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Replacement of a Chlorine with a Methylsulfonyl Group in the Metabolism of Propachlor (2-Chloro-*N*-isopropylacetanilide)

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Abstract—Urine from rats and sheep given single oral doses of [^{14}C]propachlor contained ^{14}C metabolites in which the chlorine of propachlor was replaced by a methylsulfonyl group. Methylsulfonyl-containing metabolites were also isolated from the urine of rats given an intraperitoneal dose of the cysteine conjugate of [^{14}C]propachlor; this indicated that the methylsulfonyl-containing metabolites resulted from metabolic reactions subsequent to the mercapturic acid pathway.

Introduction

LAMOUREUX and Davison[1] have shown the mercapturic acid pathway to be important in the metabolism of propachlor by rats (Scheme 1). We have isolated and identified rat and sheep urinary metabolites from propachlor in which the chlorine was replaced by a methylsulfonyl moiety.

Experimental

ANIMAL EXPERIMENTS

A ewe and rats were dosed orally with 2-chloro-*N*-isopropylacetanilide that was uniformly labeled with ^{14}C in the ring ([^{14}C]propachlor). The ewe (54.8 kg) was given 20 mg kg^{-1} of [^{14}C]propachlor as a single oral dose. The dose contained 0.5 mCi of ^{14}C. Rats (10; average weight 200 g) were each given 10 mg of [^{14}C]propachlor by stomach tube. Each rat received 2 μCi of ^{14}C. Rats (10; average weight 215 g) were each given 2.1 mg of the cysteine conjugate of [^{14}C]propachlor by intraperitoneal injection (physiological saline). Each rat received 0.7 μCi of ^{14}C. The [^{14}C]cysteine conjugate was isolated from sheep urine by the methods outlined in Fig. 1.

METABOLITE ISOLATION AND DERIVATIZATION

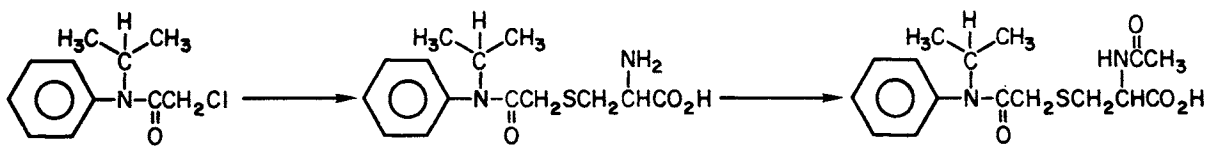
The methylsulfonyl-containing metabolites from both rat and sheep urine and the cysteine conjugate of propachlor were isolated by the methods outlined in Fig. 1. Column A was a 1.9 cm × 150 cm column of water-equilibrated Sephadex LH-20 (Pharmacia). Samples (4 ml) were applied to the column, and the radioactivity was eluted with water. Column B was prepared by first pouring water-equilibrated LH-20 to form a 0.9 cm × 110 cm column. The column was then equilibrated with two column volumes (approx. 50 ml) of 0.06 M $(NH_4)HCO_3$. Samples (1–2 ml) were applied to the column and the ^{14}C was eluted from the column with 0.06 M $(NH_4)HCO_3$. Porapak Q (Waters Assoc.) was slurried with methanol and poured to form a 1 cm × 10 cm column. The column was washed with water, the sample applied in water and the column eluted with three column volumes of water. The radioactivity was eluted with methanol.

Before gas chromatography (g.l.c.), metabolites **1**, **2** and **3** were treated with bis-(trimethylsily)trifluoroacetamide containing 1% trimethylchlorosilane (BSTFA) at room temperature for 18 h (TMS derivatives). Diazomethane was used to prepared methyl derivatives of **1** (CH_3-**1**) and **3** (CH_3-**3**).

Aglycons from sheep urine glucuronides were obtained by hydrolysis with glucuronidase.[2]

INSTRUMENTATION

For g.l.c. we utilized a 6 ft × $\frac{1}{8}$ in. i.d. glass column packed with 3% SE-30 on 60/80 mesh Chromasorb W in a Perkin–Elmer 801 gas chromatograph fitted with an effluent splitter. Ten percent of the column effluent went to the flame ionization detector and 90% was trapped in glass tubes for radioactivity detection and/or instrumental analysis. The carrier gas was helium at a



SCHEME 1. Metabolism of propachlor to the cysteine and mercapturic acid conjugates.[1]

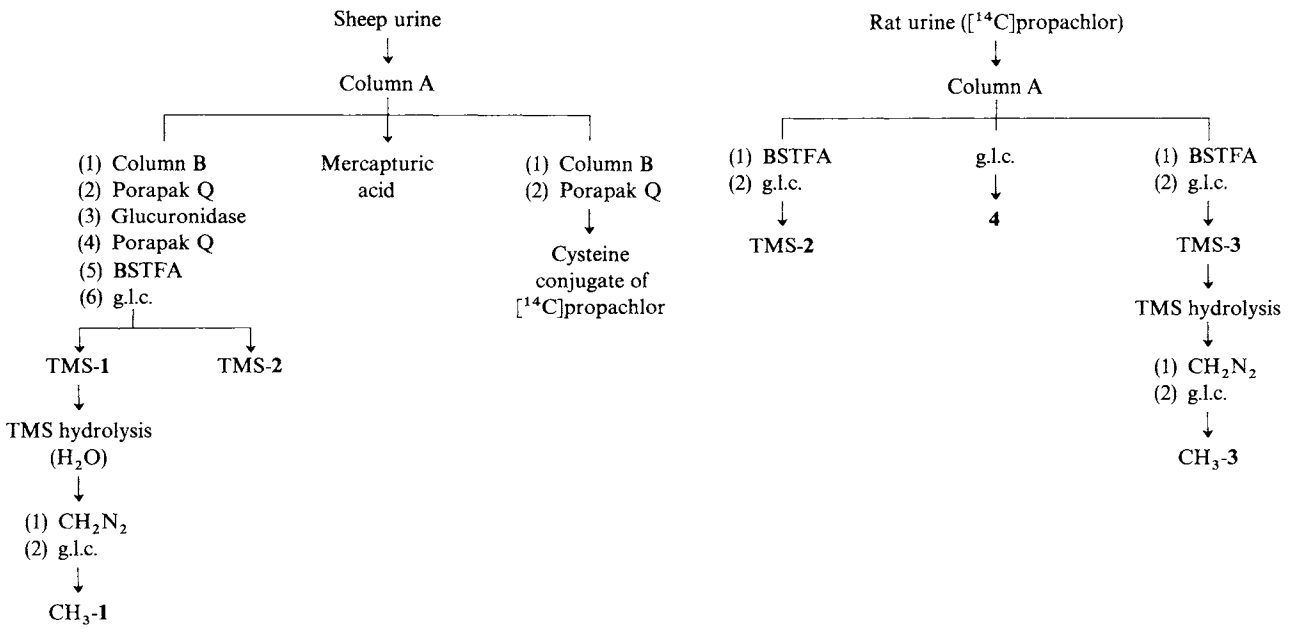


FIG. 1. Flow diagram of the methods used for the isolation of propachlor metabolites from sheep and rat urine.

flow rate of 30 ml per min. The oven temperature was programmed at 10 °C per min from 100–250 °C. The injector was maintained at 200 °C. The detector was located in the column oven.

Mass spectra were obtained using the solid sample inlet probe of a Varian MAT CH-5 DF mass spectrometer equipped with a peak matching unit.

Nuclear magnetic resonance spectra were taken with a Varian A-60A spectrometer in either $CDCl_3$ or acetone-d_6. Melting points were taken with a Thomas–Hoover capillary melting apparatus and are uncorrected.

SYNTHESIS OF METHYLSULFONYLACETAMIDES

N-Isopropylanisidines were prepared by the procedure used by Young *et al.*[3] for the preparation of *N*-isopropyl-*o*-toluidine. Distillation at reduced pressure yielded mixtures of the desired compounds and diisopropyl anisidines. These were not separated because purification was more easily accomplished after subsequent reactions.

Methylthioacetic acid was prepared by slowly adding sodium methanethiolate (16 g methylmercaptan and 33.7 g of 57% sodium hydride in 200 ml of 1:1 tetrahydrofuran + dimethylformamide) to chloroacetic acid (31.5 g in 100 ml of 1:1 THF + DMF). Normal work-up yielded material distilling at 108–112 °C at 15 Torr (Lit.[4] 107 °C at 0.5 Torr) which was oxidized by alkaline potassium permanganate[4] to yield methylsulfonylacetic acid, m.p. 112–115 °C from ethyl acetate + hexane (Lit.[4] m.p. 115 °C).

The method of Chan and Wong[5] was modified for the preparation of methylsulfonylacetamides. Silicon tetrachloride (0.17 g) was stirred into a solution of 0.09 g of aniline and 0.14 g of methylsulfonylacetic acid in 10 ml of dry pyridine. After the silicon tetrachloride was added, the mixture was stirred at 100 °C for 3 h, then cooled and poured into ether. The solid was removed by filtration and the solvents were removed at reduced pressure. The product was recrystallized from ether + hexane to yield 2-(methylsulfonyl)acetanilide, m.p. 125–127 °C. The following compounds were prepared by this method (m.p. in parentheses): 2′-methoxy-2-(methylsulfonyl)acetanilide (120–122 °C), 3′-methoxy-2-(methylsulfonyl)acetanilide (114–117 °C), 4′-methoxy-2-(methylsulfonyl)acetanilide (147–149 °C), 2′-methoxy-*N*-isopropyl-2-(methylsulfonyl) acetanilide (liquid), 3′-methoxy-*N*-isopropyl-2-(methylsulfonyl)-acetanilide (liquid), 4′-methoxy-*N*-isopropyl-2-(methylsulfonyl)acetanilide (69–72°C). Appropriate infrared absorptions were found for carbonyl (1640–1680) cm^{-1}) and sulfonyl (1115–1155 and 1305–1335 cm^{-1}) groups.[6] The ^1H n.m.r. absorptions were at δ 3.16–3.19 for SO_2CH_3, 3.69 for isopropyl–N–CO–CH_2–SO_2, and 4.10–4.34 for H–N–CO–CH_2–SO_2.

Results and discussion

In addition to the mercapturic acid and cysteine conjugates of propachlor (Scheme 1),[1] radioactive methylsulfonyl-containing metabolites (**1**, **2**, **3** and **4**, see below) were isolated from the urine excreted by a

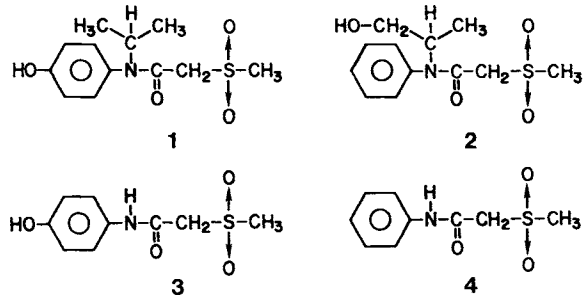
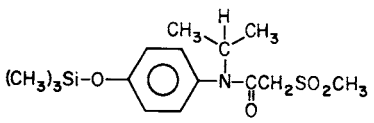
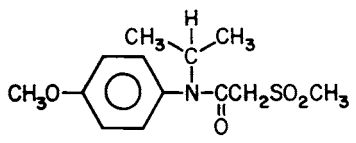
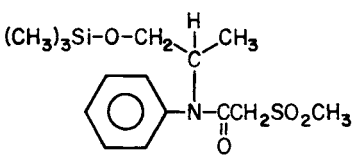
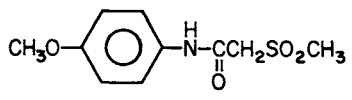
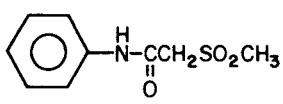


TABLE 1. Mass Spectra

m/e	Rel. int. (%)	Fragment ion description
		
		TMS-1
343.12708	50	$[M]^{\ddagger}$
328	6	$[M - CH_3]^+$
301.08048	41	$[M - CH_2{=}CH{-}CH_3]^{\ddagger}$
264.14319	23	$[M - S(O_2)CH_3]^+$
222.13093	17	$[M - C(O)CH_2S(O)_2CH_3]^+$
222.09414		$[301 - S(O)_2CH_3]^+$
208	33	$[301 - CH_2S(O)_2CH_3]^+$
206	28	
192.08453	13	$[TMS{-}O{-}C_6H_4{-}N{\equiv}CH]^{\ddagger}$
192.04760		$[(CH_3)_2Si{-}O{-}C_6H_4{-}N{=}C{=}O]^+$
182	43	
181	18	
180	16	
162	15	
137	22	
120.04426	12	$[HO{-}C_6H_4{-}N{\equiv}CH]^{\ddagger}$
79	5	$[S(O)_2CH_3]^+$
75	18	
73	100	$[(CH_3)_3Si]^+$
		
		CH_3-1
285	52	$[M]^{\ddagger}$
270	9	$[M - CH_3]^+$
243	48	$[M - CH_2{=}CH{-}CH_3]^{\ddagger}$
206	45	$[M - S(O)_2CH_3]^+$
164	90	$[243 - S(O)_2CH_3]^+$
163	10	
162	40	
150	100	$[243 - CH_2S(O)_2CH_3]^+$
149	50	
148	20	
136	75	$[NH{-}C(O){-}CH_2{-}S(O)_2CH_3]$
135	20	
134	95	$[CH_3O{-}C_6H_4{-}N{\equiv}CH]^{\ddagger}$
133	20	
124	40	
123	85	$[CH_3O{-}C_6H_4{-}NH_2]^{\ddagger}$
122	55	
121	28	
120	25	
108	45	
107	22	
95	25	
92	38	
91	50	
79	70	$[S(O)_2CH_3]^+$
		

m/e	Rel. int. (%)	Fragment ion description
		TMS-2
343 (343.12649)	0.5	$[M]^{\ddagger}$
328.10302	9	$[M - CH_3]^+$
286.09295	10	$[M - C_3H_5O]^+$
264	5	$[M - S(O)_2CH_3]^+$
240.06938	70	$[M - (CH_3)_3SiOCH_2]^+$
214.05507	9.5	
213.14755	8.5	$[M - (CH_3)_3SiOCH{=}CHCH_3]^{\ddagger}$
192	1.5	
190	1.5	
174	9	
162	3	
130	12	
120.08091	100	$[C_6H_5{-}NH{=}CHCH_3]^+$
104	14	$[C_6H_5{-}N{\equiv}CH]^{\ddagger}$
93	5	
91	8	
79	8	$[S(O)_2CH_3]^+$
77	10	$[C_6H_5]^+$
75	8	
73	70	$[(CH_3)_3Si]^+$
		
		CH_3-3
243	100	$[M]^{\ddagger}$
228	3	$[M - CH_3]^+$
164	41	$[M - S(O)_2CH_3]^+$
150	8	$[M - CH_2S(O)_2CH_3]^+$
149	14	
136	38	$[HNC(O)CH_2S(O)_2CH_3]^+$
135	4	
134	7	
133	13	
132	8	
123	85	$[CH_3O{-}C_6H_4{-}NH_2]^{\ddagger}$
122	42	
121	22	
108	89	$[CH_3O{-}C_6H_4]^+$
95	26	
94	16	
79	23	$[S(O)_2CH_3]^+$
		
		4
213	42	$[M]^{\ddagger}$
134	1.3	$[M - S(O)_2CH_3]^+$
133	0.8	
121	2.5	
120	0.8	
119	1.0	
106	7	
104	1	
93	100	$[C_6H_5{-}NH_2]^{\ddagger}$
79	12	$[S(O)_2CH_3]^+$
77	10	$[C_6H_5]^+$

sheep or rats given single oral doses of [^{14}C]propachlor. The *O*-methyl derivatives of **1** and **3** (CH_3-**1** and CH_3-**3**) and metabolite **4** gave mass spectra (Table 1) and infrared spectra identical to the spectra obtained from the synthesized standards. Comparison of the mass spectra from the TMS derivatives of metabolites **1** and **2** (Table 1) indicated that they were isomeric. Failure to react with diazomethane proved that metabolite **2** was not a phenol and suggested that the site of oxidation was the *N*-isopropyl group. The TMS derivative of metabolite **2** yielded an $[M\text{-}103]^+$ ion at *m/e* 240 $\{[M - (CH_3)_3SiOCH_2]^+\}$; this suggested the presence of a TMS ether of a primary alcohol.[7] The ion at *m/e* 213 ($[M - TMSOCH{=}CH{-}CH_3]^{\cdot+}$) provided further evidence that the TMS ether was located on the *N*-isopropyl group. The ions at *m/e* 79 ($[SO_2CH_3]^+$) and 264 ($[M - SO_2CH_3]^+$) supported the presence of the methylsulfonyl moiety. No attempt was made to synthesize **2**.

Compounds **1** and **2** were isolated from the glucuronidase hydrolyzate of a radioactive fraction separated from sheep urine; compounds **2**, **3** and **4** were isolated from radioactive fractions from rat urine (Fig. 1). These methylsulfonyl metabolites accounted for 10–17% of the dose given to the sheep and 30–35% of the dose given to rats. Therefore, these compounds resulted from a major route of metabolism in both the sheep and rat. Because the mercapturic acid pathway is important in the rat metabolism of propachlor,[1] we thought that these methylsulfonyl metabolites probably resulted from further metabolism of the mercapturic acid or cysteine conjugates of propachlor. To test this possibility, rats were given intraperitoneal doses of the cysteine conjugate of [^{14}C]propachlor. Twenty percent of the dose was present in the urine as **2** and **3** which showed that the cysteine conjugate was metabolized to methylsulfone-containing metabolites. This metabolic pathway will be investigated with ^{35}S labeled cysteine conjugate to establish the source of the sulfur in these methylsulfonyl metabolites.

REFERENCES

1. Lamoureux, G. L.; Davison, K. L. *Pest. Biochem. Physiol.* **1975**, 5, 497.
2. Paulson, G. D.; Zehr, M. V. *J. Agric. Food Chem.* **1971**, 19, 471.
3. Young, W. G.; Caserior Jr., F. F.; Brandon Jr., D. D. *J. Am. Chem. Soc.* **1960**, 82, 6163.
4. Mellander, A. *Sven. Kem. Tidskr.* **1934**, 46, 99; *Chem. Abstr.* **1934**, 28, 5408.
5. Chan, T. H.; Wong, L. T. L. *J. Org. Chem.* **1969**, 34, 2766.
6. Bellamy, L. J. *The Infra-red Spectra of Complex Molecules*, 2nd edn. Wiley: New York. **1958**, pp. 205 and 350.
7. Pierce, A. E. *Silylation of Organic Compounds*. Pierce Chemical Co.: Rockford, Illinois. **1968**, pp. 36–39.