and purified by extracting three times with 50% aqueous alcohol. The very small amount of insoluble product melted at 270–273°.

3-Sulfanilamido-4(or 5)-methylpyridazine from VII; Isomer B.—This isomer was prepared and purified as described for the compound derived from isomer A; m.p. 254–255° (sintering from 249°). A mixture with the isomer melting at 270–273° melted at 227–230°. The infrared spectrum of this compound showed marked differences throughout from that of the isomer melting at 270–273°.

Preparation of 3-N⁴-Acetylsulfanilamidopyridazines.—The following compounds were prepared by procedure B described above for the 6-methoxy compound X.

3-N⁴-Acetylsulfanilamidopyridazine was obtained from 3-sulfanilamidopyridazine[3,5] in 90% yield, m.p. 204–205° (from aqueous alcohol). *Anal.* Calcd. for $C_{12}H_{12}N_4O_3S$: C, 49.3; H, 4.1; N, 19.2. Found: C, 49.5; H, 4.3; N, 19.3.

3-N⁴-Acetylsulfanilamido-6-chloropyridazine was obtained in the same way from the corresponding sulfanilamide III[3-6] in 96% yield, m.p. 224–225°. *Anal.* Calcd. for $C_{12}H_{11}ClN_4O_3S$: C, 44.1; H, 3.4; N, 17.2; Cl, 10.9. Found: C, 44.3; H, 3.6; N, 17.0; Cl, 10.6.

Solubilities.[16]—Solubilities of various sulfanilamidopyridazines and their N⁴-acetyl derivatives were carried out at 37° by the general method of Biamonte and Schneller.[17] Determinations in duplicate of each of duplicate samples were made. Only values from such determinations that agree within 5% are reported. Equilibration was demonstrated either by the agreement of values determined at 24-hour intervals or of values from samples brought to 37° from both room temperature and 95°. Excess solid phase was present at all times and constant stirring or shaking was employed.

Citrate–phosphate buffers were used in all but one case. 3-Sulfanilamido-6-methoxypyridazine did not give reproducible results in this buffer. It was necessary in this case to use an acetate buffer (0.1 *M*) to obtain equilibrium values that agreed. The highest practical *p*H obtainable with this buffer was 6.5.

(16) We are indebted to J. J. Licari and D. S. Davies for carrying out many of these determinations.

(17) A. R. Biamonte and G. H. Schneller, *J. Am. Pharm. Assoc., A (Scient. Ed.)*, **41**, 341 (1952).

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES AND THE NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Oxindole Analogs of (5-Hydroxy)-tryptamine and -tryptophan, as Inhibitors of the Biosynthesis and Breakdown of Serotonin[1]

BY K. FRETER, H. WEISSBACH, B. REDFIELD, S. UDENFRIEND AND B. WITKOP

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Tryptamine and O-benzylserotonin were converted to the corresponding oxindole derivatives IIa, IIb, IIc *via* the symmetric disulfides Ia, Ib. As a result of interaction of the basic amino group with the lactam carbonyl of the oxindole ring, milder alkaline hydrolysis than required for unsubstituted oxindoles opened IIa to α-(*o*-aminophenyl)-γ-aminobutyric acid (V), characterized as the dicarbobenzyloxy derivative IV, which on debenzylation gave back V, and reclosed on attempted recrystallization to IIa and, as shown by electropherograms (Fig. 1), another compound possibly the isomeric pyrrolidone VI. IIa showed good competitive inhibition of the destruction of serotonin in a purified soluble monamine oxidase system; its action in intact rats, however, decreased from 100% after 15 minutes to 25% after 4 hours, when tested in liver homogenates of the sacrificed animals. IIa also inhibited 5-hydroxytryptophan decarboxylase *in vitro* as did several other oxindole derivatives of which 2,5-dihydroxytryptophan was the most active.

Recent interest in the possible role of serotonin in brain function has focused attention on the enzymes involved in the biosynthesis and metabolism of this new amine. This investigation reports the effects of oxindole analogs of (5-hydroxy)-tryptophan and -tryptamine on 5-hydroxytryptophan (5-HTP) decarboxylase and monamine oxidase (MAO).

A. Chemistry of 3-(β-Aminoethyl)-oxindoles.—The activation of peptide bonds by suitably located amino or hydroxyl groups under proper enzymatic or non-enzymatic catalysis leads to N → O acyl migration and hydrolysis, or to N → N *trans*-peptidization. Interesting recent model rearrangements of this type are the conversion of diglycinimide to glycylglycine at *p*H 5[2,3] or the alkali-catalyzed transformation of O-glycylsalicylidipeptide esters to salicyltripeptide esters.[4]

As part of an investigation on oxindolylalanine peptides the chemical part of this paper describes the synthesis of oxindole-β-ethylamines (IIa, b, c) in which the basic amino group interacts with the lactam carbonyl of the oxindole ring in analogy to similar intramolecular reactions in the oxindole series.[5]

The synthesis of 2-hydroxytryptamine by the alkylation of an unsubstituted oxindole with chloroacetonitrile and subsequent hydrogenation is not possible.[6] Hendrickson[7] was able to condense ethyl hippurate with oxindole to the largely enolic (VIIb) β-hippuryloxindole (VIIa), whose catalytic reduction in the presence of acid led to III in 90% yield. The hydrolysis of III proved difficult and gave a complicated mixture of compounds from which IIa could not be isolated. We introduced the 2-oxy function directly into tryptamine by mild reductive acid hydrolysis of the symmetric disulfide Ia, a reaction utilized before with pyrroles,[8] tryptophan[9] and lysergic acid diethylamide.[10] The use of N-carbobenzyloxytryptamine (see Experimental part) offered no advantages.

(1) Presented in part at the Conference on the Biochemistry of Mental Disease, University of British Columbia, Vancouver, B. C., June 16–18, 1957.

(2) Th. Wieland, E. Bokelmann, L. Bauer, H. V. Lang and H. H. Lau, *Ann.*, **583**, 129 (1953).

(3) Th. Wieland, H. V. Lang and D. Liebsch, *ibid.*, **597**, 227 (1955); *cf.*, Th. Wieland, *Angew. Chem.*, **69**, 362 (1957).

(4) M. Brenner, J. P. Zimmermann, J. Wehrmüller, P. Quitt and I. Photaki, *Experientia*, **11**, 397 (1955); M. Brenner, *Angew. Chem.*, **69**, 102 (1957).

(5) E. Wenkert and Th. L. Reid, *Experientia*, **10**, 417 (1954).

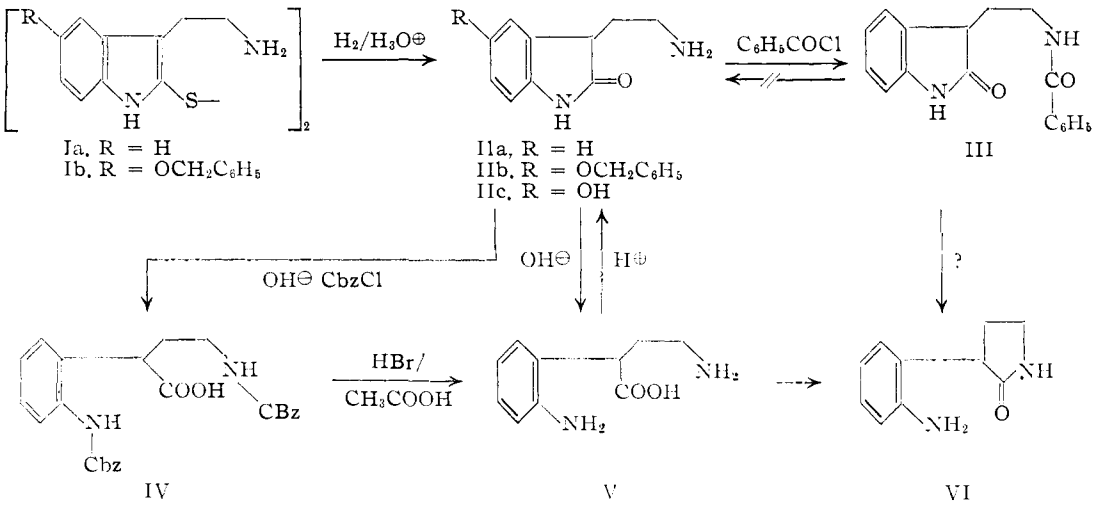
(6) E. Wenkert, private communication.

(7) James B. Hendrickson, Thesis, Harvard University, 1954.

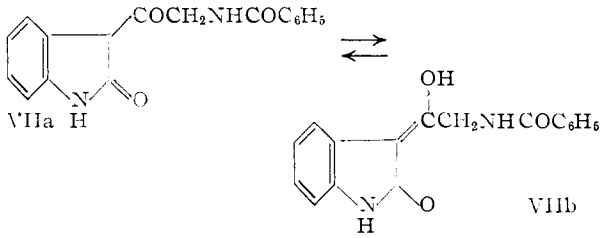
(8) H. Fischer and M. Herrmann, *Hoppe-Seyler's Z. physiol. Chem.*, **122**, 4 (1922).

(9) Th. Wieland, O. Weiberg, E. Fischer and G. Hörlein, *Ann.*, **587**, 146 (1954).

(10) K. Freter, J. Axelrod and B. Witkop, THIS JOURNAL, **79**, 3191 (1957).



The crystalline hydrochloride of α-hydroxytryptamine (IIa) was not affected by hot concentrated hydrochloric acid; however, when it was refluxed



with 2 *N* alkali the oxindole ring was opened, as shown by the formation of a diazotizable aromatic amino group. The rate of hydrolysis was followed

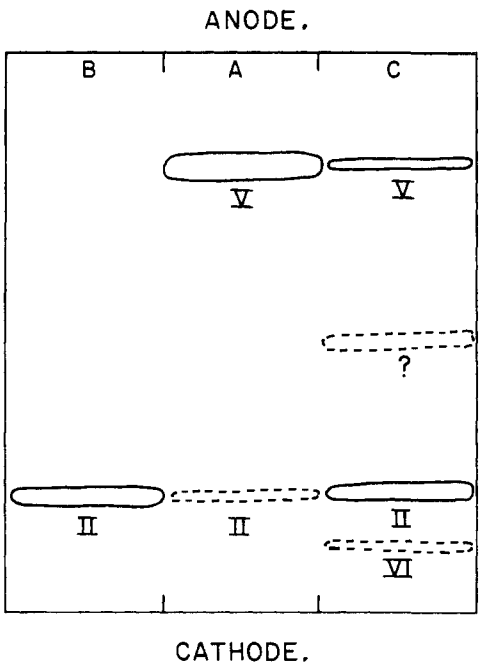
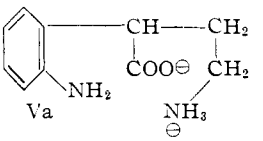


Fig. 1.—High voltage paper electropherograms (75 v./cm., 65 min.) of α-(*o*-aminophenyl)-γ-aminobutyric acid (V) and of its transformation products II and VI before (A) and after (B) recrystallization and of the total mother liquor (C); buffer system pyridine:acetic acid:water 10:1:89.

by high voltage paper electrophoresis, taking aliquots of the hydrolysate at various times. After 12 hours there was still present about 25% of the starting material. The main product was α-(*o*-aminophenyl)-γ-aminobutyric acid (V), which in neutral solution apparently was present as the zwitterion Va and showed about the same migration as toluidine in an electric field. By complete carbobenzyloxylation of the basic hydrolysate, V was con-



verted to the ether-soluble N,N-dicarbobenzyloxy derivative IV. On decarbobenzyloxylation of IV in acetic acid saturated with hydrogen bromide (or by catalytic hydrogenation) the dihydrobromide of V was obtained, which yielded a single spot on the electropherogram. Cautious recrystallization from warm methanol effected reclosure to IIa (Fig. 1). Mother liquors from IIa hydrobromide show that only a small amount of V is left and that there is a second spot (Fig. 1) of a ninhydrin-negative fluorescent compound, probably 3-(*o*-aminophenyl-α-pyrrolidone (VI).[7]

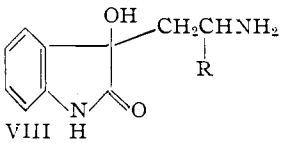
This easy reclosure of V also is observed with *o*-aminophenylacetic acid, whose formation from oxindole, however, requires very drastic conditions, namely, strong baryta at 150°.[11,12] Similar transacylations,[13] amide ketone ring-chain tautomerisms,[14–16] and amide–lactone rearrangements involving related tautomers[17,18] have been observed.

The analysis of mixtures containing an unstable compound such as V required a special rapidly working method. Ultraviolet spectrophotometry was of little help in view of the similarity of the ab-

(11) A. v. Baeyer, *Ann.*, **140**, 1 (1866).
(12) P. W. Neber, *Ber.*, **55**, 826 (1922).
(13) P. L. Julian, THIS JOURNAL, **75**, 5305 (1953).
(14) F. Ramirez and A. P. Paul, THIS JOURNAL, **77**, 3337 (1955).
(15) S. Wawzonek, H. A. Laitinen and S. J. Kwiatkowski, *ibid.*, **66**, 830 (1944).
(16) L. A. Cohen and B. Witkop, *ibid.*, **77**, 6595 (1955).
(17) E. Girod, R. Delley and F. Hafliger, *Helv. Chim. Acta*, **40**, 108 (1957).
(18) E. Walton and N. B. Green, *J. Chem. Soc.*, 315 (1945).

sorption spectra of oxindole and toluidine derivatives. Conventional paper chromatography was not fast and accurate enough. High voltage electrophoresis with the pherograph as developed by Wieland and Pfleiderer[19] proved to be the method of choice in this investigation.

The well known susceptibility of 3-alkyl substituted oxindoles to autoxidation[20-23] is noticeable with IIa whose bicarbonate-buffered solutions after aeration with oxygen showed about 6 new, partly ninhydrin-positive, spots on the electropherogram. A better method for oxidation is treatment with iodine which is decolorized slowly. Such oxidized solutions on paper electrophoresis show essentially one single spot of a new compound, presumably the dioxindolethylamine (VIII, R = H). Hendrickson[7] observed a compound of this composition (m.p. 181–184°) to which he assigned structure VIII.



VIII

Potential inhibitors of 5-hydroxytryptophan decarboxylase were sought in the tryptophan and tryptamine series. The oxindole analog of tryptophan[24] inhibited 5-hydroxytryptophan decarboxylase (Table II). This prompted the preparation of 2,5-dihydroxytryptophan which was twice as active in this respect. The dioxindole derivatives XIX (R = COOH, 2 isomers) were almost inactive. Future research will concentrate, therefore, on the synthesis of 2-substituted 5-hydroxytryptophan derivatives.

Experimental[25]

2,2'-Dithiobistryptamine (I).—Tryptamine (1.6 g.) was dissolved in 30 ml. of benzene. After addition of 3 g. of anhydrous trichloroacetic acid the solution was cooled in ice-water and treated dropwise with 0.67 g. of S_2Cl_2 in 5 ml. of chloroform. After 30 min. the yellow solution was poured into 200 ml. of petroleum ether and the flocculent precipitate washed by repeated decantation with ether. The trichloroacetate of tryptamine disulfide was dissolved in water, if necessary with addition of some acetic acid. After the addition of the same volume of ethyl acetate the base was liberated with 5 *N* NaOH and rapidly extracted. The organic layer was washed with $NaHCO_3$, water, and dried. After evaporation *in vacuo*, the base remained as a yellow amorphous powder. A sample was converted to the hydrochloride which could be recrystallized from hot water containing a few drops of concentrated HCl as yellow cubes, slightly hygroscopic, m.p. 223°. Keller's color reaction was positive, the Ehrlich reaction negative; λ_{max} 220 mμ, ε 43.000; λ_{max} 350 mμ, ε 10.900 (in ethanol).

Anal. Calcd. for $C_{20}H_{22}N_4S_2 \cdot 2HCl$: C, 52.75; H, 5.27; N, 12.30; S, 14.07. Found: C, 52.31; H, 5.52; N, 12.16; S, 14.32.

(19) Th. Wieland and G. Pfleiderer, *Angew. Chem.*, **67**, 257 (1955).

(20) E. C. Kendall, A. E. Osterberg and B. F. MacKenzie, THIS JOURNAL, **48**, 1384 (1936); **49**, 2047 (1927).

(21) P. L. Julian and J. Pikl, *ibid.*, **57**, 542 (1935).

(22) P. L. Julian, H. C. Printy and E. E. Dailey, *ibid.*, **78**, 3501 (1956).

(23) P. L. Julian, E. E. Dailey, H. C. Printy, H. L. Cohen and S. Hamashige, *ibid.*, **78**, 3508 (1956).

(24) *Cf.* A. Ek, H. Kissman, J. B. Patrick and B. Witkop, *Experientia*, **8**, 36 (1952); Th. Wieland, O. Weiberg and W. Dilger, *Ann.*, **592**, 69 (1955).

(25) The microanalyses were performed by Dr. W. C. Alford and associates, Analytical Service Laboratory, National Institutes of Health. All melting points are corrected.

N-Carbobenzyloxytryptamine was prepared in 80–90% yield by Schotten–Baumann acylation of tryptamine with carbobenzyloxy chloride. After recrystallization from water–methanol colorless leaflets, m.p. 92°, were obtained.

Anal. Calcd. for $C_{18}H_{18}N_2O_2$: C, 73.54; H, 6.16; N, 9.52. Found: C, 73.72; H, 6.30; N, 9.43.

2,2'-Dithiobis-[N,N'-carbobenzyloxytryptamine].—When 580 mg. of carbobenzyloxytryptamine was dissolved in 40 ml. of abs. benzene and treated with 135 mg. of disulfur dichloride in 1 ml. of chloroform at 0° a yellow precipitate was obtained which was washed with petroleum ether and dried. The amorphous yellow powder melted with decomposition at 120–150°.

Anal. Calcd. for $C_{36}H_{34}N_4S_2O_4$: N, 8.61; S, 9.84. Found: N, 8.41; S, 10.14.

2-Hydroxytryptamine (Oxindole-3-ethylamine, IIa) Hydrochloride.—The disulfide Ia (6 g.) was dissolved in 30 ml. of glacial acetic acid and 5 ml. of water, 1 ml. of 5 *N* HCl and 4 g. of zinc dust was added. After 6 hours heating under reflux the evolution of hydrogen sulfide had ceased. One removed the undissolved zinc by centrifugation and concentrated *in vacuo*, to a sirup. Excess free acid was then removed in the desiccator over KOH. The residue was dissolved in water (20 ml.) and H_2S bubbled through the solution until no more zinc sulfide was precipitated. After centrifugation the solution was concentrated *in vacuo*. to 5 ml. and lyophilized. The crystalline residue was recrystallized from very little methanol, yielding 0.9 g. of IIa. An additional crop of 0.4 g. could be obtained from the mother liquor by concentration and dropwise addition of ether.[26] The sample for analysis was recrystallized from methanol, m.p. 237°; λ_{max} 253 mμ, ε 4,960, shoulder at 280 mμ, ε 8.600. The Ehrlich reaction was negative. Zinc dust distillation yielded a volatile indolic fraction (odor, color reactions).

Anal. Calcd. for $C_{10}H_{12}N_2O \cdot HCl$: C, 56.47; H, 6.12; N, 13.19. Found: C, 56.59; H, 6.02; N. 12.97.

The yellow **picrate**, obtained crystalline from aqueous methanol, melted at 176°.

Anal. Calcd. for $C_{10}H_{12}N_2O \cdot C_6H_3N_3O_7$: C, 47.41; H, 3.73; N, 17.28. Found: C, 47.12; H, 4.23; N, 17.12.

Diazotization Reaction.—One milligram of IIa was dissolved in 5 drops of 1 *N* NaOH and warmed on the steam-bath for 10 min., then acidified, diazotized with 2 drops of 2 *N* $NaNO_2$ and coupled to a red dye with β-naphthol in 5 *N* NaOH. This reaction was negative without previous base treatment.

N-Benzoyl-2-hydroxytryptamine (III).—The benzoylation of 43 mg. of IIa was performed with 30 mg. of magnesium oxide as base, 35.2 mg. of benzoyl chloride in 3 ml. of water and 3 ml. of chloroform. The usual work-up yielded 40 mg. of crystalline material which after recrystallization from alcohol appeared in fine colorless needles, m.p. 194°. The infrared spectrum was identical with that published by Hendrickson.[7]

Anal. Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75. Found: C, 72.67; H, 5.52.

Hydrolysis of 2-Hydroxytryptamine (IIa).—Refluxing of IIa in ethanol saturated with hydrogen chloride or in concentrated hydrochloric acid failed to open the oxindole ring. When IIa was refluxed with 2 *N* sodium hydroxide under nitrogen the oxindole ring was opened, as shown by the positive diazo reaction. The course of the reaction was followed by high voltage paper electrophoresis. After 12 hours about 25% of the starting material was still present. The main product was α-(*o*-aminophenyl)-γ-aminobutyric acid (V). In addition a small amount of a ninhydrin-negative compound fluorescent in ultraviolet light appeared, presumably 3-(*o*-aminophenyl)-2-pyrrolidone (VI).

N,N'-Dicarbobenzyloxy-α-(*o*-aminophenyl)-γ-aminobutyric Acid (IV).—2-Hydroxytryptamine hydrochloride (130 mg.) was dissolved in 2.5 ml. of 2 *N* NaOH and refluxed for 16 hours. The solution was chilled in ice and treated with 0.2 ml. of carbobenzyloxychloride under vigorous stirring.

(26) Further addition of ethyl acetate precipitated more of an almost colorless powder which on carbobenzyloxylation under Schotten–Baumann conditions gave a benzene-soluble crystalline product, unsharp m.p. 105–115°, apparently 2,2,-bis-[N-carbobenzyloxytryptamine]-thioether [Found: C, 65.93; H, 5.60; S, 5.03. Calcd. for $(C_{16}H_{17}N_2O_2)_2S$: C, 65.6; H, 6.0; S, 5.6].

After 30 minutes the mixture was allowed to come to room temperature and stirring continued for 20 minutes. To the milky emulsion 5 ml. of water was added and the neutral products extracted three times with 5 ml. of ether. The combined ether extracts were washed, first with 1 *N* NaOH and then with water. The aqueous layers were combined and acidified with concentrated HCl under cooling and extracted with ethyl acetate. After washing and drying, the organic solution was evaporated to dryness. The residue crystallized on trituration with ether–petroleum ether, giving 35 mg. (15%) of colorless crystals, m.p. 153°. The compound was uniform on paper electrophoresis at neutral *p*H, and migrated toward the anode, as required for a carboxylic acid.

Anal. Calcd. for $C_{26}H_{26}O_6N_2$: C, 67.52; H, 5.67; N, 6.06. Found: C, 68.26; H, 5.94; N, 5.78.

β-(*o*-Aminophenyl)-γ-aminobutyric Acid (V).—The dicarbobenzyloxy derivative IV was dissolved in acetic acid saturated with hydrogen bromide and kept at room temperature for 10 min. The solution was then poured into excess ether and the colorless amorphous precipitate washed repeatedly. High-voltage electrophoresis indicated (Fig. 1) the presence of a uniform new compound (V) which contained only traces of IIa. Recrystallization from a small volume of warm methanol yielded a crystalline hydrobromide, m.p. 254°, whose electropherogram (Fig. 1, B) and analysis agreed with IIa·HBr.

Anal. Calcd. for $C_{10}H_{13}N_2OBr$: N, 10.90. Found: N, 11.03%.

The mother liquor precipitated with ether and subjected to paper electrophoresis (Fig. 1, C) contained only a small fraction of the acid V, a small amount of IIa and a new compound, presumably the pyrrolidone VI.

5-Benzyloxy-2-hydroxytryptamine (IIb) Hydrochloride.—The conversion of 5-benzyloxytryptamine to the disulfide was carried out as described for tryptamine. The crude trichloroacetate, without further purification, was reductively hydrolyzed by zinc in acetic acid containing hydrochloric acid as before. By recrystallization from methanol–water the hydrochloride was obtained as microscopic rods, m.p. 238–240°, in 20% yield. The reactions of IIb were the same as described for IIa.

Anal. Calcd. for $C_{17}H_{18}O_2N_2 \cdot HCl$: C, 64.04; H, 6.01; N, 8.79. Found: C, 63.80; H, 5.81; N, 8.77.

2,5-Dihydroxytryptamine (IIc) Hydrochloride.—Palladium black (35 mg.) was prereduced in 1 ml. of water and 15 ml. of alcohol. Then 185 mg. of 5-benzyloxy-2-hydroxytryptamine hydrochloride was added. After 1.5 hours the hydrogen uptake ceased with the uptake of the calculated volume (13.6 ml.). Removal of the catalyst and careful concentration *in vacuo*, yielded stout colorless rods, which were washed with ice-cold alcohol, then with alcohol–ether. The m.p. was 238–241°, on admixture with IIb (m.p. 238–240°) 210–220°. The yield of the combined crops (20 mg. + 30 mg. from the mother liquor) was 40%. The hydrochloride is hygroscopic and very soluble in water. With base such solutions turn rapidly brown; $\lambda_{max}^{H_2O}$ 259 (ε 6650), 307 (ε 1680); $\lambda_{max}^{0.01\ N\ NaOH}$ 280 (ε 8640), 328 (ε 3280).

Anal. Calcd. for $C_{10}H_{12}O_2N_2 \cdot HCl$: C, 52.51; H, 5.70; N, 12.28. Found: C, 51.85; H, 5.62; N, 12.46.

2,5-Dihydroxy-DL-tryptophan.—To a solution of 0.5 g. of 5-benzyloxytryptophan in 20 ml. of benzene containing enough anhydrous trichloroacetic acid to effect solution, was added 0.8 ml. of a 1 *N* solution of disulfur dichloride (S_2Cl_2) in chloroform at 0°. After 20 minutes the reaction mixture was poured into 150 ml. of petroleum ether and the yellow precipitate washed with ether. The free disulfide was liberated from the aqueous solution of the trichloroacetate by the addition of alkali, collected and dried. A solution of 0.2 g. of the disulfide in 5 ml. of glacial acetic acid was refluxed for 8 hours with 0.4 g. of zinc dust. To the cooled mixture was then added 2 ml. of glacial acetic acid saturated with hydrogen bromide. This mixture was kept at 80° for 30 minutes, then taken rapidly to dryness *in vacuo* and dried thoroughly in a desiccator. The dark residue was suspended in water and treated with H_2S. After removal of the zinc sulfide and other insoluble material the yellowish solution was taken to dryness, the residue dissolved in ethanol, filtered through cellulose powder and precipitated fractionally with ether. The crude hydrobromide of 2,5-dihydroxytryptophan was a grayish-white powder, free of sulfur, giving a negative Ehrlich reaction and was homogeneous on paper electrophoresis. The ultraviolet spectrum of an aqueous solution showed λ_{max} 257 and a shoulder at 300 mμ. After addition of alkali a shift to λ_{max} 275, shoulder at 320 mμ, was observed, indicative of a derivative of *p*-acetaminophenol.[27] The crude product gave 8.31% nitrogen (Calcd. for $C_{11}H_{13}N_2O_4Br$: N, 8.83).

B. Enzymatic Activities.—In order to influence the formation or destruction of serotonin a compound should inhibit either the enzyme which decarboxylates 5-hydroxytryptophan[28] or monamine oxidase which destroys serotonin.[29,30] The compounds reported in the preceding part were accordingly tested for such activities.

Methods.—Incubations were carried out in 20-ml. beakers on an Aminco metabolic shaker, in air at 37°. 5-Hydroxytryptophan (5HTP) decarboxylase was assayed by measuring serotonin formation essentially as described by Clark, *et al.*[28] The enzyme preparation was a high speed supernatant of guinea pig kidney. The final concentration of 5-hydroxy-DL-tryptophan employed was 2.6×10^{-3} mole.

A soluble monoamine oxidase (MAO) prepared from guinea pig liver[31] was used for most of the studies reported here. The enzyme was purified by ammonium sulfate fractionation and precipitation at *p*H 5.0–5.1. The acid-precipitated material was suspended in 0.01 *M* phosphate buffer *p*H 7.4 so that the final protein concentration was between 3–4 mg. per ml. The specific activity of this preparation (micromoles of serotonin destroyed per mg. of protein per hour) ranged between 0.2–0.5. A simple and rapid measure of MAO activity was developed by coupling the amine oxidase system with aldehyde dehydrogenase and DPN. It was possible to convert all of the aldehyde formed by the action of MAO on serotonin to 5-hydroxyindoleacetic acid (5HIAA), with a stoichiometric formation of DPNH. The DPNH could be measured spectrophotometrically by the increase in optical density at 340 mμ. The typical incubation system contained 0.5–2.0 mg. of MAO, 1 mg. of serotonin, 0.5 ml. of 0.5 *M* phosphate buffer *p*H 8.1, 0.5 μM. of DPN, 10 μM. of nicotinamide, 0.2 ml. of aldehyde dehydrogenase and H_2O to bring the volume to 3.5 ml. The reaction mixture was transferred to a Beckman quartz cuvette and the optical density increase at 340 mμ was determined against an enzyme blank (standard incubation without any substrate to correct for any endogenous reduction of DPN). A complete study describing the purification and properties of this soluble MAO will appear elsewhere.[31] MAO activity was determined in crude tissue preparations by measuring serotonin disappearance as described previously.[30]

Results

The indole analogs were tested for both MAO inhibition and 5 HTP decarboxylase inhibition. These results are summarized in Tables I and II. Although all the indole derivatives tested had some inhibitory action on MAO, only 2-hydroxytryptamine, 2-hydroxy-5-benzyloxytryptamine, oxindole-3-DL-alanine (α-hydroxy-DL-tryptophan) and the analogous 5-hydroxy derivative had any appreciable *in vitro* action on 5HTP decarboxylase (Table II). α-Hydroxytryptophan, however, had little, if any, activity when injected into guinea pigs. When 100 mg. of this compound was injected into a 500-g. guinea pig and the kidneys removed 30 minutes afterwards, activity of the enzyme prepared from this pretreated animal was comparable to the activity observed with control animals. Bifunctional catalysis by certain aminoöxindoles, *e.g.*, 3-aminoöxindole, has been claimed to be operative in

(27) *Cf.* A. L. LeRosen and E. D. Smith, THIS JOURNAL, **70**, 2705 (1948).

(28) C. T. Clark, H. Weissbach and S. Udenfriend, *J. Biol. Chem.*, **210**, 139 (1954).

(29) E. A. Zeller, J. Barsky and E. H. Berman, *ibid.*, **214**, 267 (1955).

(30) A. Sjoerdsma, T. E. Smith, T. D. Stevenson and S. Udenfriend, *Proc. Soc. Exp. Biol. & Med.*, **89**, 36 (1955).

(31) H. Weissbach, B. Redfield and S. Udenfriend, *J. Biol. Chem.*, in press.

the non-enzymatic decarboxylation of keto acids.[32] This applies only to model studies under unphysiological conditions. There is no catalytic activity at room temperature.[33]

TABLE I

INHIBITION OF PURIFIED MONAMINE OXIDASE *in vitro* BY DERIVATIVES OF OXINDOLE AND INDOLE

Compound	Final molar concn.	Inhibition, %
2-Hydroxy-DL-tryptophan[a]	1.35×10^{-4}	7
Dioxindole-3-DL-alanine[b] (low melting isomer, 232°)	2.6×10^{-4}	8
α-Iso-5-hydroxy-DL-tryptophan[c] (β-[5-hydroxyindolyl-2]-alanine)	1.35×10^{-4}	15
2-Carbethoxy-DL-tryptophan[d]	2×10^{-4}	19
α-Isoserotonin[e] (β-[5-hydroxyindolyl-2]-ethylamine)	1.35×10^{-4}	25
2,5-Dihydroxytryptamine	1.35×10^{-4}	27
2-Phenyl-DL tryptophan[d]	2×10^{-4}	35
α-Isotryptamine[e] (β-[indolyl-2]-ethylamine)	1.35×10^{-4}	50
2-Hydroxytryptamine	1.35×10^{-4}	61
2-Hydroxy-5-benzyloxytryptamine	1.8×10^{-4}	74
Tetrahydronorharmane[e]	2.7×10^{-4}	100
N-Methyltetrahydronorharmane[f]	2.6×10^{-4}	100
N-Methyltetrahydronorharmane	7×10^{-5}	55

[a] B. Witkop, *Ann.*, **558**, 98 (1946). [b] P. L. Julian, E. D. Dailey, H. C. Printy, H. L. Cohen and S. Hamashige, THIS JOURNAL, **78**, 3503 (1956); we are greatly indebted to Dr. P. L. Julian for samples of the two isomeric dioxindole-3-alanines. [c] The synthesis of the α-iso compounds has been reported by W. Schindler, *Helv. Chim. Acta*, **40**, 1130 (1957). They were supplied through the courtesy of Dr. F. Häfliger, Basle. [d] H. M. Kissman and B. Witkop, THIS JOURNAL, **75**, 1967 (1953). [e] V. Boekelheide and C. Ainsworth, *ibid.*, **72**, 232 (1950). [f] B. Witkop and S. Goodwin, *ibid.*, **75**, 3371 (1953).

TABLE II

INHIBITION OF 5-HYDROXYTRYPTOPHAN DECARBOXYLASE IN GUINEA PIG KIDNEY HIGH SPEED SUPERNATANT

The incubation mixture contained 0.5 ml. of enzyme (equivalent to 80 mg. of tissue), 0.5 ml. of 0.5 *M* phosphate buffer *p*H 8.1, 2 mg. of 5-hydroxy-DL-tryptophan, 2 mg. of inhibitor and water to make up to a total of 3.5 ml. The time of incubation was 30 min. Under these conditions about 1 μM. of serotonin was formed in the uninhibited system.

Compound	Inhibition, %
Harmine	0
Tetrahydronorharmane	0
N-Methyltetrahydronorharmane	0
2-Carbethoxy-DL-tryptophan	6
Dioxindole-3-DL-alanine, m.p. 232°	5–8
Dioxindole-3-DL-alanine, m.p. 260°	0
2-Hydroxytryptamine	27
2-Hydroxy-5-benzyloxytryptamine	25
2-Hydroxy-DL-tryptophan (oxindole-3-DL-alanine)	30
2,5-Dihydroxy-DL-tryptophan	68

Table III summarizes the effect of injected α-hydroxytryptamine on monamine oxidase activity on rat liver homogenates as a function of time. After 4 hours there was only one-fourth of the initial inhibition left.

TABLE III

INHIBITION OF MONAMINE OXIDASE IN RAT LIVER HOMOGENATES AFTER ADMINISTRATION OF 200 MG./KG. OF 2-HYDROXYTRYPTAMINE (IIa)

Two animals were sacrificed at the times indicated, the livers removed and homogenized. MAO activity was determined by measuring the rate of serotonin disappearance.[30]

Time after injection, min.	Inhibition, %
15	100
120	34
240	25
960	0

None of the amino acids tested served as substrates for 5HTP decarboxylase which emphasizes again the high specificity of this enzyme.[28] Although MAO attacks a wide variety of primary amines the oxindole amines tested did not act as substrates. The inhibition of MAO by 2-hydroxytryptamine was shown to be of a competitive nature and, therefore, could be overcome by an excess of the natural substrate, serotonin as shown when plotted according to Lineweaver and Burk (Fig. 2).[34]

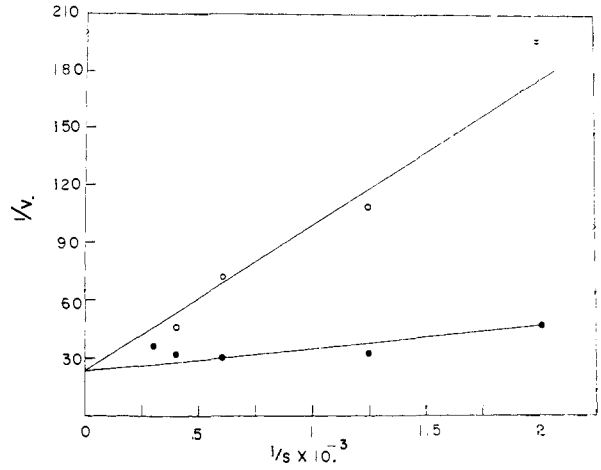


Fig. 2.—Lineweaver–Burk plot to show competitive inhibition of MAO by 2-oxytryptamine; conditions for assaying MAO are described in the text: O—O, inhibitor added, final concentration 1.6×10^{-4} *M*; ●—●, no inhibitor added.

These enzymatic tests should be valuable in determining whether the growing class of pharmacologically active compounds with antiserotonin activity[35] may act by influencing 5-hydroxytryptophan decarboxylase or monamine oxidase.

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(32) *Cf.* W. Langenbeck, "Die Organischen Katalysatoren," Springer–Verlag, Berlin, Göttingen, Heidelberg, 1949.

(33) H. von Euler, H. Hasselquist and E. Ericksson, *Ann.*, **578**, 188 (1952).

(34) H. Lineweaver and D. J. Burk, THIS JOURNAL, **56**, 658 (1934).

(35) *Cf.* D. W. Woolley, *Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol.*, **228**, 68 (1956); D. W. Woolley and E. Shaw, *Science*, **124**, 34 (1956); R. L. Clark, A. A. Pessolano, E. F. Rogers and L. H. Sarett, American Chemical Society Meeting, New York, Sept. 8–13, 1957, Abstracts, p. 18-O.