STUDIES ON IRIDOID-RELATED COMPOUNDS, II. THE STRUCTURE AND ANTIMICROBIAL ACTIVITY OF AGLUCONES OF GALIOSIDE AND GARDENOSIDE

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ABSTRACT.—Enzymatic hydrolysis of galioside (**1**) and gardenoside (**2**), epimer of **1** at C-8 position, provided the antimicrobially active aglucone (**3**) and the inactive **6(a,b)**, while acid treatment of **2** gave scandoside methylester (**8**), deacetylasperulosidic acid methylester (**9**) and 10-dehydogeniposide (**10**).

Aucubin, an iridoid glucoside, which is isolated from *Aucuba japonica* Thunb., shows antimicrobial activity against *Staphylococcus aureus* in the presence of β-glucosidase (the same effect as 600 I.U. penicillin) (1-3). Our previous paper (4) demonstrated that its unstable aglucone aucubigenin (5) was the active form for this antimicrobial activity. We also confirmed the antimicrobial activity of aglucones obtained from about 20 kinds of iridoid glucosides by treating them with β-glucosidase. Our results suggest the possibility of its defensive role against plant pathogens.

Among the iridoids we tested, galioside (**1**) (6) and gardenoside (**2**) (7), which are isomers differing at the C-8 position, gave comparatively stable aglucones when treated with β-glucosidase. In the antimicrobial test, the aglucone of **1** showed activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*, but that of **2** did not. This suggested that their activities depend upon the structural difference of aglucones of **1** and **2**. Here we wish to describe their structures.

When galioside (**1**) was treated with β-glucosidase, the aglucone (**3**) was obtained as colorless needles (mp, 110-115°) after purification on charcoal and silica gel followed by recrystallization from ethyl acetate. The acetylation of **3** under mild conditions gave the more stable diacetate (**4**). The structure of **4** was deduced by comparing its ^{1}H-nmr (360) MHz) with that of the parent iridoid glucoside acetate (**5**) and by double resonance experiments. As shown in table 1, all signals corresponding to the aglucone moiety in compound **5** appeared in the same region of compound **4**, except for the signal of C-1 proton (*i.e.*, δ 6.47 for **4** and δ 5.55 for **5**). Thus, the structure of **3** was confirmed as a product of simple cleavage of the glucoside bond.

Treatment of gardenoside (**2**) with β-glucosidase gave a mixture (1:1) of epimeric compound **6a** and **6b** (77% yield) named gardenogenin A and B, respectively. Recrystallization of the mixture from ethyl acetate gave a pure epimer **6a** (mp, 141-143°), which was transparent in the uv region, showing the absence of the conjugated C=C function. The molecular formula of **6a** was deduced to be $C_{11}H_{14}O_{16}$ from elemental analysis.

Comparison of the ^{1}H-nmr spectrum of **6a** with that of the parent iridoid (**2**) showed that the olefinic C-3 proton and glucosylic proton signals were absent in **6a**, while signals appeared for two extra protons that should be assigned to acetal C-3-H (δ 5.38) and aliphatic C-4-H (δ 2.69). These assignments were supported by ^{13}C-nmr data, in which signals of two sp^3 carbons appeared at δ 101.1 and δ 49.5 due to C-3 and C-4, respectively.

The acetylation of **6a** gave only a crystalline diacetate (**7**) with its ^{1}H-nmr spectrum ($CDCl_3$) showing a significant acetylation shift for H-3, H-9, and H-10.

On the basis of all the above data, **6a** was deduced to be the tricyclic structure. This

TABLE 1. ^{1}H-nmr data (360 MHz or 200 Mhz)[a].

	2	3[b]	4	5	6a	6b	7
H-1 . . .	5.82(d) $J_{1,9}$=2.68		6.47 $J_{1,9}$=2.88	5.55(d) $J_{1,9}$=2.92	5.50(d) $J_{1,9}$=5.76	5.55(d) $J_{1,9}$=5.75	5.53(d) $J_{1,9}$=4.88
H-3 . . .	7.36(d) $J_{3,5}$=1.71	7.45(d) $J_{3,5}$=1.44	7.41(d) $J_{3,5}$=1.62	7.37(d) $J_{3,5}$=1.46	5.38(d) $J_{3,4}$=2.63	5.18(d) $J_{3,4}$=8.64	6.34(d) $J_{3,4}$=8.79
H-4 . . .	—	—	—	—	2.69(dd) $J_{4,5}$=9.43 $J_{3,4}$=2.63	2.71(dd) $J_{3,4}$=8.64 $J_{4,5}$=5.40	2.91(dd) $J_{4,5}$=4.64 $J_{3,4}$=8.79
H-5 . . .	3.36(m)[c]	3.53(m)	3.62(dq) $J_{3,5}$=1.62 $J_{5,6}$=2.52 $J_{5,9}$=8.28	3.55(m)[d] $J_{5,6}$=2.62 $J_{5,7}$=2.05 $J_{5,9}$=8.57	3.54(tt) $J_{4,5}$=9.43 $J_{5,6}$=2.23 $J_{5,7}$=1.98 $J_{5,9}$=9.36	3.54(m)[d] $J_{4,5}$=5.40 $J_{5,9}$=8.46	3.64(m)
H-6 . . .	6.16(dd) $J_{6,7}$=6.00 $J_{5,6}$=2.69	6.08(dd) $J_{6,7}$=5.58 $J_{5,6}$=2.34	6.32(dd) $J_{6,7}$=5.85 $J_{5,6}$=2.52	6.25(dd) $J_{6,7}$=5.61 $J_{5,6}$=2.62	5.92(dd) $J_{6,7}$=5.40 $J_{5,6}$=2.23	5.81(d) $J_{6,7}$=5.76	5.92(dd) $J_{6,7}$=5.86 $J_{5,6}$=1.70
H-7 . . .	5.37(dd) $J_{6,7}$=6.00 $J_{5,7}$=1.95	5.61(dd) $J_{6,7}$=5.58 $J_{5,7}$=2.34	5.68(dd) $J_{6,7}$=5.58 $J_{5,7}$=1.98	5.66(dd) $J_{6,7}$=5.61 $J_{5,7}$=2.05	5.74(dd) $J_{6,7}$=5.40 $J_{5,7}$=1.98	5.79(d) $J_{6,7}$=5.76	6.19(dd) $J_{6,7}$=5.62 $J_{5,7}$=2.68
H-9 . . .	2.61(dd) $J_{1,9}$=2.68 $J_{5,9}$=8.54		2.63(dd) $J_{1,9}$=2.88 $J_{5,9}$=8.28	2.67(dd) $J_{1,9}$=2.92 $J_{5,9}$=8.57	2.67(dd) $J_{1,9}$=5.76 $J_{5,9}$=9.36	2.64(dd) $J_{1,9}$=5.75 $J_{5,9}$=8.46	3.16(dd) $J_{1,9}$=4.88 $J_{5,9}$=8.50
H-10 . . .	3.52(d) 3.61(d) $J_{a,b}$=11.47	3.55(m)	4.22(d) $J_{a,b}$=11.22	4.18(d) 4.23(d) $J_{a,b}$=11.42	3.54(d) 3.79(d) $J_{a,b}$=9.34	3.78(d) 3.94(d) $J_{a,b}$=9.84	4.26(d) 4.36(d) $J_{a,b}$=10.50
OCH_3 . .	3.70(s)	3.72(s)	3.77(s)	3.75(s)	3.72(s)	3.74(s)	3.77(s)
$COCH_3$.			2.12(s) 2.14(s)	1.95(s) 2.01(s) 2.03(s) 2.09(s) 2.11(s)			2.02(s) 2.05(s)

[a]Their assignments were checked by spin-decoupling experiments. The spectra of **2, 3, 6a,** and **6b** were measured in CD_3OD and those of the others in $CDCl_3$.

[b]H-1 signal overlapped to HDO and H-9 signal to CD_3OD.

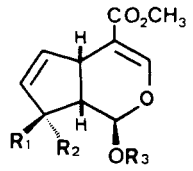
[c]This signal overlapped with CD_3OD signal.

[d]Broad signal with fine structure.

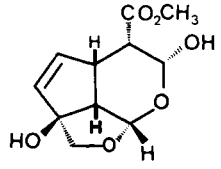
structure was confirmed by detailed analysis of the ^{1}H-nmr (360 MHz) spectrum in CD_3OD using double resonance experiments as shown in table 1.

As for the stereochemistry of **6a**, the chiral centers C-5, C-8, and C-9 obviously retained the configuration of the corresponding centers of **2**. The remarkable increase of the coupling constant $J_{1,9}$ (2.67 Hz in **2**→5.67 Hz in **6a**) and the formation of intramolecular acetal with C-10-OH confirmed C-1-OH to be α-configuration. The orientation of 3-hydroxy and 4-carbomethoxy groups can be assigned from coupling constants among C-3-H, C-4-H, and C-5-H to be α, in a half-chair conformation with C-9, C-5, C-4, and C-3 held in the same plane. Thus, the full structure of gardenogenin A (**6a**) can be depicted as in figure 1.

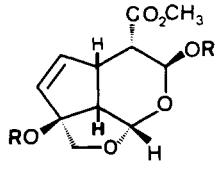
Attempted purification of **6b** from the mother liquor by silica gel chromatography gave only an equilibrium mixture of **6a** and **6b**. The signal of C-3-H of the mixture appeared at δ 5.38($J_{3,4}$=2.63 Hz, H-3 of **6a**) and δ 5.18($J_{3,4}$=8.64 Hz, H-3 of **6a**);



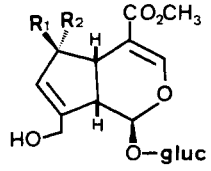
1: R_1=CH_2OH, R_2=OH, R_3=β-gluc
2: R_1=OH, R_2=CH_2OH, R_3=β-gluc
3: R_1=CH_2OH, R_2=OH, R_3=H
4: R_1=CH_2OAc, R_2=OH, R_3=Ac
5: R_1=Ch_2OAc, R_2=OH, R_3=β-gluc·4Ac



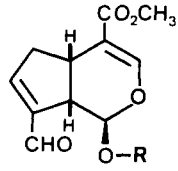
6a



6b: R=H
7: R=Ac



8: R_1=OH, R_2=H
9: R_1=H, R_2=OH



10: R=gluc
11: R=gluc·4Ac

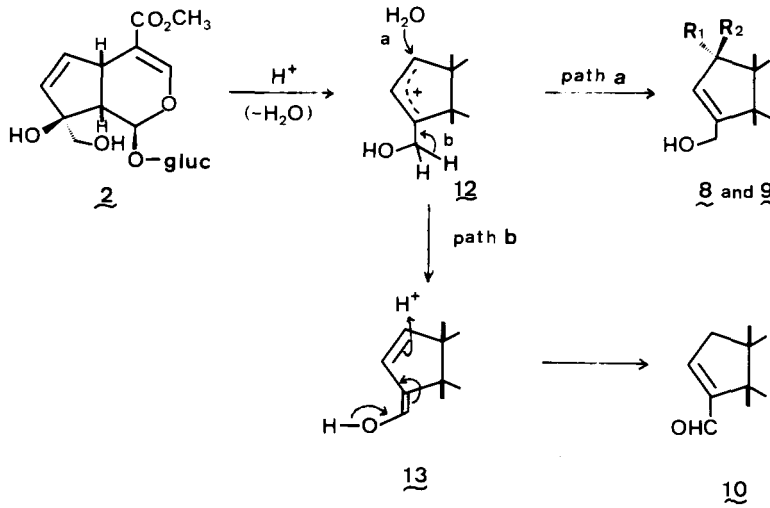
therefore, gardenogenin B (**6b**) was found to be the epimer of **6a** at C-3. Because nmr spectrum of acetate **7** of **6a** is similar to that of **6b**, the stereochemistry of acetate **7** can be deduced to be β-configuration for 3-acetoxy group and α-configuration for 4-carbomethoxy group (8).

The large change of coupling constant between **6a** and **6b** or **7** suggests the comformational alteration of a six-membered ring from a half-chair with C-9, C-5, C-4, and C-3 held in the same plane to another half-chair with C-1, C-9, C-5, and C-4 in the same plane.

In connection with enzymatic hydrolysis, we investigated the acidic hydrolysis of gardenoside (**2**). The treatment of **2** with 0.5% aqueous HCl under various conditions (reaction temperature and time) gave a mixture (1:1) of **8** and **9**. The structures of **8** and **9** were confirmed by spectrosocpic (^{1}H-nmr, ir, and uv) comparison with authentic samples of scandoside methylester and deacetylasperulosidic acid methylester, respectively. However, when **2** was hydrolyzed in 3.5% aqueous HCl, the product was neither **8** nor **9**, but 10-dehydrogeniposide (**10**) (9), the structure of which was determined with its tetraacetate (**11**) from spectroscopic data. The plausible course of these reactions is described in scheme 1. Acidic elimination of C-8-OH group forms allylic cation (**12**), then addition of water to this cation at the less-hindered C-6 position gives an epimeric mixture of **8** and **9** (path a). In another path (b), elimination of proton at C-10 affords an intermediate dienol (**13**), which will isomerize to α,β-unsaturated aldehyde **10**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Yanagimoto micro melting point apparatus and are uncorrected. The ir spectra were taken with a Shimadzu 420 spectrometer and [α] D with Nippon Bunko DIP-180. The uv absorption spectra were obtained in MeOH solution using a Hitachi 323-type instrument. The 200 MHz ^{1}H-nmr spectra for compounds **2, 3,** and **7** were measured with a JEOL 200 FX, and 360 MHz ^{1}H-nmr data for compounds **4, 5, 6a,** and **6b** were recorded with a Nicolet NT-360 using TMS as an internal standard; chemical shifts are expressed in δ units. ^{13}C-



SCHEME 1. Course of reactions.

nmr spectra were recorded with a JEOL FX-100 (25.05 MHz). Commercial Merck silica gel 60 (60-240 mesh) and Wako charcoal were used for column chromatography. Merck precoated silica gel plates were used for tlc. The chromatograms were sprayed with 0.5% anisaldehyde-H_2SO_4 and heated at 110° for 10 min to detect the spots.

GALIOSIDE AGLUCONE (**3**).—Using a sample isolated from *Vaccinium bracteatum* Thunb., identified with an authentic specimen, **1** (170 mg) was dissolved in H_2O (17 ml) and treated with β-glucosidase (MILES) (34 mg) for 1.5 h at 37°. The solution was chromatographed on a charcoal column using H_2O and EtOH as eluents. The EtOH eluate was concentrated *in vacuo* and gave a crude aglucone (**3**) (90 mg) as a white powder. The product was purified by chromatography on silica gel by means of $CHCl_3$-MeOH (15:1) as eluent and was recrystallized from AcOEt to afford a pure crystalline **3** (11 mg), mp, 110-115°, $[\alpha]^{24}D$ +52.9° (c=0.24, MeOH), ν max (KBr): 3430, 3320, 1670, 1635 cm^{-1}, λ max (MeOH): 242 nm (log ε=3.92); ^{1}H-nmr: see table 1.

DIACETATE (**4**).—**3** (40 mg) was acetylated (Ac_2O-py) for 3 h at room temperature. The amorphous residue was purified by preparative tlc with $CHCl_3$-AcOEt (1:1) and gave pure diacetate (**4**) as a colorless oil (16 mg), $[\alpha]^{24}D$ −21.7° (c=0.97, MeOH), ν max ($CHCl_3$): 3620, 3450, 1745, 1710, 1645 cm^{-1}; λ max (MeOH): 236 nm (log ε=3.90); ^{1}H-nmr: see table 1.

PENTAACETATE (**5**).—**1** (20 mg) was acetylated (Ac_2O-py) overnight at room temperature. The product was chromatographed on silica gel with $CHCl_3$-MeOH (15:1) and gave pure pentaacetate (**5**) (12 mg). This was recrystallized from Et_2O, giving pure **5** as colorless needles, mp, 149-150°, $[\alpha]^{24}D$ −79.6° (c=0.62, MeOH), ν max (KBr): 3450, 1755, 1730, 1695, 1645 cm^{-1}; λ max (MeOH): 234 nm (log ε=4.00); ^{1}H-nmr: see table 1.

Anal. Calcd for $C_{27}H_{34}O_{16}$: C, 52.76; H, 5.58. Found: C, 52.54; H, 5.65.

GARDENOGENIN A (**6a**).—Using a sample isolated from *Gardenia jasminoides* Ellis *forma gradiflora* [L.] Makino, identified with an authentic specimen, **2** (300 mg) in H_2O (30 ml) was treated with β-glucosidase (40 mg) for 2 h at 37°. The solution was chromatographed on a charcoal column with H_2O and EtOH as eluents. The EtOH eluate was concentrated *in vacuo* and gave a crude mixture (1:1) of **6a** and **6b** (131 mg). The mixture was recrystallized from AcOEt and gave pure colorless needles **6a** (31 mg); mp, 141-143°, $[\alpha]^{24}D$ +117.5° (c=1.05, MeOH), ν max (KBr): 3450, 1720 cm^{-1}; ^{1}H-nmr: see table 1, ^{13}C-nmr (CD_3OD): 172.9(s, C-11), 138.0(d, C-6), 135.5(d, C-7), 101.1(d, C-3), 93.9(s, C-8), 90.3(d, C-1), 74.7(t, C-10), 52.5(q, C-12), 49.5(d, C-4), 48.3(d, C-5), 40.5(d, C-9).

Anal. Calcd for $C_{11}H_{14}O_6$: C, 54.54; H, 5.83. Found: C, 54.22; H, 5.83.

DIACETATE (**7**).—**6a** (10 mg) in dry pyridine (0.5 ml) was treated with Ac_2O (0.5 ml) for 5 h at room temperature. After evaporation of the solvent, the residue was chromatographed on silica gel in $CHCl_3$ and gave pure **7** (6 mg), which was recrystallized from EtOH; mp, 184-188° (needles), ν max (KBr): 1735, 1725 cm^{-1}.

Anal. calcd for $C_{13}H_{16}O_7$: C, 54.93; H, 5.56. Found: C, 54.73; H, 5.59.

SCANDOSIDE METHYLESTER (**8**) AND DEACETYLASPERULOSIDIC ACID METHYLESTER (**9**).—A solution of **2** (100 mg) in 0.5% aqueous HCl (2 ml) was heated at 85° for 7 min. After cooling to room temperature, this mixture was neutralized with Ag_2CO_3, filtered, and chromatographed on a charcoal column using H_2O and EtOH as eluents. Evaporation of the EtOH eluate left a colorless oil (40 mg). This oil was chromatographed on silica gel with $CHCl_3$-MeOH (10:1) as an eluent to isolate **8** [16 mg, amorphous powder, mp, 110-114°, ν max (KBr): 3500-3200(br), 1690, 1630 cm^{-1}; λ max (H_2O): 239 nm (log ϵ=3.87); ^{1}H-nmr (CD_3OD): 7.50(1H, s, H-3), 5.79(1H, s, H-7), 5.19(1H, d, $J_{1,9}$=5.9 Hz, H-1), 4.25(2H, dd, $J_{a,b}$=13.9 Hz, H-10), 3.75(3H, s, -$COOCH_3$)] and **9** [15 mg, amorphous powder; mp, 129-133°, ν max (KBr): 3500-3200(br), 1690, 1630 cm^{-1}; λ max (H_2O): 238 nm (log ϵ=4.00); ^{1}H-nmr (D_2O): 7.65(1H, d, J=1.2 Hz, H-3), 6.01(1H, m, H-7), 5.05(1H, d, $J_{1,9}$=9.0 Hz, H-1), 4.45(2H, dd, $J_{a,b}$=15 Hz, H-10), 3.47(3H, s, -$COOCH_3$), 3.26(1H, m, H-5), 2.56(1H, bt, $J_{1,9}$=9.0 Hz, H-9)], which were identified by comparison with authentic samples by tlc ($CHCl_3$-MeOH: H_2O=7:3:0.5), uv, ir, and ^{1}H-nmr, respectively.

10-DEHYDROGENIPOSIDE (**10**).—A solution of **2** (100 mg) in 3.5% aqueous HCl (2 ml) was heated at 90° for 7 min. After cooling to room temperature, this solution was neutralized with Ag_2CO_3, filtered and chromatographed on a charcoal column using H_2O and EtOH as eluents. The EtOH eluate was evaporated, leaving a colorless oil (18 mg). This oil was chromatographed on silica gel with $CHCl_3$-MeOH (15:1) as eluent and gave pure oil **10** on tlc ($CHCl_3$-MeOH-H_2O=45:15:2). **10** was acetylated (Ac_2O-py), and the product was purified by chromatography on silica gel, with $CHCl_3$ as eluent, and recrystallized from Et_2O, giving **11** (6 mg) as needles: mp, 128-130°, ν max ($CHCl_3$): 1760, 1710, 1680, 1640 cm^{-1}; ^{1}H-nmr ($CDCl_3$): 1.90, 2.00, 2.03, and 2.13 (s, OAc × 4), 2.72(1H, m, H-6), 2.91(1H, m, H-6), 3.71(3H, s, COOMe), 6.17(1H, d, J=2.1 Hz, H-1), 6.93(1h, m, H-7), 7.36(1H, s, H-3), 9.75(1H, s, CH=O).

ANTIMICROBIAL TEST.—The *in vitro* antimicrobial activity given as the minimum inhibitory concentration (MIC) of **3**, was determined by the agar dilution method. Bacteria were cultured overnight at 37° in Trypticase soy broth (TSB: NISSUI). One loopful (2 mm in diameter) of a bacterial suspension containing about 10^6 colony-forming units (CFU)/ml was spotted on heart infusion agar (HIA: NISSUI). The MIC was defined as the lowest concentration of three that prevented visible growth after 20 h of incubation at 37°. The MIC was 125 μg/ml against *Staphylococcus aureus* and 1 mg/ml against *Klebsiella pneumoniae*.

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