Preparation and Biological Evaluation of Some *N*-Amino Acid Substituted Derivatives of Serotonin

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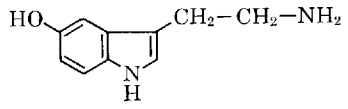
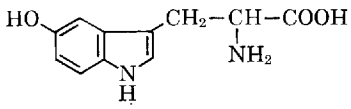
A series of *N*-amino acid substituted derivatives of serotonin was prepared. The method entailed condensing the α-halo analogs of alanine, isoleucine, phenylalanine, and valine with 5-benzyloxy-serotonin using a method adapted from Fisher's peptide synthesis. The activity of these compounds was compared with 5-hydroxytryptophan in an Actophotometer to determine their effect on generalized activity in mice. The results are presented along with some possible explanations for the biological observations.

HIGH cerebral levels of serotonin are known to produce a significant increase in central stimulation (1–3). Woolley (4) stated that hallucinations, agitation, and other signs of excitation are found in individuals with elevated cerebral levels of serotonin and that decreased levels can lead to depressive states. There is also an implication that an abnormality in serotonin metabolism may be responsible for schizophrenia (4). The biosynthesis of serotonin (I) in animals commences with the natural amino acid tryptophan which is obtained from dietary sources (5). 5-Hydroxytryptophan (II) is produced by hydroxylation of the amino acid in the 5-position of the indole ring by an enzyme system having NADP as one of the coenzymes. The latter



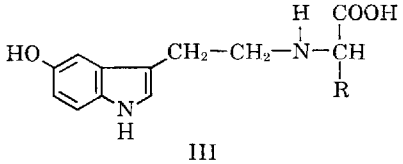
I



II

compound (II) is easily decarboxylated to the hormone serotonin (I) by 5-hydroxytryptophan-decarboxylase, which contains pyridoxal phosphate as a coenzyme. Serotonin is not produced in animals by the direct 5-hydroxylation of tryptamine (5).

Shaw and Woolley reported that exogenous serotonin does not pass from the blood into the brain very readily (6–8). Instead, the parenteral administration of serotonin produces powerful intestinal contractions, increased tissue permeability, and a rise in blood pressure. Therefore, if one wanted to observe the central effects of serotonin, a substance like 5-hydroxytryptophan (II) would be necessary. This compound can pass through the blood brain barrier where it is subsequently converted to serotonin (2, 9). Since serotonin (I) cannot pass from the blood into the brain, it appears likely that the carboxyl group of 5-hydroxytryptophan may be necessary for its transport into the brain. Evered and Randall (10) have shown that it is possible to transport cytoactive drug molecules into cells by linking them to actively transported natural amino acids as carriers. Considering these factors, it was believed that a series of compounds with unique properties could be produced if serotonin was linked to some natural amino acids. These compounds would: (*a*) have the free carboxyl group that appears to be necessary for transport across the blood brain barrier and (*b*) have natural amino acids for anchoring purposes to carrier and metabolic receptor sites. The proposed series of compounds are derivatives of structure III, where R represents the tail portion of the various amino acids used.



III

The synthesis of these compounds was initiated by treating 5-benzyloxyindole (IV) with oxalyl chloride to produce 5-benzyloxy-3-indoleglyoxalyl chloride (V). The acid chloride was converted to the amide (VI) by treatment with concentrated aqueous NH_3 and was subsequently reduced to 5-benzyloxytryptamine (VII) with $LiAlH_4$. The 5-benzyloxy derivatives of III were produced by condensing the appropriate α-

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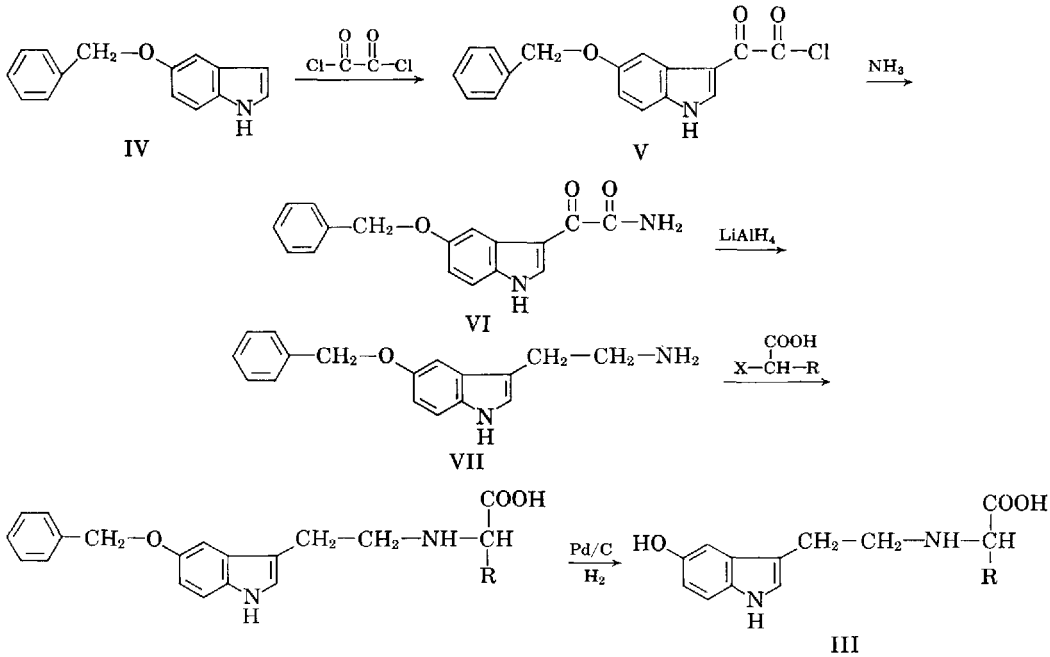
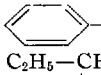


III*a*, R = CH_3—(alanine derivative)
III*b*, R = $(CH_3)_2$—CH—(valine derivative)
III*c*, R =  —CH_2—(phenylalanine derivative)
III*d*, R = C_2H_5—CH(—CH_3)—(isoleucine derivative)

Scheme I

halo analogs of the amino acids with the amine (VII). These were debenzylated by catalytic hydrogenation using Pd/C catalyst. The complete reaction is presented in Scheme I.

EXPERIMENTAL[1]

The first part of this section deals with the preparation of 5-benzyloxytryptamine (VII) which was used as the common intermediate for condensation with the α-halo analogs of the amino acids.

5-Benzyloxytryptamine (VII).—This compound was produced in 41% yield based on the starting material using the method of Lipp *et al.* (11). 5-Benzyloxytryptamine HCl (12) was converted to the base by treatment with aqueous KOH and extraction with diethyl ether.

N - (5 - Hydroxy - 3 - indole - ethyl) Alanine (IIIa).—Compounds (III*a–d*) were prepared by a modification of the method suggested by Fischer and Otto (13, 14) for the synthesis of peptides. Seventeen grams (0.064 mole) of 5-benzyloxytryptamine was dissolved in 50 ml. of recently boiled and cooled *N,N*-dimethylformamide (DMF). In a separate vessel 10.85 Gm. (0.10 mole) of α-chloropropionic acid (Eastman Red Label) was mixed with 20 ml. of DMF and enough 1.0 *N* KOH to neutralize the acid. The 2 solutions were transferred to a reflux flask and water was slowly added until the mixture became homogeneous. An additional 0.01 mole of KOH was added, and the solution was refluxed for 12 hr. During this time more base was added if necessary to maintain the pH slightly basic; however, large excesses were avoided because of the possible hydrolysis of DMF. The reaction mixture was diluted with twice its volume of 1 *N* aqueous KOH and any unreacted amine which precipitated was removed by extraction with ethyl ether. The solution was adjusted to pH 4.0 with glacial acetic acid and placed in a refrigerator for 24 hr. The product *N*-(5-benzyloxy-3-indolethyl) alanine was removed by filtration and washed with hot isopropyl alcohol. It was then suspended in 150 ml. of ethanol and water mixture (1:1) which contained 2.0 Gm. of 10% Pd/C (Matheson, Coleman and Bell). The product was debenzylated in a Parr hydrogenator at a pressure of 50 psig for 1 hr. The catalyst was removed by filtration and the solvent was removed under vacuum. The residue was crystallized from an isopropanol–water mixture and dried yielding 6 Gm. (23% yield) of *N*-(5-hydroxy-3-indole-ethyl) alanine·$1H_2O$, m.p. 238–243° dec.

Anal.—Calcd. for $C_{13}H_{18}N_2O_4$: C, 58.70; H, 6.79; N, 10.55. Found: C, 59.00; H, 6.93; N, 10.30.

N - (5 - Hydroxy - 3 - indole - ethyl) Valine (IIIb).—This compound was prepared in a similar manner to the alanine derivative (III*a*). Seventeen grams (0.064 mole) of 5-benzyloxytryptamine was reacted with 18.1 Gm. (0.10 mole) of α-bromo-

[1] All melting points were taken on a Fisher Johns apparatus and are uncorrected. Microanalyses were performed by Organic Microanalysis, Montreal, Ontario, Canada.

β-methylbutyric acid (Eastman Red Label). The *N*-(5-benzyloxy-3-indole-ethyl) valine derivative had a m.p. of 225–230° after crystallization from an ethanol–water mixture. After debenzylation and crystallization from the isopropanol–water mixture, 7.0 Gm. (25% yield) of the title compound was produced, m.p. 240–245° dec.

Anal.—Calcd. for $C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14. Found: C, 64.90; H, 7.19; N, 10.35.

N - (5 - Hydroxy - 3 - indole - ethyl) Phenylalanine (IIIc).—This compound was prepared in a similar manner to the alanine derivative (III*a*). Sixteen grams (0.06 mole) of 5-benzyloxytryptamine was refluxed with 20 Gm. (0.086 mole) of α-bromo-β-phenyl propionic acid (K & K Laboratories, New York, N. Y.) for 3 hr. The benzyloxy derivative had a m.p. of 200–205°. The debenzylated product was crystallized from an isopropanol–water mixture, and 6.5 Gm. (20% yield) of the title compound was produced, m.p. 235–238° dec.

Anal.—Calcd. for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.03; H, 6.54; N, 8.73.

N - (5 - Hydroxy - 3 - indole - ethyl) Isoleucine (IIId).—This compound was prepared in a similar manner to the alanine derivative (III*a*). Sixteen grams (0.06 mole) of 5-benzyloxytryptamine was refluxed with 20 Gm. (0.10 mole) of α-bromo-β-methyl valeric acid (K & K Laboratories, New York, N. Y.) for 3 hr. yielding the 5-benzyloxy derivative, m.p. 205–210° dec. The debenzylated product was crystallized from an isopropanol–water mixture and 5 Gm. (17.2% yield) of the title compound was produced, m.p. 212–215° dec.

Anal.—Calcd. for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65. Found: C, 65.39; H, 7.52; N, 9.81.

BIOLOGICAL TESTING

The objective of the biological testing was to determine whether these compounds had any central stimulating activity. Woolley (4) stated that high serotonin levels in the brain caused hallucinations and other signs of excitation. Since cerebral serotonin levels cannot be raised by extracerebral injections of serotonin (2, 9) a precursor substance such as 5-hydroxytryptophan (II) was used as the standard for comparison of the serotonin activity of the new compounds. The test group consisted of four 25-Gm. white mice. All the activity measurements were conducted in an Actophotometer (Metro Industries, Long Island City, N. Y.) which is designed to measure the general activity of the mice. Subcutaneous doses of normal saline solution, 2 mg. of 5-hydroxytryptophan, or the molar equivalent of compounds III*a–d* (2.26, 2.40, 2.94, and 2.62 mg., respectively) were administered, and the activity of the mice was determined periodically during a period of 5 hr. It was necessary to add minimal amounts of 0.1 *N* NaOH to solubilize some of the compounds so that the total dose was contained in 0.2 ml. The second series consisted of the simultaneous injection of equimolar mixtures of 5-hydroxytryptophan (2 mg.) and the test compounds. The results are presented in Tables I and II.

DISCUSSION

While the synthesis of serotonin and 5-benzyloxytryptamine are not new, the preparation of these *N*-substituted amino acid derivatives does represent a new concept. It is well established that natural amino acids can readily pass through cell walls with the aid of active transport systems. Serotonin, however, cannot pass from the blood into the brain very readily (6–8); therefore, the joining of the hormone to an amino acid *via* a covalent bond to assist in transporting it into the brain proposes an interesting concept.

The biological testing of these compounds suggest some reasons for their lack of central activity. The data in Table I show that a 2.0-mg. dose of 5-hydroxytryptophan per mouse slightly lowered

TABLE I.—ACTIVITY OF MICE[a] FOLLOWING SUBCUTANEOUS INJECTIONS OF THE TEST COMPOUNDS

	Time, min.										
Compd.	10	15	30	45	60	90	120	150	180	240	300
Saline control	59	80	102	110	144	157	165	185	216	227	231
5-Hydroxytryptophan[b]	19	21	59	60	74	82	82	91	91	95	140
III*a* alanine der.[c]	50	75	99	102	131	145	155	171	194	214	214
III*b* valine der.[c]	57	71	118	118	119	129	129	145	145	157	...
III*c* phenylalanine der.[c]	70	80	85	85	85	89	94	99	111	112	...
III*d* isoleucine der.[c]	58	66	104	...	149	173	173	173	175	181	189

[a] In terms of Actophotometer readings. [b] At a level of 2 mg./mouse; 15 mg./mouse produced marked excitement, convulsions, and agitation (15). [c] At an equimolar dose level with 5-hydroxytryptophan.

TABLE II.—ACTIVITY OF MICE[a] FOLLOWING SUBCUTANEOUS INJECTIONS OF MIXTURES OF 5-HYDROXYTRYPTOPHAN AND TEST COMPOUNDS

	Time, min.										
Mixture	10	15	30	45	60	90	120	150	180	240	300
Saline	59	80	102	110	144	157	165	185	216	227	231
5-HTP[b] + III*a*[c]	28	37	57	57	60	74	75	75	95	104	141
5-HTP[b] + III*b*[c]	39	51	64	67	86	90	102	102	105	132	149
5-HTP[b] + III*c*[c]	42	61	99	102	102	140	140	142	147	166	166
5-HTP[b] + III*d*[c]	31	68	100	101	118	123	151	169	174	179	185

[a] In terms of Actophotometer readings. [b] 5-Hydroxytryptophan at a level of 2 mg./mouse. [c] Test compounds at a concentration equal to the molar equivalent of 5-hydroxytryptophan.

the generalized activity when compared to that of a saline control. This is substantiated by the work of Woolley (15), who also noted that 2.5-mg. doses of 5-hydroxytryptophan per mouse caused either normal or slightly subdued activity. A possible reason for the lack of activity of small doses of 5-hydroxytryptophan is suggested by Gal *et al.* (16), who observed that intracerebral injections of labelled L-tryptophan in rats and pigeons produced a significant increase in cerebral serotonin levels; D-tryptophan, however, did not increase the serotonin levels but in fact caused a decrease. They also noted that intraperitoneal injections of L-tryptophan, at a dose level 17 times greater than the intracerebral dose did not result in the formation of any labelled serotonin in the brain. While it is true that Gal used tryptophan and not 5-hydroxytryptophan, there may be still some analogy. In the present study racemic 5-hydroxytryptophan was injected subcutaneously. Possibly some of the D-5-hydroxytryptophan entered the brain where it acted as an antagonist to the decarboxylation of L-5-hydroxytryptophan resulting in a decreased cerebral serotonin level with generalized reduction of activity. Another factor to be considered is that other organs besides the brain such as the liver can decarboxylate 5-hydroxytryptophan and bind serotonin resulting from extracerebral administration. These factors may account for the lack of stimulation demonstrated by the 2-mg. dose of 5-hydroxytryptophan. Woolley (15) also stated that large doses of 5-hydroxytryptophan (15 mg./mouse) produced marked excitement, convulsions, and agitation. It appears thus that the smaller doses are metabolized outside the brain and produced the classical symptoms on the gastrointestinal tract and blood pressure. The administration of larger amounts may be partially available to the brain because the extracerebral metabolism sites become saturated and allow some to enter the brain, thus resulting in central stimulation.

The data in Tables I and II show that the derivatives do not produce greater stimulation than 5-hydroxytryptophan when used alone or in combination with the latter compound; in fact, there is a diminution of central activity when compared to the saline control. While it is possible that the use of larger doses of the derivatives may have caused some evidence of central activity, higher doses could not be used because of the pain and tissue necrosis at the injection site. Consequently, one would not have been able to determine if the increased activity was due to central stimulation or to pain of injection.

In conclusion, therefore, the reason for the lack of central stimulation is not readily apparent at this time.

SUMMARY AND CONCLUSIONS

1. The synthesis of a series of *N*-(5-hydroxy-3-indole-ethyl) amino acid derivatives containing alanine, valine, phenylalanine, and isoleucine is described.

2. These compounds were evaluated in mice to determine their effect on generalized activity.

3. The results indicated that these compounds when used either alone or in combination with 5-hydroxytryptophan did not produce any significant increase in activity over the 5-hydroxytryptophan standard.

4. The results suggest that these compounds either are unable to enter the brain or that they are functioning as metabolic antagonists of 5-hydroxytryptophan.

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