whereas the physiological role of the hydrolase is mainly limited to the dissimilation of 3-methylcatechol and its metabolic precursors, namely *o*- and *m*-cresol. The coexistence of these two different branches in the metabolism of catechol and 4-methylcatechol, which at first sight may appear redundant, is a consequence of the low specificity of induction and action of the enzymes of the *meta*-cleavage pathway in this strain.

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Desulphation of L-Fucose Monosulphates by an Enzyme from *Patella vulgata*

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It was demonstrated earlier that sulphatase-containing preparations from the viscera of *Patella vulgata* were active against mono- and oligosaccharide sulphate mixtures derived from fucoidin (from *Fucus vesiculosus*) by graded acid hydrolysis (Lloyd & Lloyd, 1961). The enzyme, a sulphohydrolase (Lloyd, Lloyd & Owen, 1962), also showed activity towards synthetic substrates: unresolved L-fucose mono-, di- and tri-sulphates suffered partial de-esterification. Analysis of the products of these enzymic hydrolyses suggested that the sulphatase might catalyse the hydrolysis of a sulphate ester group located at one position only of the fucose molecule. To enable further studies to be made the three isomeric monosulphate esters of L-fucopyranose have been synthesized.

Direct sulphation of L-fucose with pyridine-sulphur trioxide (Baumgarten, 1926; Duff, 1960) gave a mixture of mono-, di- and tri-sulphates. The monoesters were separated from the polyesters by column chromatography on DEAE-cellulose with 1M-ammonia as eluent; this method is of general application and monosulphates of L-rhamnose, D-glucose and D-galactose have also been isolated in this way (Lloyd, Stuart & Fielder, 1968). Separation of the isomeric fucose monosulphates was effected chromatographically by using a DEAE-cellulose column with 0.1M-borate buffer, pH 10, as eluent. The fractions were desalted on columns containing Sephadex G-10. The separated L-fucose 2-, 3- and 4-sulphates (barium or sodium salts) were identified by electrophoretic, periodate oxidation and other studies.

L-Fucose 2-sulphate was also synthesized definitively by successive sulphation and de-O-acetylation of 1,3,4-tri-O-acetyl-L-fucopyranose, but yields were low. A more effective synthetic

route to the 2-sulphate furnished, in the penultimate stage, methyl- α -L-fucopyranoside 2-sulphate, which underwent hydrolysis in dilute acetic acid to give methyl- α -L-fucopyranoside and L-fucose 2-sulphate. The extreme sensitivity to acids of 2-sulphate esters of glycopyranosides has been shown to extend to other sugars (Lloyd & Forrester, 1971).

The sulphohydrolase from *Patella vulgata* de-esterified the purified L-fucose 2-, 3- and 4-sulphates at closely similar rates.

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Localization of Monomethyl- and Dimethyl-tocols on Cytoplasmic Particles in Plant Tissues

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Preliminary studies on the intracellular distribution of the tocopherols showed α -tocopherol to occur in the chloroplast, whereas β -, γ - and δ -tocopherols were found in the extrachloroplastidic part of the cell (Newton & Pennock, 1971). It was further shown that in *Fucus spiralis* the β - and γ -tocopherols appeared in a fraction that sedimented after chloroplasts and part of the δ -tocopherol could be precipitated by further centrifugation. This paper reports further studies on the localization of non- α -tocopherol in *F. spiralis*, *Coleus hybridus*, *Phaseolus vulgaris* and *Rumex sanguineus*.

After removal of chloroplasts by low-speed centrifugation of homogenates of the plants mentioned above, γ -tocopherol was found in a fraction sedimenting at 25 000g for 45 min. This heterogeneous fraction contains some fragments of nuclei, chloroplasts and cell wall (cell walls are mostly removed from the homogenate by passage through muslin) as well as mitochondria and some cytoplasmic organelles such as Golgi bodies, lysosomes, spherosomes, lomasomes and microtubules (Clowes & Juniper, 1968). Little or no γ -tocopherol was found in preparations of nuclei, mitochondria, cell walls or chloroplasts.

In earlier work δ -tocopherol was not found in

fractions sedimenting at less than 200 000g, but by using a more gentle homogenization method and the classical isolation methods of Palade & Siekevitz (1956) a microsomal fraction was obtained containing 60–70% of the δ -tocopherol. Ribosomes were separated from this fraction by solubilization of the membranous parts with 0.3% deoxycholate followed by recentrifugation to yield a ribosomal pellet. δ -Tocopherol was not in the ribosomal fraction. Of the δ -tocopherol not in the microsomal fraction (i.e. in the 'soluble' part of the cell) some could be precipitated with proteins by ammonium sulphate, and passage of the soluble fraction through Sephadex G-75 yielded δ -tocopherol in two protein-containing fractions.

Clearly α -, γ - and δ -tocopherols are separated from one another in the cell and, although the precise localization is not yet known in each case, it is hoped that these studies will throw some light on the interrelationships and functions of the tocopherols.

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The Uptake and Utilization of β -Tocopherol by Higher Plants and Algae.

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γ -Tocopherol (7,8-dimethyltocol) has been suggested as a possible precursor of α -tocopherol (5,7,8-trimethyltocol) in some plant tissues (Whistance & Threlfall, 1968). Other plants contain β -tocopherol (5,8-dimethyltocol) and α -tocopherol, and we have investigated the biosynthetic relationship between these two by using labelled β -tocopherol.

[5-Me- 14 C] β -Tocopherol was prepared by chloromethylation of δ -tocopherol with [14 C]paraformaldehyde and hydrochloric acid followed by reduction, and was taken up by shoots of wheat (3 days old) and barley (9 days old) in diluted Tween 20 or was incubated with thallus tissue of *Fucus spiralis* or cells of *Anabaena variabilis*. All these tissues have been reported to contain β -tocopherol (Brown, 1953; Green, 1958; Powls & Redfearn, 1967). The lipids were extracted and fractionated by t.l.c. and

assayed for radioactivity by scintillation counting or radioautography.

Very small amounts of radioactivity (about 100 c.p.m., 0.0002% of the administered β -tocopherol) were found in α -tocopherol in wheat and *A. variabilis*, and no radioactivity in barley and *F. spiralis*. However, seven relatively highly labelled compounds were detected, with the following by R_F values in chloroform and 18% (v/v) diethyl ether in light petroleum respectively: compound (I), 0.44 and 0.43; compound (II), 0.87 and 0.93; compound (III), 0.78 and 0.84; compound (IV), 0.70 and 0.71; compound (V), 0.67 and 0.27; compound (VI), 0.55 and 0.20; compound (VII), 0.40 and 0.15. *Fucus* contained only compound (I), which proved to be β -tocopherol; wheat and barley showed all seven, and *A. variabilis* all but compounds (II) and (VI). Compound (VII) was identified as β -tocopherolquinone, and to investigate the identities of the other materials the various dimers and trimers formed on oxidation of β -tocopherol (Nilsson, Daves & Folkers, 1968) were made for comparison. Fraction (IV) was found to migrate with synthetic diphenyl ether dimer, but although compound (VI) had chromatographic properties approximating to those of the biphenyl dimer they could be distinguished.

Unlabelled β -tocopherol (10mg) was given to barley shoots or *A. variabilis* and 0.5mg quantities of compound (III) were isolated in each case. This material proved to be identical with an as yet undescribed product of ferricyanide oxidation of β -tocopherol. Physicochemical studies (u.v., i.r., n.m.r., mass spectrometry, t.l.c. and nitrosation) suggest that the compound is a dimer with a link between the 5-methyl group of one molecule and the 5-position of the other. Rearrangement of the second ring involves formation of a dienone system.

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