Biosynthesis of Triterpenes, Ursolic Acid and Oleanolic Acid, from [2-^{13}C,2-$^{2}H_3$]Acetate in Tissue Cultures of *Rabdosia japonica* Hara†

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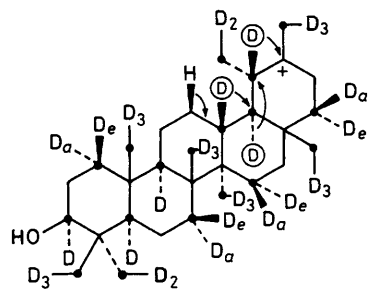
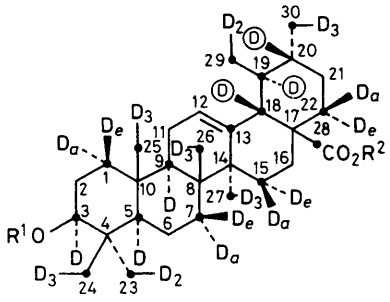
1,2-Hydride shifts in the biosynthesis of ursolic acid (**2**) and oleanolic acid (**6**), 20-H from C-19, 19-H from C-18, and 18-H from C-13 in (**2**) and 19-H from C-18 and 18-H from C-13 in (**6**), were verified in cultured cells of *Rabdosia japonica* Hara fed with [2-^{13}C,2-$^{2}H_3$]acetate.

The biogenetic isoprene rule for pentacyclic triterpenes such as the oleanene- and ursene-types includes some 1,2-hydride shifts and carbon rearrangements.[1,2] Recently, we demonstrated the occurrence of carbon rearrangements during D and E ring formation in the biosynthesis of oleanene-type and ursene-type triterpenes in cultured cells of a higher plant, *Rabdosia japonica* Hara.[3] Goodwin *et al.*[4] and Barton *et al.*[5]

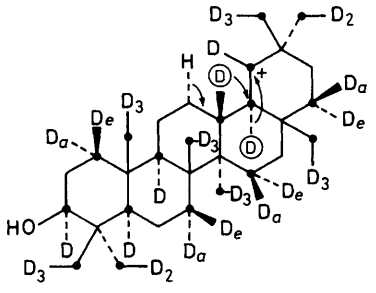
† *Rabdosia japonica* Hara was formerly called *Isodon japonicus* Hara.



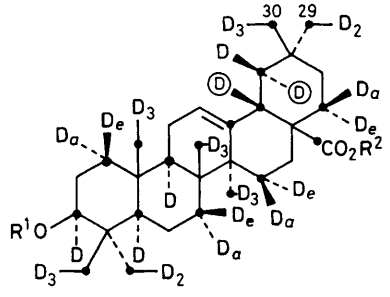
(1)



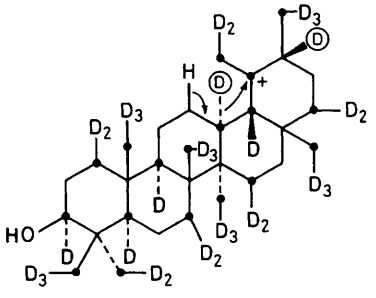
(2) $R^1 = R^2 = H$
(3) $R^1 = p\text{-}O_2NC_6H_4CO$, $R^2 = Me$
(4) $R^1 = H$, $R^2 = Me$



(5)



(6) $R^1 = R^2 = H$
(7) $R^1 = p\text{-}O_2NC_6H_4CO$, $R^2 = Me$
(8) $R^1 = H$, $R^2 = Me$



(9)

• = ^{13}C

reported two 1,2-hydride shifts, 18-H from C-13 and 19-H from C-18, in the biosynthesis of β-amyrin. We examined the stereochemistry of the hydrogen atoms at C-12 and C-13, which are the two centre carbon atoms of squalene, using [5-^{13}C,5-$^{2}H_2$]mevalonic acid and found that a 12-*pro-S* proton of (**5**) is eliminated to form the 12(13) double bond of oleanolic acid (**6**). Conversely, the 12(13) double bond of ursolic acid (**2**) is formed by a 12-*pro-R* proton elimination from (**1**).[6] An intermediate having a group X at C-13 may be proposed to rationalize this 1,2 *cis* elimination but some other mechanism *via* the C,D- and D,E-*cis*-intermediate (**9**), followed by a 1,3-hydride shift from C-13 to C-19, is conceivable for the biosynthesis of ursene-type triterpenes. However, evidence is presented here which excludes the possible intermediacy of (**9**). Three 1,2-hydride shifts are required for ursolic acid (**2**) biosynthesis.

Sodium [2-^{13}C,2-$^{2}H_3$]acetate‡ was administered to suspension cultures of *R. japonica* (grown on 9 l of Linsmeier–Skoog medium) for four weeks. The suspension cultures were worked up in the usual manner.[3] The mixture of *p*-nitrobenzoates (**3**) and (**7**) obtained was separated by h.p.l.c.[7] (TSKgel ODS-120T, methanol) followed by hydrolysis to give methyl ursolate (**4**) and methyl oleanolate (**8**).

As shown in Table 1, the 100 MHz ^{13}C-{^{1}H}{^{2}H} n.m.r. spectra of (**4**) and (**8**) showed deuterium atoms migrating to the adjacent carbon atoms because of the presence of signals which were shifted owing to the β-deuterium isotope effect ($^{2}\Delta\delta_{C(^{2}H)}$).[8] The three signals due to C-13 (δ_C 138.13), C-18

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

Table 1. ^{13}C–^{2}H Labelling patterns of methyl ursolate (**4**) and methyl oleanolate (**8**) from [2-^{13}C,2-$^{2}H_3$]acetate fed to tissue cultures of *Rabdosia japonica* Hara.[a]

	(4)			(8)				(4)				(8)			
		$^{1}\Delta\delta_{C(^{2}H)}$			$^{1}\Delta\delta_{C(^{2}H)}$				$^{1}\Delta\delta_{C(^{2}H)}$				$^{1}\Delta\delta_{C(^{2}H)}$		
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	38.66	−0.38	−0.82	38.48	−0.35	−0.79	C-16	24.25				23.10			
		−0.44			−0.43		C-17	48.09				46.73			
C-2	27.25			27.22			C-18	52.90	(−0.09)[d]			41.33	(−0.06)[d]		
C-3	78.99	−0.52		78.99	−0.52		C-19	39.06	(−0.11)[d]			45.91	−0.48		
C-4	38.74			38.76			C-20	38.88				30.68			
C-5	55.26	−0.62		55.28	−0.63		C-21	30.67				33.81			
C-6	18.32			18.36			C-22	36.63	−0.40	−0.80		32.41	−0.38	−0.76	
C-7	33.00	−0.39	−0.79	32.71	−0.36	−0.64	C-23	28.14	−0.31	−0.62		28.12	−0.31	−0.63	
C-8	39.52			39.31			C-24	15.60[b]	−0.29	−0.56	−0.85	15.58[b]	[c]	[c]	[c]
C-9	47.58	−0.51		47.67	−0.51		C-25	15.42[b]	−0.27	−0.54	−0.92	15.30[b]	−0.28	−0.56	−0.84
C-10	36.98			37.07			C-26	16.91	−0.29	−0.56	−0.83	16.85	−0.28	−0.54	−0.85
C-11	23.31			23.42			C-27	23.61	−0.30	−0.59	−0.89	25.95	−0.32	−0.62	−0.90
C-12	122.36			125.54			C-28	177.97				178.21			
C-13	138.13	(−0.05)[d]		143.77	(−0.05)[d]		C-29	17.02	−0.29	−0.59	−0.88	33.11	[c]	[c]	[c]
C-14	42.01			41.67			C-30	21.16	−0.30	−0.60		23.65	−0.31	−0.62	
C-15	28.05	−0.31	−0.71	27.73	−0.33	−0.70	OMe	51.37				51.41			
		−0.39			−0.39										

[a] ^{13}C N.m.r. spectra were recorded on a JEOL GX-400 instrument at 100 MHz with ^{1}H and ^{2}H decoupling mode in [^{2}H]chloroform (δ_C 77.000). Accuracy of δ_C is ± 0.006 p.p.m. [b] Assignments may be reversed. [c] These values were not obtained because of signal overlap. [d] $^{2}\Delta\delta_{C(^{2}H)}$ values.

(δ_C 52.90), and C-19 (δ_C 39.06) of methyl ursolate (**4**) accompanying the shifted signals owing to the β-deuterium isotope effect (shown in parentheses in Table 1) are evidence of the 1,2-hydride shifts, 18-H from C-13, 19-H from C-18, and 20-H from C-19. This result, which agrees with a recent report,[9] excludes the possibility of the intermediate (**9**).

In oleanolic acid (**6**) biosynthesis, the two 1,2-hydride shifts (18-H from C-13 and 19-H from C-18) were clearly confirmed by the β-deuterium isotopically shifted signals on C-13 (δ_C 143.77) and C-18 (δ_C 41.33). A large difference was observed in the ratio of the shifted signal to the natural abundance signal between the triterpenes (**4**) and (**8**) (*ca.* 0.5) and sitosterol (*ca.* 0.1).[10] The amplitude of $^{2}\Delta\delta_{C(^{2}H)}$ values induced by a deuterium atom on a secondary carbon (−0.06 p.p.m.) seems to be smaller than that on a tertiary carbon (*ca.* −0.1 p.p.m.). sp^2 Carbon atoms (C-13) showed −0.05 p.p.m.

The number of deuterium atoms attached directly to the ^{13}C-labelled carbon atoms was indicated by the shifted signals due to the α-deuterium isotope effect ($^{1}\Delta\delta_{C(^{2}H)}$).[8] The values of $^{1}\Delta\delta_{C(^{2}H)}$ of −0.27 to −0.32 p.p.m. for methyl groups, −0.33 to −0.43 p.p.m. for methylene groups, and −0.48 to −0.63 p.p.m. for methine groups can be useful for ^{13}C signal assignments.[8] The amplitude of an equatorial $^{1}\Delta\delta_{C(^{2}H)}$ shift was suggested to be smaller than that of an axial one.[11] Some methylene groups such as C-1 and C-15 of (**4**) and (**8**) showed two α-shifted signals for d_1. The smaller shift (−0.31 to −0.38 p.p.m.) indicates an equatorial deuterium atom and the larger shift (−0.39 to −0.44 p.p.m.) an axial one. According to the biogenetic mechanism as shown in (**5**), the deuterium atom at C-19 in (**5**) becomes equatorial (β) in (**8**), but a rather large α-shift (−0.48 p.p.m.) was observed. This might be due to an unusual magnetic effect of the 12(13) double bond[12] which is in very close proximity to the 19β-H.

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