Metabolic Fate of Epoxycholesterol in the Rat

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ABSTRACT

The rate of disappearance of intubated epoxycholesterol from the rat gastrointestinal tract has been determined. The loss of this sterol is accompanied by the appearance of a sterol metabolite. This was isolated by preparative GLC and TLC and identified by mass spectrometry as 5α-cholestan-3β-5α,6-β-triol.

INTRODUCTION

Previous experiments have shown that epoxycholesterol (5α-cholestan-5α,6α-epoxy-3β-ol) is toxic when fed to rats at the 1.5% level in their diets for 90 days (1). Analysis of the lipids from the various tissues and from the serum of these animals failed to reveal the presence of any of this sterol (2). In that preliminary study only about 50% of the ingested epoxycholesterol could be accounted for in the fecal lipids when it was intubated as a 10% solution in monoolein.

In order to obtain more information about the metabolic pathway of epoxycholesterol, the rate of disappearance was determined. Also a search was made for metabolites which might help to explain the observed biological activity (1). In lieu of a more elaborate radiotracer technique, it was hoped that this simple direct approach would provide some clues to the toxicity of the sterol. The disappearance of epoxycholesterol from the gastrointestinal (GI) tract with time after intubation was accompanied by the development of increasing amounts of an unknown sterol metabolite. This sterol has been isolated by preparative TLC and GLC and identified by mass spectrometry.

EXPERIMENTAL PROCEDURES

Sprague-Dawley male rats weighing 400-600 g were used for the studies described. Doses of 10% epoxycholesterol in monoolein equivalent to 1, 1.5, 2.0 and 2.5 g/kg were administered interperitoneally (IP); the animals were then observed during 14 days for acute effects. Also a search was made for metabolites which might help to explain the observed biological activity (1). In lieu of a more elaborate radiotracer technique, it was hoped that this simple direct approach would provide some clues to the toxicity of the sterol. The disappearance of epoxycholesterol from the gastrointestinal (GI) tract with time after intubation was accompanied by the development of increasing amounts of an unknown sterol metabolite. This sterol has been isolated by preparative TLC and GLC and identified by mass spectrometry.

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was combined with the residual soaps from the saponification and was extracted with ethyl ether for 24 hr in a liquid-liquid extractor. After concentration to about 10 ml, a portion of this ether extract was silylated. Ten to 50 µl of the silylated mixture was then injected into a Perkin Elmer Model 900 gas chromatograph equipped with a 15:1 ratio stream splitter. The conditions used were identical to those described previously. The injection was repeated about 20 times, until droplets of condensate of the desired component were visible on the sides of the collection capillary tube.

One half of the above condensate was analyzed by high resolution mass spectrometry. The instrument used was a Consolidated Electrodynamic Corp. 21-110 C equipped with a combination detector system and an electronic peak-matching accessory for precise mass measurements. The samples were introduced into the ion source by the direct introduction probe technique. The ion source was held at 210 C, and the mass spectra were recorded on plates at probe temperatures of 150-160 C. Mass measurements were made using a “Projectina” (Optical Works, Ltd., Switzerland) optical precision micrometer.

The remaining half of the sample was eluted with benzene and analyzed by GLC. The preparative TLC was done on ChromAR 500 sheet, 25 cm in length (Mallinkrodt Chemical Works). Two milliliters of the concentrate from the liquid-liquid extractor was streaked on the sheet manually and was then developed using petroleum ether-diethyl ether-acetic acid (20:80:5). After the solvent front had travelled about 20 cm, a 2 cm strip was cut and charred to locate the various bands. The band containing the unknown metabolite was extracted with methanol.

The chlorohydrin derivative of epoxycholesterol was prepared by bubbling gaseous HCl through a 3.3% solution of epoxycholesterol in chloroform for 2 hr at 30-50 C. When the solution was cooled, the product precipitated. It was recrystallized from ethyl acetate-