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2-Chloroethylstearate: An In Vivo Fatty Acid Conjugate of 2-Chloroethanol

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2-Chloroethanol is used as a solvent and an intermediate in the production of organic chemicals. The presence of 2-chloroethanol has also been reported in the food treated with ethylene oxide (Stijve et al., 1976; Pfeilsticker and Leyendecker, 1978; Heikes and Griffitt, 1979; Bruns and Currie, 1983), as well as in medical supplies (Muzeni, 1985; Merckx and Kinget, 1986; DeRudder et al., 1986). Metabolism of several compounds such as 1,2-dichloroethane, 2,2-dichloroethyl ether and 2-chloroethyl nitrosoureas [a family of anti-carcinogenic drugs] (Yllner, 1971; Guengerich et al., 1980; McCall et al., 1983; Norpoth et al., 1986; Madelmont et al., 1982; Godenech et al., 1982; Reed et al., 1985) results in the formation of 2-chloroethanol. ^{14}C-Labeled 2-chloroethanol when given to rats, was excreted mainly in urine (Grunow and Altmann, 1982) but a significant amount of radioactivity remained in liver even after four days of the treatment.

It has been shown that compounds containing hydroxyl function can be retained in the body for a longer time as a fatty acid conjugate (Leighty et al., 1976; 1980). Recently, we have shown the presence of pentachlorophenol conjugate of palmitic acid in human fat (Ansari et al., 1985) which possesses selective toxicity to pancreas (Ansari et al., 1987).

The present study was undertaken to examine if the reported retained radioactivity of 2-chloroethanol in the rat liver (Grunow and Altman, 1982) is associated with the formation of fatty acid conjugates. We report here the isolation and characterization of 2-chloroethylstearate from the liver of rats treated with 2-chloroethanol.

MATERIALS AND METHODS

2-Chloroethanol was purchased from Aldrich Chemical Company, Inc., Milwaukee, WI. Stearic acid was obtained from Sigma Chemical Company, St. Louis, MO. All the chemicals and solvents used in the extraction and chromatographic analysis were HPLC grade from Fisher Scientific Company, Fairlawn, NJ.

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Thin layer chromatography (TLC) was performed on glass coated silica gel GF plates (Analtech, Newark, DE) using hexane:diethyl-ether:methanol:acetic acid (90:20: 5:2, v/v) as the developing solvent. The spots were visualized by spraying 50% aqueous sulfuric acid followed by charring at 120°C. High performance liquid chromatography (HPLC) was carried out on a Ultrasphere ODS column (4.6 mm x 25 cm, 5 μ particle size) using a Beckman 334 gradient liquid chromatograph connected with model 165 UV detector set at 210 nm. Separation was carried out using methanol:water (40:1, v/v) at a flow rate of 1 ml/min.

Mass spectrum were obtained on a Nermag R1010C quadrapole mass spectrometer set at desorption chemical ionization (DCI) mode. Methane (CH_4) or ammonia (NH_3) was used as the reagent gas and samples were applied through solid probe.

Proton nuclear magnetic resonance spectrum was obtained on a JEOL GX-270 instrument in deutriochloroform using tetramethylsilane as the internal standard.

Authentic standard of 2-chloroethylstearate (Figure 1) was synthesized by heating a mixture of 2-chloroethanol and stearic acid in the presence of trace amounts of HCl for 2 hrs. The reaction mixture obtained gave white crystals on diluting with methanol. Crystals were filtered, dried and recrystallized from methanol to give 2-chloroethylstearate, m.p. 52°C (uncorrected), Rf 0.66, CH_4 DCI gave pseudomolecular ion peaks at m/z 347/349 (M + 1), 375/377 (M + 29) and 387/389 (M + 41). Other significant ions were at 267

(M + 1 - $ClCH_2CH_2OH$) and 122/124 ($CH_2=\overset{+OH}{\overset{\cdot|}{C}}-OCH_2CH_2Cl$, McLafferty ion). Proton NMR spectrum gave a signal at δ 4.33 t (2H,J = 5.64, 5.64 Hz, $-OCH_2CH_2-Cl$), δ 3.68 t (2H, J = 5.64, 5.64 Hz, $-OCH_2CH_2$

Cl), δ 2.35 dd (2H, J = 14.8, 7.7 Hz, $-CH_2-\overset{O}{\overset{\|}{C}}-OCH_2CH_2Cl$), δ 1.62 m

(2H,$-CH_2CH_2-\overset{O}{\overset{\|}{C}}-OCH_2CH_2Cl$), δ 0.88 t ($C_{18}-CH_3$), δ 1.26 br s (other protons).

Male Sprague-Dawley rats, weighing from 175-200 g were obtained from Timco (Houston, TX) and were acclimatized for 7 days in a constant temperature (21°C) room with an automatically controlled 12 hr light and dark cycle. Rats were fed Purina Rat Chow and water ad libitum. On the 8th day, a group of four rats received a dose of 50 mg/kg/ml of 2-chloroethanol in mineral oil while control group of rats (4) received same amount of mineral oil. Food and water were given ad libitum to both groups and were sacrificed by decapitation on the 5th day after the treatment. Livers were excised, washed, weighed and homogenized in 1.15% KCl.

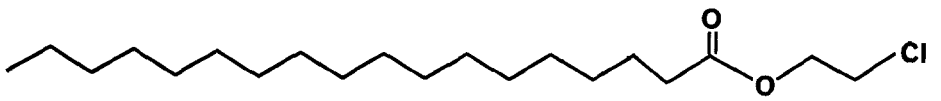

Figure 1. Structure of 2-chloroethylstearate.

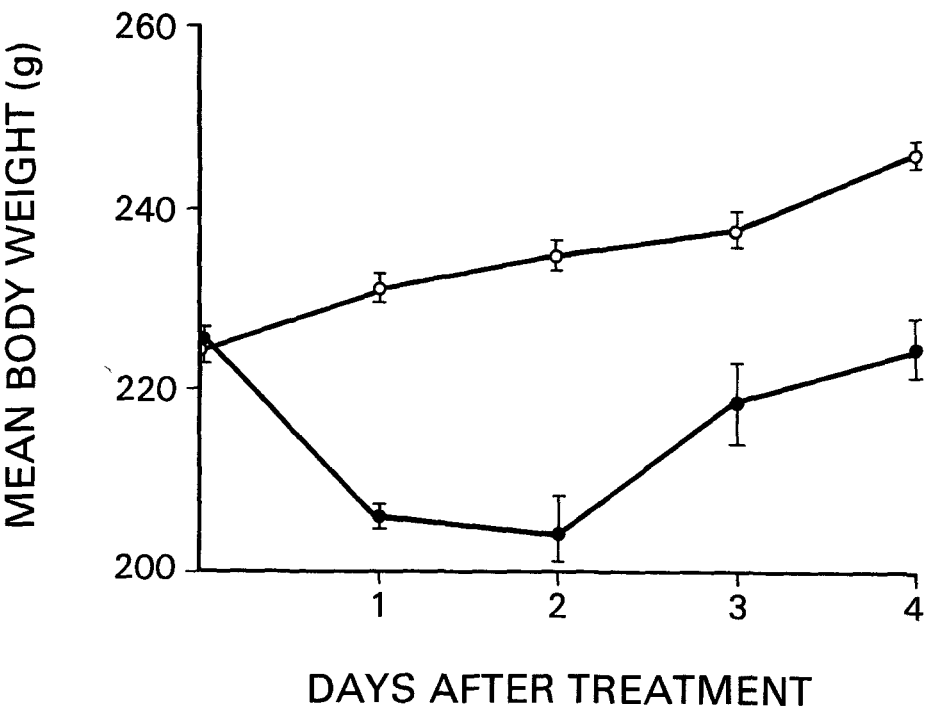

Figure 2. Changes in mean body weight of 2-chloroethanol treated (●——●) and control (O——O) rats, ± S.D. $P < 0.01$ (1 to 4 days after treatment).

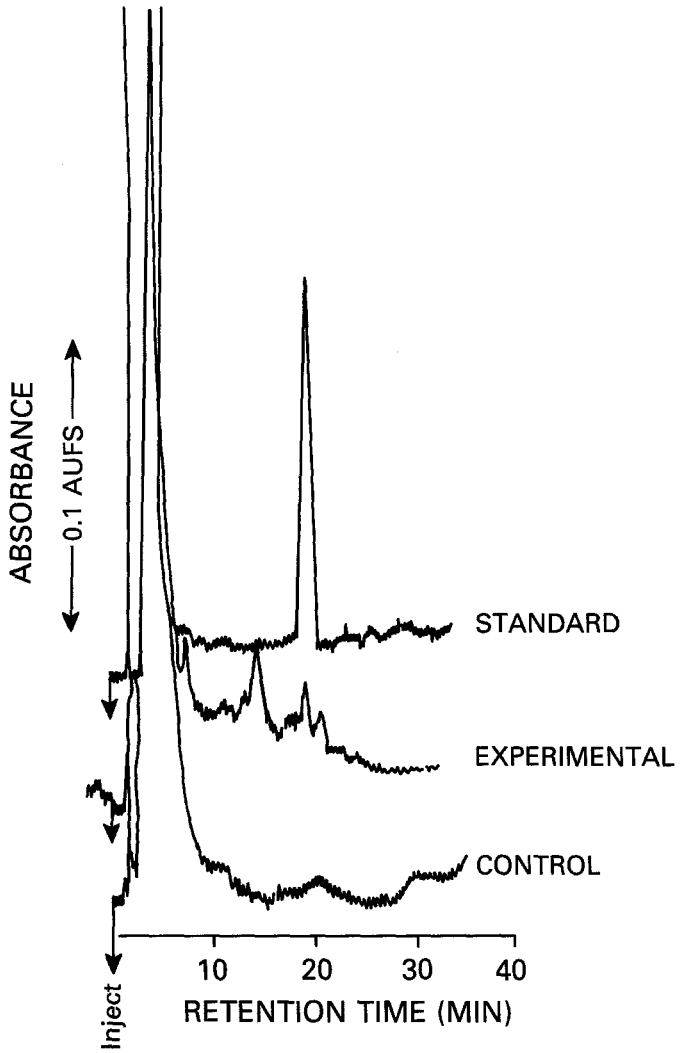

Figure 3. Separation of 2-chloroethyl stearate by high performance liquid chromatography using a reversed phase C_{18} column from hepatic microsomes of rats, standard (2-chloroethylstearate), experimental (2-chloroethanol fed) and control (mineral oil only).

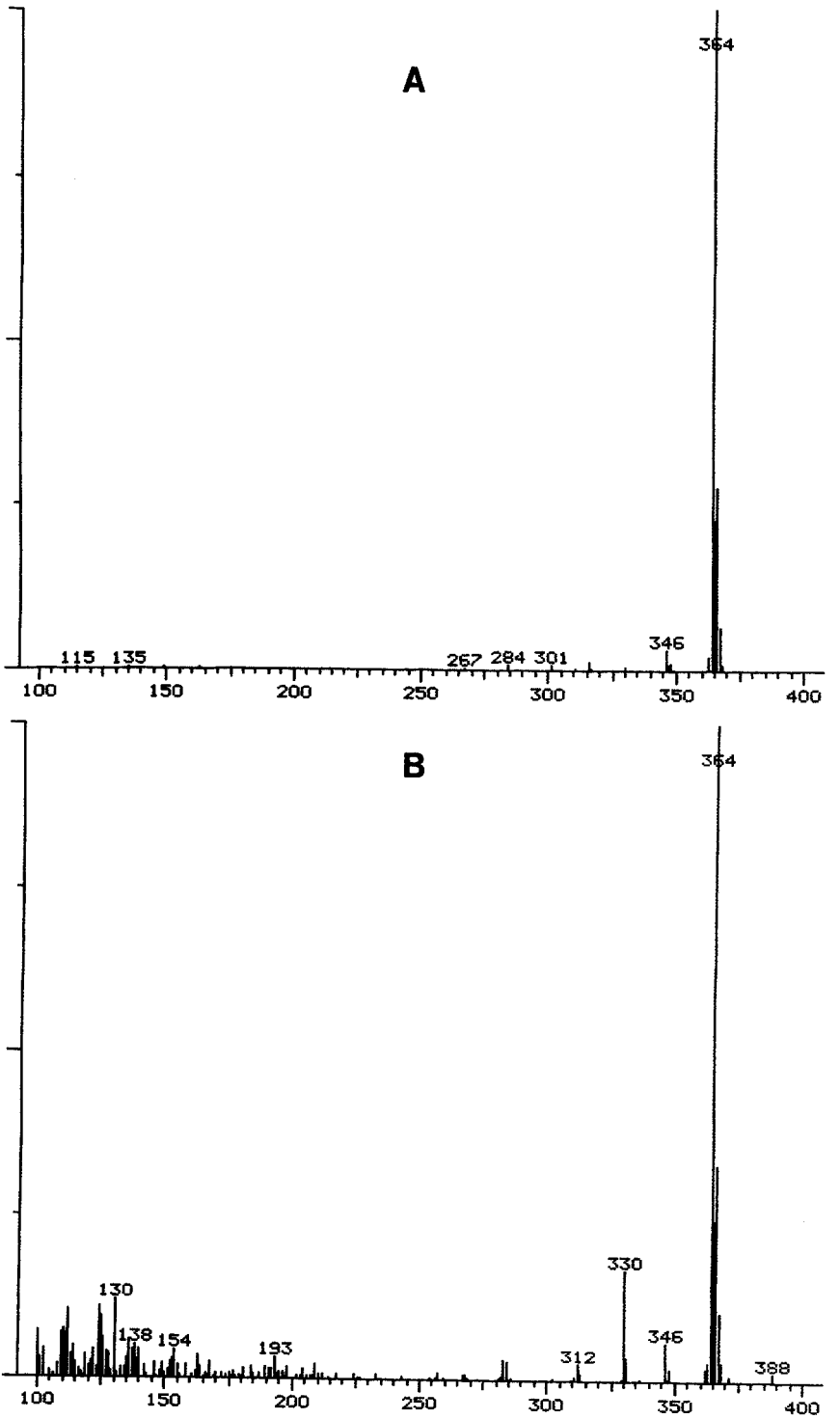

Figure 4. NH_3 DCI spectra of the authentic 2-chloroethylstearate standard (A) and compound isolated from the hepatic microsomes of rats treated with 2-chloroethanol (B).

Microsomes were prepared according to the method of Remmer et al. (1967) by centrifuging the postmitochondrial fraction at 100,000 g for 1 hr. Microsomal lipids were extracted with chloroform:methanol (2:1, v/v) (Folch et al., 1957), dried under vacuum and subjected to preparative TLC. Area corresponding to 2-chloroethylstearate was scraped and extracted with chloroform. The concentrated extract was subjected to HPLC and fractions corresponding to 2-chloroethylstearate standard were collected, evaporated to dryness and subjected to NH_3 DCI mass spectrometry.

RESULTS AND DISCUSSION

An oral dose of 50 mg/kg body weight of 2-chloroethanol in male rats caused a significant drop (∿ 9%) in their body weights on the first and second day of treatment which was recovered on the third (97%) and fourth day (99%). However, the control rats showed an apparent increase in their body weight with an average of 5 g/day as shown in Figure 2. An average difference of 24.2 ± 5.1 g in the body weights of control and experimental rats was observed from first (24 hr after dosing) to the fifth day when they were sacrificed. The mean fresh wet weight of liver was also reduced to 8.0 ± 0.60 g in experimental rats as compared to 9.5 ± 0.44 in control rats.

Lipids were extracted from microsomes and purified by thin layer chromatography. Analytical TLC of purified 2-chloroethylstearate gave an Rf of 0.69 (0.66 standard). At this Rf value, control animals did not show any spot. HPLC analyses in the experimental group showed a peak at 19.2 min while this peak was absent in the control group (Figure 3). Synthesized 2-chloroethylstearate also gave a retention time of 19.2 min (Figure 3).

NH_3 DCI spectra (Figure 4) of the isolated and standard compound gave pseudomolecular ion at m/z 364/366 ($M + \overset{+}{N}H_4$ base peak). The 3:1 ratio of 364 and 366 confirmed the presence of one chlorine in the molecule. Relatively a low abundance ion m/e 346/348 corresponds to the molecular weight of the compound.

In the present study we have concentrated our efforts to identify the in vivo formation of 2-chloroethylstearate (Figure 1) which is a novel conjugate of 2-chloroethanol. Since 2-chloroethanol decreases the mitochondrial fatty acid elongation (Andrews et al., 1983), it is possible that the acyl CoA is needed for elongation may react with 2-chloroethanol and therefore is not available for elongation reactions. A detailed study needs to be carried out where all the possible fatty acid conjugates should be analyzed both qualitatively as well as quantitatively.

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