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The presence of brassinosteroids(BRs) in primary roots of maize(*zea mays* L.) has been already demonstrated. To examine whether the endogenous BRs were biosynthesized in the roots, identification of biosynthetic precursor(s) in the roots were carried out. Gas chromatography-Mass spectrum analysis revealed that maize primary roots contained campesterol(CR) and campestanol(CN). Next, enzymatic conversion from CR to CN was undertaken.  $3\beta$ -hydroxysteroid dehydrogenase/isomerase( $3\beta$ -HSD), an enzyme converting CR to (24*R*)-24-methylcholest-4-en-3-one(4-en-3-one) via (24*R*)-24-methylcholest-4-en- $3\beta$ -ol(4-en- $3\beta$ -ol), were successfully detected in cytosolic enzyme solution, indicating that  $3\beta$ -HSD was a cytosolic soluble protein. Activity for 4-en-3-one  $5\alpha$ -reductase, an enzyme converting 4-en-3-one to 3-one, and 3-one reductase, an enzyme converting 3-one to CN, were detected in microsomal enzyme solution, indicating that the intracellular localization of the enzymes is in the membrane of ER. Together with the presence of 6 deoxocastasterone oxidase in primary roots of maize, these provide that BRs in the roots are biosynthesized by the late C6-oxidation pathway operating in the roots.

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