BIOSYNTHESIS AND STRUCTURE OF LEAF LIPIDS:
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J. L. Harwood (Cardiff)

Sterol Alkylation in Higher Plants and Micro-organisms

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The main sterol of animals is cholesterol, but in higher plants, algae and fungi it is
replaced by a diverse array of sterols with supernumerary carbon atoms in the side chain
at C-24 (see Goad et al., 1974). Fig. 1 indicates the types of substitution encountered.

Fig. 1. Types of side chains found in plant sterols

The α- and β-convention is in these cases unequivocal and simple; the R and S convention
can lead to confusion, as for example in comparing compounds (V) and (VIII).

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and it is important to note that with the methyl and ethyl derivatives both chiral species (α- and β-) exist. In algae and the fungi only 24β-isomers occur, whereas in higher plants, although in most cases the α-isomers are by far the major species encountered, small but significant amounts of 24β-methyl isomers are widespread (Mulheirn, 1973; Nes et al., 1976; Goad et al., 1977; W. J. Walker & L. J. Goad, unpublished work) and occasionally β-ethyl derivatives (e.g. clerosterol, side chain XII, Fig. 1) are encountered (Bolger et al., 1970).

The additional carbon atoms at C-24 arise by either a single transmethylation to form a C28 sterol or double transmethylation to form a C29 sterol (Castle et al., 1963). However, the detailed mechanisms involved can vary according to the organism examined; in particular the 24α-epimers are produced in a different way from the 24β-epimers. Studies which have revealed the different mechanisms have involved the use of [2-14C,4R,4-3H]mevalonic acid, [Me-2H]methionine, [Me-14C]methionine and various labelled intermediates.

In fungi, alkylation leads to the formation of a 24-methylene derivative, which is then reduced to a 24β-methyl sterol (pathway 1, Fig. 2) [see Goad et al. (1974) for a short review]. Fungi rarely synthesize C28 sterols. Our first experiments were with the Chrysophyte alga Ochromonas malhamensis. Firstly it was found that only two and five 2H atoms were incorporated from [Me-2H]methionine into the 24β-methyl and 24β-ethyl groups of brassicasterol (C-28; side chain VII; Fig. 1) and poriferasterol (C-29; side chain IX; Fig. 1) respectively (Smith et al., 1967; Goad et al., 1974). Secondly it was shown that the 3H at C-25 in cycloartenol (the first cyclic precursor of sterols in photosynthetic organisms) which arises from [4R,4-3H]mevalonic acid, is retained at C-24 in brassicasterol and poriferasterol (Smith et al., 1972). These observations led to the conclusion that mechanisms (1) and (2) (Fig. 2) were involved in alkylation in Ochromonas spp. This was supported by the demonstration that the ethylidene sterol isofucosterol (side chain III; Fig. 1) was a very effective precursor of poriferasterol (Lenton et al., 1971).

It soon became clear that the Ochromonas pathway was rather restricted in distribution, one of the first indications of this being the report that in Chlorella spp. three and five 2H atoms were incorporated into the methyl and ethyl sterols respectively from [Me-2H]methionine (Tomita et al., 1970). A pathway involving different intermediates in the formation of methyl and ethyl sterols was eventually demonstrated in the green algae Trebouxia sp. 213/3 and Scenedesmus obliquus (pathway 3; Fig. 2) (Goad et al., 1972; Wojciechowski et al., 1973). The evidence for this pathway was based on (1) the incorporation of three and five 2H atoms into the methyl and ethyl sterols respectively, (2) the isolation of both a Δ25-methylene derivative with a 24β-methyl substituent (cyclolaudanol; side chain X; Fig. 1) and a Δ24(29)-methylene derivative (methylenecycloartanol; side chain I; Fig. 1), (3) the formation of labelled cyclolaudanol and methylenecycloartanol from [Me-14C]methionine in a cell-free extract, (4) the conversion of 31-[14C]norcyclolaudanol only into methyl sterols and tritiated cycloeucalenol (31-normethylenecycloartanol) only into ethyl sterols, and (5) the conversion of 14C-clerosterol (side chain X11; Fig. 1) into poriferasterol and clionasterol (side chain VI; Fig. 1) in Trebouxia sp. 213/3 (Largeau et al., 1977).

Experiments with [2-14C,4R,4-3H]mevalonic acid in higher plants, e.g. Nicotinia tabacum, Dioscorea tokoro (Tomita & Umori, 1970), Spinacea oleracea and Medicago sativa (Armarego et al., 1973), showed that no 3H was present at C-24 or C-25 in the alkylated sterols, and thus indicated yet another mechanism for alkylation. Technical difficulties delayed the final solution of this problem, but it was solved by the use of isolated barley embryos which incorporated [Me-2H]methionine into sterols without significant dilution from endogenous sterols. This permitted the demonstration that only two and four 2H atoms were retained in the methyl and ethyl sterols respectively. It was confirmed that no 3H was present at C-24 or C-25 in the sitosterol (side chain VIII; Fig. 1) formed, but that the putative ethylidene intermediate isofucosterol (side chain III; Fig. 1) was formed by the barley embryos and that a 3H atom was still present at C-25. Finally 24-methylenelophenol and 24-ethylidenelophenol were

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Fig. 2. Alkylation pathways in sterol biosynthesis in higher plants and micro-organisms
isolated from barley embryos and it was shown that 24-[2,4-3H3]ethylidenelophenol was incorporated into sitosterol by the embryos. This led to the proposed mechanism 4 (Fig. 2) (Lenton et al., 1975) for the formation of 24α-alkyl sterols in higher plants. The pathway involves the intermediacy of Δ24(25)-methyl and -ethyl sterols: such a methyl sterol has been isolated from Withania somnifera (Lockley et al., 1974).

Thus it became clear that the mechanism involved in the biosynthesis of 24α-alkyl sterols in higher plants differed from that involved in the formation of 24β-alkyl sterols in fungi and algae. However, as indicated in the first paragraph, it has also become clear recently from 220 MHz nuclear-magnetic-resonance studies that in many higher plants small amounts of 24β-alkyl sterols exist side by side with the α-isomers, and it was thus important to know whether the two mechanisms existed side by side. It is well established that, starting with [2-14C,4R-3H]mevalonic with a 14C/3H ratio of 1:1 as substrate, the resulting Δ2-alkyl sterols formed in various sterologenic systems will have a 14C/3H ratio of 5:3 according to whether or not the side-chain 3H is lost. The ratio in stigmasterol and sitosterol in barley approximates to 5:2, as expected from pathway 4 (Fig. 2), but it is now clear that observed ratios greater than 5:2 for 24-methyl sterols are due to the presence in the samples of 24β-isomers. These presumably are being formed via the Trebouxia pathway (3, Fig. 2) and thus would retain the 3H in the side chain and would have a 14C/3H ratio of 5:3. Recent experiments with maize have borne out these arguments and shown that 14C/3H ratio of the 24-methyl sterol mixture (5:2.82) fits well with that calculated from the known relative amounts of the α- and β-isomers present as determined by nuclear-magnetic-resonance spectroscopy. Furthermore the purified stigmasterol had a ratio of 5:2. If the pathway for the synthesis of the β-isomers in higher plants is similar to that in Trebouxia spp. and Scenedesmus spp., then the early precursor cyclolaudenol should exist together with 24-methylenecycloartanol. Recently the co-occurrence of these two compounds has been demonstrated by labelling studies for the first time in maize (Goodwin et al., 1977; L. J. Goad & M. Zakelj, unpublished work).

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