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The Constituents of *Gardenia jasminoides*[1] Geniposide and Genipin-gentiobioside

Tohru Endo and Heihachiro Taguchi

Tsumura Laboratory[2]

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Two new iridoid glycosides, geniposide and genipin-1-β-D-gentiobioside, were isolated from the fruits of *Gardenia jasminoides* Ellis *f. grandiflora* (Lour.) Makino and their structures were determined to be I and II, respectively.

The fruits of *Gardenia jasminoides* Ellis f. *grandiflora*(Lour.) Makino(Rubiaceae) are used as an antiphlogistic and cholagogue under the name of Shan-zhi-i(in Chinese) or San-shi-shi-(in Japanese).

Gardenoside,[1a] shanzhiside and deacetylasperulosidic acid methyl ester[3] were isolated from this plant by Inouye, *et al.* In this paper we described on the isolation of two new iridoid glycosides from the fruits of this plant and the elucidation of their structures.

The crude methanolic extracts of the fruits was chromatographed on charcoal and silica gel to separate two crystalline substances, geniposide (I) and genipin-1-β-D-gentiobioside (II).

Geniposide (I) $C_{17}H_{24}O_{10}$ was obtained as colorless needles, mp 163—164°, $[\alpha]_D$ +7.5°. It gave a reddish violet color by heating with 10% hydrochloric acid and a blue color by the treatment with β-glucosidase (Worthington Biochemical Corp. Freehold, New Jersey) in the buffer solution, suggesting that I would be an iridoid glycoside. Acetylation of I by the usual method with pyridine and acetic anhydride afforded pentaacetate (III) $C_{27}H_{24}O_{10}$ mp 137—138°, $[\alpha]_D$ +11.0° (EtOH) as colorless needles. III showed an absorption maximum at 235 mμ (log ε 4.19) in the ultraviolet (UV) spectrum and a band of enol ether at 1640 cm^{-1} and no OH absorption in the infrared (IR) spectrum. The nuclear magnetic resonance (NMR) spectrum ($CDCl_3$) of III showed five acetyl groups at δ 1.96—2.05, a methoxy group at δ 3.70, a multiplet of an olefinic proton due to a proton of the C-7 position at δ 5.83 and a doublet (J=1.0 Hz) at δ 7.40 attributed to a proton at C-3 position of iridoid glycoside. The signal at δ 4.69 (2H, s-like) was attributed to the protons of the C-10 position. Hydrolysis of I with β-glucosidase in the buffer solution afforded, along with D-glucose, a crystalline substance (IV), $C_{11}H_{14}O_5$ mp 119—120.5°, which was identified with an authentic sample of genipin (IV).[4] Catalytic hydrogenation of III over palladized charcoal in methanol yielded a crystalline substance (V) $C_{25}H_{34}O_{13}$, mp 113—114°, $[\alpha]_D$ −75.9° (EtOH). This substance (V) was identified with an authentic sample of 7-deoxyloganin derived from asperuloside tetraacetate.[5] Thus, the structure of geniposide (I) was characterized as genipin-1-glucoside. The structure of I was finally confirmed by the comparison of its pentaacetate (III) with an authentic sample derived from monotropein.[5] As the asymmetric center at C-1 of aglucone part of I by the enzymatic hydrolysis was assumed to be remained intact, it was suggested

1) Preliminaly reports of this paper have been published. *a*) H. Inouye, S. Saito, H. Taguchi and T. Endo, *Tetrahedron Letters*, **1969**, 2349; *b*) T. Endo and H. Taguchi, *Chem. Pharm. Bull.* (Tokyo), **18**, 1066 (1970).
2) Location: *1421, Izumi, Komae-shi, Tokyo.*
3) H. Inouye, S. Saito and T. Shingu, *Tetrahedron Letters*, **1970**, 3581.
4) C. Djerassi, T. Nakano, A.N. James, L.H. Zalkow, E.J. Eisenbraun and J.N. Shoolery, *J. Org. Chem.*, **26**, 1192 (1961).
5) H. Inouye, T. Arai and Y. Miyoshi, *Chem. Pharm. Bull.* (Tokyo) **12**, 888 (1964).

that the C-1 of genipin whose configuration has remained undissolved would be R-configuration.[6]

Genipin-gentiobioside (II) $C_{23}H_{34}O_{15}\cdot 1/2H_2O$ was obtained as colorless needles mp 227—229°, $[\alpha]_D \simeq 0°$. II showed an absorption maximum at 238 mμ(log ε 4.11) in the UV spectrum and also gave a reddish violet color with mineral acid. Acetylation of II by the usual method with pyridine and acetic anhydride afforded octaacetate (VI) $C_{39}H_{50}O_{23}$, mp 167—169°, as colorless needles. Although the NMR spectrum of VI closely resembles that of III, it differs in the following points. The signals for five acetyl groups are observed at δ 1.96—2.05 in III, while the signals for eight are observed at δ 2.00—2.22 in VI (Table I and Fig. 1). The signals for a proton of C-1 position of the aglycone and four axial protons on the sugar ring, attached to C-1′∼C-4′, are around δ 4.8—5.4 in III, while in VI the signals for nine protons are found in the same region. Further, the signals for protons of C-6′ position of sugar moiety in III are observed at δ 4.20 (center, 2H, m), while in VI two methylene protons are observed at δ 3.85 (center, 2H, m) and δ 4.24 (center, 2H, m) (Fig. 1). Enzymatic hydrolysis of II with β-glucosidase afforded D-glucose and genipin (IV). Catalytic hydrogenation of II over palladized charcoal in methanol furnished 10-deoxy-7,8-dihydro derivative (VII), which was converted to the corresponding heptaacetate (VIII) $C_{35}H_{50}O_{21}$ mp 192—194°, by the acetylation. Enzymatic hydrolysis of VII with β-glucosidase followed by the acetylation gave colorless plates (IX) mp 53—56°, $[\alpha]_D$ −76°, (EtOH), which were identified with an authentic sample of IX, obtained from 7-deoxyloganin by the same procedure. Thus, the sugar moiety was proved to be linked with C-1 OH of the aglycone (IV) and to be a disaccharide as eight acetyl signals were observed in the NMR spectrum of VI as described above. Permethyl-ether (X) prepared by the Hakomori's method[7] afforded on methanolysis with 5% methanolic hydrogen chloride methyl-2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl-2,3,4-tri-O-

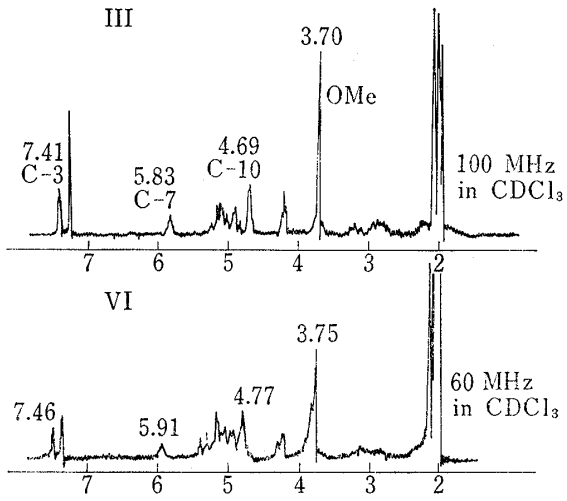

Fig. 1. NMR Spectra of III and VI

Table I. NMR Data given in δ Values, J Values in the Parentheses are given in Hz[a]

	I (D_2O, DSS)[b]	II (D_2O, DSS)[c]	III ($CDCl_3$, TMS)[b]	VI ($CDCl_3$, TMS)[c]
H-1	5.20 (d, J=7)	5.33 (d, J=7)		
H-3	7.55 (d, J=1)	7.66 (d, J=1.5)	7.40 (d, J=1)	7.46 (s)
H-7	5.82 (m)	5.95 (m)	5.83 (m)	5.91 (m)
H-10	4.22 (m)	4.32 (s-like)	4.69 (s-like)	4.77 (s-like)
$-OCH_3$	3.72 (s)	3.78 (s)	3.70 (s)	3.75 (s)
$-OCOCH_3$	—	—	1.96—2.05 (15H)	2.00—2.22 (24H)
Anomeric protons of sugar moiety	4.80 (d, J=7)	4.88 (d, J=7) 4.53 (d, J=7)		

a) d=doublet, m=multiplet s=singlet b) measured at 60 MHz c) measured at 100 MHz

6) In the preliminary paper, we proposed the configuration of C-1 of genipin as described above. Thereafter, A. Horeau and A. Nouaille suggested the opposite configuration (*Tetrahedron Letters*, **1971**, 1943). However, we believe that our assumption should be correct considering the results of the hydrolysis of geniposide with β-glucosidase giving only one aglucone together with the reaction mechanism of the enzyme.

7) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

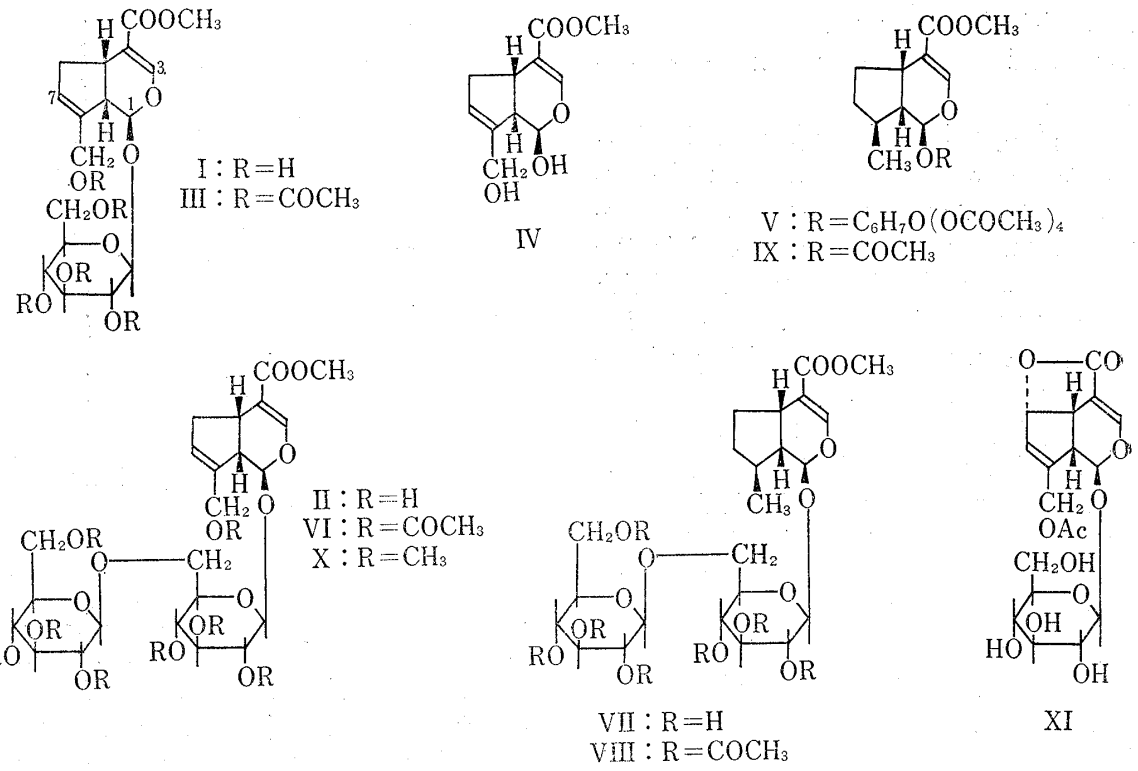

Chart 1

methyl-D-glucopyranoside in almost equal amount.[8] The NMR spectrum of II (D_2O) showed two anomeric protons of the sugar moiety at 4.53 (d, J=7 Hz) and 4.88 (d, J=7 Hz) to prove the β-linkage between two glucose moieties and also that between the sugar moiety and the aglycone. Further, it was suggested that the sugar moiety takes an quasi-equatorial conformation towards the heterocyclic ring of the aglycone from the J value of C-1 proton at δ 5.33 (d, J=7 Hz) in the NMR spectrum of II (D_2O).[8] The structure of II was thus established to be genipin-1-β-D-glucopyranosyl($1_{gul}\rightarrow6_{gul}$)-$\beta$-D-glucopyranoside(genipin-1-β-D-gentiobioside).

Experimental

All melting points were determined on a Yanagimoto Micro Melting Point Apparatus and uncorrected. IR spectra were measured with a Hitachi Model EPI-G2. NMR spectra were measured with a Japan Electron Co. Model JNM-C-60 HL and JNM-4H-100 with TMS as internal standard in $CDCl_3$ and DSS as internal standard in D_2O. Specific rotations were measured with a JASCO Model DIP-SL. Gas chromatograph used was a Hitachi Model K-53 with a hydrogen flame ionization deterctor.

Extraction——The pulverized fruits of *G. jasminoides* (1 kg) collected from the suburbs of Tokyo were first extracted with $CHCl_3$ to remove fat and oil, then extracted with MeOH for three times at room temperature. The combined MeOH extract was concentrated under reduced pressure to dryness to give a brown mass (262 g).

Separation of Glycosides——The MeOH extract was chromatographed on charcoal (600 g) using water and 10% aqueous EtOH to separate the sugars, then chromatographed using MeOH to separate the glycosides.

The glycosidic fraction (115 g) eluted with MeOH was rechromatographed on silica gel (800 g) using a mixture of MeOH and $CHCl_3$.

Geniposide (I)——On chromatography of the glycosidic fraction on silica gel, the fractions eluted with 7—10% MeOH–$CHCl_3$ were combined and concentrated under reduced pressure. The residue was recrystallized from acetone to give colorless needles (I), mp 163—164°, $[\alpha]_D^{29}$ +7.5°. UV λ_{max}^{EtOH} mμ (log ε): 236.5 (4.08).

8) *a*) H. Inouye, S. Ueda, M. Hirabayashi and N. Shimokawa, *Yakugaku Zasshi*, **86**, 943 (1966); *b*) N. Masaki, M. Hirabayashi, K. Fuji, K. Osaki and H. Inouye, *Tetrahedron Letters*, **1967**, 2367.

IR (KBr) cm^{-1}: 3400, 1710, 1700 (sh), 1640. *Anal.* Calcd. for $C_{17}H_{24}O_{10}$: C, 52.57; H, 6.23. Found: C, 52.21; H, 6.27.

Geniposide Pentaacetate (III)——I (100 mg) was acetylated by the usual method with pyridine and acetic anhydride to give pentaacetate (III) as colorless needles, mp 137.5—138°, $[\alpha]_D^{20}$ +11.0° (c=0.5, EtOH). UV λ_{max}^{EtOH} mμ (log ε): 235 (4.19). IR (KBr) cm^{-1}: 1740, 1705, 1680, no OH band. *Anal.* Calcd. for $C_{27}H_{34}O_{15}$: C, 54.18; H, 5.71. Found: C, 54.09; H, 5.69.

Hydrolysis of Geniposide (I) with β-Glucosidase——To a solution of I (400 mg) in the acetate buffer solution (80 ml, pH=5.0) was added 15 ml of 1% aqueous solution of β-glucosidase and the mixture was allowed to stand at 37° for 3 hr. The reaction mixture, colored blue, was extracted with ether. The ether extract was concentrated and the residue was crystallized with ether–MeOH to give colorless needles (IV) in a yield of 70 mg. mp 119—120.5°, $[\alpha]_D^{20}$ +136.5° (c=1.0, MeOH), UV λ_{max}^{EtOH} mμ (log ε): 240 (4.01), IR (KBr) cm^{-1}: 3400, 3200, 1700, 1640. NMR ($CDCl_3$): δ 3.72 (3H, s, OCH_3), 4.81 (1H, d, J=8 Hz, H-1), 5.88 (2H, m, H-10), 7.51 (1H, s, H-3). *Anal.* Calcd. for $C_{11}H_{14}O_5$: C, 58.41; H, 6.24. Found: C, 58.24; H, 6.10. This was identified by the mixed melting point and the comparison of IR spectra with an authentic sample of genipin (IV).

Catalytic Hydrogenation of Geniposide Pentaacetate (III)——200 mg of III in 30 ml of EtOH was shaken with H_2 in the presence of 5% Pd on charcoal (90 mg) as a catalyst. After the uptake of hydrogen corresponding to two moles per mole of III, the catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel using a mixture of benzene and n-hexane to give V as colorless needles (from n-hexane–ether). mp 113—114°. $[\alpha]_D^{20}$ −75.9° (c=0.5, EtOH). yield 120 mg. UV λ_{max}^{EtOH} mμ (log ε): 234 (4.22). IR (KBr) cm^{-1}: 1760, 1720, 1640. NMR ($CDCl_3$): δ 1.08 (3H, d, J=7 Hz, >CH-C$\underline{H}_3$), 1.92—2.08 (12H, 4×-OCOCH_3), 3.69 (3H, s, OCH_3), 7.29 (1H, s, H-3). *Anal.* Calcd. for $C_{25}H_{34}O_{13}$: C, 55.34; H, 6.32. Found: C, 55.33; H, 6.09. This was identified by the mixed melting point and the comparison of IR spectra with an authentic sample of 7-deoxyloganin (V) derived from asperuloside tetraacetate.

7-Deoxyloganin (V) from Asperulosidetetraacetate (XI)[5]——100 mg of asperuloside tetraacetate (XI) in 20 ml of MeOH was shaken with H_2 in the presence of 5% Pd on charcoal (86 mg) as a catalyst. After the uptake of hydrogen corresponding to three moles per mole of XI, the catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to yield the crude material (68 mg). 50 mg of this material in 10 ml of ether was treated with CH_2N_2 at room temperature for 2 hr. The solvent was removed under reduced pressure and the residue was purified by preparative TLC (Plate: Silica gel HF_{254}, solvent: benzene: ether=1:1) and recrystallized from EtOH to give colorless needles (V), mp 114—115°, $[\alpha]_D^{26}$ −79.2° (c=0.36, EtOH). NMR ($CDCl_3$): δ 1.06 (3H, d, J=7 Hz, >CH-C$\underline{H}_3$), 1.92—2.10 (12H, 4×-OCOCH_3), 3.72 (3H, s, OCH_3). *Anal.* Calcd. for $C_{25}H_{34}O_{13}$: C, 55.34; H, 6.32. Found: C, 55.52; H, 6.21.

Genipin-gentiobioside (II)——On chromatography of the glycosidic fraction on silica gel, the fractions eluted with 15—20% MeOH–$CHCl_3$ were combined and concentrated to dryness under reduced pressure. The residue was recrystallized from acetone to give colorless needles of II, mp 227—229°, $[\alpha]_D^{22}$ ≃0°, $[\alpha]_{577}$ +5.5°, $[\alpha]_{546}$ +18.5°, $[\alpha]_{405}$ +33.5° (c=1.0, MeOH). UV λ_{max}^{EtOH} mμ (log ε): 238 (4.11). IR (KBr) cm^{-1}: 1710, 1690, 1640. *Anal.* Calcd. for $C_{23}H_{34}O_{15}\cdot 1/2H_2O$: C, 49.40; H, 6.31. Found: C, 49.62; H, 6.31.

Genipin-gentiobioside Octaacetate (VI)——100 mg of II was acetylated by the usual method with pyridine (1 ml) and Ac_2O (1 ml) to give octaacetate of II as colorless needles (from EtOH). mp 167—169°, $[\alpha]_D^{21}$ ≃0° (c=0.5, MeOH). yield 95 mg. UV λ_{max}^{EtOH} mμ (log ε): 235 (4.22). IR (KBr) cm^{-1}: 1760, 1720, 1640. *Anal.* Calcd. for $C_{39}H_{50}O_{23}$: C, 52.84; H, 5.68. Found: C, 52.82; H, 5.55.

Hydrolysis of Genipin-gentiobioside (II) with β-Glucosidase——To a solution of II (85 mg) in the acetate buffer solution (10 ml, pH=5.0) was added 5 ml of 1% aqueous solution of β-glucosidase and the mixture was allowed to stand at 37° for 2 hr. The reaction mixture, colored blue, was extracted with ether. The ethereal layer was washed with water, dried and concentrated to dryness. The residue was crystallized from the mixture of benzene and ether to give colorless needles (IV) in a yield of 25 mg. mp 118—121°. This was identified by the mixed melting point and the comparison of IR spectra with an authentic sample of genipin (IV). The aqueous layer was evaporated to dryness. The paper chromatography of this residue (solvent, BuOH: AcOH: H_2O=4: 1: 5, upper layer), showed the presence of D-glucose only.

Catalytic Hydrogenation of Genipin-gentiobioside (II)——300 mg of II in MeOH was shaken with H_2 in the presence of Pd on charcoal as a catalyst. After the uptake of hydrogen corresponding to two moles per mole of II, the catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to give a white powder (VII). yield 220 mg. 100 mg of VII was acetylated by the usual method with pyridine and Ac_2O to give 10-deoxy-7,8-dihydro-genipin-gentiobioside-heptaacetate (VIII) as colorless needles. mp 192—193°, $[\alpha]_D^{21}$ −51° (c=0.5, MeOH). IR (KBr) cm^{-1}: 1760, 1705, 1640. *Anal.* Calcd. for $C_{37}H_{50}O_{21}$: C, 53.49; H, 6.07. Found: C, 53.19; H, 6.02.

Hydrolysis of 10-Deoxy-7,8-dihydrogenipin-1-gentiobioside (VII) with β-Glucosidase——To a solution of VII (200 mg) in the acetate buffer solution (40 ml, pH=5.0) was added 15 ml of 1% aqueous solution of β-glucosidase and the mixture was allowed to stand at 37° for 3 hr. The reaction mixture was extracted with ether. The ethereal extract was concentrated to dryness and the residue was acetylated with pyridine and Ac_2O to give 10-deoxy-7,8-dihydrogenipin-monoacetate (IX) as colorless plates (from aqueous MeOH)

mp 53—56°, $[\alpha]_D^{21}$ −76° (c=0.5, EtOH). IR (KBr) cm^{-1}: 1770, 1720, 1640. NMR ($CDCl_3$): δ 1.12 (3H, d, J=6 Hz, >CH-CH_3), 2.11 (3H, s, $OCOCH_3$), 3.70 (3H, s, OCH_3). *Anal.* Calcd. for $C_{13}H_{18}O_5$: C, 61.40; H, 7.14. Found: C, 61.43; H, 7.01. This was identified by the mixed melting point and the comparison of IR spectra with an authentic sample of IX derived from 7-deoxyloganin. The aqueous layer was evaporated to dryness. The paper chromatography of this residue (solvent: BuOH, AcOH, H_2O; 4, 1, 5: upper layer), showed the presence of D-glucose only.

Permethyl Ether of Genipin-gentiobioside——NaH (200 mg) was warmed with dimethylsulfoxide (10 ml) at 60—70° for 1 hr under stirring in N_2 gas flow. To this reagent 100 mg of II in 7 ml of dimethylsulfoxide was added and kept at 70° for 1 hr under stirring in N_2 gas flow. 2 g of CH_3I was added at 0° and the reaction mixture was allowed to stand at room temperature for 3 hr. After dilution with water the mixture was extracted with ether and the ethereal layer was washed with water and concentrated to dryness. The residue was purified by preparative thin-layer chromatography to give octa-O-methyl-ether of I (X) (20 mg) as a colorless oil. IR ($CHCl_3$) cm^{-1}: 1700, 1640, 1440, and no OH band. NMR ($CDCl_3$): δ 3.37 (3H, s), 3.43 (3H, s), 3.51 (3H, s), 3.55 (9H, s), 3.61 (3H, s) 3.66 (3H, s) 3.76 (3H, s)——($9\times OCH_3$), 4.30 (1H, d, J= 7 Hz), 4.70 (1H, d, J=7 Hz)——(anomeric protons of the sugar moiety), 5.23 (1H, d, J=6 Hz, H-1), 5.86 (1H, m, H-7), 7.56 (1H, d, J=1.5 Hz, H-3).

The above methyl ether (X) (200 mg) was heated with 5% methanolic hydrogen chloride in a sealed tube in a boiling water bath for 7 hr. The reaction mixture was concentrated and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried and concentrated. The presence of equimolecular methyl-2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl-2,3,4-tri-O-methyl-D-glucopyranoside in this residue was demonstrated by gas liquid chromatography: condition, column, 5% neopentyl-glycol-succinate on Chromosorb-w (60—80 mesh), 3 mm×2 m. Column temperature 180°, carrier gas, He (1 kg/cm^2), t_R: methyl-2,3,4,6-tetra-O-methyl-D-glucopyranoside, 5.3 (β), 7.1 (α); methyl-2,3,4-tri-O-methyl-D-glucopyranoside, 11.1 (β), 14.9 (α).

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