Phytosterol Biosynthesis in Banana Peel. Initial Removal of the 4α-Methyl Group of 24-Methylenecycloartanol during its Conversion into Cycloeucalenol in *Musa sapientum*

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Summary The 4α-methyl group and the 3α-hydrogen of 24-methylene cycloartanol are specifically removed during its conversion into cycloeucalenol in *Musa sapientum*.

THE sequence of removal of the 4α- and 4β-methyl groups from 4,4-dimethyl-triterpene intermediates during phytosterol biosynthesis is not known. Early investigations of the C-4 demethylation of lanosterol during cholesterol biosynthesis suggested initial removal of the 4β-methyl group.[1] More recent reports, however, have demonstrated that the opposite case occurs with the initial removal of the 4α-methyl group.[2] It has also been indicated that the 4α-methyl group of cycloartanol is removed during its conversion into 31-norcycloartanol in *Polypodium vulgare*.[3] We show that the same mechanism occurs in C-4 demethylation during phytosterol formation in banana peel.

The possible involvement of 3-ketonic intermediates during the conversion of lanosterol into cholesterol has been indicated.[4,5] Evidently, this also occurs during phytosterol biosynthesis.[6-9] We also demonstrate that such an intermediate is formed during the C-4 demethylation process of 24-methylenecycloartanol.

Banana-peel slices (5 g) were incubated with [2-^{14}C]-(4*R*)-[4-$^{3}H_1$]mevalonic acid (5 μc ^{14}C, 12·5 μc ^{3}H) for 24 hr.† To the lipid from these incubations was added 10 mg each of cycloartenol, 24-methylenecycloartanol, cycloeucalenol, and the 4-demethylsterols from banana peel.[10] Squalene, 4,4-dimethyl-, 4α-methyl-, and 4-demethyl-sterols were isolated by means of preparative t.l.c. The squalene was further purified by preparative t.l.c., combined with carrier (50 mg), and the hexahydrochloride then prepared. The cycloeucalenol and 4-demethyl-sterol fractions were acetylated and crystallized from methanol–ether. The 4,4-dimethyl-sterol fraction, which contained both cycloartenol and 24-methylenecycloartanol, was acetylated and the two triterpene acetates separated by preparative t.l.c. using $AgNO_3$-impregnated silica gel G plates. The purified acetates were then crystallized from methanol–ether. Calculations are based on a $^{3}H:^{14}C$ atomic ratio of 6 : 6 for squalene labelled from this substrate. There should be no loss of either label upon subsequent biological conversion of squalene into cycloartenol.[9] As indicated in the Table, the $^{3}H:^{14}C$ atomic ratio of cycloartenyl acetate is the same as that in squalene. The tritium atoms are present at the

Incorporation of [2-^{14}C]-(4*R*)-[4-$^{3}H_1$]*mevalonic acid into free triterpenes and sterols of banana peel*

	$^{3}H:^{14}C$	$^{3}H:^{14}C$ Atomic[a] ratio	Theor.
Squalene hexahydrochloride	4·10	—	6 : 6
Cycloartenyl acetate	4·02	5·88 : 6	6 : 6
24-Methylene cycloartanyl acetate	4·20	6·15 : 6	6 : 6
Cycloeucalenyl acetate ..	4·12	5·03 : 5	5 : 5
Cycloeucalenone[b]	4·11	5·02 : 5	5 : 5
4-Demethyl-steryl acetates[c] ..	2·84	3·11 : 5	3 : 5

[a] Based on a $^{3}H:^{14}C$ atomic ratio of 6 : 6 for squalene.
[b] Purified by preparative t.l.c.
[c] Consisting of stigmasteryl, campesteryl, and β-sitosteryl acetates, 92 : 2 : 6.

† The free triterpene alcohols of banana peel consist of cycloartenol, 24-methylenecycloartanol, and cycloeucalenol in the ratio 13 : 34 : 53 (F. F. Knapp and H. J. Nicholas, *Phytochemistry*, 1969, **8**, 2091). The sterols consist of stigmasterol, campesterol, and β-sitosterol, 92 : 2 : 6.

3α, 5α, 8β, 17α, 20, and 24 positions while the ^{14}C is present at positions 1, 7, 15, 22, 26 or 27, and 29 or 30.[3,9] The atomic ratio of the 24-methylenecycloartanyl acetate is also 6 : 6, indicating retention of the C-24 tritium atom of cycloartenol during the formation of the 24-methylene side-chain. It is probably retained by migration to C-25.[8,9,11] The cycloeucalenyl acetate had the same $^{3}H:^{14}C$ radioactivity ratio as squalene. This material was saponified and the resulting alcohol oxidized to cycloeucalenone. The $^{3}H:^{14}C$ ratio was unchanged, demonstrating the absence of tritium at C-3. These results show for the first time that the 3α-hydrogen of 24-methylenecycloartanol is lost upon conversion into cycloeucalenol. A ketonic intermediate must therefore have been formed at some stage of the demethylation process. Since the 3α-tritium is lost when cycloeucalenol is formed and the $^{3}H:^{14}C$ ratio of this triterpene is the same as in squalene, an atomic equivalent of ^{14}C was also removed during this transformation and the $^{3}H:^{14}C$ atomic ratio must therefore be 5 : 5. Since the carbon atom lost in this process was from C-4, radioactivity must have been associated with this methyl group.

If the 4β-methyl group of 24-methylenecycloartanol were labelled exclusively from [2-^{14}C]mevalonic acid and was specifically removed during the formation of cycloeucalenol, a ratio of 5 : 5 would be expected. Conversely, if the 4α-methyl group were labelled and the 4β-methyl group lost, the ratio would be 5 : 6. This case can be excluded since the $^{3}H:^{14}C$ ratio indicates equivalent amounts of each label to be present. Randomization of ^{14}C between the two methyl groups, a highly unlikely process, would result in a ratio of 5·5 : 6. Lastly, if the 4α-methyl group were labelled exclusively with ^{14}C and were lost during demethylation, a ratio of 5 : 5 would also be expected. Such a transformation would imply net inversion of the original 4β-methyl group of 24-methylenecycloartanol during this conversion.

Degradation of several cyclic terpenes labelled from [2-^{14}C]mevalonic acid have indicated that the 4α-methyl group is specifically labelled from this substrate. These include rosenonolactone,[12] gibberellic acid,[12] soyasapogenol A,[12] lanosterol,[13] lupeol,[14] and more recently, cycloartanol.[3] It thus seems reasonable to assume that the 4α-methyl group of 24-methylenecycloartanol is labelled from [2-^{14}C]-mevalonic acid and that this methyl group and the 3α-hydrogen are lost during the formation of cycloeucalenol.

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[1] J. L. Gaylor and C. W. Delwiche, *Steroids*, 1964, **4**, 207.
[2] K. B. Sharpless, T. E. Snyder, T. A. Spencer, K. K. Maheshwari, G. Guhn, and R. B. Clayton, *J. Amer. Chem. Soc.*, 1968, **90**, 6874.
[3] E. L. Ghisalberti, N. J. DeSouza, H. H. Rees, L. J. Goad, and T. W. Goodwin, *Chem. Comm.*, 1969, 1403.
[4] M. Lindberg, F. Gautschi, and K. Bloch, *J. Biol. Chem.*, 1963, **238**, 1661.
[5] A. C. Swindell and J. L. Gaylor, *J. Biol. Chem.*, 1968, **243**, 5546.
[6] L. J. Goad and T. W. Goodwin, *Biochem. J.*, 1965, **99**, 79P.
[7] H. H. Rees, E. I. Mercer, and T. W. Goodwin, *Biochem. J.*, 1966, **99**, 726.
[8] L. J. Goad and T. W. Goodwin, *European J. Biochem.*, 1969, **7**, 502.
[9] H. H. Rees, L. J. Goad, and T. W. Goodwin, *Biochem. J.*, 1968, **107**, 417.
[10] F. F. Knapp and H. J. Nicholas, *Phytochemistry*, 1969, **8**, 207.
[11] M. Castle, G. Blondin, and W. R. Nes, *J. Amer. Chem. Soc.*, 1963, **85**, 3306; M. Akhtar, P. F. Hunt, and M. A. Parvez, *Biochem. J.*, 1967, **103**, 616; K. H. Raab, N. J. De Souza, and W. R. Nes, *Biochim. Biophys. Acta*, 1968, **152**, 742.
[12] D. Arigoni, in "CIBA Foundation Symposium: Biosynthesis of Terpenes and Sterols," eds. G. E. Wolstenholme and C. M. O'Conner, Little, Brown and Co., Boston, Mass., 1956, p. 231.
[13] G. P. Moss and S. A. Nicolaidis, *Chem. Comm.*, 1969, 1072.
[14] L. Botta, Ph.D. Thesis, Eidgenössische Technische Hochschule, Zürich, 1968, Diss. No. 4098.