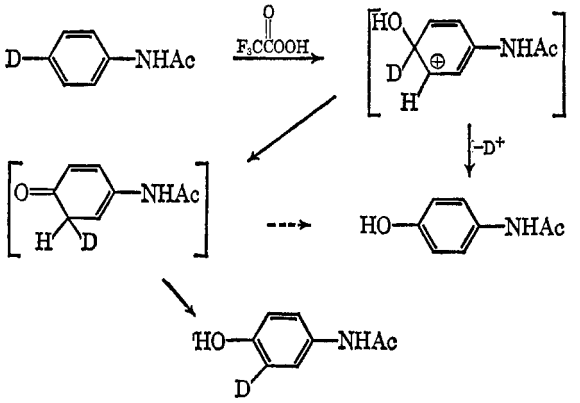
positions adjacent to the hydroxyl group by comparison of the intensity of the nmr signals of the 3,5-ring protons with those of the 2,6 protons. The retention of tritium (9.6%) and deuterium (7.5%) obtained on *para*-hydroxylation of acetanilide with trifluoroacetic acid may be compared to the retention of tritium (40–50%) and deuterium (15%) in the enzymatic hydroxylation of acetanilide.[5]

Mechanistically, the hydroxylation with trifluoroperacetic acid is likely to involve attack by "OH^+" or a related species and presumably proceeds by the pathway



The nature of the oxygenating species involved in the enzymatic hydroxylation of aromatic substrates is still a subject for speculation. The hydroxylating systems of Table II have served as models for the hydroxylases.[10] The degree of retention in other model systems currently under investigation may serve as a useful further guide for the correlation of enzymatic and nonenzymatic hydroxylations.[11]

(10) V. Ullrich and H. Staudinger, in "Biological and Chemical Aspects of Oxygenases," K. Bloch and O. Hayaishi, Ed., Maruzen Co., Ltd., Tokyo, 1966, pp 235–249.

(11) A related phenomenon, the oxidative coupling of 4-^{3}H-2,6-xylenols, gives *para*-substituted polymers with 23% retention of tritium. In this case a radical coupling mechanism has been proposed in which electron-transfer resonance stabilization leads to (rearranged) phenonium ions: W. A. Butte, Jr., and C. C. Price, *J. Am. Chem. Soc.*, **84**, 3567 (1962).

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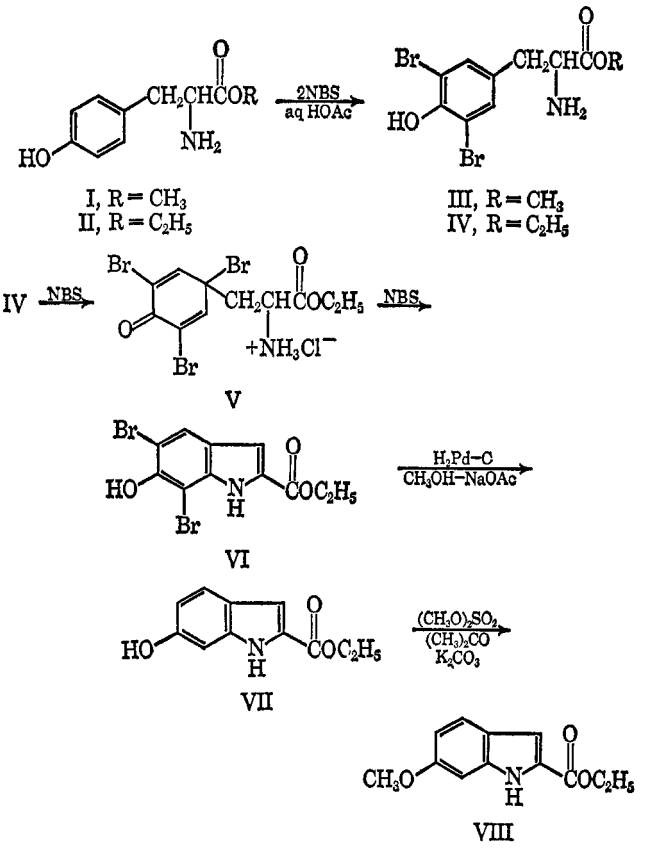
Chemical Conversion of Tyrosine to 6-Hydroxyindoles

Sir:

Silver oxide, ferricyanide, and other oxidants convert 3,4-dihydroxyphenylalanine (DOPA), 3-hydroxytyramine, noradrenaline, and related catecholamines to 5,6-dihydroxyindole derivatives.[1] The transformation of tyrosine to 5,6-dihydroxyindole-2-carboxylic acid, however, is only known as an enzymatic reaction and requires *tyrosinase* to effect the initial oxidation to DOPA.[2]

We wish to report the first direct nonenzymatic conversion of methyl or ethyl tyrosinate (I and II, respectively) to the monohydroxyindole VI by the action of



I, R = CH_3
II, R = C_2H_5

III, R = CH_3
IV, R = C_2H_5

V

VI

VII

VIII

N-bromosuccinimide (NBS). This transformation was discovered when we observed that amino-terminal tyrosine peptides failed to undergo the expected cleavage on treatment with NBS.[3]

When 1.0 m*M* solutions of the tyrosine esters I–IV in 0.25 *M* HOAc were treated with aliquots of a 20 m*M* aqueous NBS solution, the rapid appearance of the characteristic bromo dienone chromophore ($\epsilon_{260-270}$ ~8000) was observed. This absorption reached a maximum with 3 equiv of NBS on I or II and 1 equiv on the dibromo derivatives III and IV. The bromo dienone V was isolated on a preparative scale from II and from the dipeptides Tyr-Ala and Tyr-Phe. The reactive bromine in V liberated iodine in the starch–potassium iodide test. The analysis accounts for the introduction of three atoms of bromine to give the tribromo dienone hydrochloride V. This remarkable intermediate, which has been discussed previously in connection with the mechanism of cleavage,[4] is apparently stabilized by the ionized amino group in close proximity to the dienone system. In such a labile system, intramolecular Michael addition of the amino group to the dienone would be competitive with the formation of the spirodienone lactone, the reaction underlying the cleavage of peptide bonds.

This was indeed found to be the case. In contrast to the comparatively stable bromo dienone lactone of the tyrosine peptide cleavage,[4] the absorption of the labile dienone V at 260 mμ on standing decreased slowly, and a new absorption at 320 mμ began to develop. This absorption reached a maximum with 2 equiv of NBS on esters III and IV and 4 equiv on esters I and II after approximately 16 hr at room temperature or

(1) W. L. Dulière and H. S. Raper, *Biochem. J.*, **24**, 239 (1930); *cf.* R. A. Heacock, *Chem. Rev.*, **59**, 181 (1959).

(2) H. S. Raper, *Biochem. J.*, **21**, 89 (1927).

(3) M. Wilchek and B. Witkop, *Biochem. Biophys. Res. Commun.*, **26**, 296 (1967).

(4) G. L. Schmir, L. A. Cohen, and B. Witkop, *J. Am. Chem. Soc.*, **81**, 2228 (1959).

10-min heating on a steam bath. In all cases identical spectra resulted, indicative of III and IV being intermediates in the reaction of I and II with NBS. Tyrosinamide and tyrosine-N-methylamide gave similar results. Free tyrosine, 3,5-dibromotyrosine, tyramine, and N-acetyl-3,5-dibromotyrosine ethyl ester failed to produce the 320-mμ chromophore on treatment with NBS.

When the reaction was carried out on a preparative scale, starting with II, IV, or tyrosinamide in 40% aqueous acetic acid, crystalline products were isolated in 20% yield after chromatography on silica ($CHCl_3$–CH_3OH, 9:1). Infrared, ultraviolet, nmr, and mass spectra and elemental analyses suggest the structure of ethyl 5,7-dibromo-6-hydroxyindole-2-carboxylate (VI) or its amide for the 320-mμ product. The amide [mp 247° dec, λ_{max} 315 mμ (EtOH)] exhibited a triplet of parent peaks at *m/e* 331.879, 333.874, and 335.873 in the mass spectrometer consistent with the formula $C_9H_6N_2O_2Br_2$ [calcd, 331.88 (^{79}Br–^{79}Br), 333.879 (^{79}Br–^{81}Br), and 335.878 (^{81}Br–^{81}Br)].

The formation of a side product, an unstable red aminochrome, λ_{max} 480 and 320 mμ, is especially noticeable at pH values above 5. In addition, a colorless, crystalline, indolic compound [λ_{max} 315 mμ (log ε 4.33), mp 152–155°, $C_{11}H_{10}NO_4Br$, *m/e* 298.979 and 300.979 (calcd 298.979 and 300.977 for ^{79}Br and ^{81}Br)] is obtained from II or IV. The same product, ethyl 5,6-dihydroxy-7-bromoindole-2-carboxylate, was obtained from ethyl 3,4-dihydroxyphenylalanate on oxidation with NBS. NBS in this case converts tyrosine ester to products which as a rule arise only from DOPA.

The dibromoindole VI was reductively debrominated to VII with palladium on charcoal in buffered methanol [mp 169–175°; $\lambda\lambda_{max}$ (log ε) 320 mμ (4.32), 250 (3.95), 215 (4.28); parent *m/e* 205.078 (calcd for $C_{11}H_{11}NO_3$, 205.074)]. VII was methylated with dimethyl sulfate in anhydrous acetone containing fused potassium carbonate to give the known ethyl 6-methoxyindole-2-carboxylate (VIII). The ultraviolet spectrum [λ_{max}^{EtOH} 320 mμ (log ε 4.28), 250 (3.93), 215 (4.28); λ_{min}^{EtOH} 265 mμ (log ε 3.29)] and melting point (132–135°) agree with the reported data.[5]

The low-resolution mass spectrum reveals a parent peak (M) at *m/e* 219 and principal peaks at 204 (M − CH_3), 191 (M − CH_2=CH_2), 190 (M − C_2H_5), 173 (M − C_2H_5OH), 158 (M − C_2H_5OH, − CH_3), 145 (M − C_2H_5OH, − CO), 130 (M − C_2H_5OH, − CH_3, − CO), 119 (M − C_2H_5OH, − CO, − CN), 102 (M − C_2H_5OH, − CH_3, − 2CO); 76 (M − C_2H_5OH, − CH_3, − 2CO, − CN).

This transformation is now being used for the determination of amino-terminal tyrosine in proteins and for the synthesis of 6-hydroxyindole derivatives.

(5) G. Pappalardo and T. Vitali, *Gazz. Chim. Ital.*, **88**, 574 (1958).
(6) Fellow in the Visiting Program of the U. S. Public Health Service, 1966–1967, on leave of absence from the Weizmann Institute of Science, Rehovoth, Israel.

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Amino Acid Sequence around the Histidine Residue of the α-Lytic Protease of *Sorangium sp.*, a Bacterial Homolog of the Pancreatic Serine Proteases

Sir:

The elucidation of the amino acid sequence of chymotrypsinogen A,[1] chymotrypsinogen B,[2] trypsinogen,[3,4] and, in part, elastase[5] has established that the pancreatic serine proteases are homologous with respect to a subsequence in which two histidines and a cystine residue are components.[6] In chymotrypsin, for example, these components are histidine-40 and -57 and the cystine residue comprised of half-cystine-42 and -58. The discovery of this common structure, in which a disulfide bond could be considered to have the function of bringing the two histidines close together, has prompted several proposals of reaction mechanisms[7–9] which assign a catalytic function to both histidine residues. However, the kinetic evidence implicates only one.[10]

The α-lytic protease of *Sorangium sp.* is a bacterial protease which is unusual in two respects: it has the same active serine sequence as the pancreatic proteases, *i.e.*, Asp-Ser-Gly-Gly, and it has only one histidine residue.[11–13] The sequences at its three disulfide bridges are under investigation and, in this communication, we report the sequence of a histidylcystine structure which establishes a further homology with the pancreatic enzymes.

The α-lytic protease, prepared by methods previously described,[14] was digested with pepsin and submitted to the pH 6.5 diagonal procedure of Brown and Hartley.[15] The resulting peptide map is shown in Figure 1. A single peptide (B1) staining for histidine was observed off the diagonal and was paired with a more acidic peptide B2. Peptide B1 stained red and B2 yellow with the cadmium–ninhydrin reagent of Heilmann, *et al.*[16] Peptide B1, after isolation by preparative electrophoresis at pH 6.5, was found to be separable into two histidine peptides, B1A and B1B, by electrophoresis at pH 1.8 (60 v/cm for 60 min). Peptides B1A, B1B, and B2 were subjected to amino acid and sequential analysis from the N-terminal by the "dansyl–Edman" procedure.[17,18] Peptide B1A

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