Biosynthesis and Absolute Configuration of Diterpene Cryptotanshinone from *Salvia miltiorrhiza*: Stereochemistry of Methyl Migration in the Formation of the Abietane Skeleton Determined by Incorporation of D-[U-$^{13}C_6$]Glucose

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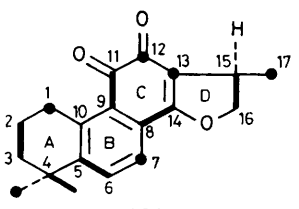
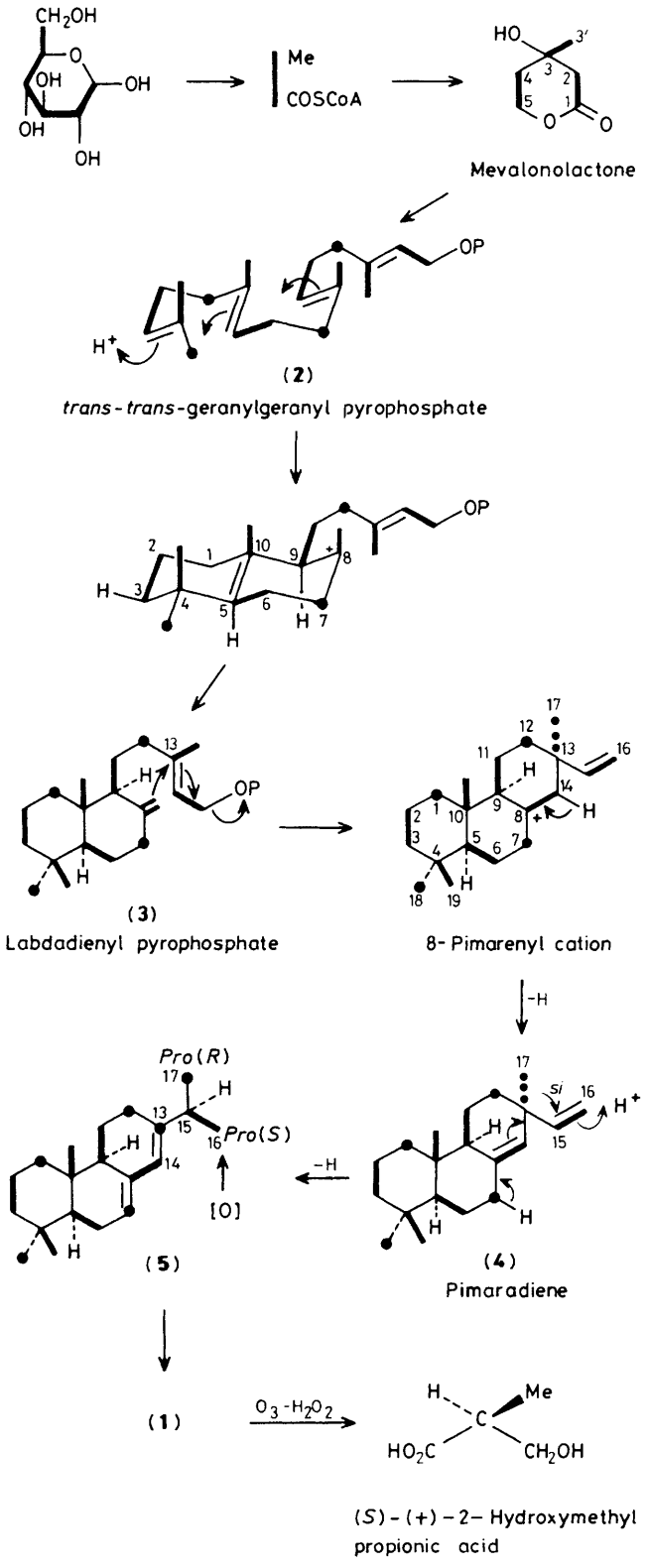
Experimental evidence is presented supporting a hypothetical pathway for the biosynthesis of diterpene cryptotanshinone (**1**); the stereochemistry of a 1,2-methyl migration in the formation of the abietane skeleton has been determined by incorporation of D-[U-$^{13}C_6$]glucose and determination of the absolute configuration at C-15 of (**1**).

Diterpene cryptotanshinone (**1**) with an abietane skeleton has been isolated from the root of *Salvia miltiorrhiza* (chinese medicine "Dan-Shen") as a potential inhibitor of platelet aggregation.[1] Its structure has been established by Takiura *et al.*[2] except for the absolute configuration at C-15. Abietane-type diterpenes are widely distributed in the plant kingdom. A generally accepted pathway for the biosynthesis of the abietane skeleton[3] is as follows (Scheme 1). Cyclization of all *trans*-geranylgeranyl pyrophosphate (**2**) having a chair–chair conformation gives labdadienyl pyrophosphate (**3**). Attack at C-13 by electrons from the exocyclic double bond accompanied by elimination of the pyrophosphate gives pimaradienyl pyrophosphate (**4**). Formation of the abietane skeleton can be explained by a 1,2-methyl migration from C-13 to C-15 in the vinyl substituent of (**4**). There is little direct evidence in support of this hypothesis of pimaradiene biosynthesis and no experimental verification of the mechanism for the formation of the abietane skeleton. In this report, direct evidence in



(**1**)

support of the pathway shown in Scheme 1 is given, along with the stereochemistry of the 1,2-methyl migration to give the abietane skeleton in cell cultures of *S. miltiorrhiza*.



Scheme 1. Proposed biosynthetic sequence to cryptotanshinone (**1**): —— 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.

In our initial biosynthetic investigation, unsuccessful attempts were made to incorporate various isoprenoid precursors such as [1,2-$^{13}C_2$]acetate and [6-^{13}C]mevalonate into (**1**). The cells rejected them, possibly owing to permeability or compartmentation effects.[4] Recently Cane *et al.*[5] demonstrated that D-[U-$^{13}C_6$]glucose is capable of penetrating the cell wall and acting as an *in vivo* precursor of [1,2-$^{13}C_2$]acetyl CoA in the biosynthesis of sesquiterpene pentalenolactone. Consequently we used D-[U-$^{13}C_2$]glucose as a labelled substrate.

The cell cultures of *S. miltiorrhiza*[6] were grown in a Linsmaier–Skoog liquid medium containing 1% sucrose, naphthyl acetic acid (NAA), and Kinetin. A solution of D-[U-$^{13}C_6$]glucose† (0.2 g; *ca.* 95% ^{13}C) in 50% ethanol (5 ml) was distributed among 5 bottles containing the cell cultures of *S. miltiorrhiza* (100 ml each). The cells were harvested after two weeks and extracted with methanol. Cryptotanshinone (**1**) was isolated and purified by preparative t.l.c. as described previously. The ^{13}C-{^1H} n.m.r. spectrum‡ of ^{13}C labelled (**1**) 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, and C-15 and -16 appeared as enhanced and coupled doublets, as expected. Carbons 1, 7, 12, 13, 17, 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-3′ of mevalonate, respectively, is attributable to the 1,2-methyl migration from C-13 to C-15 in (**4**) during the formation of the abietane skeleton.

Subsequently, the absolute configuration at C-15 of (**1**) was determined to clarify the stereochemistry of the 1,2-methyl migration. Exhaustive ozone degradation of (**1**) in acetic acid, followed by oxidation with hydrogen peroxide gave two acids, which were purified as *p*-phenylphenacyl esters. One of the acids was identical with an authentic sample of *p*-phenylphenacyl (*S*)-(+)-2-hydroxymethyl propionate§ and the other with *p*-phenylphenacyl 2,2-dimethyl malonate. It is thus evident that the absolute configuration at C-15 of (**1**) should be (*R*).

On the basis of these results, the stereochemistry of the biosynthetic pathway (**4**) → (**5**) → (**1**) was concluded to be as follows. The C-17 methyl group, in the 1,2-methyl migration from the C-13 to C-15 position, attacks C-15 on the *si*-face of the double bond, with protonation occurring at C-16 in the vinyl substituent of (**4**). Consequently the migrated methyl group becomes the C-17 methyl of (**1**) *via* the *pro*(*R*) methyl of

† D-[U-$^{13}C_6$]glucose was purchased from MSD Isotopes, Montreal, Canada.

‡ ^{13}C-{^1H} N.m.r. (50.10 MHz, int. $SiMe_4$, $CDCl_3$): (**1**) δ 25.7 (s, C-1), 19.1 (d, $^1J_{CC}$ 27 Hz, C-2), 37.9 (d, $^1J_{CC}$ 27 Hz, C-3), 34.9 (d, $^1J_{CC}$ 27 Hz, C-4), 143.7 (d, $^1J_{CC}$ 59 Hz, C-5), 132.5 (d, $^1J_{CC}$ 59 Hz, C-6), 122.5 (s, C-7), 128.7 (d, $^1J_{CC}$ 59 Hz, C-8), 126.3 (d, $^1J_{CC}$ 59 Hz, C-9), 152.4 (s, C-10), 184.5 (d, $^1J_{CC}$ 59 Hz, C-11), 175.8 (s, C-12), 118.3 (s, C-13), 170.7 (d, $^1J_{CC}$ 59 Hz, C-14), 81.4 (d, $^1J_{CC}$ 27 Hz, C-15), 34.7 (d, $^1J_{CC}$ 27 Hz, C-16), 18.8 (s, C-17), 31.9 (s, C-18), 32.0 (d, $^1J_{CC}$ 27 Hz, C-19).

§ The authentic samples were prepared in the usual manner from methyl (*R*)-(−)- and (*S*)-(+)-2-hydroxymethyl propionate (Aldrich). *p*-Phenylphenacyl (*S*)-(+)-2-hydroxymethyl propionate: $C_{18}H_{18}O_4$, M^+ *m/z* 298.1162 (calc. 298.1203); m.p. 109—110 °C; $[\alpha]_D^{23}$ + 20.83° (*c* 0.672, $CHCl_3$). *p*-Phenylphenacyl (*R*)-(−)-2-hydroxymethyl propionate: $C_{18}H_{18}O_4$, (M^+ *m/z* 298.1195 (calc. 298.1203); m.p. 109—110 °C; $[\alpha]_D^{23}$ − 20.09° (*c* 0.647, $CHCl_3$). *p*-Phenylphenacyl ester from the ozone degradation products of cryptotanshinone (**1**): $C_{18}H_{18}O_4$, M^+ *m/z* 298.1278 (calc. 298.1204); m.p. 109—110 °C; $[\alpha]_D^{23}$ + 21.89° (*c* 0.594, $CHCl_3$).

the isopropyl substituent of the generated abietane skeleton (**5**). The *pro*(*S*) methyl group of (**5**) is then oxidized to a primary alcohol to form the furan ring of (**1**).

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