The Biosynthesis of Echinulin: Origins of the Diastereotopic Methyls in the 1,1-Dimethylallyl Group

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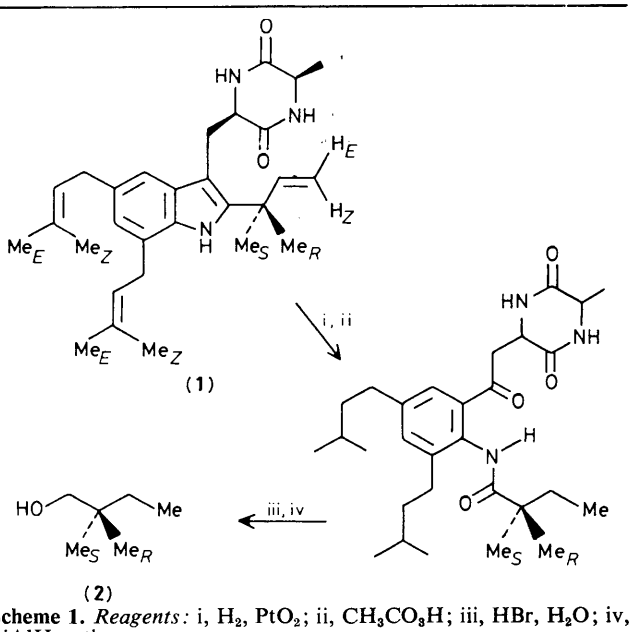
In the biosynthesis of echinulin (**1**) the *pro-S* methyl of the 1,1-dimethylallyl group is derived predominantly (89%) from the methyl group of mevalonic acid.

Echinulin (**1**) is the best known member of an important class of hemiterpenoids which contain a 1,1-dimethylallyl substituent.[1] This 'reversed' prenyl group is furnished by mevalonic acid,[2] presumably through the intermediacy of 3,3-dimethylallyl pyrophosphate. Allen *et al.* have determined the stereochemistry of the pathway by which the two 3,3-dimethylallyl substituents in echinulin are derived from mevalonic acid.[3] With regard to the 1,1-dimethylallyl group also two critical stereochemical questions may be asked: (i) which of the two diastereotopic methyl groups of echinulin (**1**), Me_R or Me_S, is derived from the methyl group of mevalonic acid, and (ii) which of the two diastereotopic vinyl protons, H_E or H_Z, is derived from the 5-*pro-R*-proton of mevalonic acid? The stereochemistry of the pathway by which mevalonic acid is converted into 3,3-dimethylallyl pyrophosphate is known.[4] Thus the solutions to these two questions would provide a complete stereochemical description of the route by which 3,3-dimethylallyl pyrophosphate furnishes the 'reversed' prenyl group of echinulin. We are exploring the answer to question (ii) by utilising methods which were developed by Cane *et al.*[5] We now report the answer to question (i) which was determined by a novel method that has potentially wide applicability.

[*Me*-$^2H_3$]Mevalonic acid lactone (650 mg) was administered to a 3-day old surface culture of *Aspergillus amstelodami* which was grown on a Czapek–Dox medium (2 250 ml) supplemented (30%) with sucrose. The mycelium (23 g) was harvested after a further 10 days and echinulin (300 mg) was isolated. The ^2H n.m.r. spectrum of the echinulin sample (in pyridine) consisted of two overlapping signals of approximately equal intensity at δ 1.57 and 1.69 p.p.m. that are assigned to the



Scheme 1. *Reagents:* i, H_2, PtO_2; ii, CH_3CO_3H; iii, HBr, H_2O; iv, $LiAlH_4$, ether.

labelled methyl of the 1,1-dimethylallyl group and the *Z*-methyls[3] of the 3,3-dimethylallyl groups respectively.† The

† [2-^{13}C]Mevalonic acid lactone was fed to *A. amstelodami* under similar conditions and echinulin was isolated. In the ^{13}C n.m.r. spectrum of (**1**) the resonances at δ 25.7 (2 × Me_E) and 28.0 p.p.m. (Me_S + Me_R) were enriched with ^{13}C to the extents of 1.6 and 1.1% respectively.

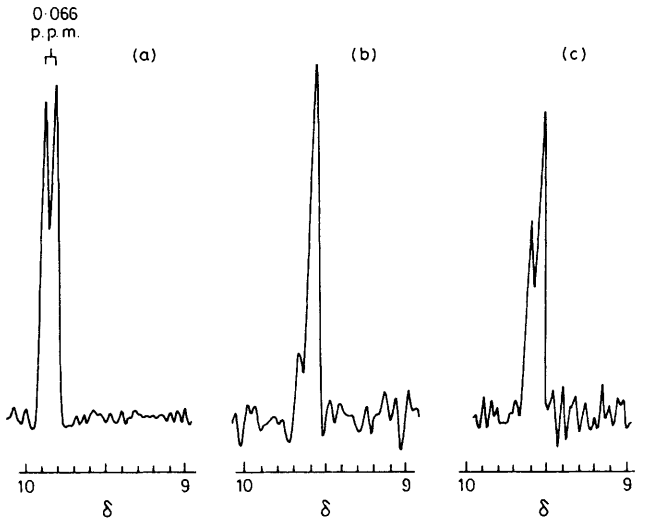


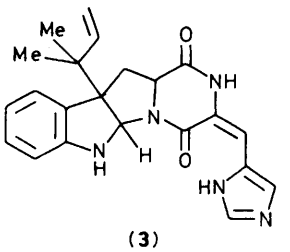
Figure 1. ^{2}H N.m.r. spectra recorded at Edinburgh University on a Bruker WH-360 spectrometer operating at 55.3 MHz. Parameters: spectral width 1 kHz; pulse width 16 μs; acquisition time 0.5 s; 1K data points. Spectra were determined with broad-band ^{1}H-decoupling and were computer resolution-enhanced. Calibration was achieved with internal [^{2}H]$CHCl_3$ reference at δ 7.25 p.p.m.

(a) 10.0 mg of (**2**) enriched (5%) in (2*RS*)-2-[$^2H_3$]methyl-2-methylbutan-1-ol, plus 155 mg of Eu(hfbc)$_3$, plus 16 mg of $CHCl_3$ enriched (2%) in [^{2}H]$CHCl_3$, in 0.8 ml of CCl_4 (9600 transients); (b) total biosynthetically labelled (**2**), plus 160 mg Eu(hfbc)$_3$, plus 10 mg $CHCl_3$ enriched (2%) in [^{2}H]$CHCl_3$, in 0.8 ml CCl_4 (28 329 transients); (c) 97 mg of sample (a) plus 633 mg of sample (b) (20 000 transients).

Each sample was dried (4 Å molecular sieves) and filtered into a 5 mm n.m.r. tube which was sealed *in vacuo* before recording the spectra. The differences between chemical shifts of corresponding resonances in spectra (a), (b), and (c) are not considered to be significant since the observed chemical shifts are extremely sensitive to traces of moisture. For each sample satisfactory agreement was obtained between the ^{2}H n.m.r. spectra that are displayed and the ^{1}H n.m.r. spectra (360 MHz) that were calibrated with internal $CHCl_3$ at δ 7.25; the ^{1}H n.m.r. spectra displayed singlets for the 2-*pro-R*- and 2-*pro-S*-methyls of (**2**) that are displaced *ca.* 0.05 p.p.m. to higher frequency than the corresponding resonances in the ^{2}H n.m.r. spectra.

deuteriated echinulin was then degraded[2] by the route which is summarised in Scheme 1 to furnish labelled 2,2-dimethylbutan-1-ol (**2**).

We have shown previously that the [$^2H_3$]methyl groups of the (*R*)- and (*S*)-enantiomers of racemic 2-[$^2H_3$]methyl-2-methylbutan-1-ol may be distinguished in the ^{2}H n.m.r. spectrum by utilizing the differential downfield shifts induced by the chiral shift reagent tris[3-heptafluorobutanoyl-(−)-camphorato]europium(III) [Eu(hfbc)$_3$] (Figure 1a). The resonance which was shifted further was assigned to the [$^2H_3$]-methyl group of the (*R*)-enantiomer.[6] The ^{2}H n.m.r. of the biosynthetically deuteriated alcohol (**2**), in the presence of the same chiral shift reagent (Figure 1b), revealed that most of the ^{2}H label (89%) was present in the 2-*pro-S*-methyl. This interpretation was confirmed by the ^{2}H n.m.r. of the mixed samples (Figure 1c). It follows that the *pro-S*-methyl of the 'reversed' dimethylallyl group of echinulin is derived predominantly (89%) from the methyl group of mevalonic acid. This result constitutes the first unambiguous determination of the biosynthetic origins of the *pro-R*- and *pro-S*-methyls in a 1,1-dimethylallyl group.



(**3**)

While this work was in progress Gorst-Allman *et al.* reported that the diastereotopic methyls of the 1,1-dimethylallyl group in roquefortine (**3**) are derived to unequal extents (2:1 ratio) from the methyl group of mevalonic acid.[7] The strategy which we have outlined here should be applicable to the determination of the absolute stereochemical origins of the diastereotopic methyls in roquefortine and in other hemiterpenoids which contain a 1,1-dimethylallyl group.

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