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Studies on Monoterpene Glucosides and Related Natural Products. XLVII.[1)] Synthesis of ^3H- and ^2H-labeled Iridodial Derivatives for Studies on the Biosynthesis of Iridoid Glucosides

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For studies on the biosynthesis of iridoid glucosides, four labeled compounds, *i.e.*, [10-^3H]-iridotrial (**4**), [10-^3H]-7,8-dehydroiridodial (**6**), [10-^3H]-7,8-dehydroiridotrial (**7**) and [10-$^2H_3$]-iridodial (**3**), along with the known [10-^3H]-iridodial glucoside (**10**) and [10-^3H]-iridodial (**3**) were prepared from geniposide (**11**). Furthermore, [11-$^2H_3$]-7,8-dehydroiridodial (**6**) was derived from mussaenoside (**14**).

Keywords——iridoid glucosides; biosynthesis; ^3H- and ^2H-labeled iridodial derivatives; iridodial; iridodial glucoside; iridotrial; 7,8-dehydroiridodial; 7,8-dehydroiridotrial; synthesis

Recently, we demonstrated that the iridoid glucosides of *Gardenia jasminoides* f. *grandiflora* suspension cultures including tarennoside (**9**) are biosynthesized through cyclization of 2*Z*- or 2*E*-10-oxocitral (**1**) to the intermediate iridodial cation (**2**), followed by randomization of the C-atoms 3 and 11.[2)] As shown in Fig. 1, two routes are conceivable for the formation of tarennoside (**9**) from the cation (**2**): one is initiated by hydride attack on the C-8 position to give rise to iridodial (**3**), which is further converted into **9** *via* iridotrial (**4**), its glucoside (**5**) and 7,8-dehydroiridotrial glucoside (**8**); the other is started by elimination of the C-7 proton to

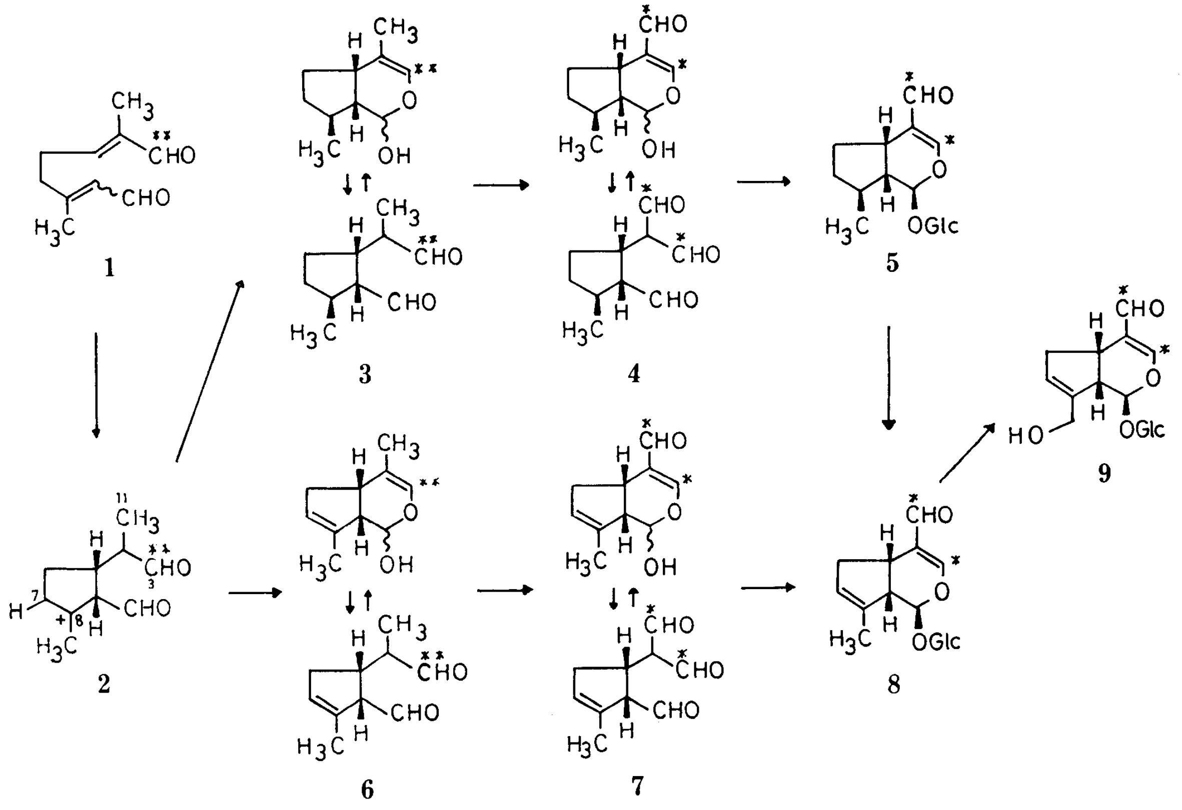


Fig. 1

generate 7,8-dehydroiridodial (**6**), which is further converted into **9** *via* 7,8-dehydroiridotrial (**7**) and 7,8-dehydroiridotrial glucoside (**8**). For feeding experiments with the aim of detailed examination of these routes, it was necessary to prepare the following isotope labeled compounds: iridodial (**3**), iridotrial (**4**), 7,8-dehydroiridodial (**6**), 7,8-dehydroiridotrial (**7**) and iridodial glucoside (**10**) labeled with tritium, as well as **3** and **6** labeled with deuterium. The present paper deals with the synthesis of these labeled compounds.

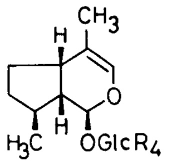
The five ^{3}H-labeled compounds were planned to be administered separately to the cell cultures. Therefore, they should be labeled at the same position, since accurate comparison of their incorporations into the iridoid glucosides would otherwise not be achieved owing to the tritium isotope effect, which might vary with positions of labeling. In addition, it would be convenient if they could be prepared from the same readily available starting material. We thus attempted to prepare all the ^{3}H-labeled compounds from [10-^{3}H]-geniposide pentaacetate (**13**). This compound was obtained through NaB^3H_4 reduction and subsequent acetylation of 10-dehydrogeniposide tetraacetate (**12**), which can easily be derived from geniposide (**11**) in two steps.[3] On the other hand, both ^{2}H-labeled compounds were planned to be administered simultaneously to the cell cultures. Therefore, they must be labeled at different positions. In addition, we wished to exclude the possibility of the deuterium isotope effect causing the preferential removal of hydrogen during the biosynthetic process. Thus, [10-$^2H_3$]-iridodial (**3**) was prepared in a manner similar to the preparation of [^{3}H]-**3**, whereas [11-$^2H_3$]-7,8-dehydroiridodial (**6**) was derived from mussaenoside[4] (**14**). The labeled compounds were prepared after model experiments with non-labeled compounds.

Conversion of 10-Dehydrogeniposide Tetraacetate (12) into [10-^{3}H]-Iridodial Glucoside (10), [10-^{3}H]-Iridodial (3), [10-^{3}H]-Iridotrial (4), [10-^{3}H]-7,8-Dehydroiridodial (6) and [10-^{3}H]-7,8-Dehydroiridotrial (7)

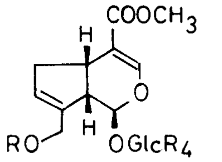
10-Dehydrogeniposide tetraacetate (**12**) derived from geniposide (**11**) was subjected to $NaBH_4$ reduction followed by acetylation. The resultant geniposide pentaacetate (**13**) gave on catalytic reduction over Pd-C deoxyloganin tetraacetate (**15**),[5] which was reduced with $LiAlH_2(OCH_3)_2$ in tetrahydrofuran under cooling with freezing mixture followed by acetylation to afford 11-hydroxyiridodial glucoside pentaacetate (**16**). The conversion of this compound into iridodial glucoside (**10**) and iridodial (**3**) has already been reported.[6] On the other hand, the deacetylation product of **16** was oxygenated over Pt in H_2O to yield iridotrial glucoside (**5**), $C_{16}H_{24}O_8$, a white powder, $[\alpha]_D$ $-114.9°$ (MeOH). Its infrared (IR) spectrum showed bands due to hydroxy groups (3300 cm^{-1}), an α,β-unsaturated aldehyde (2700 and 1655 cm^{-1}) and an acetal (1075 cm^{-1}). Additionally, its ^{1}H nuclear magnetic resonance (^{1}H NMR) spectrum exhibited a broad singlet due to the C-10 methyl protons (δ 1.08) and a singlet due to the C-11 aldehyde proton (δ 9.16). These data clearly indicated that this compound has the structure **5**. Hydrolysis of **5** with β-glucosidase yielded iridotrial (**4**), $C_{10}H_{14}O_3$, a colorless oil, bp 113°C/3.0 mmHg, $[\alpha]_D$ $-15.2°$ ($CHCl_3$). It showed IR bands due to a hydroxy group (3300 cm^{-1}), an α,β-unsaturated aldehyde (2700 and 1650 cm^{-1}) and an acetal (1145 cm^{-1}) as well as ^{1}H NMR signals due to the C-3 proton (br s, δ 7.17) and the C-11 aldehyde proton (s, δ 9.16). Thus, iridotrial was proved to exist in the enol hemiacetal form (**4**).

Geniposide pentaacetate (**13**) was subjected to hydrogenolysis with equimolecular hydrogen over Pd-C to give a product, which was purified by column chromatography on silica gel impregnated with $AgNO_3$[7] to afford 10-deoxygeniposide tetraacetate (**18**). This was reduced with $LiAlH_2(OCH_3)_2$ in tetrahydrofuran under cooling with freezing mixture followed by acetylation to give 7,8-dehydro-11-hydroxyiridodial glucoside pentaacetate (**19**). $C_{26}H_{34}O_{13}$, colorless needles, mp 120—121°C. Hydrogenolysis in the same way as described above yielded 7,8-dehydroiridodial glucoside tetraacetate (**20**), which was deacetylated to give the corresponding free glucoside (**21**), $C_{16}H_{24}O_7$. 2/3 H_2O, a white powder, $[\alpha]_D$ $-59.1°$ (MeOH). Its IR spectrum showed bands due to hydroxy groups (3350 cm^{-1}), a trisubstituted double bond

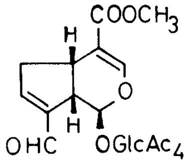
(1670 cm^{-1}) and an acetal (1075 cm^{-1}). Furthermore, its ^{1}H NMR spectrum exhibited a doublet (J=1.2 Hz) due to the C-11 methyl protons (δ 1.58), a broad singlet due to the C-10 vinyl methyl protons (δ 1.81), a multiplet due to the C-7 vinyl proton (δ 5.40) and a broad singlet due to the C-3 proton (δ 6.04). The data clearly indicate that this compound is represented by the structure **21**. Hydrolysis of **21** with β-glucosidase led to 7,8-dehydroiridodial (**6**), $C_{10}H_{14}O_2$, a colorless oil, bp 89°C (0.4 mmHg), $[\alpha]_D$ +64.5° (MeOH). Its IR spectrum showed bands due to a hydroxy group (3350 cm^{-1}), a trisubstituted double bond (1660 cm^{-1}) and an acetal (1125 cm^{-1}). Furthermore, its ^{1}H NMR spectrum showed a broad singlet due to the C-3 proton (δ 6.06), but no signal due to an aldehyde proton. Thus, 7,8-dehydroiridodial was proved to exist in the enol hemiacetal form (**6**).



10 : R=H
17 : R=Ac



11 : R=H
13 : R=Ac



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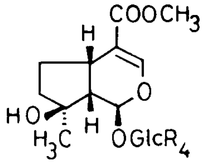
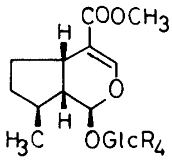
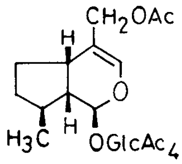


14 : R=H
24 : R=Ac



15 : R=Ac
22 : R=H



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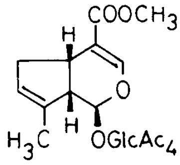

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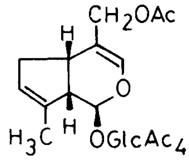

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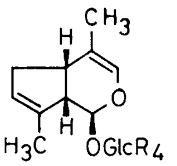
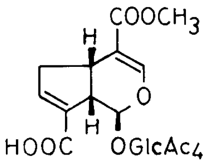


20 : R=Ac
21 : R=H



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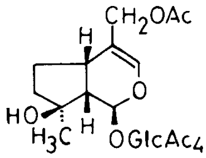

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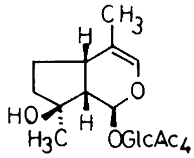

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Fig. 2

Subsequently, the deacetylation product of **19** was oxygenated over Pt in H_2O to give rise to 7,8-dehydroiridotrial glucoside (**8**), $C_{16}H_{22}O_8$. 3/2 H_2O, a white powder, $[\alpha]_D$ −8.0°. Its IR spectrum showed bands due to hydroxy groups (3350 cm^{-1}), an α,β-unsaturated aldehyde (2720 and 1665 cm^{-1}) and an acetal (1075 cm^{-1}). Additionally, its ^{1}H NMR spectrum exhibited a broad singlet due to the C-10 vinyl methyl protons (δ 1.79), a multiplet due to the C-7 vinyl proton (δ 5.50), a broad singlet due to the C-3 proton (δ 7.51) and a singlet due to the C-11 aldehyde proton (δ 9.14). These data clearly indicate that this glucoside possesses the structure **8**. Hydrolysis of **8** with β-glucosidase yielded 7,8-dehydroiridotrial (**7**), $C_{10}H_{12}O_3$, a colorless oil, bp 97°C (1.4 mmHg), $[\alpha]_D$ +196.4° (MeOH). It showed IR bands due to a hydroxy group (3250 cm^{-1}), α,β-unsaturated aldehyde (2715 and 1650 cm^{-1}) and an acetal (1145 cm^{-1}) as well as ^{1}H NMR signals due to the C-3 proton (br s, δ 7.26) and the C-11 aldehyde proton (s, δ 9.28). Thus, the enol hemiacetal structure **7** was demonstrated for 7,8-dehydroiridotrial.

Based on these preliminary experiments with the non-labeled compounds, the ^{3}H-labeled compounds were prepared in the following way. 10-Dehydrogeniposide tetraacetate (**12**) was subjected to NaB^3H_4 reduction under ice cooling followed by acetylation to give [^{3}H]-geniposide pentaacetate (**13**). For localization of the ^3H-labeling, this compound was converted into deoxyloganin (**22**), which was then subjected to Kuhn–Roth oxidation. The resultant acetic acid, captured as α-naphthylamide, was found to contain 95.2% of the total activity of the original glucoside (**22**), indicating the location of the labeling at C-10 in **13**. [10-^3H]-**13** was then converted into [10-^3H]-iridodial (**3**), [10-^3H]-iridotrial (**4**), [10-^3H]-7,8-dehydroiridodial (**6**), [10-^3H]-7,8-dehydroiridotrial (**7**) and [10-^3H]-iridodial glucoside (**10**) in the same way as described above.

Conversion of Dehydrogeniposide Tetraacetate (12) into [10-$^2H_3$]-Iridodial (3)

In order to obtain iridodial (**3**) wholly deuterated at the C-10 position, the aforementioned synthetic route for [^3H]-**3** was modified. To remove the C-10 proton, **12** was first subjected to Jones oxidation to give ixoside monomethyl ester tetraacetate (**23**)[8] which, after conversion into mixed anhydride, was reduced with $NaBH_4$ followed by acetylation, yielding geniposide pentaacetate (**13**). The next reaction, the reduction of this compound (**13**) to deoxyloganin tetraacetate (**15**) should not be carried out in one step in contrast to the synthesis of ^3H-labeled iridodial (**3**), since the C-10 position, but not the C-7 and C-8 positions, of the desired product should be deuterated. Therefore, **13** was first subjected to hydrogenolysis over Pd-C to give 10-deoxygeniposide tetraacetate (**18**), which was then hydrogenated over the same catalyst to yield deoxyloganin tetraacetate (**15**). The conversion of **15** into **3** has already been mentioned. Based on these preliminary experiments with non-labeled compounds the synthesis of deuterium labeled compounds was carried out. On NaB^2H_4 reduction followed by acetylation, the mixed anhydride of **23** gave [10-$^2H_2$]-geniposide pentaacetate (**13**), which on hydrogenolysis with deuterium gas over Pd-C, yielded [10-$^2H_3$]-10-deoxygeniposide tetraacetate (**18**). In these reactions, the ^2H-labels on the C-atom 10 in **13** and **18**, being situated at the allylic position, were found to be easily replaced by proton. Therefore, D_2O and AcOD were used during work up of the former reaction, while D_2O and DCl were employed for the preparation of Pd-C catalyst and MeOD as a solvent for the deuteration. Nevertheless, the ^1H NMR spectrum of **18** revealed that 40% of deuterium at the C-10 position was replaced by hydrogen. [10-$^2H_3$]-**18** thus obtained was again hydrogenated with hydrogen gas in MeOD over Pd-C (also prepared by using D_2O and DCl) to give [10-$^2H_3$]-**15**.[9] Its ^1H NMR spectrum showed that 50% of deuterium at the C-10 position was substituted by hydrogen. Compound **15** was further converted into **3** *via* **16**, **17** and **10** in the same way as above. The ^1H NMR spectrum of [10-$^2H_3$]-iridodial (**3**) thus obtained was in accord with that of the non-labeled counterpart except for the fact that the signal intensity of the doublet due to the C-10 methyl was only one-fifth of that of the latter.

Conversion of Mussaenoside Tetraacetate (24) into [11-$^2H_3$]-7,8-Dehydroiridodial (6)

Compound **24** was subjected to reduction with $LiAlH_2(OCH_3)_2$ in tetrahydrofuran under cooling with freezing mixture followed by acetylation to give 8β,11-dihydroxyiridodial glucoside pentaacetate (**25**), $C_{26}H_{36}O_{14}$, colorless needles, mp 129—130°C, $[\alpha]_D$ −122.1° ($CHCl_3$). Its ^1H NMR spectrum, which contained two doublets (each $J=12.0$ Hz) due to methylene protons (δ 4.23 and 4.68), indicated that the C-11 carbomethoxy group of **24** was reduced to a hydroxymethyl group. Hydrogenolysis of **25** over Pd-C in methanol afforded 8β-hydroxyiridodial glucoside tetraacetate (**26**), $C_{24}H_{34}O_{12}$· 1/4 H_2O, colorless needles, mp 58.5—60°C, $[\alpha]_D$ −119.6° ($CHCl_3$). Its ^1H NMR spectrum showed a singlet due to the C-10 methyl protons (δ 1.30) as well as a broad singlet due to the C-11 methyl protons (δ 1.47), indicating the desired formulation. Dehydration of **26** with $POCl_3$ in pyridine furnished 7,8-dehydroiridodial glucoside tetraacetate (**20**). Based on these experiments, [11-$^2H_3$]-7,8-dehydroiridodial (**6**) was synthesized in the following way. Compound **24** was reduced with $LiAl^2H_2(OCH_3)_2$ followed by acetylation to give [11-$^2H_2$]-**25**. Its ^1H NMR spectrum lacked a signal due to the C-11 methylene protons. It was then subjected to hydrogenolysis with deuterium gas over Pd-C in MeOD in the same way as for the preparation of [10-$^2H_3$]-iridodial (**3**) leading to [11-$^2H_3$]-**26**. Its ^1H NMR spectrum was consistent with that of the non-labeled counterpart except for the lack of the signal due to the C-11 methyl protons. Dehydration of **26** to [11-$^2H_3$]-**20** followed by deacetylation afforded [11-$^2H_3$]-7,8-dehydroiridodial glucoside (**21**), which, on hydrolysis with β-glucosidase, gave rise to the desired [11-$^2H_3$]-7,8-dehydroiridodial (**6**). Its ^1H NMR spectrum was consistent with that of the non-labeled counterpart except for the lack of the C-11 methyl protons.

Experimental

Melting points were measured on a Yanagimoto micro-melting point apparatus and are uncorrected. Boiling points are oven temperatures of a Büchi Kugelrohr apparatus. Optical rotations were determined with a Union PM-101 automatic digital polarimeter. Ultraviolet (UV) spectra were recorded on a Hitachi model 200-20 spectrophotometer and IR spectra on a Hitachi model 215 grating infrared spectrometer. ^1H NMR spectra were recorded on a Varian A-60 spectrometer or a JEOL JNM-PMX 60 NMR spectrometer and ^2H NMR spectra on a JEOL JNM-FX 100FT NMR spectrometer. Unless otherwise stated, NMR spectra were taken in $CDCl_3$. TMS was used in $CDCl_3$ and DSS in D_2O as internal standards. Mass spectra (MS) were determined on a JEOL JMS-01SG-2 spectrometer. Silica gel AR-100 (Mallinckrodt) and activated charcoal (Wako) were employed for column chromatography. Thin layer chromatography (TLC) and preparative layer chromatography (PLC) were performed on silica gel 60 GF_{254} (Merck) (5×20 cm, 0.25 mm in thickness) and PF_{254} (Merck) (20×20 cm, 1.00 mm in thickness), respectively. Spots and bands were visualized by UV irradiation or by exposure to I_2 vapor. Unless otherwise noted, main bands were scraped off and extracted with $CHCl_3$–MeOH (9: 1), and extracts were concentrated *in vacuo*. Ratios of solvents are expressed in volume. Radioactivities were measured in a Beckman liquid scintillation counter, model LS-230, with samples dissolved in a scintillation mixture consisting of toluene (10 ml), 2,5-diphenyloxazole (PPO) (40 mg) and 2,2′-phenylenebis(5-phenyloxazole) (POPOP) (0.5 mg).

Conversion of Deoxyloganin Tetraacetate (15) into 11-Hydroxyiridodial Glucoside Pentaacetate (16)——Dry MeOH (2.67 ml) was added over a period of 30 min to a stirred suspension of $LiAlH_4$ (1.02 g) in dry tetrahydrofuran(THF) (70 ml), keeping the mixture below -20°C under N_2. After stirring of the mixture for an additional 30 min below -20°C, a solution of **15** (1.00 g) in dry THF (50 ml) was added over a period of 30 min and stirring was continued for 2.5 h at elevated temperature (-20 to 0°C). The excess reagent was decomposed by addition of AcOEt. Insoluble materials precipitated by the successive addition of saturated aq. Na_2SO_4 were filtered off, digested in brine and extracted with *n*-BuOH (30 ml $\times$ 4). The combined extracts were washed with brine and concentrated *in vacuo* to give a syrupy residue, which was chromatographed on a charcoal (5 g) column, eluting successively with H_2O (100 ml) and EtOH (200 ml). The EtOH eluate was concentrated *in vacuo* to give a white powdery residue, which was acetylated with pyridine and Ac_2O (3 ml each). The product was recrystallized from EtOH to afford colorless needles (876 mg), mp 116—118°C. This compound was identical with an authentic sample (**16**) on the basis of mixed mp and comparisons of IR and ^1H NMR data.

Conversion of 11-Hydroxyiridodial Glucoside Pentaacetate (16) into Iridotrial Glucoside (5)——A 0.1 N methanolic MeONa solution (0.3 ml) was added to a solution of **16** (150 mg) in dry MeOH (4 ml) and the mixture was refluxed for 3 min. After cooling, the mixture was neutralized with Amberlite IR-120 (H^+-form) and concentrated *in vacuo*. A solution of the residue (105 mg) in H_2O (2 ml) was added to a suspension of Pt (prepared from PtO_2 (30 mg)) in H_2O (3 ml). After being stirring for 72 h at room temperature under O_2, the mixture was transferred to a charcoal (1 g) column and eluted successively with H_2O (50 ml) and EtOH (100 ml). Concentration of the EtOH eluate *in vacuo* gave a residue (86 mg), which was purified by PLC ($CHCl_3$–MeOH, 7: 3) to afford **5** (72 mg) as a white powder. $[\alpha]_D^{20}$ -114.9° ($c=0.43$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 250.0 (4.23). IR ν_{max}^{KBr} cm^{-1}: 3300, 2910, 2700, 1655, 1620 and 1075. ^1H NMR (D_2O) δ: 1.08 (d, $J=5.0$ Hz, 10-H_3), 3.46 (br s, 6′-H_2), 4.86 (d, $J=7.0$ Hz, 1′-H), 5.53 (d, $J=2.5$ Hz, 1-H), 7.47 (br s, 3-H) and 9.16 (s, 11-H). *Anal.* Calcd for $C_{16}H_{24}O_8$: C, 55.81; H, 7.02. Found: C, 55.89; H, 6.75.

Hydrolysis of Iridotrial Glucoside (5) with β-Glucosidase——β-Glucosidase (Miles Laboratories (PTY) Ltd.) (3 mg) was added to a solution of **5** (47 mg) in 0.1 M acetate buffer (pH 4.6, 7 ml). The mixture was allowed to stand at 35°C for 8 h and was extracted with ether (4 ml $\times$ 4). The combined extracts were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by vacuum distillation to give iridotrial (**4**) (23 mg) as a colorless oil, bp 113°C (3.0 mmHg). $[\alpha]_D^{20}$ -15.2° ($c=0.79$, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 250.5 (4.07). IR ν_{max}^{neat} cm^{-1}: 3300, 2925, 2700, 1650, 1610, and 1145. ^1H NMR δ: 1.11 (d, $J=6.0$ Hz, 10-H_3), 5.10 (d, $J=6.0$ Hz, 1-H), 7.17 (br s, 3-H), 9.16 (s, 11-H). *Anal.* Calcd for $C_{10}H_{14}O_3$: C, 65.92; H, 7.74. Found: C, 65.54; H, 7.82.

Conversion of Geniposide Pentaacetate (13) into 10-Deoxygeniposide Tetraacetate (18)——A solution of **13** (307 mg) in MeOH (10 ml) was hydrogenated over a catalyst prepared from 5% $PdCl_2$ (0.42 ml) and activated charcoal (DARCO G-60) (150 ml). After an uptake of an equimolar amount of hydrogen, the catalyst was filtered off and washed with $CHCl_3$. The combined filtrate and washings were concentrated *in vacuo* to give a residue, which was subjected to PLC (benzene–AcOEt, 85: 25). The band at around *Rf* 0.55 gave a white powder (190 mg), which was further chromatographed on a column (10 g) of silica gel impregnated with $AgNO_3$[7] with benzene and acetone (95: 5) as eluents; 55 ml fractions were collected. The combined fractions (frs) 6, 7 and 9—21 were each concentrated *in vacuo* to give residues (85.6 mg and 104.0 mg, respectively). On recrystallization from EtOH, the former afforded deoxyloganin tetraacetate (**15**) (identical with an authentic sample), whereas the latter gave 10-deoxygeniposide tetraacetate (**18**). mp 104—105°C, $[\alpha]_D^{20}$ $-41.7°$ ($c=0.53$, $CHCl_3$). UV λ_{max}^{EtOH} nm (log ε): 234.2 (4.13). IR ν_{max}^{KBr} cm^{-1}: 2940, 1740, 1700, 1635, 1440, 1370, 1225 and 900. ^1H NMR ($CDCl_3$) δ: 1.76 (s, 10-H_3), 1.97—2.08 ($OCOCH_3\times 4$), 2.74 (m, 6-H_2), 3.14 (m, 5-H), 3.70 (s, $COOCH_3$), 4.15 (dd, $J=2.4$ and 12.5 Hz, 6'-H), 4.29 (dd, $J=4.4$ and 12.5 Hz, 6'-H), 5.46 (br s, 7-H), 7.40 (s, 3-H). *Anal.* Calcd for $C_{25}H_{32}O_{13}$: C, 55.55; H, 5.97. Found: C, 55.29; H, 6.13.

Conversion of 10-Deoxygeniposide Tetraacetate (18) into 7,8-Dehydro-11-hydroxyiridodial Glucoside Pentaacetate (19)——Compound **18** (1.00 g) was reduced with $LiAlH_2(OCH_3)_2$ (prepared from $LiAlH_4$ (1.02 g) and MeOH (2.16 ml)) in THF (100 ml) in the same way as for the preparation of **16**. The resulting product was subjected to the usual acetylation followed by recrystallization from MeOH to give **19** (509 mg) as colorless needles, mp 120—121°C. $[\alpha]_D^{20}$ $-67.9°$ ($c=0.61$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2910, 1755, 1670, 1445 and 1155. ^1H NMR δ: 1.78 (br s, 10-H_3), 2.01—2.07 ($OCOCH_3\times 5$), 4.31 and 4.71 (br d, $J=12.5$ Hz, each 11-H), 5.47 (m, 7-H) and 6.39 (br s, 3-H). *Anal.* Calcd for $C_{26}H_{34}O_{13}$: C, 56.31; H, 6.18. Found: C, 56.23; H, 6.23.

Conversion of 7,8-Dehydro-11-hydroxyiridodial Glucoside Pentaacetate (19) into 7,8-Dehydroiridodial Glucoside Tetraacetate (20)——A solution of **19** (191 mg) in MeOH (5 ml) was hydrogenated over a catalyst prepared from 5% $PdCl_2$ (0.28 ml) and activated charcoal (DARCO G-60) (100 mg). After uptake of an equimolar amount of hydrogen, the catalyst was filtered off and washed with $CHCl_3$. The combined filtrate and washings were concentrated *in vacuo* to give a colorless syrupy residue (174 mg), which was subjected to PLC (ether). The band at around *Rf* 0.50 afforded the unchanged starting material (68 mg), whereas the band at around *Rf* 0.60 gave a white powdery residue (96 mg). The latter was further chromatographed on a column (10 g) of silica gel impregnated with $AgNO_3$[7] using benzene–acetone (95: 5) as the eluent; 5 ml fractions were collected. The combined frs 5—9 and 11—17 were each concentrated *in vacuo* to afford residues (34 mg and 51 mg, respectively). On recrystallization from EtOH, the former gave iridodial glucoside tetraacetate (**17**) (17 mg) as colorless needles, mp 143—144°C, whereas the latter afforded 7,8-dehydroiridodial glucoside tetraacetate (**20**) (38 mg) as colorless needles, mp 126—127°C. $[\alpha]_D^{20}$ $-89.5°$ ($c=0.87$, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2940, 1750, 1670 and 1120. ^1H NMR δ: 1.52 (d, $J=1.5$ Hz, 11-H_3), 1.76 (br s, 10-H_3), 2.00—2.07 ($OCOCH_3\times 4$), 5.43 (m, 7-H) and 5.98 (br s, 3-H). *Anal.* Calcd for $C_{24}H_{32}O_{11}$: C, 58.06; H, 6.50. Found: C, 57.85; H, 6.62.

Conversion of 7,8-Dehydroiridodial Glucoside Tetraacetate (20) into 7,8-Dehydroiridodial Glucoside (21) and 7,8-Dehydroiridodial (6)——Compound **20** (292 mg) was subjected to Zemplén reaction in the same way as 11-hydroxyiridodial glucoside pentaacetate (**16**). The usual work-up of the reaction mixture yielded a product, which was purified by PLC ($CHCl_3$–MeOH, 85: 15) to afford 7,8-dehydroiridodial glucoside (**21**) (186 mg) as a white powder. $[\alpha]_D^{20}$ $-59.1°$ ($c=0.81$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350, 2900, 1670 and 1075. ^1H NMR (CD_3OD) δ: 1.58 (d, $J=1.2$ Hz, 11-H_3), 1.81 (br s, 10-H_3), 4.68 (d, $J=7.5$ Hz, 1'-H), 4.98 (d, $J=6.0$ Hz, 1-H), 5.40 (m, 7-H) and 6.04 (br s, 3-H). *Anal.* Calcd for $C_{16}H_{24}O_7\cdot 2/3H_2O$: C, 56.46; H, 7.50. Found: C, 56.19; H, 7.78. An aliquot (61 mg) of this compound was dissolved in 0.1 M acetate buffer (pH 4.6, 10 ml) and hydrolyzed with β-glucosidase (5 mg) in the same way as iridotrial glucoside (**5**). The reaction mixture was extracted with ether (5 ml×4) and the extracts were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by PLC (*n*-hexane–ether, 3: 2) to give **6** (28 mg) as a colorless oil, bp 89°C (0.4 mmHg). $[\alpha]_D^{20}$ $+64.5°$ ($c=0.11$, MeOH). IR ν_{max}^{neat} cm^{-1}: 3350, 2890, 1660 and 1125. ^1H NMR δ: 1.60 (d, $J=1.2$ Hz, 11-H_3), 1.83 (br s, 10-H_3), 4.72 (d, $J=7.5$ Hz, 1-H), 5.47 (m, 7-H) and 6.06 (br s, 3-H). High resolution MS, Calcd for $C_{10}H_{14}O_2$ (M^+): 166.0994. Found: 166.0988.

Conversion of 7,8-Dehydro-11-hydroxyiridodial Glucoside Pentaacetate (19) into 7,8-Dehydroiridotrial Glucoside (8)——The free glucoside (222 mg) derived from **19** through the Zemplén reaction, was dissolved in H_2O (4 ml) and added to a stirred suspension of Pt (prepared from PtO_2 (50 mg)) in H_2O (5 ml). After being stirred for 72 h at room temperature under O_2, the mixture was transferred to a charcoal (1 g) column and eluted successively with H_2O (50 ml) and EtOH (100 ml). Concentration of the EtOH eluate *in vacuo* gave a residue (206 mg), which was subjected to PLC ($CHCl_3$–MeOH, 8: 2) to afford **8** (100 mg) as a white powder. $[\alpha]_D^{20}$ $-8.0°$ ($c=0.62$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 248.5 (4.07). IR ν_{max}^{KBr} cm^{-1}: 3350, 2900, 2720, 1665, 1625, 1250 and 1075. ^1H NMR (D_2O) δ: 1.79 (br s, 10-H_3), 4.86 (d, $J=7.0$ Hz, 1'-H), 5.50 (m, 7-H), 5.68 (d, $J=4.0$ Hz, 1-H), 7.51 (br s, 3-H) and 9.14 (s, 11-H). *Anal.* Calcd for $C_{16}H_{22}O_8\cdot 3/2H_2O$: C, 52.03; H, 6.82. Found: C, 51.90; H, 6.49.

Hydrolysis of 7,8-Dehydroiridotrial Glucoside (8) with β-Glucosidase——Compound **8** (65 mg) was hydrolyzed in 0.1 M acetate buffer (pH 4.6, 10 ml) with β-glucosidase (5 mg). The usual work-up afforded a product, which was purified by PLC (*n*-hexane–ether, 1: 2) to give 7,8-dehydroiridotrial (**7**) (27 mg) as a colorless oil, bp 97°C (1.4 mmHg). $[\alpha]_D^{20}$ $+196.4°$ ($c=0.47$, MeOH). UV λ_{max}^{MeOH} nm (log ε): 251.5 (4.04). IR ν_{max}^{neat} cm^{-1}:

3250, 2890, 2715, 1650, 1615, 1245 and 1145. ^{1}H NMR δ: 1.84 (br s, 10-H_3), 4.16 (br s, OH), 5.06 (d, J= 7.0 Hz, 1-H), 5.53 (m, 7-H), 7.26 (br s, 3-H) and 9.28 (s, 11-H). High resolution MS, Calcd for $C_{10}H_{12}O_3$ (M^+): 180.0786. Found: 180.0784.

Conversion of 10-Dehydrogeniposide Tetraacetate (12) into [10-^{3}H]-Geniposide Pentaacetate (13)——$NaBH_4$ (12 mg) was added to a solution of **12** (50 mg) in MeOH (3 ml) and the whole was stirred under ice-cooling for 15 min. After quenching of the mixture with phenylpropanal (200 mg), the whole was neutralized with 10% methanolic AcOH and concentrated *in vacuo*. The residue (241 mg) was subjected to PLC (*n*-hexane–ether, 3: 2). The bands at around *Rf* 0.45, 0.23 and 0.10 gave phenylpropanal (48 mg), phenylpropanol (131 mg) and a white powdery substance (45 mg), respectively. The latter was acetylated to give a product (51 mg), which was recrystallized from EtOH to afford colorless needles (35 mg), mp 137—138°C. This compound was identical with an authentic sample (**13**) on the basis of mixed mp and comparison of ^{1}H NMR data.

NaB^3H_4 (5.2 mg, 45.0 mCi) was added to a solution of the acetate (**12**) (150 mg) in MeOH and the mixture was stirred under ice cooling for 5 min. Non-radioactive $NaBH_4$ (30 mg) was then added to the mixture and the whole was stirred for a further 10 min. The same work-up as above gave [10-^{3}H]-**13** (164 mg, spec. act. 59.44 mCi/mmol).

Catalytic Reduction of [10-^{3}H]-Geniposide Pentaacetate (13) followed by Deacetylation and Kuhn-Roth Oxidation of the Resultant [10-^{3}H]-Deoxyloganin (22)——[10-^{3}H]-Pentaacetate (**13**) (164 mg) was hydrogenated over the Pd-C catalyst in the usual way to give [10-^{3}H]-deoxyloganin tetraacetate (**15**) (128 mg), which, after dilution with carrier (350 mg), was recrystallized from EtOH until the specific activity became constant (13.29 mCi/mmol). An aliquot (0.33 mg) of the radioactive **15** was diluted with carrier (129.37 mg) and deacetylated to **22** (spec. act. 7.57×10^7 dpm/mmol), which was in turn subjected to the conventional Kuhn-Roth oxidation. The acetic acid generated was converted into α-naphthylamide, which was purified by sequential PLC, rescrystallization and sublimation. Spec. act. was 7.20×10^7 dpm/mmol, which corresponded to 95.2% of the total activity in the original compound (**22**).

Conversion of [10-^{3}H]-Deoxyloganin Tetraacetate (15) into [10-^{3}H]-Iridodial Glucoside (10) and [10-^{3}H]-Iridodial (3)——The rest of the above [10-^{3}H]-deoxyloganin tetraacetate (**15**) was converted into [10-^{3}H]-**10** and [10-^{3}H]-**3** (each spec. act., 13.29 mCi/mmol) according to the reported procedure.[6]

Conversion of [10-^{3}H]-Geniposide Pentaacetate (13) into [10-^{3}H]-Iridotrial (4)——[10-^{3}H]-11-Hydroxy-iridodial glucoside pentaacetate (**16**) derived from [10-^{3}H]-**13** through $LiAlH_2(OCH_3)_2$ reduction and acetylation was subjected successively to Zemplén reaction, catalytic oxygenation, and enzymatic hydrolysis with β-glucosidase in the same way as the non-labeled counterpart to afford [10-^{3}H]-**4** (spec. act., 12.21 mCi/mmol).

Conversion of [10-^{3}H]-13 into [10-^{3}H]-7,8-Dehydroiridodial (6)——[10-^{3}H]-10-Deoxygeniposide tetraacetate (**18**) derived from [10-^{3}H]-**13** was subjected successively to $LiAlH_2(OCH_3)_2$ reduction, hydrogenolysis, Zemplén reaction and hydrolysis with β-glucosidase to furnish [10-^{3}H]-**6** (spec. act., 8.27 mCi/mmol).

Conversion of [10-^{3}H]-13 into [10-^{3}H]-7,8-Dehydroiridotrial (7)——[10-^{3}H]-7,8-Dehydro-11-hydroxy-iridodial glucoside pentaacetate (**19**) derived from [10-^{3}H]-**13** was converted into [10-^{3}H]-**7** (spec. act., 8.75 mCi/mmol) through Zemplén reaction, catalytic oxygenation and hydrolysis with β-glucosidase.

Conversion of Ixoside Monomethyl Ester Tetraacetate (23) into [10-^{2}H]-Geniposide Pentaacetate (13)——ClCOOEt (0.30 ml) was added to a stirred solution of **23** (1.800 g) and Et_3N (0.44 ml) in dry THF (10 ml) precooled to -20°C under N_2 and the mixture was stirred for 2 h at the same temperature. Then, $NaBH_4$ (0.360 g) was added over a period of 30 min with dropwise addition of H_2O (0.2 ml), and the whole was stirred for an additional 30 min at -20°C. The mixture was poured onto ice-water, neutralized with 1 N HCl and extracted with $CHCl_3$ (10 ml×4). The combined extracts were washed successively with 10% aq. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue (2.115 g) was chromatographed on silica gel (50 g) with ether as the eluent, and 15 ml fractions were collected. Concentration of the combined frs 20—25 *in vacuo* gave a residue (1.095 g), which was acetylated. The product (1.190 g) was recrystallized from EtOH to give colorless needles (1.022 g), mp 137—138°C, which were identical with an authentic sample (**13**) on the basis of mixed mp and comparisons of IR and ^{1}H NMR data. On the other hand, the starting material (**23**) (0.320 g) was recovered from the $NaHCO_3$ washing.

The acid anhydride derived from **23** (1.140 g) was reduced with NaB^2H_4 (0.228 g, 97 atom% ^{2}H) in THF in the presence of D_2O (0.15 ml) in the same way as above. After neutralization with 10% AcOD–D_2O, the mixture was worked up the same as above. The product was acetylated to [10-$^2H_2$]-**13** (0.799 g), whose ^{1}H NMR spectrum was in accord with that of the non-labeled counterpart except for a decrease of the C-10 methylene signal intensity to a level corresponding to only one-fifth of a proton.

Conversion of [10-$^2H_2$]-Geniposide Pentaacetate (13) into [10-$^2H_3$]-10-Deoxygeniposide Tetraacetate (18)——A 5% $PdCl_2$ solution (0.56 ml) prepared by using D_2O and DCl was added to a suspension of activated charcoal (DARCO G-60) (200 mg) in MeOD (2 ml) and activated under a deuterium atmosphere in the usual manner. The Pd-C catalyst thus obtained was added to a solution of [10-$^2H_2$]-**13** (1.028 g) in MeOD (3 ml) and the whole was subjected to hydrogenolysis under a deuterium atmosphere, giving rise to [10-$^2H_3$]-**18** (468 mg). Its ^{1}H NMR spectrum was in accord with that of the non-labeled counterpart except for a decrease of the C-10 methyl signal intensity to a level corresponding to two-fifths of a proton.

Conversion of [10-$^2H_3$]-10-Deoxygeniposide Tetraacetate (18) into [10-$^2H_3$]-Deoxyloganin Tetraacetate (15)

——[10-$^2H_3$]-**18** (376 mg) was hydrogenated in the usual way with hydrogen gas over the Pd-C catalyst prepared by using D_2O and DCl in MeOD (2 ml) to yield [10-$^2H_3$]-**15** (368 mg). Its ^1H NMR spectrum was in accord with that of the non-labeled counterpart except for a decrease of the C-10 methyl signal intensity to a level corresponding to half of a proton.

Conversion of [10-$^2H_3$]-Deoxyloganin Tetraacetate (15) into [10-$^2H_3$]-Iridodial (3)——[10-$^2H_3$]-**15** (368 mg) was reduced with $LiAlH_2(OCH_3)_2$, followed by acetylation to give [10-$^2H_3$]-11-hydroxyiridodial glucoside pentaacetate (**16**) (295 mg). It was then subjected to hydrogenolysis over the Pd-C catalyst in the conventional way to afford [10-$^2H_3$]-iridodial glucoside tetraacetate (**17**) (245 mg). Its ^1H NMR spectrum was in agreement with that of the non-labeled counterpart except for a signal intensity of the C-10 methyl corresponding to only half of a proton. An aliquot (100 mg) of [10-$^2H_3$]-**17** was deacetylated to [10-$^2H_3$]-iridodial glucoside (**10**) (59 mg), whose ^1H NMR spectrum was in accord with that of the non-labeled counterpart except for a decrease of the signal intensity of the C-10 methyl group to a level corresponding to half of a proton. This compound was hydrolyzed with β-glucosidase to afford [10-$^2H_3$]-3 (27 mg), whose ^1H NMR spectrum was consistent with that of the non-labeled counterpart except for a decrease of the signal intensity of the C-10 methyl to a level corresponding to half of a proton. High resolution MS: Calcd for $C_{10}H_{13}{}^2H_3O_2$ (M^+): 171.1339. Found: 171.1334.

Conversion of Mussaenoside Tetraacetate (24) into [11-$^2H_2$]-8β,11-Dihydroxyiridodial Glucoside Pentaacetate (25)——A solution of **24** (300 mg) in dry THF (10 ml) was added over a period of 30 min to a suspension of $LiAlH_2(OCH_3)_2$ [prepared from $LiAlH_4$ (420 mg) and dry MeOH (0.85 ml)] in THF (20 ml) at -20°C under N_2. The mixture was stirred at elevated temperature (-20 to 0°C) for 1 h, then the excess reagent was decomposed by addition of a few drops of water. The resulting precipitate was filtered off, digested in ice-water and neutralized with 10% aq. AcOH. Inorganic materials were filtered off and the filtrate was transferred to a charcoal (3 g) column, eluting successively with H_2O (200 ml) and EtOH (200 ml). The EtOH eluate was concentrated *in vacuo* to give a residue (208 mg), which was acetylated. The product (335 mg) was recrystallized from ether to afford **25** (307 mg) as colorless needles, mp 129—130°C. $[\alpha]_D^{20}$ $-122.1°$ (c=0.98, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500, 2940, 1745, 1670, 1380 and 1250. ^1H NMR δ: 1.33 (s, 10-H_3), 2.00—2.09 ($OCOCH_3 \times 5$), 4.23 and 4.68 (d, J=12.0 Hz, each 11-H) and 6.27 (br s, 3-H). *Anal.* Calcd for $C_{26}H_{36}H_{14}$: C, 54.54; H, 6.34. Found: C, 54.58; H, 6.62.

Compound **24** (907 mg) was reduced with $LiAl^2H_2(OCH_3)_2$ prepared from $LiAl^2H_4$ (99 atom% ^2H) in the same manner as above to give a product (579 mg), which was acetylated to [11-$^2H_2$]-**25** (850 mg). Its ^1H NMR spectrum was consistent with that of the non-labeled counterpart except for the absence of the signal of the C-11 methylene protons.

Conversion of [11-$^2H_2$]-8β,11-Dihydroxyiridodial Glucoside Tetraacetate (25) into [11-$^2H_3$]-8β-Hydroxyiridodial Glucoside Tetraacetate (26)——Compound **25** (79 mg) was subjected to hydrogenolysis over the Pd-C catalyst in the usual way. The resultant product was purified by PLC (ether) followed by recrystallization from ether to afford **26** (58 mg) as colorless needles, mp 58.5—60°C. $[\alpha]_D^{20}$ $-119.6°$ (c=0.54, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450, 2940, 1750, 1675, 1370 and 1230. ^1H NMR δ: 1.30 (s, 10-H_3), 1.47 (br s, 11-H), 1.98—2.06 ($OCOCH_3 \times 4$) and 5.87 (br s, 3-H). *Anal.* Calcd for $C_{24}H_{34}O_{12} \cdot 1/4H_2O$: C, 55.54; H, 6.70. Found: C, 55.44; H, 6.72.

[11-$^2H_2$]-**25** (500 mg) was subjected to hydrogenolysis with deuterium gas in the usual manner to give [11-$^2H_3$]-**26** (463 mg). Its ^1H NMR spectrum was in agreement with that of the non-labeled counterpart except for the absence of the signal of the C-11 methyl protons.

Conversion of [11-$^2H_3$]-8β-Hydroxyiridodial Glucoside Tetraacetate (26) into [11-$^2H_3$]-7,8-Dehydroiridodial Glucoside Tetraacetate (20)——$POCl_3$ (0.1 ml) was added to a solution of **26** (58 mg) in pyridine (0.5 ml) under ice-cooling and the whole was allowed to stand at 5°C for 16 h. The mixture was poured onto ice-water and extracted with $CHCl_3$ (5 ml × 4). The combined extracts were washed successively with 1 N HCl, 10% $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by PLC (ether) and recrystallized from EtOH to give colorless needles (39 mg), mp 126—127°C. This compound was identical with an authentic sample of **20** on the basis of mixed mp and comparisons of IR and ^1H NMR data.

[11-$^2H_3$]-**26** (463 mg) was subjected to $POCl_3$-induced dehydration in the same way as described above to yield a product (429 mg), which was chromatographed on a column (50 g) of silica gel impregnated with $AgNO_3$. The column was eluted successively with benzene–acetone (97.5: 2.5, 600 ml), (97.0: 3.0, 200 ml) and (96.0: 4.0, 200 ml), and 5 ml fractions were collected. The combined frs 114—184 were concentrated *in vacuo* and the residue was recrystallized from EtOH to afford [11-$^2H_3$]-**20** (247 mg). Its ^1H NMR spectrum was in accord with that of the non-labeled counterpart except for the absence of the signal of the C-11 methyl protons. Furthermore, its ^2H NMR spectrum showed only one signal at δ 1.49 arising from three deuterium atoms on C-11.

Conversion of [11-$^2H_3$]-7,8-Dehydroiridodial Glucoside Tetraacetate (20) into [11-$^2H_3$]-7,8-Dehydroiridodial (6)——[11-$^2H_3$]-**20** (102 mg) was subjected to Zemplén reaction in MeOD in the usual way to afford [11-$^2H_3$]-7,8-dehydroiridodial glucoside (**21**) (66 mg). Its ^1H NMR spectrum was in accord with that of the non-labeled counterpart except for the absence of the signal of the C-11 methyl protons. An aliquot (33 mg) of this compound was hydrolyzed with β-glucosidase to afford [11-$^2H_3$]-**6** (15 mg). Its ^1H NMR spectrum was in agreement with that of the non-labeled counterpart except for the absence of the signal of the C-11 methyl protons. High resolution MS: Calcd for $C_{10}H_{11}{}^{2}H_{3}O_{2}$ (M^+): 169.1182. Found: 169.1165.

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