culture in a medium containing cerelose, yeast extract, soya flour and inorganic salts, the filtered fermentation liquor was found to inhibit Gram-negative and Gram-positive bacteria and particularly certain mycobacteria in both broth-dilution and agar plate assays. From these filtrates a crystalline antibiotic with pronounced antituberculous activity has been isolated and given the generic name amicetin.

Amicetin was purified by extracting the clarified fermentation broth with *n*-butanol at *p*H 7.5, removing the solvent by distillation, leaching the activity from the residue with water, lyophilizing the aqueous solution, fractionating the freeze-dried preparation by countercurrent distribution between water and methylene chloride, and finally crystallizing from water. Amicetin is a basic compound with $pK_a$'s about 7. The crystals melted at 160–165°. On warming to 50–70° in methanol or water suspension, they were converted to a granular high-melting crystal form of the free base with the properties: m.p. 243–244°; $[\alpha]^{24}D$ +116.5° (*c*, 0.5 in 0.1 *N* hydrochloric acid); solubility in water at 25°, 1–2 mg. per ml.; only slightly soluble in common organic solvents. Amicetin exhibits characteristic ultraviolet absorption: in neutral aqueous solution at the maximum $E^{1\%}_{1\,cm.}$ = 465 at 305 mμ; in 0.1 *N* hydrochloric acid, $E^{1\%}_{1\,cm.}$ = 433 at 316 mμ; and in 0.1 *N* sodium hydroxide, $E^{1\%}_{1\,cm.}$ = 470 at 322 mμ. Titration data and analyses indicate the formula $C_{29}H_{44}N_6O_9$ for the free base. (*Anal.* Calcd. for $C_{29}H_{44}N_6O_9$: C, 56.11; H, 7.15; N, 13.54. Found: C, 55.98; H, 6.92; N, 13.18).

The *in vitro* activity of crystalline amicetin free base against several bacteria is shown in Table I.

TABLE I

ANTIBACTERIAL ACTIVITY OF CRYSTALLINE AMICETIN AS COMPARED WITH STREPTOMYCIN AND THE CULTURE BROTH FROM WHICH AMICETIN WAS EXTRACTED

Dilutions are expressed in microliters of broth or micrograms of solids per ml. required for complete inhibition of the test organism.

| Organism | Culture broth | Amicetin | Streptomycin |
|---|---|---|---|
| *Mycobacterium tuberculosis* (H37Rv)[1] | .. | 0.5 | 1.0 |
| *Mycobacterium tuberculosis* (ATCC-607) | 1.0 | 1.0 | 1.0 |
| *Staphylococcus aureus* (FDA-209) | 0.1 | 2.0 | 0.2 |
| *Bacillus subtilis* (Ill.) | 0.04 | 4.0 | 0.1 |
| *Klebsiella pneumoniae* (PCI-602) | 0.1 | >20.0 | 0.2 |
| *Escherichia coli* (ATCC-26) | 1.0 | >20.0 | 1.0 |
| Bodenheimer's cocco-bacillus (PCI-3) | 1.0 | >20.0 | >40.0 |

It is apparent that amicetin is active mainly against certain acid-fast and Gram-positive bacteria in contrast to the culture filtrates which have good Gram-negative activity as well. This is one of several indications that the *Streptomyces* sp. in question produces antibacterial activity in addition to that of amicetin.

Crystalline amicetin has been found to be active *in vivo* against *Mycobacterium tuberculosis* H37Rv when tested in infected mice.[1] However, on a weight basis, amicetin does not appear to be as effective as streptomycin sulfate when tested in this species. Preliminary studies indicate that the toxicity[2] of amicetin varies considerably with the species. The acute intravenous $LD_{50}$ of amicetin as the citrate complex at *p*H 6 in mice is approximately 90 mg. per kg.; the subcutaneous $LD_{50}$, 600–700 mg. per kg. In rats the acute intravenous $LD_{50}$ is approximately 200 mg. per kg. and the subcutaneous $LD_{50}$, about 600 mg. per kg. Amicetin is especially toxic to guinea pigs: by the subcutaneous route it is about forty times as toxic as streptomycin, but on the other hand only about one-tenth as toxic as penicillin to this same species. In contrast to streptomycin, amicetin does not appear to cause eighth-nerve damage when studied according to the procedure of Fowler and Feind.[3]

Detailed accounts of these studies are in preparation.

(1) Determined by Charles Lewis, N. D. Connor and staff, Bacteriology Research Department, The Upjohn Company.

(2) Determined by O. F. Swoap, E. S. Feenstra, R. J. Cole, and P. M. Brockie, Pharmacology Department, The Upjohn Company.

(3) E. P. Fowler and C. R. Feind, *Am. Rev. Tub.*, **60**, 39 (1949).

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## SYNTHESIS AND BIOCHEMISTRY OF 5- AND 7-HYDROXYTRYPTOPHAN AND DERIVATIVES[1]

*Sir:*

The reported occurrence of serotonin (5-hydroxytryptophan) in serum,[2] in intestinal mucosa (enteramin)[3] and blood platelets (thrombocytin)[4] as well as in the parotid glands of *Bufo marinus*[5] suggests, as mentioned previously,[6] the possibility of a direct hydroxylation of the benzene ring in tryptophan prior to any reaction on the pyrrole part of the molecule. The alternative formation of serotonin from 5-hydroxyindole *via* 2,5-dihydroxyphenylalanine[7] is unlikely, since the introduction of a β-side chain into indole is a process unknown in animals. We now have synthesized 5- and 7-hydroxytryptophan and their derivatives of actual and potential physiological importance.

Catalytic debenzylation of 5-benzyloxytryptophan (m.p. 280°, dec.), obtained *via* condensation of 5-benzyloxygramine[8] with diethylformaminomal-

(1) Oxidation Mechanisms. VIII. Labile Amino Acid Metabolites. II. *Cf. Experientia*, **8**, 377 (1952).

(2) M. W. Rapport, A. A. Green and I. H. Page, *Science*, **108**, 329 (1948).

(3) V. Erspamer and B. Ansero, *Nature*, **169**, 800 (1952); *cf. Arzneimittelforsch.*, **2**, 253 (1952).

(4) G. Reid and M. Rand, *Nature*, **169**, 801 (1952).

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

(6) *Cf.* A. Ek, H. Kissman, J. B. Patrick and B. Witkop, *ibid.*, **8**, 36 (1952).

(7) J. Harley-Mason and R. I. T. Cromartie, *Biochem. J.* (Proc. Biochem. Soc.), 51, XXIV (1952); *Chemistry and Industry*, 173 (1952); *J. Chem. Soc.*, 2525 (1952).

(8) K. E. Hamlin and F. E. Fischer, THIS JOURNAL, **73**, 5007 (1951). We used the Robertson modification of the Nenitzescu–van der Lee synthesis for the preparation of 5-benzyloxyindole and increased the yield of the gramine to 95% by carrying out the Mannich condensation in glacial acetic acid-dioxane under special precautions.

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.

(18) U. S. Public Health Postdoctoral Fellow, 1952.

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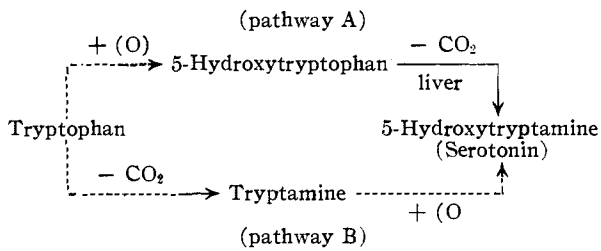
## 5-HYDROXYTRYPTOPHAN DECARBOXYLASE: A NEW ROUTE OF METABOLISM OF TRYPTOPHAN

*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.