# 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

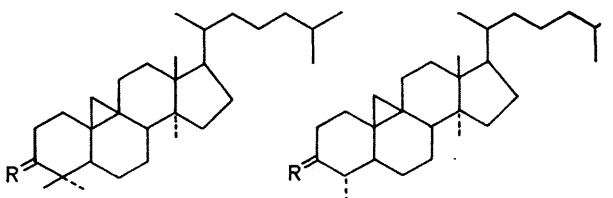
| Compound | | $^{3}H$ : $^{14}C$ ratio | Normalized $^{3}H$ : $^{14}C$ ratio | Theoretical $^{3}H$ : $^{14}C$ atomic ratio |
|---|---|---|---|---|
| Squalene | | 10·32 | 6 : 6 | 6 : 6 |
| Cycloartenyl acetate | (IV) | 10·21 | 5·94 : 6 | 6 : 6 |
| Cycloartenol | (II) | 10·29 | 5·98 : 6 | 6 : 6 |
| Cycloartenone | (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.

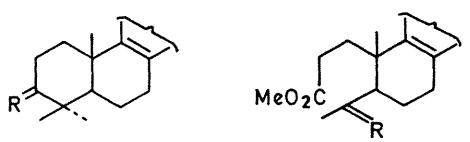
TABLE 2

| 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 |



(I) R = α-H,β-OH
(II) R = α-H,β-OH; Δ24
(III) R = α-H,β-OAc
(IV) R = α-H,β-OAc; Δ24
(V) R = O
(VI) R = O; Δ24

(VII) R = α-H,β-OH
(VIII) R = α-H,β-OAc
(IX) R = O



(X) R = α-H,β-OAc
(XI) R = α-H,β-OH
(XII) R = O
(XIII) R = NOH

(XIV) R = $CH_2$
(XV) R = OH,$CH_2OH$
(XVI) R = O

To investigate this point in the case of lanosterol.

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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