Determination of Urinary Indolic Metabolites

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Abstract □ A rapid mass spectral assay for tryptophol, 5-hydroxytryptophol, and 3-indoleacetic acid, employing stable-isotope labeled internal standards, is described. The compounds were extracted from urine or buffer with ethyl acetate and quantitatively measured by chemical-ionization mass spectrometry. The calibration curves were linear over a range of 0.06–2.8 μg/ml. The technique was applied to the analysis of urine from patients with carcinoid tumors. In addition, the first synthesis of 5-methoxy-3-indoleacrylic acid is reported.

Keyphrases □ Indole derivatives, various—mass spectrometric analyses in human urine □ Mass spectrometry—analyses, various indole derivatives in human urine □ Tryptophan metabolites—various indole derivatives, mass spectrometric analyses in human urine □ Metabolites, tryptophan—various indole derivatives, mass spectrometric analyses in human urine

The metabolism of tryptophan by carcinoid tumors to indole and 5-hydroxyindole metabolites has been under investigation in this laboratory. Carcinoid tumors are usually malignant carcinomas arising from the argentaffin cells throughout the lung and GI tract (1). Associated with this tumor are large amounts of 5-hydroxytryptamine (I) present in the circulation and excessive urinary excretion of 5-hydroxy-3-indoleacetic acid (II), which constitute the biochemical hallmark of this disease (2–6).

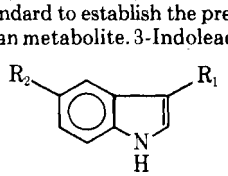
BACKGROUND

Recent investigations (7) showed that some patients with carcinoid tumors exhibit low urinary II levels and/or elevated levels of other indolic metabolites. Determination of other excreted indolic metabolites may aid disease diagnosis and treatment. A mass spectral assay for three indolic metabolites, 3-indoleacetic acid (III), tryptophol (3-indoleethanol) (IV), and 5-hydroxytryptophol (V), is presented here.

3-Indoleacetic acid is a normal urinary metabolite and is present in the urine of patients with carcinoid tumors (7–9). A previously reported (8) assay for III was considered unsatisfactory because of the high probability of interference from other indolic compounds.

5-Hydroxytryptophol and its conjugates have been identified as normal metabolites in human urine and human and rat brain homogenates (10, 11). Ethanol consumption shifts tryptophan metabolism away from the oxidation metabolite, II, toward the reduced metabolite, V, as a result of the increased ratio of reduced to unreduced nucleotide cofactors effected by ethanol metabolism (12, 13). This alternation has been demonstrated in normal humans and in patients with carcinoid tumors (14). Thus, it was necessary to assay for V and the nonhydroxylated metabolite, IV, to account for tryptophan metabolites further. Previously reported assays for IV (15) and V (16) have detection limits of 1–5 μg.

In addition, 5-methoxy-3-indoleacrylic acid (VI) was synthesized to serve as an authentic standard to establish the presence of this compound as an excreted tryptophan metabolite. 3-Indoleacrylic acid was detected in the urine of a patient with carcinoid tumor (7). Moreover, 3-indoleacrylic acid and its glycine conjugate were found as urinary metabolites in normal humans (17, 18), in those with a chromosomal abnormality (19), in patients with Hartnup disease (20, 21), in a group of East Africans who had a high intake of bananas in their diet (22), and in six children with phenylketonuria (23). Therefore, it is established that 3-indoleacrylic acid and its conjugate are urinary metabolites in normal humans and in patients with certain diseases. It is thus possible that 5-hydroxy-3-indoleacrylic acid or 5-methoxy-3-indoleacrylic acid may also be excreted as a urinary metabolite, particularly in patients with carcinoid tumors where large amounts of 5-hydroxylated indole metabolites are excreted.



I: $R_1 = CH_2CH_2NH_2$, $R_2 = OH$
II: $R_1 = CH_2COOH$, $R_2 = OH$
III: $R_1 = CH_2COOH$, $R_2 = H$
IV: $R_1 = CH_2CH_2OH$, $R_2 = H$
V: $R_1 = CH_2CH_2OH$, $R_2 = OH$
VI: $R_1 = CH{=}CHCOOH$, $R_2 = OCH_3$

EXPERIMENTAL[1]

Chemistry—*Ethyl 3-Indolylglyoxylate*—To a stirred solution of indole (3.7 g, 32 mmoles) in 50 ml of ether at 0–5° was added oxalyl chloride (4.6 g, 36 mmoles) over 20 min. After 1 hr, 60 ml of absolute ethanol and 5 ml of triethylamine were added, and the reaction mixture was refluxed for 30 min. After cooling, the precipitate was collected and recrystallized from ethanol–water to give fine white needles, 5.45 g (80%), mp 183–185° [lit. (24) mp 186°].

Tryptophol-d_4—A suspension of ethyl 3-indolylglyoxylate (5.0 g, 23 mmoles) and lithium aluminum deuteride (3.36 g, 80 mmoles) in 100 ml of tetrahydrofuran was heated under reflux for 2 hr under nitrogen. Excess hydride was decomposed by addition of 10 ml of water. The resulting precipitate was filtered and washed with tetrahydrofuran, the combined filtrates were dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo* to give an oil. Upon cooling, white crystals formed. These crystals were recrystallized from benzene–petroleum ether (bp 65–110°) to give white platelets, mp 57.5–58.5° [lit. (24) 58–59°]; chemical-ionization mass spectrum (isobutane): *m/e* 166 (MH^+).

Anal.—Calc. for $C_{10}H_7D_4NO$: C, 72.7; H, 9.1; N, 8.5. Found: C, 72.93; H, 8.88; N, 8.64.

5-Benzyloxytryptophol-d_4—To a solution of 5-benzyloxyindole (5.0 g, 22.4 mmoles) in 50 ml of ether at 0–5° was added, dropwise over 15 min, oxalyl chloride (3.3 g, 26 mmoles). The reaction mixture was stirred for 1 hr and filtered to give a red powder, which was collected and dried *in vacuo* to give 2.6 g (38%). The crude 5-benzyloxyindolylglyoxalyl chloride was added to a suspension of lithium aluminum deuteride (1.43 g, 34.1 mmoles) in 40 ml of dry tetrahydrofuran and refluxed for 2 hr under nitrogen.

The reaction mixture was decomposed with 10 ml of water, the precipitate was filtered and washed with tetrahydrofuran, and the combined tetrahydrofuran fractions were dried over anhydrous sodium sulfate and concentrated to an oil *in vacuo*. Upon standing at 5°, the oil solidified; three recrystallizations from benzene–petroleum ether (bp 65–110°) gave 875 mg (38%), mp 92–95° [lit. (25) mp 93–95°]; chemical-ionization mass spectrum (isobutane): *m/e* 272 (MH^+).

5-Hydroxytryptophol-d_4—A solution of 5-benzyloxytryptophol-d_4 (850 mg, 3.1 mmoles) in 25 ml of methanol was treated with 10% Pd/$BaSO_4$ (500 mg) under 1 atm of hydrogen. After 3 hr, the hydrogenolysis was complete; the reaction mixture was filtered, and the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate, treated with charcoal, and cooled to 5°. Petroleum ether (bp 65–110°) was added until the solution turned cloudy. The solution was allowed to stand at 5°, and fine white crystals were collected to give 110 mg (20%), mp 107–109.5° [lit. (25) mp 105–107°]; chemical-ionization mass spectrum (isobutane): *m/e* 182 (MH^+).

[1] Chemical-ionization mass spectrometry was performed on an AEI MS-9 spectrometer modified for chemical ionization and conducted under the following conditions: source pressure, 0.7 torr; and source temperature, 180°. NMR spectra were recorded on a Varian A-60 spectrometer. Melting points are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory, Berkeley, Calif.

Anal.—Calc. for $C_{10}H_7D_4NO_2$: C, 66.3; H, 8.3; N, 7.7. Found: C, 66.43; H, 8.06; N, 7.52.

3-Indoleacetic Acid-d_7—A solution of 3-indoleacetic acid[2] (1.0 g, 5.7 mmoles) and 20 ml of 4.0 *N* DCl (99% atom)[3] in deuterium oxide (99.7% atom)[3] was stirred under reflux under nitrogen for 1 hr. The solvent was then removed *in vacuo*, fresh solvent was added, and the procedure was repeated nine times. The resulting residue was treated with ethereal diazomethane. Chemical-ionization mass spectrometry (isobutane) of the methyl ester showed molecular ions at *m/e* 194 (MH + 5), 195 (MH + 6), and 196 (MH + 7).

5-Methoxy-3-indoleacrylic Acid—A solution of 5-methoxyindole (2.94 g, 20 mmoles) in 20 ml of ether was added dropwise over 15 min to a solution of methyl magnesium bromide[4] (2.77 g, 25 mmoles) in 10 ml of ether under nitrogen and cooled to −5–0° in an ice bath. After stirring for 15 min, 3-chloroacrylic acid (2.13 g, 20 mmoles) in 20 ml of ether was added dropwise over 15 min. The reaction mixture was allowed to warm to room temperature, ammonium chloride (2.8 g) in 30 ml of water was added dropwise, and the precipitate was removed by filtration.

The filtrate was separated, and the aqueous phase, at pH 7.8, was extracted with ether. The pH of the aqueous phase was adjusted to 1.6 with 6 *N* HCl. The solution was cooled, and the white precipitate was collected. Recrystallization first from ethyl acetate–hexane (1:1) with charcoal treatment and then from ethanol–water gave 5-methoxy-3-indoleacrylic acid as white platelets, 259 mg (6%), mp 197–200°; NMR (dimethyl sulfoxide): δ 6.4, 7.9 (2d, 2H, CH=CH, J = 15 Hz), 6.8–8.0 (m, 5H, N-H, 2-H, 4-H, 6-H, and 7-H), 11.85 (s, CO_2H), and 3.8 (s, 3H, OCH_3) ppm; UV (absolute ethanol): 279 and 324 nm; chemical-ionization mass spectrum (isobutane) of methyl ester: *m/e* 232 (MH^+).

Anal.—Calc. for $C_{12}H_{11}NO_3$: C, 66.3; H, 5.1; N, 6.4. Found: C, 66.18; H, 4.97; N, 6.53.

Standards—Standard solutions of tryptophol, 5-hydroxytryptophol, 3-indoleacetic acid, and their deuterium-labeled analogs consisted of known amounts weighed to 0.01 ± 0.005 mg dissolved in 0.2 *M* phosphate buffer, pH 7.4, and had the following concentrations: tryptophol, 0.87 μg/μl; tryptophol-d_4, 0.51 μg/μl; 5-hydroxytryptophol, 0.79 μg/μl; 5-hydroxytryptophol-d_4, 0.71 μg/μl; 3-indoleacetic acid, 0.70 μg/μl; and 3-indoleacetic acid-d_7, 0.99 μg/μl.

Assay Standardization—Standard curves for quantitative measurement of tryptophol, 5-hydroxytryptophol, and 3-indoleacetic acid were obtained by adding known amounts of standard solutions of these compounds and their deuterium-labeled analogs to aqueous buffer or urine and extracting with ethyl acetate after adjusting the pH to 7.0 or 1.8. Microliter quantities of the standard solutions were obtained using an adjustable microliter pipet accurate to ±0.1%. These data were reproducible and gave essentially the same results for samples prepared in urine (pH adjusted) as for those in buffer alone.

Assay—A patient's 24-hr urine was collected and stored frozen without preservative until assayed. The frozen urines were thawed, the total volume and pH were recorded, and a 100-ml aliquot was removed for assay. Known amounts of deuterium-labeled standards were added to this aliquot in amounts equivalent to 500–1000 μg/24-hr urine. The urinary pH was adjusted to 7.0 by aqueous base and extracted with three 35-ml portions of ethyl acetate; these extracts were combined, dried over anhydrous sodium sulfate, and concentrated to dryness in a nitrogen stream. This fraction, containing neutral compounds, phenols, and weak bases, was then subjected to chemical-ionization mass spectral analysis.

The remaining urine fraction was brought to pH 1.8 by addition of 6.0 *N* HCl, saturated with sodium chloride, and extracted with three 35-ml portions of ethyl acetate. The extracts were combined, dried over anhydrous sodium sulfate, and concentrated to dryness. This fraction, containing organic acids, was then subjected to chemical-ionization mass spectral analysis after treatment with ethereal diazomethane.

RESULTS AND DISCUSSION

Tryptophol-d_4 and 5-Hydroxytryptophol-d_4—These compounds were prepared according to the procedures reported for tryptophol (24) and 5-hydroxytryptophol (25) with the modification that the ester or acid chloride was reduced with lithium aluminum deuteride to give the tetradeuteroethanol side chain.

3-Indoleacetic Acid-d_7—This compound was prepared by treating 3-indoleacetic acid and 4.0 *N* DCl in deuterium oxide under reflux. Exchange of the five aromatic protons of tryptophan when treated with trifluoroacetic acid-d_1 (26, 27) and of the four aromatic protons on 5-hydroxytryptophan when treated with 4.0 *N* DCl (28) has been reported. The methylene protons of 3-indoleacetic acid, being doubly activated, were also expected to be exchanged. Isobutane chemical-ionization mass spectral analysis of the methyl ester showed the mixture composition to be 65% d_7, 29% d_6, and 6% d_5, which was sufficient deuteration for use as an internal standard.

5-Methoxy-3-indoleacrylic Acid—3-Indoleacrylic acid has been synthesized (29) in 50% yield by the Doebner modification of the Knoevenagel reaction (30), a procedure found to be unsatisfactory for preparation of 5-methoxy- or 5-hydroxy-3-indoleacrylic acid[5]. Therefore, following the model of Woodward *et al.* (32), the Grignard salt of 5-methoxyindole was reacted with 3-chloroacrylic acid to give 5-methoxy-3-indoleacrylic acid. Extensive TLC, GLC[6], and mass spectral analysis of several urines from carcinoid patients failed to produce any evidence for the presence of 5-hydroxy- or 5-methoxy-3-indoleacrylic acid.

Assay—Chemical-ionization mass spectral analysis of the ethyl acetate extracts permitted quantitative measurement from the ratio of peak heights of MH^+ 162/166 for tryptophol, MH^+ 178/182 for 5-hydroxytryptophol, and MH^+ 190/197 for 3-indoleacetic acid. Interfering peaks from other neutral, phenolic, or basic compounds were nonexistent for masses 162/166 and 178/182. Interference from acidic compounds of mass 190/197 was negligible. The ratio times the amount of deuterium-labeled compound added gave the amount of unlabeled metabolite found. Standard curves were obtained by plotting micrograms found *versus* micrograms added. The assay gave a linear correlation over the following ranges: tryptophol, 0.4–2.8 μg/ml; 5-hydroxytryptophol, 0.06–2.4 μg/ml; and 3-indoleacetic acid, 0.1–2.5 μg/ml.

A common method of obtaining a standard curve utilizes a constant amount of internal standard while varying the known amount of compound under assay (33–35). This approach was unsatisfactory because a large error is involved in measurement and ratio determination when peak heights are vastly different. Accuracy is greatest when the ratios are less than 3:1. Thus, when analyzing an unknown sample, an approximate value was obtained and the sample was assayed again with an amount of deuterium standard added to approximate a 2:1 ratio.

Patient Assay—Only two 24-hr urines from patients with carcinoid tumor were available. Patient A had the following urinary levels: 3-indoleacetic acid, 0.25 mg/24 hr; tryptophol, <17 μg/24 hr; and 5-hydroxytryptophol, <123 μg/24 hr. Patient B had the following urinary levels: 3-indoleacetic acid, 0.17 mg/24 hr; tryptophol, <12 μg/24 hr; and 5-hydroxytryptophol, <55 μg/24 hr.

REFERENCES

(1) A. J. Simpson, *N.C. Med. J.*, **30,** 399 (1969).

(2) J. Waldenstrom, *Gastroenterology*, **35,** 565 (1958).

(3) K. L. Melmon and A. Sjoerdsma, *ibid.*, **47,** 104 (1964).

(4) J. A. Oates, K. L. Melmon, L. Gillespie, A. Sjoerdsma, and D. T. Mason, *Lancet*, **1,** 514 (1964).

(5) K. L. Melmon, A. Sjoerdsma, and D. T. Mason, *Am. J. Med.*, **39,** 568 (1965).

(6) R. M. Cohen, *Calif. Med.*, **114,** 1 (1971).

(7) H. E. Williams, K. M. Wilson, W. Trager, and K. L. Melmon, *Clin. Res.*, **19,** 406 (1971).

(8) H. Weissbach, W. King, A. Sjoerdsma, and S. Udenfriend, *J. Biol. Chem.*, **234,** 81 (1959).

(9) B. J. Haverback, B. Dyce, and H. V. Thomas, *N. Engl. J. Med.*, **262,** 754 (1960).

(10) V. E. Davis, J. L. Cashaw, J. A. Huff, and H. Brown, *Proc. Soc. Exp. Biol. Med.*, **122,** 890 (1967).

(11) D. Eccleston, A. T. B. Moir, H. W. Reading, and I. M. Ritchie, *Br. J. Pharmacol. Chemother.*, **28,** 367 (1966).

(12) A. Feldstein, H. Hoagland, K. Wong, and H. Freeman, *Q. J. Stud. Alcohol*, **25,** 218 (1964).

(13) V. E. Davis, H. Brown, J. A. Huff, and J. L. Cashaw, *J. Lab. Clin.*

[2] Sigma Chemical Co.

[3] Aldrich Chemical Co.

[4] Alfa-Ventron Corp.

[5] The starting material for this synthesis, 5-benzyloxy-3-indolecarboxaldehyde, is refractory to an aldol-type reaction because of the decreased electrophilicity of the carbonyl carbon. Extensive UV and IR analyses of this compound support this hypothesis. Similar difficulties with the same reaction involving 5-benzyloxy- or 5-hydroxy-substituted benzothiophene derivatives were reported (31).

[6] GLC was performed on a Varian 2100 gas chromatograph using a 0.9-m (3-ft) column packed with 2% OV-1 on 100–200-mesh Chromosorb GHP. The column oven temperature was programmed from 100 to 250° at 2°/min.

Med., **69**, 132 (1967).
(14) V. E. Davis, *Adv. Pharmacol.*, **6-B**, 143 (1968).
(15) O. Hutzinger, *J. Chromatogr.*, **40**, 117 (1969).
(16) S. Iskric, L. Stancic, and S. Kveder, *Clin. Chim. Acta*, **25**, 435 (1969).
(17) M. D. Armstrong, K. N. F. Shaw, M. J. Gortatowski, and H. Singer, *J. Biol. Chem.*, **232**, 17 (1958).
(18) A. J. Mandell and R. T. Rubin, *Life Sci.*, **4**, 1657 (1965).
(19) W. J. Mellman, L. A. Barness, T. A. Tedesco, and D. Besselman, *Clin. Chim. Acta*, **8**, 843 (1963).
(20) J. B. Jepson and M. J. Spiro, "The Metabolic Basis of Inherited Disease," McGraw-Hill, New York, N.Y., 1960, p. 1338.
(21) J. B. Jepson, *Adv. Pharmacol.*, **6-B**, 171 (1968).
(22) I. L. Hansen and M. A. Crawford, *J. Chromatogr.*, **22**, 330 (1966).
(23) G. Strohmeyer, *Arch. Exp. Klin. Dermatol.*, **219**, 743 (1964).
(24) T. Nogrady and T. W. Doyle, *Can. J. Chem.*, **42**, 485 (1964).
(25) S. Kveder, S. Iskric, and D. Keglevic, *Biochem. J.*, **85**, 447 (1962).
(26) B. Bak, C. Dambmann, and F. Nicolaisen, *Acta Chem. Scand.*, **21**, 1674 (1967).
(27) B. Bak, J. J. Led, and E. J. Pedersen, *ibid.*, **23**, 3051 (1969).
(28) G. W. Kirby, S. W. Shah, and M. Sandler, *Chem. Commun.*, **1967**, 819.
(29) K. N. F. Shaw, A. McMillan, A. G. Gudmundson, and M. D. Armstrong, *J. Org. Chem.*, **23**, 1171 (1958).
(30) G. Jones, *Org. React.*, **15**, 204 (1969).
(31) A. Dinner and E. Campaigne, *J. Med. Chem.*, **13**, 1205 (1970).
(32) R. B. Woodward, F. E. Bader, H. Bickel, A. Frey, and R. W. Kierstead, *J. Am. Chem. Soc.*, **78**, 2023 (1956).
(33) S. H. Koslow and A. R. Green, *Adv. Biochem. Psychopharmacol.*, **7**, 33 (1973).
(34) L. Bertillson and L. Palmer, *Science*, **177**, 74 (1972).
(35) F. Cattabeni, S. H. Koslow, and E. Costa, *ibid.*, **178**, 166 (1972).

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Inhibition of Rat Liver Mitochondrial Monoamine Oxidase by Chloramphenicol and 2-Amino-1-*p*-nitrophenylpropane-1,3-diol

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Abstract □ Inhibition of rat liver mitochondrial monoamine oxidase by chloramphenicol and its hydrolytic product, 2-amino-1-*p*-nitrophenylpropane-1,3-diol, was studied. The enzyme activity and its inhibition by these two compounds were optimum at pH 7.0 after preincubation for 60 min, the time needed for maximum enzyme–inhibitor complex formation. Enzyme activity could be restored after prolonged dialysis. Monoamine oxidase inhibition by chloramphenicol and its hydrolytic product was noncompetitive and reversible. Deamination of various monoamines was not to the same degree by these compounds. Of the different antimicrobials studied, only chloramphenicol and its hydrolytic product had a strong inhibitory effect on monoamine oxidase.

Keyphrases □ Chloramphenicol—effect on *in vitro* activity of rat liver mitochondrial monoamine oxidase □ Monoamine oxidase, rat liver mitochondrial—activity *in vitro*, effect of chloramphenicol and 2-amino-1-*p*-nitrophenylpropane-1,3-diol □ Enzyme activity—rat liver mitochondrial monoamine oxidase, effect of chloramphenicol and 2-amino-1-*p*-nitrophenylpropane-1,3-diol *in vitro* □ Antimicrobials—chloramphenicol and others, effect on *in vitro* activity of rat liver mitochondrial monoamine oxidase

Chloramphenicol and its hydrolytic product, 2-amino-1-*p*-nitrophenylpropane-1,3-diol, when administered intravenously to cats, produced vasodepression followed by an overshooting rise in blood pressure (1). Monoamine oxidase inhibitor drugs also produce hypotension followed by hypertension (2, 3). Therefore, it is possible that chloramphenicol and 2-amino-1-*p*-nitrophenylpropane-1,3-diol act as monoamine oxidase inhibitors, the subject of the present investigation.

Table I—Effect of Different Antimicrobials on Rat Liver Mitochondrial Monoamine Oxidase [a]

System	Monoamine Oxidase Activity[b]	Inhibition, %
Control	46	—
Plus streptomycin sulfate	46	Nil
Plus penicillin G sodium	46	Nil
Plus tetracycline hydrochloride	36	21.7
Plus chloramphenicol	16	65
Plus 2-amino-1-*p*-nitrophenylpropane-1,3-diol	17	63

[a] Results are means of five observations. Variations in individual observations were minimal. [b] Activity was defined as aldehyde formed, micromoles per 2.5 mg of protein per 30 min of incubation at 37°.

EXPERIMENTAL

Preparation of Monoamine Oxidase—Adult male albino rats, 200 g (average weight), were killed by stunning and decapitation after an overnight fast. The livers were pooled, homogenized in ice-cold 0.25 *M* sucrose to obtain a 10% (w/v) homogenate (4), washed with 0.25 *M* sucrose at 27,000×*g* for 20 min, and suspended in 0.001 *M* phosphate buffer (pH 7.0). A 1-ml aliquot of this suspension was equivalent to 1 g of tissue, and it was used as the source of monoamine oxidase.

Assay of Monoamine Oxidase Activity—The reaction mixture for the monoamine oxidase assay contained 0.025 *M* phosphate buffer (pH 7.0), 0.0125 *M* semicarbazide hydrochloride adjusted to pH 7.0, 0.01 *M* tyramine, and 0.1 ml of mitochondrial suspension as the enzyme source in a final volume of 2 ml. The optimum concentration of tyramine for measuring monoamine oxidase activity is 0.01 *M* (5–7). A higher concentration inhibits monoamine oxidase activity (8). Monoamine oxidase activity was measured at 420 nm (9).