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BIOSYNTHESIS OF TRITERPENOIDS. THE STEREOCHEMISTRY OF THE SQUALENE FORMATION AND ITS CYCLIZATION TO β-AMYRIN

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The distribution of deuterium in squalene and β-amyrin biosynthesized from [6-D_3]-mevalonic acid in Pisum sativum has been analyzed by NMR spectroscopy. It was thus proved that the cis-terminal methyls of squalene and the 4β-methyl group of β-amyrin are stereospecifically derived from the 3-methyl group of mevalonic acid.

It has been assumed[1] that the enzymatic isomerization of isopentenyl pyrophosphate to dimethylallyl pyrophosphate proceeds in an analogous manner to the subsequent stage,[2] so that the trans-methyl group of the latter pyrophosphate is derived from C-2 of mevalonic acid. Following the proposed biogenesis of β-amyrin from squalene,[3] it is expected that the 4α- and 4β-methyls of β-amyrin are derived from the terminal methyls trans and cis, respectively, to the carbon chain of squalene. In order to prove these stereochemical relationships, the distribution of deuterium in squalene and β-amyrin biosynthesized from [6-D_3]-mevalonic acid in germinating seeds of Pisum sativum has been examined by NMR spectroscopy.

DL-[6-D_3]-Mevalonic acid (isotopic purity: 99%) was synthesized from methyl [2-D_3]-acetate in four stages.[4] Fifty grams of the dry peas were incubated at 20° with 0.55g of the sodium mevalonate dissolved in 60 ml of water for 5 days.[5] The germinating seeds, after minched in a mortar, were extracted with acetone. Unsaponifiable materials of the residue from the extract were purified by t.l.c. to give 6 mg of squalene and 15 mg of β-amyrin (mp 199-201°).

The NMR spectra of both normal and biosynthetically deuteriated squalenes showed methyl signals at δ 1.58 and 1.67 ppm,[6] which correspond to the resonance of protons of the terminal and the internal methyls cis to the carbon chain and the resonance of protons of the terminal methyls trans,[5] respectively. Only the former peak of deuteriated squalene (Fig. 1b) was depressed to less than 5% of the peak of normal squalene (Fig. 1a). This finding implies that the cis-terminal and the internal methyls of squalene were stereoselectively deuteriated as is shown in Fig. 1b. Thus it was evidenced that (i) the cis-terminal methyl group of squalene was stereospecifically derived from the 3-methyl group of mevalonic acid and (ii) the stereochemistry of the isomerization of isopentenyl pyrophosphate to dimethylallyl pyrophosphate is analogous to the condensation[2] of the allyl pyrophosphate with the pentenyl pyrophosphate.

On the other hand, biosynthesized β-amyrin was found to contain six deuteriated methyl groups on the basis of the mass spectral pattern with a molecular ion at m/e 444. As is shown in Figs. 2b and 2a, the NMR spectrum of biosynthetically

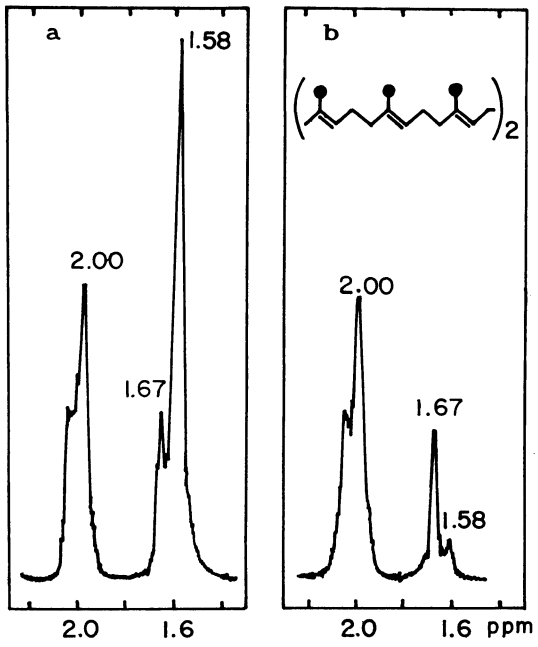

Fig. 1. Segments of NMR spectra of (a) normal, (b) biosynthetically deuteriated squalenes.

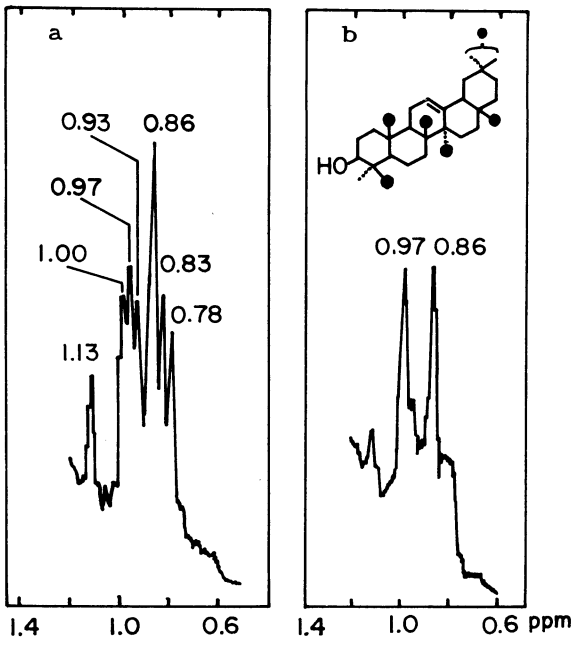

Fig. 2. Segments of NMR spectra of (a) normal, (b) biosynthetically deuteriated β-amyrins.

deuteriated β-amyrin showed the strong suppression of peaks at δ 0.78 (4β-CH_3), 0.83 (17-CH_3), 0.93 (10-CH_3), 1.00 (8-CH_3), and 1.13 ppm (14-CH_3), and a half height of a peak at δ 0.86 (20α- or 20β-CH_3), in comparison with that[7] of normal β-amyrin. These facts indicate (i) the occurrence of the deuteriation of methyl groups of β-amyrin as is shown in Fig. 2b and (ii) the derivation of the 4β-methyl group of β-amyrin from the 3-methyl group of mevalonic acid *via* the cis-terminal methyl group of squalene. These stereochemical relationships are consistent with those in the biosynthesis of lanosterol in the mammalian enzyme system.[8,9] Accordingly, the ring A of β-amyrin seems to be derived from the chair-folding form of squalene-2,3-oxide[10] which is formed from squalene. These processes were found to proceed without any inversion of the terminal methyls.

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