(0.4–0.5 mm.), yield 15 g. (13%) $\nu_{max}^{CHCl_3}$ 1650 cm.$^{-1}$ (amide C=O) no amide II band.

Anal. Calcd. for $C_{19}H_{22}N_2O$: N(basic), 4.76. Found: N(basic), 4.65 (titration).

The base was dissolved in ether and treated with 10 g. (0.11 mole) of oxalic acid in ether to form a pale yellow solid. This was recrystallized from methanol–ether to give a colorless solid of m.p. 147–149° dec.

Anal. Calcd. for $C_{19}H_{22}N_2O \cdot C_2H_2O_4$: C, 65.6; H, 6.25; N, 7.29. Found: C, 65.5; 64.8; H, 6.22, 6.10; N, 7.23.

1-Methyl-3-oxo-1-phenethyl-4-phenylpiperazinium chloride.—To an ice-cold solution of N^1-methyl-N^1-phenethyl-N^2-phenyl-1,2-ethanediamine (83.4 g., 0.328 mole) in 150 ml. of benzene and 100 ml. of 20% sodium hydroxide solution was added a solution of chloroacetyl chloride (37.1 g., 0.33 mole) in 100 ml. of benzene during 45 min. The milky solution was stirred for an additional 30 min. and the benzene layer was separated. A small amount of benzene insoluble oil was extracted with chloroform. The combined extracts were dried quickly over anhydrous magnesium sulfate and heated in an open flask on a steam bath for 5 hr. The clear solution soon became cloudy and a brown oil began to precipitate which became a colorless solid after 2 hr. heating with occasional scratching. The solid was collected, washed with ethyl acetate–ether, and dried in the air; yield 91.3 g. (78.2%), m.p. 210–211° dec. A small sample was recrystallized from a methanol–ethyl acetate–ether mixture to give a colorless solid of m.p. 212–213° dec., ν_{max}^{KCl} 1660 cm.$^{-1}$ (amide C=O).

Anal. Calcd. for $C_{19}H_{23}ClN_2O$: C, 68.9; H, 6.95; N, 8.46. Found: C, 69.0; H, 6.60; N, 8.20.

The filtrate was heated again for 5 hr. on a steam bath to give another 2.5 g. of the product of m.p. 209–210° dec.; thus the total yield was 93.8 g. (87.0%).

4-Phenethyl-1-phenyl-2-piperazinone.—The piperazinium chloride (92.0 g., 0.278 mole) was distilled pyrolytically to give a fraction boiling at 203–211° (0.7–1.3 mm.), yield 63.5 g. (81.5%). It solidified in the receiver and was recrystallized from acetone–Skelly B to give a light tan crystalline solid of m.p. 84–85°, yield 51.7 g. The filtrate was concentrated to give a milky solution which rendered an additional 8.6 g. of product on cooling. Thus, the total yield was 60.3 g. A sample of the first crop was recrystallized from acetone–Skelly B to give pale tan plates of m.p. 83–84°. $\nu_{max}^{CHCl_3}$ 1655 cm.$^{-1}$ (amide C=O), no N–H or amide II band.

Anal. Calcd. for $C_{18}H_{20}N_2O$: C, 77.2; H, 7.15; N, 10.0. Found: C, 76.8; H, 7.15; N, 10.1.

4-Phenethyl-1-phenylpiperazine.—A solution of the above piperazinone (58.7 g., 0.21 mole) in 150 ml. of THF was added dropwise to a slurry of lithium aluminum hydride (11.4 g., 0.3 mole) in 150 ml. of THF during 60 min. to give a gray suspension which was refluxed with stirring for 6 hr. and set aside overnight. The reaction mixture was treated with 12 ml. of water, 12 ml. of 20% sodium hydroxide solution, and 35 ml. of water, respectively. The inorganic salt was filtered and the light tan solution was evaporated to dryness *in vacuo* to give an amber liquid which solidified on cooling. This was distilled to give a pale yellow liquid of b.p. 162–165° (3.0–1.75 mm.), yield 48.9 g. It solidfied at once in the receiver and was recrystallized from aqueous methanol to furnish a light yellow solid of m.p. 75–77°, yield 40.3 g. It was again recrystallized to yield 32.5 g. of a colorless solid of m.p. 76–77°.

Anal. Calcd. for $C_{18}H_{22}N_2$: C, 81.3; H, 8.27; N, 10.5. Found: C, 81.6; H, 8.27; N, 10.5.

The mixture m.p. with an authentic sample (m.p. 78–79°) prepared from phenethyl bromide and 1-phenylpiperazine was not depressed (m.p. 76–79°). The infrared spectra of the two samples were identical.

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The Metabolism of Carbazole in Rats and Rabbits[1]

S. R. JOHNS AND S. E. WRIGHT

Department of Pharmacy, University of Sydney, Sydney, Australia

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3-Hydroxycarbazole, conjugated with glucuronic acid, has been shown to be the major urinary metabolite in rats and rabbits after administration of carbazole. Hydroxylation at position 3 is in accordance with an attack by oxidizing enzymes at the position of highest electron density in the molecule.

Previous studies[2] in this laboratory on the metabolism of ergometrine and lysergic acid diethylamide showed that these complex indole derivatives undergo metabolism in the rat by hydroxylation in the aromatic ring of the indole skeleton, followed by conjugation of this phenol with glucuronic acid. The major metabolite from ergometrine, after hydrolysis with dilute hydrochloric acid or β-glucuronidase, could not be separated from synthetic 12-hydroxyergometrine on a number of chromatographic systems, and it has been tentatively suggested that hydroxylation occurs at position 12.

The need for developing unambiguous methods for the synthesis of all the possible metabolites of such complicated structures, *viz.* all the possible hydroxylated ergometrine derivatives, would be reduced if the mechanism and, hence, the likely position of hydroxylation were known. This paper deals with an investigation to ascertain the position of hydroxylation in the indole nucleus by a study of more simple indoles.

Indole itself is metabolized mainly to indoxyl (3-hydroxyindole)[3] which is excreted conjugated with both glucuronic and sulfuric acids. The reactivity of position 3 in the indole nucleus to oxidation prevents extensive hydroxylation in the aromatic ring and is of little use in determining the position of hydroxylation in that ring. The increase in conjugates, however, after the administration of indole to rats[4] cannot be explained alone by indoxyl formation and 5-hydroxyindole has been suggested as a minor metabolite.

A more suitable compound for comparison with the indole alkaloids is skatole, which has the 3-position blocked to oxidation. Skatole has been extensively

(1) This work was supported by a fellowship (S. R. Johns) from Burroughs Wellcome & Co. (Aust.) Ltd. For preliminary communication see S. R. Johns and S. E. Wright, *Experientia*, **18**, 416 (1962).

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(3) E. Baumann, *Hoppe-Seyler Z.*, **1**, 60 (1877); B. Master, *ibid.*, **12**, 130 (1888).

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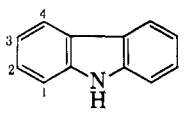
studied[5-7] and the ethereal sulfates of 5-, 6-, and 7-hydroxyskatole have all been identified in the urine of rats. The proportion of each hydroxyskatole has not been reported and a correlation between electron density about the carbon atoms and the position of hydroxylation cannot be made.

If it could be shown that some correlation exists between electron density and position of hydroxylation, then in the more complex molecules such as the indole alkaloids a knowledge of the electron density about each carbon atom would reduce the number of hydroxylated derivatives required to be synthesized in order to identify the structure of the metabolites. An accurate study of the activity of the carbazole nucleus has been carried out by Brown and Coller.[8] These workers have shown that the order of reactivity to attack by electron-deficient species in carbazole is 3 > 1 > 2 > 4. For this reason the metabolism of carbazole (I) has been studied in both rats and rabbits. The major urinary metabolite is the glucuronide of a hydroxycarbazole which has been isolated and purified as the tri-O-acetyl-methyl ester. The structure of the hydroxycarbazole, obtained after hydrolysis of the glucuronide with dilute hydrochloric acid or a β-glucuronidase preparation, has been established by both chromatographic comparison and isotopic recrystallization with the synthetic compound as 3-hydroxycarbazole, the hydroxylation occurring at the position of highest electron density in the carbazole molecule.



I

Experimental

Carbazole-C^{14}.—Aniline-C^{14} sulfate (3.1 mg., 100 μc., Radiochemical Centre, Amersham) was diluted with aniline (186 mg.) in 0.5 *N* hydrochloric acid (1 ml.) and diazotized with sodium nitrite (280 mg.) in water (0.5 ml.). The diazonium salt solution was added to a solution of sodium bisulfite [sodium hydroxide (0.2 g.) and sodium bisulfite (0.5 g.) in water (0.2 ml.)] and the resulting solution warmed on a water bath at 60° for 0.5 hr. Concentrated hydrochloric acid (0.2 ml.) was added and the mixture heated for a further 2 hr. The addition of further hydrochloric acid (2 ml.) affected the precipitation of **phenylhydrazine-C^{14} hydrochloride** (240 mg., m.p. 240–245°).

Phenylhydrazine-C^{14} hydrochloride (240 mg.) and sodium acetate (240 mg.) were dissolved in 1:1 aqueous acetic acid (10 ml.) and added to a solution of cyclohexanone (210 mg.) in water (5 ml.) and warmed on the water bath for 1 hr. After cooling in ice, **tetrahydrocarbazole-C^{14}**, colorless plates from ethanol, m.p. 117–119° (154 mg.) was obtained.

Tetrahydrocarbazole-C^{14} (154 mg.) was dissolved in boiling mesitylene (10 ml.) with palladium–charcoal (5%, 200 mg.) and refluxed for 2 hr. The palladium–charcoal was removed by filtration, the filtrate concentrated to 2 ml., then diluted with petroleum ether which afforded carbazole-C^{14} as colorless plates from benzene, m.p. 242–244° (60 mg.) with an activity of 6.0 × 10^5 d.p.m./mg.

Reference Compounds. 1-Hydroxycarbazole.—Cyclohexane-1,2-dione monophenylhydrazone (m.p. 183–185°) was prepared by the method of Coffey[9] from 2-hydroxymethylenecyclohexanone and benzenediazonium chloride. Cyclization of this monophenylhydrazone with ethanolic acetic acid gave 1,2,3,4-tetrahydro-1-oxocarbazole (m.p. 169°), which was dehydrogenated with palladium–charcoal in refluxing mesitylene[10] to 1-hydroxycarbazole (m.p. 160–162°).

2-Hydroxycarbazole.—The commercial product (K. and K. Laboratories, Inc.), m.p. 276° was used.

3-Hydroxycarbazole.—*p*-Anisidine (20 g.) was dissolved in 5 *N* hydrochloric acid (200 ml.) and diazotized with sodium nitrite (12 g.) in water (70 ml.). An ice-cold solution of stannous chloride[11] (100 g.) in hydrochloric acid (100 ml.) was added to the cold diazonium salt and the mixture allowed to stand for 1 hr. The resulting *p*-methoxyphenylhydrazine hydrochloride was filtered and recrystallized from ethanol as white plates, m.p. 198–200° (8 g.), γ_{max} 3578 (NH) and 1260 cm.$^{-1}$ (OCH_3).

p-Methoxyphenylhydrazine hydrochloride (1.75 g.), sodium acetate (1.4 g.), and cyclohexanone (1.0 g.) were dissolved in aqueous acetic acid (50%, 20 ml.) and warmed on a water bath for 1 hr. 6-Methoxy-1,2,3,4-tetrahydrocarbazole, colorless needles from ethanol, separated from the reaction mixture, m.p. 94–95° (0.5 g.).

Anal. Calcd. for $C_{13}H_{15}NO$: C, 77.6; H, 7.5; N, 7.0. Found: C, 77.5; H, 7.5; N, 7.2.

6-Methoxy-1,2,3,4-tetrahydrocarbazole (300 mg.) was dissolved in mesitylene (20 ml.) and palladium–charcoal (5%, 300 mg.) was added. The resulting mixture was refluxed for 1 hr., filtered to remove palladium-charcoal, and then concentrated to 5 ml. under vacuum. Dilution with petroleum ether gave 3-methoxycarbazole (100 mg.), colorless plates from benzene, m.p. 147–149°, γ_{max} 3405 (NH) and 1225 cm.$^{-1}$ (OCH_3).

Anal. Calcd. for $C_{13}H_{11}NO$: C, 79.2; H, 5.6; N, 7.1. Found: C, 79.0; H, 6.1; N, 7.0.

3-Methoxycarbazole (500 mg.) was dissolved in dry benzene (20 ml.) containing anhydrous aluminum chloride (750 mg.) and refluxed for 3 hr. The reaction mixture was poured into ice and extracted with 20% sodium hydroxide solution. Acidification and ether extraction gave 3-hydroxycarbazole, a brown solid, which recrystallized as off-white needles from benzene, m.p. 260–261° (120 mg.), γ_{max} 3400 (NH) and 3200 cm.$^{-1}$ (OH).

4-Hydroxycarbazole.—Cyclohexane-1,3-dione monophenylhydrazone, prepared from cyclohexane-1,3-dione (1 equiv.) and phenylhydrazine hydrochloride (1 equiv.), was condensed by the method of Clemo and Felton[12] with sulfuric acid to give 1,2,3,4-tetrahydro-4-oxocarbazole. Dehydrogenation with palladium–charcoal in boiling mesitylene gave 4-hydroxycarbazole, colorless plates from dilute hydrochloric acid, m.p. 169–170°.

Animals and Methods of Collection.—Male albino rats (200–300 g.) and a male albino rabbit were used. The rabbit was dosed orally by suspending carbazole (1 g.) in water with acacia (1 g.), administering the suspension with the aid of a stomach tube, and collecting the urine over a 3-day period, each day's collection being kept separately. The rats were dosed with carbazole in propylene glycol (0.5 ml. per rat), at dose levels of 4 mg./kg., by intraperitoneal injection and the urine collected over 3 days.

Paper and Thin-Layer Chromatography. (A) Paper Chromatography.—Whatman No. 3 or 4 paper was used depending upon the quantity of material to be chromatographed. With systems A, B, and C the chromatograms were impregnated by dipping in a 30% acetone solution of the aqueous phase and allowing the acetone to evaporate before the chromatograms were developed. With systems D and F no impregnation with the aqueous phase was required.

(B) Thin-Layer Chromatography.—Silica gel was used as adsorbent; the plates were prepared by the method of Stahl[13] and developed on solvent system E.

(C) Solvent Systems (by volume)—**(A)**, butanol–acetic acid–water, 4:1:5; **(B)**, butanol–water, saturated; **(C)**, butanol–ammonia–water (saturated), 1:1; **(D)**, toluene–isooctane–methanol–water, 15:5:16:4; **(E)**, chloroform–benzene–ethyl acetate–water, 6:2:2:5; **(F)**, ethyl acetate–pyridine–water, 3:1:1.

(D) Detection Methods.—The unhydrolyzed metabolites were visualized on paper with the aid of ultraviolet light. The

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(11) The reduction was carried out with stannous chloride in preference to sodium sulfite as the yield in the latter reduction was extremely low: J. Altschul, *Ber.*, **25**, 1842 (1892).

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hydrolyzed, phenolic bands were detected on both paper and thin-layer silica gel chromatograms by spraying with a diazotized sulfanilic acid–sodium carbonate solution.[14]

Counting Techniques.—Radioactive bands on paper chromatograms were detected with a chromatogram scanner. All radioactive estimations were carried out using a Packard Tricarb. liquid scintillation spectrometer, Model 314, d.c. The aliquots to be counted (0.1 ml.) were pipetted into a scintillation solvent (10 ml.) which contained 2,5-diphenyloxazole (PPO) (3.0 g.) and 1,4-bis-2-(5-phenyloxyazoyl)benzene (POPOP) (0.1 g.) dissolved in toluene–ethanol (1 l., 4:1, v./v.). The samples were cooled in a refrigerator to −13° and counted at a high voltage setting of 5 and with the discriminator at 10–50 v. The quenching of the aliquots were determined by adding an internal standard of benzoic acid-C^{14} to the samples and recounting.

Isolation of Metabolites. (A) Rabbit Urine.—Each day's urine was estimated for glucuronide content by the method of Fishman and Green.[15] A twofold increase in glucuronide content was observed in the first day's urine, but this had returned to normal after 3 days. The glucuronide was isolated by the method of Smith and Williams.[16] The urine was acidified to pH 4 with glacial acetic acid and treated with a saturated lead acetate solution until no further precipitation occurred. The mixture was centrifuged and the precipitate discarded. The supernatant liquid was made basic (pH 8) with ammonia and saturated basic lead acetate added. The lead was removed as lead sulfide by the addition of hydrogen sulfide and filtration. The filtrate was concentrated to dryness under reduced pressure to give a gum which could not be induced to crystallize.

(a).—A portion of this gum was dissolved in absolute ethanol and methylated with diazomethane in ether. The solvent was removed under vacuum and the residue acetylated with acetic anhydride in pyridine. After treatment with ice–water, chloroform extraction, and removal of solvent, the resulting brown oil was chromatographed on neutral alumina. Elution with benzene gave a colorless crystalline solid, m.p. 146–148°. Comparative infrared, ultraviolet, and mixture melting point identified this material as 3-methoxycarbazole. Elution with 10% chloroform–benzene gave a pale yellow powder, the tri-O-acetyl-methyl ester of a hydroxycarbazole glucuronide, which was recrystallized from ether–petroleum ether as colorless crystals; m.p. 80–81°; γ_{max} 3400 (NH), 1750 (CH_3CO), 750 (4 adjacent H in aromatic ring), 810, and 890 cm.$^{-1}$ (2 adjacent H in aromatic ring).

Anal. Calcd. for $C_{25}H_{25}NO_{10}\cdot H_2O$: C, 58.0; H, 5.3; N, 2.7. Found: C, 57.5; H, 5.6; N, 2.6.

Elution with 50% chloroform–benzene gave a small amount of a third product, an off-white powder; γ_{max} 3400 (NH), 1740 (CH_3CO), 810, and 865 cm.$^{-1}$ (2 adjacent H and a lone H in aromatic ring).

(b).—A second portion of the gum, dissolve in methanol, was purified using paper chromatography with systems A, B, and C (R_f values being 0.7, 0.8, and 0.05, respectively). The band fluorescing under ultraviolet light was eluted with methanol, the methanol removed under vacuum and the residual colorless oil hydrolyzed with a β-glucuronidase preparation[17] at 36° in acetate buffer (5 ml., pH 4.5, 0.1 *M*) according to the method of Cox.[18] After incubation for 12 hr. the solution was evaporated to dryness under reduced pressure in an atmosphere of nitrogen and the methanol-soluble material chromatographed on paper with system D, on which the phenolic product had an R_f 0.31. Comparative chromatography of the unknown phenol with the four synthetic hydroxycarbazoles on paper with system D and thin-layer with system E (Table I) showed that the unknown had R_f values identical with 3-hydroxycarbazole.

(B) Rat Urine. (a) Qualitative Analysis.—The 3-days' urine was bulked, filtered, and hydrolyzed with boiling 0.5 *N* hydrochloric acid for 2 hr. The hydrolyzed urine was concentrated to half volume and continuously extracted with ether for 6 hr. The ethereal layer was concentrated, and the residue was dissolved in methanol and purified by paper chromatography with system D. Comparative paper and thin-layer chromatography showed that the phenolic product obtained was identical with that obtained from the rabbit after β-glucuronidase hydrolysis.

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TABLE I

R_f VALUES OF HYDROXYCARBAZOLES

	R_f, System D[a]	R_f, System E[b]	Color[c]
1-Hydroxycarbazole	0.57	0.62	Red
2-Hydroxycarbazole	.26	.39	Orange
3-Hydroxycarbazole	.31	.57	Purple
4-Hydroxycarbazole	.45	.81	Pink
Hydrolyzed metabolite	.31	.57	Purple

[a] Silica gel thin-layer chromatograms. [b] Whatman No. 4 paper. [c] Reagent, diazotized sulfanilic acid.

TABLE II

URINARY EXCRETION IN RATS AFTER CARBAZOLE-C^{14} INJECTION

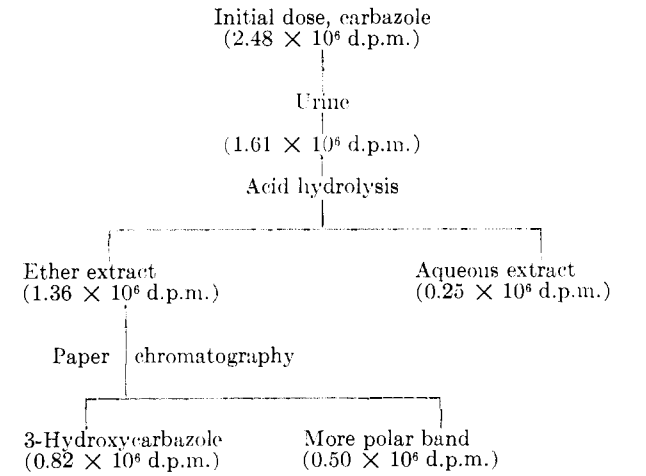
Group	Excretion of counts in urine, d.p.m. × 10^{-5} (% of initial dose)[a] Day 1	Day 2	Day 3	Total
A	4.14	0.368	0.052	4.56
	(50)	(4.5)	(0.6)	(55)
B	5.48	0.322	0.041	5.84
	(66)	(4.0)	(0.5)	(70.5)
C	5.42	0.209	0.041	5.67
	(65)	(2.5)	(0.5)	(68)
Mean excretion	5.01	0.300	0.045	5.36
	(60)	(3.5)	(0.5)	(64)

[a] Initial dose 4.13 × 10^5 d.p.m./rat, urine collected from 6 rats with 2 rats in each group.

(b) Quantitative Analysis.—After the rats were dosed with carbazole-C^{14} (4.13 × 10^5 d.p.m./rat), each day's urine was counted, and the excretion figures are tabulated in Table II. The urine was then bulked and hydrolyzed, as before, with dilute hydrochloric acid and ether extracted. Table III shows the distribution of counts in the different extracts during the purification process. The more polar band, obtained along with the monohydroxycarbazole after chromatography of the ether extract, was further separated on paper with system F to give two separate phenolic bands.

TABLE III

DISTRIBUTION OF COUNTS DURING METABOLITE PURIFICATION



(c) Isotopic Dilution Method.—The band corresponding to the monohydroxycarbazole, from the carbazole-C^{14} experiment, was eluted with methanol, concentrated to 5 ml., and divided into two portions. To one portion 3-hydroxycarbazole (20 mg.) was added, the methanol removed under vacuum, and the residual solid recrystallized from benzene–petroleum ether to constant melting point and constant radioactive count (m.p. 260–261°, 8530 ± 160 d.p.m./mg.). The second portion was concentrated to dryness, dissolved in absolute ether (5 ml.), and methylated with diazomethane. The excess diazomethane

and ether were removed by distillation, 3-methoxycarbazole (20 mg.) was added, and the resulting solid recrystallized to constant melting point and constant radioactive count from benzene (m.p. 147–149°, 6224 ± 180 d.p.m./mg.).

Results and Discussion

The lack of functional groups susceptible to enzymatic attack, together with the symmetrical nature of the carbazole molecule, suggests that an oxidative hydroxylation of an aromatic ring followed by conjugation of this phenol with either sulfuric or glucuronic acid is the most likely metabolic route of detoxication. A qualitative study of the metabolism of carbazole in rat urine gives, after acid hydrolysis of the urine followed by purification of the acidic product by paper chromatography, only one monohydroxycarbazole as the major metabolite.

To determine the structure of this hydroxycarbazole the four possible hydroxycarbazoles were synthesized by unambiguous methods. These four compounds were separated using paper and thin-layer chromatography and comparison of the unknown metabolite with these known phenols indicates that hydroxylation has taken place at position 3 in the carbazole molecule (Table I).

Estimation of the glucuronide content of rabbit urine after carbazole dosing shows a twofold increase in the first day's urine which has returned to normal after 3 days. The glucuronide was isolated from the urine by the method of Smith and Williams[16] but could not be obtained as a solid. Hydrolysis of a sample of this glucuronide with a β-glucuronidase preparation gives a phenol which cannot be separated from either the acid hydrolyzed metabolite obtained previously from rat urine or 3-hydroxycarbazole on the chromatographic systems used. The conjugate was shown to be essentially the glucuronide in preference to the ethereal sulfate (the β-glucuronidase preparation used may also contain some sulfatase activity) by preparation of the glucuronide tri-O-acetyl-methyl ester. This derivative was obtained as a solid after methylation of the glucuronide with diazomethane, acetylation with acetic anhydride in pyridine, and absorption on neutral alumina. Two other products were isolated by this method; first, a small amount of 3-methoxycarbazole formed by hydrolysis of the glucuronide during isolation and subsequent methylation with diazomethane, and second, a small amount of solid material, also a glucuronide tri-O-acetyl-methyl ester, the infrared spectrum of which suggests a symmetrically disubstituted carbazole structure, possibly a 3,6-dihydroxycarbazole.

It has been shown conclusively by administration of carbazole-C^{14} to rats that the hydrolyzed metabolite is 3-hydroxycarbazole. Carbazole-C^{14} was prepared from aniline-C^{14} sulfate by diazotization and reduction to phenylhydrazine-C^{14} followed by condensation with cyclohexanone and dehydrogenation of the 1,2,3,4-tetrahydrocarbazole-C^{14}. The radioactive metabolite was isolated, after acid hydrolysis of the urine, and purified by paper chromatography. Isotopic recrystallization of the metabolite with authentic 3-hydroxycarbazole gives a constant melting, constant counting radioactive product. Methylation of the metabolite with diazomethane and similar isotopic recrystallization with authentic 3-methoxycarbazole gives a similar, constant melting, constant counting product.

A quantitative study of the excretion of counts in urine after carbazole-C^{14} injection to rats is shown in Tables II and III. After 3 days' collection an average 64% of the original injection counts is excreted. Acid hydrolysis of the urine and continuous ether extraction gives an ether extract which possesses 85% of the excreted counts. Concentration of the ether extract and chromatography of the residue on paper with a toluene–isooctane–methanol–water (15:5:16:4) solvent system gives two major radioactive bands, both possessing phenolic properties. The less polar band, 3-hydroxycarbazole, R_f 0.31, contains 60% of the counts from the ether extract. The more polar band, which remains at the starting line with this solvent system, is a mixture of compounds and contains at least two other phenolic products which have been separated using an ethyl acetate–pyridine–water (3:1:1) solvent system. Further work is required to determine the structures of these minor metabolites but it is probable that one of the phenols is related to the minor metabolite isolated as its glucuronide tri-O-acetyl-methyl ester from rabbit urine.

These results show that the major position of attack in the carbazole nucleus by the hydroxylating enzyme in both rats and rabbits is the 3-position. Brown and Coller[8] have shown this to be the position of highest electron density in the carbazole nucleus. This suggests that the attacking agent is an electron-deficient species, hence either a hydroxyl cation or a hydroxyl free radical. Preliminary studies on the hydroxylation of carbazole[19] by Udenfriend's free radical system[20] indicate that more than one monohydroxycarbazole is formed. Further work is required to show which position is attacked predominantly by this system, but the apparent exclusive attack at position 3 *in vivo* suggests that the reaction may be heterolytic rather than homolytic.

The hydroxylation at position 3 in the carbazole nucleus further supports the assignment of position 12 as the position of hydroxylation in the metabolism of ergometrine and lysergic acid diethylamide as proposed by Wright and Slaytor.[2]

Acknowledgments.—We wish to thank Mr. L. Ladomery for assistance in isolating the glucuronide tri-O-acetyl-methyl ester derivatives of the metabolites.

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