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BIOSYNTHESIS OF IRIDOID GLUCOSIDES IN *PATRINIA GIBBOSA*[1]

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ABSTRACT.—In contrast to the case of the nepetalactone-type iridolactones in *Teucrium marum* and in *Nepeta cataria*, 10-hydroxygeraniol [**1**] is a more potent precursor than 10-hydroxycitronellol [**2**] for biosynthesis of the iridoid glucosides, gibboside [**4**] and patrinoside [**5**], in *Patrinia gibbosa*. A hydride shift such as occurs in the biosynthesis of the nepetalactone-type iridolactones does not take place during the biosynthesis of isoiridomyrmecin-type iridolactone **4**.

Regarding the biosynthesis of iridolactones, it was found by Trave and co-workers that some iridolactones such as dolicholactone in *Teucrium marum* L. (1) and nepetalactone in *Nepeta cataria* (2) are biosynthesized through somewhat different routes from those for the usual iridoid glucosides and indole alkaloids. The biosynthesis of dolicholactone and nepetalactone features the following points: (a) acyclic monoterpenes of the citronellol series are more important precursors than those of the geraniol series and (b) a hydride ion shift of the Cannizzaro type occurs from C-1 to C-3 at the stage of iridodial [**3**].

The biosynthetic pathway for iridoid glucosides having a methyl or a carboxyl group at C-4, such as those in plants of *Lamium amplexicaule* and *Gardenia jasminoides* f. *grandiflora*, was previously established as shown in Figure 1, in which 10-oxogeranial derived from 10-hydroxygeraniol [**1**] undergoes reductive cyclization to iridodial [**3**]

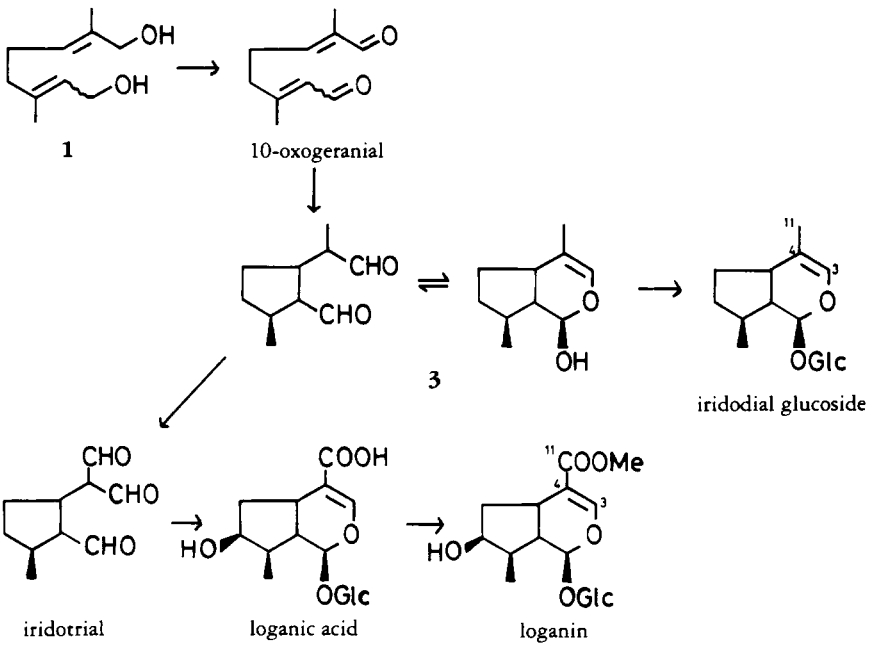


FIGURE 1. Proposed biosynthetic pathway for iridoid glucosides with a methyl or a carboxyl group at C-4.

[1]This paper is dedicated to Prof. Haruaki Yajima on the occasion of his retirement from Kyoto University in March 1989.

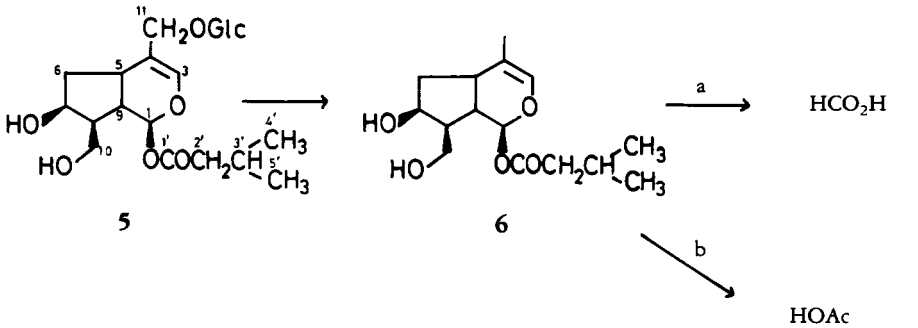
(3). On the other hand, the biosynthesis of iridoids with a C-4–CH_2OR group has not yet been elucidated.

In the present work, we investigated the biosynthesis of the glucosidic iridolactone gibboside [**4**] (isoiridomyrmecin type) (4) and the C-4-glucosyloxymethyl iridoid patrinoside [**5**], in *Patrinia gibbosa* (Valerianaceae).

RESULTS AND DISCUSSION

^{3}H-Labeled putative precursors [10-^{3}H]-10-hydroxygeraniol, (±)-[10-^{3}H]-10-hydroxycitronellol, and [6,7,8,10-$^3H_4$]-iridodial were prepared by the previously reported methods (5–8), whereas [1-^{3}H]-**1** was synthesized by a two-step reaction: 10-hydroxygeranial, prepared by SeO_2 oxidation of citral, was reduced with NaB^3H_4 to give [1-^{3}H]-**1**. These labeled compounds were then administered hydroponically to the roots of *P. gibbosa* at a flowering period in the end of September.

The distribution of ^{3}H radioactivity in the isolated patrinoside [**5**] was determined by chemical degradations of the hydrogenolysis product **6** to HCO_2H and HOAc (Scheme 1). The resulting acids were converted to α-naphthylamides and analyzed by liquid scintillation counting.



SCHEME 1. Chemical degradations of patrinoside [**5**]: (a) ozone oxidation,(b) Kuhn-Roth oxidation.

Table 1 summarizes the incorporation ratios of the labeled putative precursors into the glucosides gibboside [**4**] and patrinoside [**5**]. The incorporations of labeled **1** and **3** into **5** were very high, while that of labeled **2** was negligible. This suggests that the iridoid glucosides in *P. gibbosa* are formed not through 10-hydroxycitronellol [**2**], but through 10-hydroxygeraniol [**1**] as are the usual iridoid glucosides. The difference observed could also theoretically be due to differences in the success of the substrates getting to the relevant enzyme(s), but this is unlikely in view of the similarity of the substrates. This finding is in contrast to the results in *T. marum* and *N. cataria* (1,2).

A second observation is that the ^{3}H-label originating from [10-^{3}H]-10-hydroxygeraniol was retained at C-3 but not at C-11 of partrinoside [**5**]. This indicates that the biosynthesis of **5** most likely proceeds with retention of the nonequivalency of oxidation steps at the C-3 and C-11 carbon atoms.

Finally, [1-^{3}H]-10-hydroxygeraniol was significantly incorporated into iridolactone **4**, but [10-^{3}H]-**1** was not. This fact indicates that the hydride ion shift from C-3 to C-1 of iridodial [**3**] (the reverse direction of the shifts in dolicholactone and nepetalactone) does not take place during the biosynthesis of gibboside [**4**].

Therefore, it is concluded that the iridoid glucosides in *P. gibbosa* (Valerianaceae) are biosynthesized through 10-hydroxygeraniol [**1**] as shown in Figure 2, in contrast to the iridolactone pathway in *T. marum* and *N. cataria* (Labiatae).

TABLE 1. Incorporations of ^{3}H-Labeled Putative Precursors into Iridoid Glucosides in *Patrinia gibbosa*.

Compound fed (activity × 10^8 dpm)	Glucosides isolated				Distribution of radioactivity in HCO_2H[b] from C-3 of **6** derived from isolated **5** (%)	Distribution of radioactivity in HOAc[b] from C_4–C_{11}, $C_{3'}$–$C_{4'}$, and $C_{3'}$–$C_{5'}$ of **6** derived from isolated **5** (%)
	Gibboside [**4**][a]		Patrinoside [**5**][a]			
	Specific activity (× 10^4 dpm/mM)	Total incorporation (%)	Specific activity (× 10^4 dpm/mM)	Total incorporation (%)		
[1-^{3}H]-**1** (4.0)	20	0.014	1129	7.9	0.0	—
[10-^{3}H]-**1** (2.5)	1	0.00087	145	2.5	70.6	0.6
(±)-[10-^{3}H]-**2** (6.6)	3	0.00090	23	0.04	—	—
[6,7,8,10-$^3H_4$]-**3** (2.4)	—	—	1020	5.0	—	—

[a]Purified and calculated as pentaacetate or hexaacetate.

[b]Isolated and purified as α-formylnaphthylamide or α-acetylnaphthylamide.

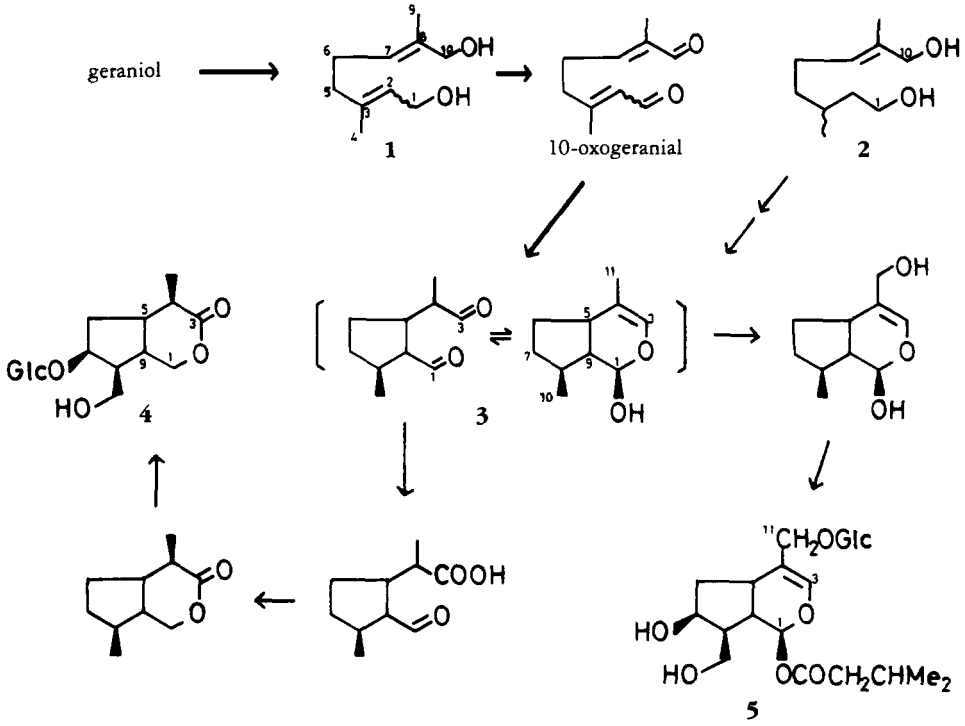


FIGURE 2. Biosynthetic pathway of gibboside [**4**] and patrinoside [**5**] in *Patrinia gibbosa*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Kieselgel 60 (Merck) and activated charcoal (Wako) were used for cc. Si gel 60 GF_{254} (Merck) and Si gel 60 F_{254} (Merck) were used for tlc and preparative tlc, respectively. Spots and bands of nonradioactive compounds were detected under uv (254 nm) or by I_2 exposure and those of radioactive compounds on an ALOKA radiochromanyzer. Unless otherwise noted, main bands were scraped off and extracted with $CHCl_3$-MeOH (9:1), and the extracts were concentrated in vacuo. Radioactivities were measured on an ALOKA-900 liquid scintillation counter with samples dissolved in scintillation solutions of 2,5-diphenyloxazole (PPO, 20 mg) and 2,2-phenylenebis(5-phenyloxazole) (POPOP, 0.25 mg) in toluene (5 ml).

Physical data of radioactive compounds are those for nonlabeled compounds prepared in preliminary model reaction.

PLANT MATERIALS.—*P. gibbosa* was collected in Sado Island, Niigata Prefecture of Japan in September 1986. A voucher specimen (S. Uesato and H. Nishimura No. 1) has been deposited in the Herbarium of the Institute of Botany, Faculty of Science, Kyoto University (KYO), Kitashirakawa-oiwake-Cho, Sakyo-Ku, Kyoto 606, Japan.

[1-^{3}H]-10-HYDROXYGERANIOL.—To a suspension of SeO_2 (89.2 mg) in CH_2Cl_2 (5 ml), solutions of citral (0.34 ml) in CH_2Cl_2 (4.8 ml) and of 70% *tert*-butyl hydroperoxide in H_2O (0.45 ml) were added. The mixture was stirred for 5 h at room temperature in the dark and filtered. The filtrate was washed with 5% $NaHCO_3$ and saturated NaCl and concentrated. The residue was subjected to preparative tlc ($CHCl_3$, multiple developments) to give 10-hydroxygeranial as an oil (210.4 mg). Subsequently, NaB^3H_4 (50 mCi) was added to a solution of 10-hydroxygeranial (10.8 mg) in EtOH (1 ml) under ice cooling, and the whole was stirred for 10 min. $NaBH_4$ (4.0 mg) was then added to the mixture. After being stirred for a further 30 min, the reaction mixture was neutralized with 10% HOAc and subjected to preparative tlc [$CHCl_3$-MeOH (9:1)] to give [1-^{3}H]-**1** (43.6 mCi).

ADMINISTRATIONS OF [1-^{3}H]- AND [10-^{3}H]-10-HYDROXYGERANIOL, (±)-[10-^{3}H]-10-HYDROXYCITRONELLOL, AND [6,7,8,10-$^3H_4$]-IRIDODIAL TO *P. GIBBOSA*.—A solution of each ^{3}H-labeled compound in MeOH (137, 150, 150, and 150 μl) was diluted with H_2O (2 ml) and administered hydroponically to the roots of *P. gibbosa* for 2 days. The roots (13.7, 43.5, 6.9, and 25.0 g) were extracted with

MeOH (200 ml × 3) under reflux, and the combined extracts were concentrated in vacuo. The residue was taken up in H_2O. The insoluble materials were filtered off over a Celite layer. The filtrate was transferred to an activated charcoal (7–14 g) column and eluted successively with H_2O (200–400 ml), 20% MeOH (200–400 ml), and MeOH (500–600 ml). The MeOH eluate was concentrated to afford a mixture of radioactive gibboside [**4**] and patrinoside [**5**]. The mixture, after acetylation, was chromatographed on a Si gel (25–40 g) column with $CHCl_3$ as eluent, giving gibboside pentaacetate (154.1, 130.8, and 93.4 mg) and patrinoside hexaacetate (1972.5, 3019.0, 817.4, and 835.0 mg). An aliqout of the respective acetate (gibboside pentaacetate 83.0, 32.2, and 93.4 mg; patrinoside hexaacetate 733.7, 285.5, 383.6, and 835.0 mg) was then recrystallized from EtOH to constant sp radioactivities.

DEGRADATIONS OF PATRINOSIDE [**5**].—Aliquots of patrinoside hexaacetate labeled from [1-^{3}H]- and [10-^{3}H]-**1** (127.8 and 119.9 mg) were separately hydrogenated over 5% Pd-C (131.7 mg) in MeOH (5 ml) followed by hydrolysis with 1 N methanolic NaOH to give crude reduction product **6** (126.5 and 123.4 mg). This compound was then ozonized in dry CH_2Cl_2 (5 ml) at $-60°$ for 3 h, and the reaction mixture was concentrated. The residue was diluted in H_2O (80 ml), and the H_2O solution was steam distilled. The distillate (50 ml), after basification with 1 N NaOH, was concentrated to give a residue. To a solution of the residue in H_2O (4 ml) (pH 4–5), α-naphthylamine (15 mg) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (50 mg) were added. The whole was stirred for 1 h at room temperature and extracted with CH_2Cl_2 (8 ml × 3). The residue of the CH_2Cl_2 extract was subjected to preparative tlc [C_6H_6-EtOAc (7:3)] to give α-formylnaphthylamide (3.51 and 1.18 mg), which was purified by repeated preparative tlc to constant sp radioactivity.

Compound **6** (48.6 mg) labeled from [10-^{3}H]-**1** was added to a 2 N H_2SO_4 solution (10 ml) containing CrO_3 (5 g), and the mixture was steam distilled for 6 h gradually adding H_2O (50 ml). The distillate (50 ml) was basified with 1 N NaOH and concentrated to give a residue, which was treated in the same way as described above to afford α-acetylnaphthylamide (5.5 mg).

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