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Note

Ester artefacts formed during isolation of ω-hydroxy fatty acids by DEAE-Sephadex ion-exchange chromatography

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Ion-exchange chromatography of complex lipids derived from natural sources provides a simple and quantitative method for separating acidic and neutral compounds[1]. The isolation of mixtures of diterpene acids from wood extracts has been achieved by use of DEAE-Sephadex ion-exchange beads, without isomerisation of labile diene acids[2]. After elution of the neutral compounds using diethyl ether–methanol–water (89:10:1), the acids were eluted quantitatively with the same solvent mixture either saturated with carbon dioxide, or containing 4% formic acid[2].

During an investigation of *Pinus radiata* needle epicuticular wax composition, it was required to separate minor amounts of ω-hydroxy fatty acids and diterpene acids from wax esters and estolides, and also to quantitatively separate C_{12}, C_{14}, and C_{16} ω-hydroxy fatty acids from *n*-alkanols and α,ω-diols obtained after basic hydrolysis of estolides[3]. DEAE-Sephadex was the ion-exchange material employed for these separations, but using chloroform–methanol–water as solvent, as the lipids were insoluble in the diethyl ether–methanol–water solvent. This paper describes the anomalous behaviour of the ω-hydroxy fatty acids during ion-exchange chromatography.

EXPERIMENTAL

AnalaR solvents (BDH, Poole, Great Britain) were used. DEAE-Sephadex A-25 (Pharmacia, Uppsala, Sweden) was treated with 2 *N* sodium hydroxide solution, immediately washed with distilled water to remove excess of base, then with methanol, finally with chloroform, and then suspended in chloroform–methanol–water (89:10:1). The slurry was poured into chromatography columns, and the DEAE-Sephadex held in place with one or two discs of Whatman GFB (Whatman, Maidstone, Great Britain). This was necessary, since the ion-exchange beads floated in this solvent mixture.

The epicuticular wax, or hydrolysate, was dissolved in the minimum volume of chloroform–methanol–water, and the solution was transferred to the column (*ca.* 100 mg lipid in 1.5 ml solvent added to 2 ml bed volume of ion-exchange resin). Neutral compounds were eluted with chloroform–methanol–water, and the acids with chloroform–methanol–water–carbon dioxide or chloroform–methanol–water–formic acid.

Thin-layer chromatography (TLC) of the methyl esters of the acid fraction was carried out on layers (0.25 mm) of silica gel G using chloroform–petrol (1:1).

For gas–liquid chromatographic (GLC) analysis, the acids were esterified (diazomethane in diethyl ether–ethanol) and residual hydroxyl groups converted [with bis(trimethylsilyl)acetamide] to trimethylsilyl (TMSi) ethers. The methyl-TMSi derivatives were chromatographed on 1.2 m × 2 mm I.D. glass columns packed with 3% OV-17 on Gas-Chrom Q, temperature programmed from 150° to 250° at 8°/min, injector temperature 180°, flame-ionization detector temperature 280°.

Infrared (IR) spectra were recorded in chloroform solutions, and proton magnetic resonance (PMR) spectra in [^{2}H]chloroform, with TMS internal standard.

RESULTS AND DISCUSSION

Neutral compounds were eluted from the DEAE-Sephadex within ten column volumes of solvent. Use of carbon dioxide-saturated solvent mixture for recovery of the acidic fraction (which was visible as a pale yellow band at the top of the column) only partially eluted the acids, even after 50 column volumes of solvent. When the formic acid-containing solvent mixture was used, the acids were readily eluted, usually within five column volumes.

GLC of the acid fraction eluted from five ion-exchange columns with chloroform–methanol–water–formic acid showed three extra peaks in addition to those of the C_{12}, C_{14}, and C_{16} ω-hydroxy fatty acid derivatives. The ratio of the total area of the three ω-hydroxy fatty acid methyl ester GLC peaks to that of the three new peaks varied from *ca.* 4:1 to 1:1. It was apparent that the ω-hydroxy fatty acids were being modified during the ion-exchange chromatography. Two classes of compounds from the acid fraction (as methyl esters) were separated by TLC on silica gel G. GLC confirmed the separation of the two classes, each containing three compounds. The IR spectrum of each class showed carbonyl absorption at 1735 cm^{-1} due to carboxylic acid ester, but only the more polar one had absorption at 3600 cm^{-1} due to hydroxyl. The PMR spectrum of the more polar compounds showed these to be ω-hydroxy fatty acid methyl esters (δ 3.62, s, OCH_3; 3.60, t, J = 7 Hz, CH_2–O; 2.32, t, J = 7 Hz, CH_2–C=O). The PMR spectrum of the less polar class showed these to be formate esters of the ω-hydroxy fatty acid methyl esters (δ 8.02, s, H–C=O; 4.18, t, J = 7 Hz, CH_2–O–C=O; 3.62, s, OCH_3; 2.22, t, J = 7 Hz, CH_2–C=O). The formate methyl esters were readily hydrolysed with base to the original ω-hydroxy fatty acids.

Formate esters were also formed (but to only *ca.* 5% of the total acid fraction) when the ω-hydroxy fatty acids were dissolved in chloroform–methanol–water–formic acid alone and kept for 12 h at 20°. To compare the behaviour of ω-hydroxy fatty acids with other hydroxy fatty acids, 2-hydroxypalmitic acid, and 12-hydroxystearic acid were committed to DEAE-Sephadex ion-exchange chromatography. Both these hydroxy fatty acids were only very slightly (< 1%) esterified compared with the ω-hydroxy fatty acids when treated in an identical manner, as shown by GLC of their methyl esters. Neither 2-hydroxypalmitic acid nor 12-hydroxystearic acid was esterified when dissolved in chloroform–methanol–water–formic acid alone.

The unusual reactivity of the primary alcohol of ω-hydroxy fatty acids towards acylation with carboxylic acid can possibly be compared with its facile alkylation with diazomethane. Treatment of di- and trihydroxy fatty acids with ethereal diazomethane

resulted in partial alkylation of the primary hydroxyl groups, but not of the secondary hydroxyl groups[4]. Similar esterification of primary hydroxyl groups of hydroxy fatty acid methyl esters, but not of secondary hydroxyl groups, has been shown to occur during their GLC on polyester phases[5].

In the present case, ready formylation of the primary hydroxyl group of ω-hydroxy fatty acids when eluted from DEAE-Sephadex columns with chloroform–methanol–water–formic acid appears due to its unusual reactivity, and possibly also due to acid catalysis by the protonated DEAE-Sephadex.

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