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Reactivity of Cyclic Peptides. II. *cyclo*-L-Tyrosyl-L-histidyl and *cyclo*-L-Tyrosyltriglycyl-L-histidylglycyl[1,2]

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The cyclic peptides named were prepared for an investigation of imidazole–hydroxyphenyl interactions. No evidence was found for significant hydrogen bonding between these side chains. Toward several phenyl acetates the peptides exhibited reactivity comparable to that of imidazole, but acylation of tyrosine hydroxyl was shown to occur, probably by intramolecular transfer from acetylimidazole.

In recent years interest has grown in the synthesis of cyclic peptides, and with present methods their synthesis is straightforward. This paper presents some initial studies of the utility of these substances as models for phenomena normally studied in proteins or poly-(amino acids).

A special stability for cyclic hexa- and decapeptides is strongly implied by the preference for cyclodimerization exhibited by linear tri- and pentapeptides,[3-5] as well as by the ready cyclization of linear peptides of appropriate length.[3,4,6,7] It has been proposed that the dimerization reaction is favored by alignment of two tripeptide (or pentapeptide) chains, held together in anti-parallel fashion by hydrogen bonds.[8] The low energy and high negative entropy of activation observed for self-condensation of triglycine methyl ester appear to agree with this contention.[9] It is reasonable to assume that, if they exist during cyclization, these hydrogen bonds persist in the cyclic product, although supporting evidence is scanty. X-Ray studies do suggest a preferred conformation containing four transannular hydrogen bonds in the cyclodecapeptide Gramicidin S,[10] and there can be written a similar, very reasonable, structure for a cyclohexapeptide with two hydrogen bridges,[8,11] but detailed analysis of a cyclic peptide structure has yet to appear. In aqueous solution it is possible that hydrophobic bonds, rather than hydrogen bridges, determine the conformation of these molecules.[12] For one reason or another, however, it is likely that even in solution cyclic hexa- and decapeptides will be relatively rigid structures.

(1) This work was supported by National Science Foundation Grant G-14324 and, in part, by U. S. Public Health Service Grant RG-7575.

(2) Paper I, *J. Am. Chem. Soc.*, **83**, 4103 (1961).

(3) G. W. Kenner, P. J. Thompson and J. M. Turner, *J. Chem. Soc.*, 4148 (1958).

(4) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **41**, 2186, 2190 (1958); R. Schwyzer and B. Gorup, *ibid.*, **41**, 2199 (1958).

(5) R. Schwyzer, B. Iselin, W. Rittel and P. Sieber, *ibid.* **39**, 872 (1956).

(6) T. Wieland and K. W. Ohly, *Ann.*, **605**, 179 (1957).

(7) D. G. H. Ballard, C. H. Bamford and F. J. Weymouth, *Proc. Roy. Soc. (London)*, **A227**, 155 (1954); *J. Am. Chem. Soc.*, **77**, 6368 (1955).

(8) R. Schwyzer, *Record Chem. Progr.*, **20**, 147 (1959).

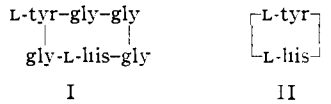
(9) P. S. Rees, D. P. Tong and G. T. Young, *J. Chem. Soc.*, 662 (1954).

(10) G. M. J. Schmidt, D. C. Hodgkin and B. M. Oughton, *Biochem. J.*, **65**, 744 (1953).

(11) Preliminary X-ray work on cyclohexaglycine, indicating a lack of hexagonal symmetry and therefore consistent with Schwyzer's structure, has been reported; E. M. Cant. *Acta Cryst.*, **9**, 681 (1956).

(12) D. T. Warner, *Nature*, **190**, 120 (1961).

As models for ordered regions in proteins, cyclic peptides would allow study of interactions of a minimal number of side chains. In principle, at least, it would not be impossible to determine with some correctness their conformation in solution. With this in mind, we have undertaken the synthesis of such molecules with only two functional side chains, to obtain information about the interaction of these side chains with each other, with solvent and with other molecules. By choice of glycine as the non-functional residue, synthetic difficulties have been held to a minimum. The synthesis and some properties of a cyclic hexapeptide containing histidine and tyrosine residues, *cyclo*-L-tyrosyltriglycyl-L-histidylglycyl (I), and for comparison, similar observations on the cyclic dipeptide, L-tyrosyl-L-histidyl (II), are reported here.



I II

Synthesis.—The path used for the synthesis of the hexapeptide I is shown in Fig. 1. Schemes utilizing histidine with unprotected imidazole did not prove successful in our hands; recourse was had therefore to N^{im}-benzylhistidine derivatives, and the benzyl group was removed as the last step. Although this blocking group can, in principle, be removed hydrogenolytically, in the present case reaction was much too slow to be practical, and the use of sodium in liquid ammonia, though it resulted in low yields, was necessary. Hydrogenolysis of the N^{im}-benzyl group was, however, an important side reaction in the terminal unblocking, by hydrogenolysis, of the linear peptide III. In this step (III to IV) the order of removal of benzyl blocking groups was shown to be the expected O-nitrobenzyl > > N-carbobenzoxy> N^{im}-benzyl (see Experimental section). It is worth noting that in the synthesis of *cyclo*-L-tyrosyldiglycyl-L-histidyldiglycyl, an isomeric peptide which will be reported on in a later paper, removal of the N^{im}-benzyl group from the cyclic peptide was readily accomplished in high yield by catalytic hydrogenolysis.

The reagent used for the coupling of the two tripeptide fragments and for the cyclization step was the recently reported water-soluble carbodiimide, N - (3 - dimethylaminopropyl) - N′ - ethylcarbo-

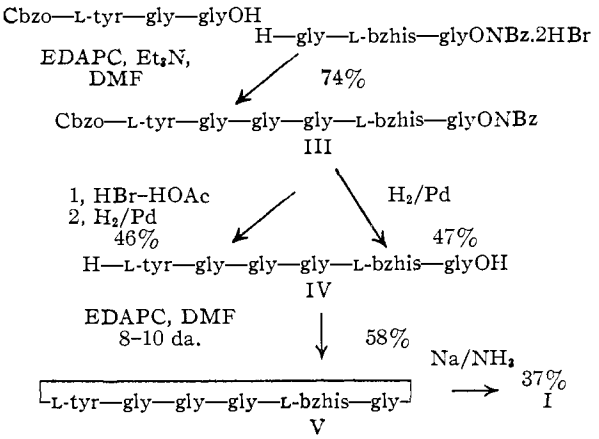


Fig. 1.—Synthesis of the cyclic hexapeptide; abbreviations: bzhis, N^{im}-benzylhistidine; Cbzo, carbobenzoxy; NBz, *p*-nitrobenzyl; DMF, dimethylformamide; EDAPC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide.

diimide,[13] which was prepared independently in the course of this work. Since this reagent was used only for steps in which the carboxyl component contained C-terminal glycine, no information has been obtained about the extent of racemization resulting from its employment. Further investigation of its utility is in order, however, because the urea formed from it is soluble in water and in organic solvents, permitting clean separation of the peptide produced, regardless of the solubility properties of the latter.

Cyclization of the hexapeptide IV was accomplished in the satisfying yield of 58% when the concentration of reacting peptide was about 0.015 *M*. Hexapeptide cyclization yields as high as that achieved here have in general been obtained only when glycine occurs at both ends of the chain.[14] The formation of some linear polymer was evident from the presence of slow-running, ninhydrin-active spots on paper chromatograms of the crude reaction product, but ready purification of the cyclic hexapeptide was achieved by cellulose powder column chromatography.

Synthesis of the diketopiperazine II was easily performed by heating an ethanolic solution of L-histidyl-L-tyrosine methyl ester, no carboxyl activation being necessary for this facile cyclization.

Ionization Constants of the Peptides.—The formation of hydrogen bonds between amino acid side chains may be examined using cyclic peptides. This interaction will affect ionization constants of groups involved, and has been discussed in detail by Laskowksi and Scheraga.[15] Molecular models of the cyclic hexapeptide I, based on the Schwyzer structure,[8] demonstrate that in it there are no steric factors operating against the formation of a tyrosine–histidine hydrogen bond, although many conformations in which O–H · · · N bonding is impossible are available to the side chains. If

(13) J. C. Sheehan, P. A. Cruickshank and G. L. Boshart, *J. Org. Chem.*, **26**, 2525 (1961). This reagent is commercially available from the Piece Chemical Co., Rockford, Ill.

(14) Discussion following paper by M. Rothe, "Special Problems of Synthesis of Cyclic Peptides," in Proceedings of the Symposium of Methods of Peptide Synthesis, Prague, 1958. Special Issue of *Coll. Czech. Chem. Comm.*, **24**, 148 (1959).

(15) M. Laskowski and H. A. Scheraga, *J. Am. Chem. Soc.*, **76**, 6305 (1954).

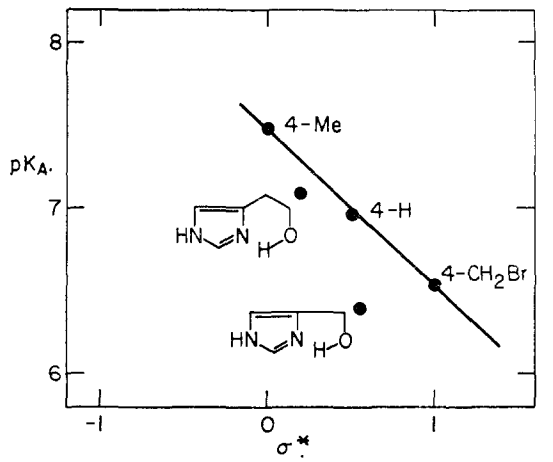


Fig. 2.—pK_A of 4-substituted imidazoles *vs.* Taft's polar substituent constant (R. W. Taft, Jr. in "Steric Effects in Organic Chemistry," M. J. Newman, ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p. 619). pK_A's of 4-CH_3-, 4-H- and 4-$HOCH_2$- are taken from A. H. M. Kirby and A. Neuberger, *Biochem. J.*, **32**, 1146 (1948). σ^* for hydroxyethyl is derived from that of hydroxymethyl by use of the attenuation factor 0.36.

it is assumed that the cyclic backbone of I is rigid, formation of the bond in question requires freezing out rotation about four single bonds. On this basis, the treatment of the authors just mentioned[15] (assuming as they do $\Delta S^\circ = -5$ e.u. per bond rotation frozen and $\Delta H^\circ = -6$ kcal./mole for the O–H · · · N bond) leads to a calculated equilibrium constant of 50 for bond formation and a consequent decrease in pK_A of the imidazole involved by 1.7 units. This prediction is not in accord with the observations recorded in Table I, where it can be seen that the imidazole and hydroxyphenyl side chains of I exhibit acidities much the same as they do in peptides where the interaction is not possible. A reason for this disagreement may lie in the choice of too large a value of $-\Delta H^\circ$.

TABLE I

pK_A OF HISTIDINE AND TYROSINE PEPTIDES, $\mu = 0.1$, 25°

Peptide	pK_A his	pK_A tyr
cyclo-L-his-L-tyr (II)	6.5	9.7
cyclo-L-tyrgly$_2$-L-hisgly (I)	6.65	10.05
cyclo-L-tyrgly$_2$-L-bzhisgly (V)	5.8	10.0
Cbzo-L-tyrgly$_2$-L-bzhisglyOH	6.3	10.1
H-L-tyrgly$_2$-L-bzhisglyOH (IV)	6.5	10.0
Cbzogly-L-hisglyOEt	6.65	
Cbzogly-L-hisglyOH	6.7	
Cbzo-L-tyrglyglyNHNH$_2$		10.0
Cbzo-L-tyrglyglyOH		10.1
Cbzo-L-pro-L-hisglyNH$_2$	6.42[a]	
ClCH$_2$CO-L-tyrOEt		10.2[b]

[a] W. L. Koltun, *et al.*, *J. Am. Chem. Soc.*, **81**, 295 (1959), $\mu = 0.16$. [b] E. S. Bruigom, cited in G. H. Beavan and E. R. Holiday, "Advances in Protein Chemistry," Vol. VII, Academic Press Inc., New York, N. Y., 1952, p. 319, extrapolated to $\mu = 0$.

From the data shown in Fig. 2, it may be estimated that an intramolecular O–H · · · N hydrogen bond in 4-hydroxymethylimidazole decreases its pK_A about 0.5 unit. For this molecule the treatment of Laskowski and Scheraga gives ΔH° −2.4

kcal./mole for hydrogen bond formation in water. About the same ΔH° can be estimated in the case of 4-hydroxyethylimidazole, but with less certainty. Use of this value for the interaction in I predicts an equilibrium constant for hydrogen bond formation of 0.14 at room temperature, and a change in pK of the ionizable groups involved of only 0.06, well within the uncertainty of our measurements. Further constraints on side chain mobility than are provided by a peptide backbone alone are therefore probably required to produce strong histidine–tyrosine side chain hydrogen bonds. A factor which could favor their formation in proteins would be the presence of hydrophobic side chains on the nearby residues. The release of water from the polar groups to solvent, occurring when the intramolecular bridge is formed, would result in a more favorable entropy change when there is hydrocarbon nearby than when the bonding groups are largely surrounded by water.[16]

The considerably heightened acidity of the imidazolium ion of the N^{im}-benzyl cyclic peptide V (pK_A 5.8) is worthy of note. Since the hydroxyphenyl moiety of V exhibits normal acidity, tyrosine–histidine hydrogen bonding cannot be involved, and since N-benzylation has only a small effect on the imidazole pK_A of the terminally unblocked peptide IV (Table I), an inductive effect of the alkyl residue is not responsible. In water the hydrophobic phenyl and hydroxyphenyl rings of V may be held in a conformation in which the imidazole ring is shielded by them and the peptide backbone. Thus imbedded in a medium of relatively low dielectric constant, imidazole would be less apt to take on a positive charge. An imidazole–peptide hydrogen bond, made favorable by the same solvent effect, would also produce this result.

Models of *cyclo*-L-tyrosyl-L-histidyl (II) indicate that in it imidazole–phenol hydrogen bonding is also sterically feasible, although some crowding of the three rings involved must occur. However, as in the hexapeptide, such an interaction can be expected to have a small effect on the pK_A of the two groups, and it is not surprising to find the pK_A for this histidine side chain in the region of other histidine peptide imidazoles. The slightly increased acidity of the hydroxyphenyl side chain is not readily interpreted, although an imidazole–phenolate hydrogen bond might be invoked as an explanation.

Reaction with Phenyl Acetates.—The choice of histidine and tyrosine to provide the functional side chains in this study was largely dictated by the desire to obtain peptides bearing acidic and basic centers which could coöperate in the catalysis of reactions such as ester hydrolysis. Several examples of coöperative nucleophilic-acid catalysis in reactions of acyl derivatives have been reported.[17–19] If coöperative catalysis occurs in reaction of I or II with phenyl acetates (imidazole-catalyzed hydrolysis) one might expect a smaller dependence of rate on phenyl substitution than occurs when imidazole itself is the catalyst. In Table II are compared second-order rate constants for liberation of phenol from phenyl acetates by the peptides and by imidazole. (The choice of esters was limited by the requirement that the ultraviolet absorption of liberated phenol and that of the peptide hydroxyphenyl not seriously overlap.) Within the group of esters employed the looked-for enhancement in reactivity of the less reactive esters is seen to be absent. Table II also indicates that the kinetic pK of the reacting group is that of the imidazole side chain, except in reaction of the diketopiperazine II with the two nitrophenyl esters. Explanation for these exceptions is at present lacking; prior equilibrium between II, substrate and a metastable complex does not seem likely.[20]

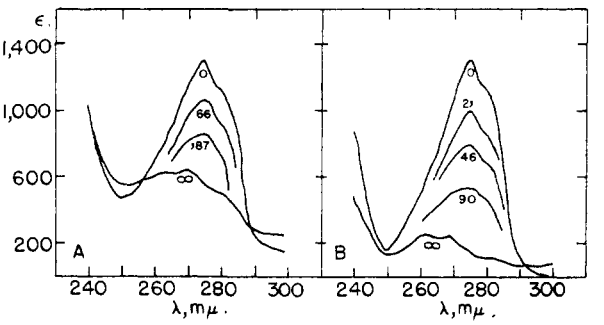


Fig. 3.—Change in ultraviolet absorption of peptides on reaction with *p*-nitrophenyl acetate, 25°; time indicated in minutes: A, cyclic hexapeptide I, 0.00065 *M*, *p*-NPA, 0.00554 *M*, *p*H 7.2; B, cyclic dipeptide II, 0.00061 *M*, *p*-NPA 0.00554 *M*, *p*H 6.5.

TABLE II

RATE CONSTANTS FOR PHENOL LIBERATION

ArOCOCH$_3$	σ	k_2, M^{-1} min.$^{-1}$ (p$K_{apparent}$) I[a]	II[a]	Imidazole[b]
2,4-$(NO_2)_2C_6H_3$–		93 (6.7)	45	170
4-$(NO_2)C_6H_4$–	1.27	7.3 (6.7)[c]	3.4 ((5.8)	21.3[d]
3-$(NO_2)C_6H_4$–	0.71		1.55 (6.2)	4.54[d]
3-$(CH_3CO)C_6H_4$–	.31	0.40 (6.7)[e]	0.20 (6.5)	1.4

[a] 0.09 *M* KCl, 0.01 *M* phosphate buffer, 9% (v./v.) ethanol, peptide 0.5 – 2.0 × 10^{-3} *M*, ester 0.5 – 4.0 × 10^{-3} *M*, 25°; values in parentheses are apparent kinetic pK's. [b] 0.2 *M* phosphate buffer, 28.5% (v./v.) ethanol, 30°. [c] 30°, k_2 = 6.0 at 25°. [d] Data of T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.*, **79**, 1663 (1957). [e] 30°.

In our previous note[2] it was reported that reaction of *p*-nitrophenyl acetate with the hexapeptide I resulted in acylation of tyrosine oxygen. The rate of this process has been followed directly. Aliquots reacting mixtures were acidified and extracted with chloroform to remove phenol and unreacted ester without loss of peptide; the peptide ultraviolet absorption was then measured. The nature of the spectral changes is shown in Fig. 3. By following the decrease in absorption at 275 mμ, acylation was shown to obey pseudo-first-order kinetics over the initial 50–70% of reaction under

(16) W. Kauzmann, "Advances in Protein Chemistry," Vol. XIV. Academic Press, Inc., New York, N. Y., 1959, pp. 37ff.

(17) H. Morawetz and J. Oreskes, *J. Am. Chem. Soc.*, **80**, 2591 (1958).

(18) J. W. Churchill, J. M. Lapkin, F. Martinez and J. A. Zaslowsky, *ibid.*, **80**, 1944 (1958).

(19) M. L. Bender, Y. L. Chow and F. Chloupek, *ibid.*, **80**, 5380 (1958).

(20) Prior formation of a peptide–ester complex to the extent required to produce the observed lowering of kinetic pK would result in less than first-order dependence on ester concentration in the range used and in a limiting reaction velocity at high ester concentration. Over the relevant range 0.5–7.0 × 10^{-3} *M* *p*-nitrophenyl acetate, reaction with II is cleanly first order in ester.

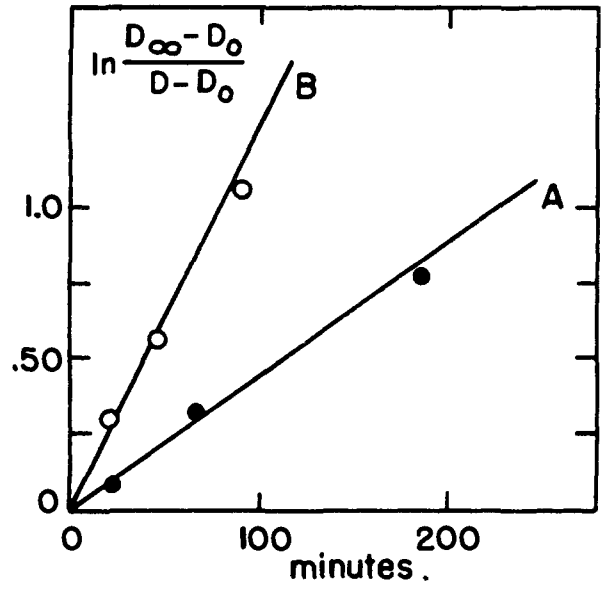


Fig. 4.—Pseudo-first-order rate plots constructed from points in Fig. 3.

the conditions used (Fig. 4). The second-order rate constants for phenol acylation so determined are compared in Table III with those for *p*-nitrophenol liberation under the same conditions.

TABLE III

RATE CONSTANTS FOR *p*-NITROPHENOL LIBERATION AND TYROSINE ACETYLATION[a]

Peptide, *M*	*p*-NPA, *M*	*p*H	k_{phenol}	k_{acyl}[b]
I, 0.00065	0.00554	7.2	4.5	0.80
I, .00032	.0038	6.9	3.9	0.60
II, .00109	.00393	7.0	3.2	2.9
II, .00061	.00554	6.5	2.8	2.3

[a] Rate constants in M^{-1} min.$^{-1}$; 0.09 *M* KCl, 0.01 *M* phosphate buffer, 9% (v./v.) ethanol, 25°. [b] From pseudo-first-order constants obtained as in Fig. 4 for at least 50% acetylation, by division by average NPA concentration.

That acylation of tyrosine hydroxyl is the reaction responsible for the spectral changes is confirmed by the similarity of the spectra of phenyl acetate (λ_{max} 255, 257 mμ, ε 190 in 0.1 *M* KCl) and those of the peptide reaction products at complete reaction (I, λ_{max} = 262, 269 mμ, ε ≈ 640; II, λ_{max} 262, 268 mμ, ε ≈ 245 for the reaction products). Acylation of the tyrosine side chain in *cyclo*-L-tyrosyl-L-histidyl (II) thus occurs almost quantitatively, while acylation of the corresponding residue in the cyclic hexapeptide I occurs in only one-fifth of the reactive collisions with *p*-nitrophenyl ester.[21]

(21) The conclusion reached in our earlier note (ref. 1) that acyl transfer to phenolic oxygen was quantitative in reaction of I with *p*-nitrophenyl acetate was based on downward curvature of pseudo-first-order plots of phenol liberation. Most of this decrease over the reaction times observed can, however, be accounted for by the build-up of catalytically ineffective acetylimidazole. Using the data of W. Jencks and J. Carriuolo, *J. Biol. Chem.*, **243**, 1272 (1959), one can estimate a rate constant for the hydrolysis of acetylimidazole in our medium. If the number so obtained (about 0.015 min.$^{-1}$) is assumed to be valid for imidazole-acetylated peptide, it can be calculated that upward of 50% of the histidine side chain should eventually be blocked by reaction with *p*-nitrophenyl acetate at the reactant concentrations employed. With 2,4-dinitrophenyl acetate virtually all of the peptide should be converted to the inactive acetylimidazole form.

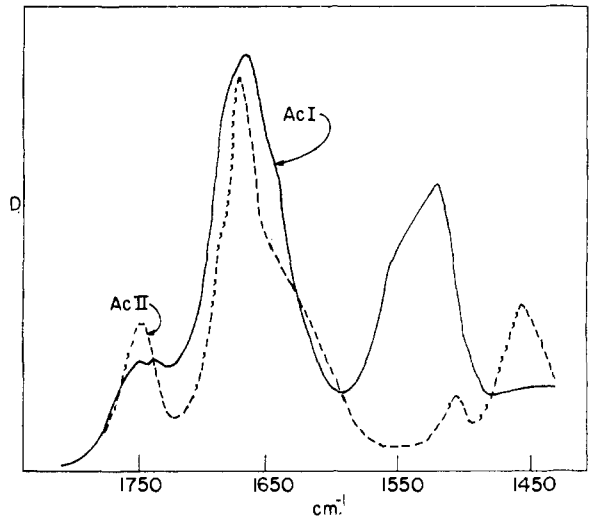
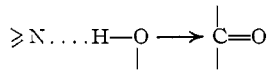


Fig. 5.—Carbonyl region infrared spectra of acetylated cyclic peptides; ordinate is an arbitrary optical density scale.

In view of the absence of a strong hydrogen bond between the tyrosine hydroxyl and imidazole, which would probably be a prerequisite for general base catalysis by imidazole of direct O-acetylation, *i.e.*



it is most likely that O-acetylation occurs by intramolecular transfer of acetyl from imidazole. To confirm this, however, demonstration will be required that rate of O-acetylation for a series of ester substrates runs parallel to the rate of phenol liberation.

Whether O-acetylation is direct or goes *via* intramolecular transfer, its occurrence requires a proximity between imidazole and hydroxyphenyl functions that molecule models of the two cyclic peptides and their acetyl derivatives do not prohibit but give no reason to predict. The ratio of O-acetylation to phenol liberation, greater for the diketopiperazine than for the cyclic hexapeptide, implies greater average side-chain proximity for the former. Whether this is a consequence of a greater rigidity of the planar diketopiperazine backbone or of the somewhat larger distance across the hexapeptide backbone is a question which may be answered by study of the same reaction using other isomers of the cyclic hexapeptide. The effect of changes in solvent on the rate ratios should also prove instructive.

Properties of the O-Acetyl Peptides.—Pure samples of the O-acetyl peptides have not been isolated from the buffered solutions in which reaction with phenyl acetates was carried out. However, acetylated peptides have been obtained by reaction of I and II with acetic anhydride or acetic anhydride in pyridine. These products possess ultraviolet absorptions identical to those of the *p*-nitrophenyl acetate reaction products. The presence of the acetoxyphenyl link is indicated by the appropriate infrared absorption: a doublet at 1747 and 1733 cm.$^{-1}$ in acetyl-I and a single sharp

band at 1750 cm.$^{-1}$ in acetyl-II (potassium bromide pellets) (see Fig. 5).

Preliminary experiments using O-acetyl-I and *p*-nitrophenyl acetate give for liberation of nitrophenol k_2 = 3.5 M^{-1} min.$^{-1}$ and an apparent kinetic pK of 6.4 – 6.5. Similar experiments with O-acetyl-II suggest that it is more reactive than its parent toward *p*-NPA.

Determination of the imidazole pK_A of the two acetylated materials gave a value of 6.4$_5$ for the derivative of *cyclo*-L-tyrosyl-L-histidyl, and 6.5$_5$ for that of the cyclic hexapeptide.

Although detailed work has not yet been carried out, it appears that the velocity of intramolecular imidazole-catalyzed hydrolysis of O-acetyl-I is negligible below pH 7.5.[22] Slow hydrolysis could be the result of exclusion of water from the appropriate site.

Experimental[23]

N-Carbobenzoxy-L-histidyl-L-tyrosine Methyl Ester.—An ethyl acetate solution of carbobenzoxy-L-histidine azide was prepared from 6.06 g. (0.02 mole) of carbobenzoxy-L-histidine hydrazide according to the method of Holley and Sondheimer,[24] and mixed with a suspension of 4.3 g. of L-tyrosine methyl ester (0.022 mole) in enough ethyl acetate to give a total volume of 250 ml. The mixture was stirred at 0° for 10 hr., at room temperature for another 12 hr., then filtered to remove traces of undissolved material, washed with 100 ml. of water, dried over magnesium sulfate and concentrated at reduced pressure to 100 ml. The solution was stored at 0–5° while crystallization occurred. Recrystallization of the product from ethanol–ethyl acetate gave 4.2 g. of analytically pure, chromatographically homogeneous dipeptide (45%), m.p. 140.5–142.5°, $[\alpha]^{25}D$ 9.0° (*c* 6, acetic acid).

Anal. Calcd. for $C_{24}H_{26}N_4O_6$: C, 61.79; H, 5.62; N, 12.01. Found: C, 61.66; H, 5.70; N, 11.94.

***cyclo*-L-Histidyl-L-tyrosyl (II).**—Carbobenzoxy-L-histidyl-L-tyrosine methyl ester (2.0 g.) was treated with 6 ml. of 5 *M* hydrogen bromide in acetic acid. Carbon dioxide evolution ceased after 20 min. and anhydrous ether was added to precipitate the dipeptide ester hydrobromide, which was triturated with ether until entirely crystalline, then washed with ether and dried in vacuum; yield 2.6 g.

The dipeptide ester hydrobromide was dissolved in 100 ml. of absolute methanol containing 2.5 ml. of 4 *M* sodium methoxide in methanol. The solution was heated under reflux 8 hr.; it then gave a negative ninhydrin reaction. Ethanol was removed under reduced pressure and the residue was taken up in 20 ml. of hot water to give an alkaline solution, which was filtered free of a small amount of amorphous material and adjusted to pH 8, then stored at 0–5° overnight while crystallization occurred. A total of 650 mg. of the diketopiperazine (51%) was collected, the last crop after concentration of the mother liquors. Recrystallization from hot water afforded an analytical sample, m.p. 274–276°dec., which gave no ninhydrin reaction and was chromatographically homogeneous. The ultraviolet spectrum of this peptide possessed a maximum at 275 mμ (ε 1300) in acidic or neutral solution (0.1 *M* KCl) and at 292 mμ (ε 2300) at pH 12.

Anal. Calcd. for $C_{15}H_{16}N_4O_3$: C, 59.99; H, 5.37; N, 18.66. Found: C, 59.85; H, 5.70; N, 18.6.

N-Carbobenzoxy-L-tyrosyldiglycine.[25]—Carbobenzoxy-L-tyrosyldiglycine ethyl ester[26] (2.75 g., 0.006 mole) was dissolved in 15 ml. of pyridine, 9 ml. of 1 *N* sodium hydroxide was added and the mixture was kept at room temperature for 1 hr. The solution was adjusted to *p*H 7–8 and concentrated under vacuum to a small volume; 100 ml. of cold water was added and the mixture made to acid to congo red by addition of 2 *N* hydrochloric acid. The precipitated acid had m.p. 214–215°, unchanged on recrystallization from ethanol–water, and was obtained in 65% yield, $[\alpha]^{24}_{4358}$ −7.5° (*c* 1, 70% acetic acid).

N-Carbobenzoxyglycyl-N^{im}-benzyl-L-histidine Hydrazide.—N^{im}-Benzyl-L-histidine[27] (12.3 g., 0.05 mole) was converted to the methyl ester dihydrochloride by treatment with dry hydrogen chloride–saturated methanol (250 ml.). The glassy product was stored in vacuum over potassium hydroxide.

A solution in 500 ml. of cold chloroform of carbobenzoxyglycine azide, prepared from 16.8 g. (0.075 mole) of carbobenzoxy glycine hydrazide,[28] was dried quickly over magnesium sulfate and combined with a solution of the above-prepared ester in 100 ml. of dry dimethylformamide. Triethylamine (14.0 ml., 0.1 mole) was added to the mixture, which was left overnight at room temperature, then washed with cold 4% bicarbonate, followed by water, dried over magnesium sulfate and concentrated to dryness at reduced pressure. The crude dipeptide ester was taken up in 100 ml. of absolute ethanol and mixed with 0.1 mole of 98% hydrazine. Crystalline hydrazide was collected by filtration after 24 hr. and a second crop was obtained upon concentration of the mother liquors. The combined crops were recrystallized from methanol to yield 13.1 g. of the dipeptide hydrazide (58%), m.p. 171–171.5°, $[\alpha]^{24}_{4358}$ −9.6° (*c* 1, 95% acetic acid).

Anal. Calcd. for $C_{23}H_{26}N_6O_4$: C, 61.32; H, 5.82; N, 18.66. Found: C, 61.72; H, 6.17; N, 18.55.

N-Carbobenzoxyglycyl-N^{im}-benzyl-L-histidylglycine-*p*-Nitrobenzyl Ester.—A solution of N-carbobenzoxy-N^{im}-benzyl-L-histidine hydrazide (9.0 g., 0.02 mole) in 60 ml. of 2 *N* hydrochloric acid was diazotized at 0° by addition of 1.5 g. of sodium nitrite; after 3 min., 50 ml. of cold 50% potassium carbonate was added. The precipitated oily azide was extracted into four 50-ml. portions of ethyl acetate; the extract was washed with a small amount of cold ether, dried over magnesium sulfate and mixed with 600 ml. of cold chloroform. Finely powdered glycine *p*-nitrobenzyl ester hydrobromide[29] (6.0 g., 0.02 mole) and 3 ml. of triethylamine were added. Held at 0° for 3 hr., the mixture rapidly cleared and then became milky. After 24 hr. at room temperature, 1 l. of chloroform was added and the solution was washed with cold water, 8% bicarbonate and again water. The solution was dried over magnesium sulfate and concentrated, at reduced pressure, to 400 ml. Upon cooling, the tripeptide crystallized and was collected by filtration, 8.6 g. An analytically pure product, m.p. 173–174°, $[\alpha]^{24}_{4358}$ −7.4° (*c* 1, 95% acetic acid), was obtained by recrystallization from 1:1 ethyl acetate–chloroform (6.9 g., 55%).

Anal. Calcd. for $C_{32}H_{32}O_8N_6$: C, 61.14; H, 5.13; N, 13.37. Found: C, 61.40; H, 5.13; N, 13.56.

N-Carbobenzoxyglycyl-N^{im}-benzyl-L-histidylglycine.—A suspension of the nitrobenzyl ester (1.6 g., 0.025 mole) in a mixture of 60 ml. of 50% ethanol and 50 ml. of 1 *N* sodium hydroxide was stirred at room temperature. After 1 hr. the resulting clear solution was adjusted to pH 6 by addition of 6 *N* hydrochloric acid and cooled. The precipitated product was collected by filtration and recrystallized from 95% ethanol to afford 1.01 g. (80%) of tripeptide acid, m.p. 202–204°, $[\alpha]^{24}_{4358}$ 1.3° (*c* 1, 95% acetic acid).

Anal. Calcd. for $C_{25}H_{27}O_6N_5$: C, 60.84; H, 5.51; N, 14.19. Found: C, 60.94; H, 5.70; N, 14.01.

Glycyl-N^{im}-benzyl-L-histidylglycine *p*-Nitrobenzyl Ester Dihydrobromide.—To a solution of carbobenzoxy-glycyl-N^{im}-benzyl-L-histidylglycine nitrobenzyl ester (5.8 g.) in

(22) T. C. Bruice and J. M. Sturtevant, *J. Am. Chem. Soc.*, **81**, 2860 (1959), report for the intramolecular reaction of phenyl 4-(4-imidazolyl)-butyrate a first-order rate constant (25°) of 2.58 min.$^{-1}$. At *p*H 8.3 and 30° the first-order rate constant is probably less than 0.02 min.$^{-1}$ for hydrolysis of acetyl I.

(23) We are indebted to Mr. William Saschek of this Laboratory for the microanalyses reported herein. All those products for which analyses are reported were chromatographically homogeneous in at least two solvent systems. All melting points are corrected.

(24) R. W. Holley and E. Sondheimer, *J. Am. Chem. Soc.*, **76**, 1326 (1954).

(25) E. Abderhalden, R. Abderhalden, H. Weidle, E. Baertich and W. Morneweg, *Fermentforsch.*, **16**, 98 (1938).

(26) G. W. Anderson, J. Blodinger and A. D. Welcher, *J. Am. Chem. Soc.*, **74**, 5309 (1952).

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

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TABLE IV

ESTIMATED CONCENTRATIONS OF HYDROGENOLYSIS PRODUCTS AT VARYING REACTION TIMES

Substance (R_f)	Reaction time, hours: 0	3	5.5	8.5	12	21	24
A (0.08)	−	+	+	+	++	+++	++++
B (.33)	−	+	++	+++	+++	++++	++++
C (.54)	−	+	+	+	+	−	−
D (.74)	−	++++	++++	++++	+++	+	−
E (.86)	++++	++++	++++	+	+	−	−

50 ml. of glacial acetic acid was added 20 ml. of 5.1 *M* hydrogen bromide in glacial acetic acid. After 20 min., 600 ml. of dry ether was added and the oil thrown down was triturated with fresh portions of ether until it solidified. This product was collected on a fritted glass funnel, washed further with ether and stored in vacuum over potassium hydroxide until used.

N-Carbobenzoxy-L-tyrosyltriglycyl-N^{im}-benzyl-L-histidylglycine *p*-Nitrobenzyl Ester (III).—In 100 ml. of cold, freshly distilled dimethylformamide were combined 7.8 g. (0.018 mole) of carbobenzoxy-L-tyrosyldiglycine, 15.0 g. of crude glycyl-N^{im}-benzyl-L-histidylglycine nitrobenzyl ester dihydrobromide (about 0.023 mole), 3.60 ml. of N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide (0.023 mole) and 3.2 ml. of triethylamine (0.023 mole). The resulting clear solution was stored in the dark at room temperature and the progress of reaction followed chromatographically. After 6 days, 300 ml. of cold water was added and the resulting amorphous precipitate was triturated with two 250-ml. portions of cold water. It was then dissolved in 200 ml. of boiling ethanol from which, upon cooling, it precipitated in an apparently amorphous, but readily filtered, form. Concentration of the ethanol mother liquors afforded a second crop of product, to give a total of 11 g. (74%).

Although this product did not possess a definite m.p., it was negative to ninhydrin and gave clean single spots on paper chromatograms using 4:1 *sec*-butyl alcohol–water (R_f 0.85) or 4:1:1 *sec*-butyl alcohol–water–acetic acid (R_f 0.86).

An analytical sample was prepared by several reprecipitations from ethanol and dried in vacuum at 100°. It had $[\alpha]^{24}_{4358}$ 3.3° (*c* 1, 95% acetic acid).

Anal. Calcd. for $C_{45}H_{47}O_{12}N_9$: C, 59.68; H, 5.22; N, 13.92. Found: C, 59.26; H, 5.39; N, 14.02.

L-Tyrosyltriglycyl-N^{im}-benzyl-L-histidylglycine (IV). A. By Hydrogenolysis Alone.—Hydrogen was bubbled for 7 hr. through an acetic acid solution of 1.0 g. of blocked hexapeptide III in which was suspended 200 mg. of 10% palladium-on-charcoal. After removal of catalyst by filtration the solution was lyophilized and the residue was dissolved in water and extracted with ether. The ether-washed aqueous solution was lyophilized and the resulting product was chromatographed on a 100 g. of cellulose powder column using 4:1 *sec*-butyl alcohol–water. Those eluate fractions corresponding to pure IV, as determined by paper chromatograms, were concentrated in vacuum; the residue was lyophilized from water to give 320 mg. (46%) of pure product, which retained one molecule of water after drying at 100° in vacuum for 48 hr.

Anal. Calcd. for $C_{30}H_{36}N_8O_8 \cdot H_2O$: C, 55.03; H, 5.85; N, 17.11. Found: C, 55.17; H, 6.25; N, 16.84.

The course of the hydrogenolysis was followed in a preliminary experiment by paper chromatography of samples removed at varying reaction times. The chromatograms were developed using 4:1:1 *sec*-butyl alcohol–water–acetic acid. Five components were detected; Table IV gives the variation in these substances with reaction time as estimated from the color produced by coupling with diazotized *p*-bromoaniline.

Component A is assigned the structure of the completely unprotected hexapeptide, tyrosyltriglycylhistidylglycine, on the basis of its low R_f value and the fact that it afforded a bright red spot with the diazo spray used. Only imidazoles with ring N–H undergo diazo coupling, and the color developed by tyrosine derivatives is orange–yellow.

Component B is the desired product IV.

Component C is the decarbobenzoxylated material, tyrosyltriglycylbenzylhistidylglycine nitrobenzyl ester, since its R_f value is identical to that of the hydrogen bromide cleavage product (see below).

Component D is the free acid, carbobenzoxytyrosyltriglycylbenzylhistidylglycine, which was isolated in a separate experiment by cellulose column chromatography (*sec*-butyl alcohol–water) from the products of brief hydrogenolysis of III. An analytical sample was prepared by recrystallization from ethanol.

Anal. Calcd. for $C_{38}H_{42}N_8O_{10}$: C, 59.23; H, 5.49; N, 14.54. Found: C, 59.25; H, 5.63; N, 14.45.

Component E is starting material.

B. By Solvolysis and Hydrogenolysis.—A solution of 9.0 g. of III in 50 ml. of glacial acetic acid was mixed with 50 ml. of 5 *N* hydrogen bromide in acetic acid. After 20 min., 600 ml. of dry ether was added and the precipitated solid was triturated with ether, collected on a glass frit funnel, washed with more ether and dried in vacuum overnight.

The resulting L-tyrosyltriglycyl-N^{im}-benzyl-L-histidylglycine *p*-nitrobenzyl ester dihydrobromide was not soluble in acetic acid alone. It was dissolved in a mixture of 150 ml. of dimethylformamide and 50 ml. of acetic acid and was hydrogenated for 2 hr. over 2 g. of 10% palladium-on-charcoal. The catalyst was removed by centrifugation and the solvent stripped by distillation at reduced pressure. Purification was achieved in a manner very similar to that described in section A above. A colorless, amorphous, but chromatographically pure, product containing 1.7 moles of hydrogen bromide was so obtained (4.12 g., 59%). The analytical sample was dried in vacuum at 100° for 24 hr.

Anal. Calcd. for $C_{30}H_{36}N_8O_8 + 1.7$ HBr: C, 46.63; H, 4.96; N, 14.50. Found: C, 46.33; H, 4.94; N, 14.04.

Cyclo-L-tyrosyltriglycyl-N^{im}-benzyl-L-histidylglycyl (V).—To a solution of IV "dihydrobromide" (2.455 g., 3.08 mmoles) in 150 ml. of dimethylformamide was added 50 ml. of the same solvent containing 2 equivalents (0.96 g.) of N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide. The solution was kept in the dark for 2 days before another 0.96-g. portion of carbodiimide was added. The progress of cyclization was followed by paper chromatography of withdrawn samples. When reaction appeared complete, after a total of 7 days, solvent was removed in vacuum and the residual oil was treated with small portions of water until it solidified. Chromatography on a 1-kg. cellulose powder column (Whatman Standard Grade) using 4:1 *sec*-butyl alcohol–water resulted in isolation of the cyclic peptide in 58% yield (1.10 g.).

This product was ninhydrin-negative and produced on paper a yellow color with diazotized *p*-bromoaniline. It gave a single spot on paper chromatography in each of seven solvent systems ranging from butanol–water–formic acid[30] (R_f 0.28) to pyridine (50)–acetic acid (35)–water(15) (R_f 0.88). Upon acid hydrolysis it yielded tyrosine, glycine and *im*-benzylhistidine. It was not detectably cleaved after storage for one week in either aqueous alcohol or 0.12 *N* perchloric acid in 90% acetic acid. The average of 5 isopiestic molecular weight determinations in trifluoroacetic acid was 305 ± 75. Assuming complete monoprotonation, the calculated molecular weight is 618/2 = 309.

An analytical sample was obtained directly from the cellulose powder column and dried 48 hr. in vacuum at 100°. It had $[\alpha]^{24}_{4358}$ −39.5° (*c* 0.2, 95% acetic acid).

Anal. Calcd. for $C_{30}H_{34}N_8O_7$: C, 58.25; H, 5.54; N, 18.11. Found: C, 58.05; H, 5.77; N, 17.62.

***cyclo*-L-Tyrosyltriglycyl-L-histidylglycyl (I).**—Into about 600 ml. of liquid ammonia, freshly distilled from sodium, was dropped 765 mg. of the N^{im}-benzyl cyclic peptide V. A small piece of sodium was added to the yellow solution. When the blue color had persisted for 3 min. excess sodium was destroyed with acetic acid and the ammonia was re-

(30) L. F. Wiggins and J. H. Williams, *Nature*, **170**, 279 (1952).

moved rapidly under reduced pressure. The residual white solid was chromatographed on a 1-kg. cellulose powder column using 4:1 *sec*-butylalcohol–acetic acid. Two passes through the column was required to effect complete separation of the desired product. Those fractions containing it were concentrated under vacuum. The residue was dissolved in water and extracted with chloroform and ethyl acetate; it was then recovered as a white powder, 242 mg. (37%) by lyophilization of the aqueous phase.

This product was negative to ninhydrin and gave a red color (on paper) with diazotized *p*-bromoaniline (unblocked imidazole). It formed single spots on paper chromatography in seven solvent systems ranging from R_f 0.05 (butanol–water–formic acid) to R_f 0.75 (pyridine–water–acetic acid). On hydrolysis it yielded glycine, histidine and tyrosine. The ultraviolet absorption spectrum possessed a maximum at 275 mμ (ε 1300) in 0.1 *N* KCl at neutrality, and at 292 mμ (ε 2150) at pH 12.

An analytical sample was dried in vacuum at 100° for 24 hr.

Anal. Calcd. for $C_{23}H_{28}N_8O_7$: C, 52.26; H, 5.34; N, 21.20. Found: C, 52.36; H, 5.73; N, 20.40.

N-Ethyl-N′-(3-dimethylaminopropyl)-carbodiimide.[31]—Ethyl isothiocyanate (25 g., 0.29 mole) in dry ether was dropped slowly into an ethereal solution of 3-dimethylaminopropylamine (28 g., 0.28 mole); the resulting solution was heated under reflux for 1 hr. and stored at room temperature overnight. A benzene solution (400 ml.) of the oil obtained upon removal of ether was warmed with 75 g. of yellow mercuric oxide and about 50 ml. of solvent was distilled off. After 30 min. the black mercuric sulfide was removed by filtration, fresh oxide was added and the process repeated. A third charge of mercuric oxide (40 g.) was introduced after 4 hr. and the mixture was boiled under reflux for 18 hr. The suspension was then centrifuged and the benzene removed from the supernatant at reduced pressure. The liquid carbodiimide was distilled at 1 mm. pressure at 45° and was obtained as 27 g. of a colorless liquid (58%).

Anal. Calcd. for $C_8H_{17}N_3$: C, 61.89; H, 11.04; N, 27.07. Found: C, 62.30; H, 11.03; N, 25.95.

N-Carbobenzoxy-L-tyrosyldiglycine Hydrazide.—Carbobenzoxy-L-tyrosyldiglycine ester[26] (2.0 g., 0.0043 mole) was dissolved in ethanol and mixed with 0.14 ml. of 98% hydrazine (2 equiv.). After 24 hr. storage at room temperature the precipitated product was collected (1.8 g., 93%) and recrystallized from water to yield a product of m.p. 167–169°.

Anal. Calcd. for $C_{21}H_{25}O_6N_5$: C, 56.87; H, 5.68. Found: C, 57.42; H, 5.84.

N-Carbobenzoxyglycyl-L-histidylglycine.—Carbobenzoxyglycyl-L-histidylglycine ethyl ester[32] (4.6 g., 0.011 mole) was dissolved in a mixture of 130 ml. of methanol, 40 ml. of water and 10.7 ml. of 0.1 *N* sodium hydroxide. The solution was kept at room temperature for 1.5 hr., then cooled and adjusted to pH 6.5 using 6 *N* hydrochloric acid. Product separated after several hours and was collected by filtration. Recrystallization from water gave a product of m.p. 170–173°, $[\alpha]^{24}_{4358}$ −7.2° (*c* 1, 95% acetic acid), yield 70%.

Anal. Calcd. for $C_{18}H_{21}O_6N_5$: C, 53.59; H, 5.25; N, 17.36. Found: C, 52.79; H, 5.56; N, 17.03.

Glycyl-L-histidylglycine.—Carbobenzoxyglycyl-L-histidylglycine ester[32] was decarbobenzoxylated using hydrogen bromide in acetic acid.[33] The tripeptide ester hydrobromide so produced was dissolved in 3.1 molar equivalents of 1 *N* sodium hydroxide and stored thus for 2 hours before the solution was cooled and adjusted to pH 7.5 by means of 2 *N* hydrochloric acid. The free tripeptide crystallized slowly and was collected after several days at 0–5°; obtained in 31% yield after recrystallization from water, it had m.p. 224° and $[\alpha]^{24}_{4358}$ −6.0° (*c* 0.25, 95% acetic acid).

Anal. Calcd. for $C_{10}H_{15}N_5O_4$: C, 44.60; H, 5.62. Found: C, 44.45; H, 5.70.

Preparation of O-Acetyl Peptides. I.—Ten mg. of cyclic peptide I was mixed with about 0.1 ml. of acetic anhydride, and the mixture was stored at room temperature for 90 min. (solution of the peptide occurred after about 30 min.). There was then added 0.4 ml. of 0.05 *N* hydrochloric acid; after a further 60 min. the solution was lyophilized. The product was again lyophilized from water.

The infrared spectrum (carbonyl region) of this product in potassium bromide pellet is shown in Fig. 5. The ultraviolet spectrum of an aqueous solution (pH 4–5) exhibited maxima at 263 and 269 mμ; when this solution was made alkaline (pH 11), stored for about 30 minutes and then reacidified, the maximum had shifted to 275 mμ and the absorption intensity doubled.

II.—Treatment of the cyclic dipeptide II with acetic anhydride alone did not result in its solution or acetylation. To a mixture of 1.5 ml. of dry pyridine and 0.4 ml. of acetic anhyride was added 90 mg. of II. The mixture was warmed on the steam-bath for 5 minutes to effect solution and the solution was then stored an hour at room temperature before concentration at 70° and 0.1 mm. The residue was stored over phosphorus pentoxide in vacuum overnight; it was then washed several times with 3-ml. portions of water (centrifugation), taken up in 2 ml. of 0.1 *N* hydrochloric acid and lyophilized. The infrared spectrum of this material is given in Fig. 5 (potassium bromide pellet). In aqueous solution it exhibited ultraviolet absorption maxima at 268 and 261 mμ, ε 250–300.

A sample of this product was dried at room temperature over phosphorus pentoxide and submitted for analysis. Agreement was obtained for O-acetyl II hydrochloride plus 7% water (about 1.5 equiv.).

Determination of pK_A's. Imidazoles.—Solutions of 0.01–0.02 mmole of peptide in 10 ml. of 0.1 *M* potassium chloride were titrated with 0.1 *N* sodium hydroxide at 24–25°, using a Radiometer SBR 2c Titrigraph. The pK_A values were taken from a plot of the derivative of the titration curve *vs.* pH. **Phenols.**—About 10^{-4} *M* solutions of peptide in 0.1 *M* potassium chloride were adjusted to various pH values by means of 0.1 *N* sodium hydroxide, the pH being measured with a Beckman model G pH meter equipped with a Type E glass electrode. The ultraviolet spectra of these solutions were measured between 2300 and 4000 Å. using a Cary Model 14 recording spectrophotometer. Measurements were made at 24–25°. The extent of phenol ionization as determined from the optical density at 2950 Å. was used in constructing titration curves, and the pK_A's determined from the derivatives of these curves. Values of pK_A's given in Table I are the result of at least two determinations.

Kinetic Measurements. A. Phenol Liberation.—Rates of phenol liberation were determined by following the ultraviolet absorption of liberated phenol using a Cary model 14 spectrophotometer with thermostated cell jacket. *p*-Nitrophenol was determined from the absorption maximum of its anion at 400 mμ, *m*-nitrophenol from absorption at the isosbestic point between it and its anion at 352 mμ, and *m*-acetylphenol from absorption at its maximum, 308 mμ. In most cases second-order rate constants were determined from pseudo-first order rate constants for initial stages of reaction, after correction for blank rates of ester hydrolysis, by division by average ester concentration over the period of measurement. In many cases true second-order plots were made, without correcting for the first-order ester blank reaction. These were usually linear out to 50% reaction based on peptide and gave second-order constants in agreement with those determined as first described. The concentration of ester used varied between 0.007 *M* and 0.002 *M*, and that of peptide between 0.004 *M* and 0.0003 *M*. **B. Acetylation.**—A solution of peptide in 0.091 *M* potassium chloride–0.0091 *M* phosphate at the appropriate pH was mixed with 0.1 its volume of an ethanol solution of *p*-nitrophenyl acetate. The mixture was stored at constant temperature. For each point a 2-ml. aliquot was pipetted into a test-tube containing one drop of glacial acetic acid; this quenched solution was extracted with four 2 ml. portions of chlorform. (Layers were separated by centrifugation and the chloroform layer withdrawn by a capillary dropper.) The aqueous solution was freed of chloroform by bubbling nitrogen through it for several minutes, and its spectrum was then measured.

Although the hexapeptide was readily soluble in water at the pH range used, the diketopiperazine was used in super-

(31) This carbodiimide has been reported (ref. 13) but was prepared independently by us by the method given here.

(32) R. F. Fischer and R. R. Whetstone, *J. Am. Chem. Soc.*, **76**, 5076 (1954).

(33) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952); D. Ben-Ishai, *ibid.*, **19**, 62 (1954).

saturated solution for all experiments, its solubility in neutral solution being less than 0.2 mg./ml. Solutions of the latter were prepared at 100°; at room temperature they persisted without crystallization for several days. That no hydrolysis occurred in their preparation was demonstrated by the absence of a ninhydrin reaction.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TEXAS, AUSTIN, TEX.]

Comparison of Ribose and Deoxyribose Nucleosides by N.m.r. and Deductions Regarding Ribose and Deoxyribose Nucleic Acids. I. Tautomeric Form

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The 60-megacycle n.m.r. spectra in dimethyl sulfoxide of the eight nucleosides found in ribose and deoxyribose nucleic acids are characterized and compared. Exchangeable proton peaks are identified. It is concluded that cytidine has the amino form but deoxycytidine has the imino form. Biological implications are outlined.

Introduction

The n.m.r. spectra of adenosine, cytidine and uridine have been studied in D_2O at varying pH.[1] Conformation of the ribose ring in these compounds was suggested[2] from the theoretical work of Karplus[3] and the observed H'_1 coupling constants.

It is apparent that considerable study of the nucleic acid derivatives, particularly with regard to ring conformation and tautomeric form, has been made with n.m.r. The importance of such studies is obvious. However, no attempt has been made to formulate a comprehensive picture of the implica-

TABLE I

CHEMICAL SHIFTS[a] AND COUPLING CONSTANTS[b] OF THE NUCLEOSIDES IN DIMETHYL SULFOXIDE

Nucleoside	H_1' ±0.01	H_2' ±0.01	H_3' ±0.01	H_4'	H_5'	OH_2' ±0.05	OH_3' ±0.05	OH_5' ±0.05	H_2 ±0.01	H_8 ±0.01	H_5 ±0.01	H_6 ±0.01	NH_2 ±0.05	NH	NH
A	0.55	2.25–2.33	1.83	2.42–2.58	2.75–2.92	*J* 4.5 1.27	0.97	*J* 5.4 1.05	−1.68	−1.87			−0.86		
dA	.09	2.75–3.00	1.92–2.08	2.50–2.67	2.75–3.00		1.12	*J* 5.6 1.16	−1.70	−1.87			− .87		
G	.71	2.25–2.33	2.00	2.53	2.75–3.00	1.25–1.42	*J* 5.7 1.02	1.25–1.33		−1.52			− .06	−4.33–4.42	
dG	.31	2.75–3.08	2.08–2.17	2.58–2.75	2.75–3.08		1.17–1.25	1.42–1.50		−1.50			− .05	−4.17–4.50	
C	.70	2.25–2.58	2.25–2.58	2.25–2.58	2.67–2.92						*J* 7.3 0.38	*J* 7.3 −1.7	− .17–.33		
dC	.39	4.22	2.18	2.60	2.75–2.92						*J* 7.9 0.22	*J* 7.9 −1.83		−2.25–2.33	−3.42–3.50
U	.70	2.42–2.67	2.42–2.67	2.42–2.67	2.75–3.08	1.33–1.58	1.08–1.25	1.33–1.58			*J* 8.2 0.82	*J* 8.2 −1.42		−4.92	−4.67
T	.31	4.40	2.17–2.25	2.58–2.83	2.75–3.08		1.31	1.53	4.72			−1.21		−4.72	

[a] All shifts in p.p.m. measured from aromatic toluene peak. The aromatic toluene peak was measured to be 6.86 p.p.m. below the peak of a tetramethylsilane internal standard. [b] *J* in c.p.s. ± 0.5 c.p.s.

A similar, more detailed analysis of the n.m.r. spectra of synthetic α- and β-thymidine in D_2O has been done.[4] Also a conformational study of the n.m.r. spectrum of deoxyuridine in D_2O has been reported with a brief, generalized reference to other deoxyribose nucleosides and nucleotides.[5] Since deoxyuridine does not occur in the nucleic acids, it is not immediately pertinent to our discussion.

Recently the 40-megacycle n.m.r. spectra of adenosine, guanosine, cytidine, uridine and thymidine in d_6-dimethyl sulfoxide were reported; but resolution and spectral analysis were limited.[6] The deoxyribose nucleosides, deoxycytidine, deoxyadenosine and deoxyguanosine, were not studied.

(1) C. D. Jardetzky and O. Jardetzky, *J. Am. Chem. Soc.*, **82**, 222 (1960).
(2) C. D. Jardetzky, *ibid.*, **82**, 229 (1960).
(3) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).
(4) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1960).
(5) C. D. Jardetzky, *J. Am. Chem. Soc.*, **82**, 2919 (1961).
(6) J. P. Kokko, J. H. Goldstein and Leon Mandell, *ibid.*, **83**, 2909 (1961).

tions of these studies concerning DNA-RNA structure and interaction.

The present work presents the first complete set of 60-megacycle n.m.r. spectra of all eight major ribose and deoxyribose nucleosides found in nucleic acids, in a common solvent (dimethyl sulfoxide), at the same pH, with the exchangeable proton peaks present. Cytidine and deoxycytidine are exceptions in that cytidine is isolated as the hemisulfate and deoxycytidine as the hydrochloride. The slight acidity thus introduced is evidenced only by the lack of exchangeable ring hydroxyl proton peaks. However, since the exchangeable NH_2 and NH protons still appear, it is felt that the comparability of these spectra has not been significantly altered. There are no significant differences in parameters and general spectral character of the nucleosides in dimethyl sulfoxide and D_2O except that the exchangeable protons do not appear in the latter. Hence conclusions are most probably applicable to biological systems.