In connection with tests on catechin and epicatechin it might be indicated that this type of compounds can also be detected in crude extract of such plants as tea. A spot test on an infusion of tea, which is known to contain several catechins, shows a distinct pink coloration suggesting their presence.

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Separation of saturated and unsaturated fatty acid esters of cholesterol by gas–liquid chromatography

While several procedures for the separation of sterols and their derivatives by gas–liquid chromatography (GLC) have been published[1], the separation of long-chain fatty acid esters of cholesterol has not been specifically reported. This communication deals with the separation of cholesteryl palmitate, stearate and oleate, prepared by trans-esterification of cholesteryl acetate and the appropriate fatty acid ester using sodium methoxide[2].

GLC analysis was carried out on an F & M model 500 temperature-programmed gas chromatographic unit with flame ionization attachment. Of several supports and stationary phases examined under a variety of conditions, the system here described gave the best separation. A 4-ft. spiral stainless steel column (0.3 in. diam.) was packed with 60/70 mesh Anakrome ABS (an acid-washed, base-washed and silicone-treated flux-calcined diatomaceous earth) coated with 2 % SE 30 (silicone rubber gum). The temperatures at the injection port and detector block were 320° and 350° respectively. Flow rates for air, helium and hydrogen were 400, 100 and 30 ml/min respectively. A mixture of cholesteryl palmitate, stearate and oleate (5 mg each) was made up to 0.3 ml with chloroform and a sample of 1–3 μl was injected. Attenuation was kept at 800. The column was programmed from 200–340° at 3°/min.

Cholesteryl palmitate was eluted at 270° and cholesteryl stearate and oleate in a single peak at 290°. The mixture of these three esters (*ca.* 20 mg) was oxidized with

permanganate–periodate[3] at room temperature for 2 h, whereby the ethylenic linkage of oleate is attacked, and the resulting free carboxyl group was esterified with diazomethane. Chromatography of this mixture under the same conditions gave an additional peak at 240° for cholesteryl azelate, while that at 290° diminished in area. Since azelate arises on oxidation of all the common unsaturated esters, oleate, linoleate and linolenate, these will be estimated together as cholesteryl azelate. A method for distinguishing these esters by preliminary separation on a silicic acid–silver nitrate column as described by DE VRIES[4] is being worked out.

Separation by GLC of serum lipids, extracted by BLOOR's method and esterified with diazomethane, was examined. Temperature programming was carried out from 100–330°. The free fatty acid esters, mono- and di-glycerides, cholesterol, cholesteryl palmitate, cholesteryl stearate/oleate and triglycerides separated into distinct peaks. Quantitative aspects will be published elsewhere.

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Notes

Chromatography on ion-exchange papers

XV. The adsorption of metal ions on cation exchangers from perchloric acid solutions

In a recent paper NELSON *et al.*[1] report the adsorption of metal ions on Dowex-50 from HCl and $HClO_4$ solutions and noted a deviation from "ideal" exchange consisting of a general trend towards increased adsorption at higher concentrations of $HClO_4$. In previous papers[2] we have compared cellulose anion exchangers with anion resin papers and thus could show the extent to which the network influences adsorption. We hence thought that it would be interesting to apply the same comparative technique to cation exchangers and the perchloric acid system as this non-ideal behaviour in concentrated $HClO_4$ has not been adequately explained so far. The