pyridoxol phosphate and of the thiazole moiety of thiamine diphosphate. The branched carbon skeleton of isoprenic units results from the rearrangement of the straight-chain DXP (refs[4,6]). Identity of the C_5 skeletons of DXP and IPP was demonstrated by incorporation of deoxyxylulose isotopomers with multiple ^{13}C labeling[7,8].

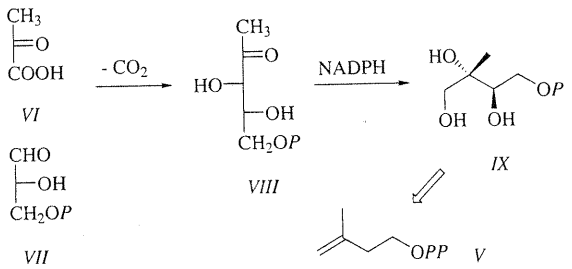

Fig. 2. Methylerythritol 4-phosphate pathway for isoprenoid biosynthesis

2-*C*-Methyl-D-erythritol cyclodiphosphate, the free tetrol or the corresponding lactone were found in several bacteria and higher plants. These tetrol derivatives fitted well in our hypothetical biogenetic scheme[4]. Feeding of ^{13}C labeled glucose to *Corynebacterium ammoniagenes* showed that the prenyl chain from dihydromenaquinone and 2-*C*-methyl-D-erythritol resulted both from the mevalonate-independent route[9]. Furthermore, the deuterium labeled D-enantiomer was incorporated into the prenyl chains of ubiquinone and menaquinone of *Escherichia coli*, indicating a possible precursor/product relationship. No other intermediates have been presently identified[10].

Two enzymes of this metabolic route were identified. The gene of the DXP synthase was cloned in *Escherichia coli*[11,12] and peppermint[13], and that of the DXP reducto-isomerase catalyzing the concomitant rearrangement of DXP and the reduction of the resulting aldehyde into 2-*C*-methyl-D-erythritol 4-phosphate (*IV*) (MEP) in *Escherichia coli*[6]. MEP with its branched C_5 carbon skeleton can be considered as an hemiterpene and might represent the first committed intermediate of this metabolic route[14].

The GAP/pyruvate route is found in many eubacteria, including pathogens and opportunistic pathogens, and in unicellular algae (Chlorophytes, Rhodophytes, Chrysophytes). In higher plants, this bacterial route is involved in the biosynthesis of all essential chloroplast isoprenoids (*e.g.* phytol, carotenoids, plastoquinone)[15] as well as in the formation of other plastid related isoprenoids of more restricted distribution such as isoprene, monoterpenes and diterpenes[14,16].

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BRASSINOSTEROID METABOLISM

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In 1979 Grove *et al.* published the structural elucidation of brassinolide, (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-6a-oxa-5α-cholestan-6-one (Fig. 1, *I*), being the first and hitherto most active member of a unique family of steroidal plant growth regulators[1].

An amount of only 4 mg of crystals of *I* were obtained from 40 kg rape pollen (*Brassica napus*), indicating occur-

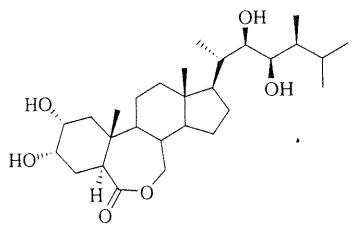

Fig. 1. Structure of brassinolide (*I*)

rence in very low concentrations. The high activity at these low concentrations on cell elongation, cell division and many other physiological processes stimulated intense research activities in many laboratories. Remarkably, the activity of brassinolide was shown to be different from that of classical phytohormones and other plant growth regulators. Many studies confirmed the ubiquitous presence of brassinolide-related compounds, collectively named brassinosteroids, in the plant kingdom. Further research efforts were directed towards chemical synthesis, biochemistry, biological mode of action, and practical application in agriculture. Despite evidence provided by extended biological studies, the status of brassinosteroids as a group of phytohormones was not generally accepted until recently. However, investigations involving a number of brassinosteroid deficient and perceptive mutants of *Arabidopsis thaliana*, *Lycopersicon esculentum* and *Pisum sativum* have established the essential role of brassinosteroids in plant growth and development (for review, see[2]).

The biosynthesis of brassinosteroids was studied by Japanese research groups, feeding tritiated or deuterated precursors to cell cultures of *Catharanthus roseus* (for review, see[3]). These studies confirmed campesterol (*II*) as precursor of brassinolide (*I*). The multistep biosynthetic pathway involves 5α-reduction of *II* to form the *trans*-fused A/B ring system followed by a sequence of oxidation steps and, in between, inversion of configuration at C-3. Two alternative routes comprising identical numbers of biosynthetic steps were found, being different only in oxidation at C-6 occurring early between campestanol (*III*) and 6-oxocampestanol (*IV*) or later converting 6-deoxocastasterone (*V*) to castasterone (*VI*) (Fig. 2).

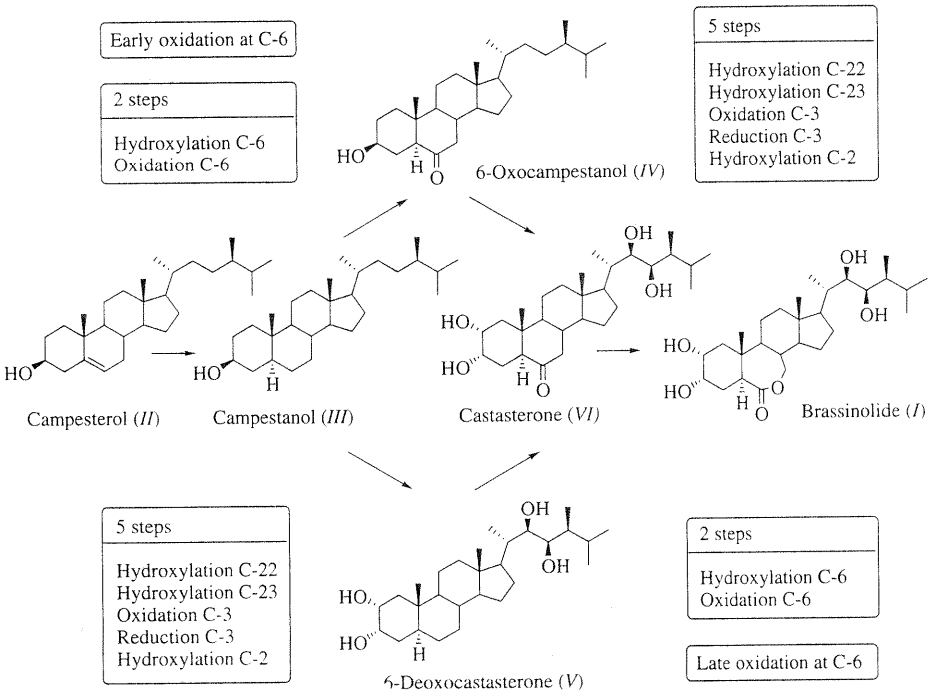

Fig. 2. Alternative early and late C-6 oxidation pathways of brassinolide biosynthesis (for review, see ref.[3])

Further variations of the functionalization sequence can be supposed. Moreover, it seems most likely that phytosterols having different side chains structures are converted to brassinosteroids in parallel pathways without altering the carbon scaffold. A number of steps in the biosynthesis of 24*R*

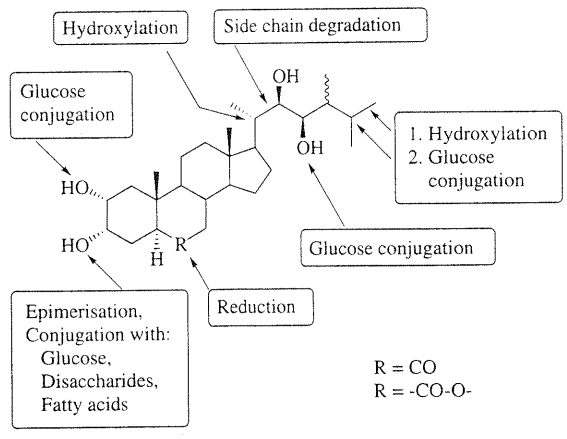

Fig. 3. Reactions observed in brassinosteroid metabolism

methyl brassinosteroids, namely epimerization of 24-epiteasterone (*VII*) at C-3 *via* 3-dehydro-24-epiteasterone (*VIII*) to afford 24-epityphasterol (*IX*), and the Baeyer–Villiger reaction converting 24-epicastasterone to 24-epibrassinolide, were demonstrated *in vitro* using enzyme preparations of *Lycopersicon esculentum* cell cultures[4]. Combination of feeding experiments and molecular analysis of brassinosteroid biosynthetic mutants, whose dwarf phenotype can be rescued to wildtype phenotype by exogenous brassinosteroid treatment, revealed the sites of lesions in the pathway. Cloning, heterologous expression, and sequence analyses indicated that at least two hydroxylation steps involved in brassinolide biosynthesis are catalysed by P450 monooxygenase type enzymes. Further mutants are deficient in enzymes catalyzing early steps of brassinosteroid biosynthesis, namely before campesterol (*II*) or between *II* and campestanol (*III*) (for review, see[5]).

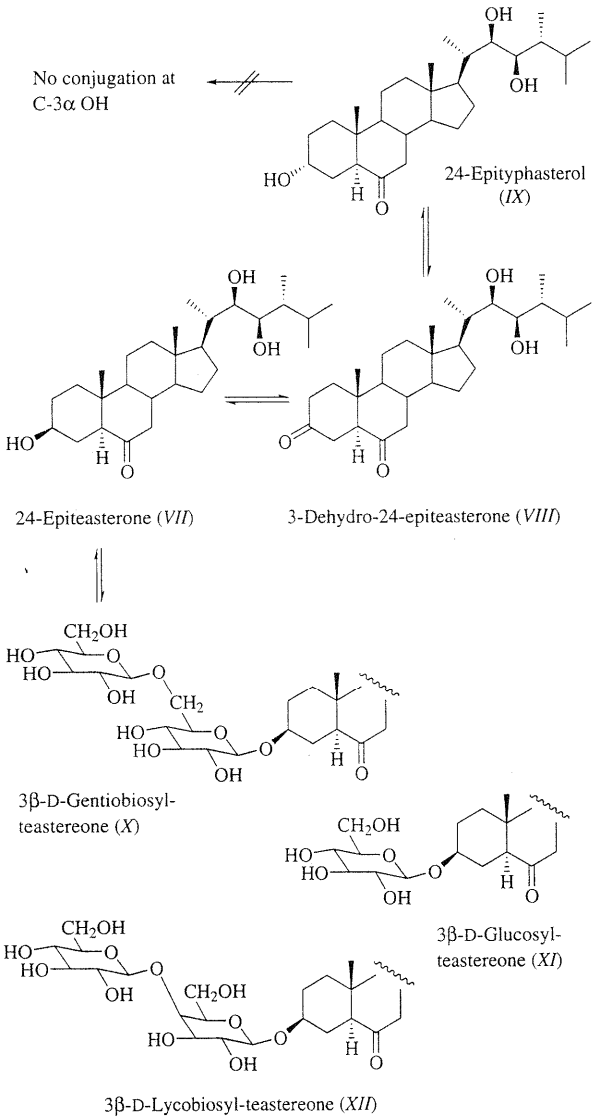

Fig. 4. Reversible epimerization and selective glycosidation of 24-epiteasterone in tomato cell cultures

Detailed studies on metabolism and conjugation of brassinosteroids were carried out using cell cultures of *Lycopersicon esculentum* and *Ornithopus sativus* (for review, see[6]). About 30 metabolites have been isolated and identified upon administration of 24-epicastasterone, 24-epibrassinolide, and 24-epiteasterone (*VII*), mostly being novel metabolites formed by hydroxylation, epimerization, side chain cleavage, reduction, and conjugation (Fig. 3). A variety of novel pentahydroxylated metabolites carrying additional hydroxyl groups at C-20, C-25, and C-26, respectively, was found. While hydroxylation at C-20 is followed by side chain removal yielding pregnane type compounds, new hydroxyl groups at C-25 and C-26 rapidly undergo glycosidation. For stereochemical reasons, conjugation at C-3 requires 3β-configuration. This was demonstrated, for example, by smooth glycosidation and acylation of 24-epiteasterone (*VII*) and other C-3β compounds. In contrast, C-3α brassinosteroids did not undergo conjugation. Selective reversible conjugation, together with reversible epimerization, may provide the opportunity to regulate the cellular level of hormonal active brassinosteroids (Fig. 4).

Biosynthetic and metabolic studies on brassinosteroids are essential to better understanding the molecular mode of action of brassinosteroids. Chemical, biochemical, physiological and molecular investigations are expected to provide information on the precise role of these of phytohormones as well as on plant growth and development in general.

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