Communications to the Editor

[Chem. Pharm. Bull. 31(11)4185—4188(1983)]

MECHANISM OF IRIDANE SKELETON FORMATION IN THE BIOSYNTHESIS OF IRIDOID GLUCOSIDES IN GARDENIA JASMINOIDES CELL CULTURES

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

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

Administration of ^{13}C-labeled acyclic monoterpenes to Gardenia jasminoides f. grandiflora suspension cultures in various combinations demonstrated that the iridoid glucosides of the suspension cultures are biosynthesized through cyclization of 10-oxocitral (**5**) to the iridodial cation (**17**) followed by randomization of the carbon atoms 3 and 11. Additionally, the possibility of 10-hydroxycitronellol (**12**) and 9,10-dihydroxycitronellol (**13**) serving as an intermediate was disproved.

KEYWORDS —— tarennoside; gardenoside; biosynthesis; iridane skeleton formation; Gardenia jasminoides; cell culture

Iridoids were originally considered to be biosynthesized via iridodial (**1**), a key intermediate, which could be formed through a Michael type cyclization of 10-oxocitronellal (**2**).[1,2] Later, however, two mechanisms, characterized by the types of iridoids concerned, were proposed for the process of iridane skeleton formation from acyclic monoterpenes. One comprises a route from 2E- or 2Z-9,10-dioxocitral (**3**) to iridotrial (**4**) (loganin, etc.),[3] the other involves a route from 2E- or 2Z-10-oxocitral (**5**) to iridodial (**1**) (asperuloside, etc.).[4] The former mechanism allows the incorporation of each of the terminal carbon atoms 9 and 10 of **3** into the 3 and 11 positions of iridoids with extensive randomization, whereas the latter does not necessarily involve such a scrambling. In addition, a route passing through 8-epideoxyloganin (**6**), has recently been inferred for some iridoids, such as lamiide.[5] Thus, there seems to be several mechanisms for iridane skeleton formation. This paper deals with the examination of the mechanism of iridane skeleton formation of iridoid glucosides, tarennoside (**7**) and gardenoside (**8**) in Gardenia jasminoides f. grandiflora suspension cultures.[6]

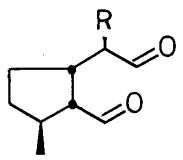
Initially, the following ^{13}C-labeled diols and triols were synthesized,[7] which correspond to dials **5** and **2** as well as trials **3** and 9,10-dioxocitronellal (**9**), probable substrates for the cyclization: [9-^{13}C]-10-hydroxygeraniol (**10**), [4-^{13}C]-10-hydroxygeraniol (**10**), [2-^{13}C]-9,10-dihydroxygeraniol (**11**), (S)-(-)-and (R)-(+)-[9-^{13}C]-10-hydroxycitronellol (**12**) and (S)-(-)-[8-^{13}C]-

NII-Electronic Library Service

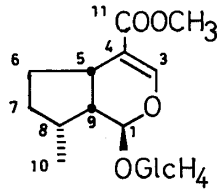
9,10-dihydroxycitronellol (**13**) (both [4-^{13}C]-**10** and [2-^{13}C]-**11** contained 20% of the corresponding nerol forms). These alcohols were administered to the cell cultures. It was expected that, in the cells, they would be oxidized to the corresponding aldehydes or isomerized to the other geometric isomers before undergoing cyclization. For the feeding experiments suspension cultures grown for two weeks after transfer were employed.

[9-^{13}C]-10-Hydroxygeraniol (**10**) corresponding to the most probable precursor **5** on the pathway depicted below was administered to the cell cultures (Exp. A). After incubation for 10 days, tarennoside (**7**) and gardenoside (**8**) were isolated and purified as their acetates **14** and **15**, respectively. In the ^{13}C NMR spectrum of tarennoside pentaacetate (**14**), the enrichment factors (EFs) of the C-3 and C-11 signals at 160.2 and 190.0 ppm were 6.0 and 6.5%, respectively. Furthermore, in the spectrum of gardenoside pentaacetate (**15**) measured in the presence of a paramagnetic reagent, tris(acetylacetonate)chromium (III), the EFs of the C-3 and C-11 signals were 0.7 and 0.6%, respectively. Thus, the precursorship of 10-hydroxygeraniol (**10**) for the biosynthesis of both glucosides **7** and **8**, as well as the almost complete randomization of the terminal carbons 9 and 10 of **10** between positions 3 and 11 of both glucosides were demonstrated.

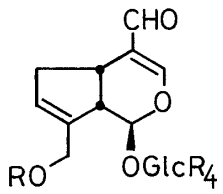
Subsequently, a mixture of [4-^{13}C]-10-hydroxygeraniol (**10**), [2-^{13}C]-9,10-dihydroxygeraniol (**11**), (S)-(-)-[9-^{13}C]-10-hydroxycitronellol (**12**) and (S)-(-)-[8-^{13}C]-9,10-dihydroxycitronellol (**13**) was administered to the cell cultures (Exp. B). The ^{13}C NMR spectrum of the acetate (**14**) of tarennoside (**7**) isolated after incubation for seven days showed that ^{13}C label of [4-^{13}C]-**10** and [2-^{13}C]-**11** had been incorporated into C-10 (EF 5.7%, 61.5 ppm) and C-9 (EF 1.4%, 46.4 ppm), respectively. In contrast, the spectrum did not indicate any incorporation of label from (S)-(-)-[9-^{13}C]-**12** and (S)-(-)-[8-^{13}C]-**13** into **7**. This could be explained by the



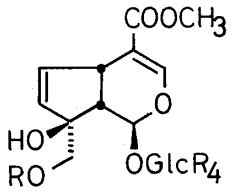
1, R = CH_3
4, R = CHO



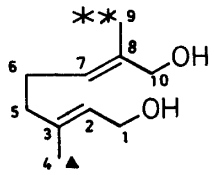
6



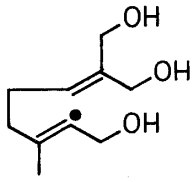
7, R = H
14, R = Ac



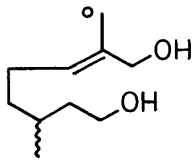
8, R = H
15, R = Ac



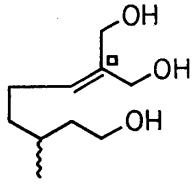
10



11



12



13

NII-Electronic Library Service

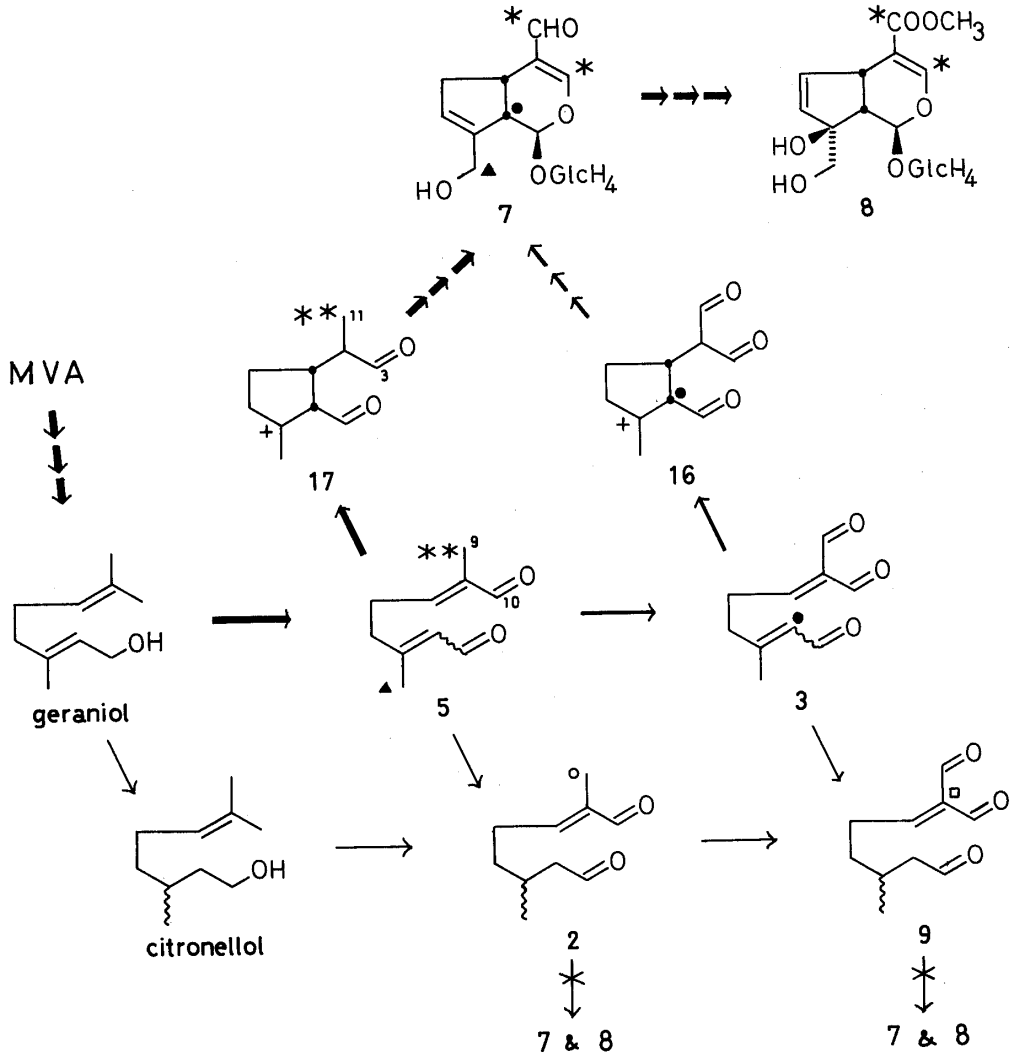


Chart 1. Biosynthetic Pathway of Tarennoside (**7**) and Gardenoside (**8**)

preferential occupation of active sites of an enzyme by aldehydic substrates **5** and **3** generated from **10** and **11**, preventing the incorporation of **12** and **13**. Therefore, in the next two experiments the cell cultures were first incubated, separately, with (S)-(-)- and with (R)-(+)-[9-^{13}C]-**12** for five days, when [4-^{13}C]-**10** was added to each, and incubation was continued for an additional seven days (Exps. C and D). In both experiments, the ^{13}C NMR spectrum of tarennoside pentaacetate (**14**) showed enrichment of only C-10 (EF, 3.0 and 3.4%), indicating incorporation only of [4-^{13}C]-**10**. The routes via (R)-(+)- and (S)-(-)-**2** generated from (R)-(+)- and (S)-(-)-**12** as well as that via (S)-(-)-**9** derived from (S)-(-)-**13** were thereby ruled out.

A further problem is the non-negligible incorporation of [2-^{13}C]-9,10-dihydroxygeraniol (**11**) into tarennoside (**7**) in the Exp. B. This may suggest the presence of a route to **7** via 9,10-dioxogeranial (**3**) and the cation **16**. If such a route exists, administration of a comparatively

NII-Electronic Library Service

large amount of non-labeled 10-hydroxygeraniol (**10**) prior to that of [2-^{13}C]-**11** should reduce the incorporation of [2-^{13}C]-**11** into **7**. An analogous administration of non-labeled **11** together with [4-^{13}C]-**10** should also reduce the incorporation of [4-^{13}C]-**10** into **7**. The cell cultures were incubated with non-labeled **10** or **11** for 5 hours and then with 0.1 eq. of labeled **11** or **10** for 7 days (Exps. E and F). Contrary to prediction, however, the results of the experiments showed that the EFs of ^{13}C in tarennoside pentaacetate (**14**) derived from labeled **11** and **10** (1.2 and 5.6%, respectively) were almost the same as those in the Exp. B. Thus the incorporation of 9,10-dihydroxygeraniol (**11**) most likely is due to a conversion by a minor route, or due to a biotransformation of an unnatural substrate by the cultured cells.

Therefore, it is concluded that the main biosynthetic pathway of tarennoside (**7**) and gardenoside (**8**) in G. jasminoides cell cultures involves the cyclization of 2E- or 2Z-10-oxocitral (**5**) to the iridodial cation (**17**), and the subsequent randomization of the carbon atoms 3 and 11.

It remains to be clarified which one of the two isomers of 2E- and 2Z-oxocitral (**5**) is the direct substrate for the cyclization, and also what iridodial congeners are the intermediates after the iridodial cation (**17**). The results of the present work provide a good basis for solving these problems by the use of ^{13}C-labeled compounds.

REFERENCES

1) K. J. Clark, G. I. Fray, R. H. Jaeger and R. Robinson, Tetrahedron, 6, 217 (1959).

2) D. A. Yeowell and H. Schmid, Experientia, 20, 250 (1964).

3) S. Escher, P. Loew and D. Arigoni, Chem. Commun., 1970, 823; A. R. Battersby, S. H. Brown and T. G. Payne, ibid., 1970, 827.

4) H. Inouye, S. Ueda and S. Uesato, Phytochemistry, 16, 1669 (1977); H. Inouye, S. Ueda, S. Uesato and K. Kobayashi, Chem. Pharm. Bull., 26, 3384 (1978).

5) S. Damtoft, J. Chem. Soc.,Chem. Commun., 1981, 228.

6) S. Ueda, K. Kobayashi, T. Muramatsu and H. Inouye, Planta Med., 41, 186 (1981).

7) Each labeled compound showed ca. 90 atom% ^{13}C in the ^{1}H or ^{13}C NMR spectrum. cf. S. Uesato, K. Kobayashi and H. Inouye, Chem. Pharm. Bull., 30, 927 (1982).

(Received August 29, 1983)

NII-Electronic Library Service