Communications to the Editor

Chem. Pharm. Bull.
32(4)1671—1674(1984)

MECHANISM FOR IRIDANE SKELETON FORMATION FROM ACYCLIC MONOTERPENES IN THE BIOSYNTHESIS OF SECOLOGANIN AND VINDOLINE IN CATHARANTHUS ROSEUS AND LONICERA MORROWII

Shinichi Uesato, Satoko Matsuda and Hiroyuki Inouye*
Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan

Administration of various tritium-labeled monoterpenes to Catharanthus roseus and Lonicera morrowii demonstrated that the loganin (**1**), secologanin (**2**) and vindoline (**10**) of these plants are biosynthesized via cyclization of 10-oxogeranial (**11a**) or 10-oxoneral (**11b**) to iridodial (**12**) and subsequent oxidation to iridotrial(**4**).

KEYWORDS —— secoiridoid glucoside; indole alkaloid; biosynthesis; iridane skeleton formation; Catharanthus roseus; Lonicera morrowii

For the iridane skeleton formation from geraniol in the biosynthesis of loganin (**1**), secologanin (**2**) and indole alkaloids of Catharanthus roseus (L.) G. Don., Arigoni et al. proposed the following mechanism: cyclization of 9,10-dioxoneral (**3a**)/9,10-dioxogeranial (**3b**) leads to iridotrial (**4**), which is further converted to **1**, **2** and indole alkaloids with extensive randomization of the carbon atoms 3 and 11.$^{1)}$ Furthermore, Tietze et al. postulated the intermediacy of 10-hydroxy derivatives of geranial and neral and of the 10-oxo derivatives of geraniol, geranial, nerol and neral.$^{2)}$ More recently, Kurz et al. observed high ratios of incorporation of the 9- and 10-oxo derivatives of geraniol, geranial, nerol and neral as well as 9,10-dihydroxygeraniol (**5**), 9-oxo-10-hydroxygeraniol (**6**) and 9,9,10,10-tetraethoxygeranial (**7**) into ajmalicine in C. roseus suspension cultures.$^{3)}$

Independently, we have been carrying out tracer experiments with two plants containing secologanin (**2**) and indole alkaloids in order to establish the iridane skeleton formation. The results, however, seem to be inconsistent with the above proposed mechanism. The details of our study are presented in this paper.

At the outset, [1-^{3}H]-10-hydroxygeraniol (**8a**),$^{4)}$ [1-^{3}H]-9,10-dihydroxygeraniol (**5**),$^{4)}$ and ($\pm$)-[10-^{3}H]-10-hydroxycitronellol (**9**)$^{4)}$ were administered separately to C. roseus (Expts. A, B and C), and radioactive vindoline (**10**) was isolated. The results summarized in Table 1 show that the total and specific incorporation ratios of 10-hydroxygeraniol (**8a**) into **10** were respectively seven and five times higher than the values of 9,10-dihydroxygeraniol (**5**). Furthermore, the incorporation ratios of (±)-10-hydroxycitronellol (**9**) into **10** were found to be negligible. Among these findings, the rather low incorporation ratio of **5** into vindoline (**10**) differs from the results obtained by Kurz et al.$^{3)}$ This substantiated the precursorship of **5**, and led us to conclude that 10-oxogeranial (**11a**)/10-oxoneral (**11b**) formed from **8a/8b** could take part directly in the cyclization to the iridane skeleton.

In order to confirm this conclusion, [10-^{3}H]iridodial (**12**)$^{5)}$ and, as a reference, a mixture of [1-^{3}H]-9-hydroxy-10-oxogeranial (**13**) and [1-^{3}H]-9-oxo-10-hydroxygeranial (**14**)$^{3,4)}$ (3:2) were administered separately to *C. roseus* (Expts. D and E) in the same way as in Expts. A, B and C. As shown in Table 1, total and specific incorporation ratios of **12** into **10** were much higher than the values of **13** and **14**. From this result, together with those of the Expts. A, B and C, it was deduced that unlike the mechanism proposed by Arigoni et al.,$^{1)}$ the loganin (**1**), secologanin (**2**) and indole alkaloids of *C. roseus* are biosynthesized via direct cyclization of 10-oxogeranial (**11a**)/10-oxoneral (**11b**) to iridodial (**12**) followed by oxidation to iridotrial (**4**) and conversion to deoxyloganic acid (**15**)$^{6,7)}$ as illustrated in Chart 1.

Table 1. Administration of Labeled Monoterpenes to *Catharanthus roseus*

	Spec. activities (dpm/mM) (amount (mg)) of substance fed	Incorporation (spec. incorp.) (%) into vindoline (**10**)
Expt. A*	[1-^{3}H]-10-hydroxygeraniol (**8a**) 6.37×10^{10} (4.9)	0.66** (0.50)
Expt. B*	[1-^{3}H]-9,10-dihydroxygeraniol (**5**) 8.46×10^{10} (4.6)	0.096** (0.10)
Expt. C*	(±)-[10-^{3}H]-10-hydroxycitronellol (**9**) 6.46×10^{10} (6.1)	0.0068** (0.0064)
Expt. D*	[10-^{3}H]iridodial (**12**) 1.31×10^{10} (8.2)	0.13** (0.34)
Expt. E*	[1-^{3}H]-9-hydroxy-10-oxogeranial (**13**) and [1-^{3}H]-9-oxo-10-hydroxygeranial (**14**) (3:2) 6.26×10^{9} (4.0)	0.0048 (0.0062)

* A series of Expts. A, B and C and Expts. D and E were carried out in September of different years.

** These values were corrected based on the assumption that the following proportions of the label of administered compounds remained in **10**: 1/2 of **5** and **8a**, 1/4 of **9** and 2/3 of **12**.

Table 2. Administration of Labeled Monoterpenes to Lonicera morrowii

	Spec. Activities (dpm/mM) (amount (mg)) of substance fed	Incorporation (spec. incorp.) (%) into secologanin (**2**)	Distribution of activity in HCHO from C-10	Distribution of activity in lactone (**17**)
Expt. F	[1-^{3}H]-10-hydroxygeraniol (**8a**) 6.37×10^{10} (12.8)	1.52* (0.26)		9.7
Expt. G	(±)-[10-^{3}H]-10-hydroxycitronellol (**9**) 6.46×10^{10} (17.8)	0.026* (0.011)		
Expt. H	[10-^{3}H]iridodial (**12**) 1.37×10^{10} (15.5)	1.13* (0.17)	92.6	
Expt. I	[1-^{3}H]-9,10-dihydroxygeraniol (**5**) 8.46×10^{10} (9.1)	0.30* (0.025)		6.5

* These values were corrected based on the assumption that the following proportion of the label of administered compounds remained in **2**: 1/2 of **5** and **8a**, 1/4 of **9** and 2/3 of **12**.

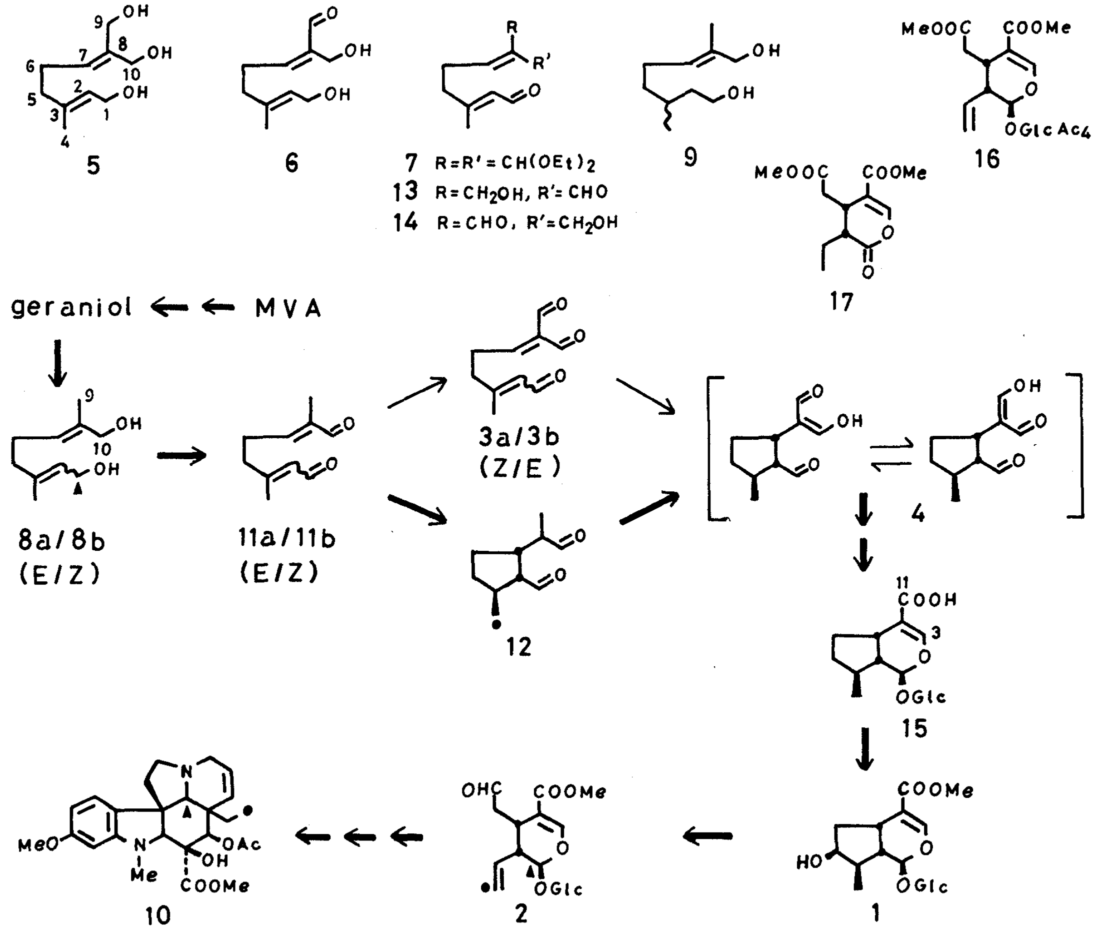

Chart 1. Biosynthetic Pathway of Loganin (**1**), Secologanin (**2**) and Vindoline (**10**) in Catharanthus roseus and Lonicera morrowii

Next, [1-^{3}H]-10-hydroxygeraniol (**8a**), (±)-[10-^{3}H]-10-hydroxycitronellol (**9**), [10-^{3}H]iridodial (**12**) and [1-^{3}H]-9,10-dihydroxygeraniol (**5**) were administered, separately, to Lonicera morrowii A. Gray (Expts. F, G, H and I). After 4 days, radioactive secologanin (**2**) was isolated and converted through acetylation, Jones oxidation and methylation to secologanoside methyl ester tetraacetate (**16**), which was recrystallized to a constant radioactivity. Compound **16** obtained in Expt. H was subjected to ozonolysis and the resulting formaldehyde was captured as the dimedone derivative. On the other hand, **16** obtained in Expts. F and I was converted, through Pd-C-catalyzed hydrogenation, Zemplén reaction, β-glucosidase-mediated hydrolysis and Jones oxidation, to lactone (**17**). The radioactivity of the formaldehyde dimedone indicated the localization of the ^{3}H label at the C-10 position of secologanin (**2**), whereas the activities of **17** showed the localization of the ^{3}H label at the C-1 position of **2**. These results are summarized in Table 2. It was found that the total and specific incorporations of both **8a** and **12** into secologanin (**2**) were much higher than those of **5** and **9**. It was therefore concluded that, like the secologanin (**2**) and vindoline (**10**) of C. roseus, the secologanin (**2**) of L. morrowii is biosynthesized via 10-oxogeranial (**11a**)/10-oxoneral (**11b**), iridodial (**12**), iridotrial (**4**), deoxyloganic acid (**15**) and loganin (**1**).

In the feeding experiments with the above two plants, the non-negligible incorporation of [1-^{3}H]-9,10-dihydroxygeraniol (**5**) into both vindoline (**10**) and secologanin (**2**) was observed. Thus, it remains to be established whether this incorporation was due to a conversion by a minor route or to a biotransformation of the compound extraneously fed. Further studies are underway.

REFERENCES AND NOTES

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

2) A. R. Battersby, M. Thomson, K.-H. Glüsenkamp and L.-F. Tietze, Chem. Ber., **114**, 3430 (1981).

3) J. Balsevich and W. G. W. Kurz, Planta Med., **49**, 79 (1983).

4) These compounds were prepared starting from geraniol, which will be described in the full publication.

5) S. Uesato, K. Kobayashi and H. Inouye, Chem. Pharm. Bull., **30**, 3942 (1982).

6) H. Inouye, S. Ueda, Y. Aoki and Y. Takeda, Tetrahedron Letters, **1969**, 2351; idem, Chem. Pharm. Bull., **20**, 1287 (1972).

7) A. R. Battersby, A. R. Burnett and P. G. Parsons, Chem. Commun., **1970**, 826.

(Received February 27, 1984)