For a number of amines including NH_3, $NH_2CH_2CH_2NH_2$, $C_2H_5NH_2$, CH_3NH_2 and $(C_2H_5)_2NH$, the rate-law was found to be

$$-d[CO]/dt = k_{exp}[CO][AgL_2^+][HL^+]^{-1} \quad (2)$$

which is kinetically equivalent to

$$-d[CO]/dt = k[CO][L\text{-}Ag\text{-}OH] \quad (3)$$

For the amines listed above, k_{exp} at 25° increased by a factor of about 140 in going along the series from NH_3 to $(C_2H_5)_2NH$. In each case the rate-determining reaction apparently was homogeneous and uninfluenced, at least over a considerable portion of the reaction, by the precipitated silver or other surface effects.

Evidence has been advanced[2] previously that the reduction of Hg^{2+} by CO in aqueous solution proceeds through an intermediate, $[—Hg—CO—OH]^+$, formed by "insertion" of CO between Hg^{2+} and a ligand water molecule, while analogous intermediates have been postulated in the Hg^{2+}- and Ag^+-catalyzed reduction of MnO_4^- by CO. It is noteworthy that the kinetics in the present instance also find a natural interpretation, according to the mechanism (4),(5), and (6), in terms of such an intermediate

$$AgL_2^+ + H_2O \overset{K}{\rightleftharpoons} L\text{-}Ag\text{-}OH + HL^+ \quad \text{(Rapid equilibrium)} \quad (4)$$

$$L\text{-}Ag\text{-}OH + CO \xrightarrow{k} L\text{-}Ag\text{-}C(=O)\text{-}OH \quad \text{(Rate-determining)} \quad (5)$$

$$L\text{-}Ag\text{-}C(=O)\text{-}OH + L\text{-}Ag\text{-}OH \longrightarrow 2Ag + CO_3^{=} + 2HL^+ \quad \text{(Rapid)} \quad (6)$$

The apparent rate constant, k_{exp}, and the rate constant, k, defined by equation 5 are thus related through

$$k_{exp} = kK = kK_dK_bK_h \quad (7)$$

where K_d is the first dissociation constant of AgL_2^+ (*i.e.*, $AgL_2 \rightleftarrows AgL^+ + L$), K_b is the basicity constant of the amine (*i.e.*, $L + H_2O \rightleftarrows HL^+ + OH^-$) and K_h is the association constant of AgL^+ with OH^- ($AgL^+ + OH^- \rightleftarrows L\text{-}Ag\text{-}OH$). Table I reveals that for the series of monodentate amines examined, the variation in k_{exp} is accounted for in large measure by the variation in K_dK_b while the value of kK_h ($\sim 2 \times 10^5$) is relatively insensitive to the nature of L (the somewhat lower value for ethylenediamine is attributable to chelation effects). If K_h (which also is probably insensitive to the nature of L) is taken to be of the same order, *i.e.*, $\sim 2 \times 10^2$, as for the uncomplexed Ag^+ ion,[3] the value of k is found to be about 10^3 M^{-1} sec.$^{-1}$.

With NH_3 as ligand some departure from the kinetics described above was observed at high NH_4^+ concentrations when the kinetics approached the form predicted by the above mechanism under

$$-d[CO]/dt \propto [CO][AgL_2^+]^2[HL^+]^{-2} \quad (8)$$

conditions when (because of lowering of the concentration of L–Ag–OH by reverse displacement of equilibrium 4) reaction 6 is slowed down to the point where competition between this step and reversal of step 5 now determines the rate.

Among other things it would appear from these considerations that the increase in pH, rather than effects associated specifically with complexing of the Ag^+ ion, is primarily responsible for the markedly enhanced reactivity of the latter toward CO on going from acidic to amine-buffered media.

Support of this work by the Alfred P. Sloan Foundation and the National Research Council of Canada is gratefully acknowledged.

TABLE I

SUMMARY OF KINETIC AND RELATED THERMODYNAMIC DATA AT 25°

Amine (L)	k_{exp}, sec.$^{-1}$	$K_d \times 10^4$,[a] M	$K_b \times 10^4$,[a] M	$kK_h \times 10^{-5}$,[b] M^{-2} sec.$^{-1}$
NH_3	5.3×10^{-4}	1.2	0.18	2.5
$NH_2CH_2CH_2NH_2$	1.1×10^{-2}	12	1.5	0.61
$C_2H_5NH_2$	1.6×10^{-2}	1.2	6.4	2.1
CH_3NH_2	3.0×10^{-2}	3.0	5.2	1.9
$(C_2H_5)_2NH$	7.6×10^{-2}	5.0	9.1	1.7

[a] Values of K_d and K_b from ref. 4 corrected[1] where necessary to 25°. [b] Computed from $kK_h = k_{exp}/K_dK_b$.

(1) A. C. Harkness and J. Halpern, *J. Am. Chem. Soc.*, **83**, 1258 (1961).

(2) H. L. Johnston, F. Cuta and A. B. Garrett, *ibid.*, **55**, 2311 (1933); J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1957, p. 69.

(4) "Stability Constants," Chemical Society Special Publications, No. 6 (1957), No. 7 (1958).

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RECEIVED AUGUST 25, 1961

REACTIVITY OF CYCLIC PEPTIDES. I. TRANSANNULAR HISTIDINE-O-ACETYLTYROSINE INTERACTION

Sir:

We have prepared *cyclo*-glycyl-L-histidylglycyl-L-tyrosylglycylglycyl as part of a program on side-chain reactivity in cyclic peptides. Reaction of this imidazole-containing peptide with one equivalent of *p*-nitro- or 2,4-dinitrophenyl acetate results in acylation of the phenolic oxygen and, as a consequence of imidazole-acetoxyphenyl coördination, virtually complete inhibition of further esterolytic activity. The interaction implied by this observation, requiring, as it does, 16-membered ring formation, would be unexpected in the absence of some secondary structure in the peptide molecule. Its occurrence in the present case suggests that cyclic peptides may be valuable models for study of protein side-chain interactions.

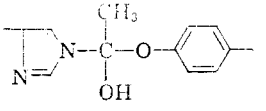
Cyclization, without especial dilution, of L-tyrosyltriglycyl-*im*-benzyl-L-histidylglycine, using ethyl-γ-dimethylaminopropylcarbodiimide in dimethylformamide, yielded 58% of the imidazole-blocked cyclic peptide, purified by partition chromatography using 2-butanol/water (4:1) on cellulose powder. Debenzylation was effected by brief treatment with sodium in liquid ammonia, and the final product was isolated as in the preceding step (37%). This material was chromatographically homogeneous in seven different solvent systems, ninhydrin inactive, and yielded only histidine, tyrosine and glycine on hydrolysis.

Anal. Calcd. for $C_{23}H_{28}N_8O_7$: C, 52.26; H, 5.34; N, 21.20; mol. wt. (in trifluoroacetic acid), 264.2. Found: C, 52.36; H, 5.73; N, 20.40; mol. wt., 254. In 0.1 potassium chloride the peptide exhibited pK_a's 6.65 ± 0.1 and 10.05 ± 0.1, within experimental error identical with those obtained for carbobenzoxyglycyl-L-histidylglycine ethyl ester and carbobenzoxy-L-tyrosyldiglycine hydrazide, respectively. There is thus no strong hydrogen-bonded interaction between histidine and tyrosine side chains of the cyclic peptide.[1]

First order rate plots of phenol liberation during reaction of cyclic peptide with nitro- and dinitrophenyl acetates are curved and asymptotic to a slope only slightly greater than the blank hydrolysis rate, indicating that the peptide is not acting catalytically. Where the contribution of the first order blank rate is negligible (up to 40% conversion of peptide at 10^{-3} *M* peptide and 3×10^{-3} *M* nitrophenyl acetate) reaction can be analyzed as second order, first order in peptide and ester. The rate constants so determined are identical with those from the initial slopes of the first order plots. Stoichiometric conversion of peptide to inactive acyl derivative is therefore indicated. The rate constants themselves (based on free imidazole) are, for *p*-nitrophenyl acetate, 6.0 M^{-1} min.$^{-1}$ and, for 2,4-dinitrophenyl acetate, 75 M^{-1} min.$^{-1}$ at 25°. These values correspond well to those for reaction of imidazoles with these esters[2] and suggest initial reaction at the imidazole site. The esterolytic activity of the acetylated peptide is <0.1 that of starting material.

Acetylated peptide may be recovered from reaction mixtures with phenyl acetates or it may be prepared directly using acetic anhydride. As the hydrochloride this substance exhibits the infrared absorption at 1740 cm.$^{-1}$ (KBr pellet) expected of a phenyl ester; this absorption is absent in the spectrum of the neutral species. The ultraviolet absorption maximum measured at *p*H 6.7 is shifted to 267 mμ (ϵ = 430) from 275 mμ (ϵ = 1330), a change comparable to that observed in going from phenol (λ_{max} 270 mμ, ϵ 1400) to phenyl acetate (λ_{max} 257 mμ, ϵ 190). In addition, the pK_a of the imidazole residue is lowered to 6.3 ± 0.1, suggesting imidazole-acetoxyphenyl coördination. Hydrolytic loss of the acetyl group is undetectable below pH 8.5.

Examination of molecular models of the cyclic peptides does not reveal any compelling requirement that the histidyl and tyrosyl side-chains be in close proximity, although a tetrahedral structure of the type is constructed readily. Bruice and



Sturtevant have reported kinetic evidence for this bonding in phenyl γ-(4-imidazolyl)-butyric esters; the resulting ring is, of course, six-membered.[3] In the present case the quantitative acyl transfer to tyrosine rather than to water, plus the heightened imidazole acidity and inhibited esterolytic activity of the acetyl derivative argue for a related interaction. For this to occur there must exist considerable rigidity of the cyclic peptide backbone[4] and perhaps a contribution from the structure of the solvent water to the orientation of the side chains.[5] A comparison of the properties of the "1,4" isomer of the present peptide and a study of the effects of variation in solvent are expected to shed more light on the matter.

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(2) T. C. Bruice and G. L. Schmir, *ibid.*, **80**, 148 (1958); M. L. Bender and B. W. Turnquest, *ibid.*, **79**, 1657 (1957).

(3) T. C. Bruice and J. M. Sturtevant, *ibid.*, **81**, 2860 (1959).

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

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

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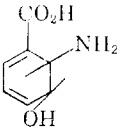
(+)*trans*-2,3-DIHYDRO-3-HYDROXYANTHRANILIC ACID. A NEW AMINO ACID PRODUCED BY *Streptomyces aureofaciens*[1]

Sir:

We wish to report the isolation of a new amino acid from the fermented mash of *Streptomyces aureofaciens* mutant, S-652. The substance has been characterized tentatively as (+)*trans*-2,3-dihydro-3-hydroxyanthranilic acid (DHAA) by its elemental analysis, ultraviolet and infrared absorption spectra, and by chemical transformations.



DHAA

DHAA originally was observed in fermentation mashes of *S. aureofaciens* mutants which were low producers of the tetracyclines. DHAA was also sometimes observed in mashes of high-producing strains grown under certain conditions of media and/or temperature which resulted in suppressed production of tetracyclines. Because of this rough reciprocal relationship we became interested in the isolation and identification of DHAA and in its possible role in the biosynthesis of the tetracyclines.

The best producer of DHAA was strain S652, a pale-tan mutant derived from *S. aureofaciens* NRRL 2209. When this strain was grown in shaker flask fermentations under conditions previously described,[2] DHAA was produced in quantities as high as 10 g./l.

DHAA was isolated from the neutral filtrate of a pilot tank fermentation run with strain S652 in a corn steep-starch-lard oil medium. Initial

(1) This paper was presented before the Division of Microbial Chemistry and Technology at the Chicago meeting of the American Chemical Society, Sept. 4–8, 1961.

(2) J. J. Goodman, M. Matrishin, R. W. Young and J. R. D. McCormick, *J. Bacteriol.*, **78**, 492 (1959).