Analogs of Amphetamine. 5.[1,2] Studies of Excretory Metabolites of 1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane (DOM) in Rats

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Metabolites of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM, STP, 1) in 24-hr rat urine and feces were studied. The major pathway of metabolism of DOM was the hydroxylation of the 4-CH_3 group to 1-(2,5-dimethoxy-4-hydroxymethylphenyl)-2-aminopropane (**2**), followed by oxidation of **2** to 1-(2,5-dimethoxy-4-carboxyphenyl)-2-aminopropane (**3**). Nearly 50% of the urinary radioactivity was present as **2** plus its glucuronide and sulfate conjugates; **3** and the unchanged DOM amounted to 28 and 8%, respectively. A trace amount of 1-(2,5-dimethoxy-4-methylphenyl)-2-propanone was also detected. In feces, **3** was essentially the exclusive metabolite; it accounted for 83% of the total activity compared to only 6% for **2**, and a very small amount of unchanged DOM.

1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane (DOM, STP, **1**) is a known hallucinogen.[3,4] Our previous studies on **1** included the tissue distribution and elimination in various species of animals,[5-7] the relation of pharmacological and behavioral effects to distribution in cat brain,[8] and the synthesis and behavioral effects of analogs of **1**.[9-11] The present paper describes the metabolites of ^{3}H-labeled **1** in 24-hr rat urine and feces.

Results and Discussion

Chromatography of the urine on paper in a solvent system of *n*-BuOH–*i*-PrOH–NH_4OH–H_2O (3:1:1:1) revealed a total of 8 radioactive bands. When each band was eluted and rechromatographed on tlc plates in three other solvent systems (Table I), most of the metabolites were identified by their R_f values, uv fluorescence, and color reactions (Table I). Bands d, f, and g were found to be 1-(2,5-dimethoxy-4-carboxyphenyl)-2-aminopropane (**3**), 1-(2,5-dimethoxy-4-hydroxymethylphenyl)-2-aminopropane (**2**), and unchanged DOM (**1**), respectively (Scheme I). Further confirmation was performed by gc; the retention time of each metabolite corresponded with the reference compounds (see Experimental Section). It was necessary to prepare the *N*-acetylpropyl ester of the 4-carboxy compound **3** for gc. Attempts to identify **3** as its trimethylsilyl derivative were unsatisfactory.

On hydrolysis of the urine with Ketodase, band a was no longer detectable on the chromatogram, and a corresponding increase in the radioactivity of band g (**2**) indicated that band a was the glucuronide conjugate of the 4-hydroxymethyl metabolite **2**.

Glusulase hydrolysis resulted in a disappearance of band b and an increase in the radioactivity of band g. Since there was no change of radioactivity on band b by the Ketodase hydrolysis, band b had to be the sulfate conjugate of **2**. No increase in radioactivity of **3** resulted from the Ketodase hydrolysis, indicating the absence of glucuronide of **3**.

Acid hydrolysis of the urine produced an artifact on paper chromatograms. Due to the instability of the benzylic OH of **2** in acid medium, the appearance of a new peak (presumably the 4-chloromethyl compound from the 4-CH_2OH group of **2**) at R_f 0.37 (solvent system A, see Table I) which superimposed with the position of **3** (R_f 0.33) made the determination of the possible glycine conjugate of the 4-carboxy compound **3** difficult. When **2** was treated with acid, the decomposition product showed quenching on paper chromatograms; this uv fluorescence characteristic differed from the purple fluorescence observed in **3** (Table I). No attempt has been made to develop a solvent system which would separate **3** from the decomposition product.

Three minor metabolites (bands c, e, and h) amounting to a total of only 7% of the urinary activity were not identified. None of the bands matched the chromatographic R_f values of *N*-acetyl DOM (**5**), or that of β-hydroxy DOM[11] (**6**), a product which could be formed by the action of β-hydroxylase. The presence of limited physical material on the chromatograms rendered the observation of uv fluorescence and color reactions of these minor metabolites impossible. Concurrently, separation of the acidic, basic, and neutral metabolites by differential pH extraction was achieved. The two basic metabolites extractable by Et_2O at pH 13 were the unchanged DOM (**1**) and 4-hydroxymethyl compound **2**. A neutral fraction, which represented a very small amount of activity, was found to contain two metabolites: an unidentified compound (R_f 0.93, solvent, EtOAc) and a trace of the keto compound (**4**, Scheme I; R_f 0.77, solvent, EtOAc). The former had the same R_f values as the band h; the latter had not been detected on chromatograms from direct application of the urine, probably due to insufficient quantity. From the

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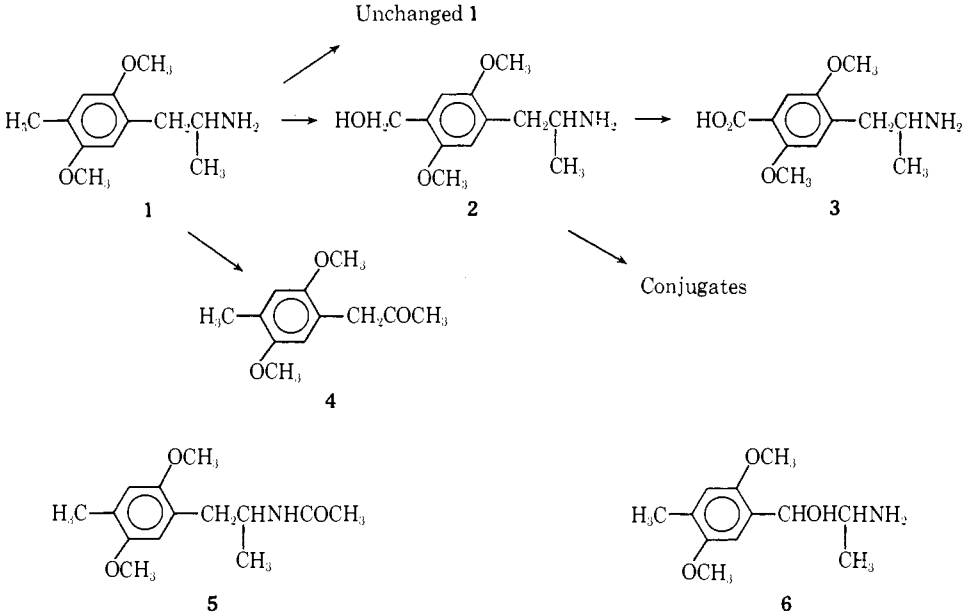
TABLE I

Compound or metabolite	R_f Solvent system[a] A[b]	B[c]	C[c]	D[b]	E[c]	Uv fluores[d]	Color reaction[f] A	B	C	D	% of metabolite in urine	% of admin dose
Characteristics of Metabolites in 24-hr Rat Urine												
a Glucuronide conjugate	0.07	0				?[e]					3.6	2.3
b Sulfate conjugate	0.17	0				B					18.9	11.8
c Unknown	0.25	0				?[e]					1.5	0.9
d **3**	0.33	0.03	0.47	0.71	0	Pu	Or	Y	NC	NC	27.8	17.4
e Unknown	0.47	0.03				?[e]					4.7	3.0
f **2**	0.87	0.57	0.52	0.73	0	BG	Or	GY	NC	G	26.9	16.8
g Unchanged 1	0.92	0.73	0.60	0.79	0	Q	Or	Y	NC	Y	7.9	5.0
h Unknown	0.94	0.90			0.93	Pu					1.2	0.8
Characteristics of Some Possible Metabolites Not Found in 24-hr Rat Urine												
4	0.94	0.91	0.80	0.90	0.77	B	P	NC	Y			
5	0.92	0.92	0.78	0.92	0.33	Q	NC	Y	NC	Y		
6	0.89	0.46	0.60		0.02	B	Pu	NC	NC			

[a] Solvent systems: A, *n*-BuOH–*i*-PrOH–NH_4OH–H_2O (3:1:1:1); B, $CHCl_3$–MeOH–*i*-PrOH–NH_4OH (90:10:95:5), C, *n*-BuOH–HOAc–H_2O (4:1:1); D, *n*-BuOH–*i*-PrOH–HOAc–H_2O (3:1:1:1); E, EtOAc. [b] On Whatman No. 1 paper. [c] On tlc. [d] Uv fluorescence: B, blue; Pu, purple; BG, bluish green; Q, quenching; NC, no color. [e] Uv fluorescence was not clearly defined. [f] Color reaction: A, with naphthoquinone (E. Stahl, "Thin Layer Chromatography," Academic Press, New York, N. Y., 1965, p 495); B, with Marquis reagent (G. F. Phillips and R. J. Mesley, *J. Pharm. Pharmacol.*, **21**, 9 (1929)); C, with 2,4-dinitrophenylhydrazine (E. Stahl, "Thin Layer Chromatography," p 490); D, with oxidizing mixture of H_2SO_4–$FeCl_3$–$HClO_4$ (W. M. McIsaac, B. T. Ho, V. Estevez, and D. Powers, *J. Chromatogr.*, **31**, 446 (1967)).

SCHEME I

METABOLIC FATE OF DOM IN RATS



acid fraction an unidentified metabolite, which accounted for less than 2% of the urinary activity, was also isolated (R_f 0.57, solvent system C; R_f 0, solvent system B). There is a possibility that this acid metabolite could well be the decomposition product of a metabolite already identified. Further characterization was not carried out. The zwitterionic metabolite **3** was not extractable in organic solvents at any pH and therefore remained in the aq solution.

Paper chromatograms of the MeOH extract of feces in solvent system A showed 83.2% of **3**, 6.2% of **2**, 1.5% of sulfate conjugate of **2**, and an unidentified metabolite (R_f 0.47) amounting to 3.7% of the fecal radioactivity. The unidentified metabolite had the same R_f value as band e on the paper chromatogram of urine (Table I). There was no significant change in radioactivity after Glusulase treatment; only a trace of hydrolysis was observed. Extraction of the aq homogenate of feces with Et_2O at pH 13, followed by tlc in solvent system B yielded unchanged **1** plus a very small amount of **2**.

Stability of **2** and **3** in media other than acid has been investigated. When the two compounds were added to urine or a basic solution, no decomposition could be detected over a period of 24 hr. The presence of 4-carboxy compound **3** in urine was therefore not due to handling. Apparently, the conjugates of **2** from bile are readily hydrolyzed in the intestine, and most of the free 4-hydroxymethyl compound **2** after being reabsorbed is either converted into the conjugated form in the liver or oxidized to the 4-carboxy compound **3**. When the latter is transported *via* the bile to the intestine, it is poorly reabsorbed, and eventually excreted to the feces.

In conclusion, the excretion of DOM and its metabo-

lites was nearly complete in 24 hr: 62.7% of the administered radioactivity in the urine and 20.2% in the feces. The major pathway of metabolism of DOM by rats was found to be the hydroxylation of the 4-CH_3 group to **2**, followed by oxidation of the benzyl alcohol to the carboxylic compound **3** (Scheme I). Of these two major metabolites, **2** and its glucuronide and sulfate conjugates accounted for nearly 50% of the urinary activity, and **3** amounted to almost 28% (Table I). Microsomal hydroxylation of a *p*-CH_3 group has been reported in a number of similar cases.[12-14] Neither acetylation of the NH_2 group of DOM nor hydroxylation of the β carbon was observed, for no **5** or **6** could be found. It is quite possible that those unidentified yet relatively minor metabolites could have resulted from the demethylation of the OCH_3 of DOM. In rats, the major route of elimination of DOM is *via* the kidneys, but the metabolite **3** from the extensive oxidation is also excreted in feces.

Experimental Section

Compounds.—1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane·HCl (DOM, 1) was labeled with ^3H by ^3H–H exchange.[8] Labile ^3H atoms were removed until a constant specific activity of 370 μCi/mg was achieved. The purity of the radioactive compound was verified by silica gel G tlc with the solvent systems A and B (Table I) and by autoradiography of the chromatogram.

The metabolites 1-(2,5-dimethoxy-4-hydroxyphenyl)-2-aminopropane (**2**), 1-(2,5-dimethoxy-4-carboxyphenyl)-2-aminopropane (**3**), and 1-(2,5-dimethoxy-4-methylphenyl)-2-propanone (**4**) were synthesized as described in the preceding paper.[1]

Collection of Samples.—Six male Sprague-Dawley rats (Cheek Jones Co., Houston, Texas), weighing 200 g, were each administered 5 mg/kg (2 mCi/kg) of the ^3H-labeled 1·HCl in saline ip. Animals were kept in metabolic cages with food and H_2O *ad libitum*. Urine and feces were collected at 24-hr postinjection and frozen until used.

Determination of Radioactivity.—The urine sample (0.1 ml) was mixed with 3 ml of MeOH in a counting vial, 15 ml of Spectrofluor (Amersham-Searle Co.) in PhMe was added, and ^3H was assayed by liquid scintillation (Nuclear-Chicago Spectrometer Model Mark 1). All values were corrected for 100% efficiency (channel ratio) and recovery.

The fecal samples were prepared as a 10% aq homogenate. In a counting vial 50 μl of the homogenate was digested, with occasional agitation, for 2 hr with 2 ml of NCS (Amersham-Searle Co.). After the addition of 15 ml of the scintillation fluid, the radioactivity was determined by liquid scintillation.

Hydrolysis of Conjugates.—Urine or the 10% aq homogenate of feces was adjusted to pH 5 or 5.5 with acetate buffer and then incubated at 37° overnight with beef liver Ketodase (β-glucuronidase, Warner Chilcott Co.: 500 units/ml) or Glusulase (β-glucuronidase and sulfatase, Endo Laboratories, Inc.: 500 units/ml). Acid hydrolysis of urine was carried out by boiling the urine in 2 *N* HCl for 2 hr.

Extraction of Metabolites.—Urine or the 10% aq homogenate of feces was adjusted to pH 13 with 10 *N* NaOH and extd 3 times with Et_2O. Basic metabolites in the combined Et_2O exts were sepd from neutral metabolites by extracting 3 times with 0.1 *N* HCl or by tlc in solvent system B (see footnote *a* of Table I). For acid metabolites, the urine was acidified to pH 2 with concd HCl, and then extracted with Et_2O.

Chromatography.—Hydrolyzed and unhydrolyzed urine samples were applied to Whatman No. 1 paper and chromatographed in solvent system A (Table I) using descending technique. Sequential sections of paper (0.5 × 1 cm^2 each) from paper chromatograms were placed in counting vials and assayed for ^3H after addn of MeOH and the scintillation fluid. Locations and amts of radioactivity of each band on chromatograms were detd by a mapping technique which involved plotting of the radioactivity *vs.* R_f value and cutting and weighing of the areas. Radioactive bands were then cut off from the remaining part of chromatograms, eluted with MeOH, and rechromatographed on silica gel G precoated tlc plates (Brinkman Co.) in solvent systems B and E. Sections of silica (0.5 × 1 cm^2) from tlc plates were also removed; the radioactivity of each section was detd and the percentage of each metabolite calcd.

Feces were extd 5 times with MeOH. The combined exts were filtered, concd, and then subjected to chromatography. The hydrolyzed fecal homogenate (see Hydrolysis of Conjugates) was treated in the same manner.

Identification of Metabolites.—Unchanged 1 and its metabolites were identified by first comparing with reference compds[1,11] for R_f values in various solvent systems and uv fluorescence (Table I). Cochromatography of metabolites and reference compds was also performed.

Eluents of the main metabolites in MeOH were further identified by gc [Varian Model 2100, F.I.D. detectors, N_2 flow rate 30 ml/min, columns: length 5 ft, i.d. 2 mm; 3% OV-17 on Chromosorb W, AW-DMCS (first column); 3% SE-30 on Varaport 30 (second column), optimal temp: for **1**, 160°; **2**, 200°; **3**, *N*-MeCO-Pr ester, 175°]. Compd **3** was converted to its *N*-acetylpropyl ester[15] and used as the gc reference. The retention times for the above metabolites correspond with those for the reference compounds.

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