Short Communication

Teasterone 3-Myristate: A New Type of Brassinosteroid Derivative in Lilium longiflorum Anthers

Seiichi ASAKAWA, Hiroshi ABE,* Yoshimasa KYOKAWA, Susumu NAKAMURA, and Masahiro NATSUME

Department of Applied Biological Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

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Teasterone 3-myristate as a new type of brassinosteroid derivative was found in lily anthers. Esterification only occurs with the hydroxyl group at C3. There is no endogenous esterified derivative of typhasterol, castasterone, or brassinolide in the lily tissues.

Brassinosteroids are widely distributed in all parts of vegetative and reproductive plant tissues. The physiological function of endogenous brassinosteroids in plant growth has not been identified. However, the very high content in reproductive tissues1,2 and promotive effects on pollen tube elongation3 and flowering4 suggest that brassinosteroids have a regulatory role in reproductive growth. During studies on the regulation of reproductive growth in higher plants, we attempted to identify endogenous brassinosteroids in the anthers and pollen grains. Here, we report the structural identification of teasterone 3-myristate as a new type of brassinosteroid derivative from lily anthers.

Anthers of a lily, Lilium longiflorum cv. Georgia, were collected immediately before and after anthesis and extracted with CHCl3−MeOH (1:1 v/v). After evaporation of the solvent, the resulting residue was dissolved in 80% CH3CN in H2O and then extracted with n-hexane to obtain less polar substances. The 80% CH3CN-insoluble residue was easily dissolved in n-hexane. The combined n-hexane solutions were successively purified chromatographically using columns of silica gel (0−100% EtOAc in n-hexane), Sephadex LH-20 (50% CHCl3 in MeOH), Develosil ODS-5 HPLC (MeOH), and aluminum oxide (20−80% EtOAc in n-hexane, 20−80% EtOH in EtOAc), yielding a biologically active extract. This extract was further purified by HPLC, using columns of Senshupak DIOL-1251-R (30−100% CHCl3 in n-hexane), Develosil ODS-5 (MeOH), and Waters Free Fatty Acid HP (THF−CH3CN−H2O, 25:50:40 v/v) before GC-MS and LC-MS analyses. During the above chromatographies a rice-lamina inclination bioassay was used for detection of the biological activity. Before bioassay a sample of each fraction was hydrolyzed with 1 N KOH in aqueous EtOH and the hydrolysate was extracted with ether.

The active extract thus obtained was hydrolyzed and the ether-soluble fraction was purified by aluminum oxide and Bond Elut column chromatography13 and furthermore by Develosil ODS-5 HPLC (45−75% CH3CN in H2O). The active fraction was analyzed by GC-MS after conversion to methanoborate-trimethylsilyl derivative as previously described.14 The retention time and major ions observed at m/z 544 (M+ : relative intensity, 44%), 529 (66), 515 (100), and 155 (28) agreed well with the values of the authentic teasterone methanoborate-trimethylsilyl derivative,15 suggesting that the active principle may be a fatty acid ester of teasterone.

Several presumable esters were synthesized, which included teasterone 3-laurate (TE-La), 3-myristate (TE-My), 3-palmitate (TE-Pa), and 3-stearate (TE-St), as found by NMR and MS measurements. When retention times on three kinds of HPLC columns described above were measured using a UV detector at 210 nm or the bioassay, the natural teasterone ester appeared at 17−19 min on Develosil ODS-5, 20−21 min on Senshupak DIOL-1251-R, and 13−16 min on Waters Free Fatty Acid HP. TE-My appeared at 18.3 min, 20−21 min, and 16.2 min on the above HPLC columns, respectively, the retention times being closest to the natural ester. The other synthetic esters had different retention times on Develosil ODS-5 column, that is, TE-La at 13.5 min, TE-Pa at 22.6 min, and TE-St at 32.8 min.

As shown in Fig. (a), GC-MS of the natural teasterone ester after conversion to methanoborate gave a molecular ion at m/z 682 (6%), a fragment ion at m/z 454 (100) resulting from elimination of fatty acid (R-COOH), and ions at 439 (30.9) and 426 (9) presumably resulting from loss of 15 and 28 mass units from the m/z 454 ion, respectively. In addition, a characteristic ion appeared at m/z 155 (21) arising by C20-C22 bond cleavage at the side chain of the methanoborate derivative of teasterone.15 These observations indicate that the fatty acid is attached to the 3β-hydroxyl group of teasterone and its molecular formula is C55H82O2, although the ion corresponding to the fatty acid moiety could not be observed in GC-MS.

In LC-MS analysis we accomplished final structure identification of the teasterone ester. A Tosoh CCPS-HPLC system was coupled with a Jeol JMS-SX102A frit-FAB-MS system. Detection was done by negative ion FAB-MS. A matrix of 1% diethanolamine in 50% CHCl3−MeOH was added at postcolumn. A column-switching LC system developed by Asakawa et al.9 was used, consisting of a trapping LC column (Inertsil C8 column, 10 mm×4 mm i.d.) and an analytical LC column (Inertsil ODS-2 column, 150 mm×1.5 mm i.d.). The mobile phase was 70% MeOH in H2O in the trapping column and 10% CHCl3 in MeOH in the analytical column. After the sample solution was injected into the trapping column, the mobile phase for trapping was allowed to flow for 30 s at the flow rate of 1 ml/min. Then the trapping column was connected to the analytical column and the mobile phase for separation was delivered at a flow rate of 0.1 ml/min. Introduction of the eluate to the frit-FAB-MS system was done by using a

* To whom correspondence should be addressed.
Fig. Mass Spectral Analysis of Teasterone Ester in the Lily Anthers.
(a) Mass spectrum of methanobronate derivative of the natural teasterone ester obtained with GC-EIMS system. (b) Mass chromatograms of the natural teasterone ester and the authentic teasterone 3-myristate. (c) Negative ion FAB-MS of the natural teasterone ester obtained with LC-frit-FAB-MS system.

splitter to reduce the flow rate to 5 μl/min. The full mass spectrum obtained (Fig. (c)) comprised two major ions at m/z 657 (a deprotonated molecular ion) and m/z 227 (a deprotonated myristic acid ion) and was identical with that of synthetic TE-My. Mass chromatograms for the natural ester and synthetic TE-My obtained by monitoring these ions were identical (Fig (b)). The evidence clearly indicates that the natural ester is teasterone 3-myristate.

As a naturally occurring brassinosteroid conjugate glucopyranosyl ethers of 25-methylcholesterol and its 2-epimer have been reported by Kim et al.15 Glucosylation only occurred with the hydroxyl group at C23. Our finding is the first evidence for the presence of an esterified brassinosteroid. The lily anthers contain brassinolide, castasterone, and typhasterol in addition to teasterone,23 but esterified derivatives of them could not be detected. Interestingly esterification only occurred with teasterone. It is suggested to result possibly from the substrate specificity of an enzyme system catalyzing such brassinosteroid esterification. In this case, the β-conformation of the hydroxyl group at C-3 could be required for substrate specificity. Such substrate specificity has been already observed in the action of acyltransferase catalyzing sterol esterification.83 Free teasterone also exists in the lily anthers, so that both forms of teasterone must be interconvertible in the reproductive tissues. The biological activity of teasterone 3-myristate is comparable to that of teasterone in the lamina inclination test. GC-MS has been widely used in the analysis of free brassinosteroids in plants. This technique is most successful but is difficult to analyze acyl esters of brassinosteroids because of partial decompositions and longer retention times even at the high column temperature used in GC. In view of this, LC-MS using the column-switching technique was very useful for analyzing such brassinosteroid esters.

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