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CYCLIZATION OF 2,3-OXIDOSQUALENE TO CYCLOARTENOL IN A CELL-FREE SYSTEM FROM HIGHER PLANTS

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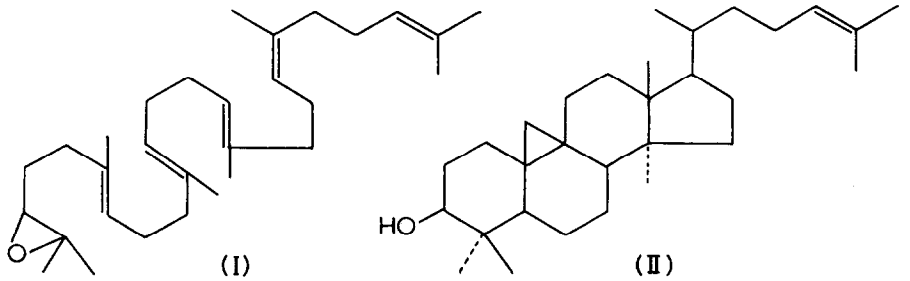
It has recently been demonstrated that the conversion of squalene into lanosterol in animal systems proceeds in two distinct steps, involving initial oxidation of the squalene to 2,3-oxidosqualene (I), which subsequently cyclizes to



(I) (II)

lanosterol (1-4). Similarly, in plants, the formation of 2,3-oxidosqualene has been demonstrated (5, W.W. Reid: personal communication) and its cyclization to β-amyrin by a cell-free system from peas has been reported (6).

It has recently been suggested (7-9) that cycloartenol (II) may replace lanosterol as the first tetracyclic triterpene formed from squalene during phytosterol biosynthesis. The present communication reports the conversion of 2,3-oxidosqualene to cycloartenol by a cell-free system from bean leaves.

[^{14}C]-Squalene was obtained by anaerobic incubation of [2-^{14}C]-mevalonic acid with rat liver homogenates prepared by the method of Bucher and McGarrahan (10). The [^{14}C]-squalene was converted into a mixture of bromohydrins by means of N-bromosuccinimide in aqueous glyme (11). The 2,3-bromohydrin was purified by thin-layer chromatography on silica gel developed with 5% ethyl acetate in benzene. The bromohydrin was transformed into racemic [^{14}C]-2,3-oxidosqualene by treatment with ethanolic base and was purified by thin-layer chromatography on silica gel with 5% ethyl acetate in hexane for development.

12 g. of newly developed bean leaves were ground in a mortar and pestle at 0°C with 20 ml. of 0.1 M phosphate buffer, pH 7.4, containing 0.01 M magnesium sulphate. The homogenate was filtered through cheesecloth and centrifuged at 10,000 g. for 20 mins. at -3°C. The resultant supernatant fraction was used for incubation.

2,3-Oxidosqualene (259, 125 d.p.m. ^{14}C) of specific activity 1,796 d.p.m./µg. was transferred in cyclohexane to a Warburg flask. After evaporation of the solvent under nitrogen, the epoxide was dissolved in acetone (1 ml.) and 5 mg. of Tween 80 added in 1 ml. of acetone. Then 0.5 ml. of the phosphate buffer was added and the acetone evaporated under nitrogen at room temperature. 2 ml. of the 10,000 g. supernatant fraction was added and the flask was flushed with nitrogen for 15 min. and then stoppered. Incubation was carried out at 37°C for 11 hr. and the mixture saponified directly. The non-saponifiable material was subjected to thin-layer chromatography on silica gel with 20% ethyl acetate in hexane for development, and 2.8% (7,355 d.p.m.) of the recovered radioactivity was found in the 4,4'dimethyl sterol band (12).

An aliquot of the 4,4'dimethyl sterol band was diluted with approximately 100 µg. of non-radioactive cycloartenol and subjected to preparative gas-liquid chromatography on 3% XE-60 at 220°C. Samples were collected at 2 min. intervals as they eluted from the column in glass capillary tubes at ambient temperature, and then assayed for radioactivity. Approximately 40% of the total radioactivity recovered from the column was associated with the cycloartenol peak.

The remainder of the 4,4'-dimethyl sterol fraction was acetylated, diluted with 15 mg. of carrier cycloartenyl acetate and recrystallized from methanol to constant specific activity (299, 235, 213, 198, 209 d.p.m./mg.). This confirms that about 65% of the radioactivity in the 4,4'dimethyl sterol band was in cycloartenol; the remainder was associated with uncharacterised compounds. This corresponds to a 2-4% conversion of one enantiomer of 2,3-oxidosqualene into cycloartenol.

The 4,4'-dimethyl sterol band from a similar incubation was diluted with carrier lanosterol and recrystallized from methanol. Two recrystallizations completely removed radioactivity from the lanosterol, which is in accord with previous reports (8, 9, 13-15) indicating the absence of detectable lanosterol in higher plant tissues.

The present results demonstrate that in common with the biosynthesis of lanosterol in animals and β-amyrin in plants, the formation of cycloartenol from squalene also involves the intermediate formation of 2,3-oxidosqualene, which then presumably undergoes proton-initiated cyclization.

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