CHROM. 4723

Sterol metabolism

XIII. Chromatographic resolution of the epimeric 24-hydroxycholesterols

Although chromatographic resolution of epimeric hydroxycholesterol derivatives bearing hydroxyl groups in the 20- (ref. 1), 22- (refs. 2–7), and 23- (ref. 8) positions has been reported, chromatographic resolution of the epimeric 24-hydroxycholesterols essential to their study in human tissues has not been achieved. Only one epimer of 24-hydroxycholesterol, named cerebrosterol, has been isolated from human brain[9–11]. Whereas this sterol may be detected readily in extracts of human brain by paper[12] and thin-layer[13] chromatography (TLC) and in human feces and meconium by gas chromatography (GC) (refs. 5, 6), the stereochemical purity of the sterol from tissues has not heretofore been demonstrated chromatographically.

We have attempted to no avail to resolve the epimers of 24-hydroxycholesterol, as the free sterols and as their diacetates and dibenzoates, by a variety of column chromatographic means, including Sephadex LH-20 (ref. 14), by direct TLC methods and by GC (ref. 1). We have now devised a TLC resolution of the epimeric 24-hydroxycholesterols as their dibenzoate esters by extended ascending irrigation for 15 h. This method, though time consuming, permits analysis for the first time of the stereochemical purity of 24-hydroxycholesterol samples isolated from human tissues.

Experimental

Both epimers of 24-hydroxycholesterol[10,11] were obtained by sodium borohydride reduction of 24-ketocholesterol. The epimer identified with the naturally occurring 24-hydroxycholesterol cerebrosterol and designated as the 24ξ^1-hydroxycholesterol initially[10,11] has been assigned absolute configuration as the 24β_F(24S)-hydroxycholesterol[15]. However, we have suggested that the assignment be reversed[8], and for the present we retain the original 24ξ^1- and 24ξ^2-nomenclature for cerebrosterol and its epimer, respectively. Cerebrosterol was isolated from human brain by modifications of previously described methods of extraction and recovery[9–13].

Crude sterol preparations and purified reference sterols were benzoylated by dissolving 1 mg of sample in 2 ml of dry pyridine and adding 1 ml of benzoyl chloride. The solution was held at room temperature overnight, whereafter 1 ml of water was added. After 4 h the mixture was extracted with 100 ml of diethyl ether. The ether extract was washed with dilute sodium hydroxide solution, with water three times, and with brine once, after which the extract was dried over anhydrous sodium sulfate and evaporated under vacuum. TLC examination of the benzoylated samples using benzene–ethyl acetate (3:2) showed that none of the free sterol remained unesterified and that only one esterified component was formed.

A 20 × 40 cm chromatoplate prepared with Silica Gel HF_{254} (E. Merck GmbH., Darmstadt, G.F.R.), 0.25 mm thick for analysis, 1 or 2 mm thick for preparative work, is washed chromatographically along the 40 cm dimension with methanol–chloroform (2:1) and redried (1 h at 110°). Samples (10–50 μg) of the benzoylated samples are spotted along the 20 cm dimension in the usual manner, with reference samples of both 24ξ^1-hydroxycholesterol (cerebrosterol) and its epimer 24ξ^2-hydroxy-

cholesterol as the 3β,24-dibenzoates. The chromatoplate is irrigated in ascending fashion with benzene–hexane (1:1) in a chromatographic chamber closed at the top so that the long dimension of the chromatoplate protrudes from the chamber into the laboratory atmosphere (about 10–12 cm is exposed). Ascending solvent evaporates from the exposed portion of the chromatoplate, and after 15 h irrigation is terminated. The chromatoplate is dried and visualized under 254 nm UV light. Visualization may also be accomplished by spraying the chromatoplate with 50% aqueous sulfuric acid in the usual fashion, with warming to full display of coloration.

For quantitation the zones detected under UV light are marked, the silica gel excised from the chromatoplate and packed into short glass columns for elution of the sterol dibenzoates with 5 ml of redistilled methanol. The methanol solution so obtained was scanned between 210–250 nm against a blank similarly prepared by methanol extraction of an equivalent area of the irrigated chromatoplate not bearing any steroid. The absorption spectrum of both dibenzoates was a symmetric peak, λ_{max} 228 nm, from which the absorption intensities were directly determined. Two known mixtures of the epimeric 24-hydroxycholesterols, carried throughout the benzoylation, chromatographic, and spectrophotometric procedure, gave very good results. A mixture containing 12.0% of the naturally occurring epimer (cerebrosterol) and 88.8% of the unnatural epimer was analyzed to contain 12.5% of cerebrosterol and 87.5% of the epimer. A mixture containing 44.2% of cerebrosterol was analyzed to contain 43.6%.

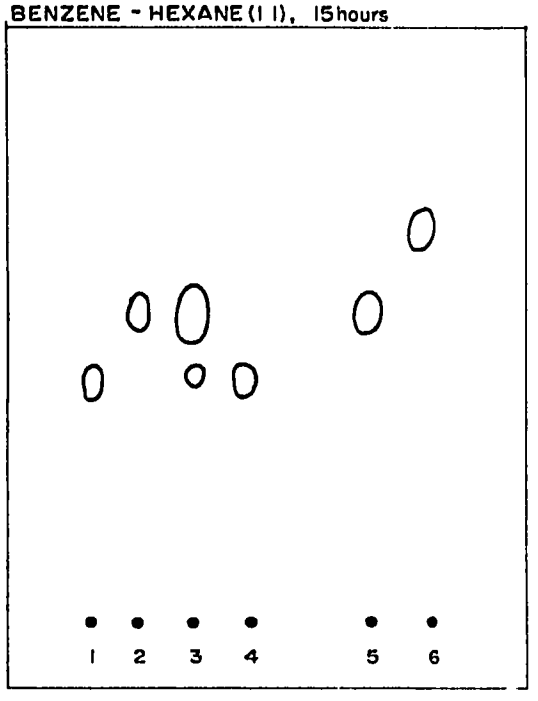

Fig. 1. Resolution of 24-hydroxycholesterol dibenzoates on Silica Gel HF_{254}, 15 h ascending irrigation with benzene–hexane (1:1). Dibenzoate esters of: (1) 24ξ^1-hydroxycholesterol from borohydride reduction of 24-ketocholesterol; (2) 24ξ^2-hydroxycholesterol by borohydride reduction; (3) a 1:2 mixture of the 24ξ^1- and 24ξ^2-hydroxycholesterol epimers; (4) 24ξ^1-hydroxycholesterol (cerebrosterol) from human brain, (5) 23R-hydroxycholesterol; (6) 23S-hydroxycholesterol.

Fig. 1 shows the resolution obtained using this system with the epimeric 24-hydroxysterols. Complete resolution is achieved. A mixture of 24-hydroxycholesterol epimers in which the unnatural 24ξ^2-hydroxycholesterol epimer predominated is also given to show the ready recognition of minor amounts of one epimer in the presence of larger amounts of the other. For comparison we also present in Fig. 1 the resolution of the epimeric 23-hydroxycholesterols as the 3β,23-dibenzoates.

We confirm with these studies that the human brain 24-hydroxycholesterol previously isolated as a single epimer is indeed stereochemically pure and that human brain preparations do not appear to have any of the other epimer present.

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