Metabolism of brassinolide in *Marchantia polymorpha* was investigated by use of in vivo suspension cultured cells. GC-MS analysis of metabolites derived from nonlabelled brassinolide and [26, 28-$^2H_6$]-brassinolide revealed that brassinolide was converted to 26-norbrassinolide while [26, 28-$^2H_6$]-brassinolide to [26,-$^2H_3$]-28-norbrassinolide. It seems that *Marchantia* cells recognized [26, 28-$^2H_6$]-brassinolide as a xenobiotic rather than brassinolide and deteriums attached to C-28 significantly affect demethylation reaction due to isotopic effect. Thus, demethylation of brassinolide in planta seems to proceed by loss of C-26 rather than C-28. The present finding is the first evidence for demethylation metabolism of brassinosteroids. The biological activity of 26-norbrassinolide was 10-fold reduced as examined by the rice lamina inclination test. However, because of its high biological activity, it remains difficult to conclude straightforward whether or not C-26 demethylation serves as an important deactivation process of brassinolide.

E204

Biosynthesis of Brassinosteroids in Primary Roots of Maize

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We have demonstrated that brassinosteroids (BRs) are involved in gravitropic response of primary roots of maize. To examine whether BRs are indeed biosynthesized in the roots, identification of BRs and their biosynthetic precussors and occurence of biosynthetic enzymes in the root were carried out. GC-MS/SIM analyses revealed that 6-deoxocastasterone and castasterone, members of the late C6-oxidation pathway, were contained in the roots. In addition, presence of campesterol and campestanol, biosynthetic precursors of 6-deoxocastasterone and castasterone, were demonstrated in the roots. These suggested that a biosynthetic pathway from campesterol to castasterone via campestanol and 6-deoxocastasterone, namely the late C6-oxidation pathway, is present to produce BRs in the roots. A microsomal fraction obtained from the roots successfully catalyzed conversion of 6-deoxocastasterone to castasterone, which provided the presence of castasterone oxidase in the roots. Taken together, it is clear that BRs are biosynthesized via the late C6-oxidation pathway in primary roots of maize to show gravitropic curvature.

E205

Cytochrome P450 Monooxygenases Catalyze Biosynthesis of Brassinolide from Typhasterol or 6-Deoxocastasterone via Castasterone in *Phaseolus vulgaris* Cells

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A microsomal fraction prepared from cultured cells of *Phaseolus vulgaris* catalyzed conversions of typhasterol to brassinolide intermediated by castasterone. This indicates that typhasterol 2a-hydroxylase and brassinolide synthase catalyzing the conversion of typhasterol to castasterone and castasterone to brassinolide, respectively, are integral proteins in the membrane of the cells. For the activity, both typhasterol 2a-hydroxylase and brassinolide synthase required NADPH and O_2. Furthermore,

commercial Cyt P450 inhibitors (Cyt c, SKF 525A, aminobenzotriazole and kentoconazole) and CO strongly inhibited both enzyme activities, and the inhibited activity by CO was clearly recovered by illumination of blue light in the presence of O_2, providing that both enzymes are Cyt P450 monooxygenases. Activity of 6-deoxocastasterone oxidase was also shown in the microsomal fraction in the presence of NADPH as a cofactor. The activity was strongly inhibited by additions of Cyt P450 inhibitors (Cyt c and SKF 525A) in the assay mixture, confirming that 6-deoxocastasterone oxidase in *Phaseolus* cells is also a Cyt P450 monooxygenase. Therefore, it is suggested that typhasterol 2a-hydroxylase, 6-deoxocastasterone oxidase and brassinolide synthase catalyzing the last two steps of the early- and late-C6-oxidation pathway to produce brassinolide are Cyt P450 monooxygenases in *Phaseolus* cells.

E206

Brassinosteroids in *Phaseolus vulgaris* are Predominantly Biosynthesized by the Early C6-Oxidation Pathway.

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Identification of endogenous brassinosteroids (BRs) in *Phaseolus vulgaris* suggested that both the early and late C6-oxidation pathway for biosynthesis of brassinolide are contained in the plant. To determine which pathway is predominantly operative, activities of campestanol oxidase and campestanol 22*R*-hydroxylase catalyzing the first step in the early and late C6-oxidation pathway, respectively, were examined. The activity of campestanol oxidase was ca 3 times higher than that of cmapestanol 22*R*-hydroxylase, indicating that campestanol is predominantly catalyzed by campestanol oxidase rather than campestanol 22*R*-hydroxylase. Next, activity of typhasterol 2a-hydroxylase and 6-deoxocastasterone oxidase responsible for the last step to produce castasterone, a direct precursor of brassinolide in the early and late C6-oxidation pathway, respectively, were investigated. Typhasterol 2a-hydroxylase also showed 3 times higher activity than that of 6-deoxocastasterone oxidase, indicating that more castasterone is produced by typhasterol 2a-hydroxylase than 6-deoxocastasterone oxidase. Therefore, it is suggested that castasterone and brassinolide in the *Phaseolus* cells were prodominantly biosynthesized by the early C6-oxidation pathway rather than the late C6-oxidation pathway.

E207

Regulation of Gravicurvature by Malformin A1 in the Primary Root of Maize

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Malformin, a small family of cyclic pentapeptides, is an active plant growth regulator isolated from the fungus *Aspergillus niger*. It has been known that maformin induces root curvature and ethylene mediated responses. We studied the action of the purified malformin A1 in the gravitropic response, and examined the possibility that this response might be related to the ethylene. Primary roots pretreated with malformin A1 vertically were placed in a humidified box in the horizontal position, and the curvature was measured using a CCD camera and a time-lapse video cassette recorder. The gravistimulated curvature was