onate,[9] yielded 5-hydroxytryptophan in 70% overall yield from the gramine as colorless stout prisms (see Table).

TABLE I

	5-Hydroxytryptophan	7-Hydroxytryptophan
M.p.	293–298° (dec.)	>330°
Rf 80% aq. pyridine	0.61	0.72
Rf 70% aq. propanol	0.31	0.34
λ max (log ε) In water (*p*H 6)	298(3.662), 291(3.662) inflections	291(3.642).
	278(3.745)	269(3.794)
In 0.1 *N* NaOH (*p*H 11)	324(3.628)	280(3.93)
Calcd. for $C_{11}H_{12}N_2O_3$: C	59.99	59.99
H	5.50	5.50
N	12.63	12.63
Found: C	59.88	59.87
H	5.71	5.58
N	12.62	12.66
Tryptophan-adapted Pseudomonas (Strain 6)[a]	Not metabolized	Not metabolized
Peroxidase–oxidase system[b]	Not metabolized	Not metabolized
Vasoconstrictor effect (0.3 mg./kg. cat.)[c]	Slight but distinct effect (16 mm. arterial pressure rise)	No effect

[a] *Cf.* R. Y. Stanier, O. Hayaishi and M. Tsuchida, *J. Bacteriol.*, **62**, 355 (1951). Dr. O. Hayaishi kindly carried out these tests. [b] *Cf.* W. E. Knox and A. H. Mehler, *J. Biol. Chem.*, **187**, 419, 431 (1950). Crude liver extract, as Dr. Mehler found, oxidizes the two amino acids to products which do not show the UV-absorption to be expected from the corresponding kynurenine derivatives. [c] Tested by Dr. I. H. Page (*cf.* ref. 14).

Of six attempted routes into the 7-hydroxyindole series the following proved to be practicable: Gibson's synthesis of 2-nitro-3-hydroxytoluene could be easily repeated[10] and gave, after acetylation on oxidation with chromium trioxide in acetic anhydride 2-nitro-3-hydroxybenzaldehyde in better yield than the direct nitration of *m*-hydroxybenzaldehyde. The following route is self-explanatory: 2-nitro-3-benzyloxybenzaldehyde (glass-clear platelets from benzene–ligroin, m.p. 85.5–86.5°, 70%) → 2′,6-dinitro-5-benzyloxystyrene (yellow rods from ethanol, m.p. 141°, 98%) → 7-benzyloxyindole (colorless platelets from ligroin, m.p. 68°, 75%)[11] → 7-benzyloxygramine (colorless needles from hexane, m.p. 145°, 93.5%) → 7-benzyloxytryptophan (from water, m.p. 234–236°, 87% from the gramine) → 7-hydroxytryptophan (see Table).

5-Hydroxyheteroauxin (needles from water, m.p. 166°) and 7-hydroxyheteroauxin (light tan platelets from water, m.p. 177°) prepared by standard methods showed only 6 and 3%, respectively, of the activity of heteroauxin in the pea slit-internode test.[12] Coupling with diazotized 2,5-dichloroaniline gave red uncrystallizable azo dyes.[13] 7-Hydroxytryptamine hydrochloride ($C_{10}H_{12}N_2O \cdot HCl$, found: C, 56.39; H, 6.26; N, 12.79, crystalline powder from alcohol–ether, m.p. 145–148°) was about eight times more active as a vasoconstrictor in various tests than tryptamine and about one-third as active as serotonin.[14,15]

The importance of 5-hydroxytryptophan as a new metabolite of tryptophan and as the precursor of serotonin is reported by Udenfriend, Clark and Titus[16] in the following communication.

The stability of 5-hydroxytryptophan to the kynurenine-forming enzymes is remarkable and emphasizes the separate nature of the two pathways in the degradation of tryptophan. Further testing of the two new amino acids in *Neurospora* and *Drosophila* is in progress. Also, the possible diabetogenic action, characteristic of many perihydroxyheterocycles (*e.g.*, 8-hydroxyquinoline, xanthurenic acid[17] etc.), is being looked for in the 7-hydroxyindole series.

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(9) Prepared by British Patent 611,600 [*C. A.*, **43**, 3445 (1949)] rather than following the procedure of A. Galat, THIS JOURNAL, **69**, 965 (1947).

(10) G. P. Gibson, *J. Chem. Soc.*, **123**, 1269 (1923); *cf.*, however, A. Butenandt, *Z. f. Naturforschung*, **5B**, 444 (1950).

(11) Catalytic debenzylation gave the known 7-hydroxyindole [R. J. S. Beer, K. Clarke, H. G. Khorana and A. Robertson, *J. Chem. Soc.*, 1605 (1948)].

(12) *Cf.* K. V. Thimann, "Plant Growth Substances," edited by F. Skoog, University of Wisconsin Press, 1951, p. 31. We are indebted to Dr. Thimann for his interest and coöperation in this investigation.

(13) *Cf.* L. Herrmanns and P. Sachs, *J. Physiol. Chem.*, **114**, 79, 88 (1921).

(14) I. H. Page, *J. Pharmacol. Exptl. Therapy*, **105**, 58 (1952).

(15) The activity of this "isoserotonin" is noteworthy in connection with the preparation of anti-metabolitess uch as 5-amino-2-methyl-3-ethylindole as well as yohimbine (ref. 2, D. W. Woolley and E. Shaw, THIS JOURNAL, **74**, 2948, 4220 (1952)).

(16) S. Udenfriend, C. Clark and E. Titus, THIS JOURNAL, **75**, 501 (1953).

(17) Y. Kotake, presented at the Spring Meeting of the Japanese Biochemical Society, Kobe, 1952.

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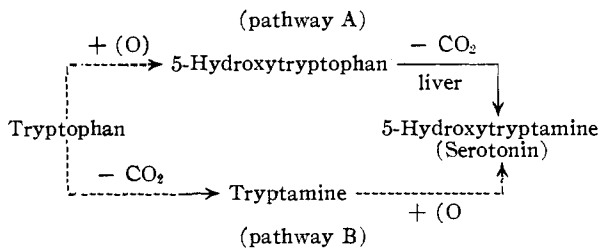
5-HYDROXYTRYPTOPHAN DECARBOXYLASE: A NEW ROUTE OF METABOLISM OF TRYPTOPHAN

Sir:

The biological significance of 5-hydroxytryptamine and its widespread occurrence in the living organism have been discussed in the preceding communication, in which are described the synthesis of 5-hydroxy and 7-hydroxytryptophan.[1] Considering tryptophan as the primary precursor of 5-hydroxytryptamine, two pathways for the conversion may be postulated.



When homogenates prepared from kidneys of dogs and guinea pigs were incubated aerobically with tryptophan, tryptamine, or 5-hydroxytryptophan, only the latter yielded 5-hydroxytryptamine. The enzyme responsible for this decarboxylation was partially purified by extraction into phosphate buffer *p*H 6.7 from acetone dried preparations of

(1) A. Ek and B. Witkop, THIS JOURNAL, **75**, 500 (1953). The authors are indebted to Drs. Ek and Witkop for making these compounds available.

guinea pig kidney. Decarboxylation of 5-hydroxytryptophan was followed by measuring the production of 5-hydroxytryptamine by a procedure developed in this laboratory. The sample was adjusted to *p*H 10, saturated with NaCl, and extracted with 4 volumes of *n*-butanol. The *n*-butanol layer was then washed three times with equal volumes of *p*H 10 borate buffer saturated with NaCl, to remove 5-hydroxytryptophan and normally occurring interfering substances. The 5-hydroxytryptamine was then returned to an aqueous phase by adding an equal volume of heptane to the *n*-butanol and shaking the mixture with a small volume of 0.1 *N* HCl. The optical density of the acid solution was measured at 275 mμ. The material measured by this procedure after incubation of 5-hydroxytryptophan with the enzyme preparation was identified as 5-hydroxytryptamine by its ultraviolet absorption spectrum and by its chromatographic properties on paper. The kidney extract did not decarboxylate tryptophan, 7-hydroxytryptophan, or tyrosine (Table I).

TABLE I

SUBSTRATE SPECIFICITY OF 5-HYDROXYTRYPTOPHAN DECARBOXYLASE

Each amino acid was incubated with guinea pig kidney acetone powder extract for 2 hours in an atmosphere of nitrogen at *p*H 6.7 and 37°

Substrate	μM added	μM of amine found after incubation[a]
5-Hydroxy-D,L-tryptophan	4.54	1.81[c]
7-Hydroxy-D,L-tryptophan[b]	4.54	<0.02
L-Tryptophan[b]	5.00	.00
L-Tyrosine[b]	5.00	< .05

[a] The amines, 5-hydroxytryptamine, 7-hydroxytryptamine, tryptamine and tyramine, were found to be perfectly stable when incubated with the enzyme preparation. [b] Chromatographic analysis showed that the bulk of the amino acid remained after incubation. [c] Represents about 80% of the theoretical value assuming that only 5-hydroxy-L-tryptophan is decarboxylated.

The presence of an enzyme which specifically catalyzes the decarboxylation of 5-hydroxytryptophan suggests that this amino acid is an intermediate in the biosynthesis of 5-hydroxytryptamine. Failure to find tryptophan decarboxylase activity in tissues makes it unlikely that the alternative pathway B is involved in 5-hydroxytryptamine formation.

Investigations with the tropical toad, *Bufo marinus*, lend further support to the existence of pathway A. Previous work has shown that the venom glands of *Bufo marinus* contain considerable amounts of 5-hydroxytryptamine[2] and its N-methyl analogs.[3] Experiments in progress show that the venom contains no tryptamine but does contain an indole compound which is chromatographically indistinguishable from 5-hydroxytryptophan.

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(2) S. Udenfriend, C. T. Clark and E. Titus, *Experientia*, **8**, 379 (1952).

(3) H. Jensen and K. K. Chen, *J. Biol. Chem.*, **116**, 87 (1936).

CHEMICAL STUDIES WITH 11-OXYGENATED STEROIDS. I. 17α-HYDROXYCORTICOSTERONE

Sir:

We wish to report a novel synthesis of 17α-hydroxycorticosterone.[1] 3α,11α,17α-Trihydroxypregnane-20-one (I),[2] now readily available from 11α-hydroxyprogesterone, was simultaneously oxidized and chlorinated with *t*-butyl hypochlorite to give 4-chloro-11α,17α-dihydroxypregnane-3,20-dione (II), m.p. 183–185° after melting at 160–165° then resolidifying (melting points are uncorrected); $[\alpha]^{23}D$ +48° (acetone). This compound was highly solvated and was difficult to dry for analysis. Oxidation of (II) with chromic acid gave 4-chloro-17α-hydroxypregnane-3,11,20-trione (III), m.p. 239.5–242°, $[\alpha]^{24}D$ +103° (acetone); (*Anal.* Calcd. for $C_{21}H_{29}ClO_4$: C, 66.16; H, 7.67; Cl, 9.31. Found: C, 66.10; H, 7.83; Cl, 9.30).

Likewise, 3α,17α-dihydroxypregnane-11,20-dione[3] was simultaneously oxidized and chlorinated with *t*-butyl hypochlorite to give 4-chloro-17α-hydroxypregnane-3,11,20-trione (III). The 3,20-bis-(ethylene glycol ketal) (IV), m.p. 238–242°; $[\alpha]^{24}D$ +55° (acetone); (*Anal.* Calcd. for $C_{25}H_{37}ClO_6$: C, 64.02; H, 7.95; Cl, 7.56. Found: C, 64.57; H, 7.86; Cl, 7.55), was formed when III was treated with ethylene glycol in the presence of an acid catalyst. Mild acidic hydrolysis of IV gave 4-chloro-17α-hydroxypregnane-3,11,20-trione 3-ethylene glycol ketal, m.p. 194–203°, $[\alpha]^{23}D$ +83° (acetone); (*Anal.* Calcd. for $C_{23}H_{33}ClO_5$: C, 65.00; H, 7.82; Cl, 8.34. Found: C, 65.16; H, 7.90; Cl, 8.37). Reduction of IV with lithium aluminum hydride gave 4-chloro-11β,17α-dihydroxypregnane-3,20-dione 3,20-bis-(ethylene glycol ketal) (V), m.p. 222–224°, $[\alpha]^{23}D$ +41° (acetone); (*Anal.* Calcd. for $C_{25}H_{39}ClO_6$: C, 63.74; H, 8.35; Cl, 7.53. Found: C, 63.80; H, 8.30; Cl, 7.53). Selective hydrolysis of the 20-ketal group with dilute acid in acetone gave the 3-monoketal (VI), m.p. 194–196°, $[\alpha]^{23}D$ +82° (acetone); (*Anal.* Calcd. for $C_{23}H_{35}ClO_5$: C, 64.70; H, 8.26. Found: C, 64.57; H, 8.13). Bromination of (VI) in chloroform solution gave the 21-bromide (VII), m.p. 199–201°; $[\alpha]^{22}D$ +91° (acetone); (*Anal.* Calcd. for $C_{23}H_{34}BrClO_5$: C, 54.50; H, 6.77; Br, 15.8. Found: C, 54.43; H, 7.01; Br, 15.3). When VII reacted with potassium acetate in acetone there was isolated 21-acetoxy-4-chloro-11β,17α-dihydroxypregnane-3,20-dione 3-ethylene glycol ketal (VIII), m.p. 232–233°, $[\alpha]^{22}D$ +96° (acetone); (*Anal.* Calcd. for $C_{26}H_{37}ClO_7$: C, 61.91; H, 7.69; Cl, 7.31. Found: C, 62.17; H, 7.73; Cl, 7.16). When VIII was treated with 2,4-dinitrophenylhydrazine or semicarbazide under acidic conditions followed by cleavage of the azone derivative by pyruvic acid there was obtained 17α-hydroxycorticosterone acetate (IX), m.p. 217–220°; $\lambda_{max.}^{EtOH}$ 242 mμ, E = 15,950. The infrared spectrum of

(1) 17α-Hydroxycorticosterone is also known as Reichstein's Compound M (T. Reichstein, *Helv. Chim. Acta*, **20**, 953 (1937)) and Kendall's Compound F (H. L. Mason, W. M. Hoehn, and E. C. Kendall, *J. Biol. Chem.*, **124**, 459 (1938)).

(2) H. L. Herzog, E. P. Oliveto, M. A. Jevnik and E. B. Hershberg, THIS JOURNAL, **74**, 4471 (1952); O. Mancera, *et al.*, *ibid.*, **74**, 3711 (1952); Kritchevsky, *et al.*, *ibid.*, **74**, 483 (1952).

(3) L. H. Sarett, *ibid.*, **70**, 1454 (1948).