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METABOLISM OF 2-(2-THIENYL)ALLYLAMINE HYDROCHLORIDE IN THE RAT: IDENTIFICATION OF A NOVEL METABOLITE

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A novel metabolite, 2-(2-thienyl)propionic acid, is formed **in vivo** from 2-(2-thienyl)allylamine hydrochloride. Mass spectral analysis suggested 2-(2-thienyl)propionic acid formation involves loss of the amine moiety followed by reduction of the olefinic group. © 1987 Academic Press, Inc.

2-(2-Thienyl)allylamine(2-TAA) is currently under investigation because it rapidly inactivates dopamine-β-hydroxylase in a mechanism-based fashion(1,-2). 2-TAA is well absorbed and extensively metabolized. We report the isolation and identification of 2-(2-thienyl)propionic acid (2-TPA) as the major metabolite of 2-TAA. We also propose mechanisms for its formation.

MATERIALS AND METHODS

2-Acetylthiophene and tosylmethyl isocyanide were purchased from Aldrich Chemical Company. All organic solvents were glass-distilled HPLC grade. BSTFA (Bis-(trimethylsilyl)trifluoroacetamide) was obtained from Regis Chemicals. 2-TAA and C-14 2-TAA were synthesized at Merrell Dow Pharmaceuticals Inc., Indianapolis, IN.

2-TPA was synthesized following the method developed by Schöllkopf (3,4) (Fig. 1). 2-Acetylthiophene (6.3 g, 0.05 mol) was treated with 1-equivalent of the anion of tosylmethyl isocyanide generated from tert-butoxide in THF. The crude reaction mixture after work-up was purified by flash chromatography (ethyl acetate:hexane, initially 1:3 then 1:1) to provide 2 (3.2 g, 20%, mp 138-140°C (EtOH)). 2 was hydrolyzed to 2-TPA with ethanol:2N HCl (1:2) at reflux for 15 h under N_2 atm. Kugelkohr distillation provided 2-TPA as a colorless oil, bp 110°C (2 mm), Lit(4) bp 116-118°C (5 mm). NMR($CDCl_3$) δ1.58 (d, 3H, J=7Hz), 3.98 (q, 1H, J=7Hz) and 6.9-7.2 (m, 3H).

Animals. Male Sprague-Dawley (CD(SD)BR) and SHR rats weighing 225 to 250 g were used for the metabolic experiments and allowed free access to food and water throughout the experiment. The rats were housed in Roth-like metabolism chambers. Single oral or intravenous 100 mg/kg doses of 2-TAA were administered to two groups of three rats. Urine, feces and volatiles were collected over a 36-h period. The urine was pooled from 0-18 h and 18-36 h, and stored at -20°C until analyzed. Urines were filtered through Millex-GS 0.22 μm filter units.

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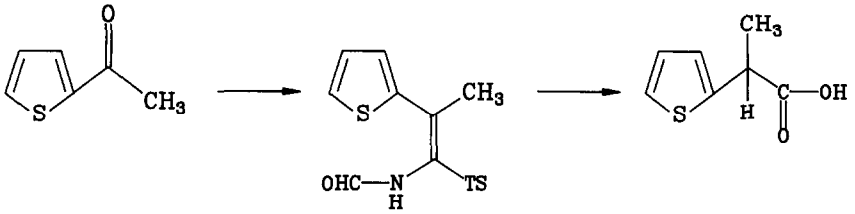

2-Acetylthiophene E/Z 2-TPA

Figure 1. Synthetic scheme for 2-TPA formation.

HPLC Assay. A gradient program was set up for the chromatographic profiling with C-14 detection. Filtered urines were injected directly onto a C-18 ODS column. The gradient conditions were:

mobile phase:	0.1 M ammonium formate A
	CH_3CN B
flow rate:	1.0 mL/min
gradient:	100% (A) to 60% (A) linearly

Isolation. The extraction of urine was carried out by acidifying with 0.5 N HCl (0.5 mL) and shaken for 10 min with ethyl acetate (4 mL). The organic layer was separated after centrifugation. The aqueous was re-extracted. The combined organic phase was back-extracted into 0.5 N NaOH (1 mL). The organic was discarded and the aqueous layer acidified with 0.5 N HCl (1.5 mL). The aqueous layer was extracted with ethyl acetate (4 mL). The organic layer was transferred and dried over Na_2SO_4 anhydrous. The organic was then evaporated to dryness under a N_2 stream at room temperature. The residue was derivatized with BSTFA (100 µL) at 60°C for 30 minutes. The solution was diluted with EtOAc (100 µL). This sample was then analyzed directly by GC/MS.

GC/MS Analysis. The derivatized sample was analyzed by a Finnigan MAT 4500 GC/MS system and on-line to an INCOS data system. Analyses were performed in the chemical ionization mode with methane as a reagent gas at 1 torr. The ion source, GLC interface and injection port temperatures were maintained at 120, 260, 250°C, respectively. Samples were introduced via the GC inlet, through which the column was interfaced directly to the ion source of the mass spectrometer. A fused silica capillary column DB-5 (J&W Scientific, Ventura, CA, 10 m x 0.32 mm ID) was used and samples were injected in the splitless mode at 50°C. For the analysis of the TMS derivative of 2-TPA, the column oven temperature was raised linearly at 10°C/min to 250°C. Retention times were measured relative to a homologous series of n-alkanes co-injected with each sample and calculated as methylene unit (MU) values.

RESULTS AND DISCUSSION

When 0-18 h urines from rats given 2-TAA either po or iv were analyzed by HPLC, one major radioactive peak was observed (Fig. 2). The major radioactive peak was extracted from the urine acidified with 0.5 N HCl. No radioactivity was extracted when the urine was basic.

The major radioactive peak was derivatized with BSTFA and positively identified as 2-TPA by its GC/MS properties (Fig. 3,4). This spectrum

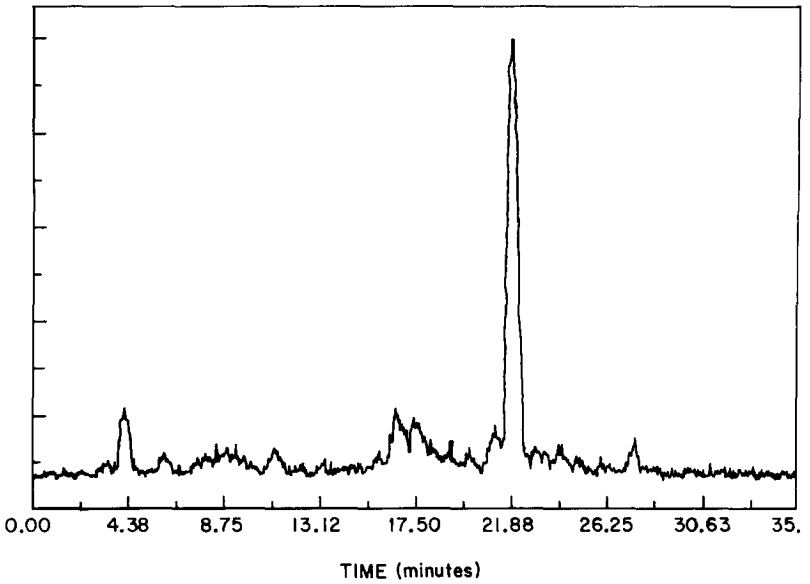

Figure 2. ^{14}C-HPLC profile of urine from a rat given ^{14}C-2-TAA.

exhibited a MH^+ ion at **m/z** 229 accompanied by prominent $[MH^+-CH_4]$ ion at **m/z** 213, $[MH^+-TMSOH]$ ion at **m/z** 139 and $[MH^+-HCO_2TMS]$ ion at **m/z** 111 (Fig. 4a). An authentic standard of 2-TPA was found to have mass spectral data, GC retention properties and partition behavior identical to the biologically-derived material (Fig. 4b).

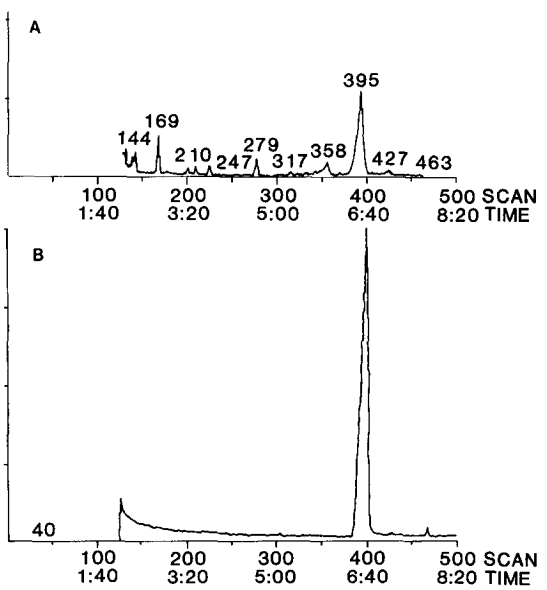

Figure 3. Total ion chromatograms of derivatized extracted urine from rat given 2-TAA A) and derivatized synthetic 2-TPA B).

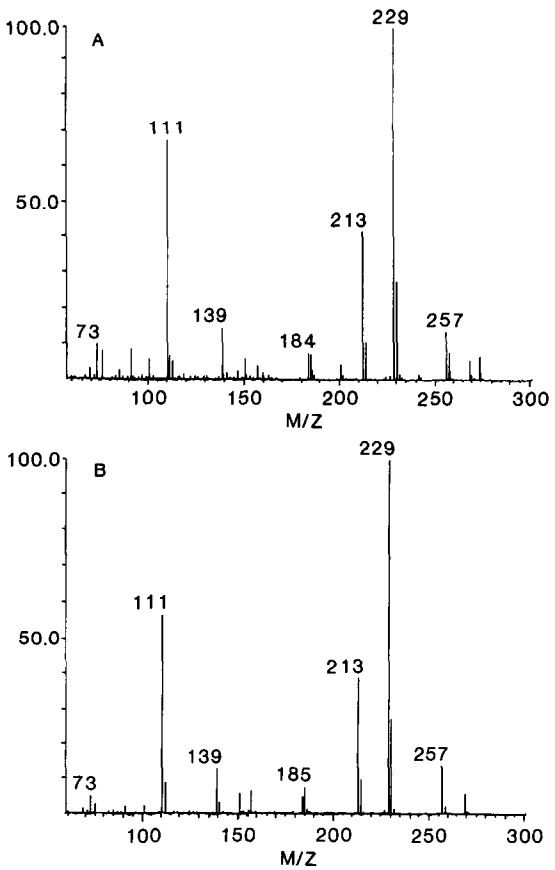

Figure 4. CI mass spectra of biologically-derived 2-TPA A) and synthetic 2-TPA B).

The isolation of 2-TPA as the major metabolite of 2-TAA presents a novel pathway for the metabolism of an allylamine. The formation of 2-TPA **in vivo** from the rat can be rationalized by the pathways presented in Fig. 5. Pathway A represents N-dealkylation of 2-TAA mediated by cytochrome P-450 giving the carbinolamine followed by a breakdown to yield the α, β unsaturated aldehyde. Pathway B represents oxidative deamination of 2-TAA mediated by an amine oxidase yielding the same intermediate as in pathway A. Further oxidation of this intermediate by aldehyde dehydrogenase (ALDH) would give the α, β unsaturated acid. Neither product has been identified but are currently under investigation. Other metabolites, such as the diols, have not been detected.

The next step in the generation of 2-TPA can be explained using an aryl propionic acid isomerase involved in lipid catabolism and anabolism(5,6).

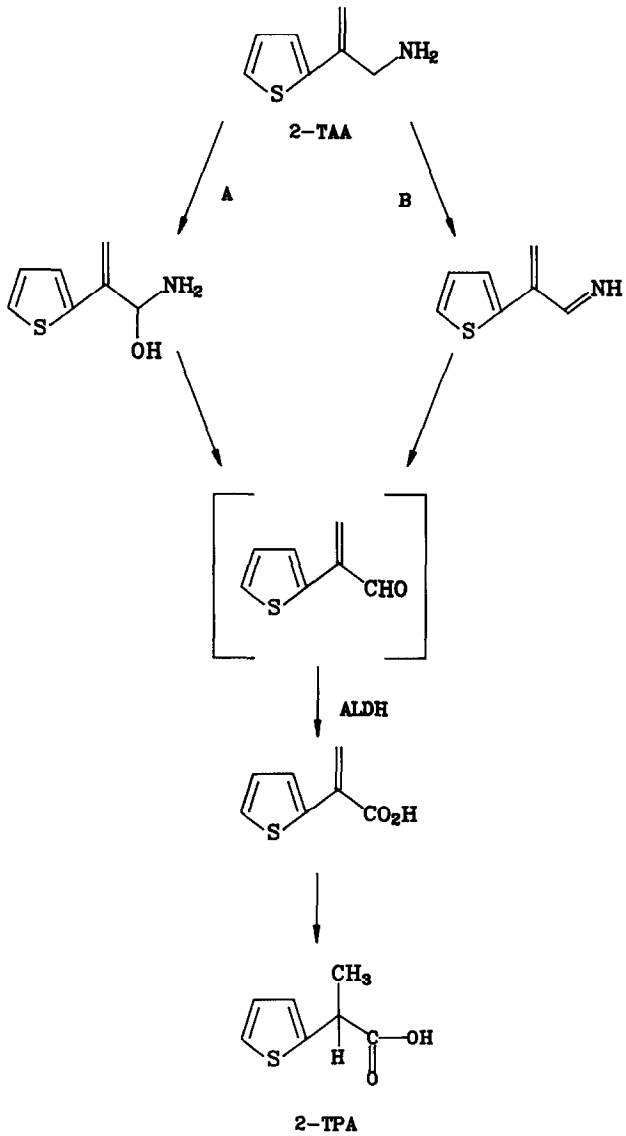

Figure 5. Proposed mechanism for the **in vivo** conversion of 2-TAA to 2-TPA.

Ibuprofen is epimerized at the saturated carbon by this isomerase. A common intermediate in this process was the α, β unsaturated acid derivative which was subject to an enoyl reductase.

We propose that enoyl reductase reduces our unsaturated acid in a similar manner to give the major metabolite, 2-TPA.

Studies on the formation of 2-TPA and several other metabolites of 2-TAA **in vitro** are currently in progress to better define these mechanisms.

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