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Absorption, Distribution and Metabolism of Epoxystearic Acid in the Rat

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This study is part of a continuing program to assess the toxic potential of chemicals present as contaminants in pulp mill effluents. cis-9,10-Epoxystearic acid, chlorinated guaiacols and resinous acids are examples of such contaminants which have been found in river water and have been shown to be toxic to fish (LEACH and THAKORE 1975). Since the organic contaminants present in river water may also appear in drinking water, it is desirable to have information on the toxicity of these chemicals in animals. Reports from our laboratories have indicated that chlorinated guaiacols and resinous acids possess a low order of toxicity (CHU et al. 1978a; VILLENEUVE et al. 1977). This communication deals with the absorption, distribution and metabolism of cis-9, 10-epoxystearic acid (abbreviated as epoxystearic acid) in the rat.

MATERIALS AND METHODS

Synthesis of Epoxystearic Acid (carboxyl ^{14}C): Oleic acid (carboxyl ^{14}C, 1 mCi), purchased from ICN Pharmaceuticals (Irvine, California), had a specific activity of 30 mCi/m mol and radiochemical purity of 98%. This chemical was diluted with unlabelled oleic acid (704 mg) and was allowed to react with m-chloroperoxybenzoic acid (509 mg, 85% pure) in chloroform solution at 10-12° for 4 h. The reaction mixture was then kept in freezer overnight to permit chlorobenzoic acid to precipitate out of the solution. The precipitate was filtered off and the solution was washed with 5% sodium sulfite solution (50 mL), followed by water. Evaporating the solvent resulted a semi-solid which, after recrystallization from hot hexane, yielded 400 mg of epoxystearic acid as a white crystal. mp. 56-58°, lit. mp. 58-59° (LEACH and THAKORE 1975). The specific activity was 1.28 μCi/mg as determined by a liquid scintillation counter. Radiochemical purity was found by TLC and radio-GC to be greater than 97%.

Male Sprague Dawley rats (200-225 g), purchased from Biobreeding Laboratories (Ottawa), were acclimatized to laboratory

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conditions for one week before experiments. All animals had free access to food (Master feed) and water during the experimental period.

The jugular veins of four rats were cannulated with Silastic tubing (0.086 cm ID, 0.165 cm OD). Three days after cannulation these animals were given single oral doses of ^{14}C-epoxystearic acid in corn oil (20 μCi/kg, 15.6 mg/kg) by a stomach tube. Serial blood samples were withdrawn from the cannulae at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 h after intubation for the estimation of radioactivity. After the last samples were taken, the animals were anesthetized with ether and exsanguinated. Tissues were excised for the analysis of radioactivity. The methods employed for radioassay of the blood, tissues, urine and feces were similar to those previously reported (CHU et al. 1978b).

For the tissue distribution studies two groups of five rats were dosed by gavage with single oral doses of ^{14}C-epoxystearic acid in corn oil (20 μ Ci/kg, 15.6 mg/kg). These animals were housed individually in metabolism cages for the separate collection of feces and urine. One group was sacrificed at 24 h and the other at 7 days after oral administration for the analysis of ^{14}C.

Seven rats were dosed with ^{14}C-epoxystearic acid (20 μ Ci/kg, 15.6 mg/kg) in corn oil by gavage and kept individually in metabolism cages (Jencons, Model Mark III, England). The urine, feces and expired air were collected for the estimation of radioactivity. The exhaled air was trapped in a flask filled with 600-mL solution of ethanolamine/cellusolve (1:2). One mL aliquot was pipetted, dissolved in Dimulune-30 and counted for radioactivity. Two autopsies for the purpose of harvesting tissues were carried out, one at 6 h (4 rats) and the other at 7 days (3 rats) after dosing.

To investigate the biliary secretion of epoxystearic acid, the common bile ducts of two rats were cannulated with Silastic tubing (0.031 cm ID, 0.064 cm OD). These animals were administered single intravenous doses via the penis veins of ^{14}C-epoxystearic acid (10 μ Ci/kg, 7.8 mg/kg) dissolved in emulphor/ethanol/normal saline (1:1:8). Hourly bile samples were collected for the estimation of ^{14}C. Urine, bile and feces samples were analyzed for possible metabolites. Techniques of separation and identification of metabolites were similar to those described elsewhere (CHU et al. 1978b).

RESULTS AND DISCUSSION

Absorption of epoxystearic acid from the gastro-intestinal tract was found to be very rapid. ^{14}C could be detected in the blood as

TABLE I

Tissue distribution of radioactivity in rats after single oral dose of ^{14}C-epoxystearic acid (20 Ci/kg, 15.6 mg/kg) in corn oil. Data represents ug equivalent of epoxystearic acid/g wet tissue (mean ± S.D.)[1].

	A	B	C	D
Liver	19.5±3.2	6.7± 0.3	5.3±1.2	1.5±0.3
Fat	10.2±5.6	30 ±19.8	18 ±6.3	3.8±3.3
Kidney	7.0±0.9	5.8± 0.43	4.0±0.93	2.1±0.30
Lung	6.5±0.7	4.8± 0.78	4.3±1.0	1.8±0.34
Spleen	5.4±0.59	4.5± 0.37	3.5±0.67	1.2±0.40
Pancreas	5.0±1.3	3.9± 0.67	3.5±0.59	1.4±0.31
Bladder	3.6±0.7	3.3± 0.73	2.7±1.2	1.3±0.61
Heart	3.4±0.13	2.7± 0.53	2.4±0.32	1.2±0.2
Skin	3.2±0.27	3.2± 1.3	3.9±2.4	1.8±0.83
Muscle	1.7±0.23	1.1± 0.1	1.5±0.55	0.76±0.21
Testes	1.4±0.21	1.1± 0.13	1.0±0.15	0.74±0.11
Brain	1.0±0.13	0.78±0.12	0.82±0.1	0.87±0.24
Blood	2	5.8 ± 2.4	2.1 ± 0.74	0.61 ± 0.24

[1]Tissues were obtained from groups of four animals killed at 6 h (A), 24 h (B), 48 h (C) and 7 days (D) after oral dosing.

[2]Blood sample was not analyzed.

early as 15 min after oral administration. The blood concentration of ^{14}C reached its peak (5.61 ± 1.37 ppm) at 1 h after dosing and rapidly declined. The radioactive material was well distributed in all tissues (Table I). It may be noted in 6 h tissues that the liver possessed the highest concentration of ^{14}C, followed by the fat, kidney, lung, spleen, pancreas, bladder, heart, skin, muscle, testes and brain. Twenty-four h after dosing the fat had accumulated the highest concentration whereas liver ^{14}C had declined. The order of accumulation of ^{14}C was the same for 24-h, 48-h and 7-day tissues with the fat having the highest concentration.

Epoxystearic acid was excreted primarily as carbon dioxide in the exhaled air. Six h following oral administration 43% of the dose was converted to CO_2. Approximately 75% was removed in the expired air in 5 days (Figure I).[2] Analysis of the urine and feces showed that 6.4 ± 1.2% and 10.6 ± 4.3% of the oral doses were excreted via the two routes respectively. Radio-assay of the bile showed that the biliary secretion accounted for 3.8 ± 0.75% of the doses, and thus this route played a minor role in the excretion of epoxystearic acid.

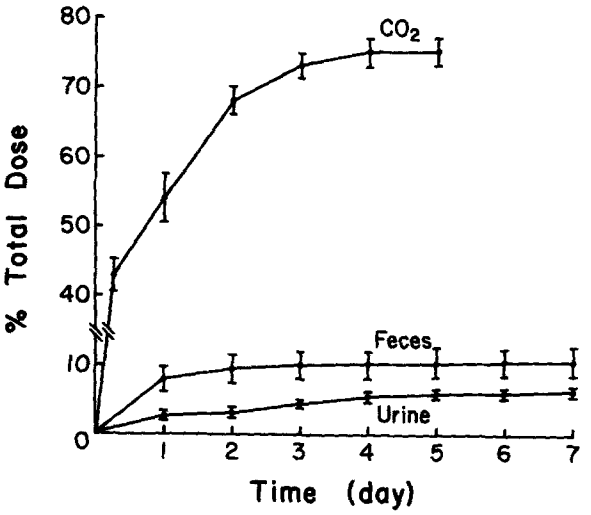
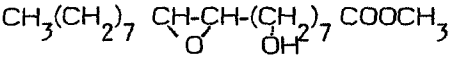


Figure I. Cumulative excretion of ^{14}C in the urine, feces and exhaled air after a single oral dose of 9,10-epoxystearic acid-^{14}C (20 μCi/kg, 15·6 mg/kg). Each point represents mean ± S.D. of three or more animals.

Carbon dioxide was the major metabolite of epoxystearic acid amounting to 75% of the dose. Radio-gas chromatography of the methylated urinary extract demonstrated two GC peaks containing radioactivity. These two peaks were further characterized by GC/MS. The mass spectrum of the major peak (90% of the total area retention time, 2.1 min) had fragments at m/e 199, 155 and 109, and the molecular ion 312 was missing. The MS characteristics were the same as those resulting from the fragmentation of the methyl ester of epoxystearic acid. Thus, this peak was unequivocally identified as epoxystearic acid. The minor peak (10% of the total area retention time, 4.8 min) had MS fragments at m/e 215 and 155. The ion at m/e 215 could be attributed to $HO(CH_2)_7COOCH_3$, which was derived from a methyl ester of hydroxylated epoxystearic acid.



The position of hydroxylation could not be ascertained in this experiment. In view of the fact that CO_2 and a hydroxylated derivative were detected, β-oxidation was likely to be responsible for the metabolism of epoxystearic acid. Only unchanged epoxystearic acid was found in both feces and bile.

CONCLUSION

Epoxystearic acid was rapidly absorbed after oral administration and well distributed in all tissues, mainly in the fat and liver of rats. Since this compound was metabolized to CO_2 and was removed in the expired air, little or no bioaccumulation could be expected.

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