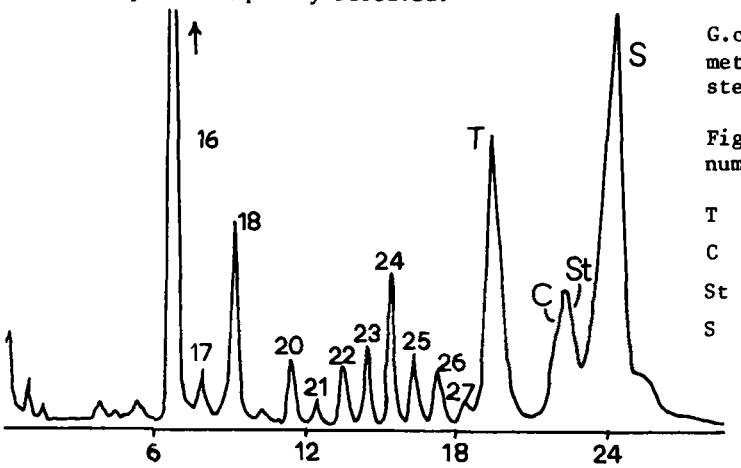
A RAPID METHOD FOR THE IDENTIFICATION OF FATTY ACID AND STEROL CONSTITUENTS OF COMPLEX STEROL ESTER MIXTURES

A.O.Adeoye, S.R.Hemingway, R.D.Waigh, Department of Pharmacy, Manchester University, Manchester M13 9PL.

Sterol esters are common plant products and are frequently encountered as complex mixtures. Three or four sterols may be esterified by ten or more fatty acids, so that most constituents are present in very small quantities. We have examined the sterol ester fraction of the petrol extract from the stem bark of Nauclea diderrichii (Rubiaceae), which presents the characteristic difficulties. Sitosterol palmitate had previously been reported from this species (King and Jurd 1953).

Examination of the intact esters by electron impact mass spectrometry was of limited value- no molecular ion was obtained. Chemical ionisation techniques allow the molecular ion to be observed when pure single esters or simple mixtures are used, (Murata et al 1975) but in the complex mixture under discussion only weak molecular ions for the major constituents could be seen. In combination with i.r. and n.m.r. spectra, which do not distinguish between the different esters, this could lead to erroneous conclusions concerning the composition of the mixture.

We have developed a double derivatisation procedure, which in conjunction with g.c./m.s. allowed the simultaneous identification of eleven fatty acids and three sterols from the sterol esters of N.diderrichii and the separation of one unidentified triterpinol. In this process the mixture was first treated with methanolic HCl, to give the methyl esters of the fatty acids and the free sterols, and the mixture after evaporation silylated by addition of excess O, N-bistrimethylsilylacetamide in pyridine. Combined g.c./m.s. on a 1% Dexsil 300 column with helium as carrier allowed the individual components to be identified. Temperature programming was used, starting at 150° for 2 min and rising by 8°/min to 270°, to give a chromatogram as below. In some runs the campesterol/stigmasterol peak was partly resolved.



G.c.of fatty acid methyl esters and sterol TMS ethers

Figures refer to carbon number of fatty acid

T triterpinol
C campesterol
St stigmasterol
S sitosterol

While sitosterol palmitate is probably the largest single component of the mixture, it may represent less than 50% of the total, depending on how extensively the sitosterol is combined with other acids. The method provides information which is otherwise inaccessible and will be useful until a technique becomes available for the separation and identification of the intact esters.

F.E.King and L.Jurd (1953) J. Chem. Soc. 1192-1195.
T.Murata, S.Takahashi and T.Takeda (1975) Anal.Chem. 47, 557-580.

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