Biosynthesis of the Abietane Type Diterpene Ferruginol in Cell Cultures of *Salvia miltiorrhiza*: Synthesis of (15*S*)- and (15*R*)-[16-$^2H_1$]12-*O*-Methylferruginol by Enzymatic Resolution of 12-*O*-Methyl-16-hydroxyferruginol and Stereochemistry of 1,2-Methyl Migration in the Formation of the Isopropyl Group

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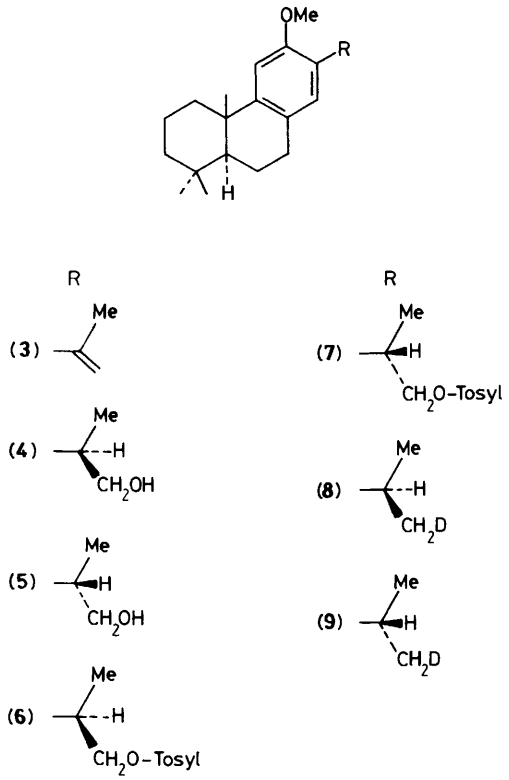
The signals for the *pro*(*R*) and *pro*(*S*) methyl groups of the isopropyl group, in the $^{13}C\{^1H\}$ n.m.r. spectrum of 12-*O*-methylferruginol, have been assigned by the synthesis of the title compounds and the stereochemistry of 1,2-methyl migration for biosynthesis of the abietane skeleton of ferruginol has been established.

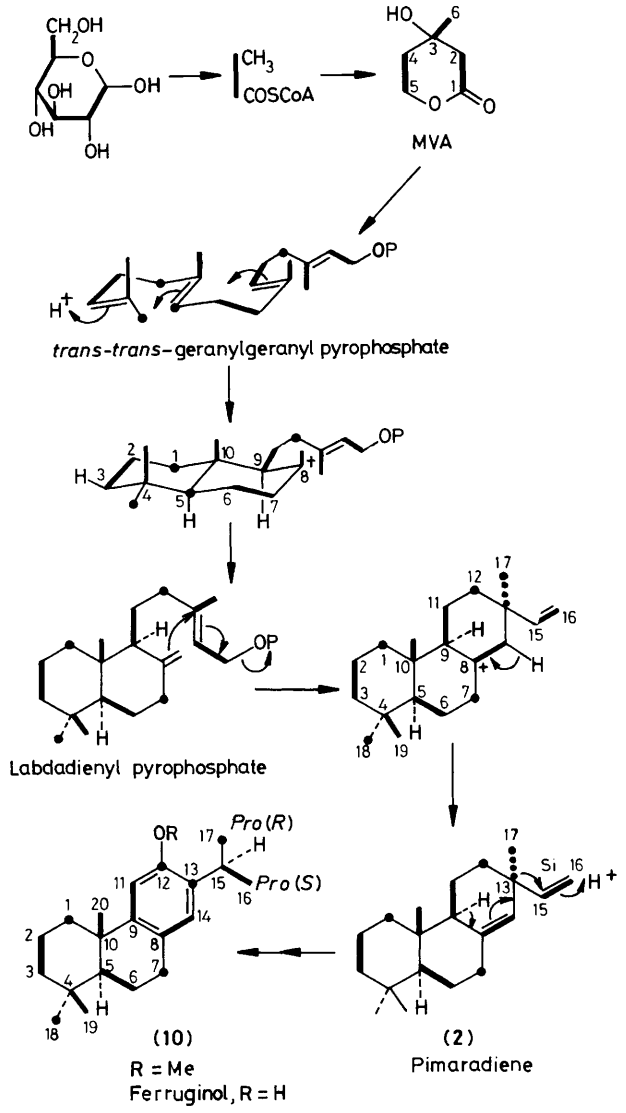
The roots of *Salvia miltiorrhiza* are a traditional chinese medicine (Dan-Shen) and contain various abietane type diterpenes which have platelet anticoagulant activity.[1] Cultured cells derived from *S. miltiorrhiza* retain their ability to synthesize these diterpenes.[2] It has previously been demonstrated[3] that the abietane skeletone of cryptotanshinone (**1**) was formed by 1,2-methyl migration from C-13 to C-15 on the *Si*-face of the double bond in the pimaradiene precursor (**2**). This was based on the $^{13}C\{^1H\}$ n.m.r. spectrum of (**1**) biosynthesized from [U-$^{13}C_6$]glucose by the *S. miltiorrhiza*



cell cultures. It was also suggested that the migrated methyl group could be the *pro*(*R*) methyl in the isopropyl group of the generated abietane skeleton. We here report the synthesis of (15*S*)- and (15*R*)-[16-$^2H_1$]12-*O*-methylferruginol (**8**) and (**9**) and provide direct stereochemical evidence of 1,2-methyl group migration occurring in the formation of the isopropyl group of ferruginol.

Hydroboration[4] of (**3**)[5] with diborane in tetrahydrofuran, followed by oxidation with hydrogen peroxide gave a mixture of (15*R*)- and (15*S*)-12-*O*-methyl-16-hydroxyferruginol (**4**) and (**5**). In the $^{13}C\{^1H\}$ n.m.r. spectrum of the mixture, the signals corresponding to C-15 appeared as two peaks at δ 35.125 and 35.198 with the same intensity. A solution of the acetate of the above stereoisomeric mixture in methanol–phosphate buffer (pH 7.0) (1 : 3) was hydrolysed with lipase at 16 °C for 18 h. A hydrolysed alcohol and an unreactive acetate were obtained after chromatographic purification. Acetylation of the alcohol, followed by treatment of the resulting acetate with lipase in the same manner gave a pure alcohol, which was identical with (15*R*)-12-*O*-methyl-16-hydroxyferruginol (**4**) {$[\alpha]_D^{22}$ + 62.86° (*c* 1.4 in $CHCl_3$); lit. $[\alpha]_D$ + 62.3° ($CHCl_3$); yield 20.1%}. After treatment of the initial unreactive acetate with lipase at 16 °C for 30 h, an unhydrolysed acetate was isolated by chromatography and treated with lithium aluminium hydride in absolute ether. The alcohol obtained was identical with (15*S*)-12-*O*-methyl-16-hydroxyferruginol (**5**) {$[\alpha]_D^{21}$ + 45.62° (*c* 1.6 in $CHCl_3$); lit.[6] $[\alpha]_D$ + 45.4° ($CHCl_3$); yield 18%}. The signals corresponding to C-15 in the $^{13}C\{^1H\}$ n.m.r. spectra of the resolved diastereoisomers (**4**) and (**5**) were each single peaks [(**4**): δ 35.198; (**5**): δ 35.125]. This result shows that diastereoisomers of diterpenes with an alcoholic hydroxy group can be resolved by treatment of their acetate with lipase.

Reduction of the tosylates (**6**) and (**7**), prepared from (**4**) and (**5**), with lithium aluminium deuteride in absolute ether gave (15*S*)- and (15*R*)-[16-$^2H_1$]12-*O*-methylferruginol (**8**) and



Scheme 1. Proposed biosynthetic sequence to ferruginol. ▬ indicates two coupled ^{13}C atoms from [1,2-$^{13}C_2$]MeCOS CoA, ● indicates uncoupled ^{13}C atom from [1,2$^{13}C_2$]MeCOS CoA.

(**9**),† respectively. In the $^{13}C\{^1H\}$ n.m.r. spectrum of (**8**), the signals corresponding to the methyl groups of the isopropyl group appeared at δ 22.70 for the singlet and δ 22.62 for the triplet: $J(^{13}C–^2H)$ 19.53 Hz. The corresponding signals, in the case of (**9**), appeared at δ 22.87 for the singlet and δ 22.40 for the triplet, with an identical coupling constant. Consequently, the singlet signals at δ 22.70 and 22.87 correspond to the *pro*(*R*)- and *pro*(*S*)-methyl group in the isopropyl group of 12-*O*-methylferruginol, respectively.

The $^{13}C\{^1H\}$ n.m.r. spectrum of 12-*O*-methyl-ferruginol (**10**), biosynthesized from [U-$^{13}C_6$]glucose by *S. miltiorrhiza* cell cultures in a similar manner to that just described, was in complete agreement with the proposed mevalonoid biosynthetic pathway (Scheme 1). The signals for C-2 and -3, C-5 and -6, C-9 and -11, C-4 and -19, C-8 and -14, C-10 and -20, and

† Colourless viscous oils: $C_{21}H_{31}DO$: (**8**), M^+ *m*/*z* 301.2548 (calc. 301.2515); $[\alpha]_D^{25}$ + 66.0° (*c* 0.5, $CHCl_3$); (**9**), M^+ *m*/*z* 301.2545; $[\alpha]_D^{20}$ + 63.4° (*c* 1.12, $CHCl_3$).

C-15 and -16 [δ 22.91, $J(^{13}C–^{13}C)$ 35.40 Hz, *pro*(*S*) methyl] appeared as enhanced and $^{13}C–^{13}C$ coupled doublets. Carbon 1, 7, 12, 13, 17 [δ 22.72, *pro*(*R*) methyl], and 18 each gave rise to enhanced singlets. The appearance of singlet signals for C-13 and C-17, originating from C-3 and C-6 of mevalonolactone respectively, is attributable to the 1,2-methyl migration from C-13 to C-15 in the precursor (**2**).

The above result demonstrates that the migrated methyl group becomes the *pro*(*R*) methyl in the isopropyl group in ferruginol. The methyl migration, therefore, occurs on the *Si*-face of the double bond of the vinyl group in (**2**).

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