[Chem. Pharm. Bull. 24(6)1216—1218(1976)]

UDC 547.918.02 : 581.192

Studies on Monoterpene Glucosides and Related Natural Products. XXXII.[1] Iridoid Glucosides of *Tarenna kotoensis* var. *gyokushinka*

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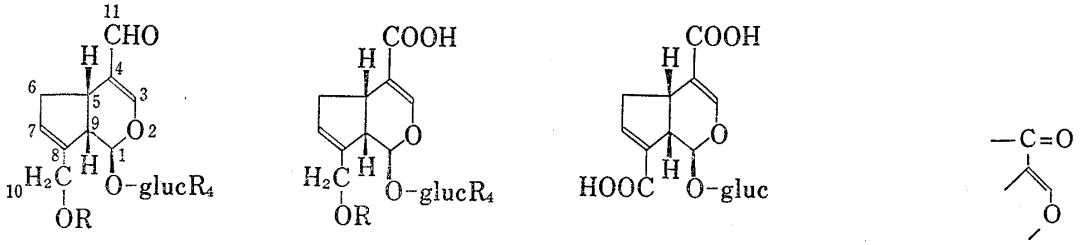
(Received September 8, 1975)

From leaves and twigs of *Tarenna kotoensis* Kan. et Sas. var. *gyokushinka*, a new iridoid glucoside, tarennoside (1) has been isolated and its structure has been established. Simultaneously, the known geniposidic acid (2) and ixoside (3) have also been isolated.

We have already isolated two new iridoid glucosides, ixoside (3) and ixoroside (4), along with the known geniposidic acid (2) from *Ixora chinensis* Lam. and have established their structures.[3] This paper deals with the iridoid glucosides of *Tarenna kotoensis* Kan. et Sas. var. *gyokushinka* (Japanese name, Gyokushinka) (Rubiaceae) which is also belonging to the tribe *Ixoreae*.

Fractionation of the methanolic extract of the leaves and twigs of *Tarenna kotoensis* var. *gyokushinka* described in the experimental part gave a new iridoid glucoside, tarennoside (**1**), along with the known geniposidic acid (**2**)[3-5] and ixoside (**3**).



1 : R=H
5 : R=Ac

2 : R=H
7 : R=Ac

3

(A)

Fig. 1

Tarennoside (**1**) has been obtained as an amorphous powder, $C_{16}H_{22}O_9$. The nuclear magnetic resonance (NMR) spectrum of **1** exhibits a singlet at δ 9.21 attributable to an aldehyde group, a singlet at δ 7.58 assignable to a proton at C-3 and a doublet (J=5.5 Hz) at δ 5.33 arising from a proton at C-1. The ultraviolet (UV) spectrum shows an absorption maximum at 250 nm (log ε=4.10) representing a bathochromic shift compared with that of iridoid glucosides bearing carboxy and carbomethoxy group at C-4. The infrared (IR) spectrum exhibits bands at 1660 and 1630 cm^{-1} attributable to the structure (A) characteristic of iridoid glucosides. Acetylation of **1** by the usual method afforded the pentaacetate (**5**), $C_{26}H_{32}O_{14}$. In the NMR spectrum of **5**, the C-3 proton signal appears at δ 7.18 as a broad singlet. Substance (**5**) was converted to the dihydrohexaacetate (**6**), $C_{28}H_{36}O_{15}\cdot 1/2H_2O$, by reduction with sodium borohydride followed by conventional acetylation, the NMR spectrum of which shows the signal due to the C-3 proton at δ 6.42 demonstrating an upfield shift compared with that from **5**. This fact, coupled with the UV spectrum of **1**, suggests that the aldehyde group locates at C-4. Considering the similarity of the NMR spectrum of **5** to that of geniposidic acid pentaacetate

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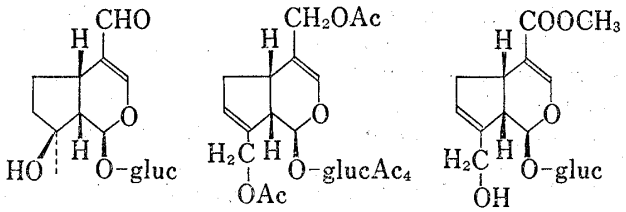

4 6 8

Fig. 2

(7)[2] except for the difference in signals assignable to the C-4 substituent and the C-3 proton, it can be presumed that tarennoside has the structure **1**, a C-11 aldehyde congener of geniposidic acid (**2**). This presumption was verified by chemical correlation between tarennoside (**1**) and geniposide (**8**).[6] Namely, the substance obtained from **8** by reduction with lithium aluminum hydride in anhydrous tetrahydrofuran under cooling followed by acetylation was found to be identical with **6** obtained from **5**. Accordingly, the absolute structure **1** is assigned to tarennoside.

As stated above, *Tarenna kotoensis* var. *gyokushinka* also contains geniposidic acid (**2**) and ixoside (**3**) besides tarennoside (**1**). Therefore, it would be reasonable to suppose that these glucosides are biosynthesized in the sequence **1**→**2**→**3** in the plant.

It has been reported on the biosynthesis of iridoid glucoside and related natural products that the radioactivity of (2-^{14}C)-mevalonic acid incorporated into plumieride,[7] sweroside, swertiamarin and gentiopicroside[8] as well as various indole alkaloids[9] was distributed almost evenly in the C-3 and C-11 carbons of the glucosides or the corresponding positions of the alkaloids, while in verbenalin,[10] nepetalactone,[11] and skytanthine,[12] such a randomization of labeling was frequently not observed depending upon the age of plants employed. Such a biosynthetic study on the iridoid glucoside bearing an aldehyde or a methyl group at C-4, however, has never been found in literature. Thus, there are many problems unsolved on the mechanism of ring closure to cyclopentane in the biosynthesis of iridoid glucosides. Since ixoroside (**4**)[3] bearing a C-11 aldehyde group has also been isolated from *Ixora chinensis* Lam. of the same tribe as the genus *Tarenna*, further occurrence of peculiar type iridoid glucosides featuring a C-11 aldehyde group in the plants of the tribe *Ixoreae* could be expected. Moreover, such glucosides will be interesting subjects for the biosynthetic studies.

Experimental[13]

Isolation Procedure——Dry leaves and twigs of *Tarenna kotoensis* var. *gyokushinka* (0.51 kg) collected in Ishigaki Island (Okinawa Pref.) in September, 1974, were extracted with 8 liters of MeOH (×3) under reflux. The MeOH extracts were combined and concentrated *in vacuo*. The residue was dissolved in H_2O (2 liters) and the insoluble material was removed by filtration through a celite layer. The filtrate was washed with

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13) All the melting points were determined on a Yanagimoto Micro Melting Point Apparatus and are uncorrected. NMR spectra were recorded on a Varian A-60 spectrometer. Chemical shifts are given in δ values (ppm) with TMS or DSS as internal standard. UV spectra were measured in a Hitachi ESP-3 spectrophotometer and IR spectra in a Hitachi Model 215 Infrared Spectrometer. Specific rotations were determined with a Rex Photoelectric Polarimeter. Silica gel G according to Stahl (Merck) was used for thin-layer chromatography (TLC) and spots were visualized by exposure to iodine 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. Preparative TLC was performed on Merck Kieselgel GF_{254} and the spots were examined under UV light (235.6 nm). Column chromatography was carried out using carbon for chromatography (Wako) or silica gel (Mallinckrodt) as adsorbent.

AcOEt (3×1 liter) and concentrated *in vacuo* to about 0.5 liter. The solution was chromatographed on a charcoal column (250 g) and eluted with H_2O–MeOH with increasing MeOH content (Chrom. 1). The fraction eluted with H_2O: MeOH 1: 9 (Chrom. 1—1) was evaporated *in vacuo* to leave a residue (1.87 g). The residue was chromatographed on silica gel (70 g) and eluted with $CHCl_3$–MeOH with increasing MeOH content. The fraction eluted with $CHCl_3$–MeOH (86: 14) was evaporated to give ixoside (3) (0.45 g) as a powder. The residue of the fraction eluted with $CHCl_3$–MeOH (84: 16) was purified by preparative TLC (0.5 mm; $CHCl_3$: MeOH 7: 3) to give tarennoside (1) (*Rf*, 0.4) (0.15 g) and geniposidic acid (2) (*Rf*, 0.15) (0.10 g). The eluate with MeOH (Chrom. 1—2) from the charcoal chromatography was evaporated *in vacuo* to leave a residue (1.54 g), which was worked up in a similar way as above to give tarennoside (1) (0.42 g) and ixoside (3) (*Rf*, 0.15) (0.35 g). The properties of glucosides isolated are as follows.

Tarennoside (1): $[\alpha]_D^{25}$ +42.1° (c=1.06, MeOH). UV λ_{max}^{MeOH} nm (log ε): 250 (4.10). IR ν_{max}^{KBr} (cm^{-1}): 3400, 1660, 1630. NMR (D_2O) δ: 4.31 (2H, s-like, C-10 H), 5.33 (1H, d, J=5.5 Hz, C-1 H), 5.97 (1H, m, C-7 H), 7.58 (1H, s, C-3 H), 9.21 (1H, s, C-11 H). *Anal.* Calcd. for $C_{16}H_{22}O_9$: C, 53.63; H, 6.19. Found: C, 53.60; H, 6.46.

Geniposidic Acid (2): $[\alpha]_D^{25}$ +16.5° (c=2.87, MeOH). UV λ_{max}^{MeOH} nm (log ε): 237 (3.64). IR ν_{max}^{KBr} (cm^{-1}): 3350, 1680, 1630. NMR (D_2O) δ: 5.25 (1H, d, J=7.0 Hz, C-1 H), 5.92 (1H, m, C-7 H), 7.56 (1H, s, C-3 H). *Anal.* Calcd. for $C_{16}H_{22}O_{10}\cdot 1/2H_2O$: C, 50.31; H, 6.05. Found: C, 50.38; H, 6.31. This substance was identified with an authentic sample of 2 by comparisons of IR (KBr) and NMR (D_2O) spectra.

Ixoside (3): $[\alpha]_D^{26}$ +30.1° (c=0.81, H_2O). UV λ_{max}^{MeOH} nm (log ε): 219 (4.16). IR ν_{max}^{KBr} (cm^{-1}): 3400, 1700, 1620. NMR (CD_3OD) δ: 4.66 (1H, d, J=7.0 Hz, C-1′), 5.83 (1H, d, J=4.0 Hz, C-1 H), 6.91 (1H, m, C-7 H), 7.51 (1H, s, C-3 H). *Anal.* Calcd. for $C_{16}H_{20}O_{11}\cdot 1/2H_2O$: C, 48.35; H, 5.33. Found: C, 48.62; H, 5.34. This substance was identified with an authentic sample of 3 by comparisons of IR (KBr) and NMR (CD_3OD) spectra.

Tarennoside Pentaacetate (5)——Tarennoside (1) (51 mg) was acetylated with Ac_2O and pyridine in the usual way and the product was recrystallized from a mixture of ether–petr. ether to give **5** (32 mg) as colorless needles, mp 127—129°, $[\alpha]_D^{25}$ −2.3° (c=0.60, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 248 (4.15). IR ν_{max}^{KBr} (cm^{-1}): 1750, 1670, 1635. NMR ($CDCl_3$) δ: 1.97—2.08 (5×OCOMe), 4.71 (2H, m, C-10 H), 5.83 (1H, m, C-7 H), 7.18 (1H, broad s, C-3 H), 9.29 (1H, s, C-11 H). *Anal.* Calcd. for $C_{26}H_{32}O_{14}$: C, 54.93; H, 5.67. Found: C, 54.65; H, 5.41.

Preparation of 11-Dihydrotarennoside Hexaacetate (6) by $NaBH_4$ Reduction of 5 followed by Acetylation——A solution of $NaBH_4$ (25 mg) in H_2O (1 ml) was added to a solution of **5** (131 mg) in dioxane (10 ml) under ice cooling. After stirring for 20 min at room temperature, AcOH was added and the solvent was removed *in vacuo*. The residue was extracted with $CHCl_3$, washed with H_2O, dried over anhyd. $MgSO_4$ and evaporated *in vacuo*. The residue (141 mg) was acetylated with Ac_2O–pyridine in a usual way and the crude product (153 mg) was purified by chromatography on silica gel (20 g) with ether as eluent. The fractions showing only the spot of *Rf* 0.53 on TLC (solvent, ether) were combined and the solvent was evaporated *in vacuo*. The residue was recrystallized from a mixture of ether–petr. ether to give **6** (95 mg) as colorless needles, mp 92—94°, $[\alpha]_D^{25}$ −33.3° (c=0.69, $CHCl_3$). IR ν_{max}^{KBr} (cm^{-1}): 1750, 1685. NMR ($CDCl_3$) δ: 2.01—2.09 (6×OCOMe), 2.85 (2H, m, C-6 H), 4.52 (2H, d, J=7.0 Hz, C-11 H), 4.73 (2H, m, C-10 H), 5.83 (1H, m, C-7 H), 6.42 (1H, m, C-3 H). *Anal.* Calcd. for $C_{28}H_{36}O_{15}\cdot 1/2H_2O$: C, 54.10; H, 6.00. Found: C, 54.38; H, 6.12.

Conversion of Geniposide (8) into Dihydrotarennoside Hexaacetate (6)——A suspension of $LiAlH_4$ (430 mg) in anhyd. THF (30 ml) was cooled to −7° and a solution of **8** (500 mg) in anhyd. THF (60 ml) was then added slowly with stirring for 40 min keeping the reaction mixture at −2—0°. The mixture was stirred at −2° for a further 16 hr. The excess of $LiAlH_4$ was decomposed by slow addition of AcOEt. Inorganic materials precipitated by adding an excess of aq. Na_2SO_4 solution was removed by filtration through a celite layer. The precipitate and the celite layer were washed with EtOH (total 200 ml). The filtrate and the washings were combined and neutralized with Amberlite IR-120 ion exchange resin (H-form). The resin was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in H_2O (100 ml) and chromatographed on a charcoal (20 g) column, eluted with H_2O (1 liter) to remove inorganic material and then with MeOH (2 liters). The eluate with MeOH was concentrated *in vacuo* to furnish a syrupy product (421 mg), which was acetylated with Ac_2O and pyridine. The reaction product was purified by chromatography on silica gel (40 g) with ether as eluent in a similar way as in the case of **6** obtained by reduction of **5** and recrystallized from a mixture of ether–petr. ether to give colorless needles (206 mg), mp 92—94°, $[\alpha]_D^{25}$ −28.2° (c=0.95, $CHCl_3$). IR ν_{max}^{KBr} (cm^{-1}): 1750, 1685. NMR ($CDCl_3$) δ: 2.01—2.09 (6×OCOMe), 2.85 (2H, m, C-6 H), 4.52 (2H, d, J=7.0 Hz, C-11 H), 4.73 (2H, m, C-10 H), 5.83 (1H, m, C-7 H), 6.42 (1H, m, C-3 H). *Anal.* Calcd. for $C_{28}H_{36}O_{15}\cdot 1/2H_2O$: C, 54.10; H, 6.00. Found: C, 54.27; H, 5.86. This substance was found to be identical with a sample of **6** derived from **1** by mixed melting point and comparisons of IR (KBr) and NMR ($CDCl_3$) spectra.

Acknowledgement This work was partly supported by Grant-in-Aid for Scientific Research from the Ministry of Education which is greatly appreciated. The authors are grateful to Dr. Y. Ikeshiro for his collaboration in collecting plant material. We are also indebted to Mrs. M. Uobe for measurements of NMR spectra and to the members of the Microanalytical Center of this University for microanalyses.