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Mass Spectrometric Studies on Bile Acids and Other Steroid Derivatives

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The mass spectra of the methyl esters of the bile acids show several characteristic features which render them useful for analytical purposes. The methyl ester of cholanic acid shows an intense peak of *m/e* 374 corresponding to the unfragmented ionized molecule $C_{25}H_{42}O_2^+$. This peak is the largest in the spectrum. When the sterol nucleus carries one or more hydroxyl groups the molecular peaks ("parent peaks") are either very small or absent because the hydroxyl groups are easily eliminated as water with the formation of double bonds. The side chain is also readily split off and in the mass spectrum there occurs a peak corresponding to the ionized unsaturated nucleus. For methyl esters of monohydroxycholanic acids such as 3-hydroxycholanic (lithocholic) acid this peak occurs at *m/e* 257. For 3,7,12-trihydroxycholanic (cholic) acid the "nuclear peak" is at *m/e* 253. For a saturated bile acid the nuclear peak thus indicates the number of hydroxyl groups present in the sterol ring system.

If the ring system contains both hydroxyl groups and double bonds the nuclear peak indicates their sum. Thus stigmasterol and β-sitosterol, which both have one hydroxyl group and one double bond in the ring system, give a characteristic nuclear peak at *m/e* 255.

The saturated monohydroxy tetracyclic triterpene tetrahydrodammaradienol [1] which possesses a trimethyl-substituted steroid nucleus gives a nuclear peak at *m/e* 299 = (257 + 3 × 14).

Peaks due to methoxycarbonyl-containing fragments are less prominent in the spectra of methyl esters of bile acids than they are in the spectra of methyl esters of aliphatic acids [2], but the intensity distribution among these peaks depends on the structure of the side chain in a manner analogous to that found in the case of methyl esters of branched chain aliphatic acids.

Positional isomers such as 3-hydroxy- and 12-hydroxycholanic acid give very different mass spectra.

The methyl ester of triacetoxycholic acid gives a very good spectrum in which the last peak observed is a strong peak at *m/e* 428 = (M—120). This fragment has evidently been formed by the elimination of two of the three acetyl groups in the form of acetic acid. The acetic acid is indicated by its strong molecular peak at *m/e* 60.

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1. Asselineau, C. and Asselineau, J. *Bull. soc. chim.* **1957** 1359.
2. Asselineau, J., Ryhage, R. and Stenhagen, E. *Acta Chem. Scand.* **11** (1957) 196.