Biosynthesis of Astellatol, a Novel Rearranged Sesterterpenoid Metabolite of *Aspergillus variecolor*

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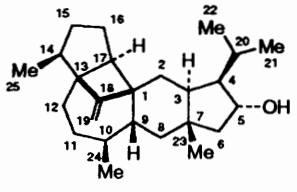
The labelling pattern arising from incorporation of [1,2-$^{13}C_2$]acetate into astellatol, a novel sesterterpenoid fungal metabolite, supports its biosynthesis *via* cyclisation and subsequent rearrangement of geranylfarnesyl pyrophosphate, and allows its relationship to a number of other fungal sesterterpenoids to be proposed.

Aspergillus variecolor (*syn. A. stellatus*) has proved to be a rich source of secondary metabolites of mixed polyketide and terpenoid origins. These include large families of xanthones[1] and meroterpenoids[2] whose structures and biosyntheses have been subject to extensive studies. During these studies, a novel sesterterpenoid astellatol was isolated and its structure **1**



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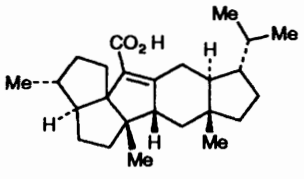

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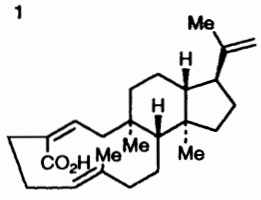

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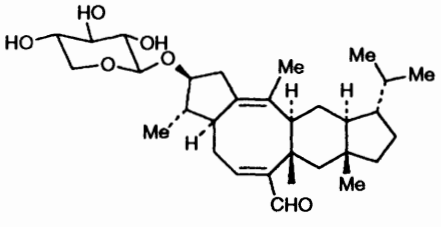

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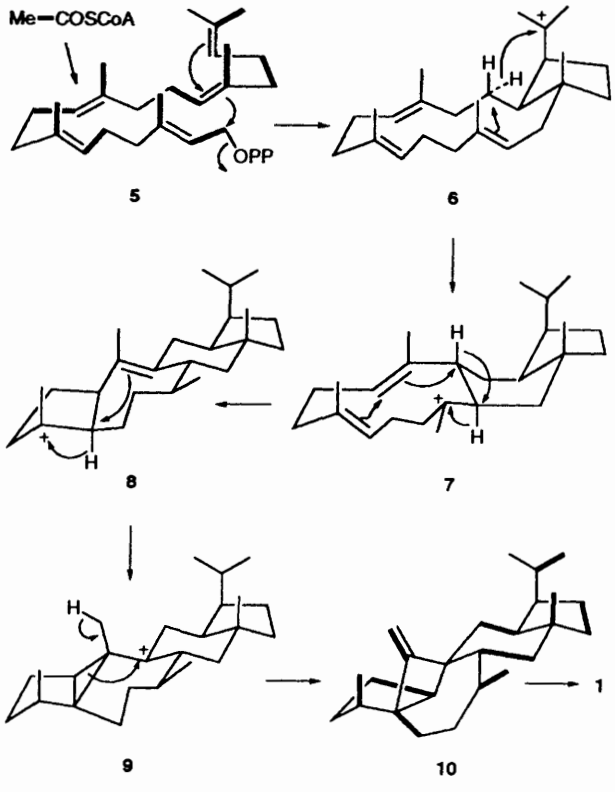
assigned by NMR spectroscopy.[3] Biosynthetic studies are now reported which indicate that astellatol **1** is formed *via* cyclisation and rearrangement of geranylfarnesyl pyrophosphate, so confirming its sesterterpenoid origin.

The ^{13}C NMR spectrum of astellatol enriched by feeding [1,2-$^{13}C_2$]acetate to cultures of *A. variecolor* showed a low overall level of enrichment. Twenty of the signals showed ^{13}C–^{13}C coupling satellites (Table 1) with intensities *ca.* 10% of the natural abundance resonance signals. The observed labelling pattern which is summarised in **10** is consistent with the biosynthetic pathway shown in Scheme 1, where it is proposed that all-*trans*-geranylfarnesyl pyrophosphate **5** undergoes initial folding and cyclisation corresponding to that proposed[4] in the biosynthesis of retigeranic acid **2** to give the bicyclic intermediate **6**. 1,5-Hydride shift from C-2 to C-20 leads to the tricyclic tertiary carbocation **7**. Analogous 1,5-hydride



Scheme 1

shifts have been observed in the biosynthesis of a number of other sesterterpenoids.[5] Subsequent hydride migrations and cyclisations lead to the cyclopropyl carbocation intermediate **9** *via* **8** as indicated. Ring expansion of the cyclopropyl intermediate then gives **10** which contains the cyclobutane ring found in astellatol.

The sesterterpenoids are the least common family of terpenoids, although they have been isolated from a wide range of sources: fungi, lichens, plants, marine organisms and insects.[5] It is noteworthy that another sesterterpenoid metabolite, stellatic acid **3**, has also been isolated from *A. stellatus*.[6] This metabolite is also present in the astellatol-producing strain, but it is clearly formed by a different mode of initial cyclisation of geranylfarnesyl pyrophosphate.[5] The tricyclic carbocation **7** is also implicated in retigeranic acid **2** biosynthesis in which it is subsequently cyclised to form the pentacyclic skeleton.[4] Aleurodiscal **4**, an antifungal sesterterpenoid isolated[7] from the basidiomycete, *Aleurodiscus mirabilis*, has a similar carbon skeleton to **8**, apart from the relative stereochemistry at C-4 and C-10.

Table 1 ^{13}C NMR data for astellatol **1** enriched from sodium [1,2-$^{13}C_2$]acetate

Carbon	δ_C(ppm)	$J_{C,C}$/Hz
1	49.8	—
2	27.0	34.2
3	45.2	34.2
4	57.1	37.0
5	77.4	37.0
6	50.8	—
7	41.7	35.1
8	43.6	32.4
9	46.1	32.3
10	37.0	36.1
11	33.9	—
12	31.0	34.2
13	59.5	34.2
14	42.6	36.1
15	35.0	—
16	24.9	35.2
17	44.5	35.2
18	161.1	71.2
19	102.1	71.2
20	29.7	34.6
21	24.0	—
22	21.8	34.6
23	20.1	35.1
24	23.0	36.1
25	13.2	36.1

Experimental

Aspergillus variecolor (Strain 212KI69) was grown in static cultures as previously described.[8] Sodium [1,2-$^{13}C_2$]acetate (1 g) was distributed among 4 culture flasks containing a 2-day growth of the organism on Czapek-Dox medium (500 cm^3; 5% sucrose) and the mycelium was harvested after a further 10 days. The dried mycelium was extracted and astellatol (13 mg) was isolated after purification by preparative thin layer chromatography. ^{13}C NMR spectra were determind on a Bruker WH 360 spectrometer in $CDCl_3$ solution.

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