Communications to the Editor

[Chem. Pharm. Bull. 32(9)3764 3767(1984)]

INTERMEDIACY OF 10-HYDROXYGERANIOL, 10-HYDROXYNEROL AND IRIDODIAL IN THE BIOSYNTHESIS OF AJMALINE AND VOMILENINE IN RAUWOLFIA SERPENTINA SUSPENSION CULTURES

Shinichi Uesato,[a] Satoko Matsuda,[a] Akira Iida,[a] Hiroyuki Inouye*,[a] and Meinhart H. Zenk[b]

Faculty of Pharmaceutical Sciences, Kyoto University,[a] Sakyo-ku, Kyoto 606, Japan and Institut für Pharmazeutische Biologie, Universität München,[b] 8000 München 2, W. Germany

Administration of ^{2}H- and ^{13}C-labeled compounds to Rauwolfia serpentina suspension cultures indicated that ajmaline (4) and vomilenine (5) produced by these cultures are biosynthesized via 10-hydroxygeraniol (6)/10-hydroxynerol (7) and iridodial (3) in the same way as secologanin (1), vindoline, etc. in Catharanthus roseus and Lonicera morrowii. Therefore, this cyclization mechanism seems likely to be common in plants containing secoiridoids and indole alkaloids.

KEYWORDS ——— Apocynaceae; Rauwolfia serpentina; suspension culture; ajmaline; vomilenine; iridodial; biosynthesis; ^{13}C-NMR; gated decoupling-without-NOE

In our previous paper,[1] we demonstrated by feeding experiments with ^{3}H-labeled monoterpenes that, in contrast to the mechanism proposed by Balsevich and Kurz,[2] secologanin (1) and vindoline of Catharanthus roseus and Lonicera

morrowii are biosynthesized via cyclization of 10-oxogeranial (2a)/10-oxoneral (2b) to iridodial (3) and subsequent oxidation to iridotrial. In order to examine the feasibility of extending this mechanism to other plants containing seco-iridoids and indole alkaloids, ^{13}C- and ^{2}H-labeled possible precursors were fed to Rauwolfia serpentina suspension cultures[3]. These cultures produce significant amounts of ajmaline (4) and vomilenine (5) and were thus expected to metabolize the administered precursors in high ratios.

A mixture of [4-^{13}C]-10-hydroxygeraniol (6), [9-^{13}C]-10-hydroxynerol (7), [2-^{13}C]-9,10-dihydroxygeraniol (8)[4a] and [10-$^{2}H_2$]iridodial (3)[4b] was administered to Rauwolfia serpentina suspension cultures five days after inoculation. The

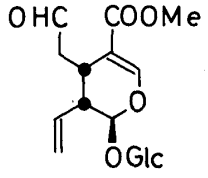
Table 1. Administration of ^{2}H- and ^{13}C-Labeled Putative Precursors to Rauwolfia serpentina Suspension Cultures

Compound fed (amount (mg))	Incorporation (spec. incorp.) (%) Ajmaline (4)	Dihydrovomilenine (9)
[4-^{13}C]-10-Hydroxygeraniol (6) (15.4)	2.3 (0.8)	7.1 (1.8)
[9-^{13}C]-10-Hydroxynerol (7) (16.0)	1.9 (0.7)*	7.2 (1.9)*
[2-^{13}C]-9,10-Dihydroxygeraniol (8) (19.4)	0.3 (0.1)	1.2 (0.3)
[10-$^{2}H_2$]Iridodial (3) (18.3)	14.4 (6.0)	10.7 (3.2)**

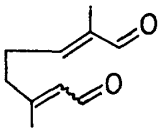
* Calculations of incorporation rates were based on the presumption that the scrambling of the carbons corresponding to the C-9 and C-10 of 7 takes place during the biosynthesis of 4 and 5.

** The values seem to be somewhat low as compared with the values for ajmaline (4). This may be due to the elimination of deuterium which would take place during the catalytic reduction of 5.

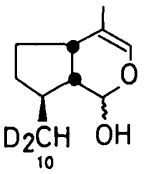
cultures were incubated for eight further days and the cells then frozen in liquid nitrogen and extracted with AcOEt containing phosphate buffer (1M, pH 8.5). The AcOEt layer was extracted with 2% H_2SO_4, and the acid layer was basified with 10% NH_4OH and extracted with $CHCl_3$. The residue of the $CHCl_3$ layer was chromatographed on silica gel to give ajmaline (4) and vomilenine (5).[3] The latter was converted, through Pd-C-catalyzed reduction, to the more stable dihydrovomilenine (9). The ^2H- and ^{13}C-NMR spectra of labeled 4 and 9 were then recorded. The ^2H-NMR spectra were taken under normal conditions, and the incorporation ratios of ^2H-labeled 3 into 4 and 5 were calculated by comparing the signal intensity at δ 7.26 (^2H due to the natural abundance of $CHCl_3$ used as the solvent) with those at δ 0.93 (18-^2H) in 4 and 0.98 (18-^2H) in 9, respectively. On the other hand, the ^{13}C-NMR spectra were recorded by using a gated decoupling-without-NOE technique[5] (acquisition time, 0.8190 s; pulse delay, 35.0000 s; temperature, 27° C) in order to integrate each signal intensity accurately. The incorporation ratios of ^{13}C-labeled 6, 7 and 8 into 4 and 5 were calculated by measuring the percentage enhancements of the signals at δ 12.21 (18-C), 77.69 (17-C) and 47.97 (20-C) in 4 as well as at δ 11.92 (18-C), 78.57 (17-C) and 47.04 (20-C) in 9. The results are summarized in Table 1.



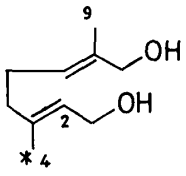
1



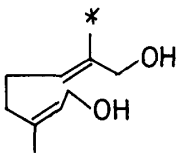
2a: *E*-form
2b: *Z*-form



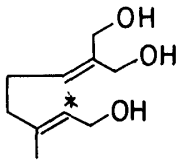
3



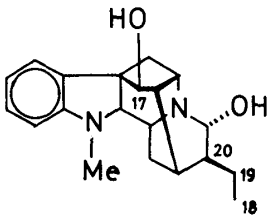
6



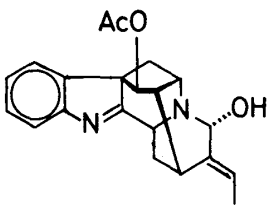
7



8



4



5

9: 19,20-dihydro

If 9,10-dihydroxygeraniol (8), which would be formed from 10-hydroxygeraniol (6)/10-hydroxynerol (7), serves as one of the intermediates in the biosynthesis of 4 and 5, the incorporation rates of 8 into 4 and 5 could be higher than those of 6/7. In practice, however, the total and specific incorporation ratios of 6 and 7 into 4 and 5 were much higher than those of 8. This finding, together with the much higher incorporation of 3 into 4 and 5, indicated that not 9,10-dihydroxygeraniol (8), but 10-hydroxygeraniol (6)/10-hydroyxynerol (7) and iridodial (3) can be the intermediates. It was therefore concluded that ajmaline (4) and vomilenine (5) in the R. serpentina suspension cultures are biosynthesized via the same cyclization process as secologanin (1) and vindoline in C. roseus and L. morrowii.

We have so far established that the secoiridoids and indole alkaloids of the above three plants are biosynthesized in the same way, via 10-hydroxygeraniol (6)/10-hydroxynerol (7) and iridodial (3). Therefore, this cyclization mechanism seems likely to be common throughout the plant species containing secoiridoids and indole alkaloids.

ACKNOWLEDGEMENT The work at Kyoto was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. The work at Munich was supported by SFB 145 of Deutsche Forschungsgemeinschaft.

REFERENCES

1) S. Uesato, S. Matsuda and H. Inouye, Chem. Pharm. Bull., 32, 1672 (1984).
2) J. Balsevich and W. G. W. Kurz, Planta Med., 49, 79 (1983).
3) J. Stöckigt, A. Pfitzner and J. Firl, Plant Cell Reports, 1, 36 (1981).
4) a) S. Uesato, K. Kobayashi and H. Inouye, Chem. Pharm. Bull., 30, 927 (1982); b) Idem, ibid., 30, 3942 (1982).
5) D. Shaw, "Fourier Transform N.M.R. Spectroscopy," Elsevier Scientific Publishing Company, Amsterdam, Oxford and New York, 1976, p. 290.

(Received July 18, 1984)