STEREOSPECIFIC INSERTION OF HYDROGEN ATOM INTO SQUALENE FROM REDUCED NICOTINAMIDE-ADENINE DINUCLEOTIDES*

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We have reported previously that during the synthesis of squalene from two molecules of farnesyl pyrophosphate (FPP) one hydrogen atom attached to C-1 of one of the two FPP molecules is lost and is replaced by another hydrogen atom derived from a reduced nicotinamide-adenine dinucleotide (NADH or NADPH) (Popják et al., 1961; 1962). Thus squalene biosynthesized either from 1-D_2-farnesyl pyrophosphate or from 5-D_2-mevalonate (C-5 of mevalonate becomes C-1 of FPP), in the presence of NADH or NADPH, contains 3 atoms of D arranged around its two central carbon atoms as shown in (I). We have suggested that the "hydrogen exchange" referred to was a stereospecific process (Popják et al., 1962). A partial proof for this assumption was provided by our observation that hydrogen from only the "B"-side of the reduced nicotinamide coenzyme (H_B in formula II; cf. Cornforth et al., 1962) was transferred to squalene (Popják et al., 1961-62). If the insertion of the hydrogen atom into squalene from the reduced coenzyme was a stereospecific process then

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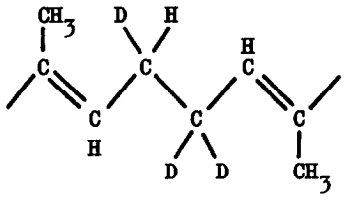
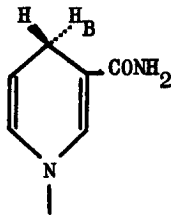


(I)



(II)

the carbon atom in the centre of squalene biosynthesized from 5-D_2-mevalonate (or from 1-D_2-FPP), which carries one deuterium and one protium atom should be an asymmetric carbon, for it is now well established that one deuterium atom attached stereospecifically to a "meso" carbon atom will impart optical activity to an otherwise optically inactive substance.

We have arranged a large scale synthesis of squalene from 5-D_2-2-C^{14}-mevalonate with enzyme preparations made from the liver of 300 rats as described previously (Popják et al., 1961). As judged from the C^{14}-counts of the squalene preparation 230 μmoles of newly synthesized squalene were obtained. This was purified (after chromatography on alumina) through the thiourea clathrate which gave 65 mg. of the pure hydrocarbon. The purified squalene was then degraded by ozonolysis and gave 75.6 mg. of a mixture of succinic and laevulinic acids. After extraction of this mixture with cold chloroform, to remove laevulinic acid, 15.1 mg. of crude succinic acid were obtained which was recrystallized from 25 μl. of water in a capillary as described (Popják et al., 1961). The yield was 11.200 mg. of recrystallized succinic acid; it was dissolved in 0.25 ml. of spectroscopic methanol and its optical rotation measured in a new spectropolarimeter manufactured by Bellingham and Stanley, London, England. It was found to be dextrorotatory.

After determination of the optical rotation, the specimen was methylated with diazomethane and the dimethylsuccinate analysed for

deuterium content by mass-spectrometry (Popják et al., 1961) and also by gas-liquid radiochromatography (Popják et al., 1959; 1962) for chemical purity and for possible content of C^{14}. A detailed analysis of the mass-spectrum according to principles previously described (Popják, et al., 1961) indicated that 88% of the molecules in the specimen contained 3 atoms of deuterium, and the remainder consisted of dideuterio (10%) and non-isotopic (2%) molecules in agreement with our previous findings.

The carbon atoms of the trideuterio succinic acid represent the four central carbon atoms of the biosynthesized squalene; the dideuterio molecules are on the other hand an artifact of ozonolysis, being derived from the oxidation of laevulinic acid. From the known reaction sequence of squalene biosynthesis from mevalonate (cf. Popják and Cornforth, 1960) it may be predicted that the ozonolysis of squalene biosynthesized from 5-D_2-2-C^{14}-mevalonate should give 2-D_2-3-C^{14}-laevulinic acid the oxidation of which in turn furnishes 2-D_2-2'-C^{14}-succinic acid. The gas-liquid radiochromatographic analysis of the dimethyl succinate on an ethylene-glycol-succinate polyester column at 140°C. showed it to be chemically pure but that it contained C^{14} in an amount indicating that 10.2% of the succinic acid must have been derived from the oxidation of laevulinic acid. This was inferred from the fact that the molar specific activity of the dimethyl succinate was 10.2% of that of the laevulinic acid (analysed by gas-liquid radiochromatography after reduction with $LiAlH_4$ and acetylation, as the di-acetate of pentane-1,4-diol). This analysis agreed very closely with the result of mass-spectrometry.

As it was reasonable to suppose that only the trideuterio molecules in the specimen of the succinic acid were optically active, the observed optical rotations were corrected accordingly and are shown in Fig. 1.

We have reported recently the preparation, by a combination of enzymic and chemical methods, of 2R-2-D_1-succinic acid (III) and

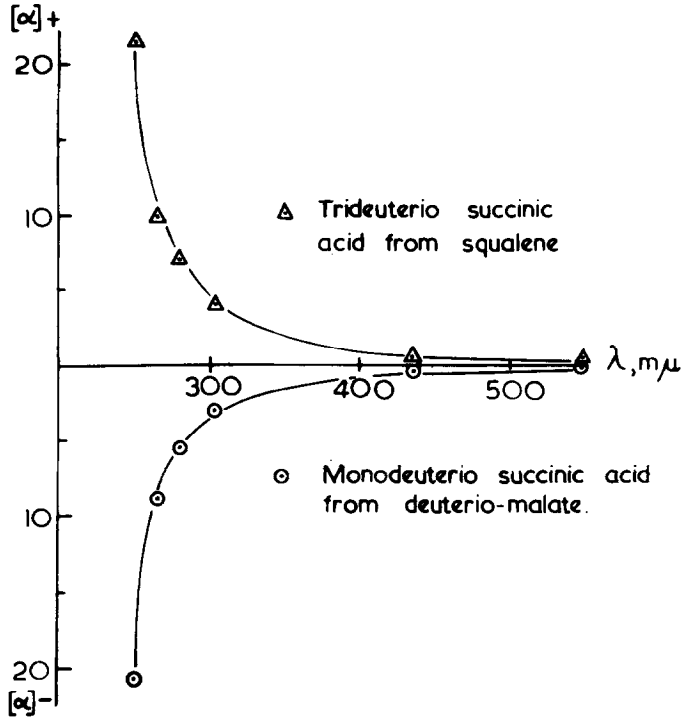
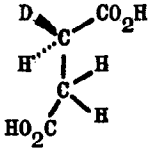
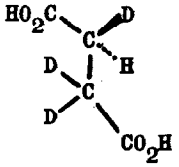


Fig. 1. Optical rotatory dispersion of deuterio-succinic acids.

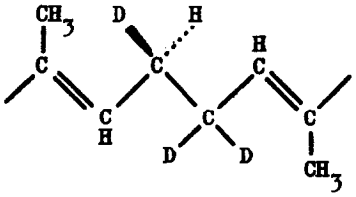
showed it to be laevorotatory (Cornforth et al., 1962). Since the optical rotatory dispersion curve for the tri-deuterio succinic acid from the squalene specimen is a mirror image of that of the reference 2R-2-D_1-succinic acid, we infer that the absolute configuration of the test specimen was S (IV). It follows that the absolute configuration of protium and deuterium atoms around the central carbons of squalene biosynthesized from 5-D_2-mevalonate with NADPH (or NADH) is as shown in formula (V).



(III)



(IV)



(V)

An implicit assumption is that substitution of hydrogen by deuterium at positions away from the centre of asymmetry should not alter the sign of rotation. It seems unlikely on general grounds that any such effect should operate; the point is to be tested, however, by preparing a trideuteriosuccinic acid of known absolute configuration.

These observations fully confirm our suggestion that the insertion of the H_B hydrogen into squalene is a stereospecific process. We have further experimental evidence at hand, to be reported later, that not only the insertion of the new hydrogen atom, but also the removal of the one attached originally to C-1 of FPP, is stereospecifically determined.

It may be predicted that if squalene were synthesized from FPP with "B"-deuterio or "B"-tritio NADPH and this squalene cyclized to lanosterol and converted to cholesterol, one would obtain either 11α-H* or 12β-H* sterol depending on whether the squalene was cyclized from one or the other end of the molecule. An independent proof of this conclusion is provided in the accompanying article by Samuelsson and Goodman (1963).

Full experimental details and further evidence on the steric course of squalene synthesis from mevalonate will be presented elsewhere.

Acknowledgments

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