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TRITERPENOID BIOSYNTHESIS. THE STEREOSPECIFICITY IN THE ENZYMATIC CYCLIZATION OF SQUALENE TO β-AMYRIN

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It was first proved that the 20α-methyl group, as well as the 4β-, 8-, 10-, 14-, and 17-methyls of β-amyrin biosynthesized from [6-D_3]-mevalonic acid in Pisum sativum are stereospecifically derived from the 3-methyl group of the acid. The result has substantiated the stereospecificity in the enzymatic cyclization of squalene to β-amyrin.

The stereospecificity in the biosynthetic formation of ring E of pentacyclic triterpenes from squalene still remains to be established.[1a] The elucidation of the stereospecificity is expected to furnish direct supports to the hypothetical mechanism[1b] proposed for the cyclization of squalene to β-amyrin and other triterpenes. NMR spectroscopic analyses of the distribution of deuterium in the 20α- and 20β-methyls of β-amyrin biosynthesized from [6-D_3]-mevalonic acid were unsuccessful,[2] because of overlapping of proton signals of the methyls in question. The present paper is the first report concerning the substantiation of the stereospecificity in the biosynthetic formation of ring E of pentacyclic triterpenes from squalene.

Deuteriated β-amyrin (mp 199-201°; M^+ at m/e 444) was biosynthesized from [6-D_3]-mevalonic acid in germinating seeds of Pisum sativum as described previously.[2] The NMR spectra[3] of biosynthetically deuteriated β-amyrin were compared with those of normal β-amyrin. The NMR spectrum of normal β-amyrin (21.6 mg) dissolved in $CDCl_3$ (0.1 ml) containing tris(dipivalomethanato)europium (III) [Eu(DPM)$_3$] (1 mol equiv.), which is an effective NMR shift reagent,[4, 5] resulted in the distinct separation of all methyl signals as shown in Fig. 1A. The co-ordinated europium ion has been speculated to approach to the hydroxyl oxygen atom from the direction of C-O bond.[4] This speculation indicates that the proximity of methyl groups to the co-ordination site is the order of the 4β-, 4α-, 10-, 8-, 14-, 17-, 20β-, and 20α-methyls. As shown in Fig. 1A, eight methyl signals were assigned such that the order of their appearance from low to high field is in accord with their spatial proximity.[4] The NMR spectrum of biosynthetically deuteriated β-amyrin (15.4 mg) in the $CDCl_3$ (0.5 ml) solution containing Eu(DPM)$_3$ (1 mol equiv.) is shown in Fig. 1B. Only two methyl signals were observed at δ 1.23 and 1.08 ppm, which were assigned to the 4α- and 20β-methyls respectively, in comparison with the spectrum of normal β-amyrin (Fig. 1A). The fact that the 4α-methyl group was not deuteriated agreed with our recent result.[2] It was thus concluded that the 4β-, 8-, 10-, 14-, 17-, and 20α-methyls were deuteriated fully and derived stereospecifically from the 3-methyl group of mevalonic acid.

It has been proved previously that the cis-terminal and the internal methyl groups of squalene were

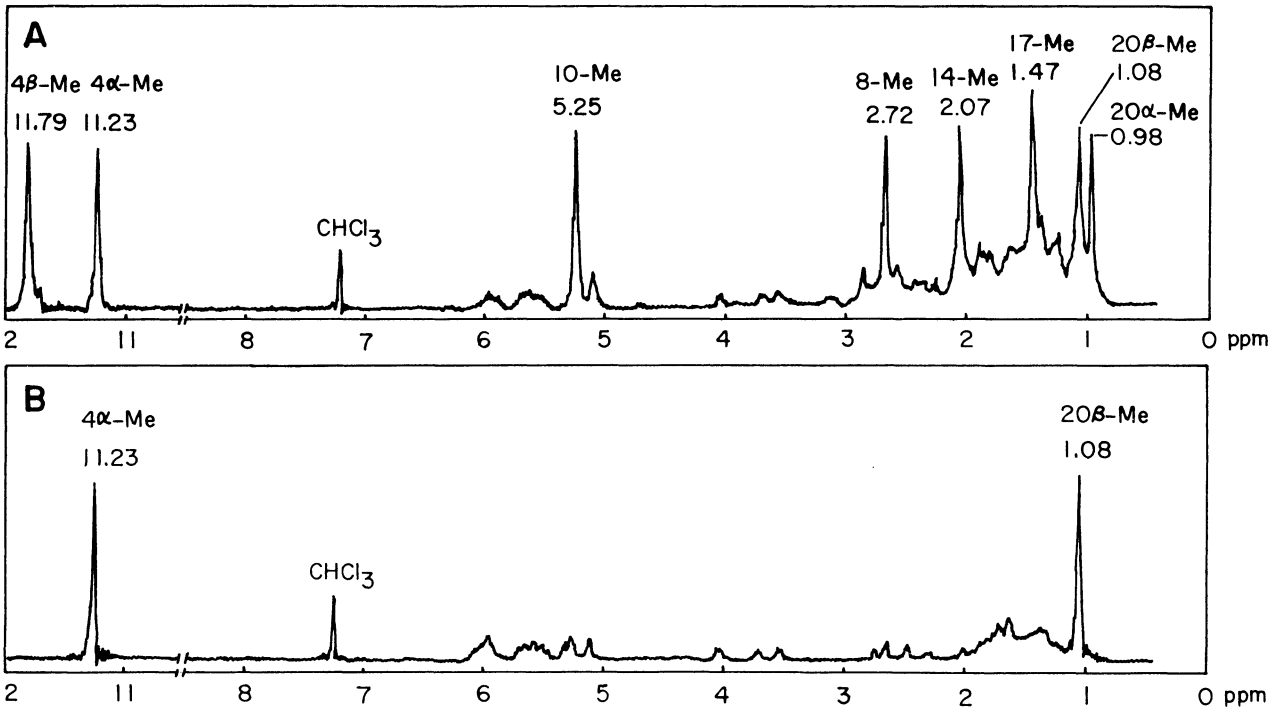
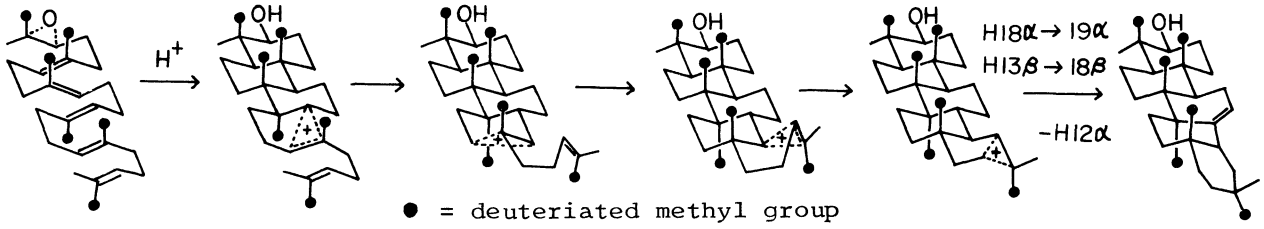


Figure 1. NMR spectra of (A) normal β-amyrin and (B) biosynthetically deuteriated β-amyrin in $CDCl_3$ containing 1 mol equiv. of $Eu(DPM)_3$.



Scheme 1. Stereospecific cyclization of 2, 3-oxidosqualene to β-amyrin.

derived specifically from the 3-methyl group of mevalonic acid.[2, 6] 2, 3-Oxidosqualene is formed without any inversion of the terminal gem-dimethyl group.[2, 6] Assuming the oxide cyclizes in the manner as shown in Scheme 1, both the 4β- and 20α-methyls of the gem-dimethyl groups at 4- and 20-positions of β-amyrin are expected to be deuteriated stereospecifically. The deuterium-labelling patterns observed for β-amyrin are exactly consistent with those expected. Thus the cyclization process was proved to proceed with the high stereospecificity. The results have hereupon supported experimentally the proposal of Ruzicka[1] for the biogenesis of pentacyclic triterpenes having the gem-dimethyl group in ring E.

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