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Studies on Monoterpene Glucosides and Related Natural Products. XXXIX.[1] Biogenetic-type Transformation of Geniposide into Plumieride

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A biogenetic-type transformation of geniposide (**9**) into plumieride (**1**) was carried out. The tetraacetyl-geniposide (**12**) prepared from geniposide (**9**) *via* 10-dehydrogeniposide (**10**) was subjected to sensitized photooxygenation to give tetraacetyl-gardenoside (**13**). Oxidation of the allylic hydroxy group of **13**, followed by condensation of the resulting aldehyde (**23**) with ethyl acetoacetate afforded tetraacetyl-13-dehydroplumieride (**24**). This compound (**24**), on reduction followed by acetylation, gave a pair of epimers, **28** and **29**, of which the major one (**28**) was deacetylated to give plumieride (**1**).

Keywords——iridoid glucosides; plumieride; geniposide; gardenoside; biogenetic-type transformation; sensitized photooxygenation

Plumieride (**1**), a bitter glucoside of the iridoid series, was first isolated in 1870 from *Plumeria lancifolia* (Apocynaceae)[3] and was also found later to occur in two other *Plumeria* plants, *P. acutifolia*[4] and *P. rubra var. alba*.[5] Although its structure remained unsolved for a long time, in 1958 Schmid *et al.*[5] assigned the absolute structure **1** to the glucoside on the basis of chemical and spectral data. As regards natural products of the same type, eleven other compounds are known, including plumericin (**2**) and isoplumericin (**3**)[6] from the plants of the genus *Plumeria*, and oruwacin (**4**)[7] from *Morinda lucida* (Rubiaceae). Among them, allamandin (**5**)[8] from *Allamanda cathartica* (Apocynaceae) and penstemide (**6**)[9] from *Penstemon deutus* (Scrophulariaceae) are of interest because of their antitumor activity. However, no reports have yet appeared in the literature on the synthesis of compounds of this type.

On the other hand, the biosynthesis of plumieride (**1**) was also investigated by Schmid *et al.*, who reported the incorporation of two units each of mevalonic acid (MVA) and acetic acid into its aglucone moiety in 1964.[10] Taking into account the findings[11] obtained in later studies on the biosynthesis of iridoids, it seemed reasonable that plumieride (**1**) could be biosynthesized from 10-dehydrogardenoside (**7**), the oxidized congener of gardenoside (**8**) derived from geniposide (**9**), or its analog after formation of the iridoid skeleton from MVA by condensation with two molecules of acetic acid—presumably as acetoacetyl CoA—followed

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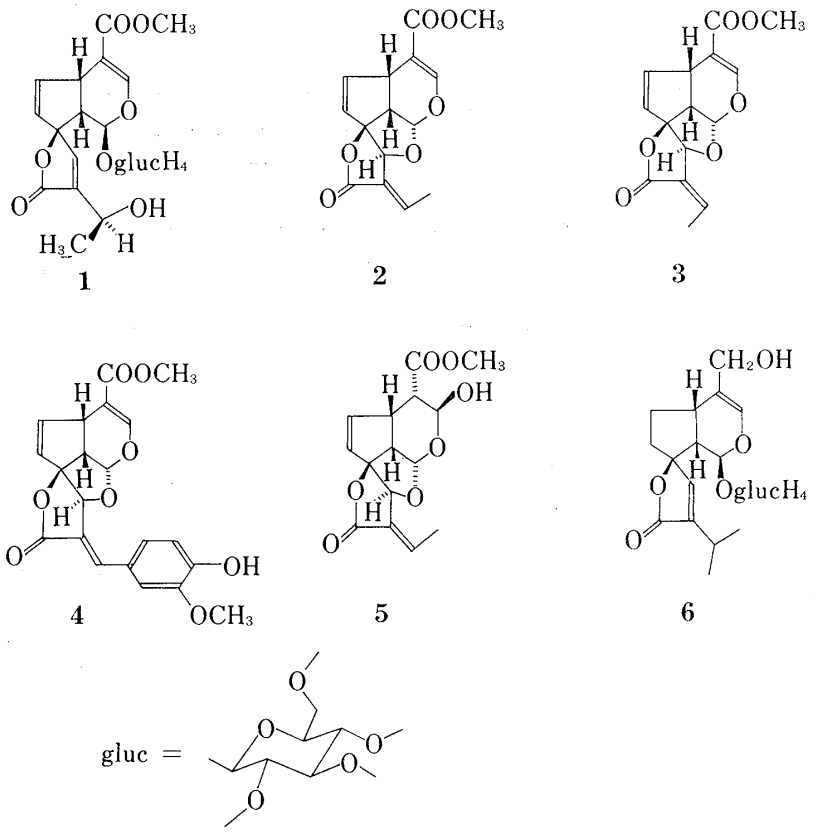

Fig. 1

by lactonization and reduction. This paper deals with a biogenetic-type partial synthesis of plumieride (**1**) from geniposide (**9**) *via* a derivative of gardenoside (**8**) based on the pathway described above. In this approach, it is essential for the preparation of the derivative of 10-dehydrogardenoside (**7**) to protect only the glucose moiety in gardenoside (**8**). However, it is very difficult to protect only the glucose moiety at this stage. We, therefore, planned to oxidize the highly reactive C-10 allylic hydroxy group of geniposide (**9**) to an aldehyde, followed by acetylation of the glucose moiety and regeneration of the C-10 alcohol group by reduction, giving rise to the 2′,3′,4′,6′-tetraacetyl derivative of geniposide (**9**). This compound should be readily convertible to the desired 2′,3′,4′,6′-tetraacetyl derivative of gardenoside (**8**).

Catalytic oxidation of **9** over Pt in 50% aq. AcOH afforded 10-dehydrogeniposide (**10**) in good yield. The compound (**10**) was acetylated and the acetate (**11**) was reduced with $NaBH_4$ to furnish 2′,3′,4′,6′-tetraacetyl-geniposide (**12**) in 85% yield. Although the biosynthetic process from geniposide (**9**) to gardenoside (**8**) has not yet been completely elucidated, such a conversion might be achieved chemically through oxidation with the aid of singlet oxygen.[12] Thus, as a model experiment for the conversion of **12** into the corresponding tetraacetyl-gardenoside (**13**), we first carried out the photooxygenation of tetraacetyl-deoxy-geniposide (**14**) obtained by the hydrogenolysis of pentaacetyl-geniposide (**15**). Namely, the compound (**14**) was oxygenated in the presence of Rose Bengal as a sensitizer in a mixture of benzene–pyridine (9: 1) to afford a peroxide, which was reduced with triphenylphosphine to yield the compound (**16**) in 53% yield. This compound was further subjected to catalytic reduction over Pd–C, affording the known 8β-hydroxy compound (**17**)[13] together

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with tetraacetyldeoxyloganin (**18**).[14] Accordingly, the photooxygenation product (**16**) of tetraacetyl-deoxygeniposide (**14**) was proved to be tetraacetyl-10-deoxygardenoside, arising from the attack of an oxygen molecule from the β-side of **14**. This seems favorable for the conversion of **12** into tetraacetylgardenoside (**13**). Therefore, **12** was subjected to photooxygenation under the same conditions, giving rise to two products, **13** and **19**. In the infrared (IR) spectrum, compound (**13**) showed a broad band due to hydroxy groups (3360 cm^{-1}), in addition to absorptions due to an ester (1740 cm^{-1}) and a chromophore –O–Ċ=Ċ(H)–COOMe (1700 and 1630 cm^{-1}). In the nuclear magnetic resonance (NMR) spectrum, signals appeared at δ 1.95—2.09 and δ 3.73 attributable to four acetoxy groups and $-CH_2OH$, as well as two double doublets (AB part of an ABX system) at δ 5.63 (J=6.0, 2.0 Hz) and δ 6.22 (J=6.0, 2.5 Hz) characteristic of gardenoside-type substances. These data suggest that **13** is 2′,3′,4′,6′-tetraacetyl-gardenoside. In fact, on acetylation it gave a product which was found to be identical with the pentaacetate (**20**)[15] of gardenoside (**8**) isolated from the plant. On the other hand, the other compound (**19**) showed IR bands due to hydroxy groups (3540, 3350 cm^{-1}) as well as an ester (1745 cm^{-1}) and a chromophore –O–Ċ=Ċ(H)–COOMe (1700 and 1630 cm^{-1}) and NMR signals due to four acetoxy groups (δ 1.93—2.11), a carbomethoxy group (δ 3.72), methylene protons of an allylic hydroxymethyl group (δ 4.42) and the C-1 proton (δ 6.33). In addition, the acetate (**21**) of **19** showed NMR signals at δ 1.92—2.09 and δ 5.69 (br d, J=7.0 Hz) attributable to six acetoxy groups and an allylic methylene bearing an acetoxy group, respectively. Based on these findings, **19** was assumed to be 2′,3′,4′,6′-tetraacetyl-8,9-dehydro-10-hydroxyloganin, while the β-configuration of the C-7 hydroxy group was deduced from the mechanism of formation of **19**.

Next, in order to oxidize the C-10 hydroxy group of **13**, it was subjected to Collins oxidation to yield the compound (**22**) instead of the desired aldehyde (**23**). The compound (**22**) showed IR bands assignable to an α,β-unsaturated ketone in a five-membered ring (1740 1580 cm^{-1}) and a chromophore –O–Ċ=Ċ(H)–COOMe (1700, 1640 cm^{-1}). The NMR spectrum showed two double doublets (AM part of an AMX system) at δ 6.18 (J=5.6, 1.8 Hz) and δ 7.98 (J=5.6, 3.0 Hz) as in the case of **13** though shifted downfield, as well as signals due to four acetoxy groups at δ 1.95—2.12, a double doublet (J=7.5, 1.2 Hz) due to the C-9 proton at δ 3.03 and a doublet (J=1.2 Hz) due to the C-1 proton at δ 5.85. Taking into account these data, compound (**22**) is assumed to contain an α,β-unsaturated cyclopentanone arising from oxidative cleavage of the bond between C-8 and C-10. This was confirmed by the finding that compound (**22**) can also be obtained by oxidation of **13** with $NaIO_4$. Conversion of **13** into an aldehyde (**23**), after various attempts, was achieved through oxidation with DMSO–Ac_2O. The oxidation product (**23**) showed an NMR spectrum very similar to that of **13**, the only difference being the absence of the signals for the hydroxymethyl group in **13** and the presence of an aldehyde proton signal (δ 9.37) in **23**.

Condensation of **23** with ethyl acetoacetate in the presence of piperidine acetate in benzene led to two products, **24** and **25**. The product (**24**) showed IR bands assignable to an α,β-unsaturated γ-lactone (1755 cm^{-1}), an ester (1740 cm^{-1}), a chromophore –O–Ċ=Ċ(H)–COOMe (1690, 1630 cm^{-1}) and a double bond (1610 cm^{-1}). The NMR spectrum, in addition to all the signals of **23** except for that of the C-10 aldehyde proton, showed signals due to a $-COOCH_3$ group at δ 2.57 and the C-10 proton at δ 7.85. Therefore, **24** was presumed to be 13-dehy-

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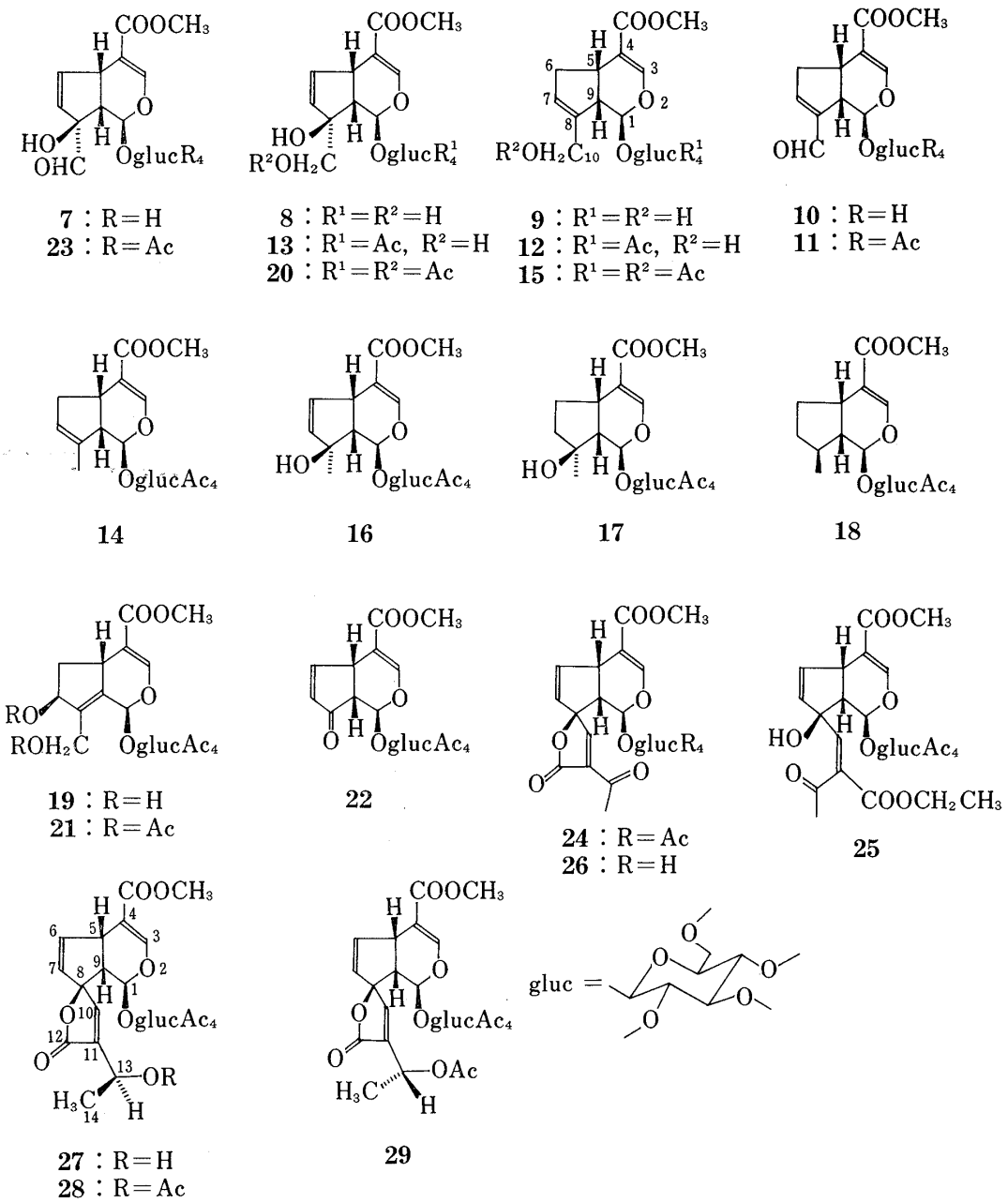

Fig. 2

droplumieride tetraacetate. This was supported by the finding that **24** was identical with the compound obtained by oxidation of plumieride (**1**) over a Pt catalyst to give the ketone (**26**), followed by acetylation. On the other hand, the other product (**25**) showed IR bands due to hydroxy groups at 3430 cm^{-1}, and an ester at 1740 cm^{-1} as well as the chromophores –O–Ċ=Ċ–COOMe and –Ċ=Ċ–COOEt (1700, 1630 cm^{-1}). NMR signals appeared corresponding to all those of **23** except for that of the C-10 aldehyde proton in addition to signals due to a methyl group in a carboethoxy moiety (t, J=7.0 Hz) at δ 1.33 and the C-10 olefinic proton (s) at δ 6.63. Accordingly, the structure **25** was assigned to the compound. This was consistent with the failure of an attempted lactonization of **25** to **24**.

Finally, in contrast to the case of **11**, the reduction of compound (**24**) with $NaBH_4$, aiming to obtain 2′,3′,4′,6′-tetraacetyl-plumieride (**27**) yielded an inseparable mixture of

reduction products formed by 1,4-hydride addition. Therefore, **24** was subjected to Meerwein-Pondorf-Verley reduction with aluminum tri-isopropoxide to yield a mixture consisting of two components in a ratio of 3: 2, which, after acetylation, were separated by high-performance liquid chromatography (HPLC). The major component, showing the more polar peak, was identical with the pentaacetate (**28**) of plumieride (**1**) isolated from the plant, while the other component (less polar) is assumed to be the 13-epimer (**29**) of **28** because of the close similarity of its NMR spectrum to that of **28**. The acetate (**28**) was easily hydrolyzed by means of the Zemplén reaction to yield plumieride (**1**). Thus, the transformation of geniposide (**9**) into plumieride (**1**) was accomplished. The absolute structure of plumieride (**1**) assumed by Schmid *et al.*[5] on the basis of chemical and spectral data as well as some stereochemical assumptions is thus unequivocally established by its chemical correlation with geniposide (**9**) (and gardenoside (**8**)) of known absolute structure.

Experimental[16]

Catalytic Oxidation of Geniposide (9)——A solution of **9** (1.0 g) in 50% aq. AcOH (10 ml) was added to a suspension of Pt (prepared from PtO_2 (200 mg)) in 50% aq. AcOH (5 ml) and the mixture was stirred for 72 hr under an O_2 atmosphere. After removal of the catalyst by filtration, the filtrate was concentrated *in vacuo*. Purification of the residue (981 mg) by PLC ($CHCl_3$–MeOH, 8: 2, *Rf* 0.37) yielded 10-dehydrogeniposide (**10**) (644 mg) as a white powder. $[\alpha]_D^{30}$ −20.8° (c=0.73, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3370 (br), 1670, 1620, NMR δ (D_2O): 3.75 (3H, s, –$COOCH_3$), 5.60 (1H, d, J=5.0 Hz, C-1 H), 7.33 (1H, m, C-7 H), 7.54 (1H, s, C-3 H), 9.66 (1H, s, –CHO). *Anal.* Calcd. for $C_{17}H_{22}O_{10}\cdot H_2O$: C, 50.50; H, 5.98. Found: C, 50.80; H, 5.91.

Acetylation of 10-Dehydrogeniposide (10)——Compound (**10**) (120 mg) was acetylated with 1.2 ml each of Ac_2O and pyridine in the usual way and the product was recrystallized from ether–petr. ether, giving tetraacetyl-10-dehydrogeniposide (**11**) (80 mg) as colorless needles, mp 130—131°, $[\alpha]_D^{27}$ +17.7° (c=0.79, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 227.5 (3.77). IR ν_{max}^{KBr} cm^{-1}: 1755, 1705, 1680, 1640. NMR δ: 1.93, 2.02, 2.05, 2.15 (each s, 4×$OCOCH_3$), 3.70 (3H, s, –$COOCH_3$), 6.13 (1H, d, J=2.0 Hz, C-1H), 6.93 (1H, m, C-7 H), 7.34 (1H, s, C-3 H), 9.77 (1H, s,–CHO). *Anal.* Calcd. for $C_{25}H_{30}O_{14}$: C, 54.15; H, 5.45. Found: C, 54.32; H, 5.72.

Reduction of Tetraacetyl-10-dehydrogeniposide (11) with $NaBH_4$——A solution of $NaBH_4$ (40 mg) in H_2O (1.5 ml) was added to a solution of **11** (201 mg) in dioxane (20 ml), and the mixture was stirred for 30 min at room temperature. Excess reagent was decomposed by adding a few drops of AcOH under ice cooling. After concentration of the mixture, the residue was extracted with $CHCl_3$, washed with H_2O and dried over $MgSO_4$. Removal of the solvent gave a residue, which was recrystallized from dil. EtOH, affording 2′,3′,4′,6′-tetraacetyl-geniposide (**12**) (170 mg) as colorless needles, mp 117—119°, $[\alpha]_D^{28}$ +6.10° (c=0.85, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450, 1750, 1705, 1640. NMR δ: 2.02—2.08 (4×$OCOCH_3$), 3.73 (3H, s, $COOCH_3$), 4.26 (4H, m, C-10 H_2 and C-6′ H_2), 5.82 (1H, m, C-7 H). 7.45 (1H, d, J=1.0 Hz, C-3 H). *Anal.* Calcd. for $C_{25}H_{32}O_{14}$: C, 53.96; H, 5.80. Found: C, 53.85; H, 6.01.

Sensitized Photooxygenation of Tetraacetyl-10-deoxygeniposide (14)——A 4: 1 mixture (800 mg) of compound (**14**) and tetraacetyl-deoxyloganin (**18**) (obtained by catalytic reduction of pentaacetyl-geniposide (**15**)) was dissolved in a mixture (80 ml) of benzene and pyridine (9: 1). Rose Bengal (60 mg) was added to this solution and the mixture was irradiated with a high-pressure mercury lamp through a Pyrex filter over a period of 10 hr in an O_2 stream under ice cooling, then concentrated *in vacuo* to about 40 ml. This solution, after addition of $(C_6H_5)_3P$ (308 mg), was stirred for 3 hr at room temperature and concentrated *in vacuo* to give a residue, which was thoroughly shaken with $CHCl_3$. The insoluble material was filtered off and washed with $CHCl_3$. After concentration of the combined filtrate and washings *in vacuo*, the residue (854 mg) was subjected to chromatography on silica gel (40 g) with $CHCl_3$ as an eluent, collecting 20 ml fractions. The combined Fractions 12—16 were concentrated and the residue was subjected to PLC (C_6H_6–EtOAc, 7: 3). The band around *Rf* 0.35 was scraped off and extracted with $CHCl_3$–MeOH (95: 5). After

16) Melting points were measured on a Yanagimoto micro-apparatus and are uncorrected. Optical rotations were taken with a Union PM 201 automatic digital polarimeter. UV spectra were recorded on a Hitachi 200-20 spectrophotometer, and IR spectra on a Hitachi 215 grating infrared spectrophotometer. Unless otherwise stated, NMR spectra were taken on a Varian A-60 or HA-100 spectrometer in $CDCl_3$ with TMS as an internal standard. HPLC was carried out on a Waters ALC/GPC-244 high-performance liquid chromatograph. Silica gel 60 GF_{254} (Merck) was used for thin-layer chromatography (TLC) and spots were visualized by exposure to I_2 vapor or by spraying with anisaldehyde–H_2SO_4 reagent followed by heating. Silica gel 60 PF_{254} (Merck) was employed for preparative thin-layer chromatography (PLC) and spots were detected under UV light. The ratios of solvents are expressed by volume.

concentration of the extract *in vacuo*, the residue (347 mg) was recrystallized from EtOH, furnishing tetraacetyl-10-deoxygardenoside (**16**) as colorless needles, mp 130—131°. $[\alpha]_D^{24}$ −119.8° (c=0.48, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3375 (br), 1745, 1735/(sh), 1695, 1640, 1620. NMR δ: 1.30 (3H, br s, C-10 H_3), 1.92, 2.00, 2.03, 2.10 (each s, 4×$OCOCH_3$), 2.63 (1H, dd, J=9.0 and 2.0 Hz, C-9 H), 3.72 (3H, s, $COOCH_3$), 5.73 (1H, d, J=2.0 Hz, C-1 H), 5.82 (1H, dd, J=6.0 and 2.0 Hz, C-7 H), 6.08 (1H, dd, J=6.0 and 3.0 Hz, C-6 H), 7.30 (1H, d, J=1.0 Hz, C-3 H). *Anal.* Calcd. for $C_{25}H_{32}O_{14}$: C, 53.96; H, 5.80. Found: C, 54.08; H, 5.74.

Catalytic Reduction of Tetraacetyl-10-deoxygardenoside (16)——A solution of **16** (150 mg) in MeOH (10 ml) was added to a suspension of Pd-C (prepared from 5% $PdCl_2$–HCl solution (0.6 ml) and activated charcoal (200 mg)) in MeOH (10 ml), and the mixture was stirred under an H_2 atmosphere. When uptake of H_2 had ceased, the catalyst was filtered off and the filtrate was concentrated. The residue (149 mg) was subjected to chromatography on silica gel (20 g) with ether as an eluent, collecting 5 ml fractions. The combined Fr. Nos. 5—7 were concentrated and the residue (117 mg) was recrystallized from EtOH to give colorless needles, mp 114—116°. This compound was identical with an authentic specimen of tetraacetyldeoxyloganin (**18**) (mixed melting point and comparisons of IR and NMR spectra). On the other hand, the residue (22 mg) from the combined Fr. Nos. 18—33 was recrystallized from ether–petr. ether to afford colorless needles, mp 86—87°. IR ν_{max}^{KBr} cm^{-1}: 3500, 1750, 1705, 1640. NMR δ: 1.33 (3H, s, C-10 H_3), 1.93—2.10 (4×$OCOCH_3$), 2.31 (1H, dd, J=9.5 and 3.0 Hz, C-9 H), 3.03 (1H, m, C-5 H), 3.71 (3H, s, $COOCH_3$), 5.33 (1H, d, J=3.0 Hz, C-1 H), 7.34 (1H, d, J=1.0 Hz, C-3 H). This compound was shown to be identical with an authentic sample of 8β-hydroxy compound (**17**) (mixed melting point and comparisons of IR and NMR spectra).

Sensitized Photooxygenation of Tetraacetyl-geniposide (12)——Rose Bengal (100 mg) was added to a solution of **12** (2.59 g) in a mixture (120 ml) of benzene, MeOH and pyridine (1:1:2), and the solution was irradiated with a high-pressure mercury lamp through a Pyrex filter over a period of 65 hr in an O_2 stream under ice cooling. After the addition of $(C_6H_5)_3P$ (400 mg) under ice cooling, the mixture was stirred for 6 hr at room temperature. The reaction mixture was then concentrated *in vacuo* and the residue was dissolved in $CHCl_3$, washed successively with 1 N HCl and H_2O, and dried over $MgSO_4$. Removal of the solvent *in vacuo* gave a residue (2.88 g), which was subjected to chromatography on silica gel (50 g), eluting with $CHCl_3$–MeOH with an increasing MeOH content. Fractions eluted with $CHCl_3$–MeOH (99.3:0.7) showing a spot of Rf 0.25 on TLC ($CHCl_3$–MeOH, 95:5) were combined and concentrated *in vacuo*. The residue (773 mg) was recrystallized from EtOH giving 2′,3′,4′,6′-tetraacetyl-gardenoside (**13**) as colorless needles, mp 183—185°. $[\alpha]_D^{30}$ −86.7° (c=0.77, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3360 (br), 1740, 1700, 1630. NMR δ: 1.95—2.09 (4×$OCOCH_3$), 2.60 (1H, dd, J=8.0 and 3.5 Hz, C-9 H), 2.84 (2H, br s, 2×OH), 3.63 (2H, m, C-10 H_2), 3.73 (3H, s, $COOCH_3$), 5.57 (1H, d, J=3.5 Hz, C-1 H), 5.63 (1H, dd, J=6.0 and 2.0 Hz, C-7 H), 6.22 (1H, dd, J=6.0 and 2.5 Hz, C-6 H), 7.36 (1H, d, J=1.0 Hz, C-3 H). *Anal.* Calcd. for $C_{25}H_{32}O_{15}$: C, 52.45; H, 5.63. Found: C, 52.19; H, 5.82. Similarly, fractions eluted with $CHCl_3$–MeOH (99; 1) showing a spot of Rf 0.15 under the same TLC conditions afforded a substance (785 mg), which was recrystallized from EtOH to furnish 2′,3′,4′,6′-tetraacetyl-8,9-dehydro-10-hydroxyloganin (**19**) as colorless needles, mp 174—176°. $[\alpha]_D^{30}$ −92.1° (c=1.11, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3540, 3350, 1740, 1690, 1620. NMR δ: 1.93—2.11 (4×$OCOCH_3$), 2.98 (2H, br s, OH, disappeared on addition of D_2O), 3.72 (3H, s, $COOCH_3$), 4.42 (2H, m, C-10 H_2), 6.33 (1H, s, C-1 H), 7.32 (1H, d, J=1.0 Hz, C-3 H). *Anal.* Calcd. for $C_{25}H_{32}O_{15}$: C, 52.45; H, 5.63. Found: C, 52.37; H, 5.82.

Acetylation of 2′,3′,4′,6′-Tetraacetyl-gardenoside (13)——Compound (**13**) (120 mg) was acetylated with Ac_2O and pyridine in the usual way and the product (118 mg) was chromatographed on silica gel (15 g), eluting with ether. Fractions showing a spot of Rf 0.20 on TLC (ether) were combined and concentrated *in vacuo* to give pentaacetyl-gardenoside (**20**) (80 mg) as a white powder. $[\alpha]_D^{30}$ −131.2° (c=0.40, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3420 (br), 1740, 1700, 1630. NMR δ: 1.91—2.09 (5×$OCOCH_3$), 2.73 (1H, m, OH, disappeared on addition of D_2O), 2.76 (1H, dd, J=9.0 and 2.0 Hz, C-9 H), 3.72 (3H, s, $COOCH_3$), 4.12 (2H, m, C-10 H_2), 5.67 (1H, d, J=6.0 and 3.0 Hz, C-6 H), 7.33 (1H, d, J=1.0 Hz, C-3 H). *Anal.* Calcd. for $C_{27}H_{34}O_{16}\cdot H_2O$: C, 51.48; H, 5.73. Found: C, 51.68; H, 5.95. This compound was identical with an authentic specimen (comparison of IR and NMR spectra and optical rotations).

Acetylation of 2′,3′,4′,6′-Tetraacetyl-8,9-dehydro-10-hydroxyloganin (19)——Compound (**19**) (100 mg) was acetylated with Ac_2O and pyridine in the usual way and the product was recrystallized from EtOH, furnishing the hexaacetate (**21**) (45 mg) as colorless needles, mp 68—70°. $[\alpha]_D^{25}$ −83.7° (c=1.85, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1750, 1710 (sh), 1625. NMR δ: 1.92—2.09 (6×$OCOCH_3$), 2.55 (1H, dd, J=7.0 and 15.0 Hz, C-6 eq. H), 5.74 (1H, br d, J=7.0 Hz, C-7 H), 6.22 (1H, s, C-1 H), 7.34 (1H, d, J=1.0 Hz, C-3 H). *Anal.* Calcd. for $C_{29}H_{36}O_{17}$: C, 53.05; H, 5.53. Found: C, 53.09; H, 5.79.

Oxidation of 2′,3′,4′,6′-Tetraacetyl-gardenoside (13) with $CrO_3(C_5H_5N)_2$——A solution of **13** (233 mg) in abs. CH_2Cl_2 (20 ml) was added to a suspension of $CrO_3(C_5H_5N)_2$ (696 mg) in abs. CH_2Cl_2 (15 ml) and the suspension was stirred for 1 hr under ice cooling. The reaction mixture was washed successively with 1 N HCl, 5% $NaHCO_3$ and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The residue (182 mg) was chromatographed on silica gel (20 g) with $CHCl_3$ as an eluent. Fractions showing a spot of Rf 0.70 on TLC (ether) were combined and concentrated *in vacuo*. The residue (55 mg) was recrystallized from EtOH, affording compound (**22**) as colorless needles, mp 129—131°. $[\alpha]_D^{30}$ −56.8° (c=1.09, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1740, 1700, 1640, 1580. NMR δ: 1.95—2.14 (4×$OCOCH_3$), 3.03 (1H, dd, J=7.5 and 1.2 Hz, C-9 H), 3.78 (3H, s,

$COOCH_3$), 3.99 (1H, m, C-5 H), 5.85 (1H, d, J=1.2 Hz, C-1 H), 6.18 (1H, dd, J=5.5 and 1.8 Hz, C-7 H), 7.31 (1H, d, J=1.0 Hz, C-3 H), 7.98 (1H, dd, J=5.5 and 3.0 Hz, C-6 H). *Anal.* Calcd. for $C_{24}H_{28}O_4$: C, 53.34; H, 5.22. Found: C, 53.59; H, 5.32.

Oxidation of 2′,3′,4′,6′-Tetraacetyl-gardenoside (13) with $NaIO_4$——A solution of $NaIO_4$ (120 mg) in H_2O (10 ml) was added to a solution of **13** (200 mg) in MeOH (20 ml) under ice cooling, then the precipitate was filtered off and washed with MeOH. The combined filtrate and washings were diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue (192 mg) was recrystallized from EtOH to give **22** (86 mg) as colorless needles, mp 129—131°. *Anal.* Calcd. for $C_{24}H_{28}O_{14}$: C, 53.34; H, 5.22. Found: C, 53.49; H, 5.19.

Oxidation of 2′,3′,4′,6′-Tetraacetyl-gardenoside (13) with DMSO-Ac_2O——Ac_2O (3 ml) was added to a solution of **13** (634 mg) in DMSO (8 ml) and the mixture was stirred for 2 hr at room temperature under N_2. The reaction mixture was poured into iced water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed successively with satd. $NaHCO_3$ and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The residue (680 mg) was chromatographed on silica gel (30 g) with ether as an eluent, collecting 5 ml fractions. The combined Fr. Nos. 11—17 were concentrated *in vacuo* and the residue (261 mg) was recrystallized from ether–petr. ether to afford tetraacetyl-10-dehydrogardenoside (**23**) as colorless needles, mp 90—92°. $[\alpha]_D^{10}$ −231.0° (c=1.00, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1750, 1710, 1640. NMR δ: 1.92—2.10 (4×$OCOCH_3$), 3.00 (1H, dd, J=9.0 and 1.5 Hz, C-9 H), 3.76 (3H, s, $COOCH_3$), 5.35 (1H, d, J=1.5 Hz, C-1 H), 5.50 (1H, J=6.0 and 1.5 Hz, C-7 H), 6.60 (1H, dd, J=6.0 and 3.0 Hz, C-6 H), 7.30 (1H, d, J=1.0 Hz, C-3 H), 9.37 (1H, s, CHO). *Anal.* Calcd. for $C_{25}H_{30}O_{15}$: C, 52.63; H, 5.30. Found: C, 52.41; H, 5.46. The MeOH eluate afforded unreacted starting material (**13**) (203 mg).

Condensation of Tetraacetyl-10-dehydrogardenoside (23) with Ethyl Acetoacetate——Piperidine acetate (20%) in abs. benzene (0.5 ml) was added to a solution of **23** (817 mg) and ethyl acetoacetate (0.1 ml) in abs. benzene (10 ml), and the mixture was stirred at 70° for 1 hr then refluxed for 3 hr. After further addition of 0.2 ml of 20% piperidine acetate in abs. benzene, the reaction mixture was refluxed for an additional 1 hr, then cooled to room temperature. The reaction mixture was washed successively with H_2O, 1 N HCl, satd. $NaHCO_3$ and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The residue (752 mg) was chromatographed on silica gel (40 g) with ether as an eluent, collecting 5 ml fractions. Fr. Nos. 8—12 were combined and concentrated *in vacuo*, and the residue (233 mg) was recrystallized from ether–petr. ether to furnish the ester (**25**) as colorless needles, mp 90—92°. $[\alpha]_D^{25}$ −120.5° (c=0.39, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3430, 1740, 1700, 1630. NMR δ: 1.33 (3H, t, J=7.0 Hz, $COOCH_2C\underline{H}_3$), 1.77 (3H, s, $-COCH_3$), 1.93—2.08 (4×$OCOCH_3$), 2.90 (1H, dd, J=9.0 and 2.5 Hz, C-9 H), 3.74 (3H, s, $COOCH_3$), 6.63 (1H, s, C-10 H), 7.38 (1H, d, J=1.5 Hz, C-3 H). *Anal.* Calcd. for $C_{31}H_{28}O_{17}\cdot H_2O$: C, 53.31; H, 5.72. Found: C, 53.14; H, 5.75. On the other hand, Fr. Nos. 13—27 from the above silica gel column chromatography were combined and concentrated *in vacuo* to give a residue (230 mg), which was recrystallized from EtOH, affording tetraacetyl-13-dehydroplumieride (**24**) as colorless needles, mp 161—163°. $[\alpha]_D^{25}$ −122.4° (c=0.34, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1755, 1740, 1690, 1630, 1610. NMR δ: 1.92—2.09 (4×$OCOCH_3$), 2.57 (3H, s, $-COCH_3$), 5.44 (1H, dd, J=6.0 and 2.0 Hz, C-7 H), 6.52 (1H, dd, J=6.0 and 3.0 Hz, C-6 H), 7.38 (1H, d, J=1.5 Hz, C-3 H), 7.85 (1H, s, C-10 H). *Anal.* Calcd. for $C_{29}H_{32}O_{16}$: C, 54.72; H, 5.07. Found: C, 54.84; H, 5.29.

Conversion of Plumieride (1) into Tetraacetyl-13-dehydroplumieride (24)——A solution of plumieride (**1**) (100 mg) in H_2O (10 ml) was added to a suspension of Pt (prepared from PtO_2 (50 mg)) in H_2O (5 ml), and the mixture was stirred for 56 hr under O_2. The catalyst was filtered off and washed with H_2O. After concentration of the combined filtrate and washings, the residue (114 mg) was subjected to PLC ($CHCl_3$–MeOH, 85: 15, 3 developments). Of the two major bands, the more polar one gave the starting material (**1**) (42 mg), while the less polar one furnished a substance (27 mg), which was recrystallized from EtOH to give 13-dehydroplumieride (**26**) as colorless needles, mp 127—129°. $[\alpha]_D^{25}$ −39.0° (c=0.61, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3600—3100, 1700, 1690, 1630. NMR δ (D_2O): 2.53 (3H, s, $COCH_3$), 3.78 (3H, s, $COOCH_3$), 5.38 (1H, d, J=5.0 Hz, C-1 H), 5.62 (1H, dd, J=6.0 and 2.0 Hz, C-7 H), 6.57 (1H, dd, J=6.0 and 2.5 Hz, C-6 H), 7.55 (1H, d, J=1.5 Hz, C-3 H), 8.44 (1H, s, C-10 H). *Anal.* Calcd. for $C_{21}H_{24}O_{12}\cdot H_2O$: C, 51.85; H, 5.39. Found: C, 51.69; H, 5.36.

Next, compound (**26**) (27 mg) was acetylated in the usual manner and the product was recrystallized from EtOH to give tetraacetyl-13-dehydroplumieride (**24**) (16 mg) as colorless needles, mp 161—163°. $[\alpha]_D^{25}$ −133.0° (c=0.41, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{32}O_{16}$: C, 54.72; H, 5.07. Found: C, 54.69; H, 5.26. This compound was identical with compound (**24**) obtained by aldol condensation of **23** with ethyl acetoacetate (mixed melting point and comparisons of the spectral data).

Reduction of Tetraacetyl-13-dehydroplumieride (24) with Aluminum Tri-iso-propoxide followed by Acetylation——A solution of **24** (178 mg) and $Al[OCH(CH_3)_2]_3$ (86 mg) in abs. benzene (28 ml) was refluxed with stirring for 17 hr under N_2. After cooling to room temperature, the mixture was washed successively with 1 N HCl and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The residue (104 mg) was subjected to PLC (ether). The band around *Rf* 0.15 gave a mixture of two components (52 mg); this material was acetylated in the usual way. The product (46 mg) was subjected to HPLC to afford pentaacetyl-plumieride (**28**) (20 mg) and its C-13 epimer (**29**) (18 mg), respectively.

Separation conditions: packing, μPorasil; column size, 30 cm×7.8 mm (preparative); solvent, isooctane–

isopropyl alcohol (9: 1); flow rate, 6.0 ml/min; detector, UV at 254 nm; retention times, 14.3 min (**28**), 11.9 min (**29**).

28, colorless needles, mp 148—149°. IR ν_{max}^{Nujol} cm^{-1}: 1740, 1715 (sh), 1635. NMR (100 MHz) δ: 1.52 (3H, d, J=7.0 Hz, -CH(OAc)C<u>H</u>$_3$), 1.92, 2.00, 2.02, 2.08 and 2.09 (each s, 5×OCOC<u>H</u>$_3$), 3.14 (1H, dd, J=9.0 and 3.0 Hz, C-9 H), 3.76 (3H, s, COOC<u>H</u>$_3$), 4.09 (1H, dd, J=12.5 and 2.0 Hz, C-6′ H), 4.33 (1H, dd, J=12.5 and 4.5 Hz, C-6′ H), 5.46 (1H, dd, J=6.0 and 2.0 Hz, C-7 H), 5.65 (1H, dq, J=1.5 and 7.0 Hz, -C<u>H</u>(OAc)-CH$_3$), 6.45 (1H, dd, J=6.0 and 3.0 Hz, C-6 H), 6.96 (1H, d, J=1.5 Hz, C-10 H), 7.39 (1H, d, J=1.5 Hz, C-3 H). This substance was identical with an authentic specimen (mixed melting point and comparisons of IR and NMR spectra).

29, a colorless syrup. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1700, 1635. NMR (100 MHz) δ: 1.49 (3H, d, J=7.0 Hz, -CH(OAc)C<u>H</u>$_3$), 1.91, 1.99, 2.02 and 2.09 (each s, 5×OCOC<u>H</u>$_3$), 3.16 (1H, dd, J=8.5 and 2.0 Hz, C-9 H), 3.75 (3H, s, COOC<u>H</u>$_3$), 4.12 (1H, dd, J=12.5 and 2.5 Hz, C-6′ H), 4.35 (1H, dd, J=12.5 and 4.5 Hz, C-6′ H), 5.45 (1H, dd, J=6.0 and 2.0 Hz, C-7 H), 5.60 (1H, dq, J=1.5 and 7.0 Hz, -C<u>H</u>(OAc)CH$_3$), 6.45 (1H, d, J=1.5 Hz, C-10 H), 7.34 (1H, d, J=1.5 Hz, C-3 H).

Zemplén Reaction of Pentaacetyl-plumieride (28)——Methanolic NaOMe (0.26 N, 0.1 ml) was added to a solution of **28** (30 mg) in abs. MeOH (1 ml) and the mixture was refluxed for 5 min. After cooling, the reaction mixture was neutralized with Amberlite IRC-50 (H^+-form) and concentrated *in vacuo*. The residue was recrystallized from EtOH, giving colorless needles (10 mg), mp 227—229°. $[\alpha]_D^{15}$ −73.1° (c=0.52, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400—3100, 1730, 1675, 1630. NMR δ (D_2O): 1.42 (3H, d, J=7.0 Hz, -CH(OH)C<u>H</u>$_3$), 2.94 (1H, dd, J=9.0 and 5.0 Hz, C-9 H), 5.27 (1H, d, J=5.0 Hz, C-1 H), 5.53 (1H, dd, J=5.5 and 2.0 Hz, C-7 H), 6.48 (1H, dd, J=5.5 and 2.5 Hz, C-6 H), 7.38 (1H, d, J=1.5 Hz, C-10 H), 7.51 (1H, d, J=1.5 Hz, C-3 H). *Anal.* Calcd. for $C_{21}H_{26}O_{12}$: C, 53.62; H, 5.57. Found: C, 53.74; H, 5.45. This compound was identical with an authentic specimen of plumieride (**1**) (mixed melting point and comparisons of spectral data).

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