7,10,13-Hexadecatrienoic acid and Hexadecanoids

Occurrence & Biosynthesis in Plants

The history of 7,10,13-hexadecatrienoic acid as a natural product dates back to 1945 in which year Shorland (1) obtained a new C16 unsaturated acid from glycerides of rape (*Brassica napus* L.) leaves. Hydrogenation of the methyl ester of the new compound afforded methyl palmitate, and bromination yielded a hexabromo derivative, thus suggesting a triunsaturated C16 fatty acid.

Subsequent structural studies employing potassium permanganate oxidation and alkali isomerization located the three double bonds at the D7, D10 and D13 positions (2). The new polyunsaturated fatty acid, *i.e.* 7,10,13-hexadecatrienoic acid or 16:3w3, was thus formally the dinor homologue of linolenic acid (9,12,15-octadecatrienoic acid). Jamieson and Reid (3) recorded the occurrence of 7,10,13-hexadecatrienoic acid in leaves from a selection of angiosperms (110 species) and found levels ranging from 2-20% in 37 species, trace-1% levels in 36 species, and zero levels in 37 species. They also noted that when present, 16:3 was enriched in the galactolipid and diacylglycerol classes of lipids in leaves. Especially the monogalactosyl diacylglycerols had a high content of 16:3. Although 16:3 like linolenic acid (18:3) appears to be generated strictly by plants, it may also occur in animal tissues as a consequence of dietary intake. Studies on rats have demonstrated that administered 16:3 can be chain-elongated to 18:3 and higher w3 fatty acids (4).

Studies of glycerolipid synthesis in leaves from higher plants have revealed the existence of two discrete pathways, only one of which results in the formation of galactolipids containing 16:3. Thus, the so-called prokaryotic pathway in chloroplasts produces diacylglycerols containing 16:0 at the *sn*-2 position and 18:1 at the *sn*-1 position. These acyl chains are further converted by stepwise desaturations to provide galactolipids containing 18:3 and 16:3 (5-8). It is believed that this pathway is dependent on the presence of a plastidial phoshatidate phosphatase activity catalyzing the formation of plastidial diacylglycerols from phosphatidic acid. In contrast, the so-called eukaryotic pathway in microsomes results in diacylglycerols having C18 acyl chains in the *sn*-2 position and either C18 or C16 chains at the *sn*-1 position. Desaturation of the C18 acyl chain produces galactolipids containing 18:3,

however, since the C16 acyl chain is not desaturated, 16:3 is absent from such galactolipids. *Arabidopsis*, potato, tobacco, rape and other plants use both of the two pathways for galactolipid synthesis, and monogalactosyl diacylglycerols from such plants ("16:3"plants) therefore contain appreciable amounts of 16:3 acyl chains at the *sn*-2 position. On the other hand, plants which synthesize galactolipids entirely by the eukaryotic pathway will lack 16:3 and are referred to as "18:3" plants. Extended studies of the distribution of 16:3 in plants by Mongrand *et al.* (9) indicated that *ca*. 12% of Angiosperm species are 16:3 plants, and further suggested that the prokaryotic pathway was gradually lost to a large extent during evolution as a consequence of disappearance of the phosphatidate phosphatase enzyme activity. Oxygenation of leaf polyunsaturated fatty acids by lipoxygenases (10) and a-dioxygenases (11) produces hydroperoxide derivatives which are further converted by secondary enzymes (10).

Studies in this area of plant lipid metabolism has focussed on linoleic (18:2) and linolenic (18:3) acid-derived compounds, the so-called "octadecanoid" subfamily of oxylipins. Weber *et al.* (12) in 1997 reported the presence of the hexadecanoid oxylipin 2,3-dinor-12-oxo-10,15(*Z*)-phytodienoic acid (dinor-12-oxo-PDA) in leaves from *Arabidopsis* and potato.

Dinor-12-oxo-PDA was absent in leaves from the *Arabidopsis* mutant *fad5*, in which synthesis of 16:3 does not take place, thus indicating that the compound is produced from 16:3 and is not formed from 12-oxo-PDA by b-oxidation (12). Furthermore, *in vitro* studies demonstrated that 16:3 is efficiently oxygenated into dinor-12-oxo-PDA in the presence of a flax extract containing lipoxygenase and allene oxide synthase (12). Subsequent studies showed that the allene oxide generated from 11(*S*)-hydroperoxy-7,9,13-hexadecatrienoic acid is a substrate for the enzyme allene oxide cyclase (13), thus demonstrating that dinor-12-oxo-PDA can be synthesized in optically active form (7(*S*),11(*S*) configuration; Scheme 1) in plant leaves. Formation of 12-oxo-PDA and dinor-12-oxo-PDA in leaves from *Arabidopsis* undoubtedly takes place starting with nonesterified 18:3 and 16:3, respectively, released from galactolipids. In agreement with this notion, 18:3 bound to monogalactosyl diacylglycerol was not converted to polar products in the presence of lipoxygenase and allene oxide synthase (14). However, a galactolipid containing 16:3-derived

dinor-12-oxo-PDA and 18:3-derived 12-oxo-PDA (Arabidopside A) has been isolated from leaves of *Arabidopsis* (15) indicating that dinor-12-oxo-PDA and 12-oxo-PDA biosynthesized as the free carboxylic acids can be re-incorporated into galactolipids. Other monogalactosyl diacylglycerols containing either two 12-oxo-PDA residues (Arabidopside B) (15) or one 12-oxo-PDA and one 16:3 residue (14), as well as a monogalactosyl monoacylglycerol containing one 12-oxo-PDA residue (16) have also been isolated.

Also the divinyl ether synthase pathway of oxylipin metabolism has been reported to produce a hexadecanoid. In this case, 11(*S*)-hydroperoxy-7,9,13-hexadecatrienoic acid generated from 16:3 was converted into 2,3-dinor-w5(*Z*)-etherolenic acid (10-[1´(*Z*),3´(*Z*)-hexadienyloxy]-7(*Z*),9(*E*)- decadienoic acid) by a particle-bound divinyl ether synthase form leaves of *Ranunculus acris* (meadow buttercup) (17). Notably, this plant, like *Arabidopsis thaliana*, belongs to the 16:3 family and has a high content of 16:3 in leaf galactolipids (3). The a-dioxygenase pathway can also generate a hexadecanoid as recently shown for tobacco (*Nicotiana tabacum*) leaves, which among other oxylipins produce 2-hydroxy-7,10,13-hexadecatrienoic acid when infected with *Pseudomonas syringae* (18). Interestingly, 16:3 is a very poor substrate for 9-lipoxygenases (Larodan, unpublished results) and so far no product of the putative 7- (w10-) hydroperoxide derivative of 16:3 has been described (Scheme 2).

The possible biological role(s) of 16:3, 16:3-containing galactolipids, or hexadecanoids are presently unknown. In this respect it may be mentioned that Weber *et al.* (12) found that dinor-12- oxo-PDA in micromolar concentrations increased the ability of extracts of *Arabidopsis* leaves to generate 13-hydroxy-12-oxo-9(*Z*)-octadecenoic acid from linoleic acid by lipoxygenase and allene oxide synthase activities. They also made the potentially important observation that leaves from the *fad5* mutant of *Arabidopsis* deficient in 16:3 had *ca*. 15-fold lowered basal levels of 12-oxo-PDA compared to leaves from wild type plants, whereas the wound-induced levels in mutant and wild type were similar (12). Furthermore, a study of the *ssi2* mutant of *Arabidopsis*, a dwarf phenotype which is deficient in a plastidic stearoyl-ACP desaturase activity, has also suggested a role of 16:3 or hexadecanoids in the manifestation of the *ssi2*-conferred phenotype (19).

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