Cryptolide, a new brassinolide catabolite with a 23-oxo group from Japanese cedar pollen/anther and its synthesis

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Based on the GC-MS direct comparison with synthetic candidates, a new brassinolide-related steroidal lactone detected in the pollen and anthers of Japanese cedar *(Cryptomeria japonica)* was identified as (22*R*,24*S*)-2α,3α,22-trihydroxy-24-methyl-*B*-homo-7-oxa-5α-cholestan-6,23-dione, being the first brassinolide catabolite with 23-oxo function in the side chain and termed as cryptolide, and furthermore, the first occurrence of 28-homobrassinolide in plants was also demonstrated by this work.

We have recently reported that pollen and anthers of Japanese cedar *(Cryptomeria japonica)* contain new 23-oxo-brassinosteroids (23-oxo-BRs), 23-dehydrobrassinolide (23-dehydroBL **1a**) or its stereoisomers.[1] GC–MS analysis indicated that these compounds are distinct from 24-epi-23-dehydroBL with respect to the retention times.[1] This finding, together with the fact that the pollen and anthers also contain typhasterol and 3-dehydroteasterone, which have the same (22*R*,23*R*,24S)-configuration as brassinolide (BL), suggests that the 23-oxo-BRs have the same C-24 configuration as BL.[1] Furthermore, four 2,3-diol stereoisomers of castasterone, a biosynthetic precursor of BL, occur naturally,[2] and some 2,3-diol stereoisomers are formed from BRs fed to plants.[3] Based on these findings, we assumed that the 23-oxo-BRs have a 24*S* stereochemistry with or without isomerized 2,3-diol. Thus we attempted to synthesize 23-dehydroBL **1a** and its three 2,3-diol stereoisomers **1b**, **1c**, and **1d** (Fig. 1) and use them as authentic specimens to characterize the 23-oxo-BRs.

Starting from four 22,23-acetonides of 2,3-diol stereoisomers of BL, the target 23-oxo-BRs, 23-dehydroBL **1a**, 2-epi-23-dehydroBL **1b**, 3-epi-23-dehydroBL **1c**, and 2,3-diepi-23-dehydroBL **1d**, were chemically synthesized via their corresponding 2,3-diacetoxy-22,23-diols by use of partial acetylation followed by Jones oxidation of the remaining hydroxyls at the C-22 and C-23 position.

The natural products contained in three HPLC fractions (fractions 19/20, 22/23, and 25/26, in which one, two, and one isomers of 23-oxo-BRs were detected, respectively, as described in our previous paper[1]) were analysed by direct GC-MS comparison with the synthetic samples, after treatment with methaneboronic acid. The natural product contained in the HPLC fraction 19/20 was found to be identical with 23-dehydroBL on the basis of Kovats retention indices (KRI)[5] and the mass spectral data. However, other natural 23-oxo-BRs detected in the other two HPLC fractions were not in agreement with any of the synthetic samples, leaving their stereochemistry unassigned.

Furthermore, rigorous KRI determination by GC-MS using authentic samples[4,6,7] in this study has led to the additional identification of 28-homoBL in the fraction 19/20 and of 28-homodolicholide in the fraction 22/23. In our previous paper,[1] occurrence of these compounds could not be demonstrated unambiguously because of the lack of the KRI data.

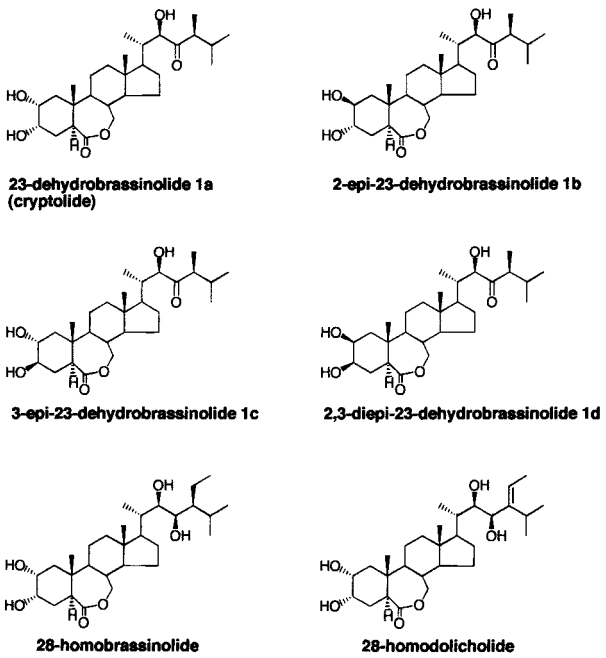

Fig. 1 Structures of 2,3-diol stereoisomers of 23-dehydrobrassinolide, 28-homobrassinolide, and 28-homodolicholide

This work is the first demonstration of the presence of 28-homoBL in plants.

In conclusion, BRs in Japanese cedar comprise 23-dehydroBL, 28-homoBL, and 28-homodolicholide, the presence of these being rigorously demonstrated by this work, as well as 3-dehydroteasterone, typhasterol, and dolicholide, which are previously identified.[1] 23-DehydroBL, (22*R*,24*S*)-2α,3α,22-trihydroxy-24-methyl-*B*-homo-7-oxa-5α-cholestan-6,23-dione, for which the name cryptolide is proposed, is the first BR with an oxo group at the C-23 position and deemed as a catabolite of BL.

Techniques used: ^{1}H-NMR, EI-HR-MS, FAB-HR-MS, GC-MS

References: 7

Figure: 1

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Schemes: 2

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Fig. 2