Biosynthesis of kelsoene in cultured cells of liverworts *Ptychanthus striatus*

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The most uncommon tricyclic sesquiterpenes, kelsoene 1 and prespatane 2, were isolated from cultured cells of liverwort *Ptychanthus striatus*, and the labelling pattern of kelsoene biosynthesized from exogenous [2-^{13}C]-mevalonate suggested that kelsoene is biosynthesized from germacradienyl cation *via* alloaromadendranyl cation.

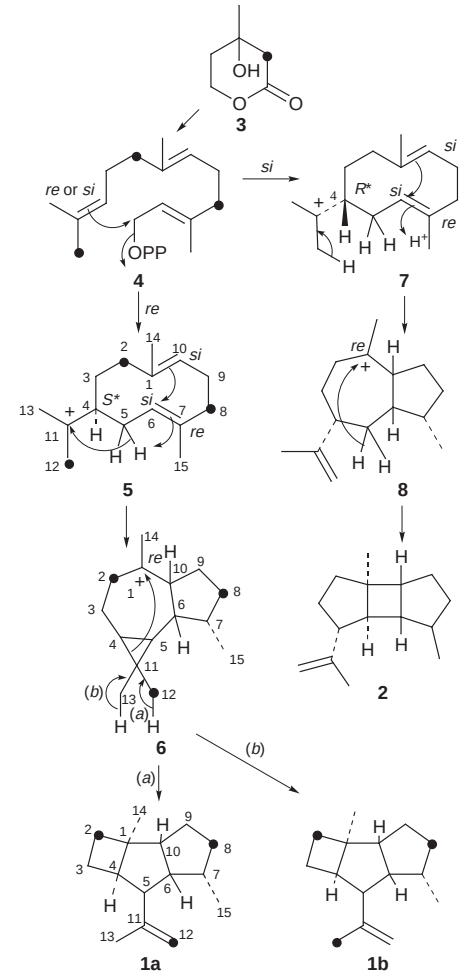
Sesquiterpenes kelsoene **1** and prespatane **2** were isolated for the first time from the tropical marine sponge *Cymbastella hooperi*.[1] Although the formation of an uncommon tricyclo[5.3.0.0^{2,5}]-decane ring system in kelsoene can be explained by the cyclization between C-1 and C-4 in alloaromadendranyl cation **6** with breaking of the C-4/C-11 bond and elimination of one proton from the *gem*-dimethyl groups, there was no experimental evidence provided regarding the biosynthesis of this compound.[1] Here we described the isolation of kelsoene **1** and prespatane **2** from suspension cultured cells of liverwort *Ptychanthus striatus* (Lejeuneaceae), which seems to have almost no phylogenetic relationship with tropical marine sponge, and the biosynthesis of kelsoene. Intact plants of *P. striatus* produce striatane-type sesquiterpenes[2] striatene, striatol and β-monocyclonerolidol.

A callus was induced from the leafy gametophytes of *P. striatus*, transfered on MSK-4 medium with 4% glucose but without 2,4-dichlorophenoxyacetic acid,[3] successively subcultured every four weeks for more than four years, and then used for suspension culture. The suspension culture was propagated routinely in 100 cm^3 of MSK-4 medium under continuous light of 3000 Lux at 25 °C for more than three years. Cells (1200 g fresh weight) were harvested and extracted with EtOAc (6.23 g). Kelsoene **1** (6.6 mg) and prespatane **2** (2.3 mg were isolated from the extract by a judicious combination of liquid chromatography (silica gel and silica gel–$AgNO_3$). Full assignment of the natural abundance ^{1}H and ^{13}C NMR spectra of (+)-kelsoene {[α]$_D$ +77.1 (lit.,[1] +78.1)} was identified by extensive 1D (^{1}H, ^{13}C and differential NOE) and 2D NMR experiments (PFG-DQF-COSY,[4] PFG-HMQC,[5] PFG-HMBC[6]). Prespetane **2** was identified by comparison of ^{1}H and ^{13}C NMR and mass spectral data with those reported previously.[1]

Table 1 ^{13}C enrichment of kelsoene incorporating [2-^{13}C]-mevalonate[a]

Carbon	δ_C	^{13}C Enrichment[b] (atom% excess)	J_{C-H}/Hz[c]
1	45.7	–	–
2	33.0	3.62	136
3	14.6	–	136
4	47.4	–	144
5	48.1	–	131
6	49.9	–	131
7	36.3	–	–
8	33.2	4.52	131
9	26.0	–	131
10	57.8	–	131
11	145.6	–	–
12	109.8	0.98	156
13	24.2	0.90	127
14	23.5	–	124
15	17.7	–	126
Average		3.34	

[a] All assignments are based on extensive 1D and 2D NMR measurements and previously reported data. [b] ^{13}C Enrichment was calculated on the basis of relative peak intensity of ^{13}C enriched peak to non-labelled carbon in biosynthetically labelled compound. [c] C–H coupling constants were determined by gated ^{1}H decoupling ^{13}C NMR analysis.



Scheme 1 Biosynthetic pathway to kelsoene **1** from [2-^{13}C]-mevalonate in cultured cells of *P. striatus*

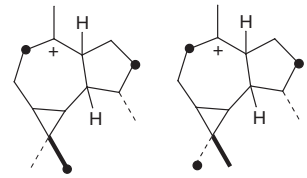
Potassium [2-^{13}C]-mevalonate (MVA, 0.5 mmol) was then fed to the culture (100 cm^3). Cells were harvested after 21 days and extracted with EtOAc. The enriched kelsoene was purified by repeated liquid chromatography as described above.

The ^{13}C{^{1}H} NMR spectrum of kelsoene derived from [2-^{13}C]-mevalonate (average ^{13}C enrichment: 3.34 atom%

excess, Table 1) showed enrichment at the C-2, C-8, C-12 and C-13 positions (4.52, 3.62, 0.98 and 0.90 atom% excess, respectively). Randomization of label between C-12 and C-13 was observed. The level of ^{13}C enrichment in each carbon was determined by comparing the relative intensities of ^{13}C enriched carbons in each carbon with those of the corresponding carbons in the non-labelled compound.

These results may be rationalized by the sequence illustrated in Scheme 1. Cyclization of farnesyl diphosphate **4** to germacradienyl cation **5** *via* the (4*S**)-isopropyl cation would be followed by concomitant intramolecular cyclization between C-6 and C-10 and between C-5 and C-11 of **5** with loss of a proton at C-5 to form a *cis* fused cyclopentane ring and a cyclopropane ring in the resultant alloaromadendranyl cation **6**. Cleavage of the cyclopropane ring and ring closure between C-1 and C-4 with loss of one proton from the *gem*-dimethyl groups generates kelsoene **1**. Observed randomization of label between C-12 and C-13 in kelsoene suggested that the protons of the *gem*-dimethyl groups on the cyclopropane ring in the cation **6** were almost equally eliminated during the conversion of **6** to **1**. Alternatively, the cation at C-11 of **5** might be attacked at both the *re* and *si* faces to form **6a** and **6b**. A proton is then eliminated specifically from a methyl group in either of the two planes of the cyclopropane ring. The co-metabolite, prespatane **2**, might be biosynthesized from a guaianyl cation **8** *via* cation **7** with a (4*R**) isopropyl cation.

Although kelsoene and prospatane were isolated from the marine sponge, they may represent a simple accumulation of constituents from a dietary source such as marine algae, since



6a **6b**

there is a hypothesis that bryophytes originate in green algae.[7]

Notes and References

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