Biological Removal of the 4α-Methyl Group during the Conversion of Cycloartanol into 31-Norcycloartanol in *Polypodium vulgare* Linn.

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Summary The 4α-methyl group of cycloartanol is derived from C-2 of mevalonic acid and this is the methyl group removed together with the hydrogen from C-3 in the biological conversion of cycloartanol into 31-norcycloartanol.

THE sequence of removal of the C-4 *gem*-dimethyl substituents during the conversion of lanosterol into cholesterol has been recently investigated.[1,2] The work of Gaylor and Delwiche[1] suggested that the 4β-methyl group of lanosterol is the first to be removed. However, the more recent results of Clayton *et al.*,[2] using a rat-liver homogenate, indicate that the 4α-methyl group is eliminated first,[2a] whilst the methyl group originally occupying the 4β-position is epimerised to give the 4α-methyl group in the resultant 4-monomethyl-sterol.[2b]

The incorporation of 3*R*-[2-^{14}C,(4*R*),4-$^{3}H_1$]mevalonic acid into the triterpenes of *Polypodium vulgare* Linn. rhizomes during an investigation of cyclolaudenol biosynthesis allowed us to examine the C-4 demethylation reaction in a plant system. We now show that it is the 4α-methyl group which is labelled from C-2 of mevalonic acid and subsequently eliminated during the conversion of cycloartanol (I) into 31-norcycloartanol (VII).

Slices of *P. vulgare* rhizomes and leaves were incubated with 3*R*-[2-^{14}C,(4*R*),4-$^{3}H_1$]mevalonic acid and the radioactive triterpenes isolated as their acetates as described previously.[3] In labelled cycloartanol (I) and cycloartenol (II), tritium is located in the 3α,5α,8β,17α,20, and 24 positions[4] and ^{14}C at positions 1,7,15,22,26 or 27, and 30 or 31. The presence of tritium in the 3α-position was confirmed by saponification of the acetates of cycloartanol (III) and cycloartenol (IV) to give the alcohols (I) and (II), respectively, followed by oxidation to the 3-oxo-compounds; (V) and (VI). The observed decrease in the ^{3}H : ^{14}C ratio in each corresponded to the loss of one tritium atom (Table 1). However, similar conversion of the 31-norcycloartanyl acetate (VIII) into the 3-oxo-derivative (IX) resulted in

TABLE 1

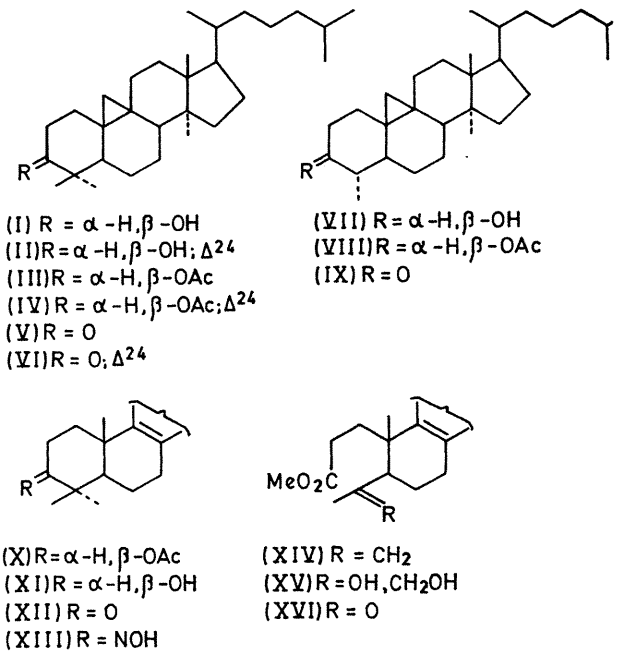
Compound		^{3}H : ^{14}C ratio	Normalized ^{3}H : ^{14}C ratio	Theoretical ^{3}H : ^{14}C atomic ratio
Squalene		10·32	6 : 6	6 : 6
Cycloartenyl acetate	(IV) ..	10·21	5·94 : 6	6 : 6
Cycloartenol	(II) ..	10·29	5·98 : 6	6 : 6
Cycloartenone	(VI) ..	8·85	5·15 : 6	5 : 6
Cycloartanyl acetate	(III) ..	10·19	5·92 : 6	6 : 6
Cycloartanol	(I) ..	10·25	5·96 : 6	6 : 6
Cycloartanone	(V) ..	8·70	5·06 : 6	5 : 6
31-Norcycloartanyl acetate	(VIII) ..	10·20	4·94 : 5	5 : 5
31-Norcycloartanol	(VII) ..	10·30	4·99 : 5	5 : 5
31-Norcycloartanone	(IX) ..	10·14	4·91 : 5	5 : 5

no change in the $^3H:^{14}C$ ratio, thus proving the absence of tritium at C-3. It was reported previously that the 3α-hydrogen is exchanged during the conversion of lanosterol into cholesterol by rat liver[5] and cycloartenol into phytosterols.[6] The present results show for the first time that the 3α-hydrogen is exchanged during the loss of the first C-4 methyl group from cycloartanol (I). Moreover, since 31-norcycloartanol (VII) had the same $^3H:^{14}C$ ratio as squalene and cycloartanol (I), it can be inferred that the labelled C-4 methyl group arising from C-2 of mevalonic acid was removed by oxidative demethylation from the cycloartanol.

The C-2 of mevalonic acid retains its identity during incorporation into squalene[7] and subsequent cyclization of squalene 2,3-oxide to give the 4α-methyl group of lanosterol[7,8a] or soyasapogenol.[9] To verify that the 4α-methyl group of cycloartenol (I) is similarly derived from C-2 of mevalonic acid, the labelled cycloartenol from the *P. vulgare* incubation was degraded by a modification of the method employing the 'abnormal' Beckmann rearrangement recently described by Moss and Nicolaidis[8] to investigate this point in the case of lanosterol.

TABLE 2

Compound		$^3H:^{14}C$ ratio	Normalised $^3H:^{14}C$ ratio	Theoretical $^3H:^{14}C$ atomic ratio
Cycloartenyl acetate	(IV) ..	10·21	6 : 6	6 : 6
Cycloartanyl acetate	(III) ..	9·86	5·73 : 6	6 : 6
Lanost-9(11)-en-3β-yl acetate	..	9·97	5·80 : 6	6 : 6
Lanost-8-en-3β-yl acetate	(X) ..	8·00	4·65 : 6	5 : 6
Lanost-8-en-3β-ol	(XI) ..	8·03	4·67 : 6	5 : 6
Lanost-8-en-3-one	(XII) ..	6·44	3·74 : 6	4 : 6
Lanost-8-en-3-one oxime	(XIII) ..	6·43	3·74 : 6	4 : 6
Methyl 3,4-secolanost-4(30),8-dien-3-oate	(XIV) ..	6·28	3·65 : 6	4 : 6
Methyl 4ξ,30-dihydroxy-3,4-secolanost-8-en-3-oate	(XV) ..	6·40	3·72 : 6	4 : 6
Methyl 4-oxo-3,4-seco-30-norlanost-8-en-3-oate	(XVI) ..	7·49	3·72 : 5·13	4 : 5



The labelled cycloartenyl acetate (IV) was diluted with carrier material and hydrogenated to give cycloartanyl acetate (III)† (Table 2). Isomerisation with gaseous hydrogen chloride produced a mixture of lanost-9(11)-en-3β-yl acetate and lanost-8-en-3β-yl acetate (X) which were separated by t.l.c. on silica gel impregnated with silver nitrate, (X) was then repeatedly recrystallized after addition of carrier material. The isolated lanost-8-en-3β-yl acetate (X) had a decreased $^3H:^{14}C$ ratio owing to loss of tritium from C-8.[4] Treatment of (X) with lithium aluminium hydride gave lanost-8-en-3β-ol (XI) which was oxidized to give lanost-8-en-3-one (XII) with the loss of one tritium atom from C-3. Formation of the oxime (XIII) m.p. 170—171°, and 'abnormal' Beckmann rearrangement[10] gave the seco-nitrile which was hydrolysed to the acid and methylated to yield the seco-methyl ester (XIV) m.p. 110—111°. Treatment of (XIV) with osmium tetroxide gave the dihydroxy-compound (XV) m.p. 167—168° which was finally cleaved with sodium periodate to yield methyl 4-oxo-3,4-seco-30-norlanost-8-en-3-oate (XVI) m.p. 116—117°. The increase in the $^3H:^{14}C$ ratio upon conversion of (XV) into (XVI) is consistent with loss of labelled carbon during removal of the methylene group from (XIV).‡ Since it has been shown[8b] that the methylene group of the seco-compound (XIV) arises from the 4α-methyl group of the parent triterpene we conclude that the 4α-methyl group of the cycloartenol was labelled and therefore arose from C-2 of mevalonic acid. It follows that the conversion of cycloartanol (I) into 31-norcycloartanol (VII) involves the loss of the 4α-methyl group whilst the 4β-methyl group is

† Cycloartenol (II) was used for the degradation because the cycloartanol (I) was insufficiently labelled. Hydrogenation of the cycloartenol (II) resulted in a small drop in the $^3H:^{14}C$ ratio, presumably due to some tritium–hydrogen exchange at C-24. The $^3H:^{14}C$ ratio in subsequent compounds in the degradation sequence are consequently lower than the theoretical value; however, this does not affect the interpretation of the results.

‡ Our results suggest that only 70–80% of the anticipated amount of radioactive carbon was eliminated in this reaction. This is in agreement with results obtained by Moss and Nicolaidis[8a] which indicate that the 'abnormal' Beckmann rearrangement is only about 70% stereospecific. We have observed that when the rearrangement is carried out under conditions which maximise the yield of the seco-compound (L. Mangoni and M. Belardini, *Gazzetta*, 1964, **94**, 382) the ring opening reaction is no longer stereospecific.

inverted to the 4α-position and is thus in agreement with the conclusions of Clayton *et al.*, who used animal tissues.

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