Synthesis and Preliminary Pharmacology of Some 1-Methylindoles

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Received January 9, 1965

Analogs methylated in the 1-position of a group of biologically interesting indoles were synthesized. One method alkylated the sodioindole in liquid ammonia and a second employed methyl *p*-toluenesulfonate in refluxing xylene. These analogs were compared with the nonmethylated ones in their effect on animal behavior, blood pressure, serotonin antagonism, and for one pair, serotonin release from human blood platelets. Many of the pairs had similar effects suggesting that the pyrrole nitrogen may be altered without essential change in biological activity. Methylreserpine was almost identical with reserpine in its ability to release serotonin from human platelets. The results also suggest that the 1-position of indoles can be used to attach groups which would modify the transport characteristics of the parent or incorporate therapeutic moieties without appreciable changes in other physiological properties. An important exception observed where the methylated and nonmethylated indoles differed was potassium 1-methylindoxyl sulfate (1-methylindican) which produced psychotropic effects on conditioned rats, whereas indican did not. 1-Methylserotonin was twice as potent as serotonin in a similar study. 5-Methoxy-1-methyltryptamine was ten times as potent as serotonin in its ability to contract isolated rat uterus. It appeared that exceptions where a methylated analog was considerably different from the nonmethylated indole seen in behavioral effects were due to transport rather than chemical function.

Indoles such as serotonin have frequently been suggested as being involved in cardiovascular and neural function.[2,3] Since the initial characterization of serotonin, it has become increasingly clear that its function in cardiovascular mechanisms is highly complex. It has been suggested that abnormal tryptamine metabolism could give rise to endogenously formed psychotropic agents which might cause some forms of mental disease. 5-Methoxytryptamine has been isolated from the urine of patients with rheumatic fever.[4] Indoxyl sulfate (urinary indican) is normally excreted in urine at the rate of about 50 mg./day along with 2–10 mg. of 5-hydroxyindole-3-acetic acid. A number of investigators have described abnormal indoles as shown by chromatograms from the urine of schizophrenic patients.[5] Other indoles have been tentatively identified in normal human urine. Characterization has often been inadequate if for no other reason than authentic compounds were not available for comparison. Therefore, the study of synthesis of new indoles can be valuable since it provides further authentic compounds for identification.

Indoles methylated in the 1-position are possible metabolites since biochemical heterocyclic N-methylation has been observed for pyridine,[6,7] histamine,[8] niacinamide,[9] quinoline, and isoquinoline.[10]

Pharmacologically, 1-methylindoles are of interest for several reasons. Adrenolutin and adrenochrome, two compounds once reported as being psychotomimetic[11] and capable of formation *in vivo* from epinephrine,[12] are methylated indolines. A series of alkylpiperidine compounds described by Biel, *et al.*,[13] produce model psychoses resembling certain mental disturbances more closely than LSD-25 or psilocybin. If the configuration of the chain of the phenethylamine moiety or catechol amines can be shaped in the form of a five-membered ring and remain biologically active, then 1-methylindoles combine some of the structural features of N-methylated catechol amines (*e.g.*, epinephrine) and tryptamines in the same molecule.

A close analog as well as a compound which could mimic the action of serotonin but, unlike serotonin, cross the blood–brain barrier would be an invaluable tool for elucidating its central role.[14] Further, study of the pharmacological effects of 1-methylindoles enables evaluation of the importance of the pyrrole nitrogen in pharmacological actions of indoles.

Analogs, methylated in the 1-position of indole, which were prepared included those of 5-methoxytryptamine, serotonin, melatonin, 5-methoxytryptophol, tryptophol, potassium indoxyl sulfate, skatole, 6-methoxy-1-methyl-1,2,3,4-tetrahydro-β-carboline, norharman (β-carboline), yohimbine, and reserpine.

Two main synthetic routes were used to prepare this series of 1-methylindoles. One procedure was a low-temperature direct alkylation of the N-sodioindole in liquid ammonia with methyl iodide.[15] A second proceeded in refluxing xylene at elevated temperatures using methyl *p*-toluenesulfonate as alkylating agent in the presence of anhydrous K_2CO_3 as catalyst.[16] The low-temperature procedure was advantageous for the reaction of heat-labile materials such as indican (indoxyl sulfate). Several substances such as 5-benzyloxytryptamine, however, underwent extensive chain N-alkylation and quaternization making the ammonia procedure impractical for the preparation of the corresponding tryptamine. In those it was advantageous to

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Table I

Pyrrole N-Methylindoles and Related Compounds

Compd.	Method[a]	Yield, %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
5-Methoxy-1-methyltryptamine hydrochloride	A	92	181.5–183[b]	$C_{12}H_{17}ClN_2O$	59.86	59.80	7.18	6.90	11.64	11.72
5-Methoxy-1-methyltryptamine hydrochloride	B	23	176–177[c]							
3-(2-Acetyl-2-methylaminoethyl)-5-methoxyindole (N-methylmelatonin)	A	8	97–99	$C_{14}H_{18}N_2O_2$	68.27	68.11	7.37	7.35	11.37	11.38
5-Methoxy-1-methyltryptophol picrate	A	80	128–129	$C_{18}H_{18}N_4O_9$	49.76	50.00	4.18	4.34	12.90	13.06
1-Methyltryptophol picrate	A	67	92–95	$C_{17}H_{16}N_4O_8$	50.50	50.75	4.00	4.12	13.86	14.12
Potasssium 1-methylindoxyl sulfate	A	40	119 dec.	$C_9H_8KNO_4S$	40.74	40.49	3.04	3.36	5.28	5.36
1,3-Dimethylindole picrate	A	90	141–143[d]							
6-Methoxy-1,9-dimethyl-1,2,3,4-tetrahydro-β-carboline hydrochloride	A	73	224.5–226.5	$C_{14}H_{19}ClN_2O$	63.03	62.77	7.18	7.20	10.50	10.45
Methylyohimbine	A[e]									
9-Methyl-β-carboline (methylnorharman)	A	52	288–290	$C_{12}H_{10}ClN_2$	65.02	64.93	6.36	6.09	12.64	12.80
Methylreserpine	A[f]									
5-(Benzyloxy)-1-methylindole	B[g]	40	131–131.5							
5-(Benzoyloxy)-1-methylgramine	B	93	44–45[h]							
5-(Benzyloxy)-1-methylindole-3-acetonitrile	B	61	86.5–87	$C_{18}H_{16}H_2O$	78.24	78.63	5.84	5.76	10.14	10.37
5-(Benzyloxy)-1-methyltryptamine hydrochloride	B	83	213–214 dec.	$C_{18}H_{21}ClN_2O$	68.23	68.05	6.68	6.70	8.84	8.51
5-Hydroxy-1-methyltryptamine picrate (1-methylserotonin)	B	66	201–202 dec.[i]	$C_{17}H_{17}N_5O_8$	48.69	48.73	4.09	4.22	16.70	16.43
5-Methoxy-1-methylindole	B	80	100.5–101[j]							
5-Methoxy-1-methylgramine	B	75	42–43[k]							

[a] Method A was a methylation employing sodium in liquid NH_3, method B was the procedure using methyl *p*-toluenesulfonate. [b] Lit.[30] m.p. 176–177°. [c] Mixture melting point with above, no depression. [d] P. E. Verkade, J. Lieste, and W. Meerburg [*Rec. trav. chim.*, **65**, 897 (1945)] report m.p. 142.5–143.5°. [e] Prepared according to the method used by C. F. Huebner, R. Lucas, H. B. MacPhillamy, and H. A. Troxell, *J. Am. Chem. Soc.*, **77**, 469 (1955). [f] Prepared according to the method used by C. F. Huebner, *ibid.*, **76**, 5792 (1954). [g] Lit.[19] m.p. 130–131°. [h] Lit.[19] m.p. 48–50°. [i] Lit.[20] m.p. 197–198°. [j] J. W. Cook, J. D. London, and P. McCloskey [*J. Chem. Soc.*, 1203 (1951)] report m.p. 103–104°. [k] Lit.[j] m.p. 43–45°.

prepare a methylated basic intermediate by the use of methyl *p*-toluenesulfonate in refluxing xylene and synthesize the tryptamine derivative from it. The preparation of 1-methylserotonin was particularly successful using this latter method and is described in Chart I.

5-Methoxytryptamine, 5-methoxytryptophol, tryptophol, skatole, 6-methoxy-1-methyl-1,2,3,4-tetrahydro-β-carboline, norharman, yohimbine, and reserpine all were readily 1-methylated in liquid ammonia. When 5-methoxytryptamine was methylated in 1-g. quantities in this manner, the 1-methylindole was the primary product. However, preparation on a larger scale resulted in considerable amounts of chain N-alkylation and quaternization.

Methylations in ammonia, where reaction failed to take place or tar formation occurred, were with 5-methoxyindole-3-acetic acid, 5-benzyloxytryptophan, and 5-benzyloxytryptamine. Some quaternary ammonium iodide was isolated during the ammonia methylation of 6-methoxy-1-methyl-1,2,3,4-tetrahydro-β-carboline. Indole N-methylation in more complex molecules such as reserpine and yohimbine was specific and good yields of the properly methylated indoles were isolated without quaternization. These methylations recommend themselves for structural studies of indole alkaloids.

The only compound in which methylation did not occur as expected was melatonin; the product isolated being the chain N-methylated derivative identified by its infrared spectrum.

The compounds prepared and their intermediates are listed in Table I. Since use can be made of some of these in characterization of metabolites, their chro-

Table II

Chromatographic Properties of Methylated Indoles

Compd.	Color reactions		R_f values in solvents		
	Ehrlich[a]	Xanthydrol[b]	A	B	C
5-Methoxy-1-methyltryptamine	Purple-blue	Blue	0.82	0.74	RAO[d]
5-Methoxytryptamine	Gray-blue	Blue	0.76	0.63	RAO
5-Methoxy-1-methyltryptophol	Blue-green	Blue-gray	0.88	0.88	0.84
5-Methoxytryptophol	Green-gray	Purple-gray	0.88	0.85	0.67
1-Methyltryptophol	Blue	Purple	0.87	0.91	0.84
Tryptophol	Blue	Purple	0.85	0.89	0.64
Potassium 1-methylindoxyl sulfate	Blue	Purple-green	0.65	0.56	RAO
Potassium indoxyl sulfate	Orange-yellow	Purple-brown	0.51	0.48	RAO
1,3-Dimethylindole	Blue	Gray-blue	0.96	0.94	0.92
3-Methylindole (skatole)	Blue	Gray-blue	0.96	0.94	0.90
1-Methylserotonin	Purple-blue		0.71	0.60	RAO
Serotonin	Purple		0.63	0.49	RAO

[a] Dimethylaminobenzaldehyde in acetone and HCl. [b] 9-Xanthenol in ethanol and HCl. [c] A, 1-Propanol–NH_4OH (8:2) on paper; B, 1-butanol–glacial acetic acid–water (4:1:5) on paper; C, $CHCl_3$–methanol (9:1) on silica thin layer. [d] RAO: remained at the origin.

matographic properties comparing the methylated analog with the parent compound are reported in Table II.

Methylation increased R_f for almost every compound over the nonmethylated analog. Previously, it has been reported that indoles substituted on the 1-nitrogen do not give a typical Ehrlich reaction or else give a negative response. This has not been confirmed with the series described here since all gave positive Ehrlich reactions with colors ranging through the blues, grays, purples, and blue-greens.

The structures of all compounds prepared were confirmed by infrared spectroscopy. On methylation the characteristic stretching peak for N–H in the region of 3.0 μ was lost. Several examples of spectra are shown in Figure 1, where methylated indoles are compared with the parent compounds. The exception, melatonin, is included showing that pyrrole N-methylation did not occur.

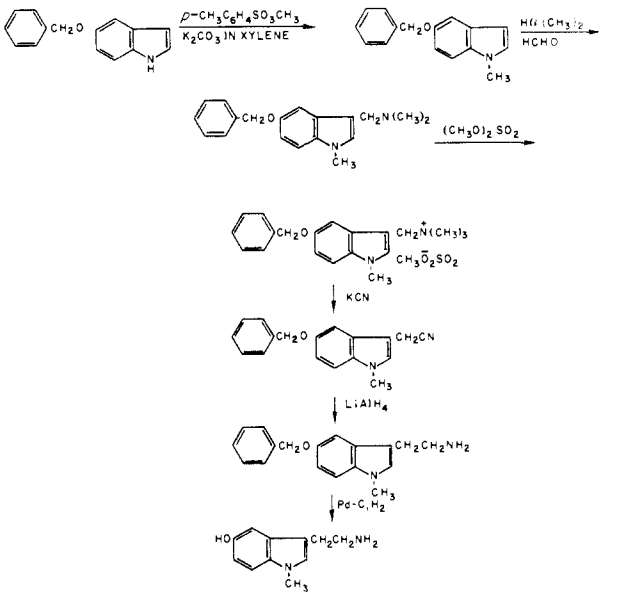
Pharmacological Activity

The materials were examined primarily for three types of activity: effects on behavior, which were judged to be a result of gross effects on the central nervous system; gross effects on the cardiovascular system; and serotonin antagonism on the isolated rat uterus (Table III). The effects on behavior consisted of studying conditioned rats on a variable-interval positive-reinforcement schedule. This schedule presented the opportunity to the animal for reward by bar pressing (food pellets) at intervals of various arbitrary lengths of time. Since it could not learn the pattern of sequences of presented opportunities, it could obtain a maximum number of rewards for the hour in the chamber by continuing to press the bar at a steady medium rate. Thus, drugs either depressed the animal and lowered its optimum work rate or stimulated it to work faster than the rate found characteristic for that animal.

Cardiovascular effects were studied by recording blood pressure of intact dogs anesthetized with pentobarbital during intravenous injection of the compounds. Serotonin antagonism was observed on isolated rat uterus muscle strips. Compounds were divided into those which were oxytocic (contracted the muscle), serotonin antagonists, or those without effect.

Chart I

Synthesis of 3-(2-Aminoethyl)-5-hydroxy-1-methylindole (1-Methylserotonin)



In an additional study the serotonin-releasing effects on platelets of methylreserpine was compared to that of reserpine (Figure 2).

Effect on Behavior (Table III).—In general, the methylated compounds were qualitatively and quantitatively similar to the nonmethylated analogs with three exceptions: 1-methylindican caused marked depression of work rates of conditioned animals at 6 mg./kg., whereas indican had no effect at 18 mg./kg. 1-Methylserotonin was about twice as potent as serotonin creatinine sulfate. It required 3.3 mg./kg. of 1-methylserotonin to reduce the work rates to less than 25% of their control values. In order for serotonin to achieve the same effects 6.6 mg. were required. The 1-methylserotonin was used as the picrate (mol. wt. 419) and the latter as the creatinine sulfate (mol. wt. 405) so the differences in potency were not artifacts due to molecular weights of the salts. Another variation was between 5-methoxy-1-methyltryptophol and 5-methoxytryptophol. The former caused moderate depression of work rates while the latter caused moderate stimulation. These results are difficult to inter-

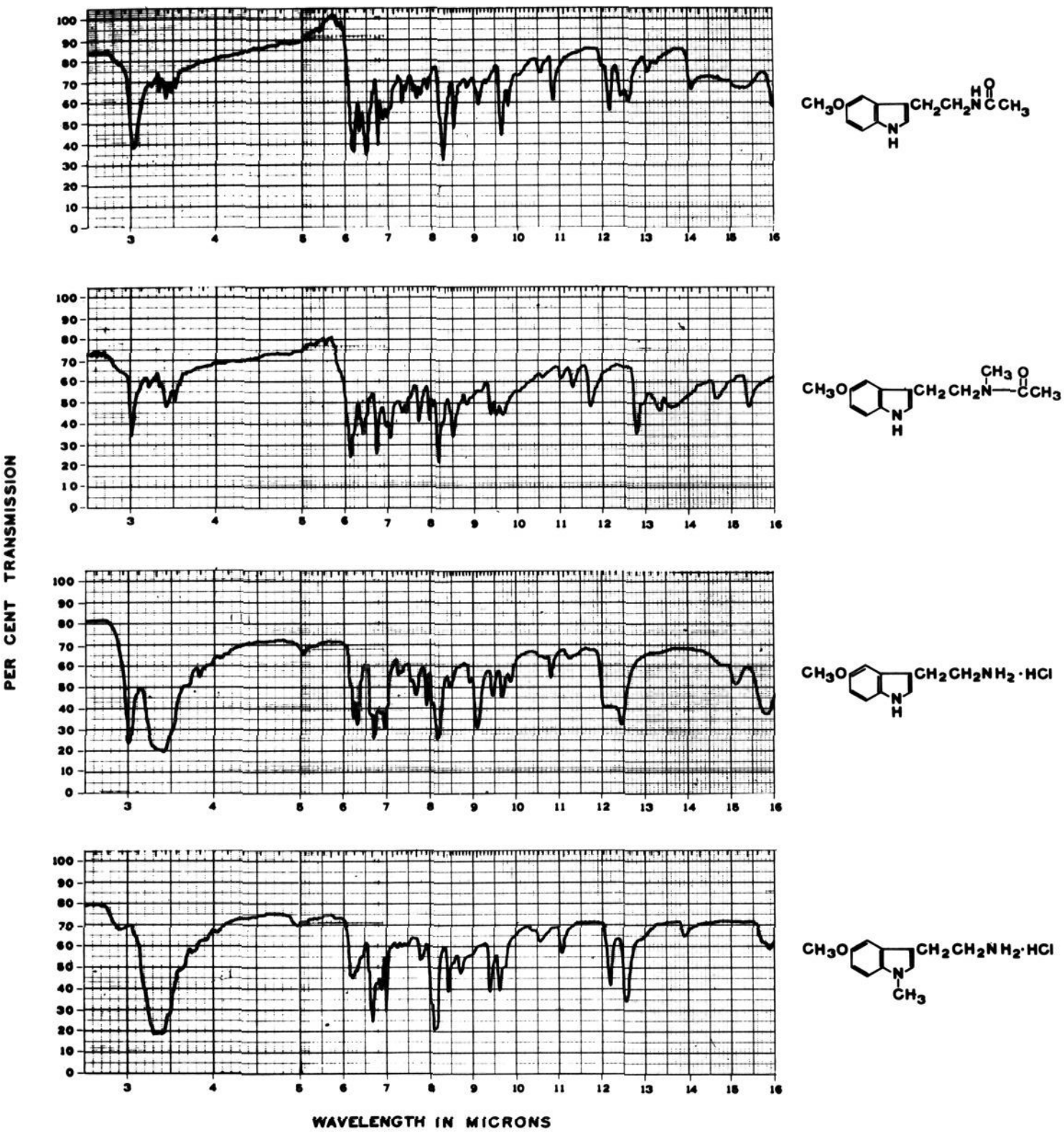


Figure 1.—A comparison of infrared spectra of two 1-methylated indoles with the nonmethylated analogs. The compounds, top to bottom, are: 3-(2-acetylaminoethyl)-5-methoxyindole (melatonin), 3-(N-acetyl-N-methyl-2-aminoethyl)-5-methoxyindole, 3-(2-aminoethyl)-5-methoxyindole hydrochloride (5-methoxytryptamine hydrochloride), and 3-(2-aminoethyl)-5-methoxy-1-methylindole hydrochloride. All spectra were determined with the use of KBr pellets.

pret; however, 5-methoxytryptophol has been demonstrated to possess hormone-like properties in influencing the estrus cycle of rats.[17] In this manner, 5-methoxytryptophol could produce increases in the animal's work rates as a general endocrinological effect. However, such interpretations must be regarded only as suggestions of a mechanism until further evidence is obtained.

Effects on Blood Pressure.—A selected number of the methylindoles were paired with the nonmethylated analogs and examined for their effect on the dog's blood pressure (Table III). In general, the methylated indoles and the nonmethylated analogs were similar in action in that some were pressor, some depressor, and some were both. There were two major exceptions to the rule of similarity of action. Although yohimbine

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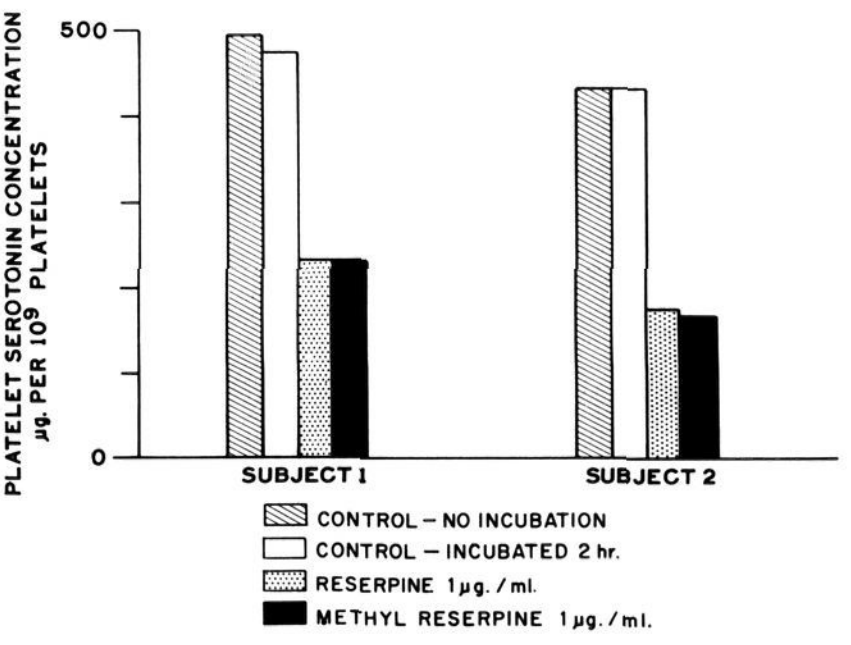

Figure 2.—Comparison of serotonin release from platelets between reserpine and methylreserpine.

Table III

Preliminary Pharmacological Characteristics of Some Methylindoles

Compd.	Effect on behavior[a] Response	Dose, mg./kg.	Effect on dogs'[b] blood pressure Response	Dose, mg./animal	Effect on smooth muscle[c] Response	Soln., M
5-Methoxy-1-methyltryptamine	S−	3.3	M+	1	Oxy.	10^{-9}
5-Methoxytryptamine	S−	3.3	S±	1	Oxy.	10^{-8}
5-Methoxy-1-methyltryptophol picrate	M−	3.3	0	2	A	$>10^{-5}$
5-Methoxytryptophol picrate	M+	3.3	W+	2	A	$>10^{-5}$
Potassium 1-methylindoxyl sulfate	S−	6.6	...	...	...	...
Potassium indoxyl sulfate	0	13.2	...	...	...	...
1,9-Dimethyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline	M−	8.6	W±	2	A	$>10^{-5}$
6-Methoxy-1-methyl-1,2,3,4-tetrahydro-β-carboline	M−	8.6	0	2	A	$>10^{-5}$
Methylyohimbine	M−	6.6	M+	2	A	$>10^{-5}$
Yohimbine	M−	6.6	0	2	A	$>10^{-5}$
Methylreserpine	S−	3.3	0	2	A	$>10^{-5}$
Reserpine	S−	3.3	0	2	A	$>10^{-5}$
1-Methylserotonin	S−	3.3	S+	1	Oxy.	10^{-8}
Serotonin	M−	3.3	S+	1	Oxy.	10^{-8}
9-Methylnorharman	M−	6.6	0	1	A	$>10^{-5}$
Norharman	M−	6.6	0	1	A	$>10^{-5}$
1,3-Dimethylindole	0	13.2	0	2	0	$>10^{-5}$
Skatole (3-methylindole)	0	13.2	0	1	0	$>10^{-5}$

[a] M, moderate effect where work rates have been changed 25% from control values; S, strong effect with changes greater than 75% from controls; +, stimulation above controls; −, decrease of work rates. [b] W, weak representing changes less than 10 mm. in blood pressure; M, medium representing changes of 10–20 mm. pressure; S, strong responses of over 20 mm.; −, lowering of pressure; +, elevation of pressure; ±, amphibaric response, where pressor responses may occur in one experiment and depressor response in another; dogs were 19–21 kg. [c] Serotonin antagonism; Oxy. means oxytocic. The concentration is that which produces the same contraction as a 10^{-8} M solution of serotonin. A indicates antagonism of 10^{-8} M solutions of serotonin by the test compound starting at concentrations of 10^{-4} M. In all data, 0 represents no activity.

was without effect on blood pressure, methylyohimbine caused moderate pressor response when 1 mg. was injected intravenously into 19–21-kg. dogs. The greatest difference seen in a pair of compounds was between 5-methoxy-1-methyltryptamine and 5-methoxytryptamine. The actions of the latter were amphibaric, at times producing marked depressor responses, whereas the methylated analog produced moderate pressor responses. Although an examination of the action of 5-methoxytryptamine was incidental to this study, it seems worthy of a thorough re-examination in light of pronounced depressor effects often observed and since it has been isolated from the urine of patients suffering from rheumatic fever.[4]

Effects on Smooth Muscle.—Oxytocic activity was quantitatively compared among the compounds (Table III). Qualitative comparisons of serotonin antagonism was also made. 5-Methoxy-1-methyltryptamine has approximately ten times as much oxytocic activity as serotonin. All compounds which were serotonin antagonists prior to 1-methylation remained so after the substitution.

Reserpine Release.—Comparison of the serotonin-releasing effect from platelets between reserpine and methylreserpine from blood of hospital patients indicated no qualitative or quantitative differences between the two compounds as can be seen in Figure 2.

General Comments.—In general 1-methylation of indoles did not produce profound changes in pharmacological activity of the parent compound suggesting that the 1-nitrogen is not functionally involved in indole receptors or enzyme sites.

The one qualitative difference seen was between indican and 1-methylindican in effects on animal behavior. That can be explained on the basis of increased lipid solubility and consequently increased penetration by the latter into the central nervous system. The higher potency of 1-methylserotonin compared to serotonin can also be attributed to transport reactions rather than changes in chemical function. These observations suggest that the 1-nitrogen position can be used for incorporation of other groups than the methyl radical to modify the transport characteristics of useful indoles. Therapeutic moieties (*e.g.*, nitrogen mustards, etc.) should also be able to substitute into the 1-nitrogen of various indole molecules, without appreciable modification of some of the physiological properties of the parent substance.

Many investigators have observed an indolic metabolite in urine of schizophrenics appearing on paper chromatograms in several systems slightly in front of urea and indican, and giving a blue reaction to Ehrlich's reagent. This compound has been provisionally identified as the sulfate ester of 6-hydroxyskatole.[18]

1-Methylindican prepared in these studies has chromatographic properties compatible with the above description. Since, unlike indican, it also has psychotropic effects in rats, it should be kept in mind in future studies concerning potential endogenously formed psychotropic agents.

Experimental

1-Methylation of Indoles.—Two different methods were employed for the preparation of compounds in this series. The first was methylation with methyl iodide in liquid ammonia. Two examples of this methylation are given below with 5-

(18) Ref. 5, p. 403.

methoxytryptamine and potassium 1-methylindoxyl sulfate. 1-Methylserotonin was prepared first by methylating 5-benzyloxyindole with methyl *p*-toluenesulfonate and then converting the methylated intermediate to the desired compound. That synthesis is described in detail below.

5-Methoxy-1-methyltryptamine Hydrochloride.—5-Methoxytryptamine (1 g., 5.1 mmoles) was pulverized and partially dissolved in 18.0 ml. of liquid NH_3, kept cold in a round-bottom flask immersed in Dry Ice–ethanol. The system was protected from atmospheric moisture. Sodium (0.15 g., 6.5 mg.-atoms), cut into small pieces was added, and the mixture was shaken for about 30 min. until all of the solids were in solution and the typical blue color disappeared. Methyl iodide (0.92 g., 6.5 mmoles) was added with shaking. A heavy white precipitate formed within 5 min. and the mixture was allowed to stand with occasional shaking for about 1 hr. more at Dry Ice temperatures. Most of the ammonia was removed by evaporation at room temperature; final traces were removed under vacuum in a desiccator.

The product was extracted from the NaI with $CHCl_3$ and filtered. The chloroform solution (about 100 ml.) was mixed with 200 ml. of ether and filtered, and about 1.0 ml. of 15% methanolic HCl was added to prepare 600 mg. of light tan 5-methoxy-1-methyltryptamine hydrochloride, m.p. 174.5–176.5°. More ether and HCl produced a second crop of 542 mg., melting at 166–170° to give a total yield of 93% of crude product. A portion was crystallized from 3:1 ethyl acetate–ethanol to give an analytical sample, m.p. 182–183°. The structure was confirmed by infrared spectroscopy which showed loss of the band characteristic for the indole pyrrole N–H group.

Potassium 1-Methylindoxyl Sulfate (1-Methylindican).—Commercial potassium indoxyl sulfate, which is usually red by indigoid impurities was used without further purification. Potassium indoxyl sulfate (1 g., 4 mmoles) was added to 20 ml. of liquid NH_3 in the manner described above. Pieces of sodium (120 mg., 5.2 mg.-atoms) were added; considerable agitation and stirring was required to dissolve all of the solids. Since the solution was highly colored, a glass rod was used to ensure that all of the solids were dissolved. Methyl iodide (0.73 g., 0.32 ml., 5.2 mmoles) was added, and the rest of the reaction was carried out as described above. However, since the product was a salt, the usual method of isolation was modified. The residue was suspended in a very small quantity of cold water which dissolved most of the NaI but left much of the product in suspension. It was filtered and washed with cold ethanol and ether to give 250 mg. of potassium 1-methylindoxyl sulfate, m.p. 122° dec., a light-colored material with a green tint. A second crop of 160 mg., m.p. 119° dec., was obtained by cooling the filtrate to give a total yield of 41%. When the material was allowed to stand or when attempts were made at crystallization in some solvents, the color deepened to a darker green and the melting point was greatly reduced (to below 100°). Ethanol was the best solvent of crystallization found which lightened the color but had little effect on improving the melting point. Most of the products were white with a light green tint immediately on preparation and sharply turned deep green at their decomposition points.

5-Benzyloxy-1-methylindole. A.—A mixture of 5-benzyloxyindole (10.0 g., 4.48×10^{-2} mole), anhydrous K_2CO_3 (4.6 g., 4.7×10^{-2} mole), methyl-*p*-toluenesulfonate (8.4 g., 4.5×10^{-2} mole), and xylene (40 ml.) was heated under reflux with vigorous stirring for 48 hr. After cooling, the reaction mixture was filtered, and the residue was leached thoroughly with hot xylene. The combined xylene solutions were reduced in volume by evaporation to *ca.* 25 ml. On cooling, yellowish crystals of the product separated out. The product was crystallized from a mixture of acetone and water to give 4.25 g. (40% yield) of material, m.p. 129.5–130.5° (lit.[19] m.p. 130–131°). A second crystallization raised the melting point to 131–131.5°.

B. 5-Benzyloxyindole (1.0 g., 4.5 mmoles) and sodium (150 mg., 6.5 mg.-atoms) were dissolved in anhydrous liquid NH_3 (30 ml.) with shaking. Methyl iodide (930 mg., 6.5 mmoles) was added in two equal portions over a period of 5 min., and the reaction mixture was allowed to stand overnight in the cold. The solvent was allowed to evaporate at room temperature with stirring, and the last traces were removed *in vacuo*. The dark solid residue was taken up in water, the product was extracted with $CHCl_3$, and the extracts were evaporated to dryness under reduced pressure. The crude product was crystallized from acetone–water to give 180 mg. (17%) of 5-benzyloxy-1-methylindole, m.p. 127–128.5°. Its identity was confirmed by the fact that it did not depress the melting point of the product from A.

5-Benzyloxy-1-methylgramine.—Aqueous dimethylamine (25%, 3.5 ml.) was added over a period of 5 min. to a mixture of 37% aqueous formaldehyde (1.3 ml.), dioxane (16 ml.), and acetic acid (16 ml.), cooled to 10°. A solution of 5-benzyloxy-1-methylindole (3.50 g., 1.48×10^{-2} mole) in 25 ml. of dioxane was added over 30 min. at 10°. The reaction mixture was stirred in the cold for 2 hr. more, allowed to stand overnight at room temperature, diluted, and filtered through Celite. An ice-cold 10% aqueous KOH solution (170 ml.) was added to the filtrate, and the mixture was cooled in an ice bath. The resulting crystals were filtered, washed thoroughly with water, and dried in the air to give 4.05 g. (1.38×10^{-2} mole, 93%) of product, m.p. 44–45° (lit.[19] m.p. 48–50°).

5-Benzyloxy-1-methylgramine Methosulfate.—A solution of 5-benzyloxy-1-methylgramine (3.8 g., 1.3×10^{-2} mole) in absolute tetrahydrofuran (THF) (30 ml.) and glacial acetic acid (0.35 ml.) was added dropwise over 30 min. to a stirred mixture of dimethyl sulfate (8.2 g., 6.5×10^{-2} mole), absolute THF (10 ml.), and glacial acetic acid (0.35 ml.) cooled to 10°. A crystalline precipitate began to settle out in a few minutes. When addition was complete, the reaction mixture was allowed to stand in the dark at room temperature for 2 hr. The product was collected, washed thoroughly with anhydrous ether, and dried *in vacuo* to give 4.5 g. (84% yield) of the quaternary methosulfate, m.p. 136–137.5°. This compound was not further purified but used directly in the next step.

5-Benzyloxy-1-methylindole-3-acetonitrile.—A solution of 5-benzyloxy-1-methylgramine methosulfate (3.5 g., 8.3 mmoles) and NaCN (1.1 g., 22 mmoles) in water (30 ml.) was heated at 80° with vigorous stirring for 3 hr. During this time the reaction mixture became turbid and a yellowish oil separated out, which solidified on cooling. The product was filtered, washed with water, and dried in air to give a yield of 1.4 g. (61%), m.p. 82–84°.

The filtrate from the above reaction was heated with stirring at 80° overnight (*ca.* 16 hr.) and worked up as before to give 0.5 g. more of the product, m.p. 77–82°. Two crystallizations from a mixture of acetone and water yielded an analytical sample, colorless plates, m.p. 86.5–87°.

5-Benzyloxy-1-methyltryptamine.—A suspension of 5-benzyloxy-1-methylindole-3-acetonitrile (1.3 g., 4.7 mmoles) in anhydrous ether (160 ml.) was added dropwise to a vigorously stirred suspension of $LiAlH_4$ (0.54 g., 11 mmoles) in anhydrous ether (40 ml.). The reaction mixture was then heated for 16 hr. under gentle reflux. The excess $LiAlH_4$ was carefully decomposed with a few milliliters of water, and a 10% aqueous NaOH solution (55 ml.) was added to the reaction flask. The ether layer was decanted off, the aqueous layer was filtered, and the filtrate was extracted with ethyl acetate. The combined ether and ethyl acetate solutions were evaporated to dryness under reduced pressure, then dried further *in vacuo* to give 1.1 g. (83%) of crude product in the form of a brownish oil. It was dissolved in 30 ml. of toluene, and anhydrous HCl bubbled through the solution. The precipitate that formed was collected, washed with acetone, and dried in the air to give 0.73 g. of yellowish crystals of 5-benzyloxy-1-methyltryptamine hydrochloride (55% yield), m.p. 208–211° dec.

Two crystallizations from a mixture of 4:1 toluene and ethanol gave an analytical sample, colorless crystals, m.p. 213–214° dec.

5-Hydroxy-1-methyltryptamine (1-Methylserotonin).—5-Benzyloxy-1-methyltryptamine hydrochloride (113 mg., 0.357 mmole) was taken up in a 10% aqueous NaOH solution. The free base was extracted with ethyl acetate, and the extract was evaporated to dryness under reduced pressure, then dried further *in vacuo*. A mixture of the free base (99 mg., 0.354 mmole), 10% palladium–charcoal catalyst (25 mg.), and methanol (25 ml.) was hydrogenated in a Parr apparatus at 2.8 kg./cm.^{2} pressure for 16 hr. The reaction mixture was filtered, and the filtrate was reduced in volume at reduced pressure to *ca.* 2 ml. The latter solution was added to a boiling solution of picric acid (70 mg.) in methanol (3 ml.). After cooling, the orange precipitate was filtered and dried in air to give 98 mg. (66% yield) of 5-hydroxy-1-methyltryptamine picrate, m.p. 194.5–195.5° dec. (lit.[20] m.p. 197–198° dec.). Two crystallizations from water

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gave an analytical sample, orange needles, m.p. 201–202° dec.

Behavior Studies.—The effect of compounds on behavior was determined in the following manner. Rats were conditioned on a variable interval (V.I.) positive reinforcement schedule, *i.e.*, bar pressing in a Skinner box at a steady medium rate which was rewarded automatically with food pellets. Faster or slower rates therefore represented less reward for effort and would indicate that behavior was not optimal. Animals were deprived of food and spent 50 min. each day in the test chamber. The mean rate of response during the test period was determined on 5 consecutive days for each individual animal. On the experimental day, the compound was administered intraperitoneally, and the increased (+) or decreased (−) response rates were computed as a percentage of the normal.

Blood Pressure.—Compounds were injected intravenously into anesthetized, whole male dogs 19–21 kg. at 10-min. intervals. Ten minutes after each injection of compound, a 1-mg. dose of serotonin creatinine sulfate was given to determine whether the animals' response remained constant to within 10% of an initial control injection given. Each compound was examined at least three times, either in the same or different animals. Long-term effects which changed the response of the animal to serotonin as anticipated were seen only with reserpine and methylreserpine. Effects of such compounds were either examined on separate dogs or at the end of the test series. Blood pressure changes were measured by means of an arterial cannula attached to a mercury manometer which operates a lever attached to a pen on a kymograph. Responses were recorded and blood pressure changes were measured in the usual manner.

Effect on Smooth Muscle.—Estrus rat uterus suspended in a Tyrode–Ringer solution muscle bath was used. The muscle was standardized to give consistent contractions to the reintroduction of a 10^{-8} *M* solution of serotonin creatinine sulfate. The potency of oxytocic activity was expressed as the molarity of a compound giving the same contraction as a 10^{-8} *M* solution of serotonin creatinine sulfate.

Antagonism to serotonin was measured by introducing the test compound into the muscle bath at concentrations starting at 10^{-5} *M*. This solution is allowed to equilibrate with the tissue for 2 min. at which time serotonin is added directly to the same bath so that it achieves a concentration of 10^{-8} *M*. Compounds reducing the normal muscle contraction of serotonin by at least 50% under these conditions were classified as antagonists.

Serotonin Release from Platelets.—Whole blood was collected by a siliconized syringe ("Monocote" treated needle) and transferred to siliconized centrifuge tubes containing EDTA-saline (1:10 w./v.). All samples were collected from "apparently normal" subjects, either laboratory and medical staff or subjects for pre-employment physicals at the Cleveland Clinic. The whole blood samples were centrifuged at 700 r.p.m. for 20 min. to prepare platelet-rich plasma samples. Platelet counts were made in the platelet-rich plasma by a bulk dilution procedure. Volumes of 2 ml. of platelet-rich plasma were incubated at 37° for 2 hr. (siliconized conical flasks), the compound under test being added in saline solution to a final concentration of 1 γ/ml. of incubation mixture. Controls (without incubation) were set up in each experiment. After incubation, the platelet-rich plasma samples were transferred to centrifuge tubes with ice-cold saline and centrifuged for 20 min. at 2500–3000 r.p.m. Supernatants were poured off, the tubes were drained, and the platelet pellets were resuspended in saline. Water was added to rupture the platelets and the proteins were precipitated with an equal volume of 20% trichloroacetic acid. Serotonin was assayed spectrophotofluorometrically[21] in the acid extract. Concentrations of serotonin were expressed as micrograms/10^{-9} platelets by reference to the platelet-rich plasma platelet count from which the platelet pellet was derived.

Acknowledgment.—The authors wish to thank Mrs. Martha Dattilo, Mrs. Catherine Rice, Mr. Jesse Green, and Mr. Rong An for their technical assistance.

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Stereochemistry of *d*-3,4-Dimethyl-2-phenylmorpholine (Phendimetrazine)[1]

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Received January 5, 1967

Acid-catalyzed cyclization of *l-erythro*-N-(β-hydroxyethyl)ephedrine to *d-threo*-3,4-dimethyl-2-phenylmorpholine (I) proceeds with inversion of the benzylic carbon atom. Inversion appears to be caused by the relief of nonbonded destabilizing interaction present in the corresponding *erythro* morpholine (II). Accordingly, acid treatment of the *erythro* morpholine (II) caused partial inversion to the *threo* diastereoisomer (I) and was reflected in change of optical rotation and decreased inhibitory effect on liver monoamine oxidase.

A number of central-stimulating appetite-depressing drugs[2] have a common phenethylamine backbone, *e.g.*, amphetamine, ephedrine, α-diethylaminopropiophenone, etc. Compounds in which this "backbone" is part of a ring system appear to retain central-stimulating and appetite-depressing properties, *e.g.*, *dl*-3-methyl-2-phenylmorpholine (phenmetrazine)[3] and its *d*-N-methyl analog (phendimetrazine).[4,5]

d-3,4-Dimethyl-2-phenylmorpholine (phendimetrazine) was first prepared by the acid-catalyzed cyclization of N-(β-hydroxyethyl)-*l*-ephedrine.[6] We have investigated this reaction, established a *threo* configuration for the cyclization product,[1] and have thus substantiated Foltz and Witkop's interpretation[7] of Otto's findings.[6b] In the meantime, similar conclusions were reached by Clarke[8] (in the N-desmethyl series) and by Drefahl, *et al.*,[9] respectively (see Scheme I).

In accordance with Otto,[6b] acid-catalyzed cyclization of N-(β-hydroxyethyl)-*l*-ephedrine gave *d*-3,4-dimethyl-2-phenylmorpholine. The identical dextrorotatory morpholine was obtained when the same procedure was

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