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BIOSYNTHETIC PATHWAY OF IRIDOID GLUCOSIDES IN GARDENIA JASMINOIDES F. GRANDIFLORA CELL SUSPENSION CULTURES AFTER IRIDODIAL CATION FORMATION

Shinichi Uesato, Shinichi Ueda, Koji Kobayashi, Masao Miyauchi and Hiroyuki Inouye*

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan

Summary Administration of ^{2}H- or ^{13}C-labeled monoterpenes to Gardenia jasminoides f. grandiflora cell suspension cultures demonstrated that iridoid glucosides of the suspension cultures are biosynthesized, after iridodial cation formation from 10-oxocitral (**3**), via 8-epiiridodial (**13**), 8-epiiridotrial (**14**), boschnaloside (8-epiiridotrial glucoside) (**12**) and dehydroiridotrial glucoside (**8**). In addition, the coexistence of a route via iridodial cation (**4**), dehydroiridodial (**6**), dehydroiridotrial (**15**) and dehydroiridotrial glucoside (**8**) is conceivable.

Several mechanisms have so far been proposed for the iridane skeleton formation from acyclic monoterpenes.[1-6] However, a comprehensive understanding of these mechanisms has not yet been obtained.

In the previous paper, we demonstrated through administration of various combinations of ^{13}C-labeled acyclic monoterpenes to Gardenia jasminoides cell suspension cultures that tarennoside (**1**) and gardenoside (**2**) of the cultured cells are biosynthesized through cyclization of 2E- or 2Z-10-oxocitral (**3**) to the iridodial cation (**4**) and subsequent randomization of the carbon atoms 3 and 11.[7] However, the pathway after iridodial cation formation remained to be established. The present paper deals with this problem.

In the following experiments, the suspension cultures grown for two weeks after transfer were incubated with ^{2}H- or ^{13}C-labeled substances for 5 to 7 days, and all the glucosides including tarennoside (**1**) were isolated as their acetates. Initially, equimolar amounts of both supposed intermediates, [10-$^{2}H_3$]iridodial (**5**) and [11-$^{2}H_3$]dehydroiridodial (**6**),[8a] were administered together to the cell cultures (Expt. A). On isolation of the glucosides, labeled tarennoside (**1**) was obtained along with [10-$^{2}H_3$]iridotrial glucoside (**7**) presumably derived from [10-$^{2}H_3$]-**5**, and [11-^{2}H]dehydroiridotrial glucoside (**8**) presumably derived from [11-$^{2}H_3$]-**6**.

The ^{2}H NMR spectrum of the acetate **9** of tarennoside (**1**) showed signals at δ 7.14 (3-^{2}H) and 9.24 (11-^{2}H) ppm in a 2:3 ratio. Both these signals originated from [11-$^{2}H_3$]dehydroiridodial (**6**), but a signal which should originate from [10-$^{2}H_3$]iridodial (**5**) was not observed. This finding suggests the intermediacy of **6** but excludes the precursorship of iridodial (**5**) in spite of the fact that [10-$^{2}H_3$]-**5** was converted to [10-$^{2}H_3$]iridotrial glucoside (**7**).

Thus, in order to examine the possibility of dehydroiridotrial glucoside (**8**) serving as one of the intermediates after **6**, dilution analysis of **8** was attempted after administering [4-^{13}C]-10-hydroxygeraniol (**10**)[8b] to the cell cultures (Expt. B). The ^{13}C NMR spectrum of the

Table 1 Administration of Supposed Labeled Precursors to G. jasminoides Cell Cultures

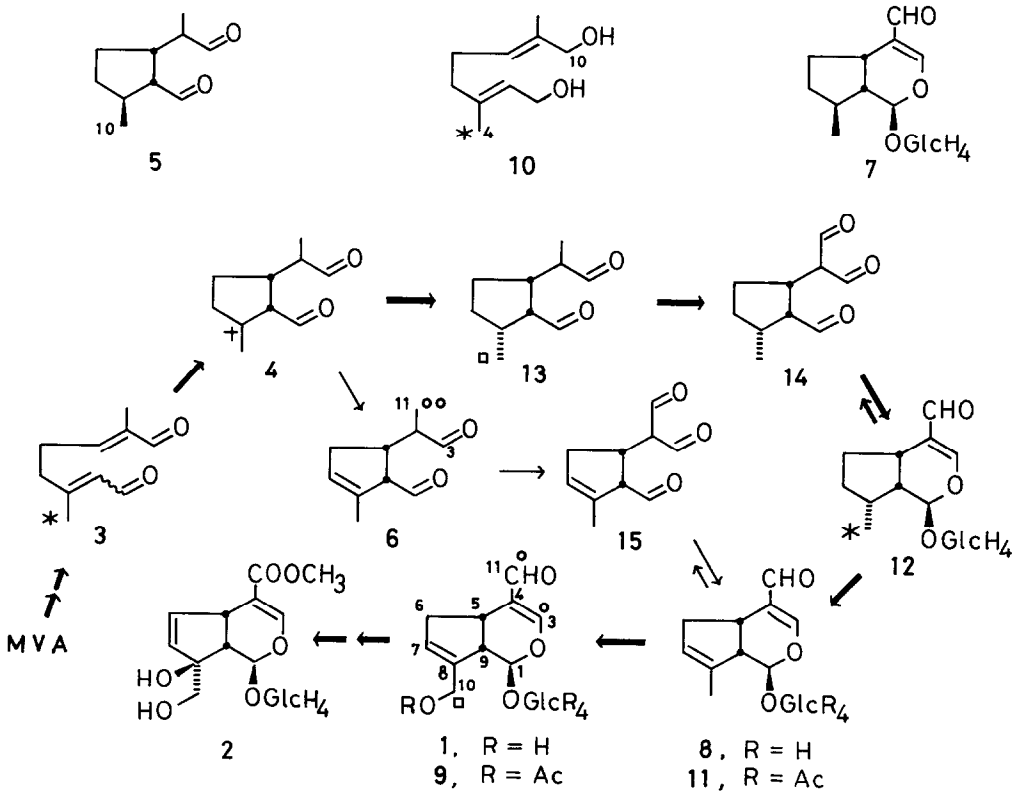
	Compounds fed*	labeled glucosides isolated or detected total incorp. (spec. incorp.) %**	ratio of signal intensities between 3-^{2}H and 11-^{2}H in tarennoside (**1**)
Expt. A	[10-$^{2}H_3$]iridodial (**5**)	[10-$^{2}H_3$]iridotrial glucoside (**7**) --- (---)	
	+		
	[11-$^{2}H_3$]dehydroiridodial (**6**)	[11-^{2}H]dehydroiridotrial glucoside (**8**) --- (---)	
		[3,11-$^{2}H_2$]tarennoside (**1**) 3.3 (23.5)	2 : 3
Expt. B	[4-^{13}C]-10-hydroxygeraniol (**10**)	[10-^{13}C]boschnaloside (**12**) --- (---)	
Expt. C	[11-$^{2}H_3$]dehydroiridodial (**6**)	[3,11-$^{2}H_2$] tarennoside (**1**) 21.0 (32.5)	1 : 2
	+		
	[10-$^{2}H_3$]-8-epiiridodial (**13**)	[10-$^{2}H_2$]tarennoside (**1**) 10.1 (13.2)	
Expt. D	[11-^{2}H]boschnaloside (**12**)	[3,11-$^{2}H_2$]tarennoside (**1**) 13.1 (41.7)	1 : 3
Expt. E	[11-^{2}H]dehydroiridotrial glucoside (**8**)	[3,11-$^{2}H_2$]tarennoside (**1**) 29.5 (70.4)	1 : 3

* Compounds **5**, **6** and **13** were administered in an enol-acetal form. cf. footnotes 8a and 9.

** Total and specific incorporations were calculated based on the assumption that there was no deuterium isotope effect causing the preferential removal of hydrogen during the biosynthetic process.

acetate **11** of the reisolated **8** did not show any ^{13}C enrichment at C-10, but indicated an unexpected enhancement at C-10 (δ 16.10) caused by the contamination with [10-^{13}C]boschnaloside (8-epiiridotrial glucoside) (**12**) which should be derived from the [4-^{13}C]-**10** administered.

To confirm this result, which suggests the intermediacy of compounds of the 8-epiiridodial (**13**) series, the following three experiments were carried out: equimolar amounts of [11-$^{2}H_3$]-dehydroiridodial (**6**) and [10-$^{2}H_3$]-8-epiiridodial (**13**)[9] were administered together to the cell cultures (Expt. C). The ^{2}H NMR spectrum of the resulting acetate **9** of tarennoside (**1**) showed incorporation of the label of **6** into H-3 and H-11 (in a 1:2 ratio) as well as of the label of **13** into H-10 (δ 4.71). Subsequently, [11-^{2}H]boschnaloside (**12**) and [11-^{2}H]dehydroiridotrial glucoside (**8**)[9] were fed separately to the cell cultures (Expts. D and E). The ^{2}H NMR spectra of **9** obtained in each run showed that the ^{2}H labels of both **12** and **8** were incorporated into H-11 and H-3 in a 3:1 ratio. Throughout the Expts. C, D and E, specific and total incorporation ratios of ^{2}H-labeled compounds into tarennoside (**1**) were remarkably high (Table 1). In both Expts. A and C, there was difference in the distribution ratio of the ^{2}H



Scheme 1 Biosynthetic Pathway of Tarennoside (**1**) and Gardenoside (**2**)

label between the 3 and 11 position of **1**, i.e., the randomization was not complete. This could be explained by assuming that some of the administered **6** with a dihydropyran structure was metabolized without opening of the dihydropyran ring and randomization after oxidation at the C-11, and that the randomization rates in the two experiments were different. The scrambling of the ^{2}H label observed in tarennoside (**1**) isolated in Expts. D and E could be explained in the same way by assuming partial equilibration between the glucosides **12** and **8** and the corresponding aglucones **14** and **15**. Furthermore, the ^{1}H NMR spectra of labeled dehydroiridotrial glucoside (**8**) recovered in Expt. E and of boschnaloside (**12**) recovered in Expt. D indicated a 1.5 and 1.4 fold dilution, respectively, of extraneous **8** and **12** by the corresponding endogenous compounds. Moreover, the spectrum of **8** showed contamination with endogenous boschnaloside (**12**). These facts strongly suggest the intermediacy of both boschnaloside (**12**) and dehydroiridotrial glucoside (**8**).

It was, therefore, concluded that tarennoside (**1**) and gardenoside (**2**) in G. jasminoides suspension cultures are biosynthesized by a route through 10-oxocitral (**3**), iridodial cation (**4**), 8-epiiridodial (**13**), boschnaloside (**12**) and dehydroiridotrial glucoside (**8**) as depicted with bold lines in Scheme 1. In view of the high incorporation of dehydroiridodial (**6**) into tarennoside (**1**), coexistence of a route via 10-oxocitral (**3**), iridodial cation (**4**), dehydroiridodial (**6**), dehydroiridotrial (**15**), dehydroiridotrial glucoside (**8**), tarennoside (**1**) and gardenoside (**2**) is also conceivable, in spite of a possibility that the incorporation of **6** into **1** might be due to a biotransformation of an extraneously given compound, as is the conversion of iridodial (**5**) into iridotrial glucoside (**7**).

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9) The synthesis of these compounds starting from geniposide will be reported elsewhere.

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