that the molecular weight of α-casein lies between 13,000 and 15,000. Thus M_β is probably between 15,000 and 25,000.

Monomer Interactions.—The interactions of the α- and β-casein monomers examined here appear to be largely dependent upon pH and temperature. At pH 12 and $\sim 0°$ both α- and β-caseins are present as monomers, the two components being distinguished electrophoretically. If the pH is gradually decreased at low temperatures, there appears in the ultracentrifuge at $\sim$ pH 10.8, in addition to the diminished peak due to monomer, a second faster peak. The sedimentation constant of this peak increases progressively, indicating a corresponding increase in the degree of polymerization. The material remaining after the formation of this second peak maintains a constant relative area and a sedimentation constant characteristic of casein monomers. At pH 7 and $\sim 0°$ a comparison of ultracentrifuge and electrophoretic patterns reveals that β-casein has remained as a separate component and that the polymers which have appeared consist primarily, if not entirely, of α-casein. The relative areas observed by either technique at this temperature are consistent and the electrophoretic patterns are equivalent to those which have been obtained from acid-precipitated casein.[6,7,14]

If the temperature at pH 7 is increased from 0°, there is a progressive disappearance of the B peak, corresponding to β-casein, until at $\sim 20°$ it is too small to measure. At the same time there is an increase in both the area and sedimentation constant of the A peak, which was due only to α-casein at 0°. At pH 7 and 30° a single peak is observed in the ultracentrifuge (see Fig. 1). Likewise, electrophoretic patterns obtained at this temperature reveal no β-casein component. Instead a single broad separated peak is observed, the minimum mobility of which corresponds to that of α-casein. It is clear that polymers containing both α- and β-casein have been formed, the electrophoretic pattern suggesting polymers which differ in their proportions of α- and β-caseins and/or frictional characteristics. The progressive changes described take place as the temperature increases from 0° to $\sim 32°$. At higher temperatures the ultracentrifuge pattern reveals marked polydispersity.

Conditions have been described for the formation of polymers of varying size, either predominantly from α-casein or through the interaction of α- and β-caseins. In all cases the polymers tend to center about a preferred size, but polymers of α-casein alone appear to approach monodispersity more closely. Clearly there exists in each case a balance of attractive and repulsive forces dependent on the physical conditions. These polymerizations bear a marked resemblance to those responsible for the formation of soap micelles, as described and analyzed by Debye.[39]

A review of the facts given above leads to the conclusion that α-casein polymerizes more readily than β-casein. This is so in spite of its higher net charge at all pH values used here. It appears that forces other than those due to charge interactions are responsible for polymer formation. A number of possibilities in this connection have been examined recently.[40] A comparison of caseins with other proteins (see ref. 40, Table III, p. 343) reveals that casein has an unusual number of non-polar residues which may be involved in casein interactions.

(39) P. Debye, *Ann. N. Y. Acad. Sci.*, **51**, 575 (1949).
(40) D. F. Waugh, *Advances Protein Chem.*, **9**, 325 (1954).

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[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

The Synthesis of Tryptamines Related to Serotonin

BY ELLIOTT SHAW

RECEIVED FEBRUARY 28, 1955

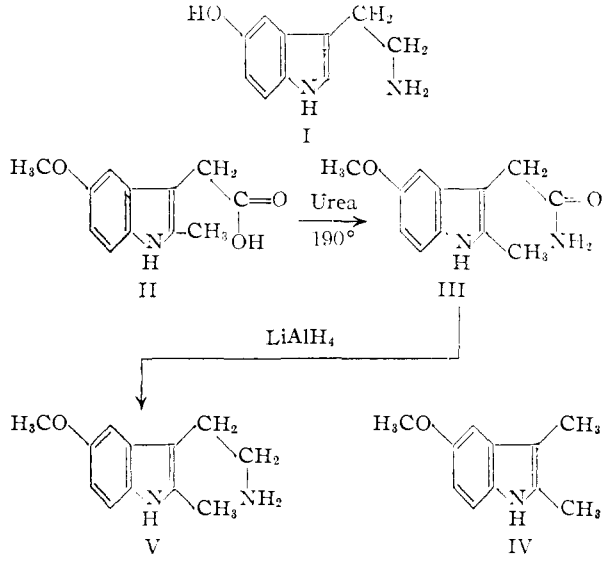
Modifications in the serotonin structure have been made by introduction of alkyl groups into the 1- and 2-positions. The Fischer rearrangement of methyl levulinate p-methoxyphenylhydrazone gave high yields (80%) of 2-methyl-5-methoxy-3-indoleacetic acid. With succinaldehydic acid as the carbonyl moiety, comparable yields were obtained with only an *asym*-N-alkyl derivative of the hydrazine. Direct amidification of 3-indoleacetic acids by heating with urea or tetramethylurea provided amides for reduction to tryptamines by means of lithium aluminum hydride. A number of related indoles have also been prepared.

The naturally occurring tryptamine, serotonin (I), has many physiological properties[1,2] in the mammalian circulatory system and apparently represents an important metabolite in tryptophan utilization in man.[3] We initially undertook the synthesis of analogs of serotonin in order to obtain substances which would prevent the pressor response characteristic of this tryptamine by acting as antimetabolites.[4] A number of contributions were subsequently made in this direction.[5,6] Meanwhile from considerations including the occurrence of serotonin in the brain and the evidence that a number of indole alkaloids may act as inhibitors at serotonin receptors[7] and are known to cause mental disturbances in man, the hypothesis was advanced[8] that serotonin is essential for normal brain function; mental disease may thus reflect

(1) I. H. Page, *Physiol. Rev.*, **34**, 563 (1954).
(2) V. Erspamer, *Rend. sci. farmitalia*, **1**, 5 (1954).
(3) E. Titus and S. Udenfriend, *Federation Proc.*, **13**, 411 (1954).
(4) D. W. Woolley, "A Study of Antimetabolites," John Wiley and Sons, Inc., New York, N. Y., 1952.
(5) D. W. Woolley and E. Shaw, *J. Pharm. Exper. Therap.*, **108**, 87 (1953).
(6) E. Shaw and D. W. Woolley, THIS JOURNAL, **75**, 1877 (1953).
(7) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **203**, 979 (1953).
(8) D. W. Woolley and E. Shaw, *Proc. Nat. Acad. Sci.*, **40**, 228 (1954).

an imbalance of this metabolite. The role of serotonin in normal physiology and in either circulatory or mental disease is still obscure. In connection with concurrent biological studies exploring the role of serotonin in the central nervous system, we desired to prepare serotonin-like substances hoping to obtain a compound more penetrable to the brain after peripheral administration and possessed of a greater duration of action than the hormone itself. For this purpose, it seemed best initially to make but small changes in the structure of serotonin to determine in which positions modifications might be made without loss of activity. Retention of the amino-ethyl side chain or its N,N-dimethyl derivative appeared advisable since, although increased resistance to enzymatic deamination may result from more elaborate alkylation of the side-chain nitrogen,[9] such changes in the 5-aminotryptamine structure lead from serotonin-like properties to antimetabolites.[10] Since the methyl ether of serotonin has serotonin activity,[11] a number of methoxytryptamines were synthesized which bear alkyl groups mainly in the 1- and 2-positions. It was appreciated that some of these substances might have antiserotonin properties.

Although the gramine route[12,13] and other novel methods[14,15] for synthesizing tryptamines are available, for some of the derivatives desired in the current work, the necessary starting material, 2-methyl-5-methoxyindole, is not conveniently obtained[16] and is reported to form a gramine base in very poor yield. The Fischer rearrangement was investigated as an approach to methoxyindoleacetic acids in the expectation that the latter could be converted to amides for reduction to tryptamines with lithium aluminum hydride. The cyclization of methyl levulinate *p*-methoxyphenylhydrazone by means of ethanolic HCl was found to proceed very readily leading, after saponification, to 2-methyl-5-methoxy-3-indoleacetic acid (II) in yields of 80–85%. Similar results were obtained with this ketoester and other hydrazines (Table I). However, the use of succinaldehydic acid instead of a levulinate gave poor results as far as the rearrangement of the *p*-methoxyphenylhydrazone was concerned. This approach was suggested by the work of Fox and Bullock.[17] The easy accessibility of the aldehyde from glutamic acid is an attractive feature of this route to indoleacetic acids. Its poor adaptability to the synthesis of the 5-methoxy acid[18] was in marked contrast to the results obtained when *asym*-N-alkyl-*p*-methoxyphenylhydrazones were rearranged. From the latter good yields of 1-alkyl-5-methoxy-3-indoleacetic acids could be obtained.[19]



The conversion of the substituted indoleacetic acids to amides was at first a source of difficulty. Although indoleacetic acid itself forms a crystalline acid chloride[7] from which amides are readily prepared, the 2-methyl-5-methoxy derivative gave poor yields of amide by this procedure. The direct conversion of an acid to its amide by heating with urea[20] at about 190° was found to be simple and effective yielding 50–60% of this III and other 3-indoleacetamides (Table I). Tetramethylurea under similar conditions led to N,N-dimethyl-3-indoleacetamides in somewhat lower yields. Decarboxylation of indoleacetic acid (II) to a skatole derivative IV was a side reaction[21] which limited the yield.

In a few cases, the dimethylamides could not be conveniently crystallized but the neutral fraction from the amidification procedure, when reduced with lithium aluminum hydride, yielded pure tryptamine derivative. The basicity of the latter permitted easier separation from neutral by-products such as skatoles (IV).[22]

The dehydration of the dibenzylamine salt of 2-methyl-5-methoxy-3-indoleacetic acid by heating at reduced pressure led to the dibenzylamide which, after hydride reduction and debenzylation, provided the tryptamine (V). However, similar dehydration of the dimethylamine salt was not achieved.

(9) W. M. Govier, B. G. Howes and A. J. Gibbons, *Science*, **118**, 596 (1953).

(10) D. W. Woolley and E. Shaw, *J. Biol. Chem.*, **203**, 69 (1953).

(11) V. Erspamer, *Nature*, **170**, 281 (1952).

(12) K. E. Hamlin and F. E. Fischer, THIS JOURNAL, **73**, 5007 (1951).

(13) A. Ek and B. Witkop, *ibid.*, **76**, 5579 (1954).

(14) M. E. Speeter and W. C. Anthony, *ibid.*, **76**, 6208 (1954).

(15) J. Harley-Mason and A. H. Jackson, *J. Chem. Soc.*, 1165 (1954).

(16) J. B. Bell and H. G. Lindwall, *J. Org. Chem.*, **13**, 547 (1948).

(17) S. W. Fox and M. W. Bullock, THIS JOURNAL, **73**, 2754 (1951).

(18) Similar experiences have been reported in the preparation of 5-benzyloxy-3-indoleacetic acid; *cf.* C. Mentzer, C. Beaudet and M. Bory, *Bull. soc. chim.*, **20**, 421 (1953), who obtained a 17% yield using phosphoric acid in methanol.

(19) The reaction of γ-keto or aldehydo acids with phenylhydrazines would be expected to lead to pyridazinones under some conditions. The improved yield of indoles from *asym*-N-alkyl phenylhydrazones in which pyridazinone formation is impossible, suggests that the latter route may be a competing reaction in other cases. A pyridazinone is formed by the action of heat on levulinic acid phenylhydrazone as shown by Fischer.[21] However, in alcoholic HCl, the pyridazinone derived from α-ketoglutaric acid underwent ring opening followed by indole formation: W. Wislicenus and M. Waldmüller, *Ber.*, **44**, 1564 (1911).

(20) E. Cherbuliez and F. Landolt, *Helv. Chim. Acta*, **29**, 1438 (1946).

(21) E. Fischer, *Ann.*, **236**, 147 (1886), observed the decarboxylation of 3-indoleacetic acids at about 220° and recommended this as a preferred route to 3-methylindoles.

(22) Dimethylformamide did not permit transamidification with displacement of formic acid by 2-methyl-5-methoxyindole-3-acetic acid. Decarboxylation of the latter resulted exclusively.

TABLE I

SOME 3-INDOLEACETIC ACIDS AND AMIDES

	Solvent	M.p., °C.	Yield, %	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
3-Indoleacetic acid									
1-Methyl-5-methoxy	c	136–138	71[a]	65.75	5.98	6.39	65.90	5.86	6.37
2-Methyl-5-methoxy	c	161–162	84[a]	65.75	5.98	6.39	65.78	6.11	6.47
2-Methyl-5-benzyloxy	c	Oil	70[a]						
1,2-Dimethyl-5-methoxy	c	169–171	59[b]	66.92	6.48	6.00	67.25	6.48	6.05
1-Benzyl-5-methoxy	c	126	51[b]	73.21	5.80	4.74	73.54	5.96	4.95
1-Benzyl-2-methyl-5-methoxy	c	174–175	86[b]	73.76	6.19	4.53	74.17	5.87	4.71
3-Indoleacetamide									
1-Methyl-5-methoxy	c	227–228	48	66.05	6.50	12.83	66.07	6.34	12.93
2-Methyl-5-methoxy	d	149–150	57	66.05	6.50	12.83	65.98	6.52	13.02
2-Methyl-5-benzyloxy	c	143–144	35	73.46	6.17	9.52	74.12	6.14	9.84
1,2-Dimethyl-5-methoxy	d	164–165	66	67.21	6.94	12.06	67.21	6.71	11.56
1-Benzyl-5-methoxy	c	156–157	60	73.46	6.17	9.52	73.31	6.15	9.10
1-Benzyl-2-methyl-5-methoxy	d	130–131	54	73.99	6.53		74.34	6.52	
3-Indole-N,N-dimethylacetamide									
2-Methyl-5-methoxy	d	134–135	40	68.27	7.37	11.37	68.72	7.06	11.54

[a] Yield from the hydrazone. [b] Yield from the substituted phenylhydrazine. [c] Ethanol. [d] Ethyl acetate with added hexane.

TABLE II

SUBSTITUTED SEROTONINS

		M.p., °C.	Yield in hydride reduction, %	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
1-Methyl-5-methoxytryptamine[a]	picrate	189–190	47	49.88	4.42	16.16	50.37	4.50	15.67
	hydrochloride	176–177		59.85	7.12		59.95	7.00	
1-Benzyl-5-methoxytryptamine	picrate	166–167	54	56.58	4.55		56.98	4.69	
2-Methyl-5-methoxytryptamine[b]	picrate	216–217	48	49.88	4.42	16.16	50.47	4.56	15.79
	hydrochloride	179–180		59.85	7.12		59.94	7.08	
2-Methyl-5-benzyloxytryptamine	picrate	207–208	40	56.58	4.55		57.01	4.63	
1,2-Dimethyl-5-methoxytryptamine	picrate	197–198		50.89	4.72		50.81	4.80	
	hydrochloride	230–232	44	61.30	7.52	10.99	60.95	7.41	11.16
1-Benzyl-2-methyl-5-methoxytryptamine[c]	hydrochloride	230–231	60	68.98	7.01	8.47	68.67	6.87	8.62
1-Methyl-5-methoxy-N,N-dimethyltryptamine									
	hydrochloride	189–190	24[e]	62.55	7.87	10.42	62.23	7.63	10.26
2-Methyl-5-methoxy-N,N-dimethyltryptamine[d]	picrate	(147)182[g]	71	52.06	5.02		52.41	4.98	
1-Benzyl-5-methoxy-N,N-dimethyltryptamine									
	hydrochloride	191–192	25[e]	70.24	7.58		70.09	7.53	
2-Methyl-5-methoxy-N,N-dibenzyltryptamine									
	hydrochloride	221–223	27[e]	74.17	6.94	6.65	74.31	6.96	6.75
1-Methyl-5-hydroxytryptamine	picrate	197–198	8[f]	48.69	4.09		48.87	4.14	
2-Methyl-5-hydroxytryptamine	picrate	216–217	74[f]	48.69	4.09		48.68	4.54	
	hydrochloride	230–231		58.25	6.66		58.03	6.58	

[a] For convenience in relating these bases to serotonin and bufotenine, simple names are being used: 1,5-dimethylserotonin. [b] 2,5-dimethylserotonin. [c] 1-Benzyl-2,5-dimethylserotonin. [d] 2,5-Dimethylbufotenine. [e] Yield for two steps from the 3-indoleacetic acid. [f] By demethylation of the ether. [g] The picrate exists in two forms. The lower melting is red; the higher, yellow. The transition of the former to the latter may be observed during m.p. determination.

The yields given in Table I represent products suitable for the subsequent synthetic procedure, *i.e.*, once crystallized and with m.p.'s within a few degrees of those listed for the analytical samples. Further purification was very wasteful of material. Slightly impure amides gave tryptamines of the same purity as did the pure amides.

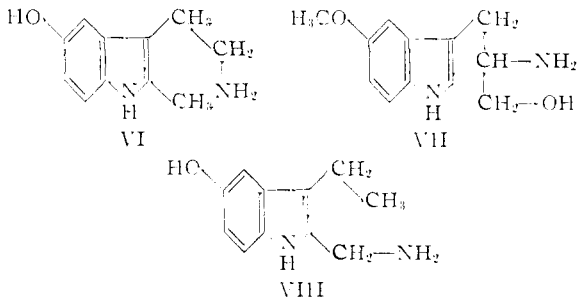
Each amide was reduced to the corresponding substituted serotonin or N,N-dimethylserotonin (bufotenine) which was isolated as a crystalline picrate or hydrochloride with the yield of pure product indicated in Table II.

For the synthesis of 2-methylserotonin (VI), the *p*-benzyloxyphenylhydrazone of methyl levulinate was taken through the previously described steps and led to 2-methyl-5-benzyloxytryptamine which underwent hydrogenolytic cleavage to the desired phenol. Demethylation of the 5-methyl ether (V) by means of aqueous HBr gave surprisingly good yields (74%) of the phenol. By contrast, the preparation of 1-methylserotonin by demethylation of its ether with HBr gave only an 8% yield. Synthesis through the benzyl ether is under study.

For the purpose of rounding out knowledge of the structure–activity relationship in the serotonin molecule, a number of additional related compounds have been prepared. 5-Methoxytryptophanol (VII) was formed from 5-methoxytryptophane[23] by hy-

(23) J. W. Cook, J. D. Loudon and P. McCloskey, *J. Chem. Soc.*, 1203 (1951).

dride reduction. 3-Ethyl-5-hydroxyindole was synthesized by the Japp-Klingemann route[24] in which the 2-carboxylic acid led, *via* the amide, to the amine VIII.



VI VII VIII

Experimental[25]

Methylation and Benzylation of *p*-Methoxyphenylhydrazine.—*p*-Methoxyphenylhydrazine was prepared from *p*-anisidine.[26] The alkylations followed the method of Audrieth, Weisiger and Carter.[27] However, the product was isolated as the hydrochloride because this salt was more stable than the free base. This was achieved by extraction of the base with ether, and, after removal of the solvent, addition of alcoholic HCl. The hydrochlorides crystallized readily in high purity on concentration.

***asym*-Methyl-*p*-methoxyphenylhydrazine hydrochloride** was obtained in a yield of 53%, m.p. 140–142°, after recrystallization from alcohol and ether.[28]

Anal. Calcd. for $C_8H_{12}N_2O\cdot HCl$: C, 50.92; H, 6.94. Found: C, 51.05; H, 6.68.

***asym*-N-Benzyl-*p*-methoxyphenylhydrazine hydrochloride** was obtained in a 50% yield, m.p. 140–142° dec.

Anal. Calcd. for $C_{14}H_{16}N_2O\cdot HCl$: C, 64.11; H, 6.35; N, 10.75. Found: C, 63.50; H, 6.09; N, 10.60.

2-Methyl-5-methoxy-3-indoleacetic Acid. General Method.—*p*-Methoxyphenylhydrazine was liberated from the tin complex,[26] dried, and used without purification. The hydrazine (22 g.) was dissolved in glacial acetic acid (45 ml.). Addition of water (150 ml.) precipitated a small amount of material which was removed by filtration. Methyl levulinate (25 ml.) was added to the filtrate. The crystalline hydrazone that formed was filtered, washed with water and dried in a desiccator. The yield, m.p. 84–86°, was 75–86%, depending on the quality of the hydrazine used.

For the Fischer rearrangement, the hydrazone (32 g.) was refluxed for one hour with 2 *N* ethanolic HCl (320 ml.) protected from moisture. The mixture was then concentrated under reduced pressure to a small volume, and the residue partitioned between water (100 ml.) and benzene (250 ml.). The organic layer was washed with aqueous sodium bicarbonate, dried over anhydrous magnesium sulfate, and concentrated at about 15 mm. to leave ethyl 2-methyl-5-methoxy-3-indoleacetate as an oil, 28.2 g.

For saponification, the ester was taken into ethanol (300 ml.) to which 6 *N* NaOH (25 ml.) was added. After three hours at room temperature, water (150 ml.) was introduced and the alcohol removed in an air stream. The aqueous solution was filtered and acidified with 6 *N* hydrochloric acid. The crystalline precipitate was filtered with suction, washed with water, and dried in a desiccator to yield 24.7 g., m.p. 157–159°.

1-Methyl-5-methoxy-3-indoleacetic Acid.—*asym*-Methyl-*p*-methoxyphenylhydrazine hydrochloride (4.4 g.) in water (50 ml.) was treated with *N* NaOH (2.3 ml.) followed by a solution of succinaldehydic acid prepared[17] from glutamic acid (0.05 mole). The *p*H was adjusted to 4–4.5 and the crystalline hydrazone which formed was filtered, washed with water, and dried in a vacuum desiccator to yield 3.7 g., m.p. 127–128°. The hydrazone was then converted to the indoleacetic acid as described for the 2-methyl isomer.

1-Benzyl-5-methoxy-3-indoleacetic Acid.—*asym*-N-Benzyl-*p*-methoxyphenylhydrazine hydrochloride (3.0 g.) was brought into solution in water (100 ml.) by stirring with 3 *N* NaOH (5 ml.) followed by glacial acetic acid (30 ml.). A solution at *p*H 4.5 of succinaldehydic acid from glutamic acid (0.03 mole) was added and the mixture left at 4° overnight. The granular precipitate, after washing, and desiccation *in vacuo*, weighed 2.7 g., m.p. 101–103°. This hydrazone was subjected to the Fischer rearrangement as described above.

1-Benzyl-2-methyl-5-methoxy-3-indoleacetic Acid.—*asym*-N-Benzyl-*p*-methoxyphenylhydrazine hydrochloride (1.32 g.) in water (100 ml.), 3 *N* NaOH (30 ml.) and glacial acetic acid (40 ml.) gave little reaction when methyl levulinate (3 ml.) was added. With increased alkali (30 ml. of 6 *N* NaOH) and cooling, separation of an oily hydrazone was completed. This was taken into benzene, washed with aqueous sodium bicarbonate, and dried. After removal of the solvent, the residue was treated with ethanolic HCl as described above.

2-Methyl-5-benzyloxy-3-indoleacetic Acid.—*p*-Benzyloxyphenylhydrazine[29] was prepared in the same way as the *p*-methoxy[26] compound. The free base (7.0 g.) was dissolved in glacial acetic acid (60 ml.) and water (30 ml.). Methyl levulinate (6 ml.) was added and the crystalline hydrazone was filtered, washed with aqueous acetic acid and dried *in vacuo* to yield 9 g., m.p. 95–98°. When this was subjected to the Fischer rearrangement as described in the general example, the acid obtained could not be crystallized. The amide obtained from it was crystalline.

2-Methyl-5-methoxy-3-indoleacetamide. General Method for Amides.—2-Methyl-5-methoxy-3-indoleacetic acid (7.0 g.) and urea (7.0 g.) were placed in a flask provided with an air condenser and heated in an oil-bath kept at 180–185° for 2.5 hours. The cooled melt was brought into solution with ethyl acetate (150 ml.) and *N* HCl (30 ml.). The organic layer was washed with aqueous bicarbonate from which unreacted starting material was obtained on acidification (5–10% recovery). After drying over magnesium sulfate, the ethyl acetate was concentrated to a small volume (*ca.* 35 ml.) and left overnight for crystallization. Slow crystallization was necessary to obtain both good yields and highest purity. The yield, based on unrecovered acid, was 3.8 g., m.p. 147–150°.

The other amides listed in Table I were prepared by this procedure. In some cases the heating was carried out at 190–195° for one and one-half hours with equal success. The amides were crystallized from a concentrated solution in the solvent indicated.

2-Methyl-5-methoxy-3-indole-N,N-dimethylacetamide, General Method for Dimethylamides.—This procedure differed from the direct amidification with urea in that, since tetramethylurea is quite soluble in organic solvents, special care was taken to ensure its removal. 2-Methyl-5-methoxy-3-indoleacetic acid (2.5 g.) and tetramethylurea[30] (2.5 g.) were heated two hours at 195°. The mixture was cooled and triturated with water containing a little hydrochloric acid to remove the excess oily urea which otherwise prevented crystallization of the amide. After decantation of the aqueous washings, the gummy residue was dissolved in ethyl acetate from which starting acid (0.51 g.) was recovered by extraction with aqueous bicarbonate. The organic layer was then dried over magnesium sulfate and concentrated to a small volume for crystallization. When, in the case of some acids, the amide was difficult to crystallize, the neutral residue was reduced without further purification.

Evidence for decarboxylation during the above procedure was obtained, for example, during the preparation of the 1-methyl isomer which was not crystallized. After reduction with lithium aluminum hydride in ether, the neutral fraction from the crude dimethylamide yielded the expected 1-methylbufotenine methyl ether, extractable with dilute

(24) W. R. Boehme, THIS JOURNAL, **75**, 2502 (1953).

(25) Melting points are uncorrected and were taken in a silicone-bath. Analyses were performed by Mr. S. Theodore Bella.

(26) K. G. Blaikie and W. H. Perkin, *J. Chem. Soc.*, **125**, 296 (1924).

(27) L. F. Audrieth, J. R. Weisiger and H. E. Carter, *J. Org. Chem.*, **6**, 417 (1941).

(28) The base has been prepared by E. Späth and O. Brunner, *Ber.*, **58**, 518 (1925). In our hands, their method, zinc dust reduction of N-nitroso-N-methyl-*p*-anisidine, was difficult to arrest at the hydrazine stage. Secondary amine was the main product. Similar experiences of Cook, *et al.*, ref. 23, were attributed to variability in zinc dust samples.

(29) A similar but not identical preparation of this compound has since been published, ref. 18.

(30) W. Miebler and C. Escherich, *Ber.*, **12**, 1162 (1879).

acid. There remained in the ether, material which crystallized completely on removal of the solvent, apparently 1,3-dimethyl-5-methoxyindole which sublimed in long needles, m.p. 61–62°,[31] 25% yield.

Lithium Aluminum Hydride Reduction of Substituted 3-Indoleacetamides.—The following rather standardized procedure was adopted. Hydride amounting in weight to about one-half that of the amide to be reduced was suspended in dry ether (roughly 500 ml. per g.). After the suspension had stirred for some time and the larger lumps dispersed, the powdered amide was added. When non-crystalline gums such as unpurified dimethylamides were to be reduced, they were first dissolved in a small amount of tetrahydrofuran for addition. Stirring was continued for two days. The excess hydride was decomposed by the very cautious and slow addition of 20% aqueous sodium potassium tartrate just sufficient to cause the ether layer to separate clearly. The ether phase was decanted from the mushy aqueous residue into a separatory funnel and extracted with 0.1 *N* HCl (1.5 equivalents per mole of amide reduced) in three portions. In some cases the hydrochloride was isolated by concentration to a glass which was crystallized by addition of a small amount of absolute ethanol. In the case of 1-benzyl-2-methylserotonin methyl ether, the hydrochloride crystallized directly in the acid extracts before concentration. Sometimes, however, the tryptamines were isolated as picrates when initially prepared. The acid extracts were warmed in an air stream to remove ether, then poured into hot 5% alcoholic picric acid (a small excess over theory was used). The precipitate was recrystallized from aqueous alcohol or acetone. The hydrochlorides were crystallized from absolute alcohol and the suspension was thinned with ether before filtration. In Table II the yield is given opposite that salt which was used for isolation.

2-Methyl-5-methoxy-N,N-dibenzyltryptamine.—A crystalline dibenzylamine salt, m.p. 141–143°, formed when 2-methyl-5-methoxy-3-indoleacetic acid and dibenzylamine were mixed in ethyl acetate solution. The salt (2.75 g.) was heated in an oil-bath at 210–220° and 15 mm. pressure for 3.5 hours. The residue was dissolved in benzene (100 ml.), filtered free of some insoluble material, and the filtrate extracted with 0.1 *N* HCl and aqueous sodium bicarbonate. There remained 1.43 g. after removal of the benzene. Crystallization was not possible. Therefore, this amide fraction was reduced directly by addition to lithium aluminum hydride (1.0 g.) in ether as described above. The hydrochloride precipitated in the acid extracts of the ether layer as crystals or a gum. When a gum separated, all liquid was decanted from the separatory funnel and the gummy hydrochloride was rinsed from the funnel with hot ethanol which rapidly induced crystallization. The yield was 0.76 g., m.p. 221–222°, unchanged on recrystallization from ethanol.

2-Methylserotonin and 1-Methylserotonin.—For demethylation, 2-methylserotonin methyl ether hydrochloride (0.20 g.) was refluxed with 48% hydrobromic acid (1.5 ml.) for 45 minutes. The solution was concentrated with reduced pressure and the residue desiccated *in vacuo* in the presence of alkali. After the excess acid had been removed, water (10 ml.) was added and the resultant solution poured into 1% aqueous picric acid (30 ml.). After the chilled solution had stood for an hour, 0.25 g. of picrate was collected, 74%, m.p. 210° dec. The hydrochloride (see Table II) was more distinctive and served better to differentiate the product from the starting material although mixtures of the picrates did give depression in m.p.

The product was identical with that obtained from the benzyl ether by catalytic debenzylation with palladized charcoal and hydrogen in ethanol in the usual way.

1-Methylserotonin methyl ether hydrochloride (70 mg.) was refluxed in 48% hydrobromic acid (2 ml.) for one-half hour and, after concentration as above, a solution of the residue in water (7 ml.) was added to hot 1% aqueous picric acid (15 ml.). A gum separated initially. When the first crystalline material appeared, the supernatant solution was decanted. The crystallization continued and yielded the picrate as nodules, 9.1 mg., m.p. 197–198°, unchanged after recrystallization from water.

5-Methoxytryptophanol (VII).—5-Methoxytryptophan[23] (0.13 g.) was refluxed with lithium aluminum hydride (0.13 g.) in tetrahydrofuran (50 ml.) for six hours. The mixture was concentrated with reduced pressure to 1/3 its original volume, dry ether (125 ml.) was added, and the excess hydride decomposed with 10% sodium potassium tartrate.[32] The ether was extracted with 0.2 *N* HCl (total 20 ml.) and the extracts were added to hot 4% alcoholic picric acid (5 ml.). An oil separated at first followed by crystals which could be collected separately to yield 30%, m.p. 192–194° after recrystallization from aqueous alcohol.

Anal. Calcd. for $C_{12}H_{16}N_2O_2 \cdot C_6H_3N_3O_7$: C, 48.10; H, 4.26. Found: C, 48.51; H, 4.43.

3-Ethyl-5-benzyloxyindole.—The Japp–Klingemann reaction was applied as described by Boehme[24] except that ethyl propylacetoacetate was used. A 50% yield of ethyl 3-ethyl-5-benzyloxy-2-indolecarboxylate, m.p. 145–148°, was obtained. A sample recrystallized from alcohol had m.p. 149–150°.

Anal. Calcd. for $C_{20}H_{21}NO_3$: C, 74.27; H, 6.55. Found: C, 74.36; H, 6.50.

The acid after recrystallization from aqueous acetic acid, had m.p. 194–195° dec.

Anal. Calcd. for $C_{18}H_{17}NO_3$: C, 73.21; H, 5.80. Found: C, 73.39; H, 5.90.

The acid (14.5 g.) was decarboxylated at 210° for one hour and the melt was taken up in ethyl acetate from which some starting material (2.3 g.) was recovered on concentration. The soluble part was dried, taken up in benzene (70 ml.), and passed through an activated alumina column (3.4 × 27 cm.).[33] On elution with benzene, 3-ethyl-5-benzyloxyindole was obtained as the first band and crystallized as thick prisms on concentration. These were thinned with benzene and hexane (1:2) for filtration, to yield 7.0 g., 70%, m.p. 76–78°. After recrystallization from ethyl acetate and hexane, the product melted at 78–79°.

Anal. Calcd. for $C_{17}H_{17}NO$: C, 81.26; H, 6.82. Found: C, 81.06; H, 6.58.

3-Ethyl-5-hydroxyindole.—The benzyl ether (8.8 g.) in absolute ethanol (100 ml.) and 5% palladized charcoal (0.8 g.) were shaken with hydrogen at 50 lb. initial pressure until the calculated pressure drop was observed. Concentration of the filtrate left a crystalline residue, 5.5 g., which was completely soluble in dilute alkali. The analytical sample was obtained by sublimation and melted at 78–79°.

Anal. Calcd. for $C_{10}H_{11}NO$: C, 74.50; H, 6.88; N, 8.69. Found: C, 75.03; H, 6.63; N, 8.76.

3-Ethyl-5-benzyloxy-2-indolecarboxamide.—The carboxylic acid (5.0 g.) was treated with phosphorus pentachloride exactly as described for 3-indoleacetic acid[7] and the chloride so obtained was left overnight in ethanol (100 ml.) half-saturated with ammonia. The residue after removal of the ethanol was stirred with water and filtered. The insoluble portion was recrystallized from 95% ethanol, yielding the amide, 1.8 g., m.p. 157–158°. Starting acid, 1.3 g., was recovered from the mother liquor. The amide melted at 162–163° after recrystallization from benzene.

Anal. Calcd. for $C_{18}H_{18}N_2O_2$: C, 73.49; H, 6.16; N, 9.52. Found: C, 73.70; H, 6.05; N, 9.54.

2-Aminomethyl-3-ethyl-5-benzyloxyindole Hydrochloride.—The amide (1.15 g.) was stirred overnight with a suspension of lithium aluminum hydride (0.6 g.) in dry ether (150 ml.). After decomposition of the excess hydride in the usual way, the ether layer was extracted with 0.1 *N* hydrochloric acid (3 × 30 ml.). Concentration of the aqueous extracts with reduced pressure left a crystalline residue, 0.9 g., m.p. 185–187°, unchanged after recrystallization from alcohol and ether.

Anal. Calcd. for $C_{18}H_{20}N_2O \cdot HCl$: C, 68.24; H, 6.36; N, 8.84. Found: C, 67.94; H, 6.75; N, 9.07.

2-Aminomethyl-3-ethyl-5-hydroxyindole Hydrochloride.—The benzyl ether hydrochloride (0.60 g.) was hydrogenated in ethanol (50 ml.) with 5% palladium on charcoal catalyst (0.5 g.) in the usual way. The residue after removal of catalyst and solvent was converted to a picrate (0.5

(31) F. E. King and R. Robinson, *J. Chem. Soc.*, 270 (1933), report m.p. 60°.

(32) These conditions were suggested by the work of O. Vogl and M. Pöhm, *Monatsh.*, **83**, 541 1952), for the reduction of amino acids.

(33) Earlier runs established the presence of an impurity not conveniently separated by distillation or crystallization but easily removed by adsorption on alumina.

g.) which separated as needles from aqueous solution. The picrate had no sharp m.p. but charred at elevated temperatures.

Anal. Calcd. for $C_{11}H_{14}N_2O \cdot C_6H_3N_3O_7$: C, 48.69; H, 4.09. Found: C, 48.91; H, 4.08.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF PENNSYLVANIA]

Metabolite Analogs. III. Preparation of Some Benzimidazoles with Substituents on the 4(7)- and 6(5)-Positions[1]

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RECEIVED MARCH 3, 1955

Benzimidazoles with chloro, nitro and amino groups on the 4(7)- and 6(5)-positions, their hydrochlorides and picrates, have been prepared for testing as potential metabolite (purine, vitamin B_{12}, folic acid) inhibitors.

The interference with purine utilization by benzimidazoles was first reported by Woolley.[2] His findings indicated that 5(6)-aminobenzimidazole is no more active in this respect than the parent compound. The information concerning the inhibitory activity of 4(7)-aminobenzimidazole, prepared by van der Want,[3] is incomplete. Benzimidazoles designed as guanine analogs have been shown to inhibit various guanine-sensitive test systems.[4] Likewise, the inhibition of virus multiplication[5] and of vitamin B_{12} utilization[6] by benzimidazoles have been reported. In view of the antagonistic properties induced in the purine series by modifying the substituents on the 2- and 6-positions, the testing of benzimidazoles containing polar substituents on the equivalent positions, especially those which result in compounds resembling the natural metabolites, appeared to be of interest. In addition to the analogs already reported, a number of derivatives containing amino, nitro, chloro, mercapto, sulfonamido and sulfonic acid groupings on the 4- and 6-positions have been prepared. In most cases, position isomers have been made for use in a study of the effect of grouping and position on the activity. These compounds have been subjected to screening tests, including vitamin B_{12} and purine inhibition. The preparation of the sulfur-containing derivatives as well as the results of the biological studies will be given in later communications.

Picramide served as the starting material for a number of the 4,6-disubstituted benzimidazoles. The complete reduction to 1,2,3,5-tetraaminobenzene, using tin and hydrochloric acid, has been described.[7] Partial reduction with ammonium sulfide results in a mixture of 1,2,3-triamino-5-nitrobenzene and 1,2-diamino-3,5-dinitrobenzene. The procedure of Horner, Schwenk and Junghanns,[8] using methyl acetate as solvent, gives predominately the dinitro compound. Adaptation of the method of Nietzki and Hagenbach (ref. 7) resulted in a more favorable yield of the triamine.

The conversion of 1,2,3,5-tetraaminobenzene to 4,6-diaminobenzimidazole was readily accomplished using Phillips' conditions.[9] Indeed, this appeared to be the method of choice for all of the cyclizations in this series except those containing an acid-labile grouping. This applies also to the preparation of 4-amino- and 4-nitrobenzimidazole previously obtained by treating the corresponding diamine with 85% formic acid.[3]

Replacement of the amino group in 4-amino-6-nitrobenzimidazole *via* the diazonium salt afforded a route to several of the sulfur-containing analogs.[10] The diazonium chloride was readily decomposed by copper to 4-chloro-6-nitrobenzimidazole. For the reduction of the nitro group to give 4-chloro-6-aminobenzimidazole, it was found that stannous chloride and hydrochloric acid gave more satisfactory results than hydrogenation over palladium catalysts. The material from catalytic hydrogenation usually contained impurities which readily underwent decomposition to colored products. These could be removed only with considerable difficulty. This was also true of the reduction of the other nitro compounds in this group.

Preparation of the reversed isomers of the latter two compounds (*i.e.*, 4-nitro-6-chloro- and 4-amino-6-chlorobenzimidazole) was accomplished by using 3-nitro-5-chloro-*o*-phenylenediamine as the starting material. The Phillips reaction was used for closing the ring and stannous chloride for reduction of the nitro group. Both of the chloroaminobenzimidazoles could be converted to 4,6-dichlorobenzimidazole by diazotization followed by decomposition of the diazonium salt with copper or cuprous chloride. This compound has been mentioned by Tamm, Folkers, Shunk and Horsfall[5d] and also by Davies, Mamalis, Petrow and Sturgeon,[11] but the preparative data are very incomplete.

The preparation of 4-nitro-6-aminobenzimidazole for comparison with the 4-amino-6-nitro isomer

(1) This investigation was supported by a research grant (C-2189) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

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(3) G. M. van der Want, *Rec. trav. chim.*, **67**, 45 (1948).

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(5) (a) R. L. Thompson, *J. Immunol.*, **55**, 345 (1947); (b) G. C. Brown, *ibid.*, **69**, 441 (1952); (c) I. Tamm, K. Folkers, C. H. Shunk, D. Heyl and F. Horsfall, *J. Expt. Med.*, **98**, 245 (1953); (d) I. Tamm, K. Folkers, C. H. Shunk and F. Horsfall, *ibid.*, **99**, 227 (1954).

(6) F. Weygand and A. Wacker, *Z. Naturforsch.*, **5**, 227 (1950).

(7) R. Nietzki and H. Hagenbach, *Ber.*, **30**, 539 (1897).

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(9) M. A. Phillips, *J. Chem. Soc.*, 2393 (1928).

(10) J. R. E. Hoover and A. R. Day, THIS JOURNAL, in press.

(11) M. T. Davies, P. Mamalis, V. Petrow and B. Sturgeon, *J. Pharm. Pharmacol.*, **3**, 420 (1951). These workers merely state that the compound was prepared from the corresponding diamine, listing its melting point as 225–226° and its nitrogen analysis as 14.9%.