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THE METABOLISM OF HYDROXYSKATOLES IN RATS

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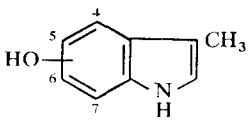
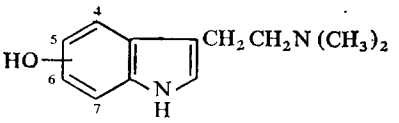
Abstract—Rats were given i.p. injections of 4-,5-,6- and 7-hydroxyskatoles (I) in propylene glycol. The average recovery of injected 4-hydroxyskatole as the free compound and its conjugates in rat urine was 3·2 per cent. Recoveries of the other isomers were considerably higher, the average value being 17·3 per cent. The relatively low recovery of the 4-hydroxyskatole suggests that this compound is not inactivated by conjugation as rapidly as its isomers. Preliminary observations indicate that 4-hydroxyskatole may cause behavioral changes in rats. The significance of these results in relation to the psychotropic activity of other substituted indoles is discussed briefly.

THE HYDROXYINDOLES are of clinical importance because of their physiological activity.[1] Interest has so far been mainly centered on the 5- and 6-hydroxylated compounds,[2–5] but the activity of the 4-substituted indoles, notably psilocin (4-hydroxy-*N*,*N*-dimethyltryptamine) (II) and its 4-*O*-phosphate ester, psilocybin, is now being recognized and investigated.[6, 7] The metabolism of the isomeric methoxyindoles[8] and the hydroxy-*N*,*N*-dimethyltryptamines[9] (II) have been compared.



I



II

High levels of excretion of 6-hydroxyskatole *O*-sulfate ester by schizophrenics have been reported.[10–12] Methods for the synthesis[13] and assay in urine of the four isomeric *O*-sulfate esters of the hydroxyskatoles have been described.[14, 15] Some physiological effects and excretion patterns of these compounds in rats are now reported.

EXPERIMENTAL

Materials. The 4-, 5-, 6- and 7-hydroxyskatoles were synthesized by the methods described in the literature.[13] Silica gel G was obtained from E. Merck A.G., Darmstadt, Germany. Glusulase‡ was obtained from Endo Laboratories, Richmond Hill, N.Y.

Animals. Female Wistar rats weighing approximately 200 g were given i.p. injections of the hydroxyskatoles (450 μg or 1000 μg) in propylene glycol (0·5 ml). Control rats were given similar injections of the propylene glycol only. The urines were collected for 24 hr.

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‡ Glusulase is an enzyme preparation containing both sulfatase and glucuronidase activity.

Assay of metabolites. The urines were adjusted to pH 2·5 with conc. sulfuric acid and extracted by water-saturated 1-butanol (5 × 10 ml); the 1-butanol was evaporated and the residue was dissolved in water (4 ml). This aqueous solution was extracted by ethyl acetate (3 × 3 ml) to remove any hydroxyskatole present and the mother liquors were then used for the assay of the conjugated hydroxyskatoles by methods described previously.[14, 15]

The ethyl acetate extract was evaporated to dryness, redissolved in methanol (0·5 ml) and an aliquot (100 μl) of this solution was applied to a silica gel thin-layer plate. The chromatogram was developed in two directions by using isopropyl ether followed by 1,2-dichloroethane-di-*iso*propylamine (6:1) (cf. ref. 16) as the developing solvents. Any hydroxyskatole present was visualized by spraying the chromatograms with *p*-*N*,*N*-bis(2-chloroethyl)aminobenzaldehyde (1%) in methanol–HCl (3:1). The appropriate zones were eluted by methanol (1·3 ml) from the chromatograms and a further quantity (0·2 ml) of the color reagent was then added. After 1 hr the intensity of the color was measured from the absorbance of this solution at 620 mμ. The concentrations of the hydroxyskatoles were estimated by comparing this absorbance with that due to an internal standard assayed at the same time. No hydroxyskatoles, other than the injected compounds, were detected on the chromatograms.

Detection of two conjugated forms of 4-hydroxyskatole. Urines from rats which had been treated with 4-hydroxyskatole were extracted with butanol as described above. The butanol was concentrated to a final volume of 1 ml; a portion (0·1 ml) of this was retained and the remainder (0·9 ml) was treated according to the procedure for the hydrolysis of the *O*-sulfate esters.[15] The aqueous solution obtained after extraction of the hydroxyskatoles by ethyl acetate was then compared chromatographically with the initial 1-butanol extract.

Chromatography was carried out in a silica gel thin-layer system with 1-butanol–ethanol–cyclohexylamine (76:3:6) as the developing solvent[16] or on Whatman No. 1 paper with isopropanol–ammonia–water (10:1:1).[17]

The ethyl acetate extract was examined for the presence of hydroxyskatoles by two-dimensional thin-layer chromatography on silica gel G with isopropyl ether followed by 1,2-dichloroethane–isopropylamine (6:1) as the developing solvents.

RESULTS AND DISCUSSION

The concentrations of the free and conjugated forms of the injected hydroxyskatoles found in the urines of rats treated with one of the four isomeric hydroxyskatoles are shown in Table 1. The levels of the other conjugated hydroxyskatoles normally present in the urines of the experimental rats and the controls are given in Table 2. The assay procedure employed[15] utilizes the resistance of the *O*-sulfate ester of 4-hydroxyskatole to hydrolysis by Glusulase[18] to obtain a separation of this isomer from the 5-, 6- and 7-*O*-sulfate esters. When the extracts of the urines from rats injected with 4-hydroxyskatole were treated with Glusulase, 4-hydroxyskatole was formed. This suggested that, in the rat, conjugation of these hydroxyskatoles is achieved to a significant degree as the glucuronides as well as the sulfates. Similar observations have been made previously with rats treated with 6-hydroxyskatole,[17] melatonin[4] and the methoxyindoles.[8] The presence of the glucuronide conjugate of 4-hydroxyskatole in the urine of rats treated with this substance was confirmed in the following way. The TLC's of 1-butanol extracts before hydrolysis showed two

intense spots (R_f values, 0·60 and 0·15) when sprayed with Ehrlich's reagent in addition to free 4-hydroxyskatole (which ran with the solvent front). One of these (R_f 0·60) has chromatographic properties similar to 4-hydroxyskatole *O*-sulfate ester. The other spot (R_f 0·15) was not present on chromatograms of samples of extracts which had been treated with the Glusulase preparation. This substance is probably the glucuronide conjugate of 4-hydroxyskatole. This is supported by the detection of 4-hydroxyskatole in ethyl acetate extracts of the aqueous solutions obtained after enzymic hydrolysis. Additional evidence for the presence of the glucuronide conjugate of 4-hydroxyskatole in the urine was obtained from the paper chromatographic examination of the urine extracts. The paper chromatograms of the initial butanol extract showed an Ehrlich positive spot (R_f 0·17) which was not present on chromatograms of samples of the same extract which had been treated with Glusulase. Glucuronic acid (R_f 0·42) was also detected on the paper chromatograms of the hydrolyzed extract. Again 4-hydroxyskatole was extracted from the hydrolysate and identified by thin-layer chromatography. The extent to which conjugation of 4-hydroxyskatole as the glucuronide occurred ranged from 14–68 per cent with the average of the four determinations being 32 per cent. In general, 90–100 per cent of all the recovered hydroxyskatoles was in the conjugated form (see Table 1).

TABLE 1. EXCRETION OF INJECTED HYDROXYSKATOLES BY RATS

No.	Isomer	Amount (μg)	Recovered (μg)			
			Free	Conjugated*		
				Total	Glucuronide	Sulfate
1	4	450	2·6	8·9	6·0	2·9
2	4	1000	1·2	22·4	3·1	19·3
3	4	1000	4·5	21·6	4·3	17·3
4	4	1000	2·8	47	11·5	35·5
5	5	450	19·0	121	—	—
6	5	1000	4·3	75·5	—	—
7	6	450	0	88·4	—	—
8	7	450	1·3	45·0	—	—

* These values include the amount normally present. These are negligible when compared to the total amount of the injected hydroxyskatole excreted.

The metabolic pictures for the hydroxyskatoles differ in only one possibly significant respect. The range of recoveries of the injected 4-hydroxyskatole was 2·3–5·0 per cent, with an average of 3·2 per cent, based on four experiments. Sohler[17] has shown that 6-hydroxyskatole is eliminated rapidly in the rat, mainly as the sulfate and glucuronide, with maximal excretion during the first 4 hr. The higher recoveries (relative to the 4-isomer) of 5-, 6- and 7-hydroxyskatole from rats treated with these compounds suggests that these latter compounds are rapidly conjugated and eliminated. On the other hand, the low recovery of 4-hydroxyskatole suggests that this compound is not inactivated by conjugation as rapidly as its isomers. This unreactivity of 4-hydroxyskatole toward conjugation is in accord with the resistance of the 4-*O*-sulfate ester toward enzymic hydrolysis.[18] Both of these properties probably result from steric shielding of the 4-position by the partially folded benzene and pyrrole rings of the

indole nucleus. However, the glucuronide derivative of 4-hydroxyskatole appears to be readily hydrolyzed by the Glusulase. The reason for this difference in the properties of the sulfate and glucuronide esters of 4-hydroxyskatole is not clear.

The 5-, 6- and 7-hydroxyskatoles did not affect the behavior of rats when compared with those treated with propylene glycol only. However, within 10 min of a 4-hydroxyskatole injection, each of the 4 rats treated with this compound had lost most of its sense of balance and tended to roll over when it moved. These preliminary observations on the behavioral effects are being extended and will be reported fully subsequently.

It has been suggested[19, 20] that the psychotropic activity of the tryptamines results from 6-hydroxylation of the indole nucleus. However, there is also evidence that this activity may result from metabolites other than the 6-hydroxylated forms.[21] Psychotomimetic activity may be associated with 4-substitution in the indole nucleus. Several such compounds, e.g. psilocin (II), psilocybin, mitragynine and lysergic acid diethylamide (LSD), are highly active in this respect. Psilocybin is probably rapidly hydrolyzed in the body to psilocin and 10–20 per cent is retained in the body for up to 7 days.[6] This indicates that it may be difficult to conjugate the free 4-hydroxyindole, i.e. psilocin. LSD is also a 4-substituted indole, although not of the hydroxylated form. This compound may also owe its activity to the inability of the body to cope with a 4-substituted indole and convert it to the inactive and excretable conjugated form.

TABLE 2. LEVELS OF CONJUGATED HYDROXYSKATOLES NORMALLY PRESENT IN RAT URINE

	Conjugated hydroxyskatole† present (μg)			
No.*	4	5	6	7
1	—	< 0·65‡	< 0·65	0
2	—	11·0	2·8	2·2
3	—	3·8	< 1·0	< 1·0
4	—	0	0	0
5	0	—	< 2·2	0
6	0	—	< 0·6	< 0·6
7	0	< 0·6	—	0
8	0	< 0·4	< 0·4	—
9	0	< 0·2	< 0·2	0
10	0	< 1·3	< 1·3	0
11	0	< 1·2	< 1·2	0

* Experiments 1–8 are the same as those in Table 1. Numbers 9–11 are the control experiments.

† Levels of conjugates of the injected hydroxyskatole which were normally present in the urine could not be obtained separately and are included in the values given in Table 1.

‡ Nonspecific values indicate that the hydroxyskatole was detected, but the amount was too small to be assayed, i.e. < 0·1 μg/ml.

Caution is necessary in extrapolating these results with rats to estimate the effects of these compounds on humans. There are two notable differences in the metabolism of skatole in humans and rats. In urines from humans the levels of hydroxyskatoles present are in the order 6->5->7-. 4-Hydroxyskatole has not been detected in urines from humans.[15] In the rat, the order is 5->6->7- (see Table 2). Furthermore,

rats excrete the hydroxyskatoles as glucuronides and sulfates while humans appear to conjugate these compounds almost entirely as the *O*-sulfate esters. A major difference in the ease of hydrolysis of these two esters forms has been noted above.

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