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THE METABOLISM OF 3-(2-ACETOXYETHYL)-5-METHOXYINDOLE(5-METHOXY-TRYPTOPHOL O-ACETATE)*

PETER DELVIGS† and ROBERT G. TABORSKY

Research Division, The Cleveland Clinic Foundation, Cleveland, Ohio, U.S.A.

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Abstract—Administration of radioactively labeled 3–(2–acetoxyethyl)–5–methoxyindole to rats produced a single metabolite. Its properties indicated that it was a hydroxyindole–3–acetic acid with hydrolysis, oxidation and hydroxylation having occurred. The product was not the expected 6–hydroxy–5–methoxyindole–3–acetic acid nor the 4–hydroxy isomer. Deduction led to the conclusion that the metabolite was 7–hydroxy–5–methoxyindole–3–acetic acid, with an apparently unusual form of indole metabolism occurring. A study of tissue distribution showed that concentration of radioactivity was 18 times as high in the pineal than in brain 1 hr after administration.

THE ABUNDANCE of the indole serotonin in peripheral tissues and in the central nervous system of the mammalian organism suggests that it has an important physiological role.[1] However, the detection of numerous indolic metabolites in urine[2] and in tissue such as the pineal gland[3] by various investigators suggests that metabolites of serotonin or other indoles may also be important. By administering various indoles to animals and learning how they are metabolized, we wish to anticipate possible naturally occurring critical indolic factors in normal and pathological functions. Because acetylation confers hormonal properties on 5-methoxytryptamine and alters its mode of metabolism, the acetate ester of 5-methoxytryptophol, which is the oxygen isostere of melatonin, was synthesized and its metabolism studied in rats.

Compounds. Acetylation of 5-methoxytryptophol-α-^{14}C with acetic anhydride gave 3-(2-acetoxy-2-^{14}C-ethyl)-5-methoxyindole (VIII in Fig. 1).[4] Since the latter is a viscous liquid, it was convenient to prepare a picrate and use this derivative in subsequent studies. 3-(2-Acetoxy-2-^{14}C-ethyl)-5-methoxyindole picrate, with a specific activity of 93·8 μc/g, was prepared by mixing concentrated boiling chloroform solutions of equimolar amounts of the free indole and picric acid.

Various compounds, hydroxylated in the 6-position of the indole ring, were prepared for chromatographic comparisons. These are shown in Fig. 1. 6-(Benzyloxy)-5-methoxyindole-3-acetonitrile (I) was prepared according to a previously described procedure.[5] Saponification of the nitrile with a propylene glycol solution of potassium hydroxide gave 6-(benzyloxy)-5-methoxyindole-3-acetic acid (II), which was converted to 6-hydroxy-5-methoxyindole-3-acetic acid (III) by debenzylation with

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hydrogen in the presence of 10% palladium-charcoal catalyst. Reduction of 6-(benzyloxy)-5-methoxyindole-3-acetic acid with lithium aluminum hydride gave 6-(benzyloxy)-5-methoxytryptophol (IV), which upon debenzylation yielded 6-hydroxy-5-methoxytryptophol (V). Acetylation of 6-(benzyloxy)-5-methoxytryptophol with acetic anhydride gave 3-(2-acetoxyethyl)-6-(benzyloxy)-5-methoxyindole (VI), which was converted to 3-(2-acetoxyethyl)-6-hydroxy-5-methoxyindole (VII) by debenzylation. Complete synthetic details are described eslewhere.[4]

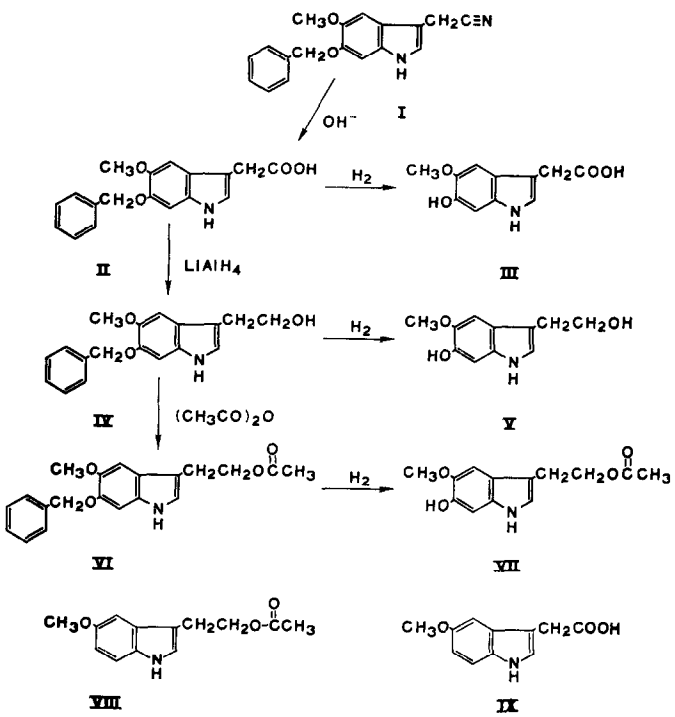

FIG. 1. Structures I–VII represent the synthetic scheme for authentic model compounds prepared in this study. Compound VIII is 3-(2-acetoxyethyl)-5-methoxyindole, and compound IX is 5-methoxyindole-3-acetic acid.

4-Hydroxy-5-methoxyindole-3-acetic acid was prepared for the first time by the procedure described below.

4-(Benzyloxy)-5-methoxyindole-3-acetic acid (5-methoxytryptamine salt). A mixture of 4-(benzyloxy)-5-methoxyindole-3-acetonitrile[6] (584 mg, 2·0 m-moles), 1·1g KOH, 2·0 ml water, and 3·0 ml ethanol was refluxed with stirring for 20 hr. After cooling, the mixture was diluted with 20 ml water and filtered. The filtrate was extracted several times with ethyl acetate. The aqueous phase was acidified to pH 3, cooled in ice, and the product collected by filtration, washed with water, and dried in air to give 313 mg of the crude acid, m.p. 108-112°. Two crystallizations from benzene : hexane (2 : 1) gave 175 mg (28 per cent) of pure 4-(benzyloxy)-5-methoxyindole-3-acetic acid, m.p. 122-123°. A solution of the acid (31 mg) in ethyl acetate (5 ml) was treated with a solution of 5-methoxytryptamine (19 mg) in ethyl acetate to give

37 mg (74 per cent) of the salt derivative, m.p. 154–155·5°. Two crystallizations from ethyl acetate yielded an analytical sample, m.p. 158·5–159·5°.

Anal. Calcd. for $C_{29}H_{31}N_3O_5$: C, 69·44; H, 6·23; N, 8·38.

Found: C, 69·25; H, 6·59; N, 8·23

Several conditions for hydrolysis of the nitrile to the acid were explored. Use of aqueous NaOH, aqueous KOH, and KOH in a mixture of water and propylene glycol gave large amounts of tar, the yield of acid being below 5 per cent; shorter reaction time (4–10 hr) gave mostly unchanged starting material

4-Hydroxy-5-methoxyindole-3-acetic acid (5-methoxytryptamine salt). A mixture of 4-(benzyloxy)-5-methoxyindole-3-acetic acid (100 mg, 0·321 m-mole), 10% palladium-charcoal (50 mg), and ethyl acetate (25 ml) was hydrogenated in a Parr apparatus at 2·8 kg/cm² for 20 hr. Filtration and evaporation of the filtrate to dryness gave 62 mg (87 per cent) of the crude product, m.p. 150–153·5°. A solution of the crude acid (60 mg) in ethyl acetate (10 ml) was treated with a solution of 5-methoxytryptamine (50 mg) in ethyl acetate (10 ml) to give 65 mg of the crude salt derivative. Crystallization of the latter from a large volume of toluene gave an analytical sample, m.p. 174° dec.

Anal. Calcd. for $C_{22}H_{25}N_3O_5$: C, 64·22; H, 6·13.

Found: C, 64·27; H, 6·06.

The free acid and the salt were unstable in air, turning dark within a few days.

Animals. Female Sprague-Dawley rats weighing 180–220 g were used for metabolic studies. Solutions of 6·8–7·8 mg of 3-(2-acetoxy-2-^{14}C ethyl)-5-methoxyindole picrate were prepared in 0·2 ml of 75% aqueous propylene glycol and administered by intraperitoneal injection. Animals were fed on a standard diet, but were deprived of food, although not water, on the day of the experiment.

Chromatographic methods. Descending paper chromatography was employed for the detection of metabolites in urine extracts. Chromatograms were scanned for radioactivity with a Scanogram RSC-5 (Atomic Accessories, Inc.). The R_f values were also compared on silica thin-layer chromatography plates. The solvents, R_f values, and color reactions of the reference compounds are given in Table 1.

TABLE 1. CHROMATOGRAPHY OF MODEL COMPOUNDS

Compound	R_f values in solvent			Color of spots on paper with reagent	
	A	B	C	1	2
3-(2-Acetoxyethyl)-5-methoxyindole	0·91	0·94	0·63	blue	none
5-Methoxytrypotophol	0·88	0·86	0·24	blue	none
5-Methoxyindole-3-acetic acid	0·32	0·83	0·40	blue	none
5-Hydroxyindole-3-acetic acid	0·23	0·80	—	blue	violet
3-(2-Acetoxyethyl)-6-hydroxy-5-methoxyindole	0·81	0·88	0·54	green–blue	purple–blue
6-Hydroxy-5-methoxytryptophol	dec.	0·76	0·46	green–blue	purple–blue
6-Hydroxy-5-methoxyindole-3-acetic acid	dec.	0·73	0·23	green–blue	purple–blue
4-Hydroxy-5-methoxyindole-3-acetic acid	dec.	0·81	0·23	pink	deep purple
Metabolite of 3-(2-acetoxyethyl)-5-methoxyindole	0·44	0·79	0·41	blue	deep blue

The solvent systems used were: solvent A, 1-propanol : NH_3 (8 : 2); and solvent B, 1-butanol : acetic acid : water (4 : 1 : 5), both employing descending paper chromatography on Whatman 1 paper; solvent C, chloroform : acetic acid (95 : 5) on silica thin-layer plates. The sprays used for detecting compounds were: 1, Ehrlich's reagent (*p*-dimethylaminobenzaldehyde, 0·5% solution in acetone plus a few drops of concentrated HCl); 2, Gibb's reagent (2,6-dichloroquinonechlorimide, 0·05% solution in ethanol, followed by saturated aqueous $NaHCO_3$ solution).

TABLE 2. TISSUE DISTRIBUTION OF RADIOACTIVITY AFTER THE ADMINISTRATION OF3-(2-ACETOXY-2-^{14}C-ETHYL)-5-METHOXYINDOLE TO RATS

Tissue	Radioactivity distribution after											
	1 hr (7·2 mg)				2 hr (6·8 mg)				6 hr (7·8 mg)			
	Rat 1		Rat 2		Rat 3		Rat 4		Rat 5		Rat 6	
	(mμc/g)	(% total)	(mμc/g)	(% total)	(mμc/g)	(% total)	(mμc/g)	(% total)	(mμc/g)	(% total)	(mμc/g)	(% total)
Urine					3910	14·3			2350	48·8	3410	52·2
Blood	27	29·6	28	28·2	22	16·9	17	17·1	3·0	2·1	3·1	2·3
Heart	4·7	0·086	8·5	0·19	4·9	0·083	4·6	0·13	0·51	0·0092	1·7	0·031
Lungs	7·7	0·22	13	0·37	5·8	0·16	7·9	0·33	0·46	0·0096	1·4	0·033
Liver	6·9	1·8	7·2	1·8	6·9	3·9	5·9	1·4	1·1	0·25	1·8	0·36
Kidney	26	1·3	17	0·89	16	0·80	16	1·1	3·2	0·13	4·5	0·18
Spleen	3·4	0·055	3·9	0·049	3·4	0·050	3·2	0·055	0·46	0·0055	1·2	0·016
Brain	0·68	0·028	0·63	0·030	2·2	0·097	1·2	0·066	0·28	0·012	0·40	0·015
Pineal	12	0·0012					9·5	0·0002			1·7	0·0001
Thyroid	6·8	0·0033	7·6	0·0049	6·3	0·0044	5·5	0·0038	1·2	0·0005	0·39	0·0016
Adrenals	11	0·046	5·6	0·010	11	0·036	3·0	0·012	0·61	0·0014	0·99	0·0023
Ovary	7·7	0·025	6·4	0·018	8·2	0·050	5·6	0·014	0·33	0·0006	1·0	0·0026
Uterus	11	0·084	14	0·10	11	0·069	7·1	0·086	0·39	0·0029	0·92	0·0070
Feces	3·2	0·39	3·9	0·28	4·3	0·41	2·9	0·28	11	1·5	16	4·3

Measurement of radioactivity in tissues. Two techniques were employed; measurements in the first method were carried out with solid samples of "infinite thickness" on nickel planchets with an end-window counter tube. The specific activities were determined by comparison with a stable polymer reference. The tissue homogenates were dried under a heat lamp until they were brittle, then ground into a fine powder in a mortar and deposited in layers on a planchet with *n*-hexane until a constant number of counts was obtained.

When amounts of dried tissues were insufficient for samples of "infinite thickness", the technique of Kalberer and Rutschmann[7] was employed. The dried tissue was burned in a sealed flask containing oxygen, the radioactive CO_2 being absorbed in 15 ml of a solution prepared by diluting 120 ml of redistilled ethanolamine with methanol (analytical grade) to 1000 ml. After addition of 10 ml of scintillator solution, the samples were counted in a Tracerlab LSC-20 liquid scintillation counter. The scintillator consisted of a solution of 4·0 g of 2,5-diphenyloxazole (PPO) and 100 mg of 1,4-bis-2(5-phenyloxazolyl)-benzene (POPOP) in 1000 ml toluene.

RESULTS

Amount of total radioactivity recovered and distribution in tissues of administered 3-(2-acetoxy-2-^{14}C-ethyl)-5-methoxyindole picrate. An average of 86·2 per cent of the administered activity was found in the urine, and an average of 3·4 per cent in the feces after 24 hr. The specific activities and percentage distributions of the radioactivity 1,2, and 6 hr after administration are given in Table 2.

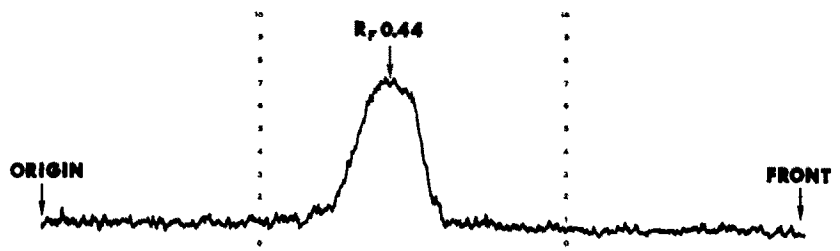

FIG. 2. Radioactive scan of paper chromatogram (1-propanol : NH_4OH, 8 : 2) of rat urine extract 24 hr after administration of 3-(2-acetoxyethyl)-5-methoxyindole.

Identification of metabolite. Paper chromatography of the 24-hr urine extracts showed the presence of a single metabolite (Fig. 2; see Table 1 for R_f values and color reactions). It could not be extracted into the organic phase at pH values above 7; however, at pH 3, extraction into the organic phase was essentially quantitative, as determined by scanning of chromatograms. The metabolite gave an immediate positive color reaction with Gibbs' and Pauly's reagents, indicating the presence of a phenolic group. Incubation of the urine with limpet viscera sulfatase and β-glucuronidase (Sigma Chemical Co.,), as well as with concentrated HCl, failed to change the R_f values on chromatography.

DISCUSSION

Indoles are usually metabolized by one of two pathways, or a combination of both. Oxidation can occur through a group on the side chain with amines or alcohols

being converted to acids.[8-12] If this pathway is inhibited by N, N-dialkylation or N-acetylation, or if the molecule does not have an oxidizable group, hydroxylation of the indole ring occurs. For example, indole, having no oxidizable group, is converted to the sulfate ester of 3-hydroxyindole (urinary indican).[13] Skatole (3-methylindole) forms several monohydroxylated isomers in the rat which are eliminated as the salts of the sulfate esters.[14] Other molecules representing important biological compounds have also been reported to be metabolized either partially or completely by hydroxylation in the 6-position.[15] These include 3-(2-acetylaminoethyl)-5-methoxyindole (melatonin), the pineal constituent and frog skin-lightening hormone,[5, 8, 10, 16] and N, N-dimethyltryptamine, a psychotropic agent.[17]

Studies of the metabolism of 5-methoxytryptophol were previously carried out in this laboratory, since it was isolated from pineal tissue.[3b] It was shown to be rapidly metabolized to the expected 5-methoxyindole-3-acetic acid.[12] When injected, 5-methoxytryptophol becomes more highly concentrated in the thyroid, adrenals, and ovaries than in other tissues.

5-Methoxytryptamine, as other tryptamines, is rapidly metabolized to the corresponding acid.[10] When the amino group in the chain is acetylated to give melatonin, the hydroxylated metabolite forms instead.[5, 10, 16] In addition to the changes of biochemical properties, acetylation alters markedly the hormonal properties of the molecule. Thus 5-methoxytryptamine is without effects on darkened frogskin, whereas the acetylated analog melatonin is a potent frog skin-lightening hormone. Therefore it was important to see whether chain acetylation of 5-methoxytryptophol (compound VIII, Fig. 1) might also produce a compound with special biological properties not seen in the precursor. For the selection of authentic compounds to be synthesized for use in chromatography, four possible metabolic transformations were anticipated initially. The model compounds synthesized and methods of synthesis are presented in Fig. 1. Complete details of the syntheses are reported elsewhere.[4]

One possible metabolic pathway was that only 6-hydroxylation could have occurred in a manner analogous to that seen for melatonin to give in this instance 3-(2-acetoxyethyl)-6-hydroxy-5-methoxyindole (VII, Fig. 1). A second was that the ester group would be hydrolyzed and metabolism would be identical with that seen for 5-methoxytryptophol to give 5-methoxyindole-3-acetic acid (IX, Fig. 1). A third was for 6-hydroxylation to occur, followed by hydrolysis of the ester group to yield 6-hydroxy-5-methoxytryptophol (V, Fig. 1). A fourth was to have 6-hydroxylation, ester hydrolysis, and oxidation to give 6-hydroxy-5-methoxyindole-3-acetic acid (III, Fig. 1).

After administering radioactive 3-(2-acetoxyethyl)-5-methoxyindole to rats, collecting urine for 24 hr, and chromatographing the urine extracts, all the radioactivity appeared as a discrete single peak in radiograms (Fig. 2), showing that only one metabolite formed.

On comparing chromatographic properties of the metabolite with 5-methoxyindole-3-acetic acid and 3-(2-acetoxyethyl)-6-hydroxy-5-methoxyindole, the incompatibilities in characteristics (Table 1) disqualified them as possible metabolites. The radioactive metabolite could not be extracted by an organic solvent from urine at pH 7 but was quantitatively removed on acidification. It therefore possessed the acidity of a carboxylic acid rather than a phenol which would be extractable at pH 7. This property in addition to its chromatographic characteristics ruled out the third alternative, 6-hydroxy-5-methoxytryptophol, as the metabolite.

The metabolite also gave positive reactions with Gibb's and Pauly's reagents, indicating the presence of a phenolic hydroxyl group. These properties were compatible with its being 6-hydroxy-5-methoxyindole-3-acetic acid. Initial chromatographic comparisons of this hydroxy acid with the metabolite indicated similarity of properties. However, on chromatography in ammoniacal systems, 6-hydroxy-5-methoxyindole-3-acetic acid underwent degradation, streaking badly, whereas the metabolite gave a discrete spot. Also, colors with Ehrlich's and Gibbs' reagents were not the same shades of blue. A variety of chromatographic systems was used to confirm that though a close similarity existed between the two, the metabolite was not the 6-hydroxy acid. The difference was best established by thin-layer chromatography with a 95 : 5 chloroform - acetic acid solvent system in which it could be shown that the R_f's of the metabolite and authentic compound were not the same (Table 1).

4-Hydroxy-5-methoxyindole-3-acetic acid was therefore synthesized as an additional standard for this study. Again its chromatographic properties differed from that of the metabolite (Table 1), ruling out its being the same. The only remaining position was at carbon-7. Attempts to synthesize 7-hydroxy-5-methoxyindole-3-acetic acid by various procedures encountered formidable obstacles and were abandoned for the present. However, all previous studies of indoles have shown that hydroxylation occurs only on the benzene ring of indoles and not at posititions 2 or on the chain. Position 3 does not have an available hydrogen for substitution. 7-Hydroxylation has been reported in the one instance of skatole.[14, 15] Therefore, it is concluded that the metabolite obtained from 3-(2-acetoxyethyl)-5-methoxyindole is 7-hydroxy-5-methoxyindole-3-acetic acid. Our studies, together with results obtained with skatole, establish that 6-hydroxylation is not a general metabolic transformation of exogenous indoles, but that 7-hydroxylation can be an exclusive option. This fact raises the interesting question why 6-hydroxylation occurs exclusively in some instances but takes place only at carbon-7 in others. A further observation is that metabolism has occurred at two sites in the molecule. A similar complex metabolism has been observed for N, N-dimethyltryptamine where one of the metabolites was 6-hydroxyindole-3-acetic acid.[17] Likewise, α-methyltryptamine yielded 6-hydroxyindole-3-acetone.[18] These complex metabolisms of some indoles may occur because the first-formed metabolites are not sufficiently water soluble to be excreted. When administered intraperitoneally to rats, 3-(2-acetoxy-2-^{14}C-ethyl)-5-methoxyindole did not concentrate in thyroids, adrenals, and ovaries as had 5-methoxytryptophol. However, after 1 hr the pineal had almost 18 times the concentration of radioactivity as the surrounding brain. The differential was still marked at 6 hr, with pineal having about 8 times the concentration of whole brain. In other studies, we found the differential of distribution for 5-methoxytryptophol to be even greater, with a ratio of concentration of radioactivity at 1 hr being about 50:1 from pineal to brain.* This distribution correlates with already defined anatomical and histological differences seen between pineal and brain and indicates that the pineal does not have the same barrier to blood that the brain has.

Rate and nature of metabolism between 5-methoxytryptophol and of its acetate ester, studied here, are very different. However, we found the latter to have very

* Unpublished results.

similar effects on reduction of incidence of estrus on maturing female rats as the unesterified alcohol had at the same dosage.[4]

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