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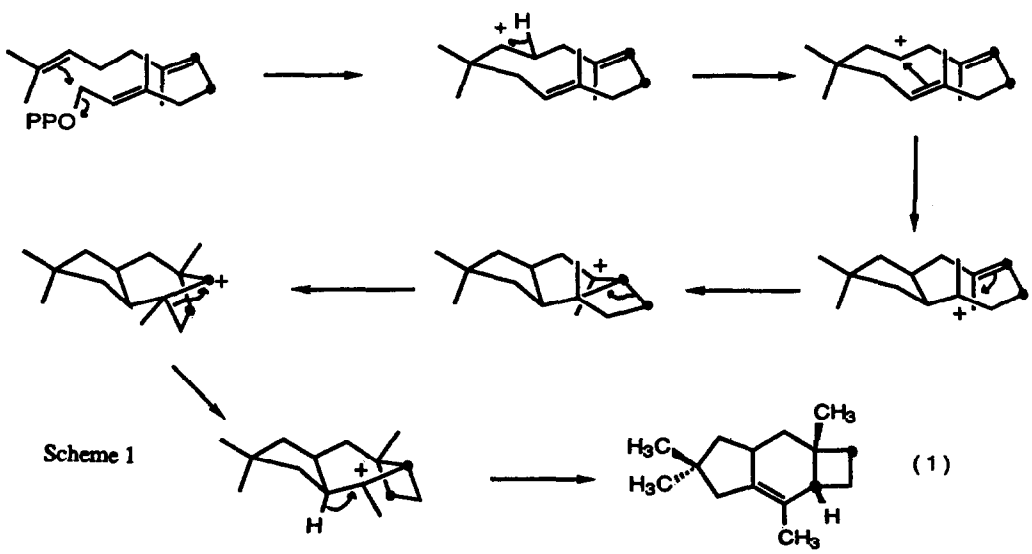
THE ABSOLUTE STEREOCHEMISTRY OF THE ENZYMIC CYCLISATION TO FORM THE STERPURENE SESQUITERPENES

Chris Abell* and Andrew P. Leech
University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW.

Incorporation studies using [1,2-$^{13}C_2$]acetate into a novel sterpurene sesquiterpene, 9,12-dihydroxysterpurene (1), have allowed the absolute stereochemistry of the enzymic cyclisation of farnesyl pyrophsphate to sterpurene to be elucidated. Observation of two-bond ^{13}C-^{13}C coupling across the cyclobutane ring confirms the derivation of these two carbon atoms from the same acetate unit.

The sterpurenes comprise a recently discovered group of sesquiterpene metabolites from the fungus *Stereum purpureum*. They are characterised by the tricyclic skeleton of the parent hydrocarbon sterpurene (1).[1] Biosynthetic experiments have established the isoprenoid nature of these compounds[2] and shown that the enzymic cyclisation of farnesyl pyrophosphate to sterpurene proceeds with carbon-carbon bond cleavage.[3] In this paper we present biosynthetic results on a novel sesquiterpene, 9,12-dihydroxysterpurene (2), which provide detailed stereochemical information on the enzymic cyclisation of farnesyl pyrophosphate and further insight into the rearrangement reaction involved in setting up the cyclobutane ring of sterpurene.

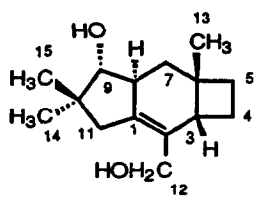


Scheme 1 (1)

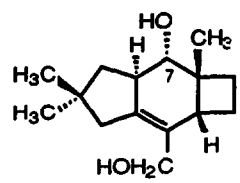
Cane has proposed that the two antipodal classes of cyclopentane sesquiterpenes are formed by enantiomeric folding of the farnesyl pyrophosphate precursor in the corresponding cyclase enzyme active sites.[4] It is proposed that attack of the C-1 cation, formed by departure of the allylic pyrophosphate, on either the *si* or *re* face of the terminal double bond generates humulene or humulyl cations in enantiomeric conformations, which

after further cyclisation on the same active site give rise to the enantiomeric cyclase products. A scheme for the enzymic cyclisation of the linear achiral farnesyl pyrophosphate to form the chiral tricyclic sterpurene structure is shown in Scheme 1. The cyclisation is initiated by electrophilic attack of the C-1 carbinyl carbon at C-11 on the *si* face of the distal double bond to generate an intermediate humulyl cation with the positive charge on C-10. Attack on the *si* face of the distal double bond is predicted for the sterpurene sesquiterpenes as it has recently been shown that they have the same absolute stereochemistry as the illudins, marasmic acid and fommanosin.[5]

It is possible to determine the stereochemistry of this cyclisation because the two faces of the distal double bond of farnesyl pyrophosphate are distinguishable by virtue of the different labelling of the two methyl groups by [1,2-$^{13}C_2$]-acetate. The *cis* methyl retains its ^{13}C-partner whereas the *trans* methyl, coming from C-2 of mevalonate, has lost its partner. In a previous paper we reported the results of incorporation studies of [1,2-$^{13}C_2$]-acetate into 7,12-dihydroxysterpurene (3). Although we observed the anticipated doublet and singlet signals in the ^{13}C NMR spectrum for the two geminal methyl carbon atoms, the corresponding signals in the ^{1}H NMR were so close (0.01 ppm apart) that we were unable to assign them or the carbon signals to the α and β methyl groups. We have subsequently isolated an isomeric minor metabolite of *Stereum purpureum*, 9,12-dihydroxysterpurene, (2) which has allowed these assignments to be made.



(2)



(3)

The structure of (2) was assigned by comparison with the isomeric 7,12-dihydroxysterpurene (3).[6] The i.r. spectrum of (2) shows only hydroxyl functionality (3610 cm^{-1}, soln. $CHCl_3$). The mass spectrum of (2) $C_{15}H_{24}O_2$ has peaks at 236 (M^+), 218 (M^+-H_2O), and 208 (M^+-C_2H_4). The loss of ethylene, from the cyclobutane ring, is characteristic of compounds with the sterpurene skeleton.

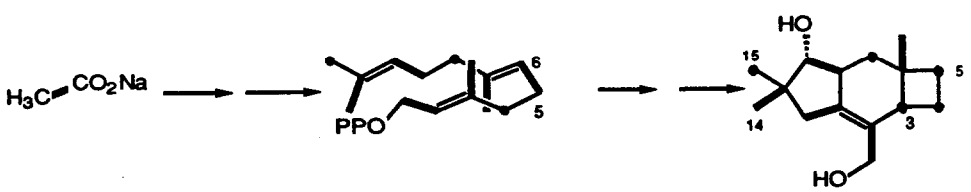
The ^{1}H NMR spectrum of (2) includes an AB quartet around 3.98 ppm (allylic primary alcohol), a doublet at 3.27 ppm (secondary alcohol) coupled to a broad multiplet at 2.50 ppm (readily assigned to H-8) and three methyl signals. A double quantum filtered COSY[7] on (2) revealed a four-spin system including the downfield hydrogen of the secondary hydroxyl group, and a five-spin system corresponding to the hydrogens on the cyclobutane ring. The determination of the structure of (2) was completed by a series of NOE experiments. The most significant of these show a path of NOEs from H-15 (methyl) to the proton adjacent to the secondary hydroxyl on C-9, from this hydrogen to H-7β, from H-7β to H-13 (methyl), and finally across to H-3. These NOEs establish the location and stereochemistry of the secondary hydroxyl group, and the *cis*-fusion of the six and four-membered rings. Corresponding NOEs on the lower face of the molecule were observed between H-14 and H-8, and H-8 and H-7α.

Most of the ^{13}C NMR spectrum of (2) was easily assigned by comparison with the partial assignment of the spectrum of (3). However this left unresolved the key assignments of the three methyl carbons, C-13, C-14 and C-15. These signals were unambiguously assigned using proton-detected ^{13}C-^{1}H correlation[8] to the methyl signals at 1.24 , 0.98, and 1.06 p.p.m. respectively, the assignments of the proton signals having already been established from the NOE study.

The ^{13}C NMR spectrum of a sample of (2) after incorporation of sodium [1,2-$^{13}C_2$]-acetate revealed as expected five intact C_2 units (C-14 — C-10, C-9 — C-8, C-13 — C-6, C-12 — C-2, and C-1 — C-11) and five carbons separated from their original partners (Scheme 2). The key observation is that C-14 (21.7 p.p.m.) retains its ^{13}C-partner from [1,2-$^{13}C_2$]-acetate, whilst C-15 does not. This labelling pattern confirms that in the enzymic cyclisation of farnesyl pyrophosphate the attack of the C-1 carbinyl carbon at C-11 does indeed occur on the *si* face of the distal double bond. This observation adds further support to the idea that the initial folding of the farnesyl pyrophosphate determines the absolute configuration of the final cyclisation product, which in turn suggests that the whole cyclisation process takes place on a single active site, as has been shown for the formation of pentalenene.[9]

The cyclisation mechanism in Scheme 1 requires that the intact acetate unit labelling C-5 and C-6 of farnesyl pyrophosphate is cleaved and the separated carbons end up at C-3 and C-5 of the sterpurene, on opposite corners of the cyclobutane ring. In the [1,2-$^{13}C_2$]-acetate feeding experiment with 7,12-dihydroxysterpurene (3) it was found that the carbons at C-3 and C-5 are not directly bonded to their ^{13}C partner as the Scheme predicts.[3] However in the standard ^{13}C NMR spectrum of (3) enriched with [1,2-$^{13}C_2$]-acetate the small two bond carbon coupling between these positions was not detected. Close inspection of the corresponding spectrum of (2) enriched with [1,2-$^{13}C_2$]-acetate showed a small coupling on C-3 and C-5. This was more clearly seen in the ^{13}C INADEQUATE spectrum where the large central peak due to natural abundance ^{13}C is suppressed. The 1,3-carbon-carbon coupling was 6.5 Hertz. Whereas the previous observation that C-3 and C-5 were not directly attached to their ^{13}C-partners showed that an intact acetate unit had been cleaved, this two-bond coupling confirms that C-3 and C-5 both derive from the *same* acetate unit.

Scheme 2



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6 ^{1}H NMR (400 MHz, CD_2Cl_2) δ: 4.02 (1H, d, 11.8 Hz, H-12), 3.96 (1H, d, 11.8 Hz, H-12), 3.27 (1H, d, 9.7 Hz, H-9), 2.50 (1H, br mult, H-8), 2.46 (1H, br d, 10 Hz, H-3), 2.40 (1H, mult, H-4β), 2.35 (1H, d, 17.3 Hz, H-11α), 2.15 (1H, d, 17.3 Hz, H-11β), 1.90 (1H, mult, H-5α), 1.71 (1H, dd, 12.5, 5.3 Hz, H-7α), 1.5 (2H, complex mult, H-4α and H-5β), 1.24 (3H, s, H-13), 1.06 (3H, s, H-15), 0.98 (3H, s, H-14), 0.73 (1H, dd, 12.6, 11.2 Hz, H-7β). Note that H-14 is the methyl group on the same face as H-8 and H-7α.
^{13}C NMR (100.6 MHz, CD_2Cl_2) δ: 134.9 (s, C-1), 134.6 (s, C-2) 85.7 (d, C-9), 62.3 (t, C-12), 44.3 (d, C-8), 41.6 (t, C-11), 41.0 (d, C-3), 40.0 (s, C-10*), 38.0 (t, C-7), 37.9 (s, C-6*), 29.8 (q, C-13), 28.5 (t, C-5), 27.4 (q, C-15), 25.4 (t, C-4), 21.9 (q, C-14). * C6 and C10 were not distinguished unambiguously.
^{13}C-^{13}C coupling constants: C-1 - C-11, 39.7 Hz; C-2 - C-12, 46.7 Hz; C-3 - C-5, 6.7 Hz; C-6 - C-13, 36.0 Hz; C-8 - C-9, 38.4 Hz; C-10 - C-14, 35.5 Hz.
High resolution mass spectroscopy on (1) for the M^+ peak gave 236.1771, $C_{15}H_{24}O_2$ requires 236.1775.

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