

Occurrence of Teasterone and Typhasterol, and Their Enzymatic Conversion in *Phaseolus vulgaris*

Tae-Wuk Kim, Seung-Hyun Park, Kwang-Seok Han, Jongkil Choo,
June Seung Lee,[†] Seongbin Hwang,[‡] and Seong-Ki Kim*

Department of Life Science, Chung-Ang University, Seoul 156-756, Korea

[†]*Department of Biological Science, Ewha Womans University, Seoul 120-750, Korea*

[‡]*Department of Molecular Biology, Sejong University, Seoul 143-747, Korea*

Received February 3, 2000

It has been demonstrated that immature seeds of *Phaseolus vulgaris* contain 6-deoxocasterone (**1**), 6-deoxodolichosterone (**2**), 6-deoxohomodolichosterone (**3**), dolichosterone (**4**), dolicholide (**5**), 25-methyldolichosterone (**6**), castasterone (**7**, CS) and brassinolide (**8**, BL).¹⁻³ In addition to these brassinosteroids (BRs), the presence of a number of unknown BRs in the seeds has been suggested by HPLC and GC-MS analysis.² Our interest in the structure and biosynthesis of BRs in *P. vulgaris* led us to re-investigate the endogenous BRs in the seed using a larger quantity of the plant materials. Two BRs, teasterone (**9**, TE) and typhasterol (**10**, TY) which are biosynthetic precursors of CS and BL, were additionally identified. Herein, identification and enzymatic conversions of these BRs are reported.

After open column chromatographies, a free-BRs fraction obtained from immature seeds (136 Kg) of *P. vulgaris* was purified by a reversed phase HPLC (Senshu Pak Develosil ODS, 20 × 250 mm, 9.9 mL min⁻¹, 45% acetonitrile for 0-40 min and 80% for 40-70 min, fractions were collected every min), giving rise to a number of biologically active fractions.

Among the HPLC fractions, fraction 47 was analyzed by a

full-scan GC-MS after methanoboronation followed by trimethylsilylation.⁴ As summarized in Table 1, mass spectrometric features of methanoboronate (BM)-trimethylsilyl (TMSi) ether of an active compound were identical to those derived from MB-TMSi ether of authentic TE (**9**). Furthermore, retention time of MB-TMSi ether of the active compound on GC was exactly the same as that of authentic TE (**9**) MB-TMSi ether. Thus, the active compound was determined to be TE (**9**).

HPLC fraction 53 corresponding to retention time of authentic TY (**10**) in the same HPLC condition was analyzed by GC-MS after methanoboronation followed by trimethylsilylation. As expected, MB-TMSi ether of an active compound in fraction 53 showed the same mass spectrum and GC retention time as those of authentic TY (**10**) MB-TMSi ether (Table 1). Thus, the active compound in the fraction 53 was identified to be TY (**10**).

TY (**10**) is biosynthesized from TE by C3-epimerization which is intermediated by a C3-keto BR, 3-dehydroteasterone (**11**, 3-DHT).⁵ To examine whether the two-step C3-epimerization also occurs in *P. vulgaris*, enzymatic conversions from TE (**9**) to TY (**10**) were subsequently investigated. To this end,

Table 1. GC-MS data for endogenous brassinosteroids in the seeds of *P. vulgaris* and authentic teasterone, 3-dehydroteasterone and typhasterol

Compound	Retention Time on GC ^c	Prominent ions (<i>m/z</i> , relative intensity %)
Fraction 47 ^a	18.06	544 (M ⁺ , 30), 529 (55), 515 (100), 454 (8), 300 (8), 155 (39)
Fraction 53 ^a	15.12	544 (M ⁺ , 100), 529 (79), 515 (57), 454 (68), 300 (13), 155 (60)
Teasterone ^a	18.06	544 (M ⁺ , 28), 529 (60), 515 (100), 454 (8), 300 (8), 155 (35)
3-Dehydroteasterone ^b	17.76	470 (M ⁺ , 64), 399 (8), 357 (11), 316 (19), 298 (11), 287 (11), 245 (19), 155 (100)
Typhasterol ^a	15.12	544 (M ⁺ , 100), 529 (80), 515 (55), 454 (67), 300 (12), 155 (58)

^aThe sample was analyzed as a derivative of methanoboronate-trimethylsilyl ether. ^bThe sample was analyzed as a derivative of methanoboronate. ^cGC condition: HP-5 (0.25 mm × 30 m, 0.25 μm film thickness), 1 mL min⁻¹ He, on-column injection mode, temp. program; 175 °C for 2 min, thermal gradient 40 °C min⁻¹ to 290 °C, and then 290 °C.

Table 2. Identification of enzyme product(s) of teasterone dehydrogenase and 3-dehydroteasterone reductase prepared from suspension cultured cells of *P. vulgaris*

Enzyme	Product	Retention time on GC ^c	Prominent ions (<i>m/z</i> , relative intensity %)
Teasterone dehydrogenase	3-Dehydroteasterone ^a	17.76	470 (M ⁺ , 63), 399 (7), 357 (5), 316 (21), 298 (10), 287 (11), 245 (11), 155 (100)
	Teasterone ^b	18.06	544 (M ⁺ , 29), 529 (53), 515 (100), 454 (5), 300 (8), 155 (39)
3-Dehydroteasterone reductase	Typhasterol ^b	15.12	544 (M ⁺ , 100), 529 (81), 515 (55), 454 (72), 300 (10), 166 (60)

^aThe sample was analyzed as a derivative of methanoboronate. ^bThe sample was analyzed as a derivative of methanoboronate-trimethylsilyl ether. ^cGC condition: HP-5 (0.25 mm × 30 m, 0.25 μm film thickness), 1 mL min⁻¹ He, on-column injection mode, temp. program; 175 °C for 2 min, thermal gradient 40 °C min⁻¹ to 290 °C, and then 290 °C.

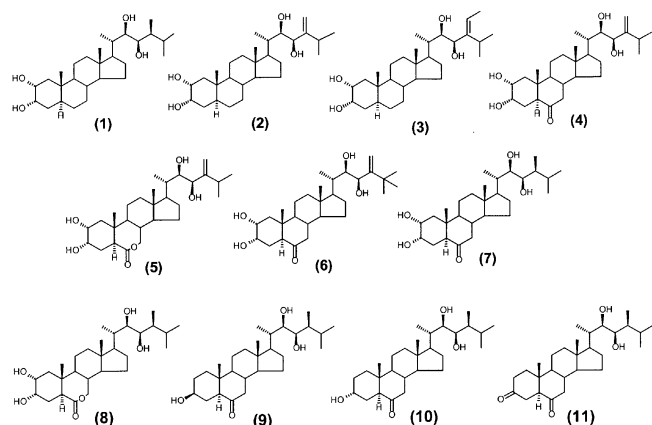


Figure 1. Structure of brassinosteroids described in this paper. 6-Deoxocastasterone (1), 6-Deoxodolichosterone (2), 6-Deoxohomololichosterone (3), Dolichosterone (4), Dolicholide (5), 25-Methyl-dolichosterone (6), Castasterone (7), Brassinolide (8), Teasterone (9), Typhasterol (10) and 3-Dehydroteasterone (11).

suspension cultured cells (20 g) of *P. vulgaris* were homogenized with Tris-HCl buffer (50 mM, pH 7.4). After discarding cell debris by centrifugation at $6,000 \times g$ for 10 min, the supernatant was re-centrifuged at $15,000 \times g$ for 20 min. The resulting supernatant was used as a crude enzyme solution.

Because endogenous amount of BRs in the cultured cells (20 g) was negligible, TE (2 μg) as a substrate was added to a reaction mixture (1 mL) containing the crude enzyme solution, 0.2 mM NADP and 1 mM MgCl_2 . After incubation for 30 min at 37 °C, the product was analyzed by a capillary GC-MS after methanoboronation. MB of the product gave a molecular ion at m/z 470 and prominent ions at m/z 399, 357, 316, 298, 287, 245 and 155 which were identical with those of MB of authentic 3-DHT (11). Furthermore, the retention time of MB of the product on GC was the same as that of authentic 3-DHT (11) MB (Table 1 and 2). Thus, the product was characterized to be 3-DHT (11).

Next, 3-DHT (2 μg) was added to the crude enzyme solution (1 mL) containing 0.2 mM NADPH and 1 mM MgCl_2 , and the reaction mixture was incubated for 30 min at 37 °C. The products were analyzed by a full scan GC-MS after methanoboronation followed by trimethylsilylation. On total ion chromatogram, two peaks of the enzyme product were detected at 15.12 and 18.06 min. The former peak corresponding to retention time of authentic TY (10) MB-TMSi ether showed the same mass spectrum as that of MB-TMSi of authentic TY (10, Table 1 and 2). On the other hand, the latter peak provided identical mass spectrum and GC retention time with those of authentic TE (9) MB-TMSi ether (Table 1 and 2). The amount of both enzyme products measured by GC-selected ion monitoring analysis was approximately 2% of the substrate. In consequence, TE (9) and TY (10) as enzyme

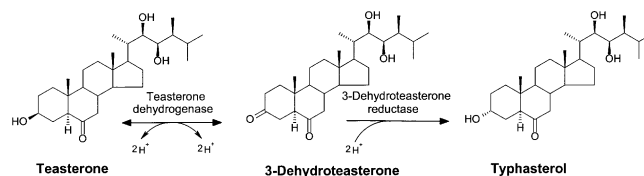


Figure 2. The two-step C3-epimerization from teasterone to typhasterol via 3-dehydroteasterone in *P. vulgaris*.

products of 3-DHT (11) were identified, indicating that 3-DHT (11) was converted into TY (10) by 3-DHT reductase and reversibly into TE (9) by TE dehydrogenase. Thus, the presence of the C3-epimerization from TE (9) to TY (10) via 3-DHT (11) was demonstrated in *Phaseolus* cells (Figure 2).

The C3-epimerization of TE (9) to TY (10) is important for increasing biological activity of BRs.⁶ Recently, the significance of this epimerization in the regulation of BRs biosynthesis has also been reported.⁷ In several plants, it is verified that the C3-epimerization from TE (9) to TY (10) is carried out by a two-step reaction, dehydrogenation and reduction via 3-DHT (11).⁵ Although the presence of 3-DHT in *Phaseolus* cells has not been reported yet, the involvement of 3-DHT in the conversion from TE to TY in the cells is confirmed by enzymatic conversions. Moreover, the fact that the conversion of TE to 3-DHT is reversible, is established. The enzymes responsible for the epimerization, namely TE dehydrogenase and 3-DHT reductase, are recently characterized to be soluble proteins in a liverwort, *Marchantia polymorpha*.⁴ Now, properties including intracellular localization of these enzymes in *P. vulgaris* are under investigation.

Acknowledgment. This work was supported by the Basic Science Research Institute Program, Ministry of Education of Korea, Grant No. BSRI-99-4422.

References

- Yokota, T.; Morita, M.; Takahashi, N. *Agric. Biol. Chem.* **1983**, *47*, 2149.
- Yokota, T.; Koba, S.; Kim, S.-K.; Okada, K.; Mori, K.; Takahashi, N. *Agric. Biol. Chem.* **1987**, *51*, 1625.
- Kim, S.-K.; Yokota, T.; Takahashi, N. *Agric. Biol. Chem.* **1987**, *51*, 2303.
- Park, S.-H.; Han, K.-S.; Kim, T.-W.; Shim, J.-K.; Takatsuto, S.; Yokota, T.; Kim, S.-K. *Plant Cell Physiol.* **1999**, *40*(9), 955.
- Sakurai, A. In *Brassinosteroids; Steroidal Plant Hormones*; Sakurai, A., Yokota, T., Clouse, S. D., Eds.; Springer-Verlag: Tokyo, 1999; p 91.
- Kim, S.-K.; Abe, H.; Little, C. H. A.; Pharis, R. P. *Plant Physiol.* **1990**, *94*, 1709.
- Kolbe, A.; Schneider, B.; Porzel, A.; Adam, G. *Phytochemistry* **1998**, *48*, 467.