E238 Biosynthesis of Brassinosteroids in a Liverwort, *Marchantia polymorpha* : From Teasterone to Castasterone

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The presence of C3-epimerization from teasterone (TE) to typhasterol (TY), a part of biosynthetic pathway for brassinosteroids (BRs) has been previously demonstrated in suspension cultured cell of *M. polymorpha*. To get further information on biosynthesis of BRs in the cells, TY was fed to the cells, and its metabolites were investigated. Four biologically active peaks (I, II, III and IV) obtained after a reversed phase HPLC were analyzed by a capillary GC-MS. The bismethaneboronate (BMB) of active compound in I and IV gave the same mass spectrum and GC Rt as those of BMB of castasterone (CS) and 3-Dehydroteasterone (3DHT), respectively. The MB-trimethylsilly ether of active compound in II and III gave the same GC Rt and mass spectrum as did the MB-trimethylsilly ether of TE and TY, respectively. Thus the active compounds in I, II, III and IV were characterized to be CS, TE, TY and 3DHT, respectively. The identification of CS indicates that TY is converted to CS by 2α-hydroxylation in the cell. The identification of 3DHT and TE indicates a reverse conversion from TE to TY via 3DHT is also present in the cells. Thus a sequence for biosynthesis of BRs in the cells, TE ↔ 3DHT ↔ TY → CS is established..

E239 Metabolism of Brassinolide in Suspension Cultured Cells of *M. polymorpha*

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Brassinolide (BL) is the most active and wide-distributed brassinosteroid (BR) in plant kingdom. In order to elucidate metabolism of BL, deterium-labelled ([$^2H_6$]) BL was fed to suspension cultured cells of a liverwort, *M. polymorpha*, and a metabolite of [$^2H_6$]BL was investigated. After purifying the metabolite by C_{18} bulk and LH-20 column chromatography, a weak peak was obtained by a reversed phase HPLC. An active compound in the peak was analyzed by GC-MS after methaneboronation. The bismethaneboronate (BMB) of the compound gave characteristic ions at *m/z* 517[M+], 374, 344, 321, 177, 163, 144 (base peak), suggesting that the chemical structure of the compound is a demethylated [$^2H_6$]BL at C-26 or C-28. To determine the position of demethylation, [$^2H_0$]BL was re-fed to the cells, and the metabolite was analyzed by GC-MS. The Rt of BMB of the metabolite was quite different from that of authentic 28-demethylBL (28-norBL) BMB, indicating that the position of demethylation is not at C-28 but at C-26 of BL. Thus the metabolite of BL in the cell was tentatively characterized to be 26-demethylBL (26-norBL). This is the first evidence for the presence of 26-norBL in plant kingdom.