1,2-Hydride Shift in the Biosynthesis of Pinguisane-type Sesquiterpenes

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A 1H-NMR analysis of 6α-hydroxy-3-oxo-pinguis-5(10)-ene-11,6-olide produced by axenic culture of liverwort Aneura pinguis in the presence of [2-3H2]-labeled mevalonate clarified the presence of a 1,2-hydride shift and retention of deuterium at the C-4 position in the biosynthesis of pinguisane-type sesquiterpenes from FPP.

Key words: Aneura pinguis; Hepaticae; pinguisone; sesquiterpene; biosynthesis

Pinguisone (1)1 is interesting for biosynthetic studies since the formation of its structure cannot be simply explained in terms of the isoprene rule. This interest led us to investigate the structures and biosyntheses of pinguisane-type sesquiterpenes. In a previous work, we isolated three new pinguisane-type sesquiterpenes from axenic-cultured gametophytes of Aneura pinguis as coexisting compounds of pinguisone,2 and proposed a biosynthetic pathway leading to 1 that is based on feeding [2-13C] acetate.3 In a recent communication on pinguisane biosynthesis in cultured gametophytes of A. pinguis, we administered the labeled mevalonates, [4,5-13C2], [5-13C], [2-13C]- and [5-13C]-mevalonic acid (MVA), to cultured gametophytes of A. pinguis. The results revealed the previously proposed biosynthetic route.4 A cyclic cation (2) may be formed via the formation of a C-6/C-10 bond from farnesy lpyrophosphatase (FPP) without the elimination of diphenolate. Cation 2 is converted to diphenolate 3 with the migration of a 1,2-methyl and a 1,2-hydride shift. Formation of the C-3/C-10 bond in 4 with the elimination of diphenolate gives a bicyclic cation (5). Rupture of the C-9/C-10 bond and recycloization to a cyclopenta ring in 5 give a cation (6), which converted to the pinguisane skeleton by a 1,2-methyl shift. In this report, we present direct proof of a 1,2-hydride shift in the biosynthesis of pinguisane-type sesquiterpene by using [4-2H2] MVA and [4-13C] MVA.

[4-2H2] MVA and [4-13C] MVA were prepared as previously reported.5 Axenic cultures of gametophytes of A. pinguis were grown in a Gamborg B5 liquid medium6 containing 2% sucrose and the respective labeled potassium MVAs in a 10.0 mM concentration under continuous light. 6α-Hydroxy-3-oxo-pinguis-5(10)-ene-11,6-olide (1) was isolated from the Et2O extracts of axenically cul-

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A recent study on the biosynthesis of striatol, which was derived from 4 in liverwort Psychanthus striatus, also verified a 1,2-methyl migration, a 1,2-hydride shift and the retention of a deuterium atom at C-10 in 3' in the formation of striatol.7 While the pathway shown in Fig. 1 is in agreement with the labeling studies, the sequence of steps involved needs to be proven by enzymatic studies.

Experimental

General details. 1H-NMR: 270 MHz in CDCl3; 1H-NMR: MHz in CHCl3; 13C-NMR: 67.8 MHz in CDCl3, solvent peaks as the int. standard. IR: KBr pellet; UV and optical rotation: EtOH and CHCl3, respectively. The isolation and culture of A. pinguis have been previously reported.4 [4-2H2] and [4-13C] MVA were prepared by the procedures previously reported.5
Fig. 1. Incorporation of [4-\textsuperscript{2}H\textsubscript{2}] and [4-\textsuperscript{13}C] MVA into Compound 1.
While the carbons in compounds 2-6 are numbered according to FPP, those in pinguisane-type sesquiterpene 1 are trivially numbered.

**Labeling experiment on [4-\textsuperscript{2}H\textsubscript{2}] MVA.** Axenic cultures of the gametophytes of *A. pinguis* were grown in a Gamborg B5 medium, to which had been fed 10.0 mm potassium [4-\textsuperscript{2}H\textsubscript{2}] MVA in 5 x 3 ml of the B5 liquid medium with 2\% sucrose, under continuous light at 20\° C. The gametophytes (1.92 g dry weight) were grown for 28 days in the medium with [4-\textsuperscript{2}H\textsubscript{2}] MVA, and then harvested by filtration, air-dried and pulverized. The powdered material was extracted with Et\textsubscript{2}O (20 ml x 3) and then concentrated *in vacuo* to dryness. The Et\textsubscript{2}O extracts were separated by HPLC (SiO\textsubscript{2}; n-hexane- EtOAc, 1:1) to afford \textsuperscript{2}H-labeled 6a-hydroxy-3-oxo-pinguis-5(10)-ene-11,6-olide (1, 5.2 mg). The \textsuperscript{2}H enrichment (9.40 atom\% excess) was estimated by a GC-MS selected ion monitoring analysis.

**Feeding experiment on [4-\textsuperscript{13}C] MVA.** Axenic cultures of the gametophytes of *A. pinguis* were grown in a Gamborg B5 medium, to which had been fed 10.0 mm potassium [4-\textsuperscript{13}C] MVA in 5 x 3 ml of the B5 liquid medium with 2\% sucrose, under continuous light at 20\° C. The gametophytes (1.17 g dry weight) were grown for 28 days in the medium with [4-\textsuperscript{13}C] MVA and then harvested by filtration, air-dried and pulverized. The powdered material was extracted with Et\textsubscript{2}O (20 ml x 3), each Et\textsubscript{2}O solution then being concentrated *in vacuo* to dryness. The Et\textsubscript{2}O extracts were separated by HPLC (SiO\textsubscript{2}; n-hexane-EtOAc, 1:1) to afford \textsuperscript{13}C-labeled 6a-hydroxy-3-oxo-pinguis-5(10)-ene-11,6-olide (1, 9.0 mg). The \textsuperscript{13}C enrichment (2.40 atom\% excess) was estimated by \textsuperscript{13}C-NMR measurement.

**References**