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# Studies on Monoterpene Glucosides and Related Natural Products. XXXIV.[1] Two Further New Glucosides from the Fruit of *Gardenia jasminoides* Ellis forma *grandiflora* (Lour.) Makino[2]

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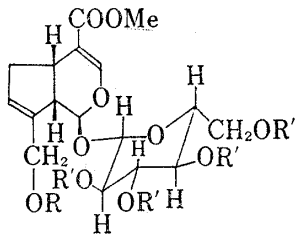
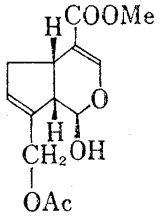
From the fruit of *Gardenia jasminoides* forma *grandiflora*, two new glucosides, 10-acetylgeniposide (**1**) and picrocrocinic acid (**2**) were isolated and their structures have been established.

Eight iridoid glucosides have already been isolated from the fruits of *Gardenia jasminoides* Ellis forma *grandiflora* (Lour.) Makino by this group[2,4] and Taguchi's group[5] and their structures have been established. This paper decrsibes the structural elucidation of a new iridoid glucoside, 10-acetylgeniposide (**1**) and an acidic non-iridoid glucoside, picrocrocinic acid (**2**), which were isolated during examination of the minor glucosides of the fruits of this plant.

10-Acetylgeniposide (**1**) was obtained as colorless prisms, mp 173—175°, $C_{19}H_{26}O_{11}$, $(\alpha)_D$+22.1° (MeOH), exhibiting an absorption maximum at 239 nm (log $\varepsilon$=4.06) in the ultraviolet (UV) spectrum and absorptions at 3700—3130, 1720, 1695, 1635 $cm^{-1}$ in the infrared (IR) spectrum. The nuclear magnetic resonance (NMR) spectrum (in $CD_3OD$) of **1** shows a singlet at $\delta$ 2.04 due to an acetyl group, a singlet at $\delta$ 3.68 arising from a carbomethoxy group, a multiplet at $\delta$ 5.83 assignable to the C-7 proton. Acetylation of **1** gave the tetraacetate, $C_{27}H_{34}O_{15}$, the NMR spectrum of which displays the signals ($\delta$ 1.98—2.08) assignable to five acetyl groups and is superimposable to that of geniposide pentaacetate (**3**). The identity of the above-mentioned tetraacetate of **1** with **3** was also confirmed by mixed melting point and IR spectral comparison. Therefore, it was concluded that **1** is the monoacetate of geniposide (**4**). Hydrolysis of **1** with $\beta$-glucosidase gave the aglucone (**5**), $C_{13}H_{16}O_6$, the NMR spectrum of which displays a singlet at $\delta$ 2.10 assignable to an acetyl group. Accordingly, it was confirmed that **1** is 10-acetylgeniposide which becomes the ninth iridoid glucoside isolated from this plant.



**1** : R=Ac;R′=H
**3** : R=R′=Ac
**4** : R=R′=H



**5**

Fig. 1

Picrocrocinic acid (**2**) was obtained as an amorphous hygroscopic powder, $C_{16}H_{26}O_8 \cdot H_2O$, $(\alpha)_D$—28.2° (MeOH). Acetylation of **2** gave the tetraacetate (**6**), $C_{24}H_{34}O_{12}$, mp 137—138°, which, upon methylation, gave the tetraacetate methyl ester (**7**), $C_{25}H_{36}O_{12}$, mp 135—136°. The NMR spectrum of **7** shows two singlets at $\delta$ 1.05 and 1.19 due to a *gem*-dimethyl group

1) Part XXXIII: H. Inouye, S. Tobita, and M. Moriguchi, *Chem. Pharm. Bull.* (Tokyo), **24**, 1406 (1976).
2) Part III in the series "On the Constituents of *Gardenia* species". For Part II see H. Inouye, Y. Takeda, and H. Nishimura, *Phytochemistry*, **13**, 2219 (1974).
3) Location: *Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto.*
4) H. Inouye, Y. Takeda, S. Saito, H. Nishimura, and R. Sakuragi, *Yakugaku Zasshi*, **94**, 577 (1974).
5) T. Endo and H. Taguchi, *Chem. Pharm. Bull.* (Tokyo), **21**, 2684 (1973).

at a quaternary carbon, a singlet at $\delta$ 1.66 assignable to an olefinic methyl group, signals arising from four acetyl groups at $\delta$ 2.00—2.07 and a singlet at $\delta$ 3.74 due to a carbomethoxy group. Hydrolysis of **2** with $\beta$-glucosidase gave the aglucone (**8**), $C_{10}H_{16}O_3$, mp 155°, which shows the parent peak at *m/e* 184 in the mass (MS) spectrum. The composition of **8** coupled with the degree of unsaturation indicates that **8** should be a monocyclic compound. From these facts, it could be presumed that the original substance has the structure **2**, that is, a carboxylic acid corresponding to picrocrocin (**9**). Then, the teraacetate (**10**) of picrocrocin (**9**) with the definite absolute stereochemistry[6] was subjected to the oxidation with AgO giving an oxidation product which was found to be identical with picrocrocinic acid tetraacetate (**6**). Accordingly, it was clarified that the absolute structure of the original acid can be represented as **2**.

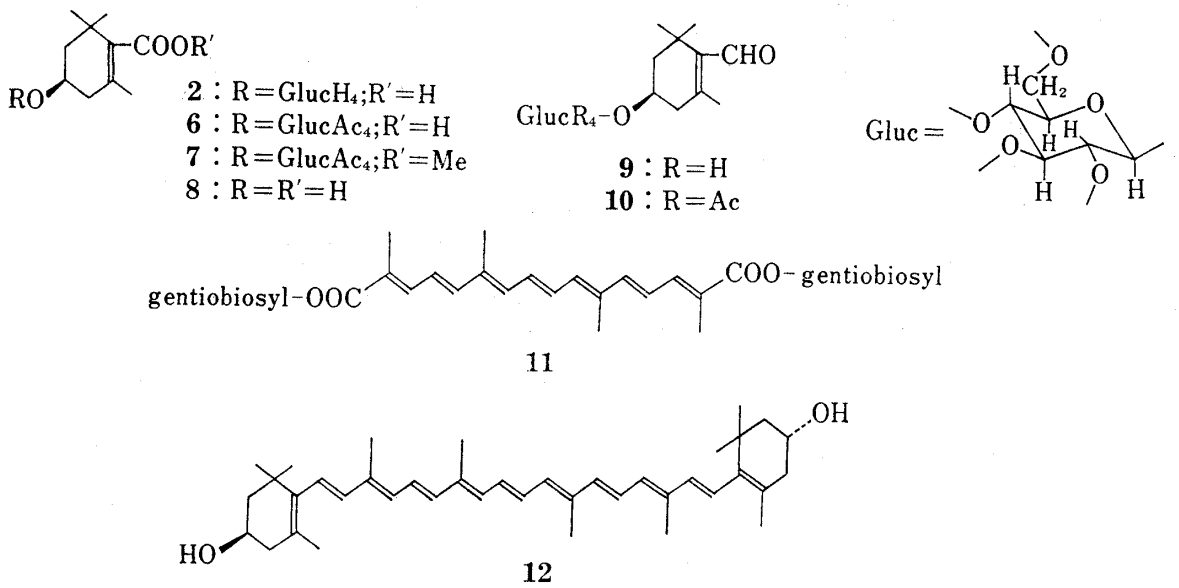



Fig. 2

Taking into consideration of the existence of crocin (**11**) in the *Gardenia* plant[7,8] picrocrocinic acid (**2**) would be formed by an oxidative fission of zeaxanthin (**12**) or the corresponding glucoside in the plant as in the case of **9** in *Crocus sativus* Lin.[9]

## Experimental[10]

**Isolation Procedures**——The fruits of *Gardenia jasminoides* forma *grandiflora* (7 kg) were extracted with MeOH (3×15 liters) under reflux. The methanolic extracts were combined and the solvent was removed *in vacuo*. $H_2O$ (3 liters) was added to the residue and the insoluble material was filtered off through a Celite layer. After washing the filtrate with AcOEt (3×2 liters), the aqueous layer was concentrated *in vacuo* to about 1 liter and poured on a charcoal column (800 g) and eluted with $H_2O$–MeOH with increasing MeOH content (Chrom. 1). The aqueous eluate was concentrated *in vacuo* and the residue was rechromatographed on a charcoal column (300 g) in the same way as above (Chrom. 1-1). The eluate with $H_2O$–MeOH (9: 1) was evaporated *in vacuo* to furnish a residue (3.3 g), a portion of which (2.4 g) was subjected to counter current distribution (solvent: *n*-BuOH–$H_2O$ 1: 1). Transfer No. 536. Fr. No. 240—270 were combined and concentrated *in vacuo* to give picrocrocinic acid (**2**) (350 mg) as a white powder. $(\alpha)_D^{33}$ −28.2° (*c*=1.13, MeOH): UV

6) R. Buckecker and C.H. Eugster, *Helv. Chim. Acta*, **56**, 1121 (1973).
7) Fr. Rochleder and L. Mayer, *J. Prak. Chem.*, **72**, 394 (1857); *idem*, *ibid.*, **74**, 1 (1858).
8) R. Kuhn, A. Winterstein and W. Wiegand, *Helv. Chim. Acta*, **11**, 718 (1928).
9) R. Kuhn and A. Winterstein, *Ber.*, **67**, 344 (1934).
10) For general procedures, see the footnote 13 in the XXXII. paper of this series; Y. Takeda, H. Nishimura, and H. Inouye, *Chem. Pharm. Bull.* (Tokyo), **24**, 1216 (1976). UV spectrum of **2** was recorded on a Hitachi Double Beam Spectrophotometer Model 124. Counter current distribution was performed in a Mitamura full automatic all-glass apparatus with 300 tubes.

$\lambda_{max}^{H_2O}$ nm (log $\varepsilon$): 194 (3.74); IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3500—3100, 1700, 1645; NMR ($CD_3OD$) $\delta$: 1.10, 1.12 (each 3H, singlets $\begin{matrix}Me\\Me\end{matrix}\!>\!C\langle$), 1.73 (3H, s, olefinin Me), 4.44 (1H, d, $J$=7.0 Hz, $>C\langle\begin{matrix}H\\OH\end{matrix}$). *Anal.* Calcd. for $C_{16}H_{26}O_8\cdot H_2O$: C, 52.74; H, 7.75. Found: C, 52.36; H, 7.82.

The eluate with 100% MeOH from Chrom. 1 was concentrated *in vacuo* and the residue was chromatographed on a silica gel column (450 g) with $CHCl_3$–MeOH as eluent with increasing MeOH content (Chrom. 1—2). Of the eluates with $CHCl_3$–MeOH 90: 10—88: 12, all the fractions showing single spot at *Rf* 0.47 on TLC (solvent: $CHCl_3$–MeOH 8: 2) were combined and the solvent was removed *in vacuo*. The residue was recrystallized from EtOH to give 10-acetylgeniposide (**1**) (1.18 g) as colorless prisms, mp 173—175°. $(\alpha)_D^{29.5}$ +22.1° ($c$=2.74, MeOH); UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 239 (4.06); IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3700—3130, 1720, 1695, 1635; NMR ($CD_3OD$) $\delta$: 2.04 (3H, s, OCOMe), 3.68 (3H, s, COOMe), 5.13 (1H, d, $J$=8.0 Hz, C-1H), 5.83 (1H, m, C-7H), 7.51 (1H, d, $J$=1.0 Hz, C-3H). *Anal.* Calcd. for $C_{19}H_{26}O_{11}$: C, 53.02; H, 6.09. Found: C, 52.96; H, 5.82.

**Acetylation of 10-Acetylgeniposide (1)**——10-Acetylgeniposide (**1**) (253.4 mg) was acetylated with $Ac_2O$–pyridine and the reaction product was recrystallized from EtOH to give **3** as colorless needles, mp 137—138°. $(\alpha)_D^{28}$ +4.4° ($c$=2.49, $CHCl_3$); UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 237 (4.10); IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 1750, 1710, 1640; NMR ($CDCl_3$) $\delta$: 1.98—2.08 (5×OCOMe), 3.72 (3H, s, COOMe), 4.70 (2H, diffused s, C-10H), 5.83 (1H, m, C-7H), 7.42 (1H, d, $J$=1.0 Hz, C-3H). *Anal.* Calcd. for $C_{21}H_{34}O_{15}$: C, 54.18; H, 5.73. Found: C, 54.06; H, 5.52. This substance was identified with an authentic sample of geniposide pentaacetate (**3**) by mixed mp and comparisons of IR (KBr) and NMR ($CDCl_3$) spectra.

**10-Acetylgenipin (5)**——$\beta$-Glucosidase (Miles Laboratory (PTY) Ltd.) (50 mg) was added to a solution of **1** (201.3 mg) in acetate buffer (0.1 M, pH 4.8) (15 ml) and the mixture was allowed to stand overnight at 37°. The reaction mixture was extracted with ether (4×30 ml) and the combined ether extracts were dried over anhyd. $MgSO_4$ and evaporated. The residue was purified by chromatography on a silica gel column (15 g) with ether as eluent to give 10-acetylgenipin (**5**) (74.3 mg) as a colorless syrup. $(\alpha)_D^{33}$ +48.2° ($c$=1.46, $CHCl_3$); UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 240 (3.97); IR $\nu_{max}^{CHCl_3}$ ($cm^{-1}$): 3400—3250, 1730, 1700, 1630; NMR ($CDCl_3$) $\delta$: 2.10 (3H, s, OCOMe), 3.71 (3H, s, COOMe), 5.75 (1H, m, C-7H), 7.52 (1H, d, $J$=1.0 Hz, C-3H). High resolution Mass spectrum: Calcd. for $C_{13}H_{16}O_6$: 268.0947; Found: 268.0943.

**Picrocrocinic Acid Tetraacetate (6)**——Picrocrocinic acid (**2**) (43.2 mg) was acetylated with $Ac_2O$–pyridine. The reaction product was recrystallized from a mixture of ether–petr. ether to give **6** (34.9 mg) as colorless needles, mp 137—138°. $(\alpha)_D^{32}$ −21.3° ($c$=1.15, $CHCl_3$); IR $\nu_{max}^{Nujol}$ ($cm^{-1}$): 1760—1720, 1680; NMR ($CDCl_3$) $\delta$: 1.15, 1.25 (each 3H, singlets, $\begin{matrix}Me\\Me\end{matrix}\!>\!C\langle$), 1.80 (3H, s, olefinic Me), 2.02—2.08 (4×OCOMe). *Anal.* Calcd. for $C_{24}H_{34}O_{12}$: C, 56.03; H, 6.66. Found: C, 55.71; H, 6.67.

**Picrocrocinic Acid Tetraacetate Methyl Ester (7)**——Picrocrocinic acid tetraacetate (**6**) (90 mg) was treated with an excess ethereal $CH_2N_2$ solution. The reaction product was recrystallized from dil. MeOH yielding **7** (50 mg) as colorless needles, mp 135—136°. $(\alpha)_D^{31}$ −25.9° ($c$=1.18, $CHCl_3$); IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 1760—1730, 1710; NMR ($CDCl_3$) $\delta$: 1.05, 1.19 (each 3H, singlets, $\begin{matrix}Me\\Me\end{matrix}\!>\!C\langle$), 1.66 (3H, s, olefinic Me), 2.00—2.07 (4×OCOMe), 3.74 (3H, s, COOMe). *Anal.* Calcd. for $C_{25}H_{36}O_{12}$: C, 56.81; H, 6.87. Found: C, 56.81; H, 6.86.

**Aglucone (8) of Picrocrocinic Acid (2)**——Picrocrocinic acid (**2**) (1.15 g) was treated with $\beta$-glucosidase (80 mg) in the same way as in the case of 10-acetylgeniposide (**1**) to give **8** (148 mg) as colorless needles, mp 155°. $(\alpha)_D^{33}$ −72.4° ($c$=1.13, pyridine); IR $\nu_{max}^{Nujol}$ ($cm^{-1}$): 3500—3200, 1670, 1640; NMR ($d_5$-pyridine) $\delta$: 1.38, 1.52 (each 3H, singlets, $\begin{matrix}Me\\Me\end{matrix}\!>\!C\langle$), 1.95 (3H, s, olefinic Me), 4.43 (1H, m, OH–C–H). High resolution mass spectrum: Calcd. for $C_{10}H_{16}O_3$: 184.1100. Found: 184.1100.

**AgO Oxidation of Picrocrocin Tetraacetate (10)**——AgO (22 mg) was added to a solution of **10** (21.2 mg) in THF–$H_2O$ (9: 1) (3 ml) and the mixture was stirred at room temperature for 4 days. The insoluble material was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by chromatography on a silica gel column (5 g) with $CHCl_3$ as eluent and recrystallized from a mixture of ether–petr. ether to give **6** (11.6 mg) as colorless needles, mp 137—138°. $(\alpha)_D^{30}$ −29.1° ($c$=0.75, $CHCl_3$); IR $\nu_{max}^{Nujol}$ ($cm^{-1}$): 1760—1720, 1680; NMR ($CDCl_3$) $\delta$: 1.15, 1.25 (each 3H, singlets, $\begin{matrix}Me\\Me\end{matrix}\!>\!C\langle$), 1.80 (3H, s, olefinic Me), 2.02—2.08 (4×OCOMe). *Anal.* Calcd. for $C_{24}H_{34}O_{12}$: C, 56.03; H, 6.66. Found: C, 56.27; H, 6.42. This substance was identified with an authentic sample of picrocrocinic acid tetraacetate (**6**) by mixed mp and comparisons of IR (Nujol) and NMR ($CDCl_3+D_2O$) spectra.

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