Gas chromatography of squalene, sterols and bile acid methyl esters

The application of high-temperature gas chromatography to the separation of sterols[1], of steroids and sterols[2–4], of squalene and cholesterol[5], and of bile acids[6,7] has provided a quick, sensitive, and reproducible method of studying mixtures of these solutions in biological materials. The present communication describes columns coated with thin films of General Electric silicone gum SE-52* which give quick and excellent separations of squalene and several sterols found in mammalian tissues. In addition these columns give good separations of bile acid methyl esters merely by changing the operating temperature of the column.

The method of preparation of the columns has been found to be quite critical and, therefore, is described in detail. The solid support Gas Chrom (100–140 mesh)** is washed with acid and then with alkali as described by FARQUHAR, INSUL, ROSEN, STOFFEL AND AHRENS[8]. After drying overnight at 110°, 25 g of the washed Gas Chrom is added to 125 ml of 0.1% dimethyldichlorosilane in toluene, and the resulting slurry stirred for 5 min. The mixture is then filtered with suction through a sintered-glass funnel until 70–75% of the toluene is removed. The material in the funnel is thoroughly mixed, 25 ml of methanol is added with mixing and the resulting slurry is allowed to stand for 5 min. After removal of the methanol by filtration with suction, the Gas Chrom is washed to neutrality with several portions of methanol, and the product then is dried at 110° for at least 1 h. This treatment gives a completely siliconized product. The use of higher concentrations of dimethyldichlorosilane for presiliconizing the solid support has been found to increase tailing of the sterol peaks.

The stationary phase, SE-52, is prepared by adding a precalculated amount to 150 ml of toluene the evening before use, and allowing it to dissolve overnight. The following day the 25 g of presiliconized Gas Chrom is slurried with this SE-52 solution, and allowed to stand for 15 min with occasional stirring. The filtration method of HORNING, MOSCATTELI AND SWEELEY[9] then is used to ensure an even coating. The slurry is transferred to a coarse sintered-glass funnel and the excess SE-52 solution is allowed to filter off under gravity. The hold-up volume of the packing has been found to be 50–60% when 150 ml of solvent are used. The per cent SE-52 on the column packing is calculated from the hold-up volume. After thorough mixing in the funnel the product is spread on a preheated glass tray and placed in an oven at 85°. The material in the tray is thoroughly mixed every 5 min until it looks quite dry, usually for about a total of 30 min. The last traces of toluene are removed by drying overnight at 85° or for at least 2 h at 110°. The columns are packed in the conventional manner and conditioned by baking at 250–260° for a period of 24–48 h.

A Barber–Colman Model No. 10 gas chromatograph equipped with sapphire insulated ^{90}Sr detectors was used for these studies. The operating conditions were: flash heater, 290–300°; detector compartment, 260–270°; amplifier sensitivity, $1 \cdot 10^{-7}$ A; cell voltage, 1000 V, and column temperature and gas flow rate as indicated in the figure legends.

The results obtained with a mixture of squalene and four sterols on a 6 ft × 6 mm I.D. column are shown in Fig. 1.

Since a tendency for the ^{90}Sr detectors to lose sensitivity was noted, unless a

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suitable bake-out period (30 min) was allowed between runs, an effort was made to reduce the retention time of the sterols. The use of a 4 ft. × 2 mm I.D. column reduced the retention time by a factor of three and still gave satisfactory resolution of all the components in the sterol mixture (Fig. 2). Excellent separation of five bile acid methyl esters was obtained on this 4 ft column by the simple expedient of elevating the temperature to 250° (Fig. 3). Somewhat better resolution of the bile acids is obtained at 225–230°; however, one then encounters some skewing of the methyl cholate and methyl dehydrocholate peaks.

Both the 4-ft. and 6-ft. columns have been used extensively during the past

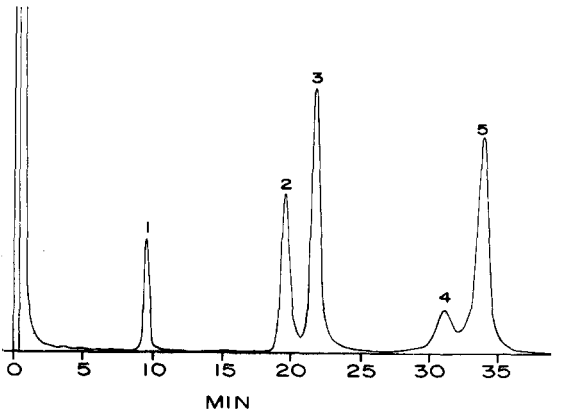

Fig. 1. Separation on a 0.49% SE-52 column. Column temperature, 225°; argon flow rate, 56 ml/min. The peaks are: (1) squalene, (2) cholesterol, (3) desmosterol, (4) 4,4-dimethyl-Δ^8-cholestenol, and (5) lanosterol.

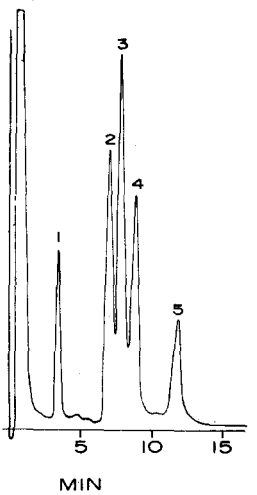

Fig. 2. Sterol separation on 0.56% SE-52 column. Temperature, 227°; flow rate, 18 ml/min. Peaks are: (1) squalene, (2) cholesterol, (3) desmosterol, (4) zymosterol, and (5) lanosterol.

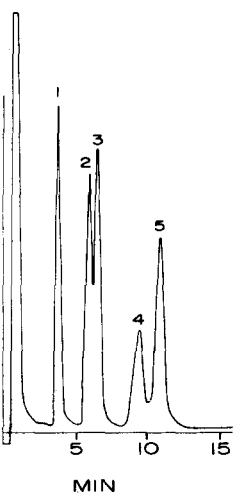

Fig. 3. Separation of bile acid methyl esters on 0.56% SE-52 column. Temperature, 250°; flow rate, 17 ml/min. Peaks are: (1) methyl lithocholate, (2) methyl deoxycholate, (3) methyl chenodeoxycholate, (4) methyl dehydrocholate, and (5) methyl cholate.

several months to investigate the sterol content of the non-saponifiable fraction of a wide variety of tissues. Satisfactory results have been obtained by evaporating petroleum ether extracts of saponified tissues to dryness, dissolving the residue in a suitable solvent (chloroform) and applying the crude mixture directly to the column. Preliminary studies have produced good results with methyl esters of mixtures of bile acids isolated from bile. Both the 4-ft and 6-ft columns have given excellent service for many weeks; their actual lifetime has not been determined.

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Isolation of 6-deoxy-D-altrose from chemically reduced hygromycin

In a previous publication[1,2], we have reported that 5-keto-6-deoxy-D-arabohexose, a component of hygromycin A, arises from D-glucose probably without rearrangement of the carbon skeleton. In the course of this work, it was necessary to reduce the antibiotic, and isolate the sugar as its L-fucose diethylmercaptal derivative. However, one would also expect to find the other isomer of the reduction, namely 6-deoxy-D-altrose. We would now like to report the isolation and characterization of this compound.

Hygromycin A was reduced as previously described[3]. The reduced antibiotic was purified by charcoal-column chromatography[4]. It was then hydrolyzed by refluxing in 1.5 *N* H_2SO_4 for 2 h. After neutralization with $BaCO_3$, the filtrate was passed through Dowex-50 (H^+) and Dowex-1 (CO_3^{2-}) to remove any remaining ions, and concentrated *in vacuo*. Sugars were then purified by paper chromatography on Whatman No. 1 paper in an isopropanol–water solvent (9:1). Three methylpentoses were observed after application of the nitroprusside spray reagent[5]. One of these has previously been identified as L-fucose[3]. The second methylpentose was chromatographed along with an authentic sample of 6-deoxy-D-altrose (kindly supplied by Dr. V. GINSBURG, National Institutes of Health, Bethesda, Md., U.S.A.) as shown in Table I. The