Biosynthesis of Sitosterol in Tissue Cultures of *Rabdosia japonica* Hara and Ergosterol in Yeast from [2-^{13}C,2-$^{2}H_3$]Acetate†

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The fate of the hydrogen atoms originating from [2-^{13}C,2-$^{2}H_3$]acetate was investigated in the biosynthesis of sitosterol (**5**) in cultured cells of *Rabdosia japonica* Hara and ergosterol (**6**) in yeast and the 1,2-hydride shifts, 20-H from C-17 and 17-H from C-13, were verified.

The biosynthesis of cholesterol from acetic acid was suggested more than 40 years ago.[1] Since the biogenetic isoprene rule was proposed,[2,3] there have been many reports concerning the fate of the hydrogen atoms 2-H, 4-H, and 5-H, of mevalonic acid (MVA).[4—6] Here we report on the fate of the hydrogen atoms originating from acetic acid in the biosynthesis of sitosterol (**5**) in tissue cultures of *Rabdosia japonica* Hara and ergosterol (**6**) in yeast (*Saccharomyces cereviceae* IFO 1346)-fed [2-^{13}C,2-$^{2}H_3$]acetate.

Sodium [2-^{13}C,2-$^{2}H_3$]acetate‡ was administered for four weeks to suspension cultures of *R. japonica*. Sitosterol (**5**) was isolated from the cells in the usual way[7] together with triterpenes such as ursolic acid and oleanolic acid.[8] Ergosterol (**6**) was isolated from yeast-fed [2-^{13}C,2-$^{2}H_3$]acetate.§ We have already reported the ^{13}C n.m.r. signal assignments of (**5**)[9] and (**6**)[10] based on the labelling patterns from [1,2-$^{13}C_2$]acetate. Using these assignments, we investigated the ^{13}C and ^{2}H labelling patterns in the 100 MHz ^{13}C-{^{1}H}{^{2}H} n.m.r. spectra. A carbon atom which has a deuterium atom migrating to the adjacent carbon atom appears with an accompanying signal which is shifted by the β-deuterium isotope effect[11] ($^{2}\Delta\delta_{C(^{2}H)}$ shown in parentheses in Table 1). The number of deuterium atoms attached directly to the ^{13}C-labelled carbon is indicated by the signals shifted by the α-deuterium isotope effect ($^{1}\Delta\delta_{C(^{2}H)}$ −0.27 to −0.32 p.p.m. for primary and −0.34 to −0.42 p.p.m. for secondary carbon atoms).

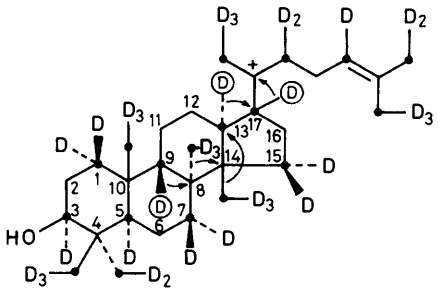
As shown in Table 1, the signals due to C-13 [δ_C 42.28 for (**5**) and 42.74 for (**6**)] and C-17 [δ_C 56.12 for (**5**) and 55.67 for (**6**)] have β-deuterium isotopically shifted signals (−0.09 to −0.11 p.p.m.). Thus, it follows that 17-H and 20-H shift from C-13 and C-17, respectively, in (**1**) as postulated in the biogenetic isoprene rule[2,3] to form a cationic intermediate (**2**). To complete the backbone rearrangement, a cyclopropyl ring is formed with the loss of a hydrogen from C-19 to give cycloartenol (**3**) in higher plants [(**1**)→(**2**)→(**3**)→(**5**)].[4,5] In yeast a 8(9) double bond is formed to afford lanosterol (**4**) [(**1**)→(**2**)→(**4**)→(**6**)]. Unfortunately, the number of deuterium atoms at C-19 of (**5**) could not be observed owing to signal overlapping.

The absence of a deuterium atom at C-3 in (**5**) and (**6**) supports the existence of a 3-oxo intermediate during extrusion of the two methyl groups at C-4.[4,5] One of the deuterium atoms at C-7 was displaced by a hydrogen in (**5**), which

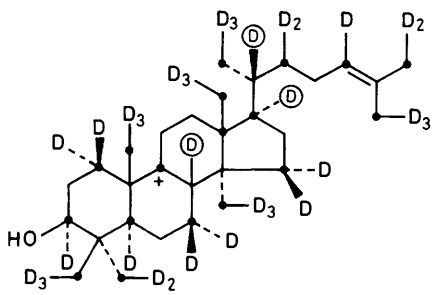
† *Rabdosia japonica* Hara was formerly called *Isodon japonicus* Hara.

‡ A mixture of labelled acetate (630 mg) and non-labelled acetate (1.26 g) in 9 l of medium.

§ A mixture of labelled acetate (150 mg) and non-labelled acetate (300 mg) in 1 l of medium.



(1)



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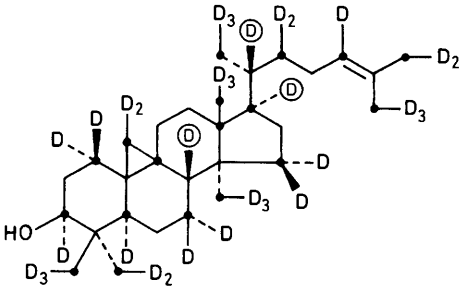

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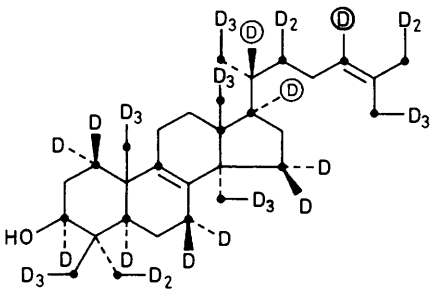

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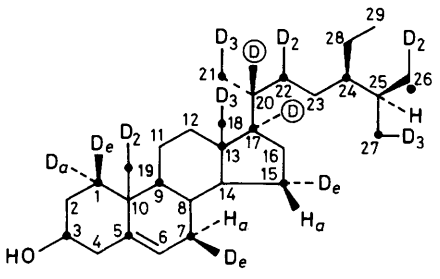
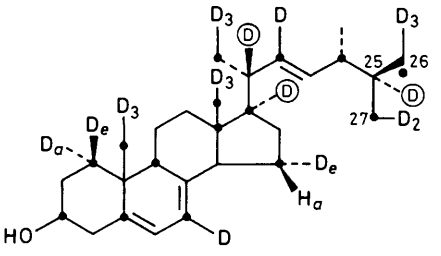


(5)



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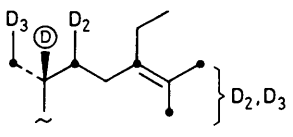

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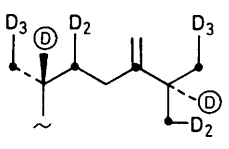

(8)

• = ^{13}C

supports 7(8) double bond formation followed by migration to the 5(6) position. The 7α hydrogen atom was suggested to originate from NADH, and the 7β hydrogen atom from the 2-*pro-R* hydrogen atom of MVA.[6] This is also indicated by $^{1}\Delta\delta_{C(2H)}$ (−0.37 p.p.m.) because the value of one of the two shifted signals due to an α-deuterium atom at C-1 is −0.37 p.p.m. and the amplitude of an equatorial α-deuterium isotope effect was suggested to be smaller than that for an axial effect.[12] Displacement of one of the deuterium atoms by a hydrogen at C-15 in (**5**) and (**6**) agrees with 14(15) double bond formation during extrusion of the methyl group at C-14 followed by hydrogenation to 14-H_α and 15-H_β.[6]

As for the side-chain formation including alkylation at C-24, the deuterium atom at C-24 in (**3**) was lost in (**5**). In contrast, the deuterium atom at C-24 in (**4**) was located at C-25 in (**6**) ($^{2}\Delta\delta_{C(2H)}$ −0.12 p.p.m.). This agrees with the mechanism that sitosterol (**5**) is formed *via* an intermediate (**7**) having a 24(25) double bond followed by reduction[13] and that ergosterol (**6**) is

Table 1. ^{13}C N.m.r. data of sitosterol[a] (**5**) and ergosterol[b] (**6**) biosynthetically labelled from [2-^{13}C,2-$^{2}H_3$]acetate.[c]

	(5)			(6)				(5)				(6)			
		$^1\Delta\delta_{C(^2H)}$			$^1\Delta\delta_{C(^2H)}$				$^1\Delta\delta_C$				$^1\Delta\delta_C$		
Carbon	δ_C	d_1	d_2	δ_C	d_1	d_2	Carbon	δ_C	d_1	d_2	d_3	δ_C	d_1	d_2	d_3
C-1	37.30	−0.37	[e]	38.33	−0.37	−0.80	C-15	24.32	−0.34			22.99	−0.34		
		−0.41			−0.42		C-16	28.25				28.19			
C-2	31.69			31.75			C-17	56.12	(−0.11)[f]			55.67	(−0.10)[f]		
C-3	71.78			69.98			C-18	11.87	−0.29	−0.57	−0.86	12.06	−0.28	−0.57	−0.85
C-4	42.25[d]			40.54			C-19	19.40	[e]	[e]		16.27	−0.27	−0.56	−0.83
C-5	141.77			139.32			C-20	36.17				40.24			
C-6	121.66			119.09			C-21	18.81	−0.33	−0.61	−0.91	21.07	−0.30	−0.61	−0.91
C-7	31.94	−0.37		115.87	−0.29		C-22	34.00	−0.42	−0.81		135.03	−0.37		
C-8	31.94			140.67			C-23	26.19				131.51			
C-9	50.19			46.21			C-24	45.89				42.68	(−0.12)[f]		
C-10	36.52			37.00			C-25	29.24				33.04			
C-11	21.11			21.14			C-26	19.81	−0.31	[e]		19.89	−0.28	−0.60	−0.90
C-12	39.82			39.06			C-27	19.09	[e]	[e]	[e]	19.61	−0.32	−0.61	
C-13	42.28[d]	(−0.10)[f]		42.74	(−0.09)[f]		C-28	23.12				17.57			
C-14	56.81			54.42			C-29	12.01							

[a] Biosynthesized in tissue cultures of *Rabdosia japonica* Hara. [b] Biosynthesized in yeast (*Saccharomyces cereviceae* IFO 1346). [c] ^{13}C N.m.r. spectra were recorded on a JEOL GX-400 spectrometer operated at 100 MHz with ^1H and ^2H decoupling mode in [^2H]chloroform (δ_C 77.000). [d] These signals were assigned by INEPT. [e] These signals were not observed owing to overlap with other signals. [f] $^2\Delta\delta_{C(^2H)}$ values.

biosynthesized *via* the $\Delta^{24(28)}$ intermediate (**8**).[14] Carbon-26 (*pro-R* methyl group at C-25) of (**6**) retained three deuterium atoms, while C-27 (*pro-S* methyl group at C-25) of (**6**) retained only two. This fact confirms that C-26 and C-27 originate from C-6 and C-2, respectively, of MVA.[10]

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