tri-5β-cholanoic acid (Carlstrom et al., 1981). Comparison of the spectra however reveal that the two A-ring cleavage ions m/z 142 and 143 are very weak in our spectrum, suggesting perhaps some slight structural difference in this region of the molecule. This is confirmed by the fact that our compound appears to form a cyclic boronate ester on treatment with 1-butyl boronic acid, implying a cis-diol structure, whereas the 1β,3α,12α-trihydroxy-5β-cholanic acid (1,3-trans-diol) is reported not to (Carlstrom et al., 1981). It is not possible to further assign the configuration of the hydroxyl substituents on the basis of the mass spectrum, and there was insufficient material for nuclear magnetic resonance, or additional microchemical reactions. We would suggest however on biochemical grounds that the more plausible structure is 1β,3β,12α-trihydroxy-5β-cholanoic acid, which would be generated by either microbial or hepatic 1α-hydroxylation of 3β,12α-dihydroxy-5β-cholanoic acid, which was present in significant amounts in the faeces of the same subject, whereas 1α-hydroxylation of the steroid nucleus is not usually observed.

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The use of fast atom bombardment mass spectrometry to identify and study urinary acylcarnitines in disorders of organic acid metabolism

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Recent work has shown that many inherited disorders of organic acid metabolism ('the organic acidurias') are characterized by greatly increased urinary excretion of acylcarnitines (Chalmers et al., 1984) and new roles for t-carnitine, both in intramitochondrial detoxification of accumulating acyl moieties in these diseases and in the maintenance of metabolic homeostasis through modulation of key mitochondrial processes, have been proposed (Bieber et al., 1982; Chalmers et al., 1983). Acylcarnitines are highly polar water-soluble quaternary ammonio compounds and positive ion fast atom bombardment mass spectrometry (FAB-MS) offers an ideal method for their analysis and characterization in mixtures. In the present work FAB-MS was carried out on acylcarnitines extracted using Dowex 50 (Tracey et al., 1986) from urine from patients with propionic acidemia, methylmalonic aciduria, isovaleric acidemia, medium-chain acyl-CoA dehydrogenase (MCAD) deficiency and 3-hydroxy-3-methylglutaric aciduria. All patients have been previously diagnosed on the basis of their organic aciduria and enzymology on cultured skin fibroblasts. FAB-MS measurements were made using both Varian MAT 731 and Jeol DX-303 high-resolution double
**Fig. 1. Fast atom bombardment mass spectrum of urinary acylcarnitines in 3-hydroxy-3-methylglutaric aciduria**

Extracted from urine using Dowex 50 and obtained using a xenon beam at 3 kV and a glycerol matrix (background-subtracted spectrum shown). Acylcarnitines were identified from ions at m/z 204, 218, 232, 246, 262, 286 and 290 based on their accurate masses shown in the Figure; identities were confirmed by alkaline hydrolysis of similar extracts with the liberated acids studied using capillary GC-MS.
The identification of $\delta$-tocopherol in cyanobacteria using mass-analysed ion-kinetic energy spectroscopy

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In eukaryotic plants the non-$\alpha$-tocopherols are located predominantly outside the chloroplast, while the $\alpha$-tocopherol is found within the plastid (Newton & Pennock, 1971a,b). The reported absence of non-$\alpha$-tocopherols from most cyanobacteria (Powls & Redfearn, 1967; Dasilva & Jensen, 1971), which biochemically can be considered to resemble free-living chloroplasts, was thus predictable from an evolutionary viewpoint. The presence of $\delta$-tocopherol in Gloeocapsa (Gloeothece) LB795 (Newton et al., 1977; Mullins et al., 1985) is consequently of considerable evolutionary and biosynthetic significance, as is the occurrence of $\beta$-/y-tocopherol in Synechocystis sp. 6714 (Mullins et al., 1985).

Identification of these non-$\alpha$-tocopherols in cyanobacteria has to date been largely based upon the putative tocochromanol exhibiting chromatographic and chemical properties indistinguishable from those of the authentic meroterpenoid. The technique of mass-analysed ion-kinetic energy spectroscopy applied to the molecular ion and characteristic fragments of tocopherols produced by electron impact mass spectrometry provides a means for their unequivocal identification, and has further established the presence of $\delta$-tocopherol in cyanobacteria by a direct physical method.

Putative $\alpha$- and $\delta$-tocopherol were extracted from cells of Synechocystis sp. 6714 and Gloeothece LB6909 respectively, and purified and provisionally identified essentially as described previously (Mullins et al., 1985). Mass spectra and mass-analysed ion-kinetic energy spectra (m.i.k.e.s.) were obtained on a VG ZAB-2F mass spectrometer. Electron impact mass spectra of tocopherols (1–10 $\mu$g) were determined at 70 eV using a direct inlet probe, heated to 55°C, and an accelerating voltage of 6 kV. Collision induced dissociation spectra were generated with N$_2$ as the collision gas in the second field free region gas cell at a pressure of 800 $\mu$Pa (6 $\mu$Torr). M.i.k.e.s. of ions selected using the magnetic sector were determined by scanning the electric-sector under data system control; normally three to five sweeps were signal averaged.

In the respective tocopherol electron impact mass spectra the molecular ion ($m/z$ 430; $\gamma$-tocopherol) and $\delta$-tocopherol ($m/z$ 402) was the base peak, and was accompanied by a strong $\alpha$-cleavage ion ($M^+ - 255$) resulting from loss of the isoprenoid side chain and a prominent ion arising by cleavage of the non-aromatic ring ($M^+ - 265$) in a fragmentation pattern generally similar to that previously reported (Scheppel et al., 1972). M.i.k.e.s. were obtained from the molecular ions of standard $\alpha$-, $\gamma$-, and $\delta$-tocopherol. In each case the major ions observed corresponded to fragmentation processes formally analogous to those in the electron impact spectra ($\alpha$-tocopherol $m/z$ 205 and $m/z$ 165; $\gamma$-tocopherol $m/z$ 191 and $m/z$ 151; $\delta$-tocopherol $m/z$ 177 and $m/z$ 137). The m.i.k.e.s. of the molecular ion of putative cyanobacterial $\alpha$- and $\delta$-tocopherol were essentially superimposable with their respective standard determined under identical operating conditions (Fig. 1), providing direct, physical evidence for the natural occurrence of non-$\alpha$-tocopherols in cyanobacteria.

It is anticipated that m.i.k.e.s. fragmentation patterns of ions which represent the tocol nucleus and which retain the aromatic ring substituents of the original tocopherol will be characteristic and thus enable not only distinction between rings containing different extents of methylation, but also between positional isomers, and preliminary support for this view has come from studies of such fragment ions selected from the electron impact mass spectra of $\alpha$-, $\gamma$-, and $\delta$-tocopherol. M.i.k.e.s. of the ion at $M^+ - 265$ in the electron impact mass spectra of $\alpha$-tocopherol $m/z$ 165; $\gamma$-tocopherol $m/z$ 151; $\delta$-tocopherol $m/z$ 137) which is derived essentially from the aromatic system of the tocochromanol nucleus (Scheppel et al., 1972), were determined. In the case of each tocopherol, fragment ions, assigned to loss of CO and $-C\equiv H\cdot O$ ($\alpha$-tocopherol $m/z$ 137 and $m/z$ 110, $\gamma$-tocopherol $m/z$ 123 and 96, $\delta$-tocopherol $m/z$ 109 and $m/z$ 82), were prominent. In addition, in the m.i.k.e.s. of the 5,7,8-trimethyltocol nucleus ($m/z$ 122 and 92, $\alpha$-tocopherol) and 7,8-dimethyltocol nucleus ($m/z$ 108 and $m/z$ 78, $\gamma$-tocopherol), further strong ions were observed which are assigned to multiple loss of neutral fragments, whereas analogous ions at the corresponding $m/z$ values were absent from m.i.k.e.s. of the ion representing the 8-monomethyl tocol nucleus derived from $\delta$-tocopherol.

The application of the mass spectrometry/m.i.k.e.s. technique will thus be a valuable tool in distinguishing closely related meroterpenoid structures in future studies of tocopherol biosynthesis, an area in which controversy has been renewed by the demonstration of 5- and 7-monomethyl tocol, together with 5,7-dimethyl tocol (Janiszewska & Pennock, 1976; Pennock, 1983).