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TWO NEW IRIDOID GLUCOSIDES FROM *MUSSAENDA PARVIFLORA* AND *MUSSAENDA SHIKOKIANA**

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Key Word Index—*Mussaenda parviflora*; *Mussaenda shikokiana*; Rubiaceae; iridoid glucosides; mussaenoside; shanzhiside methyl ester.

Abstract—From the leaves of *Mussaenda parviflora*, two new iridoid glucosides, mussaenoside and shanzhiside methyl ester were isolated and their structures established. The occurrence of mussaenoside and shanzhiside methyl ester in the leaves and fruits of *Mussaenda shikokiana* was also demonstrated.

INTRODUCTION

In the course of the systematic investigation of iridoid glucosides of Rubiaceous plants, we previously isolated many new iridoid glucosides from *Gardenia jasminoides* forma *grandiflora* [1–3], *Ixora chinensis* [4] and *Tarenna kotoensis* var. *gyokushinka* [5] of the subfamily Ixoroideae and established their structures.

This paper describes the structure elucidation of two new iridoid glucosides isolated from plants of the tribe *Mussaendeae* and the subfamily Cinchonoideae.

RESULTS AND DISCUSSION

The new iridoid glucosides, mussaenoside (**1**) and shanzhiside methyl ester (**2**) were obtained from the methanolic extract of leaves of *Mussaenda parviflora* (Japanese name: Konronka).

The first glucoside, mussaenoside (**1**) was obtained as a white powder, $C_{17}H_{26}O_{10}\cdot 1/2\ H_2O$, $[\alpha]_D^{30}$ −106.0° (MeOH, *c* 0.61). It showed an absorption maximum at 238 nm (log ε = 4.04, MeOH) in the UV spectrum and bands at 3400, 1695, 1640 cm^{-1} in the IR (KBr) spectrum. The NMR spectrum (D_2O) of **1** showed a singlet at δ 1.32 assignable to a tertiary methyl group attached to a hydroxyl bearing carbon, a double doublet (*J* = 10.3 and 3.3 Hz) at δ 2.36 due to the C-9 proton, a singlet at δ 3.75 attributable to a carbomethoxy group, a doublet (*J* = 3.3 Hz) at δ 5.55 arising from the C-1 proton and a doublet (*J* = 1.0 Hz) at δ 7.48 characteristic of the C-3 proton. Acetylation of **1** with acetic anhydride–pyridine gave the tetraacetate (**3**), $C_{25}H_{34}O_{14}$. The NMR spectrum showed signals of four acetyl groups (δ 1.93–2.10) and of a tertiary hydroxyl group which disappeared on addition of D_2O. Further acetylation of **3** with acetic anhydride–boron trifluoride gave the pentaacetate (**4**), $C_{27}H_{36}O_{15}$, together with a dehydration product (**5**) which was also obtained by the dehydration of **3** with phosphoryl chloride–pyridine. The location of the tertiary hydroxyl group at C-8 was concluded from the fact that the NMR signal of the tertiary methyl group shifted from δ 1.33 in **3** to δ 1.51 in **4** and from the spectrum of **5** which showed a broad singlet at δ 1.77 assignable to an olefinic methyl group and a multiplet at δ 5.47 due to the C-7 olefinic proton. Except for the C-8 configuration, the absolute structure of **1** was established by the identification of **5** with 10-deoxygeniposide tetraacetate which was obtained by methylation of the corresponding acid tetraacetate (**6**) derived in turn by partial catalytic hydrogenation of asperuloside tetraacetate (**7**) of defined absolute structure. Previously, we assigned the configuration at C-8 of several iridoid glucosides bearing a hydroxyl group at that position by comparison of chemical shifts of the C-1 and C-9 protons in the 8-hydroxy and the corresponding 8-acetoxy compounds [1]. On the basis of this assignment, the down-field shifts of the NMR signals of the C-1 and C-9 protons from δ 5.33 and 2.31 of the 8-hydroxy compound (**3**) to δ 5.71 and 2.66 of the pentaacetate (**4**) suggest that the tertiary hydroxyl group in **1** assumes the β-configuration. In fact, the tetraacetate (**3**) was identical with the hydrogenation product [4] of the β-epoxy compound (**8**) [6] of known absolute stereochemistry over Pd-C catalyst in the presence of perchloric acid. Accordingly, the absolute structure **1** was assigned to mussaenoside.

The second glucoside, shanzhiside methyl ester (**2**) was obtained as a white powder, $C_{17}H_{26}O_{11}\cdot H_2O$, $[\alpha]_D^{30}$ −110.8° (MeOH, *c* 0.42). This substance showed a UV absorption maximum at 238 nm (log ε = 3.93, MeOH), IR (KBr) bands at 3400, 1690, 1640 cm^{-1}. The NMR spectrum (D_2O) of **2** showed a singlet at δ 1.28 assignable to a tertiary methyl group attached to a carbon bearing a hydroxyl group, a singlet at δ 3.78 arising from a carbomethoxy group and a doublet (*J* = 1.0 Hz) at δ 7.48 due to the C-3 proton. Acetylation of **2** with acetic anhydride–pyridine gave the pentaacetate (**9**), $C_{27}H_{36}O_{16}$, which was identical with shanzhiside pentaacetate methyl ester.

Although the configuration at C-8 of shanzhiside (**10**) was tentatively assigned, as mentioned above, according to NMR spectral evidence [1], unequivocal assignment has not yet been made. Chemical confirmation of this stereochemistry was accomplished as follows. Shanzhi-

*Part 35 in the series 'Studies on Monoterpene Glucosides and Related Natural Products.' For Part 34 see ref. [3].

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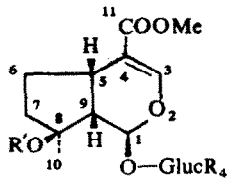
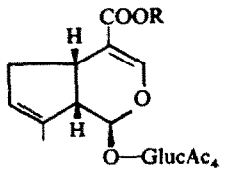
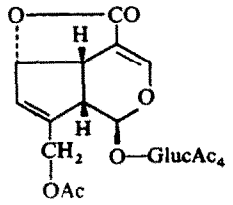


1: R = R′ = H
3: R = COMe; R′ = H
4: R = R′ = COMe



5: R = Me
6: R = H



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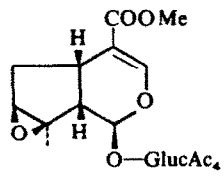

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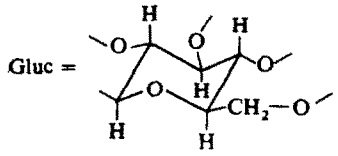


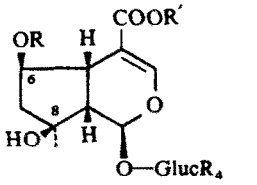
side methyl ester (2) was treated with benzaldehyde and zinc chloride to give 4′,6′-benzylideneshanzhiside methyl ester (11), $C_{24}H_{30}O_{11}$. 1/2 H_2O, which was successively reacted with phenylboronic acid in acetone, then with benzoyl chloride–pyridine. After treatment with 1,3-propanediol, the product was acetylated with acetic anhydride–pyridine to furnish 6-*O*-acetyl-4′,6′-benzylideneshanzhiside methyl ester-2′,3′-diphenylpyroboronate (12), $C_{38}H_{40}O_{13}B_2$ [7, 8]. The fact that 12 was obtained by this series of reactions suggests the intermediary formation of the cyclic 6,8-phenylboronic acid ester (13), which was cleaved by 1,3-propanediol to 14 and then acetylated to give 12. It is evident from inspection of Dreiding models that neither cyclic 6,8-phenylboronic acid ester nor 6,8-diphenylpyroboronate can be formed when the C-6 and C-8 hydroxyl groups have a *trans*-orientation. As the absolute stereochemistry at C-6 in compound 2 has been determined as the *R*-configuration by chemical correlation with scandoside hexaacetate methyl ester (15) [1], the above result definitely indicates the *S*-configuration at position C-8.

We also examined the iridoid constituents of *Mussaenda shikokiana* (Japanese name: Hirohanokonronka) which is closely related to *M. parviflora*. This revealed the occurrence of mussaenoside (1) and shanzhiside methyl ester (2) in the leaves and fruits.

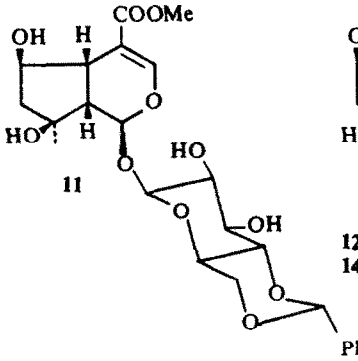
The coexistence of mussaenoside (1) and shanzhiside methyl ester (2) in both plants suggests that 2 is biosynthesized by hydroxylation of 7-deoxyloganin (16) at C-8 followed by hydroxylation at C-6 in these plants.

EXPERIMENTAL

General procedures. All mp's were uncorr. NMR spectra were recorded at 60 MHz. Chemical shifts are given in δ values (ppm) with TMS or DSS as the internal standard. Si gel G was used for TLC and spots visualized by exposure to I_2 vapour or spraying with a mixture of anisaldehyde (0.5 ml), conc H_2SO_4 (0.5 ml), AcOH (few drops) and 95% EtOH (9 ml) followed by heating. For column chromatography, Si gel (Mallinckrodt) or carbon for chromatography (Wako) was used.



2: R = H; R′ = Me
9: R = COMe; R′ = Me
10: R = R′ = H



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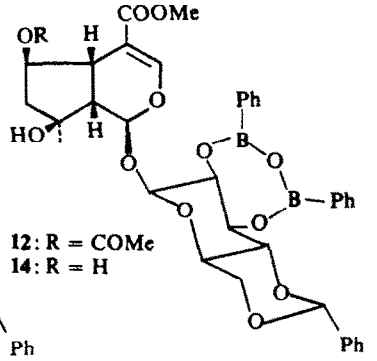
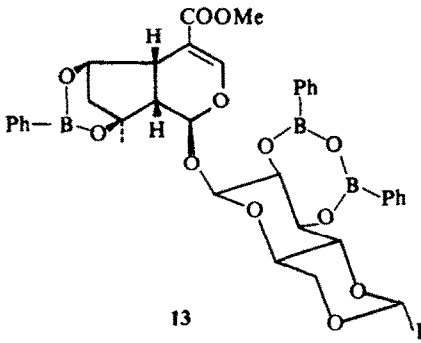


12: R = COMe
14: R = H



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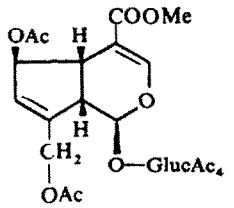

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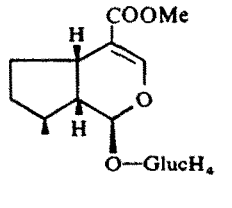

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Plant materials. Mussaenda parviflora Miq. was collected in Ishigaki Island (Okinawa Pref.) and *Mussaenda shikokiana* Makino was collected in the Botanical Garden of Faculty of Agriculture, Kagoshima University (Kagoshima Pref.) in August, 1974. Plant materials were identified by Mr. G. Murata of Faculty of Sciences, Kyoto University. Voucher specimens of *Mussaenda parviflora* (Y. Takeda, Y. Ikeshiro and H. Nishimura, No. 6) and *Mussaenda shikokiana* (idem., No. 1) were deposited in the herbarium of the Institute of Botany, Kyoto University (KYO), Kitashirakawa-oiwake-cho, Sakyo-ku, Kyoto, 606, Japan.

Isolation of glucosides from Mussaenda parviflora. Dry leaves of *M. parviflora* (900 g) were extracted with hot MeOH (14 l. × 3). After concn. of the combined methanolic extracts *in vacuo*, H_2O (3 l.) was added and the insoluble material filtered off through a celite layer. The filtrate was rinsed with EtOAc (1 l. × 3) and evapd *in vacuo*. The residue was chromatographed on a charcoal (400 g) column and eluted with H_2O–MeOH with increasing MeOH content (Chrom. 1). The fraction eluted with H_2O–MeOH (1:1) was evapd *in vacuo* to leave a residue (11.85 g). A portion (2.37 g) of this residue was chromatographed on a Si gel (60 g) column and eluted with $CHCl_3$–MeOH with increasing MeOH content (Chrom. 1-1). The fractions eluted with $CHCl_3$–MeOH (9:1 ~ 22:3) were combined and evapd *in vacuo* to give mussaenoside (**1**) (1.71 g). (Found: C, 50.94; H, 7.14. $C_{17}H_{26}O_{10}$. 1/2 H_2O requires: C, 51.12; H, 6.81%). The fractions eluted with $CHCl_3$–MeOH (17:3) gave shanzhiside methyl ester (**2**) (0.24 g). (Found: C, 47.91; H, 6.90. $C_{17}H_{26}O_{11}$. H_2O requires: C, 48.11; H, 6.65%). The fractions eluted with H_2O–MeOH (3:7) from the above charcoal column (Chrom. 1) were evapd *in vacuo* to leave a residue (4.83 g). This residue was purified by chromatography on Si gel (Chrom. 1-2) as above to give **1** (4.21 g).

Mussaenoside tetraacetate (**3**). Mussaenoside (**1**) (100 mg) was acetylated with Ac_2O–Py and the product recrystallized from MeOH to give **3** (55 mg) as colourless needles, mp 124–126°. $[\alpha]_D^{30}$ −92.5° ($CHCl_3$, *c* 1.18); UV λ_{max}^{MeOH} nm (log δ): 237 (3.99); IR ν_{max}^{KBr} cm^{-1}: 1750, 1705, 1640; NMR ($CDCl_3$): δ1.33 (3H, *s*, 10-H), 2.31 (1H, *dd*, *J* = 9.5, 3.0 Hz, 9-H), 3.03 (1H, *m*, 5-H), 3.71 (3H, *s*, COOMe), 5.33 (1H, *d*, *J* = 3.0 Hz, 1-H), 7.34 (1H, *d*, *J* = 1.0 Hz, 3-H). (Found: C, 54.02; H, 6.43). $C_{25}H_{34}O_{14}$ requires: C, 53.76; H, 6.14%).

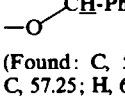
Treatment of mussaenoside tetraacetate (**3**) *with* Ac_2O–BF_3. To a soln of **3** (166.1 mg) in Ac_2O (4 ml) was added BF_3-etherate (4 drops) and the mixture was allowed to stand at room temp. for 2 min. Ice H_2O was added to the reaction mixture and the resulting ppt. was extracted with $CHCl_3$ (30 ml × 3). The $CHCl_3$ extracts were washed with aq. 10% $NaHCO_3$ and then with H_2O, dried ($MgSO_4$), and evapd to give a residue (187.6 mg), which was chromatographed on a Si gel (20 g) column with $CHCl_3$ as eluent. The faster eluate was concd *in vacuo* and the residue recrystallized from EtOH to give **5** (35 mg) as colourless needles, mp 110–112°, $[\alpha]_D^{30}$ −16.3° ($CHCl_3$, *c* 0.43). UV λ_{max}^{MeOH} nm (log ε): 238 (3.95); IR ν_{max}^{KBr} cm^{-1}: 1745, 1705, 1635; NMR ($CDCl_3$): δ1.97–2.08 (4 × OCOMe), 3.71 (3H, *s*, COOMe), 7.42 1H, *d*, *J* B 1.0 Hz, 3-H). Found: C, 55.38; H, 5.70. $C_{25}H_{32}O_{13}$ requires: C, 55.55; H, 5.97%). The slower eluate was concd *in vacuo* and the residue recrystallized from EtOH to give **4** (40 mg) as colourless needles, mp 116–118°, $[\alpha]_D^{30}$ −75.4° ($CHCl_3$, *c* 0.66). UV λ_{max}^{MeOH} nm (log ε): 236 (3.96); IR ν_{max}^{KBr} cm^{-1}: 1750, 1710, 1650; NMR ($CDCl_3$): δ1.51 (3H, *s*, 10-H), 1.90–2.11 (5 × OCOMe), 2.66 (1H, *dd*, *J* = 8.8, 2.0 Hz, 9-H), 2.95 (1H, *m*, 5-H), 3.71 (3H, *s*, COOMe), 5.71 (1H, *d*, *J* = 2.0 Hz, 1-H), 7.38 (1H, *d*, *J* = 1.0 Ha, 3-H). (Found: C, 53.72; H, 6.03. $C_{27}H_{36}O_{15}$ requires: C, 54.00; H, 6.04%).

Dehydration of mussaenoside tetraacetate (**3**). To a soln of **3** (217 mg) in dry Py (2 ml) was added $POCl_3$ (2 ml) at 0° and the mixture allowed to stand overnight at 0–4°. The reaction mixture was poured dropwise into ice H_2O and the resulting ppt. extracted with $CHCl_3$. After washing with 5% HCl, 5% $NaHCO_3$ and then H_2O, successively, the $CHCl_3$ extract was dried ($MgSO_4$) and evapd *in vacuo* to leave a residue (227 mg), which was purified on a Si gel (20 g) column with Et_2O as eluent and recrystallized from EtOH to give **5** (120 mg) as colourless needles, mp 110–112°, $[\alpha]_D^{30}$ −15.2° ($CHCl_3$, *c* 0.79). IR ν_{max}^{KBr} cm^{-1}: 1745, 1705, 1635; NMR ($CDCl_3$): δ1.97–2.08 (4 × OCOMe), 3.71 (3H, *s*, COOMe), 7.42 (1H, *d*, *J* = 1.0 Hz, 3-H). (Found: C, 55.29; H, 6.09. Calc. for $C_{25}H_{32}O_{13}$: C, 55.55; H, 5.97%). This substance was identical with a sample of **5** obtained by treatment of **3** with Ac_2O–BF_3 by mmp, IR (KBr) and NMR ($CDCl_3$).

Conversion of asperuloside tetraacetate (**7**) *to the dehydration product* (**5**). A mixture (210 mg) of 10-deoxygeniposidic acid tetraacetate (**6**) and its 7,8-dihydro compound in a ratio 4.5:1 (evaluated from the NMR spectrum), which was obtained by partial catalytic hydrogenation of asperuloside tetraacetate (**7**) [6], was separated by chromatography on $AgNO_3$–Si gel (11:89) (40 g) with C_6H_6–Me_2CO (9:1) as eluent. The slower eluate was recrystallized from dil. EtOH to give **6** as colourless needles (20 mg), mp 180–181°. NMR ($CDCl_3$): 1.78 (3H, *br. s*, 10-H), 1.98–2.10 (4 × OCOMe), 5.48 (1H, *m*, 7-H), 7.53 (1H, *s*, 3-H). This substance was methylated with an ethereal CH_2N_2 soln and the reaction product recrystallized from EtOH to give the methyl ester (15 mg) as colourless needles, mp 110–112°, $[\alpha]_D^{30}$ −16.8° ($CHCl_3$, *c* 0.45). IR ν_{max}^{KBr} cm^{-1}: 1745, 1705, 1635; NMR ($CDCl_3$): δ1.97–2.08 (4 × OCOMe), 3.71 (3H, *s*, COOMe), 7.42 (1H, *d*, *J* = 1.0 Hz, 3-H). (Found: C, 55.30; H, 6.16. Calc. for $C_{25}H_{32}O_{13}$: C, 55.55; H, 5.97%). This substance was identical with a sample of **5** derived from **3** by mmp and comparison of IR (KBr) and NMR ($CDCl_3$).

Shanzhiside methyl ester pentaacetate (**9**). Substance (**2**) (50 mg) was acetylated with Ac_2O–Py and the reaction product recrystallized from EtOH to give **9** (42 mg) as colourless needles, mp 173–175°, $[\alpha]_D^{30}$ −111.9° ($CHCl_3$, *c* 0.67). UV λ_{max}^{MeOH} nm (log ε): 237 (4.04); IR ν_{max}^{KBr} cm^{-1}: 3500, 1750, 1720, 1640; NMR ($CDCl_3$): δ1.32 (3H, *s*, 10-H), 1.91–2.10 (5 × OCOMe), 2.65 (1H, *dd*, *J* = 9.3, 2.5 Hz, 9-H), 3.20 (1H, *m*, 5-H), 3.70 (3H, *s*, COOMe), 5.40 (1H, *d*, *J* = 2.5 Hz, 1-H), 7.36 (1H, *d*, *J* = 1.0 Hz, 3-H). (Found: C, 52.51; H, 5.70. Calc. for $C_{27}H_{36}O_{16}$: C, 52.60; H, 5.89%). This substance was identical with an authentic sample of shanzhiside pentaacetate methyl ester according to mmp, IR (KBr) and NMR ($CDCl_3$).

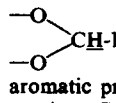
4′,6′-Benzylideneshanzhiside methyl ester (**11**). A mixture of $ZnCl_2$ (2.1 g). C_6H_5CHO (10 ml) and shanzhiside methyl ester (**2**) (1.05 g) was stirred 18 hr at room temp. After addition of H_2O (50 ml), the reaction mixture was extracted with EtOAc (50 ml × 3). The EtOAc extract was washed with H_2O, dried ($MgSO_4$) and concd *in vacuo*. The residue was chromatographed on a Si gel (20 g) column with $CHCl_3$–MeOH as eluent with increasing MeOH content. Concn of the eluate with $CHCl_3$–MeOH (97:3) gave **11** (459 mg) as a white powder. $[\alpha]_D^{29}$ −116.9° (MeOH, *c* 1.04); IR ν_{max}^{Nujol} cm^{-1}: 3650–3100, 1680, 1635; NMR (CD_3OD): δ1.25 (3H, *s*, 10-H), 3.71 (3H, *s*, COOMe), 5.46 (1H, *d*, *J* = 3.0 Hz, 1-H), 5.57 (1H, *s*,



CH-Ph), 7.17–7.63 (6H, 3-H + aromatic protons).

(Found: C, 56.99; H, 6.48. $C_{24}H_{30}O_{11}$. 1/2 H_2O requires: C, 57.25; H, 6.21%).

6-O-Acetyl-4′,6′-benzylideneshanzhiside methyl ester-2′,3′-diphenylpyroboronate (**12**). Phenylboronic acid (31 mg) was added to a soln of **11** (126.1 mg) in dry Me_2CO (7 ml) and the reaction mixture was refluxed for 4 hr. Evapn of the solvent under N_2 gave a residue which was treated with C_6H_5COCl (0.5 ml)–Py (1 ml) in the usual way. The reaction product was dissolved in Me_2CO (3 ml). To this soln was added 1,3-propanediol (0.2 ml) and the soln concd *in vacuo*. The residue was acetylated with Ac_2O–Py and the product chromatographed on a Si gel (20 g) column with $CHCl_3$–MeOH as eluent with increasing MeOH content. Evapn of the eluate with $CHCl_3$–MeOH (49:1) gave a residue which was recrystallized from MeOH to give **12** (19.0 mg) as colourless needles, mp 233–235°, $[\alpha]_D^{29}$ −159.3° (Py, *c* 1.02). IR ν_{max}^{Nujol} cm^{-1}: 3500–3350, 1720, 1700, 1640; NMR ($CDCl_3$): δ1.30 (3H, *s*, 10-H), 2.07 (3H, *s*, OCOMe), 3.32 (3H, *s*, COOMe), 5.49 (1H, *d*, *J* = 2.5 Hz, 1-H), 5.55 (1H, *s*,


CH-Ph), 7.06 (1H, *d*, *J* = 1.5 Hz· 3-H), 7.15–8.07 (15 aromatic protons). (Found: C, 63.36; H, 5.59. $C_{38}H_{40}O_{13}B_2$ requires: C, 62.84; H, 5.55%).

Isolation of iridoid glucosides from Mussaenda shikokiana. (i) Isolation of mussaenoside (1) and shanzhiside methyl ester (2) from the fruits. The methanolic extracts of dry fruits of *M. shikokiana* (31 g) were treated in the same way as in the case of *M. parviflora*. Glucoside 1 (26.0 mg) and 2 (17.5 mg) were obtained as white powders. Both glucosides were identified as their acetates with authentic specimens by mmp, IR (Nujol) and NMR ($CDCl_3$), respectively. (ii) Isolation of mussaenoside (1) and detection of shanzhiside methyl ester (2) from the leaves. The methanolic extract of dry leaves (18 g) of the plant were separated as above to give 1 (67 mg). The acetate of 1 was identical with sample 3 in all respects. As scarcity of the material prevented the isolation of 2, it was only examined by GLC [9] after trimethylsilylation. GLC (detector: FID; carrier gas: N_2 at 50 ml/min; column: 2 m × 3.5 mm i.d. packed with 1.5% OV-1 on Shimalite W AW/DMCS; column temp. 260°). The gas chromatogram showed the peaks due to mussaenoside (1) (R_t 8.25 min) and shanzhiside methyl ester (2) (R_t 9.35 min).

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