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Note

Anomalies in the gas–liquid chromatography of cholesterol heptafluorobutyrate

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The formation of cholesterol heptafluorobutyrate and its subsequent GLC has been widely reported[1–5]. We believe in all these cases the single peak obtained on GLC with detection by a flame ionization detector (FID) is in fact cholesta-3,5-diene obtained by thermal elimination of the free acid.

An initial GLC–FID investigation of cholesterol heptafluorobutyrate gave a single peak whose retention time was less than that of cholesterol as would be expected. However, its electron capture detector (ECD) response was poor and GC–MS indicated a molecular weight of 368. Dual monitoring of the column effluent by FID and ECD produced two peaks of different retention time, the ECD giving a poor response to a compound eluting earlier than that shown by the FID.

The ester was prepared by heating 100 mg of cholesterol in 1.0 ml of hexane at 60° for 1 h with 0.75 ml of heptafluorobutyric anhydride. The acid was extracted with 8% aqueous sodium bicarbonate and the hexane layer dried with anhydrous sodium sulphate. The solvent was removed with nitrogen and the ester recrystallized from methanol containing a trace of benzene to give cholesterol heptafluorobutyrate; m.p., 116–117°; composition, 63.9% C, 7.5% H ($C_{31}H_{45}O_2F_7$ requires 63.8% C, 7.7% H); IR, ν (C=O) 1770 cm^{-1}; MS, temperature 170°, ionizing energies 80 eV, 3.0 KeV; peaks at m/e 582 (M^+ relative intensity 10%), 567 (8%), 469 (7%), 426 (14%), 368 (100%).

Thermal decomposition was suspected and the injection port heater on a Pye Series 104 gas chromatograph was varied in the range 200–275°. A column temperature of 190–230° is normally required to produce a retention time in the range 5–20 min on a 5 ft. column of 1% OV-101. In all cases, and on several columns, the FID and ECD responded to products of different retention times from the same sample of cholesterol heptafluorobutyrate. An 80-mg sample of the authentic cholesterol heptafluorobutyrate was pyrolysed at 240°/10 mmHg for 20 min. TLC on freshly activated silica gel, eluting with light petroleum (b.p. 40–60°) and spraying with 20% aqueous sulphuric acid and heating at 110° gave two spots corresponding to the pyrolysed product (R_F=0.68) and unchanged heptafluorobutyrate (R_F=0.41). A visual estimate suggested greater than 95% conversion to the decomposition product. The pyrolysis product was purified by PLC* and the product recrystallized from ethanol to give cholesta-3,5-diene; m.p., 79–80° (ref. 6: 80°); composition,

* PLC = Preparative thin-layer chromatography.

88.1% C, 12.1% H ($C_{27}H_{44}$ requires 88.0% C, 12.0% H); UV, $\lambda_{max.}$ 235 nm, $\varepsilon_{max.}$ 22,700 (ref. 7: $\lambda_{max.}$ 235 nm, $\varepsilon_{max.}$ 21,000); MS, 368 (M^+). GLC–FID of the cholesterol heptafluorobutyrate and the cholesta-3,5-diene had identical retention times on a 5 ft. column of 1% OV-101, flow-rate 60 ml/min, column oven temperature 220° (924 plates/ft. cholesterol).

Even under mild thermal chromatographic conditions cholesterol heptafluorobutyrate decomposes. FID traces of the heptafluorobutyrate correspond to cholesta-3,5-diene whereas the ECD traces are either a small amount of the cholesterol heptafluorobutyrate or alternatively the heptafluorobutyrate of an impurity present in the cholesterol. The pyrolysis of cholesterol heptafluorobutyrate explains the low ECD response recorded for the compound[3] and the fact that other authors have not reported the FID and ECD responses together.

The heptafluorobutyrates of steroids show excellent ECD responses (as little as 0.005 ng can be detected in favourable circumstances) but many of the esters are unstable to TLC or GC. Simple steroids which can not be analysed as their heptafluorobutyrates after silica gel TLC include cholesterol, lumisterol, ergosterol, 5,7-cholestadiene-3β-ol, 20α-hydroxypregn-4-en-3-one, oestradiol, oestriol and pregnanetriol. For detection of cholesterol by vapour phase chromatography at the nanogram level we suggest the use of pentafluorophenyldimethylsilyl ethers[8] or the less volatile bromomethyldimethylsilyl ethers.

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