Biochemical Pharmacology, Vol. 19, pp. 2763–2773. Pergamon Press. 1970. Printed in Great Britain

METABOLISM OF TYRAMINE-1-^{14}C BY THE RAT*

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(*Received* 21 *October* 1969; *accepted* 23 *February* 1970)

Abstract—Rats given tyramine-1-^{14}C intraperitoneally excrete over 98 per cent of the administered radioactivity within 24 hr. Thirteen of the 15 urinary metabolites which account for almost 99 per cent of the radioactivity excreted in 24 hr were identified and estimated. *p*-Hydroxyphenylacetic acid, the major urinary metabolite, is excreted free (77 per cent of the urinary radioactivity), conjugated with glycine (10·5 per cent), with sulfate and glucuronic acid (4·2 per cent), and with both glycine and sulfate (0·7 per cent). About 2 per cent is excreted as free tyramine and tyramine-*O*-glucuronide. The neutral metabolites *N*-acetyltyramine and tyrosol are excreted conjugated with glucuronic acid or sulfate. Less than 0·2 per cent is excreted as free *N*-acetyltyramine. No free tyrosol was detected. A third neutral radioactive compound was tentatively identified as *p*-hydroxyphenylacetaldehyde (0·2 per cent). Two metabolites, which together constitute 1·1 per cent, were not identified.

TYRAMINE [TA(I)],‡ *p*-hydroxyphenethylamine, is a biogenic amine structurally related to dopamine and norepinephrine. It is present in several common foodstuffs such as bananas, avocados,[1] cheese,[2] and red wine[3] and is also synthesized in the gut by the decarboxylation of tyrosine by bacteria.[4] In 1910, Ewins and Laidlaw[5] identified *p*-hydroxyphenylacetic acid [pHPAA(II)] as a urinary metabolite of TA (I) given to dogs in the first study of the metabolism of this amine. In 1952 Schayer[6] made the first quantitative study of the metabolism of TA (I) in which rats were injected with TA(I)-^{14}C. He reported that the only radioactive compounds in acid-hydrolyzed urine were pHPAA(II) (88 per cent) and TA(I) (9 per cent). Nakijama and Sano[7] identified tyrosol [TOH(III)] and *N*-acetyltyramine [NAT(IV)] and tentatively identified the glycine conjugate of pHPAA(II), *p*-hydroxyphenylacetylglycine [pHPAG(V)] in the urine of rats given a large dose of TA(I) (200 mg/kg). Lemberger *et al.*[8] made a quantitative study of the acidic and neutral metabolites of TA(I)-^{14}C in the acid-hydrolyzed urine of the rabbit and identified small amounts of *p*-hydroxymandelic acid, vanilmandelic acid, and homovanillic acid.

Most of the previous investigations of the urinary metabolites of TA(I) have been carried out on acid-hydrolyzed urine and little is known about the excretion of conju-

*Supported by the Grant MH-29689 from the U.S.P.H.S. and by the Britton Fund. Martha Tacker received a predoctoral fellowship from the U.S.P.H.S.

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‡The abbreviations used are: TA(I): *p*-tyramine; pHPAA(II): *p*-hydroxyphenylacetic acid; TOH-(III): tyrosol, *p*-hydroxyphenylethyl alcohol; NAT(IV):*N*-acetyltyramine; pHPAG (V): *p*-hydroxyphenylacetylglycine; pHPAld(VI): *p*-hydroxyphenylacetaldehyde.

gates after dosage with this amine. No previous study has included estimation of the basic as well as the acidic and neutral metabolites. The results of a detailed, quantitative study of the metabolism of TA(I)-1-^{14}C in the rat are reported here.

MATERIALS AND METHODS

Chemicals. TA(I)-1-^{14}C HBr (5·3 mc/m-mole, New England Nuclear Corp.) was shown to be radiochemically pure by two-dimensional paper chromatography and autoradiography. pHPAG(V) (m.p. 155·0–155·5°) was generously provided by Dr. B. T. Ho. TOH(III) was synthesized by reduction of pHPAA(II) with lithium aluminum hydride and recrystallized from chloroform to yield white crystals, m.p. 90·5–91·5°.[9] *Anal.* Calcd. for $C_8H_{10}O_2$: C, 69·56; H, 7·25. Found: C, 69·80; H, 7·16. The compound produced a single spot on paper chromatography in six solvent systems and thin-layer chromatography in one solvent system when sprayed with diazotized sulfanilic acid (see Table 1). NAT(IV) was synthesized by acetylation of TA(I) with acetic anhydride and recrystallized from ethylene dichloride to yield white crystals, m.p. 131·0–131·5°.[7] *Anal.* Calcd. for $C_{10}H_{13}O_2N$: C, 67·03; H, 7·26; N, 7·82. Found: C, 66·94; H, 7·47; N, 7·68. The compound produced a single spot on paper chromatography in six solvent systems and thin-layer chromatography in one solvent system when sprayed with diazotized sulfanilic acid and no reaction with ninhydrin spray (see Table 1). Acid hydrolysis of the acetylated compound produced a compound which was identified as TA(I) by paper chromatography and reaction with spray reagents.

Animals, dosage and collection of urine. Male Sprague–Dawley rats weighing 200 g were housed in metabolism cages and given water *ad lib.* but no food during the first 24 hr of the experiment. Numerous experiments have shown that the conventional practice of removing the food for a relatively short period of time to eliminate the possibility of spurious results because of contamination of the urine with pieces of food pellets does not significantly alter the metabolism. TA(I)-1-^{14}C HBr (1·9 mg/kg, 5·3 mc/m-mole) was given as a single injection (i. p.). A larger dose (150 mg/kg, 0·05 mc/m-mole) was given in experiments in which spray reagents were used to identify metabolites.

Urine was collected in test tubes containing 0·5 ml of sodium acetate buffer, pH 4·0. Aliquots were taken for measurement of radioactivity and the collections through 24 hr were combined for estimation and identification of the metabolites. Urine was brought to pH 2 with 12 N HCl and stored at −10°.

Measurement of radioactivity. Radioactivity was measured by liquid scintillation (Nuclear Chicago model 725) using a scintillation solution (Liquifluor, Nuclear Chicago) consisting of 4 g PPO, 50 mg dimethyl–POPOP and 70 g naphthalene per litre of toluene. Aqueous solutions (0·1 ml) were dissolved in methanol (3 ml) and liquifluor (10 ml).

Estimation of urinary metabolites. Urine collected during the first 24 hr after administration of TA(I)-1-^{14}C was chromatographed on Whatman 3MM paper in two dimensions (Solvent A and Solvent B, Table 1). The radioactive areas were detected by autoradiography, cut out and eluted with methanol, and the radioactivity counted. Three of these radioactive areas found to contain more than one radioactive compound were cut out of duplicate chromatograms, hydrolyzed and the parent compounds separated by thin-layer chromatography. The relative amount of each metabolite

TABLE. 1. R_f VALUES AND COLOR REACTIONS OF SOME POSSIBLE METABOLITES OF TYRAMINE

Compound*	Solvent systems†:	R_f Values (Paper chromatography) A	B	C	D	E	F	R_f Values (Thin-layer chromatography) F	G	H	Color reactions Ninhydrin	Diazotized sulfanilic acid
Tyramine		0·78	0·59	‡	0·45	0·62	0·30	0·07	0·57	0·49	purple	orange
Octopamine		0·55	0·53	‡	0·35	0·47	0·12	0·05	0·45	0·51	purple	yellow
3-Methoxy-4-hydroxy-phenylethylamine		0·73	0·55	‡	0·30	0·30	0·58	0·08	0·53	0·39	purple	pink
Normetanephrine		0·48	0·38	0·005	0·45	0·19	‡	0·10	0·40	0·45	purple	gold
p-Hydroxyphenylacetic acid		0·34	0·84	0·40	0·71	0·86	0·55	0·60	0·21	0·97		orange
p-Hydroxyphenylacetyl glycine		0·27	0·78	0·04	0·57	0·88	0·28	0·10				orange
Homovanillic acid		0·33	0·82	0·61	0·82	0·80	0·32	0·69	0·17	0·90		pink
Vanilmandelic acid		0·21	0·68	0·06	0·55	0·65	0·21	0·08	0·05	0·64		gold
p-Hydroxymandelic acid		0·19	0·76	0·02	0·55	0·89	0·22	0·08		0·75		yellow
Tyrosol		0·84	0·83	0·40	0·76	0·86	0·65	0·50	0·85	0·86		orange
N-Acetyltyramine		0·84	0·84	0·42	0·82	0·87	0·68	0·30				orange

*Compounds were chromatographed by descending chromatography on Whatman 3MM paper or on thin-layer plates of silica gel H, as described under Methods.

†Solvent systems: A—1-butanol–2-propanol–ammonia–water (3:1:1:1); B—1-butanol–acetic acid, glacial–water (4:1:1); C—benzene–acetic acid, glacial–water (2:2:1, upper phase); D—chloroform–acetic acid, glacial–water (2:2:1, lower phase); E—1-butanol saturated with 1 N HCl; F—benzene–propionic acid–water (2:2:1, upper phase); G—2-butanol–ethyl acetate–ammonia (3:3:1); H—2-butanol–formic acid–water (40:1:6).

‡Streaked.

present was determined by eluting the material from the silica gel with methanol and counting the radioactivity.

Chromatography. Metabolites and reference compounds were chromatographed on Whatman 3MM paper and sprayed with ninhydrin spray reagent and diazotized sulfanilic acid. Solvent systems, R_f values and color reactions are shown in Table 1. Radioactivity on one-dimensional chromatograms was located by autoradiography, scanning the chromatogram with a 4-π strip scanner (Vanguard Instrument Corp. model 880) or by cutting the chromatogram into 1-cm bands and counting the radioactivity. Radioactivity on two-dimensional chromatograms was located by autoradiography.

Thin-layer plates coated with silica gel H (Brinkman Instruments, Inc.) were activated at 110° for 1 hr and stored in a drying cabinet. The R_f values of reference compounds in three solvent systems are shown in Table 1. Radioactivity was located by scanning with a 2-π strip scanner (Vanguard Instrument Corp. model 885 glass plate scanner) or by scraping 1-cm bands of silica gel into counting vials containing methanol (3 ml) and counting the radioactivity after addition of liquifluor (10 ml).

The urinary amine fraction was separated from the acidic and neutral fractions by ion-exchange column chromatography (Dowex 50-X2, 1 × 4 cm) according to the procedure outlined by Kakimoto and Armstrong[10] as modified by Takesada *et al.*[11] Urine (2 ml) was diluted with water (2 ml), adjusted to pH 5 with 12 N HCl and added to the resin in the H^+ form. The compounds were eluted successively with 10 ml of water, 10 ml of 0·1 N sodium acetate, 10 ml of water and 40 ml of 1 N NH_4OH in 65% ethanol. The eluate was collected in 150 drop fractions and the radioactivity in aliquots (0·1 ml) of each fraction was counted. The total amount of radioactivity in the eluate was always greater than 95 per cent of that added to the column.

Spray reagents. Phenolic compounds were detected with diazotized sulfanilic acid, primary amines with ninhydrin-pyridine and glucuronic acid from β-glucuronide conjugates with naphthoresorcinol spray reagent and heating.[12] Sulfate conjugates were detected by the method of Dodgson *et al.*[13]

Hydrolysis of conjugates. Urinary β-glucuronide conjugates were hydrolyzed specifically with a commercial preparation of β-glucuronidase (Ketodase, 500 units β-glucuronidase/ml, Warner-Chilcott). Urine was taken to pH 5 with 12 N HCl and one-tenth volume of 0·05 M sodium acetate buffer, pH 5, was added. Ketodase (0·05 ml/ml urine) was added to the mixture which was incubated in an open tube at 37° for 18 hr.

Urinary β-glucuronide and sulfate conjugates were hydrolyzed simultaneously with a mixture of β-glucuronidase and sulfatase (Gluculase, 130,500 units β-glucuronidase and 20,000 units sulfatase/ml, Endo Laboratories). Urine was taken to pH 11 with 10 N NaOH and solid barium chloride was added to remove the phosphates.[14] The mixture was centrifuged and the supernatant removed and taken to pH 5 with 12 N HCl. To 1 ml of the solution was added 0·05 M sodium acetate buffer, pH 5·5 (0·1 ml), ascorbic acid (10 mg), EDTA, 2% (0·3 ml) and Gluculase (50 μl) and the mixture incubated in an open tube at 37° for 18 hr.

Acid hydrolysis of conjugates was accomplished by adding one-sixth volume of 12 N HCl to the urine which was then heated in a sealed tube at 100° for 2 hr.

Incubation of TA(I)-^{14}C with monoamine oxidase (MAO). TA(I)-^{14}C was incubated with beef liver MAO according to the procedure of Ho *et al.*[15] The enzymatic reaction was stopped by addition of 12 N HCl (0·5 ml) and the acidic and neutral products

were extracted with 3 vol. each of ethyl acetate and ether. The combined organic extracts were dried over anhydrous sodium sulfate, filtered and the volume reduced under vacuum. The residue was subjected to paper and thin-layer chromatography.

Identification of metabolites. The components of the radioactive areas separated for quantitative analysis by two-dimensional paper chromatography were identified as described below. Preparative paper chromatography of horizontal bands of urine in Solvent B (Table 1) separated several bands of activity which were cut out and eluted three times with methanol. The three eluates from each band were combined and the volumes reduced under vacuum. Paper chromatography in Solvent A (Table 1) of the eluate from each band produced bands of radioactivity which could be correlated with the radioactive areas in the two-dimensional chromatograms.

The metabolites thus separated were eluted and identified by further paper and thin-layer chromatography and, in some cases, by isotope dilution with known compounds and recrystallization to constant specific activity. Cold carrier was not added since the radioactive metabolites were either present in amounts sufficient for colorimetric detection with spray reagents or were detected by autoradiography. Conjugates were identified by changes in R_f values and color reactions after enzymatic or acid hydrolysis.

pHPAA(II). The material at R_f 0·34 in Solvent A and R_f 0·85 in Solvent B was identified as pHPAA(II). Aliquots of the eluate of this area separated by preparative paper chromatography were rechromatographed in Solvents C, D, E and F on paper, and Solvents F and G on thin-layer plates. The material gave the R_f values and color reactions of pHPAA(II). Enzymatic or acid hydrolysis did not alter the chromatographic characteristics of the eluate. Identification was confirmed by isotope dilution with pHPAA(II) and recrystallization to constant specific activity.

pHPAA(II)glucuronide. The material at R_f 0·04 in Solvent A and R_f 0·30 in Solvent B gave a blue color with naphthoresorcinol spray reagent but no color with diazotized sulfanilic acid. Incubation of the material with ketodase produced a radioactive compound which corresponded to pHPAA(II) on paper and thin-layer chromatograms after spraying with diazotized sulfanilic acid. This metabolite was identified as pHPAA-(II) ether glucuronide.

pHPAA(II)sulfate. The material at R_f 0·10 in Solvent A and R_f 0·30 in Solvent B was treated with ketodase which did not alter the chromatographic characteristics of the material. However, incubation with gluculase produced a compound with R_f values and color reactions to spray reagents identical with those of pHPAA(II). The unhydrolyzed material produced no color with diazotized sulfanilic acid but gave a positive reaction to the sulfate spray sequence. The metabolite was identified as the sulfate conjugate of pHPAA(II).

pHPAG(V). The material at R_f 0·28 in Solvent A and R_f 0·78 in Solvent B corresponded in all solvent systems to authentic pHPAG(V). It gave no color with ninhydrin but produced an orange color with diazotized sulfanilic acid. Incubation with ketodase or gluculase did not alter the chromatographic characteristics of the metabolite, but acid hydrolysis produced two compounds: a radioactive compound which corresponded chromatographically to pHPAA(II) and a nonradioactive compound which gave a purple color with ninhydrin and whose R_f values in Solvents A and B corresponded with those of glycine. Upon chromatography the products of acid-hydrolyzed synthetic pHPAG(V) gave the same color reactions and R_f values as reported above. Isotope dilution of the acid-hydrolyzed and unhydrolyzed aliquots of the radioactive eluate

with pHPAA(II) and recrystallization to constant specific activity showed that pHPAA(II)-^{14}C was present after but not before acid hydrolysis. This metabolite was identified as pHPAG(V).

pHPAG(V)sulfate. The material at R_f 0·10 in Solvent A and R_f 0·20 in Solvent B gave no color with diazotized sulfanilic acid or ninhydrin spray. Its chromatographic properties were not altered by incubation with ketodase, but treatment with gluculase produced pHPAG(V) identified as described above. This metabolite was identified as the sulfate conjugate of pHPAG(V).

Glucuronide conjugates of TOH(III) and NAT(IV). The material at R_f 0·27 in Solvent A and R_f 0·30 in Solvent B gave no reaction with diazotized sulfanilic acid but produced a blue color with naphthoresorcinol spray reagent. Incubation of the eluate with ketodase produced a material which could be resolved by thin-layer chromatography in Solvent F into two aglycones which corresponded to TOH(III) and NAT(IV) and recrystallization to constant specific activity confirmed the identities and relative amounts of these two compounds. The presence of a positive reaction to the phenolic spray only after hydrolysis indicated that the glucuronide moiety was attached to the phenolic oxygen. The metabolites were identified as the glucuronide conjugates of TOH(III) and NAT(IV).

Sulfate conjugates of TOH(III) and NAT(IV). The material at R_f 0·60 in Solvent A and R_f 0·40 in Solvent B gave no reaction with diazotized sulfanilic acid or with ninhydrin spray reagent but gave a positive reaction with the sulfate spray sequence. Treatment with ketodase did not alter the chromatographic properties or the reactions to spray reagents of this material. However, treatment with gluculase produced a material which could be resolved by thin-layer chromatography in Solvent F into two compounds which were identified as TOH(III) and NAT(IV), as described above. The urinary metabolites were identified as the sulfate conjugates of TOH(III) and NAT(IV).

Tentative identification of p-hydroxyphenylacetaldehyde (pHPAld(VI)). The material at R_f 0·93 in Solvent A and R_f 0·88 in Solvent B had R_f values similar to those of TOH(III) and NAT(IV) and, therefore, was subjected to thin-layer chromatography in Solvent F. However, most of the radioactivity was at R_f 0·90, whereas TOH(III) has R_f 0·50 and NAT(IV) has R_f 0·30 in this solvent system. The metabolite was rechromatographed on paper in toluene–ethyl acetate–methanol–water (1:1:1:1, upper phase). The radioactivity was located at R_f 0·93 by scanning the chromatogram. This R_f value corresponded to that reported for pHPAld (VI) in this solvent system.[16] The radioactivity in the chromatograms corresponded with an orange spot produced by spraying with diazotized sulfanilic acid and with a faintly colored spot from 2,4-dinitrophenylhydrazine.

The tentative identification of this metabolite as pHPAld(VI) was supported by the separation of a radioactive compound formed during the incubation of TA(I)-^{14}C with beef liver MAO as described above. Paper chromatography of the residue in Solvent A separated a radioactive compound at R_f 0·90 which was eluted with methanol. On rechromatography on paper in the toluene–ethyl acetate–methanol–water system and on a thin-layer plate in Solvent F, the R_f values of the radioactive compound corresponded to those of the tentatively identified pHPAld(VI). This aldehyde is known to be a product of oxidative deamination of TA(I) by MAO[17] and has been identified as a product of the incubation of TA(I) with partially purified amine oxidase from bovine

plasma.[18] It was therefore considered probable that the radioactive metabolite in the rat urine was pHPAld(VI).

TA(I). The material at R_f 0·79 in Solvent A and R_f 0·60 in Solvent B gave a purple color with ninhydrin and an orange color with diazotized sulfanilic acid and corresponded to TA(I) in all solvent systems. Isotope dilution of the eluate with TA(I) and recrystallization to constant specific activity confirmed identification of the metabolite as TA(I). This identification was also supported by ion-exchange chromatography of the urine; one radioactive compound collected in the amine fraction corresponded to TA(I) in percentage of urinary radioactivity, and on paper and thin-layer chromatograms and in reactions to spray reagents.

TA(I)glucuronide. The material at R_f 0·14 in Solvent A and R_f 0·09 in Solvent B gave a positive reaction with ninhydrin and with naphthoresorcinol spray reagents but no color with diazotized sulfanilic acid. It appeared in the amine fraction when urine was subjected to ion-exchange chromatography. Treatment of this material with ketodase produced a compound with the chromatographic properties of TA(I). This metabolite, therefore, was identified as TA(I)-*O*-glucuronide.

Unidentified metabolites. Two urinary metabolites of TA(I) separated by paper chromatography have not yet been identified. The R_f values on paper chromatography of one metabolite (R_f 0·37 in Solvent A and R_f 0·20 in Solvent B) were not altered by treatment with ketodase but were changed after incubation with gluculase (R_f 0·63 in Solvent B on paper, R_f 0·65 on thin-layer chromatography). The chromatographic characteristics of the other metabolite (R_f 0·41 in Solvent A and R_f 0·37 in Solvent B) were unchanged by treatment with ketodase. The small amount of radioactive compound recovered from this chromatogram was insufficient for further characterization.

RESULTS

Excretion of radioactivity. Rats given a single injection (i. p.) of TA(I)-1-^{14}C HBr (1·5 mg/kg in saline) excrete virtually all of the radioactivity in the urine. Two-thirds of the administered radioactivity is excreted within the first 3 hr and over 98 per cent within 24 hr (Table 2).

Urinary metabolites of TA(I)-1-^{14}C. Thirteen of the 15 urinary metabolites of TA(1)-1-^{14}C have been identified (see Fig. 1) and account for 98·9 per cent of the

TABLE 2. URINARY EXCRETION OF RADIOACTIVITY AFTER ADMINISTRATION OF TYRAMINE-^{14}C

Time (hr)	Cumulative percentage of dose*
3	66·59 ± 22·50
6	88·80 ± 4·88
9	93·27 ± 5·06
12	94·69 ± 4·78
24	96·31 ± 4·05
48	96·67 ± 3·97
96	96·69 ± 3·96

*Mean ± S. D. based on four animals.

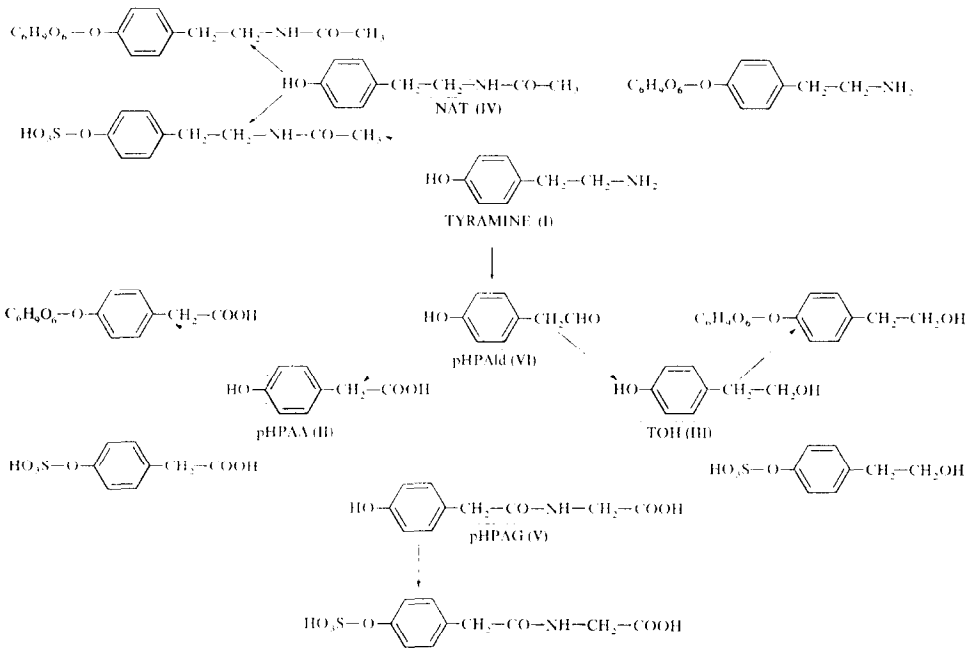

FIG. 1. Urinary metabolites of tyramine excreted by the rat.

TABLE 3. URINARY METABOLITES OF TYRAMINE-^{14}C IN THE RAT

Metabolite	Percentage of urinary radioactivity*
pHPAA(II)	77·0 ± 2·2
pHPAA(II)sulfate	3·19†
	4·2 ± 0·4
pHPAA(II)glucuronide	0·53†
pHPAG(V)	10·5 ± 0·7
pHPAG(V)sulfate	0·7 ± 0·0
TA(I)	1·4 ± 0·3
TA(I)glucuronide	0·6 ± 0·2
NAT(IV)	0·2 ± 0·2
NAT(IV)sulfate	1·5 = 0·2
NAT(IV)glucuronide	1·4 ± 0·2
TOH(III)sulfate	0·6 ± 0·1
TOH(III)glucuronide	0·1 ± 0·1
pHPAld(VI)	0·2 ± 0·2
Unidentified metabolites	
No. 1	0·9 ± 0·4
No. 2	0·2 ± 0·1

*Mean ± S. D. based on four animals.
†Based on one determination.

urinary radioactivity excreted within the first 24 hr (95·6 per cent of the dose). The R_f values in solvents A and B and the average percentage of urinary radioactivity for each metabolite are given in Table 3.

pHPAA(II) and its conjugates account for over 92 per cent of the urinary radioactivity (Table 3). The free acid is the major metabolite and constitutes over three-fourths of the total radioactivity excreted. The sulfate and glucuronide conjugates of pHPAA(II) were incompletely separated by two-dimensional chromatography and, therefore, were estimated together (4·2 per cent of the urinary radioactivity). In one experiment an aliquot of urine was treated with ketodase to hydrolyze the glucuronide before chromatography on paper in two-dimensions. The percentage of urinary radioactivity in the area corresponding to both conjugates of pHPAA(II) was decreased from 4·4 per cent in untreated urine to 3·7 per cent, indicating that the sulfate conjugate was the major metabolite in this area.

The second most abundant metabolite of TA(I) is the glycine conjugate of pHPAA (II), pHPAG(V). This metabolite constitutes 10·5 per cent of the urinary radioactivity and its sulfate conjugate, 0·7 per cent. Other acidic compounds such as homovanillic acid, *p*-hydroxymandelic acid and vanilmandelic acid (metabolites of dopamine, octopamine and norepinephrine respectively) were not detected as urinary metabolites of TA(I).

The neutral metabolites TOH(III) and NAT(IV) are excreted mainly conjugated with sulfate or glucuronic acid. A trace of free NAT(IV) was found but no free TOH-(III) was detected. The total amount of NAT(IV) excreted is over four times that of TOH (III).

Unchanged TA(I) constitutes 2·1 per cent of the urinary radioactivity, 1·5 per cent as the free base and 0·6 per cent as the ether glucuronide. No radioactivity which corresponded to other basic metabolites such as octopamine, dopamine, 3-methoxy-4-hydroxyphenethylamine or norepinephrine could be detected.

DISCUSSION

Rats given TA(I)-1-^{14}C excrete virtually all of the radioactivity in the urine within the first 24 hr, but less than 2 per cent of this radioactivity is unchanged TA(I). Fourteen other compounds, 12 of which have been identified, account for the remaining 98 per cent of the urinary radioactivity. Of these 12 compounds 6 are conjugates which have not been previously identified as metabolites of TA(I), and 3 are conjugates which have been identified only tentatively.

It has been firmly established that pHAA(I) is the major urinary metabolite of TA(I) and our results support those of earlier studies.[6, 8, 19] However, the observation by Schayer that the rat excretes this acid partly conjugated has received little attention. We found that the rat excretes the pHPAA(II) derived from TA(I) mostly as the free acid accompanied by four conjugates: pHPAG(V), pHPAG(V) sulfate, pHPAA(I)-glucuronide and pHPAA(II) sulfate. Our identification of pHPAG(V), which constitutes two-thirds of the amount of conjugated pHPAA(II) excreted, confirms the tentative identification of this compound by Nakijama and Sano.[7] The other three conjugates of pHPAA(II) have not previously been identified as metabolites of TA(I), although pHPAA(II) sulfate has been found as a metabolite of tyrosine-*O*-sulfate in the rat.[13] We found no evidence for the formation of an ester glucuronide of pHPAA-(II).

The aldehyde produced by oxidative deamination of TA(I) by MAO can either be oxidized to pHPAA(II) by aldehyde dehydrogenase or reduced to the corresponding alcohol, TOH(III), by alcohol dehydrogenase. The rat metabolized less than 1 per cent of a dose of TA(I) to TOH(III). This is in contrast to the metabolism of epinephrine[20] and norepinephrine[21] in the rat which metabolizes these amines principally to 3-methoxy-4-hydroxyphenylglycol. Recent evidence shows that β-hydroxylated-phenethylamines such as epinephrine and norepinephrine are preferentially metabolized to the corresponding alcohol by the rat, whereas phenethylamines without the β-hydroxyl group such as TA(I) are metabolized mainly to the corresponding acid.[22] The excretion of TOH(III) after a dose of TA(I) is greatly increased by pretreatment with ethanol.[23]

A very small amount of the urinary radioactivity (less than 0·2 per cent) has been identified tentatively as pHPAld(VI), the aldehyde produced by oxidative deamination of TA(I) by MAO. Previous accounts of the identification of aldehydes in biological fluids include that of protocatechuic aldehyde (3-methoxy-4-hydroxy-phenylacetaldehyde), isolated from the urine of a patient with a pheochromocytoma[24] and 5-hydroxyindoleacetaldehyde sulfate, tentatively identified in the perfusate from an isolated rat liver perfused with a solution containing serotonin-^{14}C.[25] Goodall and Alton[26] found protocatechuic aldehyde in the urine of normal human subjects infused with 3,4-dihydroxymandelic acid but showed that the aldehyde was a degradation product rather than a metabolic product.

β-Hydroxylation of TA(I) produces octopamine, but we were unable to detect this amine or its acidic metabolite, *p*-hydroxymandelic acid, as a metabolite of TA(I) in rat urine. Musacchio *et al.*[27] showed that the rat heart, spleen and salivary glands can β-hydroxylate TA(I)-^{3}H *in vivo*, but the total amount of radioactivity present in these tissues as octopamine was less than 1 per cent of the dose. Masuoka *et al.*[28] also detected octopamine-^{14}C in the tissues of one rat given TA(I)-^{14}C but could not identify octopamine-^{14}C in the urine. However, Lemberger *et al.*[8] reported that rabbits excrete 7·2 per cent of a dose of TA(I) as *p*-hydroxymandelic acid. The difference between our results in the rat and those of Lemberger *et al.* in the rabbit may be related to the relative activity in these two species of the enzyme which catalyzes this reaction; the the rate of β-hydroxylation of TA(I) by a rat tissue homogenate was lower than that from any other species examined by Pisano *et al.*[29] Alternatively, the rat might metabolize octopamine, a β-hydroxylated phenethylamine, to the corresponding alcohol, *p*-hydroxyphenylglycol, rather than to *p*-hydroxymandelic acid after the pattern of norepinephrine metabolism in this species. It is possible that one of the two unidentified metabolites of TA(I) in the rat urine is a conjugate of this glycol; neither of these, however, accounts for more than 1 per cent of the urinary radioactivity.

The aromatic ring of TA(I) can be hydroxylated to produce dopamine, but we were unable to detect this amine or its principle urinary metabolite, homovanillic acid, as a metabolite of TA(I) in the rat, although 0·55 per cent of a dose of TA(I) given to rabbits has been reported to be excreted as homovanillic acid.[30]

Acknowledgements—The authors wish to thank Mrs. Annie Rougeaux for her skilful technical assistance and Dr. B. T. Ho, Section of Medicinal Chemistry, Texas Research Institute of Mental Sciences, Houston, Texas, for supplying us with pHPAG(IV).

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