solvent for titrations with $HClO_4$ in dioxane and NaOMe in benzene–methanol. Crystal violet (CV) served as indicator and glacial acetic acid as solvent for titrations with $HClO_4$ in glacial acetic acid. [b] Y = yellow; R = red; V = violet; G = green. [c] tr = trace (about 0.02 ml.). [d] Prepared according to O. Gerngross, ref. 14.

of sodium methoxide was added to neutralize the hydrochloric acid present, prior to the titration with perchloric acid of the parent imidazole compounds formed as a result of the base-catalyzed alcoholysis. The titration data given in no. 14 show that, as mentioned previously, no base-catalyzed alcoholysis takes place in the case of dicarbobenzoxybenzimidazoline derivatives.

The discussed alcoholysis reaction was also followed spectrophotometrically in the case of 1,N-dicarbobenzoxyhistidine (Ia) (see Experimental).

Experimental

All melting points are uncorrected.

1,N-Dicarbobenzoxy-L-histidine (I).—Benzyl chloroformate (17 ml.) was added in ten portions to an ice-cooled solution of L-histidine hydrochloride hydrate (10.5 g.) in 2 *N* sodium hydroxide (50 ml.). After the addition of each portion of the reagent the mixture was vigorously shaken and its *p*H adjusted to 9–10 by the addition of 2 *N* sodium hydroxide. A total volume of approximately 60 ml. of alkali was consumed and the coupling was completed within 20 minutes. The mixture was then acidified to *p*H 2.4 with cold 4 *N* hydrochloric acid. The white precipitate formed was caused to coagulate by shaking, the supernatant was discarded and the crude product (I) was immediately recrystallized from methanol. The 1,N-dicarbobenzoxy-L-histidine crystallized with one molecule of methanol (Ia); yield 16 g. (70%), m.p. 105–107° dec. when heated quickly, $[\alpha]^{26}D$ +15.3° (*c* 6.3, in acetone–methanol 4:1 by volume).

The crystalline material was air-dried at room temperature before analysis.

Anal. Calcd. for $C_{22}H_{21}N_3O_6 \cdot CH_3OH$: C, 60.6; H, 5.5; N, 9.2; CH_3O, 6.8; neut. equiv., 455. Found: C, 60.5; H, 5.5; N, 9.2; CH_3O, 6.7 (prolonged drying *in vacuo* at room temperature resulted in a marked decrease in methoxyl); neut. equiv., 455 determined by anhydrous titration in methanol with 0.1 *N* sodium methoxide using thymol blue as indicator, or in glacial acetic acid with 0.1 *N* perchloric acid in glacial acetic acid using crystal violet as indicator. No titratable groups could be detected on titration in dioxane with 0.1 *N* perchloric acid in dioxane using thymol blue as indicator. A solution of 1,N-dicarbobenzoxy-L-histidine in methanol shows an absorption maximum at 2380 Å. with a molar absorption coefficient $\epsilon = 3200$.

When the crude 1,N-dicarbobenzoxy-L-histidine was crystallized from ethanol, crystals with one molecule of ethanol, Ib, were obtained.

Anal. Calcd. for $C_{22}H_{21}N_3O_6 \cdot C_2H_5OH$: C, 61.4; H, 5.8; C_2H_5O, 9.6; neut. equiv., 469. Found: C, 62.0; H, 5.7; C_2H_5O, 9.5; neut. equiv., 471 determined as above.

Ia and Ib are soluble in acetone and acetic acid, sparingly soluble in cold ethanol and insoluble in ether and petroleum ether. Methanol and ethanol were the only two solvents found from which crystals of I could be obtained. Thus it could not be crystallized from propanol, butanol, benzene or ethyl acetate.

When the crystalline dicarbobenzoxy-L-histidine preparations containing methanol (Ia) or ethanol (Ib) were kept in closed vessels at room temperature for several weeks decomposition occurred. From the resulting viscous material N-carbobenzoxy-L-histidine could be isolated in a 70–85% yield by trituration with ether and acetone and crystallization of the solid residue from ethanol; m.p. 163°, not depressed by admixture with a sample of N-carbobenzoxy-L-histidine (m.p. 166–167°) prepared as described below. A similar decomposition occurred within several hours when the crystallization of I from alcohol was delayed. Decarbobenzoxylation also occurred when crude preparations of I were heated on a water-bath in ethyl acetate or glacial acetic acid for half an hour. In these cases partial racemization took place (see footnote 16).

1,N-Dicarbobenzoxy-DL-histidine.—1,N-Dicarbobenzoxy-DL-histidine was prepared from DL-histidine analogously to the respective L-compound. The DL-dicarbobenzoxy derivative crystallized with one molecule of methanol from methanol; yield 70%, m.p. 86° dec.

Anal. Calcd. for $C_{22}H_{21}N_3O_6 \cdot CH_3OH$: C, 60.6; H, 5.5; N, 9.2; CH_3O, 6.8; neut. equiv., 455. Found: C, 60.3; H, 5.5; N, 9.5; CH_3O, 6.8; neut. equiv., 456, determined by anhydrous titration with 0.1 *N* sodium methoxide as above.

N-Carbobenzoxy-L-histidine (II).—Alcoholic potassium hydroxide (1.00 *N*, 10.0 ml.) was added to a suspension of Ib (4.69 g.) in ethanol (10 ml.) and the mixture left at room temperature for five minutes. The mixture was neutralized with 1.00 *N* aqueous perchloric acid (10.0 ml.), and the potassium perchlorate formed was filtered off. The filtrate was concentrated *in vacuo* to a volume of about 5 ml., alcohol (20 ml.) and benzene (20 ml.) were added and water removed by azeotropic distillation *in vacuo*. The residue was treated with hot alcohol (15 ml.) and any insoluble potas-

sium perchlorate was filtered off. Crystallization of N-carbobenzoxy-L-histidine in the filtrate started after the addition of acetone (10 ml.). The crystalline product was collected after two days. Yield 2.3 g. (80%), m.p. 164–165° dec.; after recrystallization from ethanol m.p. 166–167° dec.,[16] $[\alpha]^{22}D$ −25.0° (*c* 6.0, in 6 *N* hydrochloric acid).

Anal. Calcd. for $C_{14}H_{15}N_3O_4$: C, 58.1; H, 5.2; N, 14.5; neut. equiv., 289.3. Found: C, 58.0; H, 5.2; N, 14.2; neut. equiv., 290, determined by anhydrous titration in ethanol with 0.1 *N* sodium methoxide or with 0.1 *N* perchloric acid in dioxane using thymol blue as indicator. When II (500 mg.) was hydrolyzed with boiling 6 *N* hydrochloric acid (10 ml.) for 1.5 hours, the hydrolysate gave a specific rotation of $[\alpha]^{25}D$ +13.0° calculated for a quantitative yield of histidine (26.8 mg.).[17]

N-Carbobenzoxy-L-histidine is soluble in alcohol and water and is insoluble in acetone, benzene and ether.

N-Carbobenzoxy-DL-histidine.—N-Carbobenzoxy-DL-histidine was prepared from 1,N-dicarbobenzoxy-DL-histidine analogously to the L-compound; recrystallized from ethanol, m.p. 209–210° dec.

Anal. Calcd. for $C_{14}H_{15}N_3O_4$: C, 58.1; H, 5.2; N, 14.5; neut. equiv., 289. Found: C, 58.1; H, 5.4; N, 14.2; neut. equiv., 290, determined by anhydrous titration with 0.1 *N* sodium methoxide.

N-Carbobenzoxy-DL-histidine is soluble in boiling alcohol and boiling water; it is sparingly soluble in cold water and alcohol and is insoluble in acetone, benzene and ether.

Decarbobenzoxylation of 1,N-Dicarbobenzoxy-L-histidine. a. By Aniline.—1,N-Dicarbobenzoxy-L-histidine (Ia) (0.86 g.) was dissolved in aniline (2 ml.) and the solution was heated on a water-bath for half an hour. N-Carbobenzoxy-L-histidine was precipitated by the addition of acetone (20 ml.) and recrystallized from alcohol–acetone; yield 52% of the theoretical, m.p. 162° dec. After recrystallization from ethanol m.p. 164–165° dec., not depressed by admixture with II prepared as described above; neut. equiv., 290, determined by titration in alcohol with perchloric acid in dioxane using thymol blue as indicator. From the mother liquor N-phenyl benzylcarbamate was isolated in 85% yield, m.p. 75–76°, after recrystallization from ethanol. The m.p. was not depressed on admixture with an authentic sample of N-phenyl benzylcarbamate.[18]

b. **By Ammonia.**—1,N-Dicarbobenzoxy-L-histidine (Ib, 0.5 g.) was dissolved in concentrated ammonia (2 ml.) whereupon crystallization of benzylcarbamate began. The mixture was heated on a water-bath for five minutes to bring the decarbobenzoxylation to completion. Benzylcarbamate (0.132 g., 82%) crystallized out on cooling, m.p. 85–86°, not depressed on admixture with an authentic sample of benzylcarbamate.[19] The mother liquor was brought to dryness *in vacuo* and the residue dissolved in ethanol (3 ml.). N-Carbobenzoxy-L-histidine (m.p. 163–164°) crystallized on standing at room temperature.

c. **By Sodium Methoxide.**—In this case the extent of decarbobenzoxylation was followed spectrophotometrically at 2380 Å. where 1,N-dicarbobenzoxy-L-histidine (Ia) has a molar absorption coefficient of $\epsilon = 3200$, whereas the resulting N-carbobenzoxy-L-histidine and the methyl benzylcarbamate each have molar absorption coefficients of about $\epsilon = 200$. In a 0.014 *M* solution of Ia in methanol, in the presence of two equivalents of sodium methoxide, decarbobenzoxylation at room temperature was complete within a few minutes.

The above quantitative decarbobenzoxylation reaction also could be demonstrated titrimetrically. Thus when a 0.1 *M* solution of Ia in methanol was neutralized with one equivalent of 0.1 *N* sodium methoxide to the blue end-point of thymol blue it consumed two equivalents of 0.1 *N* perchloric acid in dioxane on back titration to the red end-point (see Table I, no. 6).

1,N-Dicarbobenzoxy-L-histidyl Chloride Hydrochloride (IV).—1,N-Dicarbobenzoxy-L-histidine (Ib, 1.0 g.) was dissolved in thionyl chloride (1.0 ml.) at 0° and the solution diluted with ethyl acetate (20 ml.). The crystalline 1,N-dicarbobenzoxy-L-histidyl chloride hydrochloride separated out within half an hour on seeding or scratching. The crystals were washed with ethyl acetate and dried *in vacuo* over phosphorus pentoxide and potassium hydroxide; yield 0.5 g. m.p. 98–100° dec.

Anal. Calcd. for $C_{22}H_{21}O_5N_3Cl_2$: C, 55.2; H, 4.4; N, 8.8; Cl, 14.8; mol. wt., 478.4. Found: C, 55.9; H, 5.0; N, 9.4; Cl, 14.8; equiv. wt., 244 determined by anhydrous titration in aniline with 0.1 *N* sodium methoxide using thymol blue as indicator. This corresponds to a molecular weight of 488 assuming the presence of two acidic groups per molecule. The presence of an acid chloride group was demonstrated by titration with sodium methoxide as above after hydrolysis in moist pyridine at room temperature. Three equivalents of sodium methoxide were consumed per mole of the starting chloride hydrochloride IV.

IV is soluble in chloroform and is insoluble in ethyl acetate and benzene.

1,N-Dicarbobenzoxy-L-histidine Methyl Ester Hydrochloride (III).—Crude 1,N-dicarbobenzoxy-L-histidine (I) obtained from 10.5 g. of L-histidine hydrochloride hydrate, was dissolved in ethyl acetate (50 ml.), and the solution was washed with water and dried over sodium sulfate. To the dried solution an excess of diazomethane in ether was added. When evolution of nitrogen had ceased, dry hydrogen chloride was passed through the solution, and the crystals formed were collected and dried *in vacuo* over potassium hydroxide; yield 17.1 g. (72%), m.p. 113° dec.; after recrystallization from acetone m.p. 117–118° dec.

Anal. Calcd. for $C_{23}H_{24}O_6N_3Cl$: C, 58.3; H, 5.1; Cl, 7.5; CH_3O, 6.5; neut. equiv., 474. Found: C, 58.5; H, 5.4; Cl, 7.6; CH_3O, 6.4; neut. equiv., 473, determined by anhydrous titration in ethanol with 0.1 *N* sodium methoxide to the blue end-point using thymol blue as indicator. The ester hydrochloride gives a red color with the indicator which turns yellow when about half of the titrant has been added. This indicates that partial decarbobenzoxylation had occurred. III also can be titrated with 0.1 *N* tributylamine in dioxane to the yellow end-point of thymol blue, consuming one mole of base. In this case no decarbobenzoxylation occurs.

Alternatively III was prepared by direct esterification of 1,N-dicarbobenzoxy-L-histidine (Ia). Ia (1.0 g.) was suspended in anhydrous methanol (20 ml.) and the mixture saturated with dry hydrogen chloride at 0°. After 12 hours at room temperature the clear solution was brought to dryness *in vacuo* at a water-bath temperature of 35°, and the crystalline residue was triturated with anhydrous ether, collected and dried *in vacuo* over potassium hydroxide; yield 0.80 g. (77%), m.p. 115–117° dec.; neut. equiv., 471, determined as above.

III also was obtained from the chloride hydrochloride IV (0.1 g.) by treatment with methanol (0.5 ml.) containing one drop of pyridine. The resulting solution was diluted with ether (10 ml.) and saturated with dry hydrogen chloride. The methyl ester hydrochloride III which separated out was recrystallized from acetone–ether; m.p. 118° dec.; neut. equiv., 470, determined as above.

1,N-Dicarbobenzoxy-L-histidine methyl ester (IIIa) was prepared by treatment of an alcoholic solution of III with an excess of diazomethane in ether. The reaction mixture was brought to dryness and the oily residue left to crystallize at room temperature; m.p. 45–46°.

Anal. Calcd. for $C_{23}H_{23}N_3O_6$: C, 63.1; H, 5.3; N, 9.6; neut. equiv., 437. Found: C, 63.2; H, 5.4; N, 9.7; neut. equiv., 440, determined by titration in glacial acetic acid with 0.1 *N* perchloric acid using crystal violet as indicator. No sodium methoxide or perchloric acid was consumed when thymol blue was used as indicator (see Table I, no. 7).

(16) Bergmann and Zervas (ref. 6) give m.p. 210° for N-carbobenzoxy-L-histidine but do not report an optical rotation. In the preparation of this compound the authors used a procedure similar to that employed by us for the preparation of I, followed by heating of the intermediate in acetic acid. When a solution of I in glacial acetic acid was evaporated to dryness and the residue crystallized from alcohol we obtained a small yield (15%) of material with m.p. 210° identical with N-carbobenzoxy-DL-histidine. It seems therefore that some racemization occurs during the decarbobenzoxylation of I in acetic acid and that the DL-compound only was isolated by the above authors owing to its low solubility in alcohol.

(17) M. S. Dunn, E. H. Frieden, M. P. Stoddard and H. V. Brown, *J. Biol. Chem.*, **144**, 487 (1942), give for L-histidine $[\alpha]^{25}D$ +13.3° (*c* 4.05, in 6.08 *N* hydrochloric acid).

(18) H. v. Soden and W. Rojahn, *Ber.*, **34**, 2809 (1901).

(19) H. E. Carter, R. L. Frank and H. W. Johnston, *Org. Syntheses*, **23**, 13 (1946).

The free ester is soluble in the usual organic solvents and is insoluble in petroleum ether and water.

1-Carbobenzoxyimidazole Hydrochloride (V). a. In Non-aqueous Medium.—Benzyl chloroformate (0.75 ml.) was added to an ice-cold solution of imidazole (0.68 g.) in anhydrous benzene (10 ml.) and the mixture was left at room temperature overnight. Imidazole hydrochloride, which had separated out, was removed by filtration, and the filtrate was saturated with dry hydrogen chloride at 0°. The crystalline 1-carbobenzoxyimidazole hydrochloride formed was collected, washed with ether and dried *in vacuo* over potassium hydroxide and phosphorus pentoxide; yield 1.05 g. (88%), m.p. 74–76° dec.

Anal. Calcd. for $C_{11}H_{11}O_2N_2Cl$: C, 55.3; H, 4.65; N, 11.7; Cl, 14.9; neut. equiv., 238.7. Found: C, 54.3; H, 4.8; N, 11.9; Cl, 14.9; neut. equiv., 242, determined by titration with 0.1 *N* sodium methoxide in ethanol using thymol blue as indicator; neut. equiv., 246, determined by titration with 0.1 *N* tributylamine in dioxane using thymol blue as indicator. The change in color at the end-point is from red to yellow.

V is soluble in hot alcohol, hot acetone and is insoluble in ether. On heating or prolonged standing at room temperature V decomposes yielding benzyl chloride and carbon dioxide. Similarly to the 1,N-dicarbobenzoxy-L-histidine (I), V is decarbobenzoxylated by sodium methoxide. Thus imidazole could be isolated as the picrate (67 mg., m.p. 207–208°) from a reaction mixture of V (72 mg.) in methanol (1 ml.) and 0.1 *N* methanolic sodium methoxide (3.0 ml.).

b. **In Aqueous Medium.**—Imidazole (0.68 g.) dissolved in water (10 ml.) was coupled at 5° with benzyl chloroformate (1.5 ml.) in benzene (10 ml.) at *p*H 9–10. Approximately 10 ml. of 1 *N* sodium hydroxide was consumed during the course of the reaction, which was completed within 20 minutes. The benzene layer was separated, and the aqueous layer extracted twice with benzene (40 ml.). The combined benzene solutions were dried over anhydrous sodium sulfate and saturated at 0° with hydrogen chloride. The oil which separated was dissolved in acetone and crystals were obtained by the addition of ether (50 ml.); yield 0.3 g., m.p. 74–75° dec., not depressed on admixture with a sample of V prepared as described in the preceding paragraph.

1-Carbobenzoxybenzimidazole Hydrochloride (VI). a. In Non-aqueous Medium.—Benzyl chloroformate (1.6 ml.) was added to a suspension of finely powdered benzimidazole (2.36 g.) in dry benzene (50 ml.). The mixture was shaken for one hour and the resulting benzimidazole hydrochloride was filtered off. The benzene solution was diluted with dry ether (50 ml.) and saturated with dry hydrogen chloride at 0°. The crystalline 1-carbobenzoxybenzimidazole hydrochloride (VI), was collected, washed with ether and dried *in vacuo* over potassium hydroxide and phosphorus pentoxide; yield 2.4 g. (83%), m.p. 99–100° dec.

Anal. Calcd. for $C_{15}H_{13}O_2N_2Cl$: C, 62.4; H, 4.5; N, 9.7; Cl, 12.3; neut. equiv., 289. Found: C, 62.2; H, 4.2; N, 9.2; Cl, 12.2; neut. equiv., 289, determined by titration with 0.1 *N* sodium methoxide in ethanol using thymol blue as indicator.

VI is soluble in ethanol and is insoluble in acetone and ether.

b. **In Aqueous Medium.**—Finely powdered benzimidazole (1.18 g.), suspended in water (20 ml.), was coupled at 0° with benzyl chloroformate (1.6 ml.) in the presence of 1 *N* sodium hydroxide (10 ml.) and benzene (20 ml.). The benzene layer was separated and the aqueous solution extracted with benzene (15 ml.). The combined benzene solutions were dried over sodium sulfate, saturated with dry hydrogen chloride and left at room temperature overnight. The crystals of 1-carbobenzoxybenzimidazole hydrochloride which had separated out were collected, washed with ether and dried *in vacuo* over potassium hydroxide and phosphorus pentoxide; yield 2.36 g. (82%), m.p. 97–99° dec.; neut. equiv., 287, determined as above.

1,3-Dicarbobenzoxy-2-hydroxybenzimidazoline (VII).—Finely powdered benzimidazole (2.36 g.) was suspended in water (60 ml.) and coupled at 0°, at *p*H 8, with benzyl chloroformate (6.4 ml.) dissolved in benzene (60 ml.). The *p*H was maintained during the course of the reaction (1 hr.) by the addition of 1.0 *N* sodium hydroxide (approximately 35 ml. of base were consumed). The benzene layer was separated, and the aqueous solution extracted with benzene (40 ml.). The combined benzene solutions were dried over sodium sulfate and concentrated *in vacuo* to a volume of 10 ml. Petroleum ether was added (50 ml.) precipitating an oil which solidified on trituration with petroleum ether; yield 5.5 g. (68%), m.p. 115–117°.

Anal. Calcd. for $C_{23}H_{20}O_5N_2$: C, 68.3; H, 5.0; N, 6.9; active H, 0.25. Found: C, 68.6; H, 4.9; N, 7.2; active H, 0.28, determined by the Zerewitinoff method. A strong absorption band at 3.0 μ due to the hydroxyl group was observed in the infrared spectrum of the substance in chloroform.

1,3-Dicarbobenzoxy-2-methoxybenzimidazoline (VIII).—Methanol (100 ml.) was added to a concentrated benzene solution of VII obtained from benzimidazole (2.36 g.), and the mixture was refluxed for five minutes and allowed to cool to room temperature. Crystallization was complete within two hours. The product was filtered, washed with cold methanol and dried *in vacuo* over sulfuric acid; yield 6.1 g. (73%), m.p. 125–129°. It was recrystallized from benzene before analysis.

Anal. Calcd. for $C_{24}H_{22}O_5N_2$: C, 68.9; H, 5.3; N, 6.7; CH_3O, 7.4. Found: C, 68.8; H, 5.2; N, 6.6; CH_3O, 7.6.

When a methanolic solution of VIII containing 0.1 equivalent of sodium methoxide was boiled for one minute, the intact starting material could be recovered in almost quantitative yield.

1,3-Dicarbobenzoxy-2-benzyloxybenzimidazoline (IX) was prepared analogously to the corresponding methoxy derivative VIII using benzyl alcohol (30 ml.) instead of methanol. The benzyl alcohol solution was heated for five minutes on the water-bath whereupon the product crystallized. It was collected, washed with methanol and dried *in vacuo* over sulfuric acid; yield 75%, m.p. 135–139°; after recrystallization from benzene m.p. 142–145°.

Anal. Calcd. for $C_{30}H_{26}O_5N_2$: C, 72.8; H, 5.3; N, 5.7. Found: C, 73.0; H, 4.9; N, 5.7.

Decarbobenzoxylation of VII, VIII and IX by Anhydrous Hydrogen Chloride.—A solution of VIII (270 mg.) in a mixture of benzene (3 ml.) and ether (2 ml.) was saturated with hydrogen chloride at 0°. The crystals of 1-carbobenzoxybenzimidazole hydrochloride which formed overnight were collected, washed with ether and dried *in vacuo* over phosphorus pentoxide and potassium hydroxide; yield quantitative, m.p. 101–103° dec., not depressed on admixture with an authentic sample of VI; neut. equiv., 289.

To the filtrate, which had a distinct odor of benzyl chloroformate, 25% aqueous ammonia (1 ml.) was added and the mixture was brought to dryness *in vacuo*. Crystallization of the residue from water yielded benzyl carbamate (60%); m.p. 84–86°, not depressed on admixture with an authentic sample of benzyl carbamate, m.p. 86°.[19]

Similar yields of VI and benzyl chloroformate were obtained from VII or IX when treated as above.

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