escence arise not from binding of the mineral cation to the phospholipid, but that they are due to increasing cation charge density near to the lipid–water interface.

In the absence of added salt, the fluorescence of α-tocopherol was not changed between pH 4 and pH 11 when the α-tocopherol-lecithin dispersion was titrated against HCl or NaOH (Fig. 1a). At pH 4 or pH 11, α-tocopherol fluorescence increased, probably due to the millimolar cationic concentrations of the added HCl or NaOH respectively. In the presence of KCl, α-tocopherol fluorescence was strongly pH-dependent, showing a minimum at pH 4 and a maximum at pH 8. Thus lowering the pH within this range increased the quenching of α-tocopherol by the lecithin. Between pH 4 and pH 8 the curve was sigmoidal and closely resembled the titration curve of a weak acid. It is proposed that the pH effect arises directly from the pH-dependent reversible ionization of the phosphate group in the phospholipid. If this is the case, the mid-point of the titration curve should be at a considerably lower pH than the observed value of 5.5. This apparent discrepancy can, however, be explained by distortion of the curve as a result of the increased fluorescence due to high cation (H+) charge density at low pH.

Fig. 1(b) shows the same data presented as a series of ‘binding’ isotherms (ΔF against [KCl]) at different pH values. At pH 8 the isotherm approaches the form of a hyperbola, while at pH 4 the isotherm is distinctly sigmoidal. The curves for pH 5, 6 and 7 have intermediate forms. Using our hypothetical model for the α-tocopherol-phospholipid interaction (Baig & Laidman, 1983), the curve at pH 4 can be seen to represent a positively co-operative process in which increasing K+ concentration encourages dissociation of the phosphate and increases its affinity for K+.

These data provide further evidence a priori that a polar interaction occurs between the phenolic headgroup of the tocopherol and the phosphate group of the phospholipid. A study using space-filling molecular models further shows that the steric interaction between α-tocopherol and phospholipid described by Diplock & Lucy (1973) can occur at the same time as the polar interaction. Thus, when the phytyl sidechain of α-tocopherol and a polyunsaturated acyl chain at C-2 in the phospholipid lie in the correct juxtaposition for their steric interaction, the phenolic hydroxy group of α-tocopherol lies close to the phosphoryl oxygen in the phospholipid so that interaction, possibly by hydrogen bonding, can occur.


Analysis of steroid conjugates by fast atom bombardment mass spectrometry

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The involatility and thermal lability of steroid conjugates has largely prevented analysis of the intact molecules by conventional mass spectrometry in which samples are volatilized by heat prior to ionization. However, there are mild methods of ionization in which the sample is ionized directly from the liquid or solid phase at ambient temperature (Rose & Johnstone, 1982). Molecules can be ionized on impact with a beam of rapidly moving ions (secondary ion mass spectrometry, SIMS) or atoms (fast atom bombardment, FAB). Steroid sulphates and glucuronides have been analysed successfully by SIMS and FAB (Liehr et al., 1982; Shackleton & Straub, 1982). The mass spectra were dominated by quasi-molecular ions, [M–X]− or [M+X]+ where X = H, Na, etc. Quasi-molecular ions and fragment ions arising from consecutive losses of sugar units of plant saponins have been observed with field desorption mass spectrometry (Komori et al., 1979; Encarnacion et al., 1981; Kitajima et al., 1982; Schulten et al., 1982). We report here the application of negative ion and positive ion FAB mass spectrometry (Barber et al., 1981) to a range of underivatized and intact steroid and triterpenoid conjugates from simple sugars and glucuronides to complex plant glycosides (saponins) such as digitonin.

Analysed as solutions in glycerol, sulphates and glucuronides are well suited to the FAB method. Abundant quasi-molecular ions are observed in either the positive ion or negative ion mode, though few fragment ions diagnostic of steroid structure occur. For example, nearly all of the negative ion current for sodium dehydroepiandrosterone sulphate was accounted for by SO3− (m/z 80, 49%), HSO3− (m/z 97, 100%) and [M–Na]− (m/z 367, 17%). All sulphates examined to date afford large peaks at m/z 80 and 97. Whilst sulphates and phosphates of the same sugar have the same molecular weight, they are readily differentiated by negative ion FAB mass spectrometry because phosphates yield abundant ions at m/z 79 (PO4−) and 97 (H2PO4−) (R. E. Isaac, M. E. Rose, H. H. Rees & T. W. Goodwin, unpublished results). In the positive ion mode, quasi-molecular ions are of the type [M+X]+ and [M–H–2X]+ where X = H, Na, K, etc. As with others (Liehr et al., 1982), we conclude that, for sulphate and glucuronide conjugates, FAB mass spectrometry is readily applicable to molecular weight determination, purity assessment, determination of counter-cations (positive ion mode), and identification of the type of conjugate (negative ion mode).

Recording of useful FAB spectra of glycosides was more difficult. For example, as solutions in glycerol, digitonin and even cholesterol glucoside did not afford [M–H]+ ions. For such compounds the solvent is critical. The relative abundances of [M–H]+ ions of cholesterol glucoside in glycerol, α-thioglycerol and poly(ethylene glycol) 200 were 0, 5.2 and 15.9%, respectively. When dissolved in poly(ethylene glycol) 200 the pentaglycoside, digitonin, yielded the negative ion FAB spectrum shown in Fig. 1. Branching of the carbohydrate chain is indicated by elimination of the abundant [M–H]+ ion of both hexose (162 a.m.u.) and pentose residues (132 a.m.u.) to give peaks at m/z 1065 and 1095. The [M–H–hexose−] ion ejected a further hexose (m/z 903) or pentose (m/z 933). After two hexose and one pentose residue were lost, the pattern of sequence ions became simple (single peak at m/z 771) because a simple carbohydrate chain remained; thus the branch point was defined. The two remaining hexose units were then ejected successively (m/z 609, 445), the latter with a 2H shift (see Fig. 1). Ions that define molecular weight and sugar sequence were less pronounced in the positive ion FAB spectrum of digitonin lecithin, absent when glycerol was the solvent. The aglycone structure was represented by abundant ions at m/z 449 (aglycone+H+4) and three successive losses of water therefrom (m/z 431, 431, 395).

The utility of poly(ethylene glycol) 200 as a solvent for FAB mass spectrometry is a general phenomenon for glycosidic compounds. Compared with glycerol solutions, saponins and di- and trisaccharides in poly(ethylene glycol) 200 gave more abundant ions indicative of molecular weight and sugar sequence. 

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In summary, FAB mass spectrometry forms a useful part of a strategy for structure elucidation of steroid conjugates. Since the glycosidic and sulphate moieties accommodate a negative charge more readily than a positive one, negative ion FAB mass spectra are preferred.

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