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Biosynthesis of (+)-Epicubenol

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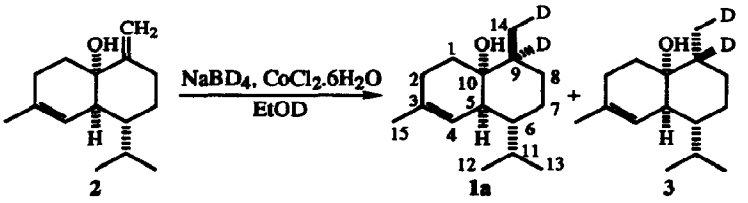
Abstract: Incubation of [6-^{2}H]FPP (**14a**) with epicubenol synthase isolated from *Streptomyces* sp. LL-B7 gave epicubenol (**1c**) labeled at D-9 as established by ^{2}H NMR. These results confirm the involvement of a predicted 1,2-hydride shift in the mechanism of formation of **1**.

(+)-Epicubenol (**1**), a cadinene-type sesquiterpene alcohol, has been isolated from *Streptomyces* sp. LL-B7, sp. LL-B5a, and sp. LL-100-1 (Eren)[1] and shown to be the enantiomer of (-)-epicubenol isolated from cubeb oil[2a] and a variety of other plants sources.[2b,c] In the preceding paper we have reported the first total synthesis of (±)-epicubenol.[3] Earlier biosynthetic studies in our laboratory, using cell free extracts obtained from *Streptomyces* sp. LL-B7, have established that **1** is derived by the cyclization of farnesyl diphosphate and provided evidence for a mechanism of formation involving a germacradienyl cation intermediate and the operation of a 1,3-hydride shift.[4] In this communication we wish to report the involvement of a 1,2-hydride shift in the formation of **1**.

Exploration of the mechanism of the cyclization reaction using ^{2}H NMR analysis required the unambiguous assignment of ^{1}H NMR resonances of **1**.[4] Of particular interest was the assignment of the resonance for the methine proton, H-9. The ^{1}H NMR assignment of **1** in $CDCl_3$ showed that the chemical shifts of the methine proton at H-9 (δ 1.58) and that of the methylene protons at H-1 (δ 1.58 and 1.63) were not cleanly resolved. However, in C_6D_6 the chemical shifts of H-9 (δ 1.62) and H-1 (δ 1.55) were separated by δ 0.07 ppm. Since the precise chemical shifts of the epicubenol protons in C_6D_6 are known to be concentration dependent, we decide to synthesize epicubenol labeled with ^{2}H at C-9 and C-1 (**1a**, **1b**).

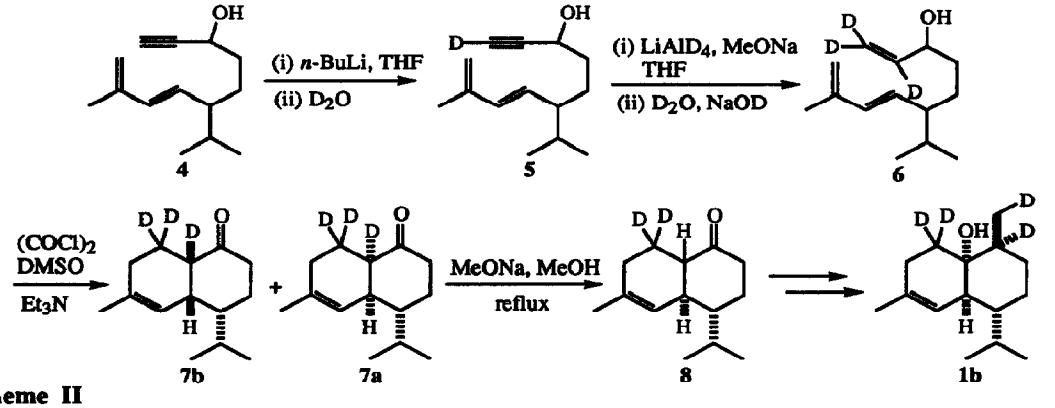


2 **1a** **3**

Scheme I

The compound **1a** was synthesized by reducing the exocyclic double bond of the α-hydroxyalkene **2** with a mixture of $NaBD_4$ (3.0 equiv) and $CoCl_2.6H_2O$ (1.0 equiv) in EtOD (rt, 14 h) (Scheme I).[3] The reduction resulted in the formation of **1a** and its diastereomer syn-epicubenol (**3**), labeled at D-9 and D-14 in a 1:1 ratio with a combined yield of 75% for the two isomers.[5] They were separated by repeated SiO_2 flash column chromatography and subjected to ^2H NMR analysis. The ^2H NMR spectrum of [9,14-$^2H_2$]epicubenol (**1a**) recorded in $CHCl_3$ gave rise to signals at δ 1.59 (D-9) and 0.97 (D-14) whereas the spectrum recorded in C_6H_6 displayed signals at δ 1.57 and 1.07.

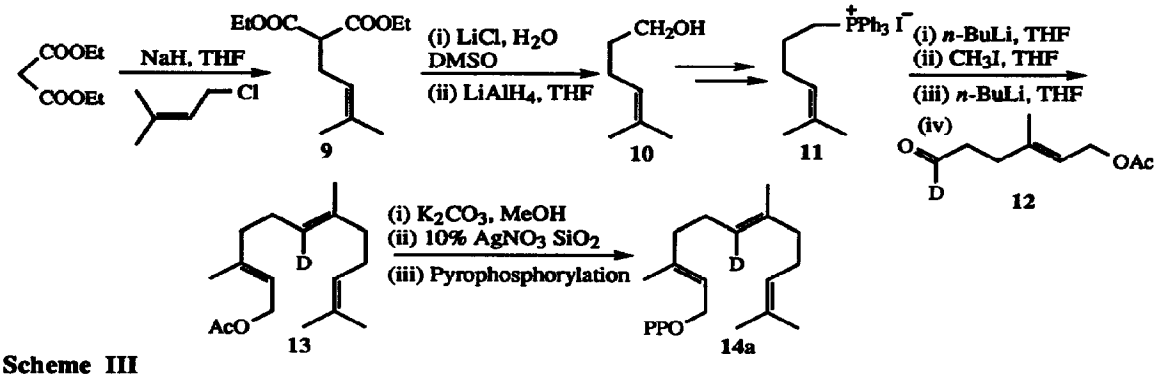
For the synthesis of [1,1,9,14-$^2H_4$]epicubenol (**1b**), first the alkyne **5** was synthesised in 93% yield by generating the anion of **4** with *n*-BuLi (2.0 equiv, THF, 0 °C, 5 min, rt, 1 h) and quenching with D_2O (Scheme II). The alkyne moiety in **5** was reduced to the alkene by refluxing with lithium aluminum deuteride (3.0 equiv) and sodium methoxide (6.0 equiv) in THF (12 h).[6] The reaction was quenched with D_2O followed by successive addition of NaOD and D_2O resulting in the formation of the triene **6** in 92% yield. Swern oxidation of the triene gave the trideuterated bicyclic ketones **7a** and **7b** *via* an intramolecular Diels-Alder reaction, as described by Taber et al.[7] The correct isomer **7a** was separated and converted into the dideuterated bicyclic ketone **8** by refluxing with sodium methoxide (0.05 equiv) in MeOH (3 h) in 94% yield. The compound **8** was converted into **1b** labeled at D-1, D-9 and D-14 using methods described previously.[3] The ^2H NMR spectrum of **1b** recorded in $CHCl_3$ gave rise to signals at δ 1.64 (D-1), 1.58 (D-1, D-9) and 0.96 (D-14) and the spectrum recorded in C_6H_6 displayed signals at δ 1.57 (D-9), 1.46 (D-1) and 1.05 (D-14).



Scheme II

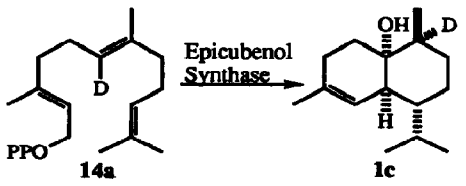
With the above data in hand, we proceeded with the synthesis of [6-^2H]FPP (**14a**) (Scheme III). Diethyl malonate (1.0 equiv) was deprotonated with NaH (1.1 equiv, 0 °C, 30 min) in THF followed by the addition of isoprenyl chloride (1.0 equiv, rt, 2 h), resulting in the formation of diester **9** in 81% yield. The diester **9** was monodecarboxylated[8] in 95% yield by refluxing with LiCl (3.0 equiv) and H_2O (1.0 equiv) in DMSO for 5 h, followed by the reduction of the resulting monoester with $LiAlH_4$ to alcohol **10** in 56% yield.[9] The alcohol **10** was converted to **11** with a known procedure.[10] The ylid of **11**, generated by addition of *n*-BuLi (1.0 equiv, -78 °C, 10 min, rt, 45 min) was first methylated with iodomethane (1.0 equiv, -78 °C, 5 min, rt, 45 min). The ylid of the resulting methylated Wittig reagent was generated as before and reacted with the

deuterated aldehyde 12 (rt, 3 h).[11] Both *cis,trans* and *trans,trans* isomers of [6-^{2}H]farnesyl acetate (13) were obtained in an overall yield of 46% yield for both isomers. The acetate group was hydrolysed with K_2CO_3 (1.5 equiv) in MeOH (rt. 1 h) in 90% yield,[12] resulting in the formation of two isomeric [6-^{2}H]farnesols, which were separated by repeated flash column chromatography on 10% $AgNO_3$ impregnated SiO_2. The *trans,trans*-[6-^{2}H]farnesol was then converted into its diphosphate 14a.[12]



Scheme III

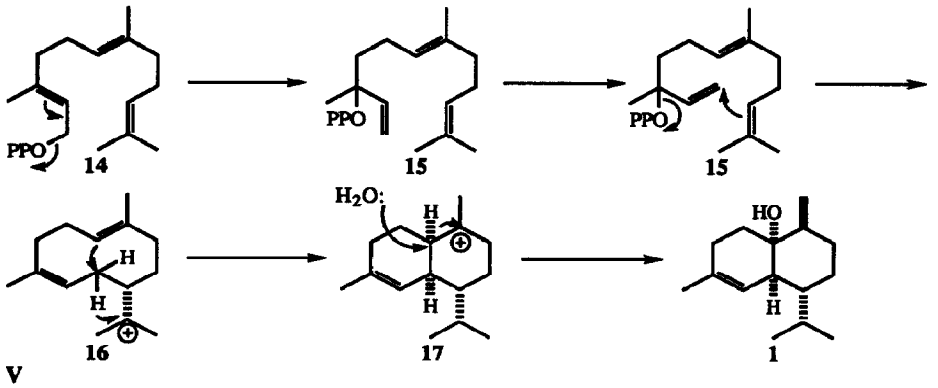
The preparative scale incubation of [6-^{2}H]FPP (14a) with epicubenol synthase was then performed (Scheme IV). Cell free extracts of *Streptomyces* sp. LL-B7 were prepared from the mycelium obtained from six 600-mL batches of a 42-h fermentation culture by grinding with glass beads. After centrifugation of the broken cell suspension, the resulting supernatant was used directly for enzymatic cyclization. The crude cell free extract (630 mL) was incubated with [6-^{2}H]FPP (14a) (5 μM) containing [1-^{3}H]FPP as the internal standard (final specific activity 42 nCi/μmol) in the presence of 30 mM $MgCl_2$ for 120 min at 30 °C in a glass flask. After addition of 3 mg of carrier (±)-epicubenol, the resulting products were rigorously purified by flash column chromatography on SiO_2. The purified epicubenol (1c) (1.5 mg, 56 nmol of deuterated 1c from the enzymatic reaction) displayed a single methyl peak at δ 1.56 in $CHCl_3$ and at δ 1.55 in C_6H_6 .[13] This result unambigously showed that the deuterium in 1c is located at D-9 and is derived from D-6 of FPP thereby establishing that a 1,2-hydride shift is involved in the formation of epicubenol.



Scheme IV

The labeling results obtained to date are consistent with the earlier proposed mechanism involving an initial rearrangement of FPP to the tertiary allylic isomer, nerolidyl diphosphate (15),[14] followed by ionization and electrophilic attack on C-10 of the distal double bond (Scheme V). A 1,3-hydride shift, followed by a

second electrophilic cyclization of the germacradienyl cation **16** would generate the cadinanyl cation (**17**) which upon 1,2-hydride shift and *syn* capture of water will generate epicubenol (**1**). The predicted 1,2-hydride shift in the biosynthesis of this major class of cadinene metabolites is demonstrated here for the first time at the enzymatic level. Further investigations to establish the intermediacy of **15** and the stereochemical detail of the cyclization are in progress.



Scheme V

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