give 10.3 g. (98.2%) of benzyltriphenyllead, m.p. 91–93°. A mixed melting point with an authentic sample[5] was not depressed.

In a run in which all the prepared triphenylleadlithium was added to benzyl chloride, the benzyl derivative was obtained in a 78% yield.

Carbonation of Triphenylleadlithium.—The second portion of the above triphenylleadlithium solution was poured onto a slurry of Dry Ice and ether. After acid hydrolysis, the layers were separated and filtered to give 3.9 g. of a solid which did not melt below 360°. The organic layer was extracted with dilute sodium hydroxide. Acidification of the basic extracts gave no products. The organic layer was dried over magnesium sulfate and the solvent was evaporated. The residue was treated with petroleum ether (b.p. 60–70°) to afford 2.95 g. (16.8%) of hexaphenyldilead. Recrystallization from chloroform gave 2.7 g. (15.4%) of the pure dilead compound which turned black at 153° and completely melted at 220°. There was no change in these properties when admixed with an authentic sample, which also showed these melting characteristics.

Stability of Triphenyltinlithium.—A tetrahydrofuran (THF) solution containing triphenyltinlithium was prepared from 11.5 g. (0.03 mole) of triphenyltin chloride and 0.7 g. (0.1 g.-atom) of cut lithium wire in 75 ml. of THF. After stirring the reaction mixture 3 hr. at room temperature, the excess lithium was removed by pouring the suspension through a glass-wool plug into a second flask. The solution was then refluxed 8 hr. After cooling, the solid was removed by filtration under nitrogen and recrystallized from petroleum ether (b.p. 60–70°)–benzene mixture to give 2.5 g. (19.5%) of tetraphenyltin, m.p. and mixed m.p. 229–230°.

The filtrate was added dropwise to 3.4 g. (0.03 mole) of benzyl chloride in 20 ml. of THF. The mixture was refluxed and hydrolyzed. The usual work-up afforded no benzyltriphenyltin.

In another test of the stability of the tin-lithium compound, the triphenyltinlithium solution was shaken in a sealed tube under nitrogen for 1 week at room temperature, and then was allowed to stand 8 hr. The solid that had formed was filtered and identified as tetraphenyltin (18.2%). The filtrate was added to benzyl chloride and treated as described above. A mixture of hexaphenylditin and tetraphenyltin were the only products isolated.

N(Im)-Carbobenzoxyhistidine Derivatives as Intermediates for the Synthesis of Histidine Peptides[1]

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Received March 22, 1962

When the esters of N(α),N(Im)-dicarbobenzoxy-L-histidine or -L-histidyl peptides are treated with hydrogen bromide in glacial acetic acid or in dioxane, the action of the acid reagent leads to selective decarbobenzoxylation at the α-position under the ordinary conditions. The resulting N(Im)-carbobenzoxy-L-histidine derivatives can be smoothly coupled with the carboxyl functions by the carbodiimide method to give peptide bonds at the α-position of histidine. The N(Im)-carbobenzoxy group can be easily removed by alkaline hydrolysis or catalytic hydrogenation. By this procedure a variety of histidine peptides, some of which have the sequences occurring in insulin, corticotropin, and hypertensin, have been synthesized with excellent yields without any difficulties.

Histidine occurs widely in biologically active proteins and peptides, such as ribonuclease, insulin, corticotropin (ACTH), melanocyte-stimulating hormone (MSH), hypertensin, and so on. Since it has recently been suggested that in some instances the histidine residue occupies the site of biological activity,[2] development of methods for the synthesis of histidine peptides is very desirable for biochemical studies on proteins and peptides.

In recent years, Akabori, *et al.*,[3,4] have reported that N(α),N(Im)-dicarbobenzoxy-L-histidine[4,5] is an excellent starting material in the synthesis of histidyl peptides. Thus, the peptide containing a histidine residue at the amino end of the molecule can be obtained in high yield without any difficulties. On the other hand, it is rather troublesome to prepare the peptide containing histidine at the carboxyl end or in the middle of the molecule. This is mainly due to the fact that there has been lacking a simple and satisfactory method to protect selectively the basic imidazole group of histidine. Although in this respect N(Im)-benzyl-L-histidine[6] and its derivative[7] are valuable intermediates,[7,8] they are not easily accessible enough to be used for practical purposes. Of late, Zervas, *et al.*,[9] have obtained N(Im)-trityl-L-histidine methyl ester hydrochloride by heating a methanolic solution of dityrityl-L-histidine methyl ester hydrochloride. This compound may, however, be useless because of its labile nature.

It has now been found that the carbobenzoxy group linked at the imidazole nitrogen of histidine is unexpectedly resistant to treatment with hydrogen bromide in glacial acetic acid or in dioxane. Thus treatment of an N(α),N(Im)-dicarbobenz-

(1) This investigation was presented in part at the Vth International Congress of Biochemistry, Moscow, August, 1961, and at the Japanese Symposium on Protein Structure, Kanazawa, November, 1961. K. Inouye and H. Otsuka, *J. Org. Chem.*, **26**, 2613 (1961).

(2) For example: H. G. Gundlach, W. H. Stein, and S. Moor, *J. Biol. Chem.*, **234**, 1754 (1959).

(3) S. Akabori, K. Okawa, and F. Sakiyama, *Nature*, **181**, 772 (1958).

(4) F. Sakiyama, K. Okawa, T. Yamakawa, and S. Akabori, *Bull. Chem. Soc. Japan*, **31**, 926 (1958).

(5) A. Patchornik, A. Berger, and E. Katchalski, *J. Am. Chem. Soc.*, **79**, 6416 (1957).

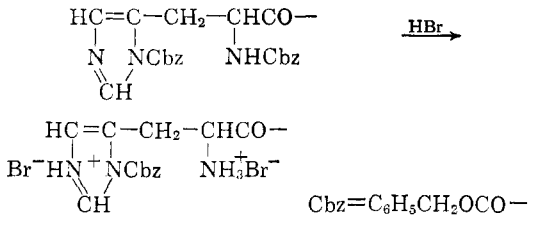
(6) V. du Vigneaud and O. K. Behlens, *J. Biol. Chem.*, **117**, 27 (1937).

(7) A. H. Cook, I. Heilbron, and A. P. Mahanderan, *J. Chem. Soc.*, 1061 (1949); B. G. Overell and V. Petrow, *ibid.*, 232, (1955).

(8) For example: D. Theodoropoulos, *J. Org. Chem.*, **21**, 1550 (1956); ref. 18.

(9) G. C. Stelakatos, D. M. Theodoropoulos, and L. Zervas, *J. Am. Chem. Soc.*, **81**, 2884 (1959).

oxy-L-histidyl compound with the acid reagent gave the corresponding N(Im)-carbobenzoxy derivative as the dihydrobromide as follows:



In the present paper the preparation of various N(Im)-carbobenzoxy-L-histidine derivatives and their applications to the synthesis of histidine peptides will be described.

N(α),N(Im)-Dicarbobenzoxy-L-histidine methyl ester hydrochloride (II) was prepared by esterification of dicarbobenzoxy-L-histidine (I) with thionyl chloride in methanol, and N(α),N(Im)-dicarbobenzoxy-L-histidyl peptide esters (III to V) were obtained by the Sheehan's carbodiimide method.[10] Treatment of these N(α),N(Im)-dicarbobenzoxy-L-histidine and -L-histidyl peptide esters with 30 to 40% (w./w.) hydrogen bromide in glacial acetic acid or in dioxane gave the corresponding monocarbobenzoxy derivatives as the dihydrobromides in almost quantitative yields. The reaction, accompanied by the evolution of carbon dioxide, was completed within five to fifteen minutes at room temperature. In the case of N(α),N(Im)-dicarbobenzoxy-L-histidyl-L-phenylalanine methyl (III) or benzyl ester (IV), the reaction had no sooner stopped than the product separated in crystalline state from the medium.

The decarbobenzoxylated derivatives give positive ninhydrin tests and reacted rather slowly with the Pauly reagent. This apparent reactivity to the Pauly reagent is presumed to be due to a secondary reaction; splitting of the N(Im)-carbobenzoxy group with the alkaline reagent.

The ultraviolet and infrared absorption analyses further confirmed this character of the reaction, that it involved a selective cleavage of the N(α)-carbobenzoxy bonding. Compound III, for example, shows a maximum absorption at 236 mμ with a molar absorption coefficient of ε 3600, which decreases to about 400 within a few minutes by treatment with sodium methoxide in nonaqueous medium. Katchalski, *et al.*,[5] have reported a similar phenomenon in regard to N(α),N(Im)-dicarbobenzoxy-L-histidine (Ia) and have shown that it occurred as a result of cleavage of the N(Im)-carbobenzoxy bonding which is sensitive to nucleophilic reagents such as sodium methoxide and amines. Fig. 1 shows that the nature of the compound derived from III by the action of the acid reagent is almost the same as that observed in the preceding dicarbobenzoxy compound.

(10) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

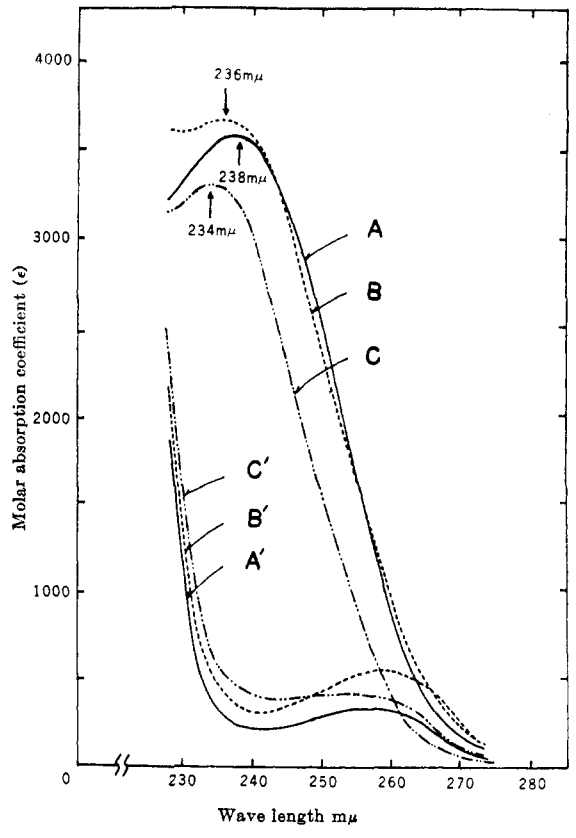


Fig. 1.—Ultraviolet absorption spectra: A, N(α),N(Im)-dicarbobenzoxy-L-histidine (I); B, N(α),N(Im)-dicarbobenzoxy-L-histidyl-L-phenylalanine methyl ester (III); C, N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine methyl ester dihydrobromide (VIII). A′, B′, and C′ are those after treatment of A, B, and C with CH_3ONa, respectively. All in absolute methanol.

Infrared absorption spectra of compound II and its decarbobenzoxylated derivative are shown in Fig. 2. The bands at 1695 cm.$^{-1}$ and 1783 cm.$^{-1}$ in the former may correspond to N(α) - acyl —CO—NH— and N(Im)-acyl —CO—N< groups, respectively. In the latter compound the band at 1800 cm.$^{-1}$ may correspond to the N(Im)-acyl bond, whereas the band equivalent to 1695 cm.$^{-1}$ is entirely missing.

These observations have led to the conclusion that decarbobenzoxylation takes place only at the α-position of histidine and the N(Im)-carbobenzoxy group is still present unattacked with the action of the hydrogen bromide reagent under ordinary conditions.

The N(Im)-carbobenzoxy-L-histidine derivatives are stable in an acid solution, but are rather susceptible to alkaline hydrolysis and aminolysis. However, the ester dihydrobromide of N(Im)-carbobenzoxy-L-histidine or -L-histidyl peptide can be safely converted into the corresponding free ester by dilute aqueous ammonia or sodium bicarbonate in a methylene chloride solution at 0°. The resulting free ester in methylene chloride is coupled with

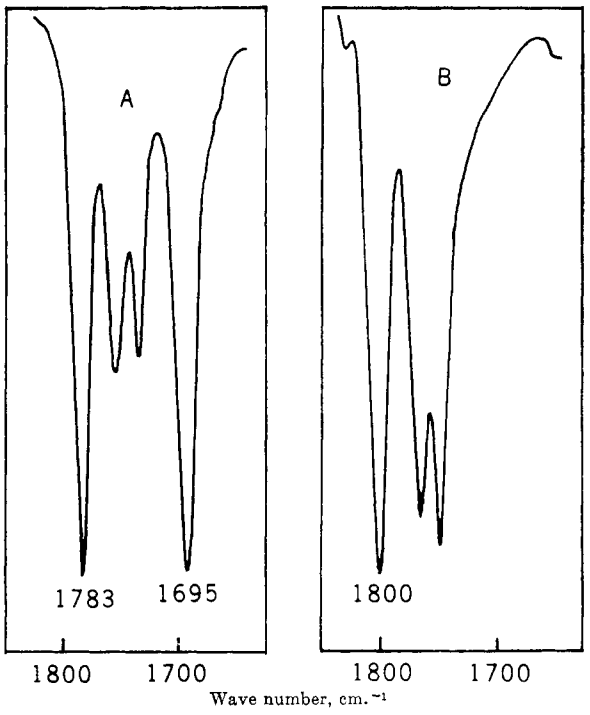


Fig. 2.—Comparison of infrared absorption spectra between A, N(α),N(Im)-dicarbobenzoxy-L-histidine methyl ester hydrochloride (II), and B, N(Im)-carbobenzoxy-L-histidine methyl ester dihydrobromide (VI).

the appropriate carboxyl function by the carbodiimide method, affording a peptide derivative which contains a histidine residue at the carboxyl end or in the middle of the molecule.

By this procedure the various peptide derivatives with the N(Im)-carbobenzoxy-L-histidine residue were synthesized in 80 to 90% yields; for example, carbobenzoxyglycyl - N(Im) - carbobenzoxy - L-histidine methyl ester (X), carbobenzoxyglycyl-N(Im) - carbobenzoxy - L - histidyl - L - leucine methyl ester (XI), carbobenzoxyglycyl-N(Im)-carbobenzoxy - L - histidyl - L - phenylalanine methyl ester (XII), and N(α),N(Im)-dicarbobenzoxy-L-histidyl-N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine benzyl ester (XIII). Furthermore, this procedure was applied to the synthesis of some histidine peptides occurring in biologically active substances. When formyl-γ-methyl-L-glutamic acid was coupled with N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine benzyl ester (IXa), formyl-γ-methyl - L - glutamyl - N(Im) - carbobenzoxy - L-histidyl-L-phenylalanine benzyl ester (XIV) was obtained in a 87% yield and, similarly, carbobenzoxy - γ - *t* - butyl - L - glutamyl - N(Im) - carbobenzoxy - L - histidyl - L - phenylalanine methyl ester (XV) was synthesized from carbobenzoxy-γ-*t*-butyl-L-glutamic acid and N(Im)-carbobenzoxy-L - histidyl - L - phenylalanine methyl ester (VIII) in an exceedingly good yield of more than 90%. Both tripeptide derivatives have the sequence appearing in the positions 5 to 7 of corticotropin[11] and α-MSH[12] molecules. Condensation of carbobenzoxy-L-prolyl-L-phenylalanine with N-(Im) - carbobenzoxy - L - histidyl - L - leucine methyl ester (VII) afforded a tetrapeptide, carbobenzoxy - L - prolyl - L - phenylalanyl - N(Im)-carbobenzoxy - L - histidyl - L - leucine methyl ester (XVIII), the sequence occurring in hypertensin-I.[13] In a similar fashion, the tri-(positions 9 to 11) and tetra-(positions 8 to 11) peptide sequences in the B-chain of insulin[14] were synthesized by coupling of compound VII with carbobenzoxy-O-benzyl-L-serine and formylglycyl-O-benzyl-L-serine, respectively.

These peptide derivatives are negative to both ninhydrin and the Pauly reagent. The susceptibility to alkaline hydrolysis of the N(Im)-carbobenzoxy bonding in these N(α)-substituted compounds and compound I seems to be not so remarkable as compared with that of the N(α)-free compounds, which are more or less reactive to the Pauly reagent. Each of these peptide derivatives has a maximum absorption at 236 mμ with a molar absorption coefficient of about 3600 to 3900 for an N(Im)-carbobenzoxy group, and as shown in Fig. 3, this characteristic absorption spectrum decreases instantly with the treatment of sodium methoxide. These features are the same as those found in the N(α),N(Im) - dicarbobenzoxy - L - histidine derivatives (Fig. 1). Infrared spectrum of the compound XIV, as an example, is also shown in Fig. 4 in comparison with that of formyl-γ-methyl-L-glutamyl-L-histidyl-L-phenylalanine benzyl ester.[15] It can be confirmed from Fig. 4 that the band at 1764 cm.$^{-1}$, which is missing in the latter compound, corresponds to the N(Im)-acyl bond, even though there is a small degree of difference from Otting's observation that the characteristic absorptions of several acylimidazoles appear in the region of 1744–1748 cm.$^{-1}$.[16] In addition, the band at 1019 cm^{-1}, which is also missing in the latter compound, might be characteristic to the N(Im)-carbobenzoxy group, since all of the N(Im)-carbobenzoxy compounds mentioned above have this sharp band in the region of 1010–1020 cm.$^{-1}$.

Lengthening at the amino end of the peptide chain containing a histidine residue, of which the imidazole function is blocked with a carbobenzoxy group, may be effectively carried out by repeating the above-mentioned procedure. In this case, however, acid susceptible groups, such as carbobenzoxy and *t*-butyloxycarbonyl groups, have to be

(11) P. H. Bell, *J. Am. Chem. Soc.*, **76**, 5565 (1954).

(12) J. I. Harris and A. B. Lerner, *Nature*, **179**, 1346 (1957); J. I. Harris, *Biochem. J.*, **71**, 451 (1959).

(13) D. F. Elliot and W. S. Peart, *Nature*, **177**, 527 (1956).

(14) A. P. Ryle, F. Sanger, L. F. Smith, and R. Kitai, *Biochem. J.*, **60**, 541 (1955).

(15) This compound was synthesized by condensation of formyl-γ-methyl-L-glutamic acid[19] with L-histidyl-L-phenylalanine benzyl ester by the carbodiimide method; yield 46.2%, m.p. 176–177° dec. *Anal.* Calcd. for $C_{29}H_{33}N_5O_7$: C, 61.9; H, 5.90; N, 12.40. Found: C, 62.26; H, 6.46; N, 12.08. N,N′-Dicyclohexylurea is contained in a slight amount.

(16) W. Otting, *Ber.*, **89**, 1940 (1956).

used for protecting the terminal amino function. In a preliminary experiment, compound XII could be converted into glycyl-N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine methyl ester dihydrobromide (XIX), having a molar absorption coefficient of 3640 at 234 mμ, by the action of the hydrogen bromide reagent.

Since the N(Im)-carbobenzoxy group is immediately split by alkaline hydrolysis,[4] saponification of the peptide ester accompanies a cleavage of the N(Im)-acyl bonding. Compounds XII and XV were converted with two equivalent amounts of alkali into carbobenzoxyglycyl-L-histidyl-L-phenylalanine (XX) and carbobenzoxy-γ-*t*-butyl-L-glutamyl - L - histidyl - L - phenylalanine (XXII), respectively. In addition the N(Im)-carbobenzoxy group is removed by catalytic hydrogenation.[17] Therefore formyl-γ-methyl-L-glutamyl-L-histidyl-L-phenylalanine (XXI) could be obtained from the preceding benzyl ester (XIV) by the catalytic reduction.

The ionic nature or basicity of the imidazole nucleus blocked with the acyl group is considerably lower than that of the free imidazole. Consequently, in the formation of the peptide bond, the use of an N(Im)-acylhistidine in place of histidine should be very effective in eliminating the unwanted side reactions which might be caused by the basicity of the free imidazole function. As a matter of fact, it has been demonstrated that the N(Im)-carbobenzoxy-L-histidine derivatives are of considerable utility as reagents for introducing histidine into a peptide chain to avoid side reactions. Furthermore the N(Im)-carbobenzoxy-L-histidine peptide derivatives described above have moderate solubilities in common organic solvents, such as alcohols, ethyl acetate, and chloroform, and in most cases they can be crystallized with ease in high purities. These properties are very advantageous in carrying out the reactions and for purification of the products.

In preparation of the N(Im)-carbobenzoxyhistidine derivatives, there is some difficulty when the acid treatment is attempted to a compound which has a hydroxyl or a carboxyl function in its molecule. In the case of N(α),N(Im)-dicarbobenzoxy-L-histidine (I), L-histidine was the only product that could be isolated after treatment with the acid reagent. Moreover, attempts to isolate N(Im)-carbobenzoxy-L-histidyl-L-threonine methyl ester hydrobromide have still been unsuccessful.[18] The nonprotected hydroxyl or carboxyl function adjacent to the N(Im)-acyl group seems to make the latter susceptible to cleavage by the acid reagent.

Despite these observations, the new procedure described above has been shown to be one of the most promising methods for synthesis of histidine peptides.

(17) H. Kappeler, *Helv. Chim. Acta*, **44**, 476 (1961).
(18) K. Narita and F. Sakiyama, private communication.

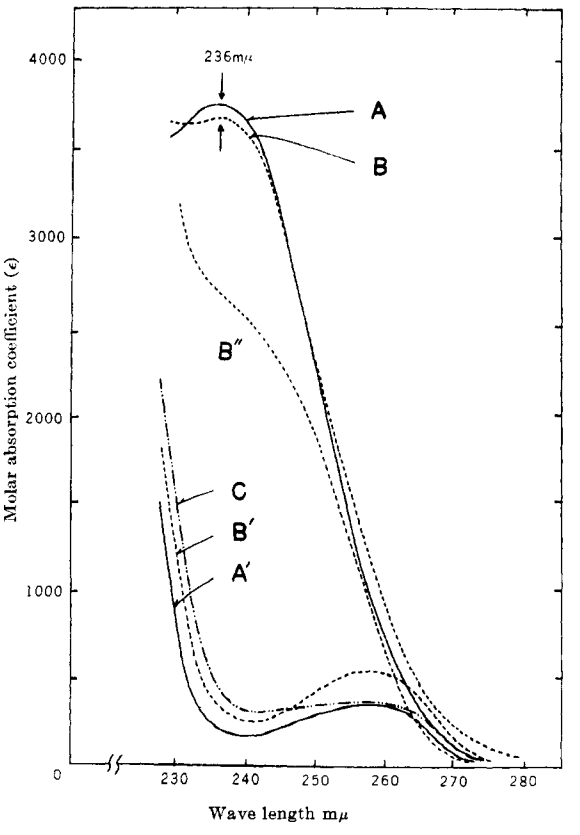


Fig. 3.—Ultraviolet absorption spectra: A, carbobenzoxyglycyl-N(Im)-carbobenzoxy-L-histidine methyl ester (X); B, carbobenzoxyglycyl-N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine methyl ester (XII); C, carbobenzoxyglycyl-L-histidyl-L-phenylalanine (XX). A′ and B′ are those after treatment of A and B with CH_3ONa, respectively, and B″, after standing B at room temperature for 72 hours showing about 30% of decomposition (in 0.188 mmolar soln.). All in absolute methanol.

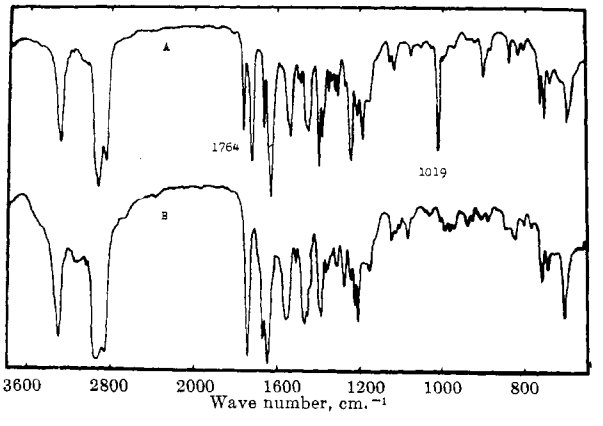


Fig. 4.—Comparison of infrared absorption spectra between A, formyl-γ-methyl-L-glutamyl-N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine benzyl ester (XIV), and B, formyl-γ-methyl-L-glutamyl-L-histidyl-L-phenylalanine benzyl ester.

Experimental

All melting points are uncorrected.

N(α),N(Im)-Dicarbobenzoxy-L-histidine (I).—To a solution of 8.38 g. (0.04 mole) of L-histidine monohydrochloride monohydrate in 20 ml. of 4 *N* sodium hydroxide were added

42.4 ml. of 5% sodium carbonate and 30 ml. of dioxane. The solution was cooled in an ice bath and then treated with 14.35 g. (0.084 mole) of benzyl chloroformate and 21 ml. of 4 *N* sodium hydroxide in five equal portions within 40 min. The mixture was stirred for an additional 20 min. at 0°. The sodium salt of the product often separated as a crystalline precipitate. After about 100 ml. of ethyl acetate had been added, the reaction mixture was neutralized with 6 *N* hydrochloric acid. The aqueous phase was extracted twice with ethyl acetate. The combined extract was concentrated *in vacuo* to remove dioxane and the resulting sirup was redissolved in ethyl acetate. The solution was washed with water, dried over sodium sulfate, and concentrated to about 20 ml. at 30° *in vacuo*. Scratching under refrigeration below −10° caused the product to separate in a crystalline state. After addition of 50 to 60 ml. of anhydrous ether the crystals were filtered off, washed with anhydrous ether, and dried; wt. 12.78 g. (76%), m.p. 87–90° dec. Recrystallization from ethyl acetate–ether gave lustrous granules; m.p. 90.5–92° dec., $[\alpha]^{19}D$ +29.1± 0.1° (*c*, 1.655 in ethyl acetate), λ_{max}^{MeOH} 238 mμ (ε 3600); lit.,[5] λ_{max}^{MeOH} 238 mμ (ε 3200). Soluble in ethyl acetate, chloroform; slightly in ether; insoluble in petroleum ether, water.

Anal. Calcd. for $C_{22}H_{21}N_3O_6$: C, 62.5; N, 5.01; N, 9.93. Found: C, 62.17; H, 5.06; N, 9.86.

These crystals are readily soluble also in methanol, and from the solution soon separated a second form of crystal in fine needles which has one molecule of methanol (Ia); m.p. 105–107° dec., $[\alpha]^{21}D$ +13.5 ± 2° [*c*, 1.025 in methanol–acetone (1:4) by volume]; lit.,[4,5] m.p. 105–107° dec., $[\alpha]^{20}D$ +15.3° [*c*, 6.3 in methanol–acetone (1:4) by volume].[5]

Anal. Calcd. for $C_{22}H_{21}N_3O_6 \cdot CH_3OH$: C, 60.8; H, 5.55; N, 9.25. Found: C, 60.67; H, 5.50; N, 9.39.

N(α),N(Im)-Dicarbobenzoxy-L-histidine Methyl Ester Hydrochloride (II).—To 40 ml. of absolute methanol previously cooled below −10° were added 0.78 ml. (0.011 mole) of thionyl chloride and 4.23 g. (0.01 mole) of I. The resulting clear solution was stirred for 3 hr. and upon standing overnight at 0° the hydrochloride of the ester separated in colorless needles. To complete separation, 40 ml. of anhydrous ether was added and after refrigeration the crystals were filtered off and washed with methanol–ether (1:2) and with ether; wt. 3.20 g. (67.6%), m.p. 121.5–122.5° dec., $[\alpha]^{21}D$ −20.0 ± 2° (*c*, 3.073 in methanol); lit.,[5] m.p. 117–118° dec.

Anal. Calcd. for $C_{23}H_{24}N_3O_6Cl$: C, 58.3; H, 5.11; N, 8.87; Cl, 7.49. Found: C, 58.38; H, 5.09; N, 9.05; Cl, 7.39.

From the mother liquor was isolated an additional 0.57 g. of the crystals which had m.p. 116° dec. after recrystallization.

N(α),N(Im)-Dicarbobenzoxy-L-histidyl-L-phenylalanine Methyl Ester (III).—A suspension of 1.30 g. (0.006 mole) of L-phenylalanine methyl ester hydrochloride in 40 ml. of ether was cooled in an ice–salt bath and shaken vigorously with 12 ml. of ice-cold 50% potassium carbonate. The aqueous phase was once extracted with ether and the ether extracts were combined and dried over sodium sulfate at 0°. After removal of the solvent *in vacuo* the resulting oil of the free ester was dissolved in 20 ml. of methylene chloride. To this solution were added 2.12 g. (0.005 mole) of I and 1.03 g. (0.005 mole) of N,N′-dicyclohexylcarbodiimide. The mixture was allowed to stand overnight at room temperature and then refrigerated. The separated dicyclohexylurea was filtered off (1.07 g., 95.7%) and the filtrate was washed with *N* hydrochloric acid, water, ice-cold 5% sodium bicarbonate and with water, successively, and dried over sodium sulfate. Concentration *in vacuo* gave a clear sirup, which was then dissolved in 20 ml. of ethyl acetate. Upon addition of 20 ml. of ether the product was slowly separated in silky needles. After refrigeration overnight the crystals were filtered off, washed with ether, and dried; wt. 2.61 g. (89.2%), m.p. 135.5–136°. Recrystallization did not alter the melting point; $[\alpha]^{29}D$ +54.5 ± 0.7° (*c*, 3.261 in chloroform), λ_{max}^{MeOH} 236 mμ (ε 3685).

Anal. Calcd. for $C_{32}H_{32}N_4O_7$: C, 65.8; H, 5.52; N, 9.60. Found: C, 65.59; H, 5.54; N, 9.60.

N(α),N(Im)-Dicarbobenzoxy-L-histidyl-L-phenylalanine Benzyl Ester (IV).—To a mixture of 6.35 g. (0.015 mole) of I and L-phenylalanine benzyl ester [prepared from 5.25 g. (0.018 mole) of the hydrochloride as in the case of the methyl ester] were dissolved in 70 ml. of acetonitrile was added 3.10 g. (0.015 mole) of N,N′-dicyclohexylcarbodiimide dissolved in acetonitrile. The reaction mixture was stirred for 5 hr. and upon standing overnight at room temperature the whole solidified. The solvent was removed by suction, and the crystalline residue was washed three times with acetonitrile and dissolved in about 60 ml. of hot acetonitrile. The insoluble dicyclohexylurea was filtered off (3.11 g., 92.5%), and the filtrate was diluted with 80 ml. of ethyl acetate and refrigerated. The crystals were collected (6.04 g., m.p. 125.5–126.5°) and the filtrate was concentrated at 35° in vacuo. The residue was redissolved in ethyl acetate, washed with *N* hydrochloric acid, water, 5% sodium bicarbonate, and finally with water, dried over sodium sulfate, and concentrated to afford crystals (2.55 g., m.p. 124–125.5°); total yield 8.92 g. (90.2%). Recrystallization from ethyl acetate–ether (3:5) gave 8.70 g. (87.9%) of colorless needles; m.p. 126–127°, $[\alpha]^{28}D$ −7.2 ± 0.1° (*c*, 1.720 in methanol), which were readily soluble in chloroform, hot ethyl acetate; soluble in ethyl acetate, methanol; slightly in ether, acetonitrile; insoluble in petroleum ether, water.

Anal. Calcd. for $C_{38}H_{36}N_4O_7$: C, 69.1; H, 5.47; N, 8.49. Found: C, 68.98; H, 5.68; N, 8.49.

N(α),N(Im)-Dicarbobenzoxy-L-histidyl-L-leucine Methyl Ester (V).—A 1.45-g. sample (0.0034 mole) of I and L-leucine methyl ester [prepared from 0.76 g. (0.0042 mole) of the hydrochloride as in the case of L-phenylalanine methyl ester] were coupled in methylene chloride by 0.72 g. (0.0035 mole) of the carbodiimide almost as in the case of III to give 1.70 g. (90.0%) of the peptide; m.p. 102–103.5°. Recrystallization from ethyl acetate–ether gave silky needles having m.p. 102.5–103.5°, $[\alpha]^{18}D$ −7.6 ± 2° (*c*, 3.327 in methanol), $[\alpha]^{28}D$ +14.1 ± 0.6° (*c*, 3.089 in ethyl acetate); lit.,[4] m.p. 101–103°, $[\alpha]^{14}D$ +21.44° (*c*, 29.0 mg. in 1 ml. of ethyl acetate).

Anal. Calcd. for $C_{29}H_{23}N_4O_7$: C, 63.3; H, 6.23; N, 10.17. Found: C, 64.01; H, 6.23; N, 10.54.

N(Im)-Carbobenzoxy-L-histidine Methyl Ester Dihydrobromide (VI).—To 1.14 g. (0.0024 mole) of II was added 6 ml. of 40.4% (w./w.) hydrogen bromide in dioxane and the mixture was allowed to stand at room temperature. The evolution of carbon dioxide had ceased within 15 min., after which time 30 ml. of anhydrous ether was added. The deposited oil soon solidified and the precipitate was washed with methylene chloride and with ether, and dried; wt. 1.05 g. (94.3%), m.p. 167–167.5° dec., λ_{max}^{MeOH} 234 mμ (ε 3140). Soluble in methanol, water; insoluble in methylene chloride, ethyl acetate, ether.

Anal. Calcd. for $C_{15}H_{19}N_3O_4Br_2$: C, 38.75; H, 4.12; N, 9.04; Br, 34.4. Found: C, 37.23; H, 4.44; N, 9.43; Br, 35.03.

N(Im)-Carbobenzoxy-L-histidyl-L-leucine Methyl Ester Dihydrobromide (VII).—A mixture of 0.55 g. (0.001 mole) of V in 3 ml. of 37.5% (w./w.) hydrogen bromide in dioxane was stood at room temperature for 30 min. Upon addition of 20 ml. of anhydrous ether an oil was deposited which soon solidified. The precipitate was washed with ether and dried; wt. 0.55 g. (94.3%), m.p. 95° dec. Soluble in methanol, methylene chloride, water; insoluble in ethyl acetate, ether.

Anal. Calcd. for $C_{21}H_{30}N_4O_5Br_2$: C, 43.7; H, 5.23; N, 9.70; Br, 27.7. Found: C, 40.94; H, 5.60; N, 10.62; Br, 27.91.

N(Im)-Carbobenzoxy-L-histidyl-L-phenylalanine Methyl Ester Dihydrobromide (VIII).—A 0.585 g. sample (0.001

mole) of III was treated with 3 ml. of 37.5% (w./w.) hydrogen bromide in dioxane at room temperature. As soon as the solid had disappeared, the product started to separate and the whole solidified. After 30 min. the crystals were filtered off with the aid of 15 ml. of ether, washed with methylene chloride and with ether, and dried; wt. 0.60 g. (98.7%), colorless needles, m.p. 133–134° dec., λ_{max}^{MeOH} 234 mμ (ε 3300). Soluble in methanol, water; slightly in methylene chloride; insoluble in ethyl acetate, ether.

Anal. Calcd. for $C_{24}H_{28}N_4O_5Br_2$: C, 47.15; H, 4.61; N, 9.15; Br, 26.15; Found: C, 46.87; H, 4.75; N, 9.05; Br. 26.26.

When the treatment was carried out with 29.3% (w./w.) hydrogen bromide in glacial acetic acid, the dipeptide ester dihydrobromide was obtained as fine needles in a 99.3% yield; m.p. 135.5–136° dec.

Anal. Calcd. for $C_{24}H_{28}N_4O_5Br_2$: C, 47.15; H, 4.61; N, 9.15; Br, 26.15. Found: C, 46.58; H, 4.61; N, 8.90; Br, 26.28.

N(Im)-Carbobenzoxy-L-histidyl-L-phenylalanine Benzyl Ester Dihydrobromide (IX).—A mixture of 2.98 g. (0.0045 mole) of IV with 15 ml. of 35% (w./w.) hydrogen bromide in dioxane was stood at room temperature for 30 min. The crystalline precipitate which separated was filtered off, washed with ether, and dried; wt. 3.08 g. (99.7%), m.p. 127–127.5° dec.

Anal. Calcd. for $C_{30}H_{32}N_4O_5Br_2$: C, 52.3; H, 4.69; N, 8.15; Br, 23.25. Found: C, 53.75; H, 5.09; N, 8.59; Br, 23.61.

A suspension of 10.02 g. of IX in 100 ml. of methylene chloride was cooled in an ice bath and shaken with 30 ml. of about 3 *N* ammonia at 0°. The aqueous phase was twice extracted with methylene chloride and the extracts were combined. The methylene chloride solution was concentrated at 20° *in vacuo* and the residue was redissolved in 100 ml. of ethyl acetate and shaken with 30 ml. of ice-cold *N* hydrochloric acid to separate the ester monohydrochloride monohydrate (IXa) as needles; wt. 7.13 g. (83.5% of recovery), m.p. 136.5–137° dec., λ_{max}^{MeOH} 236 mμ (ε 3390). Soluble in methanol, cold methylene chloride; slightly in ethyl acetate, water; insoluble in ether.

Anal. Calcd. for $C_{30}H_{30}N_4O_5 \cdot HCl \cdot H_2O$: C, 62.1; H, 5.38; N, 9.66; Cl, 6.11. Found: C, 61.90; H, 5.65; N, 9.90; Cl, 5.84.

Treatment of N(α),N(Im)-Dicarbobenzoxy-L-histidine with Hydrogen Bromide in Acetic Acid.—A mixture of 2.12 g. (0.005 mole) of I and 15 g. of 34% (w./w.) hydrogen bromide in glacial acetic acid was allowed to stand at room temperature for 30 min., after which time 75 ml. of anhydrous ether was added. The resulting precipitate was filtered off, washed with ether, and dried; wt. 2.44 g. This product gave three spots reactive to ninhydrin on a paper chromatogram (*n*-butyl alcohol–glacial acetic acid–water–pyridine in 30:6:24:20 by volume). The main spot (R_f 0.14) was identified with histidine, but the other spots, which were very faint, have not been identified.

A 1.76-g. sample of this crude product was dissolved in about 10 ml. of water. The cloudy solution was thoroughly washed with ether and concentrated *in vacuo*. After the washing and concentration had been repeated until the concentrate no longer had a nasty odor, about 10 ml. of methanol was added to the sirupy residue. The resulting crystalline precipitate of histidine monohydrobromide monohydrate was collected, washed with methanol, and dried; wt. 0.427 g. (*ca.* 47% based on I).

Anal. Calcd. for $C_6H_9N_3O_2 \cdot HBr \cdot H_2O$: C, 28.35; H, 4.76; N, 16.5; Br, 31.45. Found: C, 28.88; H, 5.08; N, 16.71; Br, 32.06.

The mother liquor gave two distinct spots reactive to both ninhydrin and the Pauly reagent on a paper chromatogram (*n*-butyl alcohol–glacial acetic acid–water in 4:1:1 by volume). One of these spots was identical with histidine and another has not been identified.

On the other hand, a solution of 0.45 g. of the crude product in water was, after washing with ether, shaken with Amberlite IR-45 (OH^- form) for 10 min. The filtrate was concentrated to a few milliliters *in vacuo* and about a five times volume of acetone was added. After refrigeration overnight the precipitate was filtered off, washed with acetone, and dried; wt. 0.147 g. (*ca.* 77% as histidine dihydrate based on I). Recrystallization from acetone-water gave 0.127 g. of lustrous crystals. By infrared absorption analysis, this compound was shown to be identical with the material which was quantitatively obtained from L-histidine monohydrochloride monohydrate by the treatment with Amberlite IR-45.

Carbobenzoxyglycyl-N(Im)-carbobenzoxy-L-histidine Methyl Ester (X).—To 1.03 g. (0.0022 mole) of VI in 20 ml. of methylene chloride and 10 ml. of water was added 1 ml. of 28% ammoniacal water with vigorous shaking at 0°. The aqueous phase was saturated with sodium chloride and then extracted with methylene chloride. The combined extract was dried over sodium sulfate at 0° and concentrated to a small volume *in vacuo*. To this solution of the free ester were added 0.42 g. (0.002 mole) of carbobenzoxyglycine and 0.41 g. (0.002 mole) of N,N′-dicyclohexylcarbodiimide with methylene chloride to a total volume of 20 ml. The reaction mixture was allowed to stand at room temperature overnight and then refrigerated. After removal of the dicyclohexylurea (0.43 g., 96.5%), the filtrate was concentrated *in vacuo* and was dissolved in ethyl acetate. The solution was cooled in an ice bath, washed with ice-cold *N* hydrochloric acid, 5% sodium bicarbonate, and water, dried over sodium sulfate, and evaporated to a sirup, which was crystallized from ethyl acetate–petroleum ether; wt. 0.84 g. (85.3%), m.p. 74–75.5°. Recrystallization from ethyl acetate–ether gave fine rods without change in the m.p., $[\alpha]^{20}D$ +3.0 ± 0.5° (*c*, 3.102 in methanol), $[\alpha]^{29}D$ +24.5 ± 0.7° (*c*, 2.827 in ethyl acetate), λ_{max}^{MeOH} 236 mμ (ε 3755). Soluble in ethyl acetate, chloroform, methanol; slightly in ether; insoluble in petroleum ether, water.

Anal. Calcd. for $C_{25}H_{26}N_4O_7$: C, 60.7; H, 5.31; N, 11.3. Found: C, 60.61; H, 5.45; N, 11.43.

Carbobenzoxyglycyl-N(Im)-carbobenzoxy-L-histidyl-L-leucine Methyl Ester (XI).—To a solution of 0.477 g. (0.825 mmole) of VII in 20 ml. of methylene chloride was added 5 ml. of ice-cold 5% sodium bicarbonate with vigorous shaking at 0°. The aqueous phase was saturated with sodium chloride and extracted with 10-ml. portions of methylene chloride. The extracts were combined and evaporated *in vacuo* after drying over sodium sulfate. The resulting free ester was coupled with 0.157 g. (0.75 mmole) of carbobenzoxyglycine, as in the case of X, yielding 0.371 g. (81.6%) of the tripeptide; m.p. 147.5–149°. Recrystallization from ethyl acetate gave a sample for analyses with m.p. 152–152.5°, $[\alpha]^{30}D$ −9.2 ± 1° (*c*, 2.062 in methanol), λ_{max}^{MeOH} 236 mμ (ε 3610). Soluble in chloroform, methanol; sparingly in ethyl acetate; insoluble in ether, petroleum ether, water.

Anal. Calcd. for $C_{31}H_{37}N_5O_8$: C, 61.3; H, 6.14; N, 11.5. Found: C, 61.39; H, 6.31; N, 11.44.

Carbobenzoxyglycyl-N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine Methyl Ester (XII).—N(Im)-Carbobenzoxy-L-histidyl-L-phenylalanine methyl ester [prepared from 0.522 g. (0.85 mmole) of VIII with ammonia as in the case of X] was dissolved with 0.162 g. (0.775 mmole) of carbobenzoxyglycine in methylene chloride and to this mixture was then added 0.160 g. (0.775 mmole) of the carbodiimide. Separation of the crystalline tripeptide started within a few hours. After standing overnight the reaction mixture was gently warmed at 40° and filtered to remove the dicyclohexylurea (0.156 g. 89.7%). The filtrate was refrigerated to separate the product in needles; wt. 0.445 g. (89.6%), m.p. 151–152°. Recrystallization from methylene chloride gave fine needles with m.p. 154–154.5°, $[\alpha]^{18}D$ −8.6 ± 2° (*c*, 2.336 in methanol), λ_{max}^{MeOH} 236 mμ (ε 3655). Soluble in warm methylene chloride; sparingly in methylene chloride,

ethyl acetate, methanol; insoluble in ether, petroleum ether, water.

Anal. Calcd. for $C_{34}H_{35}N_5O_8$: C, 63.7; H, 5.51; N, 10.9. Found: C, 63.69; H, 5.63; N, 10.84.

N(α),N(Im)-Dicarbobenzoxy-L-histidyl-N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine Benzyl Ester (XIII).—A mixture of 0.423 g. (0.001 mole) of I, N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine benzyl ester [prepared from 0.70 g. (0.0012 mole) of IXa with ammonia] and 0.206 g. (0.001 mole) of the carbodiimide in methylene chloride was stirred at room temperature for 2.5 hr., stood overnight and then concentrated *in vacuo*. The resulting sirup was dissolved in about 5 to 6 ml. of acetonitrile and after removal of the insoluble urea (0.212 g., 94.6%) the product was precipitated with the addition of ether; wt. 0.754 g. (81.0%), m.p. 132–133°. Recrystallization from ethyl acetate afforded fine crystals; m.p. 138–140°, $[\alpha]^{22}D$ −17.9 ± 2° (*c*, 3.047 in methanol), λ_{max}^{MeOH} 236 mμ (ε 7830). It was readily soluble in methylene chloride, hot ethyl acetate; soluble in acetonitrile, methanol; slightly in ether; insoluble in petroleum ether, water.

Anal. Calcd. for $C_{52}H_{49}N_7O_{10}$: C, 67.1; H, 5.79; N, 10.5. Found: C, 67.05; H, 5.41; N, 10.69.

Formyl-γ-methyl-L-glutamyl-N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine Benzyl Ester (XIV).—To a solution of N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine benzyl ester [prepared from 1.162 g. (0.002 mole) of IXa with ammonia] in 10 ml. of methylene chloride and 5 ml. of acetonitrile were added 0.417 g. (0.0022 mole) of formyl-γ-methyl-L-glutamic acid[19] dissolved in 20 ml. of acetonitrile and 0.455 g. (0.0022 mole) of the carbodiimide. The reaction mixture was stirred at room temperature for 3 hr. and refrigerated. The crystalline precipitate which separated was filtered off and redissolved in 20 ml. of hot acetonitrile. After the insoluble urea (0.414 g., 92.3%) was carefully removed, the filtrate was allowed to stand at room temperature and then refrigerated to complete separation of the peptide; wt. 1.215 g. (87.2%), m.p. 165–165.5° dec., $[\alpha]^{24}D$ −12.1 ± 3° (*c*, 2.032 in dimethylformamide), λ_{max}^{MeOH} 236 mμ (ε 3415).

Anal. Calcd. for $C_{37}H_{39}N_5O_9$: C, 63.7; H, 5.63; N, 10.0. Found: C, 63.79; H, 5.90; N, 10.36.

Carbobenzoxy-γ-*t*-butyl-L-glutamyl-N(Im)-carbobenzoxy-L-histidyl-L-phenylalanine Methyl Ester (XV).—A solution of carbobenzoxy-γ-*t*-butyl-L-glutamic acid{prepared from 0.260 g. (0.5 mmole) of the dicyclohexylammonium salt [m.p. 140–141°, $[\alpha]^{23}D$ +7.3 ± 1° (*c*, 2.395 in methanol)] by treatment with Dowex-50 (H^+ form)}[20] in methylene chloride was mixed with a solution of N(Im)-carbobenzoxy-L-histidiyl-L-phenylalanine methyl ester [prepared from 0.294 g. (0.48 mmole) of VIII as in the case of X with ammonia] in methylene chloride. To the solution at 0° was added 0.103 g. (0.5 mmole) of the carbodiimide in methylene chloride to a total volume of about 15 ml. and the mixture was allowed to stand overnight at room temperature. After refrigeration, the separated dicyclohexylurea was filtered off (0.103 g., 96%) and the filtrate was concentrated to dryness *in vacuo* to afford a solid mass which was washed with ethyl acetate–ether and dried; wt. 0.351 g. (95.2%), m.p. 158–159°. Recrystallization from acetonitrile gave a sample for analysis; m.p. 161–162°, $[\alpha]^{24}D$ −11.6 ± 1° (*c*, 1.557 in dimethylformamide).

Anal. Calcd. for $C_{41}H_{47}N_5O_{10}$: C, 64.0; H, 6.15; N, 9.10. Found: C, 63.94; H, 6.21; N, 9.18.

Often the product combines with one molecule of water, most likely owing to the presence of a trace of water in the reaction medium; m.p. 132–133°, $[\alpha]^{24}D$ −11.1 ± 1° (*c*, 1.510 in dimethylformamide).

Anal. Calcd. for $C_{41}H_{47}N_5O_{10}\cdot H_2O$: C, 62.5; H, 6.27; N, 8.89; H_2O, 2.29. Found: C, 62.99; H, 6.45; N, 8.57; H_2O, 1.65.

Carbobenzoxy-O-benzyl-L-seryl-N(Im)-carbobenzoxy-L-histidyl-L-leucine Methyl Ester (XVI).—To a mixture of 0.576 g. (1.75 mmoles) of carbobenzoxy-O-benzyl-L-serine[21,22] and N(Im)-carbobenzoxy-L-histidyl-L-leucine methyl ester [prepared from 1.118 g. (1.94 mmoles) of VII with ammonia] in methylene chloride was added 0.362 g. (1.75 mmoles) of the carbodiimide. The solution was stirred for 3 hr. and allowed to stand overnight at room temperature. After removal of the urea (0.370 g., 94.5%) the filtrate was concentrated *in vacuo*. The resulting sirup was redissolved in ethyl acetate, washed successively with *N* hydrochloric acid, water, 5% sodium bicarbonate and with water and after drying over sodium sulfate concentrated to a sirup which was precipitated from ethyl acetate–ether to give 1.118 g. (86.1%); m.p. 102–105°. Recrystallization from the same solvent gave a sample for analyses; m.p. 105–106°, $[\alpha]^{30}D$ +7.8 ± 1° (*c*, 1.990 in ethyl acetate).

Anal. Calcd. for $C_{39}H_{45}N_5O_9$: C, 64.3; H, 6.23; N, 9.62. Found: C, 64.04; H, 6.29; N, 9.51.

Formylglycyl-O-benzyl-L-seryl-N(Im)-carbobenzoxy-L-histidyl-L-leucine Methyl Ester (XVIII).—O-Benzyl-L-serine methyl ester [prepared from 1.81 g. (0.0074 mole) of the hydrochloride[22]] and 0.68 g. (0.0066 mole) of formylglycine[23,24] were condensed by the carbodiimide method in methylene chloride to formylglycyl-O-benzyl-L-serine methyl ester; wt. 1.714 g. (88.4%), m.p. 104–105°. Recrystallization from methanol–ether gave silky needles with m.p. 106.5–107°.

Anal. Calcd. for $C_{14}H_{18}N_2O_5$: C, 57.2; H, 6.17; N, 9.53. Found: C, 57.91; H, 6.48; N, 9.48.

Formylglycyl-O-benzyl-L-serine was obtained in an 80% yield by saponification of the preceding ester; m.p. 136–137°.

Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C, 55.8; H, 5.76; N, 10.0. Found: C, 56.02; H, 6.00; N, 9.88.

N(Im)-Carbobenzoxy-L-histidyl-L-leucine methyl ester [prepared from 1.118 g. (1.94 mmoles) of VII with ammonia] and 0.491 g. (1.75 mmoles) of formylglycyl-O-benzyl-L-serine were coupled, almost as in the case of XVI, to the desired tetrapeptide; wt. 0.815 g. (66.8%). Recrystallization from methanol afforded needles with m.p. 131–132°, $[\alpha]^{30}D$ +1.6 ± 1° (*c*, 2.046 in ethyl acetate).

Anal. Calcd. for $C_{34}H_{42}N_6O_9$: C, 60.1; H, 6.24; N, 12.35. Found: C, 59.85; H, 6.24; N, 12.32.

Carbobenzoxy-L-propyl-L-phenylalanyl-N(Im)-carbobenzoxy-L-histidyl-L-leucine Methyl Ester (XVIII).[25]—Coupling of 2.49 g. (0.01 mole) of carbobenzoxy-L-proline[23,26] with L-phenylalanine methyl ester [prepared from 2.59 g. (0.012 mole) of the hydrochloride] by the carbodiimide method yielded 4.36 g. of carbobenzoxy-L-prolyl-L-phenylalanine methyl ester as a sirup. The methanolic solution of the crude ester obtained above was shaken with 5 ml. of 2 *N* sodium hydroxide for 1 hr. to afford 2.54 g. of carbobenzoxy-L-prolyl-L-phenylalanine (64.2% based on carbobenzoxy-L-proline used), m.p. 131–132°. Recrystallization from acetone–petroleum ether gave long plates with m.p. 132°. $[\alpha]^{29.5}D$ −58.4 ± 0.8° (*c*, 2.510 in chloroform); lit.,[27] m.p,

(19) This was prepared by formylation of γ-methyl L-glutamate after Sheehan's method for preparation of optically active formylamino acids [J. C. Sheehan and D-D. H. Young, *J. Am. Chem. Soc.*, **80**, 1154 (1958)]; yield 71.7% after recrystallization from methanol–ether, m.p. 112–114°, $[\alpha]^{19}D$ +10.3 ± 0.1° (*c*, 1.991 in methanol). *Anal.* Calcd. for $C_7H_{11}NO_5$: C, 44.45; H, 5.87; N, 7.42. Found: C, 44.63; H, 5.97; N, 7.68.

(20) H. Kappeler and R. Schwyzer, *Helv. Chim. Acta*, **44**, 1136 (1961); R. Schwyzer and H. Kappeler, *ibid.*, **44**, 1991 (1961).

(21) K. Okawa, *Bull. Chem. Soc. Japan*, **29**, 488 (1956).

(22) K. Inouye and H. Otsuka, *ibid.*, **34**, 1 (1961).

(23) K. Inouye and H. Otsuka, *ibid.*, **34**, 4 (1961).

(24) E. Fischer and O. Warburg, *Ber.*, **38**, 3997 (1905).

(25) Schwyzer, *et al.*,[27] has synthesized the same tetrapeptide sequence, carbobenzoxy-L-prolyl-L-phenylalanyl-L-histidyl-L-leucine methyl ester from carbobenzoxy-L-prolyl-L-phenylalanine and L-histidyl-L-leucine methyl ester by the carbodiimide method; yield, 61% after purification by means of the countercurrent distribution.

(26) A. Berger, J. Kurtz, and E. Katchalski, *J. Am. Chem. Soc.*, **76**, 5552 (1954).

(27) R. Schwyzer, B. Iselin, H. Kappeler, B. Riniker, W. Rittel, and H. Zuber, *Helv. Chim. Acta*, **41**, 1273 (1958).

126–127°, $[\alpha]^{22}D$ −49 ± 2° (*c*, 2.46 in absolute chloroform).

Anal. Calcd. for $C_{22}H_{24}N_2O_5$: C, 66.65; H, 6.10; N, 7.08. Found: C, 66.65; H, 6.29; N, 7.37.

N(Im)-Carbobenzoxy-L-histidyl-L-leucine methyl ester [prepared from 1.39 g. (0.0024 mole) of VII with ammonia] and 0.793 g. (0.002 mole) of carbobenzoxy-L-prolyl-L-phenylalanine were coupled almost as in the case of the compound XVI to carbobenzoxy-L-prolyl-L-phenylalanyl-N(Im)-carbobenzoxy-L-histidyl-L-leucine methyl ester; wt. 1.267 g. (80.0%). Recrystallization from acetone-ether gave a sample for analysis; m.p. 144.5–145° dec., $[\alpha]^{24}D$ −56.4 ± 1° (*c*, 2.544 in methanol).

Anal. Calcd. for $C_{43}H_{50}N_6O_9$: C, 65.1; H, 6.35; N, 10.6. Found: C, 64.42; H, 6.47; N, 10.71.

Glycyl-N(Im)-carbobenzoxy-L-histidyl-L-phendylalanine Methyl Ester Dihydrobromide (XIX).—A 0.642-g. sample of the compound XII was treated with 3 ml. of 37.5% (w./w. hydrogen bromide in dioxane as in the case of VII to give 0.421 g. of the tripeptide ester dihydrobromide, λ_{max}^{MeOH} 234 mμ (ε 3640).

Anal. Calcd. for $C_{26}H_{31}N_5O_6Br_2$: C, 46.7; H, 4.68; N, 10.0; Br, 23.9. Found: C, 45.73; H, 4.99; N, 10.40; Br, 22.59.

Carbobenzoxyglycyl-L-histidyl-L-phenylalanine (XX).—To a suspension of 0.642 g. (0.001 mole) of XII in 15 ml. of methanol was added 2.2 ml. of *N* sodium hydroxide and the mixture was shaken at room temperature for 30 min. The resulting clear solution was allowed to stand for additional 30 min., cooled in an ice bath, neutralized with 2.2 ml. of *N* hydrochloric acid, and then concentrated *in vacuo* to dryness. The crystalline residue was washed with ice-cold water and dried; wt. 0.382 g. (77.5%), m.p. 200° dec., $[\alpha]^{30}D$ +15.4 ± 1.4° (*c*, 1.424 in methanol).

Anal. Calcd. for $C_{25}H_{27}N_5O_6$: C, 60.85; H, 5.52; N, 14.2. Found: C, 60.51; H, 5.65; N, 14.11.

Formyl-γ-methyl-L-glutamyl-L-histidyl-L-phenylalanine (XXI).—A suspension of 0.349 g. (0.5 mmole) of XV in 20 ml. of methanol was submitted to hydrogenation in the presence of palladium black catalyst at room temperature. After the crystal had completely dissolved the hydrogenation was carried on for an additional 8 hr. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The resulting sirup was dissolved in water and after removal of a slight amount of insoluble precipitate the aqueous solution was concentrated *in vacuo*. Crystallization was effected by repeated concentration with methylene chloride, the product was filtered off, washed with methylene chloride, and dried; wt. 0.212 g. (89.5%), m.p. 185–189° dec., $[\alpha]^{25}D$ −12.2 ± 2° (*c*, 2.210 in methanol).

Anal. Calcd. for $C_{22}H_{27}N_5O_7 \cdot 2H_2O$: C, 51.9; H, 6.13; N, 13.7. Found: C, 51.28; H, 5.77; N, 13.48.

Carbobenzoxy-γ-*t*-butyl-L-glutamyl-L-histidyl-L-phenylalanine (XXII).—To a suspension of 2.00 g. (0.0026 mole) of XVI in 26 ml. of methanol was added 5.7 ml. of *N* sodium hydroxide at 0°, and the mixture was shaken at room temperature for 15 min. and then cooled in an ice bath. After addition of about 40 ml. of water the solution was neutralized with 5.7 ml. of *N* hydrochloric acid to separate the product as a partially crystallized solid. The precipitate was filtered off, washed with cold water, and dried; wt. 1.41 g. (85.0%). The suspension of this crude product in 20 ml. of acetonitrile was boiled for a few minutes to ensure crystallization. After refrigeration, the crystals were filtered off, washed with acetonitrile and ether, and dried; wt. 1.24 g. (75.0%), m.p. 172–173°, $[\alpha]^{24}D$ −3.7 ± 1° (*c*, 2.409 in dimethylformamide).

Anal. Calcd. for $C_{32}H_{39}N_5O_8 \cdot H_2O$: C, 60.1; H, 6.46; N, 10.9. Found: C, 59.94; H, 6.44; N, 10.75.

Conformations. I. Synthesis, Proton Magnetic Resonance Spectra, and Ultraviolet Spectra of Substituted 1-Phenylcyclohexenes

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Received April 16, 1962

The synthesis of a number of substituted 1-phenylcyclohexenes is described. Proton magnetic resonance and ultraviolet spectra of these compounds are reported.

This is the first of several papers to be published concerning the determination of substituent conformations in cyclohexenes. The synthesis of a number of substituted 1-phenylcyclohexenes will be discussed here. Proton magnetic resonance and ultraviolet spectra of these compounds have been determined and these data are summarized in Table I.

Compounds 1–11 and 13–16 were prepared by dehydration of the corresponding tertiary arylcyclohexanols at room temperature employing usually a sulfuric acid in acetic acid dehydration mixture.[2] In this manner, dehydrations of 2-methyl-1-phenylcyclohexanol,[3] 2-isopropyl-1-phenylcyclohexanol,[3] and 1,2-diphenylcyclohexanol[3] led to mixtures of 6- and 2-substituted—1-phenylcyclohexenes (compounds 6–9 and 13) which were readily separated by conventional methods. Dehydration of 2-*tert*-butyl-1-phenylcyclohexanol[3] afforded only 6-*tert*-butyl-1-phenylcyclohexene (compound 10) as evidenced by a single gas-liquid chromatography band.[4]

The synthetic routes to alkenes 12 and 17–21 are schematically described in Chart I. The preparations of the 6-nitro-1-phenylcyclohexene derivatives (compounds 22–27) have been reported.[5]

(1) N.S.F. Postdoctoral Fellow, 1961–1962. Present address: Department of Chemistry, University of Chicago, Chicago 37, Ill.

(2) E. W. Garbisch, Jr., *J. Org. Chem.*, **26**, 4165 (1961).

(3) Carbinols are of unknown configurations.

(4) It has not been definitely established whether or not the dehydrations afforded equilibrium mixtures of the possible alkenes.

(5) E. W. Garbisch, Jr., Ph.D. thesis, Northwestern University, 1961. This work will be published shortly. Compound 23 was prepared in the same manner as described for *trans*-4-*tert*-butyl-6-nitro-1-phenylcyclohexene. Compound 26 was furnished by Robert L. Arnold of Northwestern University.