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Biosynthesis of phomactins: common intermediate phomactatriene and taxadiene†

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The stereochemical course of GGDP cyclization in the biosynthesis of phomactins is proposed by the stereochemistry of a cyclization product, phomacta-1(14),3,7-triene, isolated from *Phoma sp*. and the results of incorporation experiments with [1-^{13}C]- and [1,2-$^{13}C_2$]acetates.

Phomactins are known as platelet-activating factor (PAF) antagonists and are structurally unique diterpenes having a bicyclo[9.3.1]pentadecane ring system. To date, seven phomactins have been isolated from a marine fungus, *Phoma sp*.,[1] and total syntheses of phomactin A[2] and D[3] have been achieved. With an interest in a key enzyme responsible for construction of the carbon framework, we started a biosynthetic study of phomacta-1(14),3,7-triene (**1**) which has the same carbon framework as that of phomactins and is regarded as a biosynthetic precursor. Herein we describe the determination of the relative configuration of **1**, a cyclization mechanism based on incorporation experiments, and a proposal of the stereochemical course in geranylgeranyl diphosphate (GGDP) cyclization toward **1** *via* a verticillen-12-yl cation (**8**).

Chu *et al*. isolated Sch 47918 (phomactin C) along with structurally related diterpene hydrocarbon Sch 49026 (**10**). They proposed the stereochemistry of **10** to be the same as that of Sch 47918 without any experimental data.[1b] Since hydroxylation in secondary metabolites usually proceeds with retention of configuration,[4] the C15 stereochemistry of **10** is not consistent with that of phomactin B (**9**). To clarify this problem, we isolated **1** from *Phoma sp*., and determined the relative configuration by NOE experiments in C_6D_6. The observed NOEs established the stereochemistry of **1** as shown in Fig. 1. The NOE between 12-Me and H15 clearly showed *trans*-relationship between 12-Me and 15-Me. Since all spectral data of **1** including ^{1}H, ^{13}C NMR and $[\alpha]_D$ are the same as those of **10**, the stereochemistry of **10** should be revised as that of **1**.

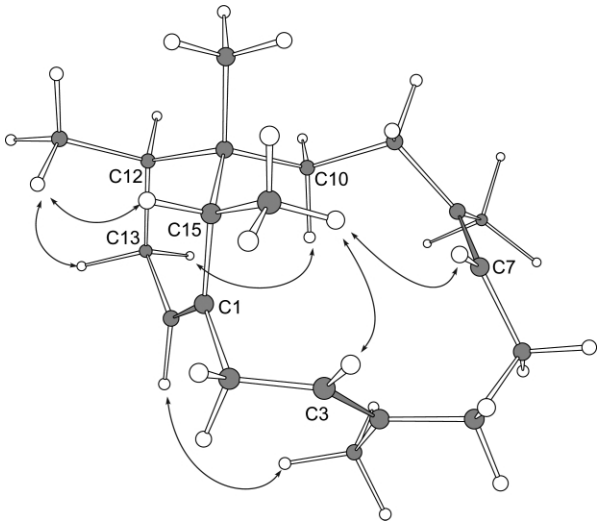

Fig. 1 NOE correlations of phomacta-1(14),3,7-triene (**1**).

In order to prove the origin of the carbon framework of **1**, incorporation experiments were performed with *Phoma sp*. using [1-^{13}C]acetate and [1,2-$^{13}C_2$]acetate. The results are summarized in Table 1 and Fig. 2. The ^{13}C NMR analysis of labelled **1** exhibited typical labelling patterns for the isoprene unit derived from the mevalonate pathway. These results established that 11-Me migrated from C15 without loss of ^{13}C-^{13}C coupling, indicating that 11-Me originated from **a** in the corresponding dimethylallyl diphosphate (Fig. 2). The finding that no significant decrease of the intensity on the coupled signal at 37.69 ppm (C15) was observed[5] indicated that the free rotation of the C1–C15 bond, *e.g.* from **6** to **7** (Fig. 3), is prohibited by the prefixed conformation in the active site of the plausible terpene synthase.

Based on these observations, only two biosynthetic pathways are possible leaving the labelling at C15 and 15-Me (Fig. 3). Two-step cyclizations of GGDP can occur *via* chair- and boat-like transition

Table 1 ^{13}C NMR data of **1** derived from ^{13}C-labelled acetates

			[1,2-$^{13}C_2$]acetate	
	δ_C (ppm)	[1-^{13}C]acetate enrichment[a]	enrichment[a]	J_{C-C} (Hz)
1	139.08		3.0	71
2	35.44	19.8	1.6	43
3	127.21		2.6	43
4	136.10	20.8	2.4	42
5	40.27		2.2	
6	25.07	19.1	3.0	45
7	123.08		2.6	45
8	134.07	14.9	4.2	42
9	33.79		2.5	
10	34.34	15.8	2.6	35
11	38.86		3.0	35
12	41.60	11.7	2.7	36
13	31.63		2.4	
14	121.58	19.2	2.7	71
15	37.87	13.9	2.6	35
4–Me	15.64		3.5	42
8-Me	17.18		4.0	42
11-Me	22.32		2.2	
12-Me	17.74		3.1	36
15-Me	14.11		2.8	35

[a] The values of enrichments were determined by comparison of the relative peak intensities of the corresponding carbons in labelled and unlabelled spectra.

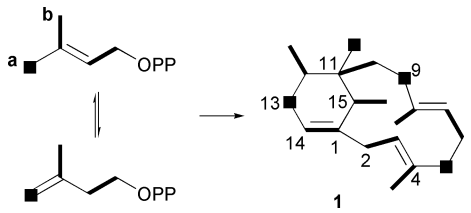

Fig. 2 Incorporation of ^{13}C-labelled acetates into **1**

† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra for labelled and unlabelled **1**.See http://www.rsc.org/suppdata/cc/b4/b401377h/

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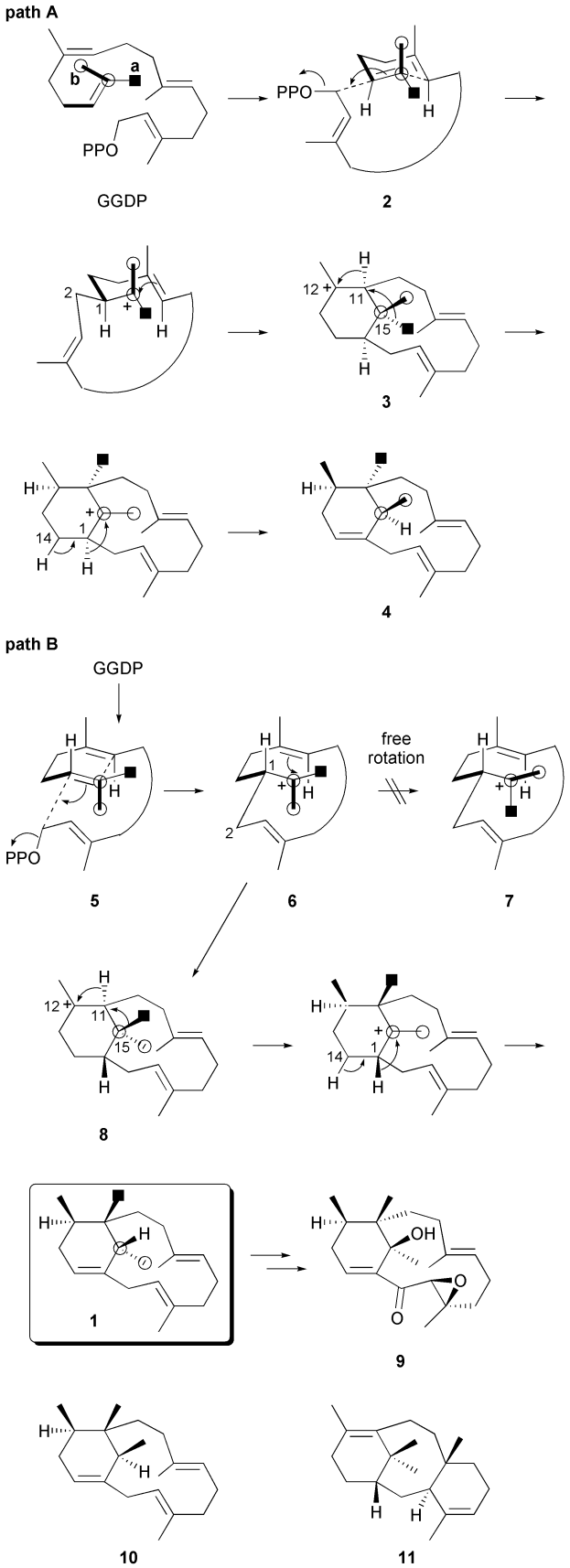

Fig. 3 Biosynthetic pathway of phomactins

states (**2** and **5**, respectively), and give diastereomeric verticillen-12-yl cation intermediates **3** and **8**. Since 1,2-shifts would occur in a stepwise manner, and be possible from both α- and β-faces, the stereochemistry of the product is simply determined by stereogenic centers attached to migrating groups. Migration of 11-H, followed by 1,2-rearrangements of 15α-Me/1α-H and deprotonation of 14-H afford **4** (path A). On the other hand, similar sequential rearrangements including 15β-Me/1β-H provide **1** (path B). Thus, the actual biosynthetic route should be path B *via* boat-like transition state.

Recently, details of a proton transfer and a cyclization in taxadiene synthase have been investigated,[6] and verticillen-12-yl cation **8** is proposed as an intermediate of taxa-4(5),11-diene (**11**). However, no data was provided for the cyclization of GGDP to **8**. We assume that the proposed cyclization mechanism in this communication can be applied to that of the taxadiene synthase reaction.

It is interesting that the common cationic intermediate **8** provides either **1** by sequential rearrangements or **11** by intramolecular proton transfer and further cyclization. Currently, we are studying stereochemical control in the conversion of **8** to **1** to obtain a molecular basis for the diterpene synthase reaction.

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