Biosynthesis of 24β-Ethylsterols in Cultured Cells of *Trichosanthes kirilowii* (Cucurbitaceae) Fed with [1,2-$^{13}C_2$]- and [2-$^{13}C^2H_3$]-Acetate: Reinvestigation of the Stereochemistry at C-25

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[^{13}C]- and [$^{13}C^2$H]-Enriched 24β-ethylsterols, 24β-ethyl-25-dehydrolophenol (**14**) and (**18**), 22-dihydro-25-dehydrochondrillasterol (**15**) and (**19**), 22-dihydrochondrillasterol (**16**) and (**20**), and chondrillasterol (**17**) and (**21**) have been isolated from cultured cells of *Trichosanthes kirilowii* Maxim. var. *japonica* fed with [1,2-$^{13}C_2$]- and [2-$^{13}C^2H_3$]-acetate. The most probable biosynthetic mechanism of the 24β-ethyl side-chain (**27**) from the 24-methylene side-chain (**24**) is that (i) the second methyl group attacks the C-28 of 24-methylenecycloartanol (**24**), (ii) 25-H migrates back to the 24-*Re*-face, (iii) 26-H is eliminated to form the 25(26) double bond (**26**), and (iv) the protonation at C-25 of the Δ^{25}-sterols takes place from the *Si*-face to form the 24β-ethyl-25-saturated side-chain (**27**). The labelling patterns also indicate that reduction of the 22(23) double bond is not a primary pathway in the formation of 22-saturated sterols and that 24β-ethylsterols are formed *via* cycloartenol (**1A**).

Plants and micro-organisms produce sterols with a methyl or an ethyl group at C-24 with α or β orientation.* The alkyl groups are introduced by methyl transformatiom from *S*-adenosylmethionine. Both of the 24-diastereoisomers have been found and the configuration at C-24 and the side-chain formation mechanism seem to have phylogenetic significance. In general, most vascular plants produce 24α-ethylsterols, one example being sitosterol (**12B**). The mechanism for side-chain formation of 24α-ethylsterols was proposed to involve a mechanism in which the 24-H of cycloartenol (**1A**)[1] is removed *via* a $\Delta^{24(25)}$-intermediate (**11**).[2] Recently, we confirmed the elimination of the 24-H in the biosynthesis of sitosterol (**12B**) by ^{13}C n.m.r. spectroscopy in cultured cells of *Rabdosia japonica* fed with [2-$^{13}C^2H_3$]acetate.[3] Algae generally produce 24β-ethylsterols; poriferasterol is one example. The biosynthetic mechanism of the side-chain of the 24β-ethylsterol can differ for various classes of algae and vascular plants. In the chrysophyte *Ochromonas malhamensis*[4] and in the alga *Monodus subterraneous*,[5] the side-chain formation of clionasterol (**9B**) and poriferasterol (**10B**) involves and 1,2-hydride migration from C-24 to C-25 and the hydrogen is retained there as shown in (**9**) and (**10**). The 1,2-hydride migration was reported to take place on the *Si*-face of the 24(25) double bond,[4] but it should be *Re*-face as we reported.[6] In contrast, in the alga *Treboxia* sp.,[7] a reverse 1,2-hydride migration (from C-24 to C-25 and then from C-25 back to C-24) has been proposed in the biosynthesis of the same sterols, clionasterol (**6B**) and poriferasterol (**7B**). This has been also found to occur in the formation of Δ^{25}-poriferasterol in the vascular plant *Clerodendrum campbelli* (Verbenaceae),[8] although it seemed to be an exceptional example.

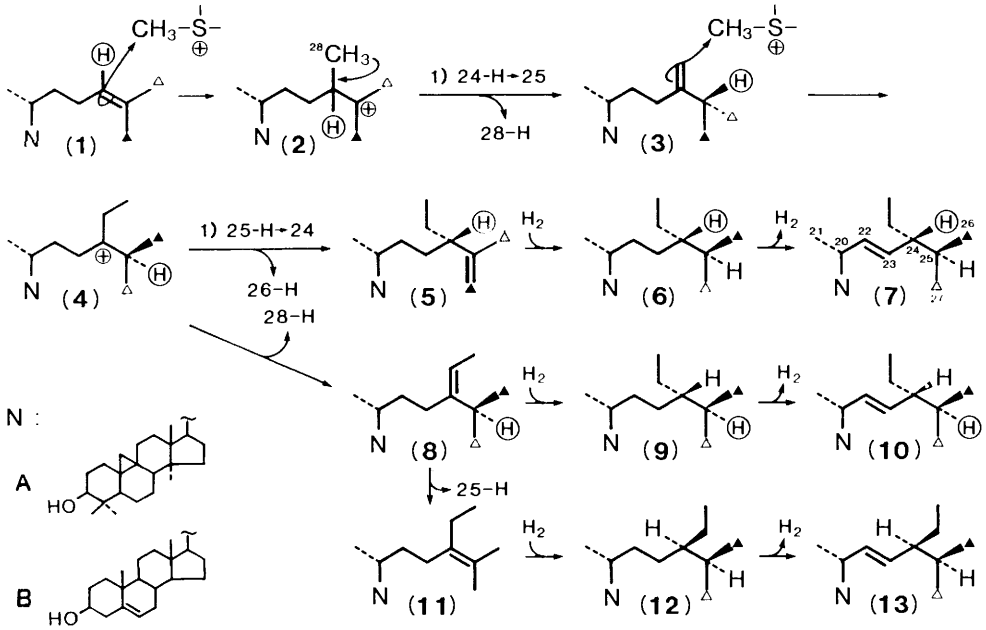
* Nomenclature: 24β-ethyl-25-dehydrolophenol = (24*S*)-24-ethyl-4α-methyl-5α-cholesta-7,25-dien-3β-ol; 22-dihydro-25-dehydrochondrillasterol = (24*S*)-24-ethyl-5α-cholesta-7,25-dien-3β-ol; 22-dihydrochondrillasterol = (24*S*)-24-ethyl-5α-cholest-7-en-3β-ol; chondrillasterol = (22*E*,24*R*)-24-ethyl-5α-cholesta-7,22-dien-3β-ol.

Some plants such as *Cucurbita pepo* and *Trichosanthes kirilowii* belonging to the family Cucurbitaceae were recently found to have characteristic sterol profiles. The seeds of these plants were reported to contain principally 24β-ethylsterols.[9] However, tissues from the mature plants mainly synthesize 24α-ethylsterols.[10] These findings suggest that the sterol biosynthesis in these plants are an intermediate type between algae and tracheophytes and may show the evolutionary recapitulation during development. We found that the cultured cells of *T. kirilowii* Maxim. var. *japonica* produce the 24β-ethylsterols, 24β-ethyl-25-dehydrolophenol, 22-dihydro-25-dehydrochondrillasterol, 22-dihydrochondrillasterol, and chondrillasterol together with 24-methylenecycloartanol. The biosynthesis of 24-methylenecycloartanol (**3A**) involves a stereospecific 1,2-hydride migration from C-24 to the 25-*Re*-face of the 24(25) double bond in cycloartenol (**1A**), as we reported.[3]

Since we determined the ^{13}C n.m.r. signals of the C-26 (the *pro-R*) and the C-27 (the *pro-S*) methyl groups at C-25 of 24β-ethylsterols together with 24α-isomers,[6] here, we report on the biosynthetic mechanism of 24β-ethylsterols. The mechanism involves the second transmethylation at C-28 of (**3A**) and then a stereospecific 1,2-hydride migration from C-25 back to C-24 followed by a hydrogen elimination from one of the two methyl groups at C-25 to form the 24β-ethyl-Δ^{25}-side-chain (**5**) and then a stereospecific protonation on the 25-*Si*-face of the 25(26) double bond. To the best of our knowledge, this is the first study of the stereochemistry of the reduction of the 25(26) double bond in sterol biosynthesis.

Results

The callus of *T. kirilowii* Maxim. var. *japonica* was grown in Linsmeier-Skoog liquid medium fortified with 2,4-dichlorophenoxyacetic acid (2,4-D, 10^{-6}M) and kinetin (0.02 p.p.m.). Sodium [1,2-$^{13}C_2$]acetate (91 and 91 atom% of ^{13}C) diluted with a twofold amount of unlabelled specimen and sodium [2-$^{13}C^2H_3$]acetate (90 and 98 atom% of ^{13}C and ^{2}H, respectively)



Scheme 1. Possible pathways for the biosynthesis of 24-ethylsterols. △, ▲: Carbons derived from C-2 and C-6 of MVA, respectively

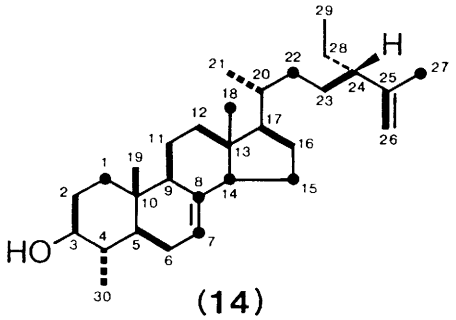
were added individually. After *ca.* 2 weeks incubation, [^{13}C]- and [^{13}C^{2}H]-enriched 24β-ethylsterols, 24β-ethyl-25-dehydrolophenol (**14**) and (**18**), 22-dihydro-25-dehydrochondrillasterol (**15**) and (**19**), 22-dihydrochondrillasterol (**16**) and (**20**), and chondrillasterol (**17**) and (**21**) were isolated by successive chromatography of methanol extracts of the cells. We assigned the configuration at C-24 by comparison with the ^{13}C n.m.r. chemical shifts of the corresponding side-chain carbons and those of their 24-epimers.[6] Only ^{13}C data of the 24β-isomer of the Δ^{25}-side-chain have been reported.[11] In order to determine the C-24 configuration of the 25-unsaturated sterols, (**15**) was hydrogenated over Adams' catalyst. The product, analysed by ^{13}C n.m.r. spectroscopy, consisted of 22-dihydrochondrillasterol (>97%) and a trace amount of the 24α-isomer (<3%). The ^{13}C chemical shifts of the side-chain of (**14**) were identical with those of (**15**).

As shown in Table 1, the [^{13}C]-labelling patterns from [1,2-$^{13}C_2$]acetate were determined by ^{13}C-{^{1}H} n.m.r. spectroscopy. The carbons C-26 and C-27 showed different multiplicity in enriched signals due to their biosynthetic origin. The enriched singlet and doublet methyl group arise from C-2 and C-6 of mevalonate (MVA), respectively. The Δ^{25}-sterols have the enriched singlet methyl group, C-27, [δ_C 17.80 for (**14**) and δ_C 17.78 for (**15**)] and the enriched doublet olefinic carbon, C-26, [δ_C 111.39, *J* 71 Hz for (**14**) and δ_C 111.39, *J* 72 Hz for (**15**)]. In the case of the 25-saturated sterols, the labelling patterns of the methyl groups were the singlet C-27 [δ_C 19.61 for (**16**) and δ_C 18.95 for (**17**)] and the

Table 1. ^{13}C N.m.r. data[a] of 24β-ethyl-25-dehydrolophenol (**14**), 22-dihydro-25-dehydrochondrillasterol (**15**), 22-dihydrochondrillasterol (**16**),[b] chondrillasterol (**17**),[c] and 24-methylenecycloartanol (**3A**)[3] biosynthesized from [1,2-$^{13}C_2$]acetate in tissue cultures of *T. kirilowii* Maxim. var. *japonica*

Carbon	(14)[d] δ_C	*J*/Hz	(15) δ_C	*J*/Hz	(16)[e] δ_C	*J*/Hz	(17)[f] δ_C	*J*/Hz	(3A)[3] δ_C	*J*/Hz
1	37.02	s	37.15	s	37.16	s	37.17	s	31.98	s
2	31.00	37	31.47	36	31.47	36	31.48	36	30.41	37
3	76.25	37	71.05	36	71.05	36	71.06	36	78.85	37
4	40.28	36	37.98	s	37.97	s	38.00	s	40.50	36
5	46.68	35	40.26	34	40.27	34	40.28	34	47.13	35
6	26.66	35	29.66	34	29.67	34	29.66	34	21.13	35
7	117.46	s	117.43	s	117.43	s	117.47	s	26.02	s
8	139.17	s	139.60	s	139.59	s	139.56	s	48.00	s
9	49.67	34	49.46	34	49.47	34	49.47	34	20.02	43
10	34.86	36	34.21	37	34.21	36	34.23	36	26.10	12
11	21.39	34	21.56	34	21.57	34	21.56	34	26.50	43
12	39.57	36	39.58	36	39.58	36	39.49	36	32.92	35
13	43.38	36	43.39	36	43.38	36	43.29	36	45.32	35
14	54.97	s	55.04	s	55.05	s	55.14	s	48.83	s
15	22.93	s	22.96	s	22.99	s	23.02	s	35.58	s
16	27.92	33	27.89	33	27.97	33	28.41	34	28.17	33
17	56.09	33	56.09	33	56.08	33	55.90	34	52.29	33
18	11.84	s	11.84	s	11.85	s	12.07	s	18.04	s
19	14.16	36	13.03	37	13.04	36	13.05	36	29.90	12
20	36.00	35	35.99	35	36.73	35	40.78	34	36.13	35
21	18.79	35	18.77	35	18.98	35	21.35[f]	34	18.32	35
22	33.65	s	33.63	s	33.88	s	138.12	s	35.02	s
23	29.51	34	29.50	34	26.52	35	129.50	44	31.33	41
24	49.55	34	49.53	34	46.07	35	51.24	44	156.92	41
25	147.57	71	147.53	72	28.97	35	31.84	36[g]	33.82	35
26	111.39	71	111.39	72	18.98[h]	35[h]	20.93	36	22.01	35
27	17.80	s	17.78	s	19.61[h]	s[h]	18.95	s	21.88	s
28	26.54		26.53		23.01		25.40		105.94	
29	12.07		12.07		12.33		12.45			
30	15.16	36							25.45	s
31									14.01	36
32									19.33	s

[a] Chemical shifts (δ_C in p.p.m.), s indicating enriched singlet and figures in *J*/Hz indicating the ^{13}C–^{13}C coupling constants of the enriched doublet. [b] This compound contained *ca.* 20% of the 24α-epimer. [c] This compound contained *ca.* 10% of the 24α-epimer. [d] The signal assignments are based on the data in ref. 11. [e] The signal assignments are based on the data in ref. 6. [f] The signal assignments of carbons other than C-21, C-26, and C-27 are based on ref. 20. The closed signals due to C-11 and C-21 were assigned by INEPT method. [g] One of the doublet peaks overlapped with other signals. [h] The assignment and the labelling patterns in ref. 17 are revised based on ref. 6.



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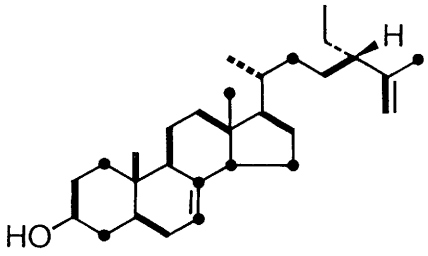

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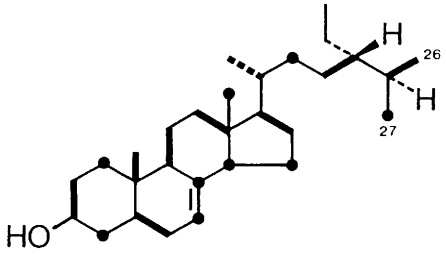

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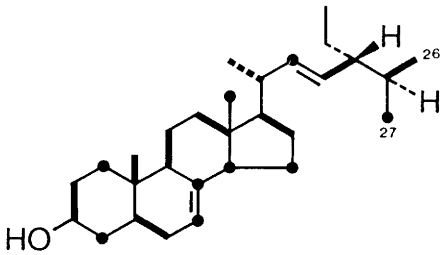

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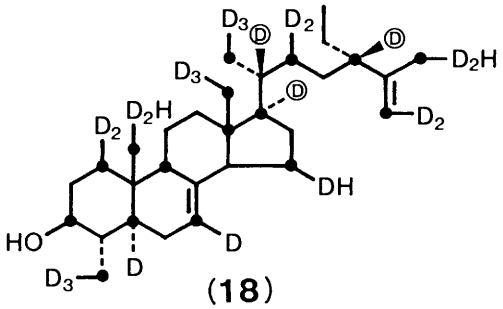

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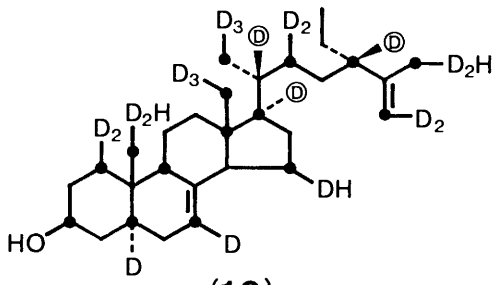

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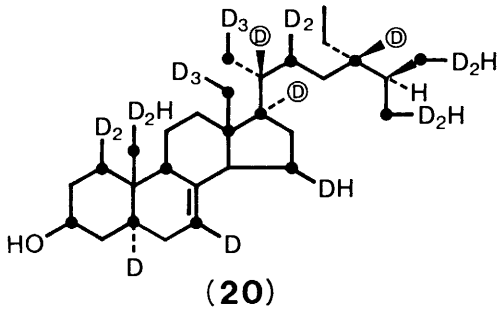

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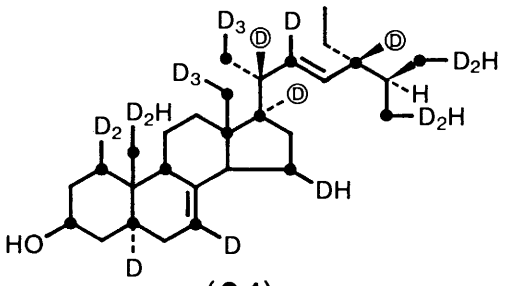


(21)

doublet C-26 [δ_C 18.98, J 35 Hz for (**16**) and δ_C 20.93, J 36 Hz for (**17**)].

The [$^{13}C^2H$]-labelling patterns were determined by ^{13}C-{^1H}{^2H} n.m.r. spectroscopy and are shown in Table 2. The C-27 of (**18**) and (**19**), arising from C-2 of MVA, was labelled as [$^{13}C^2H_2$] ($^1\Delta\delta_{C(^2H_2)}$ −0.56 and −0.54). The methyl group arising from C-6 of MVA should be labelled as [$^{13}C^2H_3$], but the C-26 of (**21**) were clearly observed to have [$^{13}C^2H_2$]-labelling ($^1\Delta\delta_{C(^2H_2)}$ −0.62), although that in (**20**) showed overlap with other signals. This indicated that one of the three expected deuterium atoms at C-26 in 24-methylenecycloartanol (**3A**) has been replaced by a hydrogen atom.

The deuterium atom directly attached at C-24 was clearly observed at C-24, δ_C 49.55 in (**18**), δ_C 49.53 in (**19**), δ_C 46.07 in (**20**), and δ_C 51.24 in (**21**) with $^1\Delta\delta_{C(^2H)}$ (−0.52, −0.53, −0.61, and −0.57, respectively).

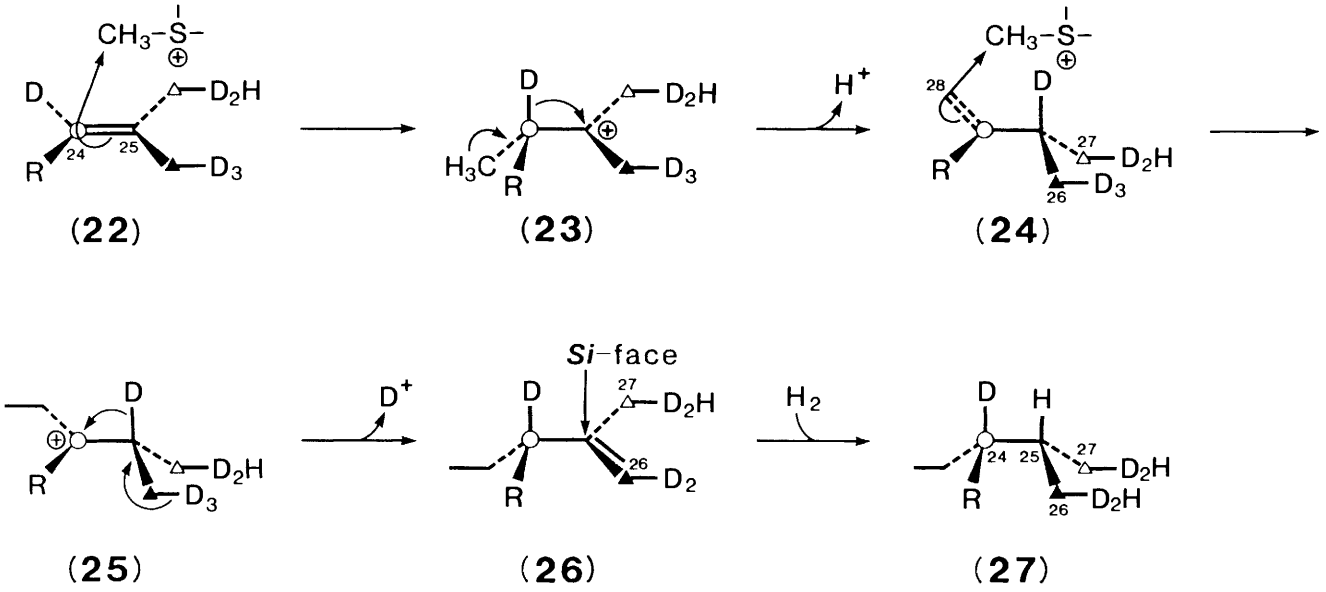
Discussion

The deuterium atom originally present at C-24 in cycloartenol (**22**)[1] was demonstrated to migrate to C-25 on the *Re*-face of the double bond after the first transmethylation followed by a hydrogen elimination from C-28 to form 24-methylenecycloartanol (**24**).[3] The observation of a deuterium atom at C-24 in (**18**) and (**19**) confirms the proposed mechanism in which the deuterium atom at C-25 in (**24**) migrates back to C-24 [(**24**)→(**25**)→(**26**)] followed by elimination of a deuterium atom from a methyl group to form the 24β-ethyl-Δ^{25}-sterols (**26**). The

Table 2. [$^{13}C^{2}H$]-Labelling patterns[a] of 24β-ethyl-25-dehydrolophenol (**18**), 22-dihydro-25-dehydrochondrillasterol (**19**), 22-dihydrochondrillasterol (**20**),[b] chondrillasterol (**21**), and 24-methylenecycloartanol (**24**)[4] biosynthesized from [2-$^{13}C^{2}H_3$]acetate in tissue cultures of *T. kirilowii* Maxim. var. *japonica*

Carbon	(18) $^{1}\Delta\delta_C$			(19) $^{1}\Delta\delta_C$			(20) $^{1}\Delta\delta_C$			(21) $^{1}\Delta\delta_C$			(24)[3] $^{1}\Delta\delta_C$		
	$^{2}H_1$	$^{2}H_2$	$^{2}H_3$	$^{2}H_1$	$^{2}H_2$	$^{2}H_3$	$^{2}H_1$	$^{2}H_2$	$^{2}H_3$	$^{2}H_1$	$^{2}H_2$	$^{2}H_3$	$^{2}H_1$	$^{2}H_2$	$^{2}H_3$
1	−0.44	−0.85		−0.42	−0.80		*c*	−0.80		−0.40	*c*		−0.33	−0.77	
													−0.44		
3													−0.54		
5	−0.63			−0.54			−0.55			−0.53			−0.64		
7	−0.32			−0.34			−0.35			−0.32			−0.41	*c*	
9													(−0.09)[d]		
13	(−0.08)[d]			(−0.09)[d]			(−0.08)[d]			(−0.10)[d]			(−0.09)[d]		
15	−0.36			−0.35			−0.35			−0.35			−0.39	−0.77	
17	(−0.11)[d]			(−0.11)[d]			(−0.11)[d]			(−0.10)[d]			(−0.11)[d]		
18	−0.29	−0.57	−0.86	−0.28	−0.57	−0.83	−0.31	−0.57	−0.88	*c*	−0.58	−0.85	−0.31	−0.60	−0.85
19	−0.29	−0.58		−0.26	−0.57		−0.31	−0.60		−0.28	*c*		−0.44	−0.86	
21	−0.32	−0.60	−0.92	−0.31	−0.61	*c*	−0.32	−0.61	−0.92	−0.30	−0.50	−0.91	*c*	*c*	*c*
22	−0.42	−0.85		−0.41	−0.82		−0.44	−0.88		−0.34			−0.42	−0.80	
24	−0.52			−0.53			−0.61			−0.57			(−0.03)[d]		
26	−0.30	−0.57		−0.29	−0.56		−0.32	−0.61		−0.31	−0.62		−0.31	−0.60	*c*
27	−0.28	−0.56		−0.28	−0.54		−0.31	*c*		−0.30	−0.61		−0.31	−0.61	
30	−0.30	−0.59	−0.88										−0.31	−0.61	
31													−0.29	−0.57	−0.88
32													−0.29	−0.58	−0.93

[a] The signals due to C-2, C-4, C-6, C-8, C-10, C-11, C-12, C-14, C-16, C-20, C-23, and C-25 were not labelled from [2-$^{13}C^{2}H_3$]acetate in any compounds consistent with them originating from C-1 of the acetate. C-28 and C-29 Were also not labelled. [b] This compound contained about 20% of the 24α-epimer. [c] These signals overlapped with other signals. [d] These signals are β-deuterium isotopically shifted signals ($^{2}\Delta\delta_{C(^{2}H)}$), which were observed as singlets in ^{13}C-{^{1}H} n.m.r. spectra.



Scheme 2. △, ▲, and ○: Carbons derived from C-2, C-6, and C-4 of MVA, respectively

deuterium migration (25-^{2}H→C-24) must take place stereospecifically to the *Re*-face of the double bond of (**24**), because the ^{13}C signals due to the 24α-epimers of (**18**) and (**19**) could not be observed. The deuterium elimination occurs regiospecifically from the *pro-R* methyl group which originates from C-6 of MVA. The regiospecificity is the same as that of clerosterol biosynthesis in *Clerodendrum*.[8] The 24α-epimer of (**18**) has been isolated from the fern *Polypodium niponicum*.[12] In this case, the 1,2-hydride migration from C-25 to C-24 might occur with opposite stereospecificity. It is noteworthy that the deuterium atom retained at C-24 is characteristic of the 24β-ethylsterols in higher plants and also of cyclolaudenol in *Polypodium*.[13] In other cases, the deuterium atom at C-24 in (**22**) migrates to C-25 as seen in isofucosterol in *Pinus pinea*,[14] poriferasterol (**10B**) in *O. malhamensis*,[4] ergosterol in yeast,[3,15] and 24-methylencycloartanol (**3A**) in higher plants[3,16] but it is lost in the 24α-ethylsterols (**12B**) and (**13B**) in vascular plants.[2,3]

The signals due to [$^{13}C^{2}H_2$]-labelling of both methyl groups C-26 and C-27 of (**21**) indicate that the 24β-ethylsterols with a 25-saturated side-chain are formed by reduction of the 25(26) double bond of (**19**). From the stereochemical viewpoint, a hydrogen can attack the double bond in two ways, from the *Re*-face or from the *Si*-face. The former should give the carbon originating from C-6 of MVA to become the *pro-S* methyl group

Table 3. ^{1}H N.m.r. data[a] of 24β-ethyl-25-dehydrolophenol (**14**), 22-dihydro-25-dehydrochondrillasterol (**15**), 22-dihydrochondrillasterol (**16**), and chondrillasterol (**17**)

Proton	(**14**)	(**15**)	(**16**)	(**17**)
18-H	0.526 (3 H) (s)	0.527 (3 H) (s)	0.536 (3 H) (s)	0.550 (3 H) (s)
19-H	0.824 (3 H) (s)	0.793 (3 H) (s)	0.796 (3 H) (s)	0.798 (3 H) (s)
21-H	0.909 (3 H) (d, *J* 6.5 Hz)	0.908 (3 H) (d, *J* 6.2 Hz)	0.931 (3 H) (d, *J* 6.1 Hz)	1.028 (3 H) (d, *J* 6.5 Hz)
26-H	4.645 (1 H) (br d, *J* 2.8 Hz) 4.731 (1 H) (dq, *J* 2.8, 1.4 Hz)	4.645 (1 H) (br d, *J* 2.8 Hz) 4.730 (1 H) (dq, *J* 2.8, 1.4 Hz)	0.813 (3 H) (d, *J* 6.7 Hz)	0.845 (3 H) (d, *J* 6.4 Hz)
27-H	1.567 (3 H) (dd, *J* 1.4, 0.8 Hz)	1.567 (3 H) (dd, *J* 1.4, 0.8 Hz)	0.833 (3 H) (d, *J* 6.9 Hz)	0.792 (3 H) (d, *J* 6.6 Hz)
29-H	0.802 (3 H) (t, *J* 7.5 Hz)	0.801 (3 H) (t, *J* 7.2 Hz)	0.854 (3 H) (t, *J* 7.4 Hz)	0.815 (3 H) (t, *J* 7.5 Hz)
3-H	3.129 (1 H) (m)	3.594 (1 H) (m)	3.604 (1 H) (m)	3.600 (1 H) (m)
7-H	5.180 (1 H) (dd, *J* 6, 1.5 Hz)	5.160 (1 H) (br s, $W_{\frac{1}{2}}$ 9 Hz)	5.169 (1 H) (br s, $W_{\frac{1}{2}}$ 8 Hz)	5.161 (1 H) (br s)
30- or 22-,23-H	0.986 (3 H) (d, *J* 6.5 Hz) (30-H)			5.040 (1 H) (dd, *J* 15, 8 Hz) (23-H) 5.160 (1 H) (dd, *J* 15, 8 Hz) (22-H)

[a] ^{1}H Chemical shifts (δ_H), signal intensity, signal multiplicities, and ^{1}H–^{1}H coupling constants in Hz. Abbreviations indicate dd, a doublet of doublets; dq, a doublet of quartet; m, multiplet; $W_{\frac{1}{2}}$, line width at half height.

(C-27) and the latter the *pro-R* methyl group (C-26). The enriched singlet C-27 and the enriched doublet C-26 of (**16**) and (**17**) suggest that the hydrogen atom attacks the *Si*-face of the 25(26) double bond as shown in Scheme 2 [(**26**)→(**27**)]. The labelling patterns at C-26 and C-27 of (**16**) and (**17**) and the stereochemistry of the double bond reduction in the preliminary report[17] should be revised because the ^{13}C n.m.r. signal assignments of these carbons were reversed.[6]

In the spectra of (**16**) and (**20**), small peaks (1/5 in height) due to the 24α-epimers were observed, but no signal corresponding to a deuterium atom at C-24 or C-25 was observed. These findings indicate that the 24α-ethylsterols simultaneously synthesized in this callus are formed by an alternative mechanism such as that *via* an intermediate with the 24-ethyl-Δ^{24}-side-chain (**11B**) similar to sitosterol (**12B**) formation.

Two deuterium atoms retained at C-22 suggest that the 22-saturated side-chain is not predominantly synthesized from the Δ^{22}-sterol (**21**). The labelling patterns of sterol nucleus agree with the well established mechanism[3] including the 'back bone rearrangement'.[18] The singlet C-14 and C-18 showing a methyl migration from C-14 to C-13 and the β-deuterium isotopically shifted signals $^2\Delta\delta_{C(^2H)}$ observed at C-13 and C-17, as shown in parentheses in Table 2, indicate the occurrence of the 1,2-hydride migrations (13-H to C-17 and 17-H to C-20). The two deuterium atoms observed at C-19 ($^1\Delta\delta_{C(^2H_2)}$ −0.57 to −0.60) verify the intermediacy of cycloartenol for the 24β-ethylsterols. The 4α-methyl group (C-30) observed as a doublet (δ_C 15.16, *J* 36 Hz) in (**14**) and as [$^{13}C^2H_3$] ($^1\Delta\delta_{C(^2H_3)}$ −0.88) in (**18**) indicated that it originated from C-6 of MVA; no deuterium atom was observed at C-3 of (**18**). These findings agree with the accepted oxidative removal mechanism for C-30 of lanosterol in cholesterol biosynthesis.[19]

Experimental

^{13}C-{^{1}H} N.m.r. spectra were recorded on a Varian XL-200 instrument at 50.309 MHz in the ^{1}H decoupling mode in [^{2}H]chloroform using tetramethylsilane (TMS) as an internal standard (δ_C 0). Typical F.t. n.m.r. conditions were: spectral width (s.w.), 9 090.9 Hz; acquisition time (a.t.), 1.760 s; and pulse flip angle, 28° or 47°. ^{13}C-{^{1}H}{^{2}H} N.m.r. spectra were determined on a JEOL GX-400 instrument at 100.40 MHz in [^{2}H]chloroform (δ_C 77.000) under the following conditions: s.w., 24 038.5 Hz; a.t., 0.682 s; pulse delay, 3 s; pulse width, 4.7 μs; and number of transients, 2 749. ^{1}H N.m.r. spectra were recorded on a Varian XL-200 instrument at 200.057 MHz in [^{2}H]chloroform with TMS as an internal reference (δ_H 0) under the following conditions: s.w., 2 137 Hz; a.t., 5 s; and pulse flip angle, 37°. The accuracies of δ_C and δ_H are ±0.02 and ±0.005 p.p.m., respectively and J_{CC} and J_{HH} are ±1 and ±0.4 Hz, respectively. E.i. mass spectra were obtained on a Hitachi RMU-8GN spectrometer, and $[\alpha]_D$ was measured on a Hitachi Perkin-Elmer 141 instrument. M.p.s were determined on a Yanagimoto micro melting point apparatus and are uncorrected. H.p.l.c. was performed with a Waters 600 multisolvent delivery system or a Knauer HPLC pump 64 equipped with a UVILOG-5IIIA u.v. detector at 205 nm or 210 nm and a column (a) YMC-pack SH343 (S-15) ODS (25 cm × 20 mm i.d.) eluted with methanol (6.0 ml/min) or (b) TSK-gel ODS-120T (25 cm × 20 mm i.d.) eluted with methanol (4.0 ml/min) or (c) Develosil ODS-T-5 (25 cm × 10 mm i.d.) eluted with methanol (2.4 ml/min). Induction and culture conditions of *T. kirilowii* Maxim. var. *japonica* were given in our previous report.[3]

Feeding of Sodium[1,2-$^{13}C_2$]*Acetate.*—To precultured cells of *T. kirilowii* Maxim. var. *japonica* at 25 °C for 14 days in Linsmaier-Skoog liquid medium (10.2 l) supplemented with 2,4-D (10^{-6}M) and kinetin (0.02 p.p.m.) were added sodium [1,2-$^{13}C_2$]acetate (714 mg, 91 and 91 atom% of ^{13}C) and unlabelled sodium acetate (1.428 g) through a membrane filter (0.22 μm). After incubation for 17 days, cells were collected (5.4 kg fresh weight) and extracted with hot methanol (4 × 3 l) The butanol-soluble part (12 g) of the methanol extracts (56 g) was chromatographed on silica gel (150 g deactivated with 15 g of water) and eluted successively with hexane (600 ml), chloroform (1.2 l), acetone (300 ml), and then methanol (800 ml) to give a fraction containing sterols and triterpenes (366 mg); this was acetylated with acetic anhydride and pyridine and then chromatographed on silica gel (Lobar B) with hexane–chloroform–ethyl acetate (15:1:1) as eluant to give a mixture of acetates (355 mg). The acetate mixture was hydrolysed with 1% potassium hydroxide in methanol (10 ml)

to give a mixture of sterols and triterpenes (300 mg), which was chromatographed on silica gel (Lobar B) with hexane–chloroform–ethyl acetate (4:1:1) as eluant. This afforded crude 24-methylenecycloartanol (33 mg), 24β-ethyl-25-dehydrolophenol (11.6 mg), and a mixture of Δ^7-sterols (250 mg). 24β-Ethyl-25-dehydrolophenol (**14**) (4 mg) purified by h.p.l.c. (a) had m.p. 162—164 °C (from MeOH); *m/z* 426 (M^+). The acetate of (**14**) had m.p. 170—172 °C (from AcOEt) (lit.,[11] 170—172 °C). ^{13}C N.m.r. chemical shifts of the acetate agreed well with the reported data.[11] Repeated h.p.l.c. (*a*) of the Δ^7-sterol mixture gave 22-dihydro-25-dehydrochondrillasterol (**15**) (26.4 mg), m.p. 147—150 °C (from MeOH); $[\alpha]_D^{24}$ −1.0°, $[\alpha]_{365}^{24}$ −31.3° (*c* 1.0 in $CHCl_3$); *m/z* 412 (M^+), a mixture of chondrillasterol (**17**) and a small amount of its 24α-epimer (10%) (28 mg), m.p. 165.5—166.5 °C (from MeOH), and a mixture of 22-dihydrochondrillasterol (**16**) and a small amount of its 24α-epimer (20%) (54 mg), m.p. 147—148 °C (from MeOH). The ^1H n.m.r. data are shown in Table 3.

Catalytic Reduction of 22-Dihydro-25-dehydrochondrillasterol (**15**).—The Δ^{25}-sterol (**15**) (11 mg) in ethanol–benzene (1:1, 2.4 ml) was stirred at room temperature for 1 h with Adams' catalyst under hydrogen. Filtration and solvent evaporation gave a product which was purified by h.p.l.c. (*c*), yielded 22-dihydrochondrillasterol (8 mg), m.p. 147—149 °C (from AcOEt); $[\alpha]_D^{23}$ +4.9° (*c* 0.66 in $CHCl_3$); *m/z* 414 (M^+). The ^{13}C n.m.r. spectrum of this compound was identical with that of (**16**) except for the enriched signals due to C-26 and C-27 and very small signals (< 3% in height) corresponding to the 24α-isomer.

Feeding of Sodium [2-$^{13}C^2H_3$]*Acetate*.—To cells precultured for 16 days under the same conditions (7.8 l), sodium [2-$^{13}C^2H_3$]acetate (1.01 g, 90 and 98 atom% of ^{13}C and ^2H, respectively) was added through a membrane filter (0.22 μm). After incubation for 12 days the cells (3.7 kg fresh weight) were collected and sterols were isolated in the same way as described above. 24β-Ethyl-25-dehydrolophenol (**18**) (16.8 mg) had m.p. 164.5—165 °C (from MeOH); $[\alpha]_D^{25}$ −3.8° (*c* 0.8 in $CHCl_3$). 22-Dihydro-25-dehydrochondrillasterol (**19**) (28 mg) showed *m/z* 412 (M^+). Chondrillasterol (**20**) (13 mg) purified by h.p.l.c. (c) had m.p. 170—172.5 °C, $[\alpha]_D^{23}$ −2.9° (*c* 0.76 in $CHCl_3$); *m/z* 412 (M^+). A mixture of 22-dihydrochondrillasterol (**21**) and a small amount of its 24α-epimer (20%) (73 mg) had m.p. 147—148 °C; $[\alpha]_D^{24}$ +6.5 (*c* 0.99 in $CHCl_3$); *m/z* 414 (M^+).

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Received 21*st April* 1988; *Paper* 8/01578C