evaporated, and the oily residue was stirred into 900 ml. of 6% HCl. This mixture was slowly heated and then refluxed for 6 hr. It was then cooled to room temperature and stirring was continued overnight. Then the solution was boiled with activated charcoal and the filtrate was extracted three times with ether. The acidic aqueous layer was separated, made alkaline, and extracted with chloroform. The organic layer was dried and evaporated, and the oily residue was distilled *in vacuo* to yield 19 g. of oily **15**.

4-(1-Hydroxypropyl)-4-piperidinopiperidine (24).—To a heated solution (40°) of 6.7 g. (0.03 mole) of 4-propionyl-4-piperidinopiperidine in 100 ml. of 2-propanol was added portionwise 1.3 g. of $NaBH_4$. The whole was stirred for 6 hr. at the same temperature. After cooling in an ice bath, the reaction mixture was decomposed by dropwise addition of 60 ml. of 5 *N* HCl. The solution was filtered and evaporated, the residue was dissolved in 100 ml. of water, and the aqueous solution was made alkaline and extracted with chloroform. The organic layer was dried, filtered, and evaporated. The residue was triturated with diisopropyl ether to yield 4.3 g. of **24**.

1-[γ-(4-Fluorobenzoyl)propyl]-4-propionyl-4-piperidinopiperidine (45).—A mixture of 5.6 g. (0.028 mole) of γ-chloro-4-fluorobutyrophenone,[12] 4.4 g. (0.02 mole) of 4-propionyl-4-piperidinopiperidine, 6.4 g. of Na_2CO_3, and some crystals of KI in 250 ml. of methyl isobutyl ketone was refluxed with stirring for 48 hr. The solution was filtered hot and evaporated. The residue was crystallized from diisopropyl ether to yield 5.1 g. of **45**, m.p. 95–96.5°. This product was converted to its dihydrochloride which, after recrystallization from 2-propanol, melted at 208–210°.

1-(3-Carboxamido-3,3-diphenylpropyl)-4-propionyl-4-piperidinopiperidine (36).—A solution of 6.2 g. (0.012 mole) of **35** in 8 ml. of 90% H_2SO_4 was heated for 3 hr. at 100°. After cooling, the reaction mixture was poured onto 30 g. of ice. The whole was made alkaline with NH_4OH and extracted with chloroform. The organic layer was dried (Na_2SO_4), filtered, and evaporated. The solid residue was crystallized twice from acetone to yield 3.7 g. of **36**, m.p. 156–157°.

1-Methyl-4-propionyl-4-piperidinopiperidine (54).—A mixture of 4.5 g. (0.02 mole) of 4-propionyl-4-piperidinopiperidine, 0.7 g. of paraformaldehyde, 23.5 g. of formic acid, and 250 ml. of 2-propanol was stirred and refluxed for 2 hr. The reaction mixture was concentrated to 20 ml., and to this residue was added 20 ml. of water. This solution was made alkaline with NaOH and extracted with ether. The ethereal solution was dried (K_2CO_3) and filtered, and gaseous HCl was introduced into it. The precipitated hydrochloride was filtered off and recrystallized from ethanol to yield 2.5 g. of **54**, m.p. 260–263°.

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6-Hydroxyindoles and the Metabolism of Melatonin

ROBERT G. TABORSKY, PETER DELVIGS, AND IRVINE H. PAGE

Research Division of the Cleveland Clinic Foundation, Cleveland 6, Ohio

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Different published data on investigations of the metabolism of melatonin are incongruous and one out of three metabolites formed has not been identified. One purpose of this study was to resolve the apparent ambiguities in the literature and to identify the third, unknown metabolite. 3-(2-Acetylaminoethyl)-6-hydroxy-5-methoxyindole (6-hydroxymelatonin) was synthesized and a study of the metabolism of melatonin was repeated. Comparison of chromatographic properties of metabolites confirmed earlier data that the major radioactive peak seen on chromatograms was 6-hydroxymelatonin sulfate. A previously unidentified spot was shown to be free 6-hydroxymelatonin by comparing it with our synthetic compound of unequivocal structure. Because of the past suggestion that 6-hydroxylated metabolites of psychotomimetic tryptamines should be more psychoactive than the nonhydroxylated parent compounds, 6-hydroxy-5-methoxytryptamine was synthesized. It was found less effective in depressing work rates of conditioned rats than 5-methoxytryptamine, thus failing to support the hypothesis in this instance.

Two representatives of indoles hydroxylated in the 6-position were synthesized to examine some of their biological and chemical properties. Such compounds are of interest for several reasons. Szara and Hearst[1] suggested that 6-hydroxylated metabolites of psychotomimetic tryptamines should be more psychoactive than the parent nonhydroxylated compounds.

Hydroxylation is an important means by which mammals detoxify aromatic compounds.[2] Indications are that indoles which cannot be metabolized through other functional groups are hydroxylated and eliminated by the kidney as glucuronides or sulfate esters.[3] Although tryptamines[4] and even chain N-methyltryptamines[5] are metabolized to the corresponding acids, chain N-acetylation[6] and chain N,N-dialkylation[7] prevent or slow down biochemical oxidation to the acids. In these instances an alternative pathway of aromatic hydroxylation can prevail.

The syntheses of compounds prepared in this study are given in Chart I. Reacting 6-benzyloxy-5-methoxyindole (I) with aqueous formaldehyde and dimethylamine produced the substituted gramine (II) in good yield. Since it has been shown previously that the quaternary salts react more efficiently in the following reaction than gramine itself,[8] 6-benzyloxy-5-methoxygramine methosulfate (III) was prepared. Reaction of the quaternary salt with sodium cyanide in aqueous

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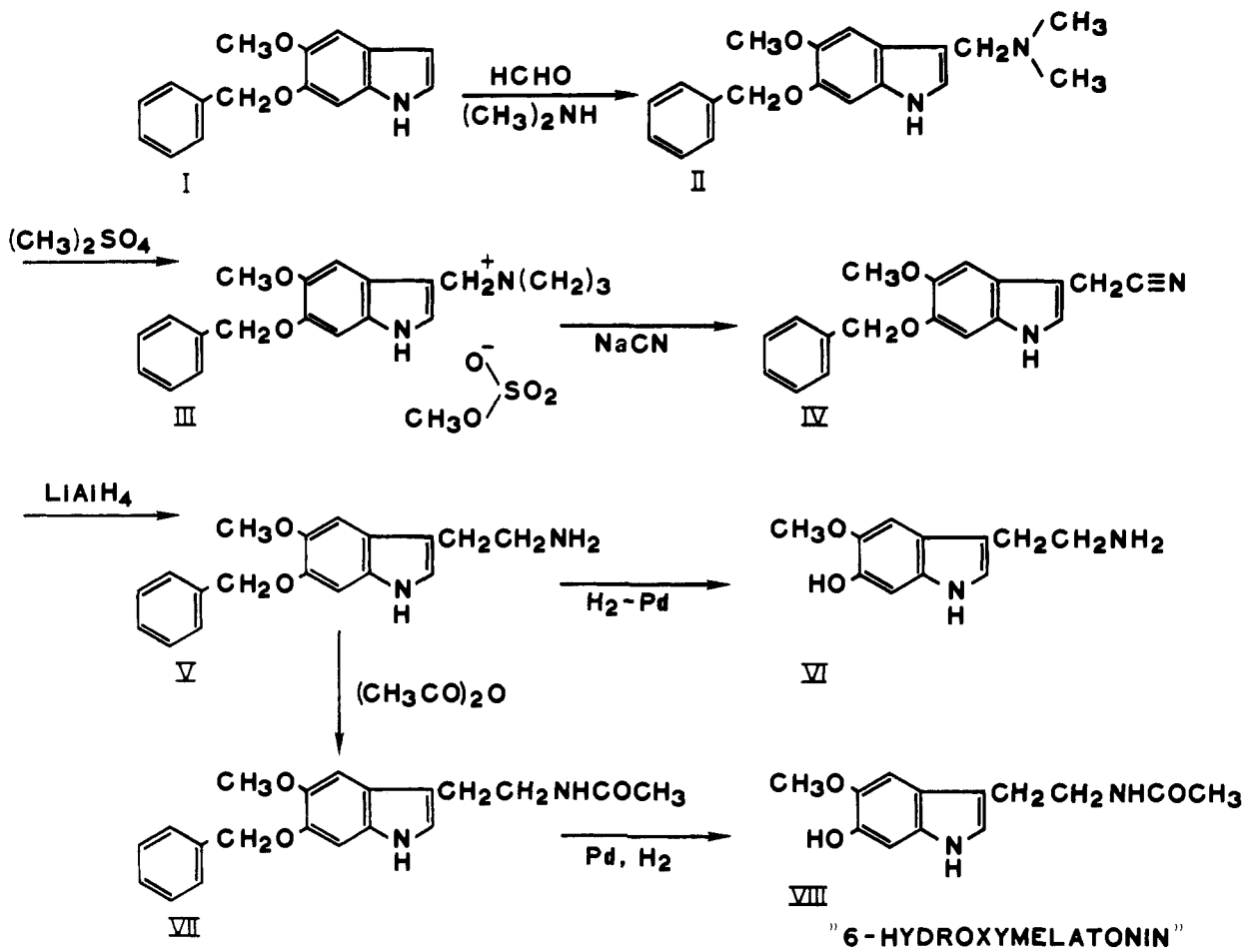
CHART I



solution yielded a solid nitrile (IV) in 90% yield. Reduction with $LiAlH_4$ yielded 6-benzyloxy-5-methoxytryptamine (V) which was isolated as its hydrochloride salt. Part of the amine salt was debenzylated by hydrogen over palladium to give 6-hydroxy-5-methoxytryptamine (V) hydrochloride, a white, stable salt. Another portion was converted to the amine base and acetylated yielding 3-(2-acetylaminoethyl)-6-benzyloxy-5-methoxyindole (VII). Debenzylation of the latter gave 3-(2-acetylaminoethyl)-6-hydroxy-5-methoxyindole (6-hydroxymelatonin). The indolic picrate complex was prepared and crystallized to give material of analytical purity. Crude, free 6-hydroxymelatonin and material liberated from the picrate had identical chromatographic properties.

Several studies of the metabolism of melatonin have been carried out previously. Results describing the chromatographic values of the major metabolite from four published studies are summarized in Table I. First, it can be seen that some chromatographic values for the sulfate ester of 6-hydroxymelatonin in similar solvent systems are very different.[9-11] Interpretations of some of the data are further complicated by the fact that the chromatographic standards, so called authentic 6-hydroxymelatonin, used for identification were products of a liver enzyme reaction or of uncertain chemical hydroxylating systems. Thus, the structural identity of the standard had not been proved in an unequivocal manner.

Therefore, the chromatographic properties of our synthetic 6-hydroxymelatonin of unequivocal structure were compared with those previously reported[9,11] for that compound in connection with studies on the metabolism of melatonin. R_f values of our synthetic material and some from the literature[11] for 6-hydroxymelatonin were markedly different (Table I). For this reason, a metabolic study was made using labeled melatonin-α-C^{14} in 36% aqueous ethanol. Attempts were not made to repeat other aspects of previous metabolism studies involving tissue distribution and quantitation of recovered radioactivity. The melatonin solution was administered by intraperitoneal injection to female Sprague–Dawley rats and 24-hr. urine samples were collected. Considerable amounts of radioactivity could be extracted initially with ethyl acetate from the urine at pH 2 (radiograms I and II, Figure 1). Urine which remained from the extraction contained the major metabolite (R_f 0.52, 4:1:1 1-butanol–glacial acetic acid–water, radiogram II, Figure 1) which had chromatographic values compatible with those previously reported for the sulfate ester of 6-hydroxymelatonin (around R_f 0.52).[6,9,11] The chromatographic properties of the metabolite in the ethyl acetate extract were compatible with our synthetic 6-hydroxymelatonin and the product reported by Kopin, *et al.*,[9] (Table I) obtained after the metabolite had been treated with sulfatase. Our spot at R_f 0.70 from the extract

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TABLE I
CHROMATOGRAPHIC CHARACTERISTICS OF METABOLITES OF MELATONIN REPORTED IN THE LITERATURE

Description[a]	Lit. ref.	R_f value, Solvent[b] A	B	C	D	E	F	Color reaction, Gibbs[c]	Color reaction, Ehrlich[d]
Major metabolite	10		0.22				0.40	—	+
Major metabolite 80% of activity	9			0.54					+
Sulfatase-treated major metabolite (above)	9					0.67			Blue
Major metabolite 80% of activity	6				0.55				
Major metabolite	11	0.55					0.35		Blue
Major metabolite (above) after acid hydrolysis	11	0.37					0.26	—	
Major metabolite without treatment	Present	0.55				0.54		Blue	Blue
Synthetic 6-hydroxymelatonin	Present	0.80				0.70	0.76	Blue	Blue

[a] The "major metabolite" has been described as 6-hydroxymelatonin sulfate in every previous investigation. [b] A, 1-propanol–NH_4OH (8:2); B, 1-propanol–NH_4OH (9:1); C, 2-propanol–5% NH_4OH (8:2); D, 2-propanol–5% NH_4OH (4:2); E, 1-butanol–acetic acid–water (4:1:1); F, 1-butanol–acetic acid–water (4:1:5). [c] Ref. 13. [d] Ref. 12.

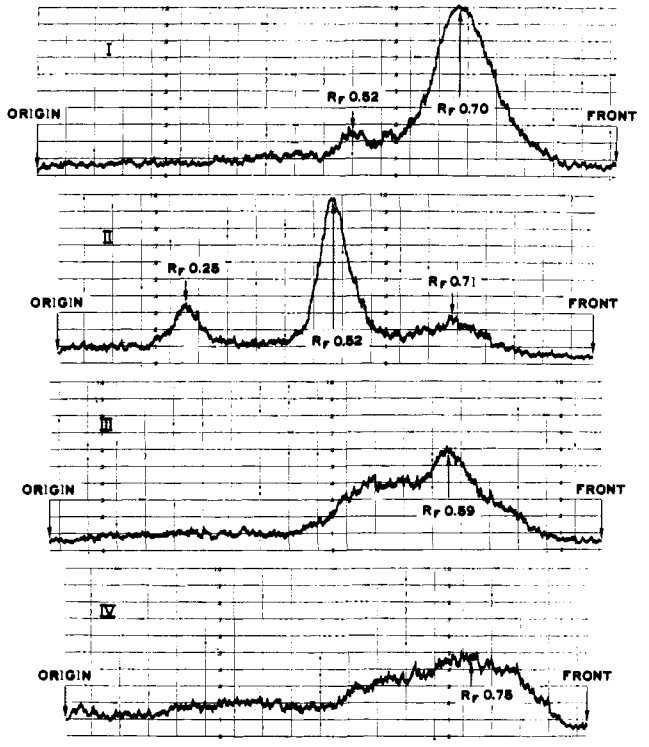

Figure 1.—Radiogram I (top) is of the ethyl acetate extract of a 24-hr. urine sample. Radiogram II is of the urine remaining after the ethyl acetate extraction. Radiogram III is the ethyl acetate extract of a portion of the above urine (II) after incubation with sulfatase. Radiogram IV is the ethyl acetate extraction of a portion of the urine mentioned above (II) on treatment with HCl.

produced a blue Ehrlich[12] color positive for indoles and gave a positive Gibbs[13] reaction for the phenolic group.

Urine remaining from the ethyl acetate extraction was treated in two ways. In one experiment a sample was incubated with sulfatase for 18 hr. at pH 5.5. In another it was adjusted to pH 2 with HCl and incubated at 90° for 18 hr. Ascorbic acid was added as antioxidant to each. Both treatments resulted in complete conversion of the compound found at R_f 0.52 (4:1:1 1-butanol–acetic acid–water) into an ethyl acetate extractable material. This material was identical chromatographically (R_f 0.69, 0.73) with our synthetic 6-hydroxymelatonin (R_f 0.70, Table I). The acid hydrolysis product showed evidence of having undergone more degradation than the product from the enzymatic reaction, indicated by its broader radioactive peak on chromatography (radiogram IV, Figure 1).

The above results confirm the data of Kopin, *et al.*,[9] and the interpretation that conjugates of 6-hydroxymelatonin are two of the metabolites of melatonin. These investigators found 70–80% of the excreted radioactivity associated with the sulfate conjugate of 6-hydroxymelatonin, 5% as glucuronide, and about 12% as the unknown third peak.

From our investigations the third unknown material obtained initially and seen at R_f 0.70 in 4:1:1 1-butanol–glacial acetic acid–water and 0.80 in 8:2 1-propanol–ammonium hydroxide was proven to be free 6-hydroxymelatonin. This product is much more likely to be formed according to current biochemical theory than 6-methoxyharmalan which has been postulated as a third metabolite of melatonin.[11]

6-Hydroxy-5-methoxytryptamine was also synthesized in this study. In other investigations,[14] we found that 5-methoxytryptamine exerted a moderate depressant effect on the behavior of conditioned rats. Therefore, the latter compound was compared with the 6-hydroxylated analog.

Rats were conditioned on a variable-interval positive-reinforcement schedule by the free operant technique of Skinner.[15] This schedule presented the opportunity for reward (food pellets) to the animal at the end of various arbitrary lengths of time for 1 hr. If they pressed a bar at these times, the animals were rewarded. Since the rats could not learn the sequences of presented opportunities, they obtained a maximum number of rewards only by continuing to press the bar at a steady medium rate.

5-Methoxytryptamine hydrochloride at 3.3 mg./kg. i.p. consistently caused a slowing of work rates to below 25% of the control values of the same animals. 6-Hydroxy-5-methoxytryptamine hydrochloride at 8.0 mg./kg. i.p. did not produce any significant changes. Therefore, in these preliminary behavioral studies, the 6-hydroxylated analog was less potent than the parent compound. This subject is currently being investigated more extensively.

(12) *p*-Dimethylaminobenzaldehyde, 10% in concentrated HCl with 4 vol. of acetone.

(13) 2,6-Dichloroquinonechloroimide (0.2%) in absolute ethanol followed by saturated aqueous $NaHCO_3$.

(14) R. G. Taborsky, P. Delvigs, I. H. Page, and N. Crawford, *J. Med. Chem.*, **8**, 460 (1965).

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Experimental Section

Reagents.—6-Benzyloxy-5-methoxyindole was purchased from the Regis Chemical Co., Chicago, Ill.

6-Benzyloxy-5-methoxygramine.—A solution of 8.1 g. (32 mmoles) of 6-benzyloxy-5-methoxyindole in 70 ml. of dioxane was added dropwise to a stirred mixture of 40 ml. of dioxane, 40 ml. of glacial acetic acid, 3.0 ml. of 36% aqueous formaldehyde, and 7.0 ml. of 25% aqueous dimethylamine and cooled at 5° with ice. After the addition was complete, the reaction solution was kept at 5° for 2 hr. and allowed to warm and stand for 18 hr. at room temperature in darkness. The mixture was diluted with 400 ml. of water, charcoaled, filtered, and made alkaline with 20% NaOH solution. On standing overnight, 8.5 g. (79% yield) of needle-like crystalline product, m.p. 131–134°, was obtained on filtration and drying. Crystallization from toluene and hexane yielded an analytical sample, m.p. 135–136°.

Anal. Calcd. for $C_{19}H_{22}N_2O_2$: C, 73.53; H, 7.10; N, 9.13. Found: C, 73.66; H, 7.08; N, 9.45.

6-Benzyloxy-5-methoxygramine Methosulfate.—A solution of 8.5 g. (28 mmoles) of crude 6-benzyloxy-5-methoxygramine in 50 ml. of dry tetrahydrofuran containing 0.5 ml. of glacial acetic acid was added dropwise over 0.5 hr. to a stirred, ice-cooled solution of 30 g. (0.24 mole) of dimethyl sulfate in 20 ml. of dry tetrahydrofuran. The solution was stirred at 5° for 1 hr. and allowed to stand at room temperature in darkness for 18 hr. The precipitate that formed was collected by vacuum filtration and washed with anhydrous ether to give 9.3 g. (83%) of crystalline salt, m.p. 136–138°. A portion was crystallized from toluene to give an analytical sample, m.p. 145–146°.

Anal. Calcd. for $C_{21}H_{28}N_2O_6S$: C, 57.75; H, 6.43; N, 6.45. Found: C, 57.36; H, 6.31; N, 6.27.

5-Benzyloxy-6-methoxyindole-3-acetonitrile.—Four grams (82 mmoles) of powdered NaCN was added to a stirred solution of 8.5 g. (20 mmoles) of 6-benzyloxy-5-methoxygramine methosulfate in 120 ml. of water. The mixture was heated at 67° during which time cream-colored solid appeared. At the end of 3 hr., the mixture was cooled at 5° for 1 hr., filtered, and oven dried (80°) to obtain 5.1 g. (90% yield) of cream-colored product, m.p. 148–150°. Crystallization from ethanol–water yielded an analytical sample, m.p. 163–164°.

Anal. Calcd. for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.58; N, 9.58. Found: C, 73.67; H, 5.58; N, 9.11.

5-Benzyloxy-6-methoxytryptamine Hydrochloride.—A suspension of 3.8 g. (13 mmoles) of 6-benzyloxy-5-methoxyindole-3-acetonitrile in 200 ml. of anhydrous ether was added with stirring to a mixture of 2.5 g. of $LiAlH_4$ in 50 ml. of anhydrous ether at such a rate as to cause gentle refluxing. After the addition was complete, the mixture was stirred and refluxed for 18 hr. Excess hydride was decomposed under a nitrogen atmosphere with water and 200 ml. of 10% NaOH was added to destroy the resultant lithium amine compound. The layers were separated and the aqueous portion was extracted twice more with ether. The combined ether portions were dried (Na_2SO_4) and filtered. Anhydrous HCl gas was passed into the ether solution with stirring to obtain the hydrochloride. It was recovered by filtration and crystallized from chloroform to give 2.0 g. (57% yield) of white salt, m.p. 183–184°.

Anal. Calcd. for $C_{18}H_{21}ClN_2O_2$: C, 64.92; H, 6.15; N, 8.40. Found: C, 65.02; H, 6.43; N, 8.58.

6-Hydroxy-5-methoxytryptamine Hydrochloride.—A solution of 500 mg. (1.5 mmoles) of 6-benzyloxy-5-methoxytryptamine hydrochloride in 25.0 ml. of methanol with 80 mg. of 10% Pd–C was hydrogenated in a Parr low-pressure hydrogenator at 2.8 kg./cm.^{2} for 4 hr. at room temperature. The catalyst was filtered off and the volume of the filtrate was reduced to 5 ml. under vacuum. The addition of excess ether to the alcohol solution resulted in precipitation of the product which on filtering and drying amounted to 320 mg. (88% yield) of white powder, m.p. 277–278°. Crystallization from 1-butanol to give an analytical sample did not change the melting point.

Anal. Calcd. for $C_{11}H_{15}ClN_2O_2$: C, 54.48; H, 6.30; Cl, 14.55; N, 11.54. Found: C, 54.24; H, 6.42; Cl, 14.31; N, 11.36.

3-(2-Acetylaminoethyl)-6-benzyloxy-5-methoxyindole.—One gram (3 mmoles) of 6-benzyloxy-5-methoxytryptamine hydrochloride was dissolved in 25 ml. of water, and the solution was charcoaled and filtered. Addition of 10% Na_2CO_3 solution to the filtrate to achieve pH 9 yielded 835 mg. (2.8 mmoles) of the base, m.p. 55–60°, on filtering and drying. A portion of the dried amine (635 mg., 2.1 mmoles) was rapidly stirred into 2.0 ml. of acetic anhydride in a test tube to obtain momentary solution followed by total solidification of the contents. The mixture was allowed to stand 15 min. and 10 ml. of ethyl acetate followed by 35 ml. of heptane was added. The insoluble precipitate was the desired product amounting to 572 mg. (81% yield), m.p. 148–150°, on filtering and drying. Crystallization from methanol–water yielded an analytical sample, m.p. 151–152°.

Anal. Calcd. for $C_{20}H_{22}N_2O_3$: C, 70.97; H, 6.55; N, 8.30. Found: C, 71.22; H, 6.55; N, 8.30.

3-(2-Acetylaminoethyl)-6-hydroxy-5-methoxyindole (6-Hydroxymelatonin) Picrate.—3-(2-Acetylaminoethyl)-6-benzyloxy-5-methoxyindole (200 mg., 0.59 mmole) was debenzylated in methanol over 80 mg. of 10% Pd-C at 2.8 kg./cm.^{2}. The catalyst was filtered off and the filtrate evaporated to dryness under vacuum. The residue was redissolved in 3.0 ml. of chloroform, and picric acid (136 mg., 0.59 mmole) in 3.0 ml. of chloroform was added to give an instant red coloration. The solution was then reduced in volume and cooled to give 410 mg. (86% yield) of red picrate. Crystallization from chloroform yielded an analytical sample, m.p. 145–146°.

Anal. Calcd. for $C_{19}H_{18}N_5O_{10}$: C, 47.81; H, 4.01; N, 14.88. Found: C, 47.88; H, 4.27; N, 14.88.

The picrate is a useful derivative of 6-hydroxymelatonin since it protects the latter against decomposition and it completely dissociates on chromatography so that 6-hydroxymelatonin runs as the free phenol far from picric acid and its chromatographic properties can be observed. Its values were the same as those obtained for a crude product melting at 154–164°, isolated from one experiment.

Melatonin Metabolism Study.—Radioactive melatonin with a specific activity of approximately 250 μc./g. was synthesized according to the method described in the literature.[13] Descending paper chromatography was used with 4:1:1 1-butanol–glacial acetic acid–water as solvent. Radiograms were obtained by scanning paper chromatogram strips in an Atomic Accessories, Inc., Seaborgram, RSC-5.

Three milligrams of radioactive melatonin was injected into female, Sprague–Dawley rats in metabolite cages and 24-hr. urines samples were collected. Volumes averaged 8.0 ml. of urine for 24 hr. Radiograms in Figure 1 are referred to below.

In the first experiment the urine was extracted with three 5-ml. portions of ethyl acetate. The combined ethyl acetate portions were reduced to 1 ml. in a rotary evaporator below 50°. Chromatography of this concentrate yielded radiogram I. The aqueous portion from the extraction on running in the same system gave radiogram II.

The aqueous portion (8.0 ml.) was incubated with 250 mg. of limpet viscera sulfatase (Sigma Chemical Co.) at 37° for 18 hr. That solution was again extracted as above with ethyl acetate. A chromatogram of the solvent concentrate gave radiogram III.

Another portion of urine was extracted with ethyl acetate to remove all of the R_f 0.70 material. It was then adjusted to pH 2 with HCl and incubated at 90° with 1% ascorbic acid for 18 hr. Chromatography of the ethyl acetate extracts yielded radiogram IV.

Behavior Studies.—The effect of compounds on behavior was determined in the following manner. Rats were conditioned on a variable-interval 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 efforts 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 each quarter of the test period was determined on five consecutive days. On the experimental day, the compound was administered intraperitoneally and the increased or decreased response rates were computed as a percentage of normal for that animal.

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