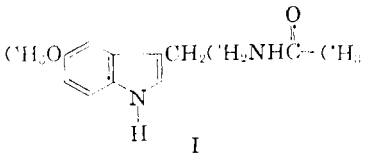

I

I was eluted from a silicic acid column and subjected again to countercurrent and column purification; 40 μg. of I was obtained from 100 g. of lyophilized bovine pineal glands.[6]

I and II possessed similar ultraviolet spectra (I, λ_{max} 2780 Å., shoulders at 2970, 3090 Å.; II, λ_{max} 2760 Å., shoulders at 2960, 3080 Å.); fluorescence spectra (when $\lambda_{excitation}$ = 304 mμ, I λ_{max} = 333 mμ, II λ_{max} = 338 mμ). These data and similar blue color with Ehrlich reagent suggested that I is also a substituted 5-hydroxyindole. O-Methylation at position 5 was suggested by: failure to migrate as a cation in electrophoresis at *p*H 11; lack of acid–base shift of ultraviolet absorption maxima; greatly increased lightening ability of many 5-methoxyindoles over their parent 5-hydroxyindoles.

The presence of an ester or amide in the side chain at position 3 was indicated by: detection of a carbonyl group (by infrared)[7] not conjugated with the indole nucleus (by ultraviolet absorption and fluorescence); neutrality of I and negative color reactions for aldehydes and ketones. An alcohol ester was unlikely because on hydrolysis of I with acid or base II was not detected. From these findings, together with observations that acetylcholine but not choline has lightening ability, we guessed that I might be N-acetyl-5-methoxytryptamine.

Synthetic N-acetyl-5-methoxytryptamine (III, 40 mg.) was prepared by reducing 100 mg. of 5-methoxyindole-3-acetonitrile[7] with 160 mg. of sodium and 2 ml. of ethanol,[8] then acetylating the product with 4 ml. of both glacial acetic acid and acetic anhydride at 100° for 1 minute. Purification was achieved by countercurrent distribution and silicic acid chromatography as for I.

III and I were found identical with respect to: countercurrent distribution (peak tube 12); elution curve from silicic acid column; ultraviolet and fluorescence maxima; biologic activity (minimal lightening of isolated frog skin at 10^{-12} gram/ml.); R_f in descending chromatography systems 2-propanol:concd. NH_3:water 16:1:3 (R_f 0.83); 1-butanol:acetic acid:water 4:1:5 (R_f 0.9); 1-butanol:acetic acid:water:pyridine 15:5:12:10 (R_f 0.9); heptane:pyridine 7:3 (R_f 0.10); heptane:pyridine 6.5:3.5 (R_f 0.80); benzene:ethyl acetate:water 20:1:20 (R_f 0.39).

The increased lightening ability of I over N-acetyl - 5 - hydroxytryptamine[9] suggests that O-methylation of hydroxyindoles forms substances of increased biologic activity, in contrast to O-methylation of catechol amines. The exquisite sensitivity of frog melanocytes to I indicates a neurohormone function, since melanocytes reflect their neural origin by responding to compounds, including acetylcholine and noradrenaline, which stimulate neurons.

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(6) We are grateful to the Armour Laboratories for supplying us with many kilograms of bovine pineal glands.

(7) We wish to thank Drs. A. Krivis and J. Szmuszkovicz and Mr. W. C. Anthony of The Upjohn Company for carrying out microinfrared studies, preparing numerous model compounds, supplying us with 5-methoxyindole 3-acetonitrile and offering many helpful suggestions.

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WATER SUBEXCITATION ELECTRONS IN AQUEOUS FORMIC ACID RADIOLYSIS[1]

Sir:

The sole γ-ray radiolysis and 1860 Å. photolysis products of dilute formic acid are equal amounts of carbon dioxide and hydrogen.[2,3] Carbon monoxide, however, is produced by formic acid excitation when the light-absorbing species is changed from water to formic acid at 1860, 2537 and 2669 Å.[3,4] Although electron capture mechanisms have been suggested[5,6,7] and may account for carbon monoxide, water subexcitation electrons[8,9] having a kinetic energy $\epsilon < E_w$, the lowest excitation potential of water, provide a more likely exciting species for this reaction.

$G(CO)$ for pure 26.6 M formic acid irradiated by γ-rays is 1.25 and consequently direct ionization and dissociation also produce carbon monoxide.[4] But direct ionization cannot account for the unexpectedly high $G(CO)$ of 0.25 and 0.50 in 0.1 and 1.0 M formic acid respectively. Over this concentration range, $G(CO_2)$ increases and $G(H_2)$ remains unchanged. Direct action $G(CO_2)$ here will be below 0.005 and 0.05. In view of carbon monoxide formation by light, its absence in dilute solution radiolysis and the expected negligible direct ionization action in 0.1 M solutions, direct dissociation of excited formic acid by water subexcitation electrons is postulated.

Although the probability of molecular excitation is proportional to the appropriate optical constant only for fast electrons,[10,11] the ratio of probabilities of two different excitations is approximately equal to the ratio of oscillator strengths down to fairly low values of ϵ.[12] Therefore we assume that all electrons including the subionization electrons excite water and formic acid in the ratio of $k_w c_w$ to $k_f c_f$ at any given energy transfer (k_w, k_f; molar

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

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(3) M. S. Matheson, Proc. of the 2nd Intl. Conf., Geneva, 1958, **29**, 385, 195.

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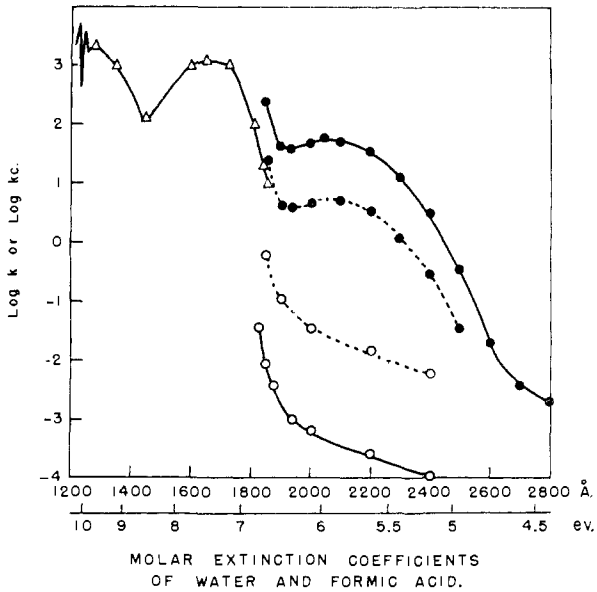

Fig. 1.—Δ, water vapor[13]; O, liquid water (solid line); 55.5 k (dotted line)[14]; ● aq. formic acid (solid line); 0.1 k (dotted line).[15]

extinction coefficient and c_w, c_f; concentrations of water and formic acid, respectively.) In Fig. 1, note that formic acid excitation dominates water excitation above 1800 Å. even in 0.1 M formic acid. Here $k_f c_f$ exceeds $k_w c_w$ by a factor of 100, which is far greater than the expected departure of the ratio of corresponding excitation probabilities from theory.[10]

The limits of this excitation effect can be estimated in 0.1 M formic acid by dividing G(CO) by the quantum yield, ϕ(CO). One finds that the number of excitations/100 ev. varies from 1.7 for ϕ(CO) equal to 0.14 at 2669 Å. to 0.40 for ϕ(CO) equal to 0.58 at 1860 Å. Thus this effect is appreciable and supports Platzman's prediction[8] that the subexcitation electron effect becomes significant at molar concentrations of the order of 0.1 and can attain a G value of the order of one.

If carbon monoxide originates from dissociation of formic acid by water subexcitation electrons, one may conclude that water contains no transitions below 6.85 ev. A transition to a low-lying level, if allowed for these slow electrons (even though optically forbidden) would channel energy very rapidly from the electron and formic acid would be largely unaffected in solutions as dilute as 0.1 M.

Acknowledgment.—The author wishes to thank M. S. Matheson, J. L. Weeks and R. L. Platzman for their coöperation and helpful discussions.

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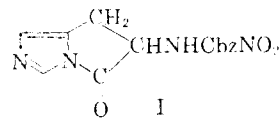
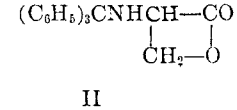
ACTIVATED CYCLIC DERIVATIVES OF AMINO ACIDS

Sir:

Many biologically important amino acids contain "extra" functional groups (*e.g.*, hydroxyls, thiols, and potentially reactive heterocycles) which may complicate peptide synthesis. These functions may be blocked as a separate operation or a "selective" condensing agent may be used. This communication reports attractive examples of reagents in which the "extra" group is "protected" while the carboxyl function is "activated."



I



II

Interaction of N-(*p*-nitrocarbobenzyloxy)-L-histidine[1] [m.p. 202–204°, $\alpha^{27}D$ −22.6° (*C*, 1.6 in 6 *N* hydrochloric acid)] and N,N′-diisopropylcarbodiimide in dioxane solution afforded 60% of the crystalline cyclized product I [found: C, 53.00; H, 3.91; N, 17.41]; $\alpha^{25}D$ −14.9° (*C*, 1.36 in tetrahydrofuran). Crystallization from tetrahydrofuran–hexane gave m.p.186–187°; the infrared spectrum shows a strong band at 1775–1780 cm.$^{-1}$ attributable to an activated amide. The benzylamide, m.p. 190–191° (ethanol–ether), [found: C, 59.65; H, 5.33; N, 16.33], forms in nearly quantitative yield at room temperature on treatment with benzylamine; there is no band at 1775–1780 cm.$^{-1}$.

Although a large number of β-lactones are known, no authentic synthesis from the corresponding hydroxy acid has been reported[2] until the past year.[3]

We have cyclized N-trityl-L-serine[4] to a fully characterized crystalline β-lactone (II), using N,N′-diisopropylcarbodiimide, in 15% isolated yield; m.p. 193–194°, $[\alpha]^{28}D$ −62° (*C*, 0.5 in chloroform), ν_{max}^{KBr} 1820 cm.$^{-1}$, attributable to a β-lactone group, and no hydroxyl absorption band (chloroform) [found: C, 79.83; H, 5.78; N, 4.36; mol. wt., 309 (Rast)]. Treatment of II with benzylamine gave, in 93% yield, the benzylamide, m.p. 146–147°, $[\alpha]^{27.5}D$ −114° (*C*, 0.5 in chloroform) [found: C, 79.62; H, 6.45; N, 6.29] identical with the benzylamide obtained directly from the condensation of N-trityl-L-serine and benzylamine in the presence of N,N′-diisopropylcarbodiimide. Treatment of II with L-alanine methyl ester hydrochloride[5] in the presence of triethylamine gave, in 66% yield, N-trityl-L-seryl-L-alanine methyl ester, m.p. 149–150°, $[\alpha]^{30}D$ −41° (*C*, 1 in ethanol) [found: C, 72.27; H, 6.58; N, 6.51].

The possible utility of derivatives I and II as monomers is being investigated.

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(1) Prepared by the procedure described for the racemate, D. T. Gish and F. H. Carpenter, THIS JOURNAL, **75**, 950 (1953).
(2) H. E. Zaugg, "Organic Reactions," Vol. VIII, R. Adams, Editor, John Wiley and Sons, Inc., New York, N. Y., 1954, p. 307.
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(5) Prepared by Fischer esterification, m.p. 110–110.5°, $[\alpha]^{25}D$ +41° (*C*, 1 in methanol) [found: C, 34.17; H, 7.16; N, 10.00].