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Teikaside A, a Pregnane Glycoside of *Trachelospermum asiaticum*

FUMIKO ABE and TATSUO YAMAUCHI*

Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma 11, Nishi-ku, Fukuoka

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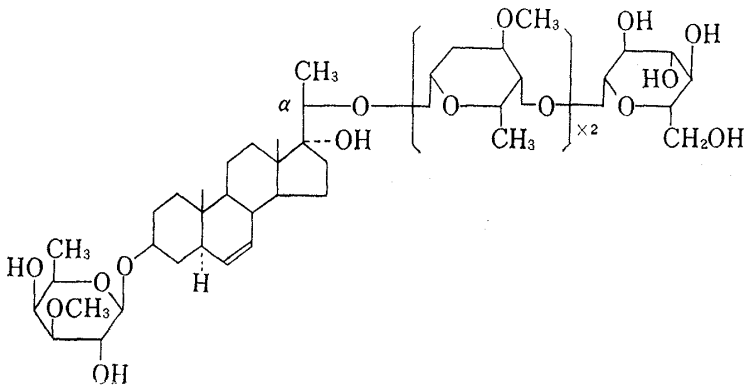
Teikaside A, one of the pregnane glycosides of *Trachelospermum asiaticum* (Apocynaceae), was isolated and its structure was determined to be 3-O-(β-D-digitalosyl)-20-O-(β-D-glucosyl-β-D-sarmentosyl-β-D-sarmentosyl)-3β,17α,20α-trihydroxy-5α-pregn-6-ene. In view of the structural similarity to the glycosides in Bei-Wujiapi, which are known to induce the potentiation of the nerve growth factor, similar physiological activity is expected in teikaside A.

Keywords——Apocynaceae; *Trachelospermum asiaticum*; pregnane; pregnane-glycoside; Δ^6-steroid; 3β,17α,20α-trihydroxy-5α-pregn-6-ene-3,20-bis-O-glycoside; 3-O-digitalosyl-pregnane-20-O-glycosyl-bis-sarmentoside

Trachelospermum asiaticum NAKAI (Japanese name: Teikakazura) is a climbing shrub, indigenous to the south-western area of Japan, and the stem and leaves have been used as a tonic component in oriental crude drugs. The lignans of this plant were studied by Takano *et al.*[1] and by Nishibe *et al.*,[2] and the presence of a trihydroxy-pregnane compound with one olefinic linkage was reported by the latter group.[3] In this paper, we describe the isolation and structure determination of teikaside A (I), a major pregnane glycoside of this plant.

The stem and leaves of this plant were percolated with methanol, and the concentrate of the whole percolate was successively partitioned with hexane, benzene and chloroform. The chloroform extract was fractionated by column chromatography on polyamide and silica gel. The fraction showing blue-violet spots with $SbCl_3$ reagent on thin-layer chromatography (TLC) was treated with β-glucosidase in order to remove the lignan glucosides which were found in a large amount in the same fraction. The remaining unhydrolyzed glycosides were again chromatographed on a silica gel column. I was obtained as a homogeneous solid, and I-acetate was crystallized, mp 235—245°, in an overall yield of 0.002%.



teikaside A

On hydrolysis of I with 0.05 N H_2SO_4–50% EtOH, two less polar compounds (II and III) were obtained. When III was treated with 1% HCl in acetone for 24 hr at room temperature, II and digitalose, together with the acetonides of II (IV) and of III (V), were produced. II was considered to be the aglycone of I on the basis of its molecular formula, $C_{21}H_{34}O_3$. On

acetylation, II afforded a diacetate which exhibited an unacetylated hydroxyl group in the infrared (IR) spectrum.

The nuclear magnetic resonance (PMR) spectrum of IV indicated one methyl group at δ 1.37 (d) with a coupling constant of 6 Hz, ascribable to methyl protons of C-21 adjacent to the 20-carbinyl proton, together with two angular methyl groups, six protons of one acetonide residue, two carbinyl protons at δ 3.65 (m) and 4.26 (q, J=6 Hz) and two protons at 5.26 and 5.48, coupled to each other with a constant of 10 Hz, of one di-substituted double bond. The presence of a glycol system was obviously indicated by the acetonide formation in IV, and its location was presumed to be C-17 and C-20, since the carbinyl proton observed at δ 4.26 in IV could be assigned to C-20-H on the basis of its coupling pattern. The remaining carbinyl proton at δ 3.65 was tentatively assigned to C-3-H.

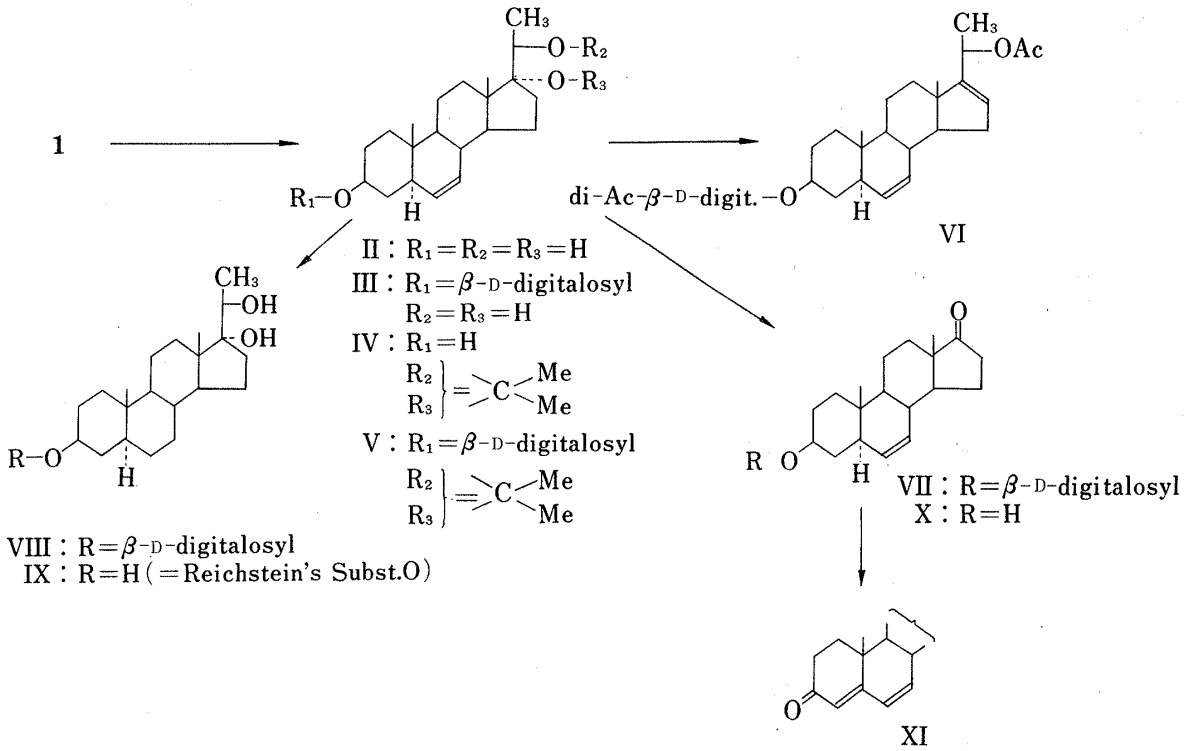


Chart 1

III, $C_{28}H_{46}O_7$, the digitaloside of II, seems to correspond to Subst. I, previously reported by Inagaki *et al.*[3] as a digitaloside of trihydroxy-pregnene isolated from the hydrolysate of the methanol extract of the same plant materials. The PMR spectrum of III-acetate showed the resonances of one methoxyl at δ 3.38, three acetyls at δ 2.08, 2.10 and 2.18, and one anomeric proton at δ 4.51 (d, J=7 Hz), along with two angular methyls (0.73 and 0.81), 21-methyl and 6′-methyl at δ 1.23 (J=6 Hz), and five protons at δ 4.95—5.50. The glycol system at C-17 and C-20 was confirmed by the reaction of III-acetate with thionyl chloride to form an anhydro-compound (VI), and by periodate oxidation to give VII, in which the PMR resonance due to 21-methyl protons was no longer apparent. The α-configuration of the hydroxyl groups at C-17 and C-20 as well as the presence of 3β-hydroxyl in a 5α-steroid framework was proved by direct comparison of the hydrogenated aglycone (IX) which was prepared by the catalytic reduction of III, followed by hydrolysis of the dihydro-derivative of III (VIII), with the authentic 5α-pregnane-3β,17α,20α-tiol (Reichstein's Subst. O).[4] Since III formed the acetonide, the digitalose in III is linked at the 3β-hydroxyl group.

With respect to the olefinic linkage observed as an AB quartet at δ 5.26 and 5.48 in IV,

four possible locations such as Δ^1, Δ^6, Δ^{11} and Δ^{15} can be considered. Among them, Δ^{15} was excluded since there was no UV absorption of a 15-en-17-one moiety in VII. The molecular rotation difference between II-acetate and Subst. O acetate (IX-acetate) (−486°) is closer to that of Δ^6-5α-steroids (−417°) than to that of a Δ^1 (−41°) or Δ^{11} derivative (+33°).[5] On CrO_3 oxidation of the 3-hydroxyl group, followed by SeO_2 oxidation, the aglycone of VII (X) was converted into androsta-4,6-diene-3,17-dione (XI), which showed absorption at 282 nm, and coincided well with an authentic sample on gas–liquid chromatography (GLC). The structure of II, therefore, was identified as 3β,17α,20α-trihydroxy-5α-pregn-6-ene, and that of III was identified as the 3-O-digitaloside of II.

On hydrolysis of I, three sugars, sarmentose, digitalose and glucose were detected. Since I, on acetylation, afforded the hexaacetate which showed the presence of three methoxyl and three methyl protons ascribable to C-3 and C-6 of digitalose and sarmentose along with the C-21 methyl protons, the sugar moiety appeared to be composed of one digitalose, two sarmentose and one glucose. The glucose was located at the terminal position on the basis of the mass spectral (MS) peak at *m/e* 331$^+$, due to the peracetylated glucosyl residue. Although the coupling pattern of the anomeric protons in the sugar linkages was obscure, except for that of digitalose in III-acetate, all the sugars are tentatively regarded as being of the D-series with β-glycosidic linkages, since only the D-forms of these sugars have been found in nature. The sarmentose-sarmentose-glucose unit was considered to be linked to the 20α-hydroxyl, since one hydroxyl remained unacetylated in I-acetate and the methyl permethyl digitaloside was obtained on methanolysis of I-permethylate, along with methyl sarmentoside and methyl permethyl glucoside. Consequently, the structure of I was considered to be 3-O-(β-D-digitalosyl)-20-O-(β-D-glucosyl-β-D-sarmentosyl-β-D-sarmentosyl)-3β,17α,20α-trihydroxy-pregn-6-ene.

Since pregnane glycoside with similar structures, such as 3,20-bis-O-glycosides of Δ^5-pregnene-3β,20α-diol (Glycosides K and H_1), -3β,17α,20α-triol (-E) and -3β,16α,20α-triol ($-H_2$), were isolated from Bei-Wujiapi, a Chinese crude drug originating from Asclepiadaceae plant, *Periploca sepium* Bunge, and found to cause potentiation of nerve fiber outgrowth mediated by NGF (nerve growth factor,)[6] I is expected to show a similar biological activity. It is noteworthy that this is the first example of the isolation of a pregnane glycoside with 2,6- and 6-deoxy-3-O-methyl sugars in Apocynaceae, though pregnenolone glucosides were isolated from the root bark of *Nerium odorum*.[7] Several pregnanes with characteristic structure have been found in this family,[8] and in addition, many pregnane glycosides are known in Asclepiadaceae.

Experimental

All melting points were measured on a Kofler block and are uncorrected. PMR spectra were taken with a Hitachi R-22 spectrometer in $CDCl_3$, and MS were obtained with a JEOL JMS-01SG spectrometer. GLC was run on a Shimadzu GC-3BF machine. The following solvent systems were used for column, thin-layer (TLC) and paper chromatographies; (PC) solv. 1: $CHCl_3$–MeOH–H_2O (7: 3: 1), solv. 2: $CHCl_3$–MeOH (10: 1—5: 1), solv. 3: BuOH–AcOH–H_2O (4: 1: 5), solv. 4: benzene–BuOH (1: 9) saturated with H_2O. $SbCl_3$ in $CHCl_3$ and aniline hydrogen phthalate reagents were used for detection of pregnanes and sugars, respectively.

Extraction and Isolation of Teikaside A (I)——The dried powdered stem and leaves (10 kg) were percolated with MeOH, and the whole MeOH solution was concentrated *in vacuo* to 5 l. The concentrate was diluted with 5 l of H_2O and extracted with hexane (ext. 100 g), benzene (30 g) and then with $CHCl_3$ (50 g). The $CHCl_3$ ext. was dissolved in solv. 1 and passed through a silica gel column. The fraction in which lignan glycosides were detected was then chromatographed on a polyamide column and eluted with H_2O and MeOH–H_2O. The column chromatographies were repeated several times, alternately. On each column chromatography, a portion of each fraction was hydrolyzed with acid and the pregnane content was monitored by TLC (solv. 1 or 2). The pregnane glycosides-rich fraction was dissolved in H_2O and treated with a crude enzyme preparation containing β-glucosidase (Kokulase, Sankyo Co., Ltd.) to hydrolyze lignan glucosides which were included in the same fraction. The mixture of unhydrolyzed pregnane- and lignan glycosides was again fractionated on a silica gel column with solv. 1 and solv. 2 several times, and finally one of the pregnane glycosides was isolated as a colorless homogeneous solid (I) which, on acetylation with Ac_2O/

pyridine at room temp., followed by crystallization from hexane–EtOAc, gave 200 mg of prisms (I-acetate), mp 235—245°, $[\alpha]_D^{25}$ −69.2° (c=0.11, $CHCl_3$), *Anal.* Calcd for $C_{60}H_{92}O_{24}\cdot H_2O$ (I-hexaacetate): C, 59.29; H, 7.80. Found: C, 59.53; H, 7.63. MS: m/e 331$^+$, PMR δ: 0.72 (6H, s, 18 and 19-Me), 1.11, 1.24, 1.32 (3H, 6H, 3H, d, J=6 Hz, 21-Me, 3×6-Me of 6-deoxy sugars), 1.98, 2.01, 2.04, 2.07 (3H, 6H, 6H, 3H, s, 6×OAc), 3.34, 3.36, 3.40 (3H of each, s, –OMe).

Hydrolysis of Teikaside A (I)——I (150 mg) was refluxed with 10 ml of 0.05 N H_2SO_4–50% EtOH for 1 hr. The solution was concentrated *in vacuo*, diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ ext. (78 mg) was then fractionated by column chromatography to give two compounds, II (2 mg) and III (41 mg).

II (3β,17α,20α-trihydroxy-5α-pregn-6-ene) was crystallized from hexane–EtOAc to give prisms, mp 230—233°, $[\alpha]_D^{25}$ −159.6° (c=0.075, MeOH), MS: Calcd for $C_{21}H_{34}O_3$ m/e 334.2508; Found: 334.2506. II-acetate was obtained as prisms after usual acetylation, followed by crystallization from dil. EtOH, mp 220—223°, $[\alpha]_D^{25}$ −145.2°. MS: Calcd for $C_{25}H_{38}O_5$ m/e 418.2719; Found: 418.2756. IR ν_{max}^{KBr} cm^{-1}: 3475 (–OH). $M_{D\cdot II\text{-acet.}} - M_{D\cdot IX\text{-acet.}} = -606° - (-120°)^{5)} = -486°$.

III (3-O-β-D-digitaloside of II) was crystallized from acetone to give prisms, mp 253—257°, $[\alpha]_D^{25}$ −106.7° (c=0.21, MeOH). *Anal.* Calcd for $C_{28}H_{46}O_7$: C, 67.98; H, 9.37. Found: C, 67.89; H, 9.35. III-acetate was obtained as prisms by usual acetylation of III, followed by crystallization from MeOH, mp 245—250°, $[\alpha]_D^{25}$ −88.9° (c=0.09, MeOH). PMR: 0.73, 0.81 (3H of each, s, 18 and 19-Me), 1.23 (6H, d, J=6 Hz, 21-Me and 6′-Me), 2.08, 2.10, 2.18 (3H of each, s, 3×OAc), 3.38 (3H, s, 3′-OMe), 3.25—3.80 (3H, m), 4.51 (1H, d, J=7 Hz, 1′-H), 4.95—5.50 (5H, m).

Hydrolysis of III——III (100 mg) was allowed to stand with 20 ml of 1% HCl in acetone (Mannich's reagent) at room temp. for 24 hr. The mixture was then diluted with H_2O and extracted with $CHCl_3$ to give II (8 mg), and the acetonides of II (IV) (20 mg) and III (V) (35 mg). Digitalose was detected in H_2O layer by PC and GLC; PC: *Rf* 0.46 (solv. 3: D-digitalose 0.45, L-thevetose 0.55, D-glucose 0.16, L-rhamnose 0.37), GLC (5% 1,4-butanediol succinate, 1.7 m, column temp.: 169°, N_2: 1.0 kg/cm^2): t_R (min) 6.95, 10.30 (methyl digitaloside: 6.95, 10.30).

IV was crystallized from hexane to give needles, mp 175—178°, $[\alpha]_D^{25}$ −162.5° (c=0.08, MeOH), MS: Calcd for $C_{24}H_{38}O_3$ m/e 374.2821; Found: 374.2770. PMR: 0.80, 0.85 (3H of each, s, 18 and 19-Me), 1.37 (3H, d, J=6 Hz, 21-Me), 1.42 (6H, s, acetonide-$(Me)_2$), 3.65 (1H, m, 3-H), 4.26 (1H, q, J=6 Hz, 20-H), 5.26, 5.48 (1H of each, d, J=10 Hz, 6 and 7-H). V was crystallized from hexane–EtOAc to give prisms, mp 198—200°, $[\alpha]_D^{25}$ −128.4° (c=0.185, MeOH). *Anal.* Calcd for $C_{31}H_{50}O_7$: C, 69.63; H, 9.43. Found: C, 69.22; H, 9.62. PMR: 0.80, 0.86 (3H of each, s, 18 and 19-Me), 1.38 (6H, d, J=6 Hz, 21-Me and 6′-Me), 1.40, 1.42 (3H of each, s, acetonide-$(Me)_2$), 3.21 (1H, dd, J=4, 10 Hz, 3′-H), 3.52 (3H, s, 3′-OMe), 3.67 (1H, dd, J=10, 8 Hz, 2′-H), 3.86 (1H, dd, J=4, 2 Hz, 4′-H), 4.27 (1H, q, J=6 Hz, 20-H), 4.37 (1H, d, J=8 Hz, 1′-H), 5.27, 5.50 (1H of each, d, J=10 Hz, 6 and 7-H).

$SOCl_2$ Reaction of III-Acetate——III-acetate (20 mg) was dissolved in pyridine and a small amount of $SOCl_2$ was added. The reaction mixture was allowed to stand at 0° for 2 hr, then diluted with ice-water. The mixture was extracted with $CHCl_3$. The $CHCl_3$ ext. was crystallized from hexane–ether to give 10 mg of prisms (VI), mp 230—233°; no absorption maximum was observed in the range of 210—350 nm. IR (KBr) cm^{-1}: $\nu_{C=O}$ 1730, $\nu_{-\dot{C}=\dot{C}-H}$ 1660, $\delta_{-\dot{C}=\dot{C}-H}$ 840, 825; no absorption band at *ca.* 3500 cm^{-1}. MS: m/e 602 M$^+$), 560 (M−CH_3CO), 542 (M−CH_3COOH), 527 (M−$CH_3COOH-CH_3$).

Periodate Oxidation of III——III (120 mg) was dissolved in 20 ml of EtOH, then 90 mg of HIO_4 in 1.5 ml of H_2O was added. The solution was allowed to stand in the dark for 6 hr. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$, followed by crystallization from hexane–EtOAc to give 40 mg of prisms (VII) (digitaloside of 3β-hydroxy-5α-androst-6-en-17-one), mp 165—169°, $[\alpha]_D^{25}$ −56.4° (c=0.11, MeOH); no absorption maximum was observed in the range of 210—350 nm, PMR: 0.82, 0.90 (3H of each, s, 18 and 19-Me), 1.37 (3H, d, J=6 Hz, 6′-Me), 3.21 (1H, dd, J=4, 10 Hz, 3′-H), 3.53 (3H, s, 3′-OMe), 3.86 (1H, d, J=4 Hz, 4′-H), 4.37 (1H, d, J=7 Hz, 1′-H), 5.37, 5.58 (1H of each, d, J=10 Hz, 6 and 7-H).

Hydrogenation of III——A solution of III (100 mg) in EtOH was shaken for 3 hr with PdO (30 mg) as a catalyst under an atmosphere of hydrogen. The product (VIII) (digitaloside of 5α-pregnane-3β,17α,20α-triol) was crystallized from EtOH–EtOAc–ether to give prisms (70 mg), mp 273—275°, $[\alpha]_D^{25}$ −20.0° (c=0.20, MeOH). *Anal.* Calcd for $C_{28}H_{48}O_7$: C, 67.71; H, 9.74. Found: C, 67.89; H, 9.35. VIII was subjected to hydrolysis with 1% HCl in acetone to give the aglycone of VIII (IX), mp 229—231° and IX-acetonide, mp 207—209°. On admixture of IX with authentic 5α-pregnane-3β,17α,20α-triol (mp 225—227°),[4] no melting point depression was observed, and the IR spectra of both samples were in good agreement.

CrO_3 and SeO_2 Oxidation of the Aglycone of VII (X) (3β-Hydroxy-5α-androst-6-en-17-one)——VII (180 mg) was hydrolyzed with 1% HCl in acetone at room temp. to give an aglycone which was crystallized from hexane–EtOAc to give X (73 mg) as prisms, mp 186—188°, $[\alpha]_D^{25}$ −50.0° (c=0.176, $CHCl_3$), MS: Calcd for $C_{19}H_{28}O_2$ 288.2089; Found: 288.2045. X (50 mg) was dissolved in pyridine (3 ml) and subjected to oxidation with CrO_3/pyridine (120 mg/12 ml) complex for 23 hr. The mixture was diluted with H_2O and extracted with $CHCl_3$. Without purification, the $CHCl_3$ ext. (*ca.* 20 mg) was then refluxed with SeO_2 (10 mg) in *t*-BuOH (3 ml)–HOAc (0.5 ml) for 2 hr. The product (XI) was isolated from the reaction mixture with $CHCl_3$ in the usual manner, and purified by preparative TLC with benzene–acetone. UV λ_{max}^{MeOH} 282 nm. GLC

(1.5% SE-52, 2.1 m, column temp.: 216°, N_2: 1 kg/cm²): t_R of SeO_2 oxidation product: 8.1 min, t_R of CrO_3/pyridine oxidation product (Δ^6-androstene-3,17-dione): 6.0, (authentic 5α-androstane-3,17-dione: 6.45; Δ^4-androstene-3,17-dione: 7.9; $\Delta^{4,6}$-androstadiene-3,17-dione: 8.1). GLC (1.5% QF-1, 2.1 m, column temp. 219°, N_2: 1 kg/cm²): t_R of SeO_2 oxidn. product: 22.1 min, t_R of CrO_3/pyridine oxidn. product: 12.9 min, (5α-androstane-3,17-dione: 5.0, Δ^4-androstene-3,17-dione: 24.3, $\Delta^{4,6}$-androstadiene-3,17-dione: 22.0).

Detection of Sugar Moiety——1) I was hydrolyzed with 0.5 N H_2SO_4–50% EtOH for 1 hr, then the aglycone moiety was removed by $CHCl_3$ extraction. The H_2O layer was neutralized with IR-410 and concentrated. The residue was examined by TLC and PC, and sarmentose, digitalose and glucose were identified by comparison with the corresponding authentic samples; $R_{digin.}$ 1.05 (TLC; solv. 1: cymalose 1.18, oleandrose 0.93, sarmentose[9] 1.05, diginose 1.00), $R_{digin.}$ 1.18 (TLC; solv. 3: cym. 1.17, ole. 1.22, sarm. 1.18, digin. 1.00). *Rf* 0.78 (PC; solv. 4: cym. 0.75, sarm. 0.78, digin. 0.77), *Rf* 0.29, 0.14 (PC; solv. 3: digitalose 0.29, glucose 0.14), *Rf* 0.41, 0.07 (PC; solv. 4: digit. 0.41, gluc. 0.07).

2) A small amount of I was methylated with MeI and Ag_2O in dimethylformamide (Kuhn's procedure). The I-permethylate was refluxed with 1% methanolic HCl for 2 hr, then, deacidified with IR-410, and the methylated sugar was examined by GLC (5% 1,4-butanediol succinate, 1.7 m, column temp.: 163°, N_2: 0.8 kg/cm²): t_R (min) 1.8, 2.1, 2.7, 3.8 (methyl sarmentoside: 1.8, 2.1; methyl 2,4-di-O-methyl digitaloside: 2.7, 3.8; methyl 2,3,4,6-tetra-O-methyl-α-D-glucoside: 3.8).

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