[Chem. Pharm. Bull. 33(10)4228—4234(1985)]

Studies on Monoterpene Glucosides and Related Natural Products. LV.[1] Iridane Skeleton Formation from Acyclic Monoterpenes in the Biosynthesis of Iridoid Glucosides in *Gardenia jasminoides* f. *grandiflora* Cell Suspension Cultures

KOJI KOBAYASHI, SHINICHI UESATO, SHINICHI UEDA and HIROYUKI INOUYE*

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

(Received February 15, 1985)

Administration of ^{3}H- or ^{13}C-labeled acyclic monoterpenes to *Gardenia jasminoides* f. *grandiflora* cell suspension cultures showed that tarennoside (**20**) and gardenoside (**21**) were biosynthesized in the cell cultures *via* the cyclization of 10-oxogeranial (**5a**)/10-oxoneral (**5b**) to iridodial cation (**14**), followed by extensive randomization of the carbon atoms 3 and 11. Furthermore, the intermediacy of (*R*)-(+)- and (*S*)-(−)-10-hydroxycitronellol (**24a**, **24b**) and (*R*)-(+)- and (*S*)-(−)-9,10-dihydroxycitronellol (**28a**, **28b**) was disproved.

Keywords——iridoid glucoside; biosynthesis; iridane skeleton formation; 10-oxocitral; iridodial cation; tarennoside; *Gardenia jasminoides*; cell culture

Several mechanisms have so far been proposed for iridane skeleton formation from acylic monoterpenes in the biosynthesis of iridoids: cyclization of 9,10-dioxogeranial (**1a**) (or 9,10-dioxoneral (**1b**)) to iridotrial (**2**) followed by further elaboration with randomization of C-3 and C-11 of the latter would lead to loganin (**3**), secologanin (**4**), *etc.*[2,3] Furthermore, cyclization of 10-oxogeranial (**5a**) (or 10-oxoneral (**5b**)) to iridodial (**6**), followed by further elaboration not involving scrambling of C-3 and C-11 of the latter would lead to deutzioside (**7**), asperuloside (**8**), *etc.*[4] On the other hand, mechanisms involving cyclization of (*S*)-(−)-10-oxocitronellal (**9b**)[2,3,5] (which was shown not to be a precursor of secoiridoids and indole alkaloids[2,3]) or (*S*)-(−)-9-hydroxy-10-oxocitronellal (**10**)[5] have recently been proposed for the biosynthesis of some non-glycosidic iridoids including dolichodial (**11**), dolicholactone (**12**) and nepetalactone (**13**). Besides the above-mentioned iridoid glucosides, there are many others with cyclopentane rings of diverse oxidation levels, suggesting the existence of a variety of cyclization mechanisms.

If iridodial cation (**14**),[6] which might be formed from **5a** or **5b**, is postulated as a common key intermediate of iridoid glucosides, their cyclization mechanisms can be systematically interpreted: one initiated by a hydride ion attack on C-8 of **14** from the α- or β-side, yielding iridodial (**6**) (route a) or 8-epiiridodial (**15**) (route b); one starting by elimination of the proton on C-7 or C-9 giving 7,8-dehydroiridodial (**16**) (route c) or 8,9-dehydroiridodial (**17**) (route d), respectively.

Based on this premise, loganin (**3**) should be biosynthesized *via* route a. However, the iridoid glucosides, for which the precursorship of 8-epideoxyloganic acid (**18**) or its methyl ester (**19**) has recently been proved,[7,8] should be formed *via* route b.

In order to examine in detail the various iridane skeleton formation mechanisms, including the above possibilities, we administered various ^{3}H- or ^{13}C-labeled putative precursors to *Gardenia jasminoides* suspension cultures,[9] which produce tarennoside (**20**) and

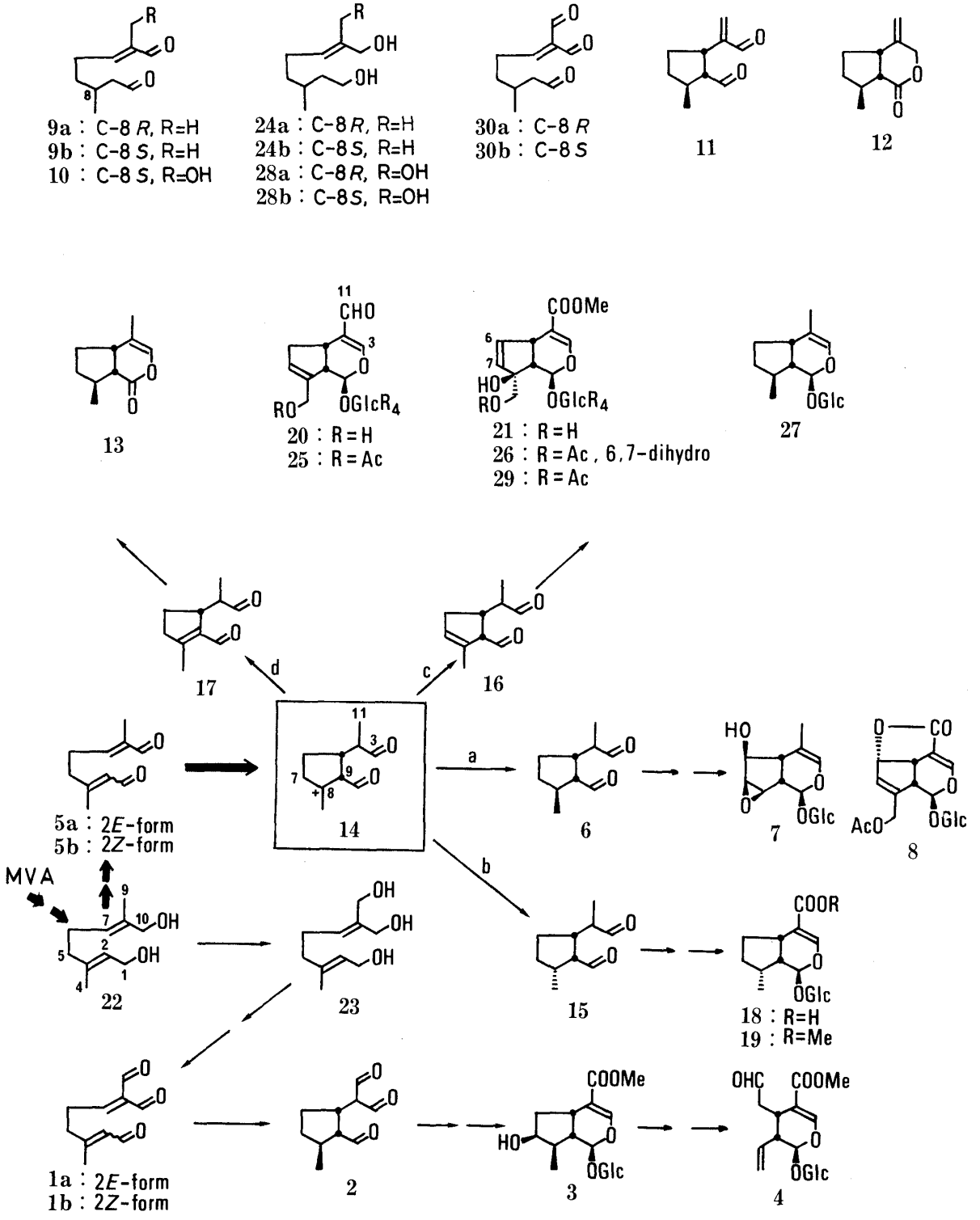

Fig. 1

gardenoside (**21**) in fairly good yields and are thus expected to convert the fed precursors to the glucosides in high ratios.

Prior to experiments with ^{13}C-labeled compounds, the following ^{3}H-labeled acyclic monoterpenes were fed separately to the suspension cultures four weeks after inoculation: [10-^{3}H]-10-hydroxygeraniol (**22**), [1-^{3}H]-9,10-dihydroxygeraniol (**23**) and (*RS*)-(±)-[10-^{3}H]-10-hydroxycitronellol (**24a**, **b**).[10] After 10 d of incubation, tarennoside (**20**) and gardenoside (**21**) were isolated and purified after conversion to the pentaacetate (**25**) and dihydropentaacetate

TABLE I. Results of Administration of ^{3}H-Labeled Acyclic Monoterpenes to *Gardenia* Cell Suspension Cultures

Compounds administered Total act. (mCi) and amount (mg)	Incorporation (spec. incorp.)[a] into tarennoside (**20**)	gardenoside (**21**)	% distribution of radioactivity of tarennoside (**20**) in CH_3COOH from C-4 and C-11	HCOOH from C-3
[10-^{3}H]-10-Hydroxygeraniol (**22**)	5.45	0.122	50.4	46.2
0.176 2.19	(6.64)	(0.141)		
[1-^{3}H]-9,10-Dihydroxygeraniol (**23**)	3.95	0.0607	—	—
0.311 1.52	(1.10)	(0.0067)		
(*RS*)-(±)-[10-^{3}H]-10-Hydroxycitronellol (**24a, b**)	0.577	0.0273	—	—
	(0.913)	(0.0136)		
0.474 2.08				

a) Loss of ^{3}H due to oxidation was disregarded.

(**26**), respectively. The acetate **25**, obtained after the administration of [10-^{3}H]-**22**, was hydrogenated over Pd–C followed by deacetylation to give iridodial glucoside (**27**). This compound was subjected to the Kuhn–Roth oxidation, and the resultant acetic acid, originating from C-4,11 and C-8,10 of **27**, was converted into acetyl-α-naphthylamide. Compound **27** was also subjected to ozonolysis and the resultant formic acid, corresponding to C-3 of **27**, was converted into the formyl-α-naphthylamide. These results indicated that both [10-^{3}H]-**22** and [1-^{3}H]-**23** were incorporated at much higher rates than (*RS*)-(±)-[10-^{3}H]-**24a, b** into **20** and **21** and that the label originating from **22** was located half on C-3 and half on C-11 of **20** (Table I). Thus, tarennoside (**20**) and gardenoside (**21**) were concluded to be biosynthesized *via* a process involving extensive randomization of the terminal carbons 9 and 10 of **22**. However, it remained to be examined whether the geraniol or citronellol series is on the main pathway and whether dioxo or trioxo derivatives of both series are involved in the cyclization. Examination of this problem seemed likely to be hindered by the fluctuation in the growth rates and enzyme activities of the cell cultures used for the individual administrations with the ^{3}H-labeled compounds. This fluctuation made it difficult to determine which of the normal and secondarily induced pathways caused the conversion to **20** and **21**. Thus, simultaneous administrations of various combinations of the following ^{13}C-labeled acyclic monoterpenes[11,12] were attempted: [4-^{13}C]-10-hydroxygeraniol (**22**), [2-^{13}C]-9,10-dihydroxygeraniol (**23**), (*S*)-(−)- and (*R*)-(+)-[9-^{13}C]-10-hydroxycitronellol (**24a**, **24b**) and (*S*)-(−)-[8-^{13}C]-9,10-dihydroxycitronellol (**28b**).

Before simultaneous administrations of these compounds, [9-^{13}C]-10-hydroxygeraniol (**22**) was fed to the cell cultures two weeks after inoculation, and tarennoside (**20**) and gardenoside (**21**) were isolated as their pentaacetates (**25** and **29**). In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **25**, the enrichment factors (EFs)[13] of C-3 (δ 160.2) and C-11 (δ 190.0) were 6.0 and 6.5%, respectively. Furthermore, in the ^{13}C-NMR spectrum of **29** measured in the presence of a paramagnetic reagent, tris(acetylacetonate)-chromium (III),[14] the EFs of C-3 (δ 149.1) and C-11 (δ 166.3) were 0.7 and 0.60%, respectively. Therefore, the feeding experiment using ^{13}C-labeled compound corroborated the incorporation of 10-hydroxygeraniol (**22**) into tarennoside (**20**) and gardenoside (**21**) as well as the randomized distribution of the terminal carbons 9 and 10 of **22** in the 3 and 11 positions of both glucosides.

Subsequently, a mixture of [4-^{13}C]-10-hydroxygeraniol (**22**), [2-^{13}C]-9,10-dihydroxygeraniol (**23**), (*S*)-(−)-[9-^{13}C]-10-hydroxycitronellol (**24b**) and (*S*)-(−)-[8-^{13}C]-9,10-di-

hydroxycitronellol (**28b**) was fed to the cell cultures two weeks after inoculation. After 5 d of incubation, tarennoside (**20**) was isolated as its acetate (**25**). Its ^{13}C-NMR spectrum showed that ^{13}C-labels originating from [4-^{13}C]-**22** and [2-^{13}C]-**23** were incorporated into C-10 (δ 61.5) and C-9 (δ 46.4) (EFs, 5.7 and 1.4%), respectively. However, enrichments of the signals of C-11 (δ 190.0) and C-4 (δ 124.5), which should be derived from (*S*)-(−)-[9-^{13}C]-**24b** and (*S*)-(−)-[8-^{13}C]-**28b**, respectively, were not observed. In view of the non-negligible incorporation of (*RS*)-(±)-[^{3}H]-**24a, b** into **20** and **21**, the above results could be explained by the preferential occupation of the enzymes by 10-oxogeranial (**5a**)/10-oxoneral (**5b**) and 9,10-dioxogeranial (**1a**)/9,10-dioxoneral (**1b**) formed from **22** and **23**, respectively, which might have prevented the incorporation of (*S*)-(−)-**24b** and (*S*)-(−)-**28b**. Thus, in the next experiment, the cell cultures were first incubated with (*S*)-(−)-[9-^{13}C]-10-hydroxycitronellol (**24b**) for 5 d and then with [4-^{13}C]-10-hydroxygeraniol (**22**) for an additional 7 d. An analogous experiment was also carried out with (*R*)-(+)-[9-^{13}C]-10-hydroxycitronellol (**24a**) and [4-^{13}C]-10-hydroxygeraniol (**22**). In both cases, the ^{13}C-NMR spectrum of the acetate (**25**) of tarennoside (**20**) showed only enrichment of the C-10 signal (EFs, 3.0 and 3.4%), which should originate from [4-^{13}C]-10-hydroxygeraniol (**22**). Therefore, the intermediacy of (*R*)-(+)- and (*S*)-(−)-10-oxocitronellal (**9a**, **9b**) and (*R*)-(+)- and (*S*)-(−)-9,10-dioxocitronellal (**30a**, **30b**), which should be derived from (*R*)-(+)- and (*S*)-(−)-10-hydroxycitronellol (**24a**, **24b**) and (*R*)-(+)- and (*S*)-(−)-9,10-dihydroxycitronellol (**28a**, **28b**), was ruled out regardless of the results of the experiments with the ^{3}H-labeled compounds.

The next problem was the possibility of 9,10-dihydroxygeraniol (**23**) as an intermediate.[15] In experiments with [1-^{3}H]-**23** and with the four ^{13}C-labeled compounds, **23** was incorporated into tarennoside (**20**) at non-negligible rates. Therefore, the possibility still remains that **20** would be formed *via* **5a**/**5b** and **1a**/**1b** (equivalent to **22** and **23**, respectively), though the discrepancy between the EFs of **22** and **23** makes it unlikely. If such a route exists, the specific incorporation ratio of [2-^{13}C]-**23** into **20** should be reduced by administration of a comparatively large amount of non-labeled **22** prior to [2-^{13}C]-**23**. An analogous administration of a large amount of non-labeled **23** together with [4-^{13}C]-**22** should also reduced the incorporation ratio of [4-^{13}C]-**22** into **20**. In practice, however, these experiments showed that the specific incorporation ratios of ^{13}C-labeled **22** and **23** into tarennoside (**20**) ($\fallingdotseq$EFs, 5.6 and 1.2%, respectively) were almost the same as those in the simultaneous administration of four ^{13}C-labeled monoterpenes. Thus, the intermediacy of 9,10-dihydroxygeraniol (**23**) between 10-hydroxygeraniol (**22**) and tarennoside (**20**) was ruled out. The constant non-negligible incorporations of **23** into **20** could be interpreted in terms of a biotransformation through a minor route *via* **1a**/**1b**,[15] or of a conversion of an unnatural substrate by the cultured cells. From the results mentioned so far, it seems most likely that the main synthetic pathway of tarennoside (**20**) and gardenoside (**21**) in *G. jasminoides* cell cultures involves the cyclization of **5a**/**5b** to iridodial cation (**14**) as depicted by bold lines in Fig. 1. The pathway after **14** is considered to start from iridodial (**6**), 8-epiiridodial (**15**) or 7,8-dehydroiridodial (**16**) following the oxidation of the 11-methyl group and randomization of the carbon atoms 3 and 11, as we shall describe in a separate paper.

Experimental

General Procedures——Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM-PMX 60 spectrometer with tetramethylsilane (TMS) as the internal standard. ^{13}C-NMR spectra were taken on a JNM FX 100 FT NMR spectrometer at 25.0 MHz under the following conditions: 8K data points, 45° pulse, repetition 2.5 s; samples in $CDCl_3$ with TMS as the internal standard. Active charcoal (Wako) was used for column chromatography. Preparative thin layer chromatography (TLC) was carried out on silica gel plates (Kieselgel 60 PF_{254}, Merck, 20 × 20 cm, 1.0 mm thick). Unless otherwise noted, the main bands were scraped off and extracted with $CHCl_3$–

MeOH (9 : 1), and the extracts were concentrated *in vacuo*. Radioactivity was measured in a Beckman liquid scintillation counter, model LS 230, with samples dissolved in a scintillation cocktail consisting of toluene (10 ml), 2,5-diphenyloxazole (PPO) (40 mg) and 2,2′-*p*-phenylenebis(5-phenyloxazole) (POPOP) (0.5 mg). Specific activities are expressed as values before dilution.

Administration of [10-^{3}H]-10-Hydroxygeraniol (22) to *G. jasminoides* Cell Cultures——[10-^{3}H]-10-Hydroxygeraniol (**22**) (2.19 mg, 0.176 mCi) dissolved in EtOH (2 ml) containing Tween 80 (2 drops) was administered to the cell cultures (200 ml) 4 weeks after inoculation. After incubation for 10 d, the cultured cells (fresh weight 38 g) were collected by filtration and extracted with MeOH (50 ml × 4) under reflux. The combined extracts were concentrated *in vacuo*. The residue was dissolved in H_2O (5 ml), poured onto an active charcoal (5 g) column and eluted successively with H_2O (200 ml), H_2O–MeOH (9 : 1, 8 : 2, 200 ml each) and MeOH (400 ml). Concentration of the MeOH eluate *in vacuo* gave a residue, which was subjected to preparative layer chromatography (PLC) ($CHCl_3$–MeOH, 8 : 2, 3 developments) to give tarennoside (**20**) (4.00 mg) and gardenoside (**21**) (4.25 mg).

Acetylation of Tarennoside (20)——Radioactive **20** (4.00 mg) thus obtained was acetylated with 0.5 ml each of Ac_2O and pyridine. The product was sujbected to PLC (ether) to give tarennoside pentaacetate (**25**) (5.70 mg), which was diluted with the carrier (54.37 mg) and recrystallized from EtOH to constant specific activity (2.13×10^9 dpm/mmol).

Conversion of Radioactive Gardenoside (21) to Dihydrogardenoside Pentaacetate (26)——Radioactive **21** (4.25 mg) was diluted with the carrier (47.62 mg) and acetylated with 1 ml each of Ac_2O and pyridine. The product was subjected to PLC (ether) to give gardenoside pentaacetate (**29**) as a white powder (53.97 mg), which was hydrogenated over 5% Pd–C (50 mg) in MeOH (10 ml) until 1 mol of H_2 had been taken up. The catalyst was filtered off and the filtrate was concentrated *in vacuo* to give a residue, which was purified by PLC (ether) to give **26** as colorless needles (29.70 mg). The acetate **26** was diluted with the carrier (26.47 mg) and recrystallized from ether to constant specific activity (4.52×10^7 dpm/mmol).

Conversion of Radioactive Tarennoside Pentaacetate (25) to Iridodial Glucoside (27)——i) Cold Run: Compound **25** (253.5 mg) was hydrogenated over 5% Pd–C (100 mg) in AcOEt (10 ml) until 3.8 mol of H_2 had been taken up. After filtration, the filtrate was concentrated *in vacuo* to give a residue (198.4 mg), which was subjected to PLC (ether) to give colorless needles (91.0 mg), mp 143—144.5 °C. This compound was dissolved in dry MeOH (2 ml) containing 0.1 N methanolic $NaOCH_3$ (0.2 ml). The solution was refluxed for 5 min, neutralized with Amberlite IR-120 (H^+-form) and then concentrated *in vacuo* to yield colorless needles (**27**) (56.7 mg), mp 167—169 °C. This compound was identified as iridodial glucoside (**27**) by mixed mp and comparison of the ^{1}H-NMR spectrum with that of an authentic sample. ^{1}H-NMR (CD_3OD) δ: 1.06 (d, J=5.8 Hz, 10-H_3), 1.52 (d, J=10 Hz, 11-H_3), 5.06 (d, J=4.2 Hz, 1-H), 5.97 (m, 3-H).

ii) Hot Run: A mixture of radioactive **25** (10.23 mg, spec. activity 2.02×10^8 dpm/mmol) and the carrier (75.04 mg) was hydrogenated over 5% Pd–C (50 mg) in AcOEt (10 ml) in the same way as above to give colorless needles (19.98 mg), which were diluted with the carrier (369.9 mg) and recrystallized repeatedly from EtOH to give colorless needles (275.1 mg) of constant specific activity (1.24×10^6 dpm/mmol). This acetate was deacetylated with $NaOCH_3$ in dry MeOH (5 ml) to afford **27** as colorless needles (176.8 mg).

Kuhn–Roth Oxidation and Ozonolysis of Radioactive Iridodial Glucoside (27)——An aliquot (66.0 mg) of radioactive **27** (176.8 mg) was subjected to Kuhn–Roth oxidation and the generated AcOH was converted to acetyl-α-naphthylamide according to the procedure reported earlier by us.[4] This amide was purified by PLC (benzene–AcOEt, 7 : 3), recrystallization (from benzene–petr. ether) and vacuum distillation to a constant specific activity of 3.13×10^5 dpm/mmol, which corresponds to 25.2% of the radioactivity of the original **27**. The rest (110.8 mg) of the radioactive **27** was subjected to ozonolysis, and liberated HCOOH was converted to formyl-α-naphthylamide according to the earlier procedure.[4] This amide was purified in the same way as above to a constant specific activity of 5.73×10^5 dpm/mmol, which corresponds to 46.2% of th radioactivity of the original **27**.

Administration of [1-^{3}H]-9,10-Dihydroxygeraniol (23) to Cell Cultures——A solution of [1-^{3}H]-**23** (1.52 mg, 0.311 mCi) in EtOH–Tween 80 (2 ml–2 drops) was administered to the cell cultures (200 ml) 4 weeks after inoculation. After incubation for 10 d, the cells (fr. wt. 24 g) were extracted with MeOH (50 ml × 4) under reflux. The combined extracts were concentrated *in vacuo* to give a residue, which was subjected to active charcoal column chromatography and PLC in the usual way to afford a residue containing **20** and **21**. Acetylation and purification by PLC gave tarennoside pentaacetate (**25**) (16.68 mg) and gardenoside pentaacetate (**29**) (45.48 mg). The former was diluted with the carrier (30.80 mg) and recrystallized from EtOH to constant specific activity (9.28×10^8 dpm/mmol). The latter and the carrier (39.80 mg) were hydrogenated over 5% Pd–C in the conventional way to give dihydrogardenoside pentaacetate (**26**), which was recrystallized from ether to constant specific activity (5.65×10^6 dpm/mmol).

Administration of (*RS*)-(±)-[10-^{3}H]-10-Hydroxycitronellol (24a, b) to Cell Cultures——A solution of (*RS*)-(±)-[10-^{3}H]-**24a, b** (2.08 mg, 0.474 mCi) in EtOH–Tween 80 (2 ml–2 drops) was administered to the cell cultures (200 ml) 4 weeks after transfer. After incubation for 10 d, the cells (fr. wt. 14 g) were worked up in the usual manner to give the pentaacetate (**25**) (4.35 mg) of **20** and pentaacetate (**29**) (14.97 mg) of **21**. The former, mixed with the carrier (33.20 mg), was recrystallized from EtOH to constant specific activity (7.94×10^8 dpm/mmol). The latter, mixed with the carrier (51.48 mg), was converted into dihydrogardenoside pentaacetate (**26**) (45.07 mg), which was recrystallized

from ether to constant specific activity (1.18×10^7 dpm/mmol).

Administration of [9-^{13}C]-10-Hydroxygeraniol (22) to Cell Cultures——A solution of [9-^{13}C]-**22** (15.0 mg) in EtOH–Tween 80 (2 ml–2 drops) was administered to the cell cultures (600 ml) 2 weeks after inoculation. After incubation for 10 d, the cells (fr. wt. 70 g) were worked up in the usual way to afford **20** (32.4 mg) and **21** (44.4 mg). These glucosides were acetylated and purified by PLC (ether) to afford tarennoside pentaacetate (**25**) (40.0 mg) and gardenoside pentaacetate (**29**) (43.8 mg), respectively.

25: ^{13}C-NMR δ: 31.7 (d, C-5), 37.4 (t, C-6), 46.4 (d, C-9), 61.5 (t, C-10, C-6′), 68.3 (d, C-4′), 70.7 (d, C-2′), 72.2 (d, C-3′), 72.4 (d, C-5′), 96.3 (d, C-1), 96.4 (d, C-1′), 124.5 (s, C-4), 131.5 (d, C-7), 136.7 (s, C-8), 160.2 (d, C-3), 190.0 (d, C-11).

29: ^{13}C-NMR δ: 37.3 (d, C-5), 50.9 (d, C-9), 61.7 (t, C-6′), 68.1 (d, C-4′), 70.6 (d, C-2′), 72.2 (d, C-3′), 72.4 (d, C-5′), 83.5 (s, C-8), 92.5 (d, C-1′), 95.5 (d, C-1), 111.1 (s, C-4), 134.0 (d, C-6), 135.4 (d, C-7), 149.1 (d, C-3), 166.3 (s, C-11).

Simultaneous Administration of [4-^{13}C]-10-Hydroxygeraniol (22), [2-^{13}C]-9,10-Dihydroxygeraniol (23), (*S*)-(−)-[9-^{13}C]-10-Hydroxycitronellol (24b) and (*S*)-(−)-[8-^{13}C]-9,10-Dihydroxycitronellol (28b) to Cell Cultures——A solution of [4-^{13}C]-**22** (17.2 mg), [2-^{13}C]-**23** (18.6 mg), (*S*)-(−)-[9-^{13}C]-**24b** (17.2 mg) and (*S*)-(−)-[8-^{13}C]-**28b** (18.8 mg, 0.10 mmol) in EtOH–Tween 80 (4 ml–4 drops) was administered to the cell cultures (800 ml) 2 weeks after inoculation. After incubation for 7 d, the cells (fr. wt. 75 g) were worked up in the usual way to yield two acetates, **25** (24.7 mg) and **29** (45.0 mg).

Administration of (*S*)-(−)-[9-^{13}C]-10-Hydroxycitronellol (24b) and [4-^{13}C]-10-Hydroxygeraniol (22) to Cell Cultures——A solution of (*S*)-(−)-[9-^{13}C]-**24b** (17.2 mg) in EtOH–Tween 80 (2 ml–2 drops) was administered to the cell cultures (800 ml) 2 weeks after transfer and incubated for 5 d. Then, a solution of [4-^{13}C]-**22** (17.0 mg) in EtOH–Tween 80 (2 ml–2 drops) was fed and cultivation was continued for a further 7 d. The cells (fr. wt. 148 g) were worked up in the usual manner to give tarennoside pentaacetate (**25**) (34.7 mg) and gardenoside pentaacetate (**29**) (42.2 mg).

Administration of (*R*)-(+)-[9-^{13}C]-10-Hydroxycitronellol (24a) and [4-^{13}C]-10-Hydroxygeraniol (22) to Cell Cultures——Two monoterpenes, (*R*)-(+)-[9-^{13}C]-**24a** (17.2 mg) and [4-^{13}C]-**22** (17.0 mg), in EtOH–Tween 80 (2 ml–2 drops) were administered to the cell cultures (800 ml) in the same way as above. The usual work up (fr. wt. of cells, 154 g) yielded tarennoside pentaacetate (**25**) (15.8 mg) and gardenoside pentaacetate (**29**) (28.8 mg).

Administration of Non-labeled 10-Hydroxygeraniol (22) and [2-^{13}C]-9,10-Dihydroxygeraniol (23) to Cell Culturers——A solution of non-labeled **22** (170 mg, 1.0 mmol) in EtOH–Tween 80 (4 ml–4 drops) was administered to the cell cultures (800 ml) 2 weeks after inoculation. After incubation for 4 h, a solution of [2-^{13}C]-**23** (18.6 mg, 1.10 mmol) in EtOH–Tween 80 (2 ml–2 drops) was fed and cultivation was continued for a further 7 d. The cells (fr. wt. 111 g) were worked up in the usual way to give tarennoside pentaacetate (**25**) (54.5 mg) and gardenoside pentaacetate (**29**) (50.5 mg).

Simultaneous Administration of [4-^{13}C]-10-Hydroxygeraniol (22) and Non-labeled 9,10-Dihydroxygeraniol (23) to Cell Cultures——A solution of [4-^{13}C]-**22** (10.9 mg, 0.064 mmol) and non-labeled **23** (119.0 mg, 0.64 mmol) in EtOH–Tween 80 (4 ml–4 drops) was administered to the cell cultures (800 ml) 2 weeks after inoculation. After incubation for 7 d, the cells (fr. wt. 111 g) were worked up in the usual manner to yield tarennoside pentaacetate (**25**) (77.0 mg) and gardenoside pentaacetate (**29**) (46.4 mg).

Acknowledgement This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture. We thank Miss F. Higuchi of the Institute for Chemical Research of this University for measurements of ^{13}C-NMR spectra.

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6) Compound **14** may not exist as such; the cyclization may proceed concertedly, or a cyclic intermediate of **5a**/**5b** may be bound to an enzyme.
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12) Each labeled compound showed *ca.* 90 atom% ^{13}C in the ^{1}H- or ^{13}C-NMR spectrum.

13) Enrichment factor (EF) $=(i_{\text{lab.}}/i_{\text{nat. abund.}}-1)\times 1.1\%$, $i_{\text{lab.}}=$ peak intensities of labeled compounds, $i_{\text{nat. abund.}}=$ peak intensities of natural abundance.
14) $(acac)_3Cr$ (13.0 mg) was dissolved in a solution of gardenoside pentaacetate (**29**) (30.2 mg) in $CDCl_3$ (0.25 ml). *Cf.* M. Tanabe, K. T. Suzuki and W. C. Jankowski, *Tetrahedron Lett.*, **1973**, 4723.
15) Evidence supporting the intermediacy of 9,10-dihydroxygeraniol (**23**) and 9,10-dioxogeranial (**1a**) in the biosynthesis of indole alkaloids in *Catharanthus roseus* suspension cultures was recently reported. *Cf.* J. Balsevich and W. G. W. Kurz, *Planta Medica*, **49**, 79 (1983).