The Synthesis of 5*S*-5-[$^3H_1$]Mevalonic Acid Lactone

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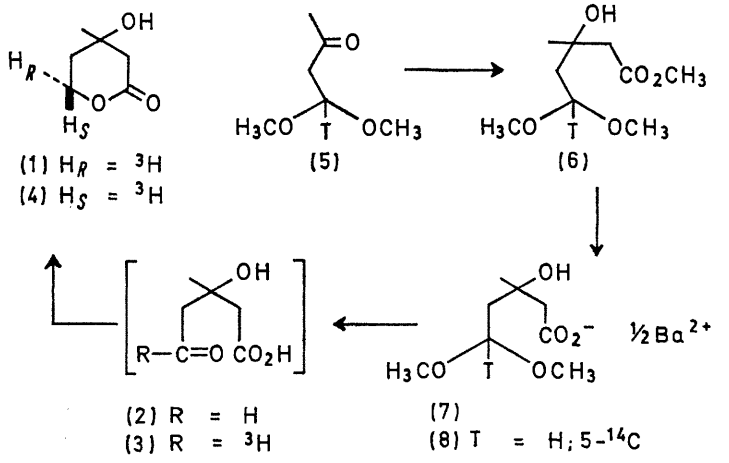
Summary The synthesis of 5*S*-5-[$^3H_1$]mevalonic acid lactone and the proof of its structure by enzymatic conversion into squalene are described.

As part of a study of the biosynthesis of various mevalonic-derived alkaloids, we required a sample of 5*S*-5-[$^3H_1$]-mevalonic acid lactone. Since this compound may be of use to many researchers in the terpenoid field, and since the 5*S*-5-[$^3H_1$]-isomer is the only unknown asymmetrically labelled mevalonate,[1] we report details of its synthesis and proof of structure.[2]

The introduction of asymmetry to position 5 of mevalonic acid has been achieved by Donninger and Popják[3] in the synthesis of 5*R*-5-[$^3H_1$]mevalonic acid (**1**). Their procedure involved the enzymatic reduction of mevaldic acid (**2**) with 4*R*-4-[$^3H_1$]-TPNH and rat liver mevaldate reductase. By preparing 5-[^{3}H]mevaldic acid (**3**) and incubating it with the same enzyme and TPNH, the 5*S*-5-[$^3H_1$]-isomer of mevalonic acid (**4**) should result.

5[^{3}H]Mevaldic acid was synthesised as outlined in Scheme 1. Ten millicuries of sodium [^{3}H]formate was converted into the methyl ester by heating with dimethyl sulphate.[4] In a modification of a published procedure,[5] the ester was condensed with acetone in the presence of sodium hydride to give the sodium salt of 1-[^{3}H]-3-oxobutyraldehyde. The crude salt was then treated with methanolic hydrogen chloride to afford 1-[^{3}H]-3-oxobutyraldehyde dimethylacetal (**5**). Condensation of acetal (**5**) with methyl iodoacetate and granular zinc under Reformatsky conditions[6] produced methyl 5-[3H]-5,5-dimethoxy-3-hydroxy-3-methylvalerate (**6**). Hydrolysis of the ester with aqueous $Ba(OH)_2$ solution gave the barium salt of mevaldic acid dimethylacetal (**7**) in an overall radiochemical yield of 2·5%. The corresponding 5-[^{14}C]mevaldic acid dimethylacetal (**8**) was prepared by the same route starting with sodium [^{14}C]-formate.

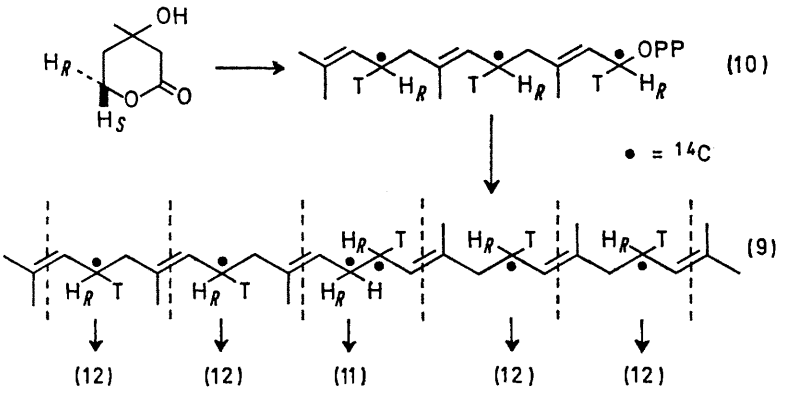
Hydrolysis of acetal (**7**) with dilute sulphuric acid was followed by incubation with rat-liver mevaldate reductase.[3] The enzyme solution was acidified and lyophilized, and the residue was continuously extracted with chloroform to isolate 5*S*-5-[$^3H_1$]mevalonic acid lactone (**4**). The corresponding 5-[^{14}C]-isomer was prepared by sodium borohydride reduction.[7] Both compounds were purified to



SCHEME 1

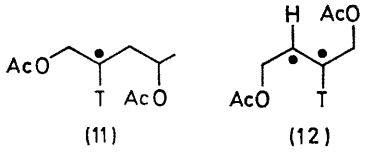
constant activity by thick-layer chromatography on silica gel.

The stereochemical purity of (**4**) was determined by the method Popják and Cornforth[8] developed for the 5*R*-5-[$^3H_1$]mevalonic acid isomer (**1**). They found that the conversion of six molecules of mevalonic acid into squalene (**9**) by an anaerobic rat-liver homogenate resulted in the elimination of *one* 5*S*-hydrogen, all six 5*R*-hydrogens being retained. The hydrogen which is lost comes from C-1 of farnesyl pyrophosphate (**10**) during its head-to-head coupling to form squalene (see Scheme 2).



SCHEME 2

Ozonolysis of a sample of squalene obtained from 5*S*-5-[$^3H_1$]-5[^{14}C]mevalonic acid followed by reduction of the ozonide with lithium aluminium hydride and acetylation of the alcoholic products with acetic anhydride gave a mixture of butane-1,4-diol diacetate (**11**) and pentane-1,4-diol diacetate (**12**). The diacetates were separated by gas chromatography and their $^3H/^{14}C$ ratios measured. As recorded in the Table, the butane-1,4-diol diacetate had a $^3H/^{14}C$ ratio of 8·75 : 1 (9·4 : 1) as compared to the pentane-1,4-diol diacetate ratio of 15·2 : 1 (17·0 : 1). From Scheme 2, each molecule of (**11**) should have one ^3H and two ^{14}C, while each molecule of (**12**) should have one ^3H and one ^{14}C.



That is, the $^3H/^{14}C$ ratio of (**11**) should be one half that of (**12**). The observed ratios of 0·57 (0·55) indicate that, within experimental error, the synthetic 5*S*-5-[$^3H_1$]mevalonic acid lactone is stereochemically pure.

A note of warning should be added at this point. The $^3H/^{14}C$ ratios obtained by mixing enzymatically prepared substrates with synthetic ones are not always reliable. The $^3H/^{14}C$ ratio of (**4**) should be the same as that of the pentane-1,4-diol diacetate sample. That it is not is due to the partial specificity of mevaldate reductase for 3*R*-mevaldate at the expense of the 3*S*-isomer.[9] In the synthesis of squalene, one of the intermediate enzymes, mevalonate kinase, is completely specific for 3*R*-mevalonate. Since the [^{3}H]mevalonate is richer in the 3*R*-isomer than the completely racemic ^{14}C compound, the $^3H/^{14}C$ ratio of (**12**) is correspondingly higher than that of (**4**).

TABLE

Compound	Run 1 c.p.m.	Run 1 $^3H/^{14}C$	Run 2 c.p.m.	Run 2 $^3H/^{14}C$
5*S*-5-[$^3H_1$]5[^{14}C]mevalonic acid lactone	$5·0 \times 10^5$	9·6	1×10^6	10·1
Squalene	$1·6 \times 10^5$	12·6	3×10^5	13·9
Pentanediol 1,4-diacetate (**12**)		15·2		17·0
Butanediol 1,4-diacetate (**11**)		8·75		9·4

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