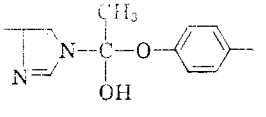
Anal. Calcd. for $C_{23}H_{28}N_8O_7$: C, 52.26; H, 5.34; N, 21.20; mol. wt. (in trifluoroacetic acid), 264.2. Found: C, 52.36; H, 5.73; N, 20.40; mol. wt., 254. In 0.1 potassium chloride the peptide exhibited pK_a's 6.65 ± 0.1 and 10.05 ± 0.1, within experimental error identical with those obtained 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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(1) M. Laskowski, Jr., and H. A. Scheraga, *J. Am. Chem. Soc.*, **76**, 6305 (1954).

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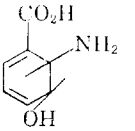
(+)*trans*-2,3-DIHYDRO-3-HYDROXYANTHRANILIC ACID. A NEW AMINO ACID PRODUCED BY *Streptomyces aureofaciens*[1]

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).

purification was accomplished by ion-exchange chromatography of the acidified filtrate. DHAA was taken up on a column of Amberlite IR-120 (hydrogen form) and was then eluted with 1 *N* sulfuric acid. The quantity of product contained in the various eluate fractions was determined by spectrophotometric assay. Isolation of DHAA from the rich cuts was accomplished by removal of sulfate as barium sulfate and crystallization of the product from the resulting solution by concentrating and cooling. The crude product was recrystallized from glacial acetic acid to give (+)*trans*-**2,3-dihydro-3-hydroxyanthranilic acid,** m.p. (dec.) 190–191°. *Anal.* Found for $C_7H_9NO_3$: C, 53.78; H, 5.95; N, 8.82; neut. equiv., 156; pK_a (water), 8.6; $[\alpha]^{22}D$ (0.5% in water), +445°; ultraviolet absorption, $\lambda_{max.}$ (0.1 *N* hydrochloric acid) 278 mμ, ε 9350; infrared absorption: strong $-NH_3^+$ absorption at 2860–3000 cm.$^{-1}$ (broad), and 2125 cm.$^{-1}$, and $-CO_2^-$ absorption at 1590 and 1390 cm.$^{-1}$.

The infrared spectrum of crystalline DHAA suggested that it was an amino acid. The ultraviolet absorption could be accounted for by the carboxyl and two linearly conjugated double bonds.[3] Catalytic hydrogenation of DHAA resulted in the uptake of two moles of hydrogen to yield **hexahydro-3-hydroxyanthranilic acid,** m.p. (dec.) 270–276°. *Anal.* Found for $C_7H_{13}NO_3$: C, 52.68; H, 8.43; N, 8.59. Thus the original substance, DHAA, was indicated to be a cyclic diene amino acid. Confirmation was seen in the ready conversion under vigorous acidic conditions to anthranilic acid, and under vigorous alkaline conditions to *m*-hydroxybenzoic acid. The presence of acylable amino and hydroxyl groups in DHAA was confirmed by acetylation to **2-acetamido-3-acetoxy-2,3-dihydrobenzoic acid,** m.p. 177–178°. *Anal.* Found for $C_{11}H_{13}NO_5$: C, 55.15; H, 5.54; N, 6.11; acetyl, 35.5. Final proof of the constitution of DHAA as a cyclohexadienoic acid and relative placement of the carboxyl, amino, and hydroxyl groups was found in the very facile catalytic disproportionation of DHAA to 3-hydroxyanthranilic acid and hexahydro-3-hydroxyanthranilic acid. In addition, since DHAA was optically active, gave no ketone reaction with dinitrophenylhydrazine and produced no ammonia on mild alkaline hydrolysis, the structure must be one of the four possible stereoisomeric 2,3-dihydro-3-hydroxyanthranilic acids. The vigorous conditions (4 hours in 12*N* hydrochloric acid at 60°) required for dehydration of DHAA to anthranilic acid strongly suggested a *trans* relationship for the amino and hydroxyl groups.

The possibility that DHAA was involved as an intermediate in the biosynthesis of the tetracyclines was investigated by radiotracer techniques.[4] Labeled DHAA was prepared by an S-652 fermentation of uniformly C^{14}-labeled tobacco starch and was isolated from the mash filtrate by means of preparative paper chromatography. The isolated material was added to a BC-41 fermentation in the active 7-chlorotetracycline (CTC)-producing phase. At the end of the BC-41 fermentation period, CTC was isolated by paper chromatographic means and was shown to contain less than 1% of the total radioactivity added as labeled DHAA. We therefore have concluded that DHAA is not an intermediate in the pathway from carbohydrate to the tetracyclines.[5]

(3) E. A. Braude in "Determination of Organic Structures by Physical Methods," E. A. Braude and F. C. Nachod, Ed., Academic Press, Inc., New York, N. Y., 1955, pp. 131–185.

(4) P. A. Miller, J. R. D. McCormick and A. P. Doerschuk, *Science*, **123**, 1030 (1956).

(5) The close chemical relationship of DHAA to anthranilic acid suggests a possible role for DHAA as an intermediate in the biological conversion of shikimic acid to anthranilic acid (B. D. Davis, "Advances in Enzymology," **16**, 247 (1955); E. L. Tatum, S. R. Gross, G. Ehrensvärd and L. Garnjobst, *Proc. Natl. Acad. Sci.*, **40**, 271 (1954); P. R. Srinivasan, *J. Am. Chem. Soc.*, **81**, 1772 (1959).

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ELECTROPHILIC REACTIONS OF ARGENTOUS AND MERCURIC FLUORIDES WITH FLUOROOLEFINS IN HYDROGEN FLUORIDE

Sir:

We wish to report (1) the addition of mercuric fluoride to 1,1,3,3,3-pentafluoropropene and to hexafluoropropene in hydrogen fluoride to form isopropylmercury derivatives, and (2) the silver fluoride catalyzed addition of hydrogen fluoride to hexafluoropropene and to octafluoro-2-butene. Our results demonstrate electrophilic attack on perfluoroölefins by metal cations and point to electrophilic reactions of π-electron systems as a new area of carbon–fluorine chemistry.

Hexafluoropropene rapidly adds hydrogen fluoride at 25° by reaction with potassium fluoride in formamide with initial nucleophilic attack by fluoride ion[1] but is stable to anhydrous hydrogen fluoride at 200°. Less than 1% CF_3CHFCF_3 was formed from $CF_3CF{=}CF_2$ with excess HF at 200°.[2] Potassium fluoride in HF is ineffective as a nucleophilic reagent. An 8 mole per cent. solution of KF in HF yielded only 8% CF_3CHFCF_3 at 200°; at 125°, less than 1% HF addition took place with $CF_3CF{=}CF_2$ and with $CF_3CF{=}CFCF_3$.[2] The strong solvation of F^- to form $H_nF_{n+1}^-$ ions promotes dissociation but inhibits nucleophilic reaction. Hydrogen fluoride is thus a useful solvent for reactions between metal cations and perfluoroolefins.[3]

Reaction between HgF_2 and the fluoropropenes proceeds smoothly at 85° in hydrogen fluoride without HF addition.[2,4–7] From 16 g. of $CF_3CH{=}$

(1) W. T. Miller, Jr., J. H. Fried and H. Goldwhite, *J. Am. Chem. Soc.*, **82**, 3091 (1960).

(2) Reaction mixtures were heated for 24 hr. in rocker-type stainless steel bombs.

(3) Reactions have also been carried out in aqueous solutions.[4]

(4) The experiments in aqueous solutions and with $CF_3CF{=}CFCF_3$ in HF were carried out by Nathan Edelson.

(5) At 11.9° HgF_2 forms a 0.5% soln. in HF.[6] Equimolar amounts of HgF_2 and $CF_3CF{=}CF_2$ without a solvent gave <5% addition in 12 hr. at 140°.

(6) A. W. Jache and G. H. Cady, *J. Phys. Chem.*, **56**, 1106 (1952).