

## Biosynthesis of Triterpenes, Ursolic Acid and Oleanolic Acid, from [2-<sup>13</sup>C,2-<sup>2</sup>H<sub>3</sub>]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-<sup>13</sup>C,2-<sup>2</sup>H<sub>3</sub>]acetate.

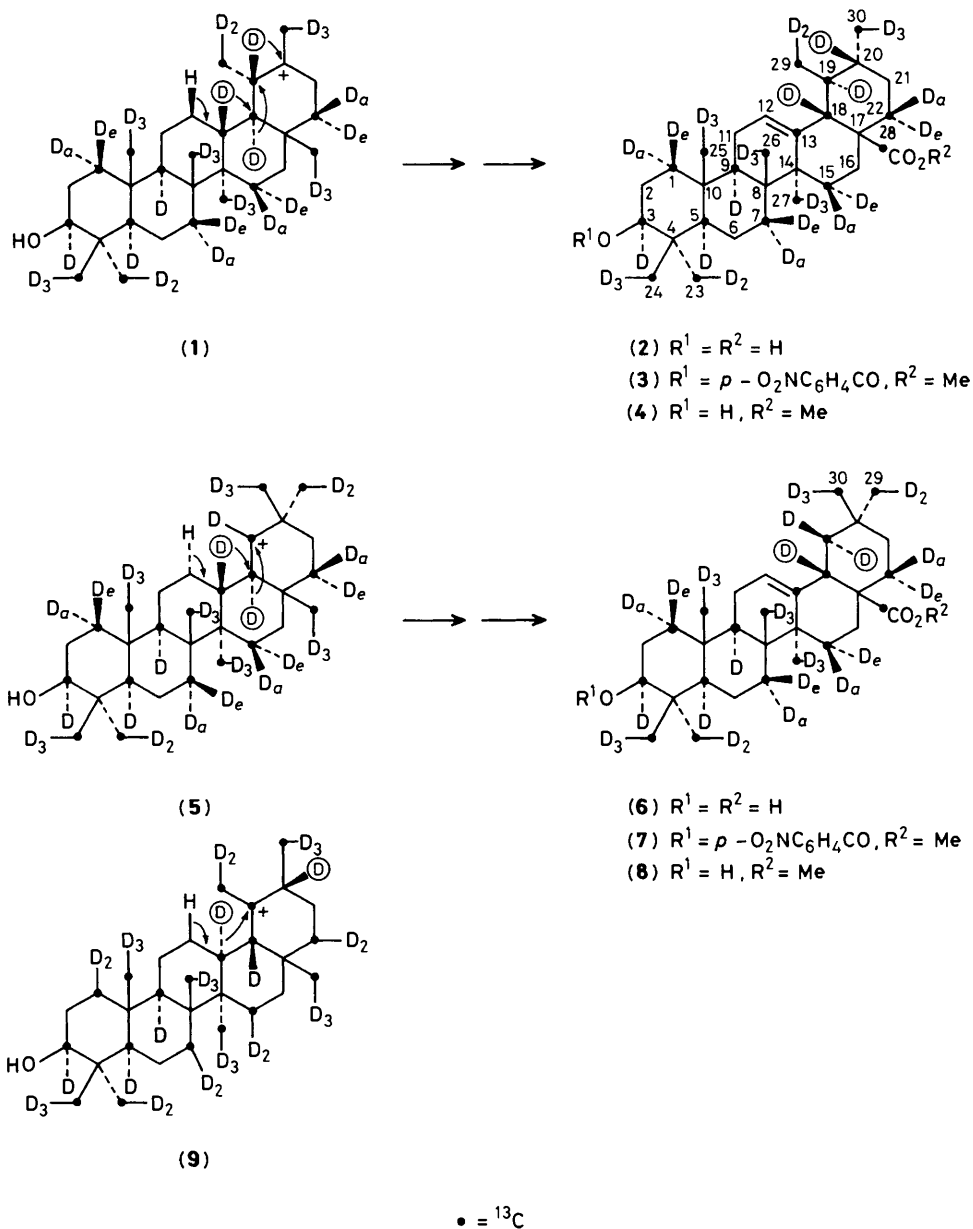
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The biogenetic isoprene rule for pentacyclic triterpenes such as the oleanene- and ursene-types includes some 1,2-hydride

shifts and carbon rearrangements.<sup>1,2</sup> 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.<sup>3</sup> Goodwin *et al.*<sup>4</sup> and Barton *et al.*<sup>5</sup>

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



reported two 1,2-hydride shifts, 18-H from C-13 and 19-H from C-18, in the biosynthesis of  $\beta$ -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- $^2H_2$ ]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).<sup>6</sup> 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- $^2H_3$ ]acetate $\ddagger$  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.<sup>3</sup> The mixture of *p*-nitrobenzoates (3) and (7) obtained was separated by h.p.l.c.<sup>7</sup> (TSKgel ODS-120T, methanol) followed by hydrolysis to give methyl ursolate (4) and methyl oleanolate (8).

As shown in Table 1, the 100 MHz  $^{13}C$ - $\{^1H\}$ - $\{^2H\}$  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  $\beta$ -deuterium isotope effect ( $^2\Delta\delta_{C(2H)}$ ).<sup>8</sup> The three signals due to C-13 ( $\delta_C$  138.13), C-18

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

**Table 1.**  $^{13}\text{C}$ - $^2\text{H}$  Labelling patterns of methyl ursolate (4) and methyl oleanolate (8) from  $[2\text{-}^{13}\text{C}, 2\text{-}^2\text{H}_3]\text{acetate}$  fed to tissue cultures of *Rabdosia japonica* Hara.<sup>a</sup>

Carbon	(4)			(8)			Carbon	(4)				(8)			
	$\delta_{\text{C}}$	$^1\Delta\delta_{\text{C}(2\text{H})}$ d <sub>1</sub>	d <sub>2</sub>	$\delta_{\text{C}}$	$^1\Delta\delta_{\text{C}(2\text{H})}$ d <sub>1</sub>	d <sub>2</sub>		$\delta_{\text{C}}$	d <sub>1</sub>	$^1\Delta\delta_{\text{C}(2\text{H})}$ d <sub>2</sub>	d <sub>3</sub>	$\delta_{\text{C}}$	d <sub>1</sub>	$^1\Delta\delta_{\text{C}(2\text{H})}$ d <sub>2</sub>	d <sub>3</sub>
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) <sup>d</sup>			41.33	(-0.06) <sup>d</sup>		
C-3	78.99	-0.52		78.99	-0.52		C-19	39.06	(-0.11) <sup>d</sup>			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 <sup>b</sup>	-0.29	-0.56	-0.85	15.58 <sup>b</sup>	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>
C-9	47.58	-0.51		47.67	-0.51		C-25	15.42 <sup>b</sup>	-0.27	-0.54	-0.92	15.30 <sup>b</sup>	-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) <sup>d</sup>		143.77	(-0.05) <sup>d</sup>		C-29	17.02	-0.29	-0.59	-0.88	33.11	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>
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										

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

( $\delta_{\text{C}}$  52.90), and C-19 ( $\delta_{\text{C}}$  39.06) of methyl ursolate (4) accompanying the shifted signals owing to the  $\beta$ -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,<sup>9</sup> 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  $\beta$ -deuterium isotopically shifted signals on C-13 ( $\delta_{\text{C}}$  143.77) and C-18 ( $\delta_{\text{C}}$  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).<sup>10</sup> The amplitude of  $^2\Delta\delta_{\text{C}(2\text{H})}$  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.).  $\text{sp}^2$  Carbon atoms (C-13) showed  $-0.05$  p.p.m.

The number of deuterium atoms attached directly to the  $^{13}\text{C}$ -labelled carbon atoms was indicated by the shifted signals due to the  $\alpha$ -deuterium isotope effect ( $^1\Delta\delta_{\text{C}(2\text{H})}$ ).<sup>8</sup> The values of  $^1\Delta\delta_{\text{C}(2\text{H})}$  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  $^{13}\text{C}$  signal assignments.<sup>8</sup> The amplitude of an equatorial  $^1\Delta\delta_{\text{C}(2\text{H})}$  shift was suggested to be smaller than that of an axial one.<sup>11</sup> Some methylene groups such as C-1 and C-15 of (4) and (8) showed two  $\alpha$ -shifted signals for d<sub>1</sub>. 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 ( $\beta$ ) in (8), but a rather large

$\alpha$ -shift ( $-0.48$  p.p.m.) was observed. This might be due to an unusual magnetic effect of the 12(13) double bond<sup>12</sup> which is in very close proximity to the 19 $\beta$ -H.

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