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Two Glycosides of a Novel Dammarane Alcohol from *Gynostemma pentaphyllum*

Masahiro Nagai,* Kazuo Izawa, Seiji Nagumo, Nobuko Sakurai,
and Takao Inoue

Hoshi College of Pharmacy, Ebara 2-4-41, Shinagawa-ku, Tokyo, 142, Japan

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From the aerial parts of *Gynostemma pentaphyllum* Makino (Cucurbitaceae), two glycosides were isolated, namely gynosaponin TN-1 (III), $C_{36}H_{62}O_9 \cdot 3/2H_2O$, mp 168—173°, $[\alpha]_D+34.5°$ and gynosaponin TN-2 (IV), $C_{42}H_{72}O_{13} \cdot 1/2H_2O$, mp 236—240°, $[\alpha]_D+11.6°$. On acid hydrolysis, gynosaponin TN-1 (III) yielded glucose as its sugar moiety and 2α-hydroxypanaxadiol (II), $C_{30}H_{52}O_4$ as an artificial genin, while gynosaponin TN-2 (IV) afforded rutinose and II. The structure of II was elucidated to be 20, 25-epoxydammarane-2α,3β,12β-triol on the basis of its mass spectrum, molecular optical rotation and nuclear magnetic resonance (NMR) spectra (^1H, ^{13}C) in comparison with those of panaxadiol (I). Carbon 13 NMR spectra of III and IV indicated that their aglycone moiety has a (20*S*)-dammar-24-en-20-ol partial structure. Their chemical structures were established as (20*S*)-dammar-24-ene-2α,3β,12β,20-tetraol 20-*O*-β-D-glucopyranoside (III) and (20*S*)-dammar-24-ene-2α,3β,12β,20-tetraol 20-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (IV).

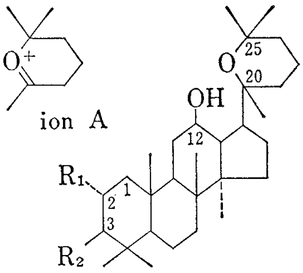
Keywords——*Gynostemma pentaphyllum*; Cucurbitaceae; triterpene glycoside; dammarenetetraol glycoside; glucoside of triterpenol; rutinoside of triterpenol; glycoside of triterpenol; gynosaponin TN-1; gynosaponin TN-2; 2α-hydroxypanaxadiol

Gynostemma pentaphyllum Makino (Cucurbitaceae), a perennial creeping herb distributed in Japan, Korea, China and Southeast Asia, was once used as a sweetener in Japan, and has been used as a folk medicine in China. No study on its chemical constituents had been reported before 1976, when we isolated panaxadiol (I) and 2α-hydroxypanaxadiol (II) as sapogenins after acid hydrolysis of a saponin mixture obtained from the plant.[1] Panaxadiol[2] (I) is a dammarane-type triterpenoid first isolated from Korean ginseng as an artificial sapogenin. Afterwards, Takemoto and his co-workers[3] reported the isolation and structure elucidation of twenty-one "gynosaponins" from the plant, though the separation procedures were difficult. In the course of our investigation of the sweetening components of the plant, we isolated two tasteless glycosides designated here as gynosaponin TN-1 (III), and gynosaponin TN-2 (IV), whose genin is a novel triterpene alcohol dammar-24-eneteraol. This report deals in detail with the elucidation of their structures.

Gynosaponin TN-1 (III), $C_{36}H_{62}O_9 \cdot 3/2H_2O$, mp 168—173°, $[\alpha]_D$ +34.5° and gynosaponin TN-2 (IV), $C_{42}H_{72}O_{13} \cdot 1/2H_2O$, mp 236—240°, $[\alpha]_D$ +11.6° were separated from a less polar saponin fraction contained in the aerial parts of *G. pentaphyllum*. On acid hydrolysis, TN-1 (III) and TN-2 (IV) commonly afforded an artificial aglycone named 2α-hydroxypanaxadiol (II), $C_{30}H_{52}O_4$, mp 287—289°, $[\alpha]_D$ −6.0°. As regards the sugar moiety, gynosaponin TN-1 (III) afforded glucose, while gynosaponin TN-2 (IV) gave rutinose together with smaller amounts of rhamnose and glucose. Thus, gynosaponin TN-1 (III) and TN-2 (IV) were concluded to be a glucoside and a rutinoside of a triterpene alcohol, respectively.

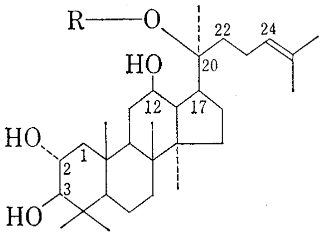
The aglycone (II) and panaxadiol (I), the latter of which was isolated in addition to II from the sapogenin fraction on acid hydrolysis of a crude mixture of *Gynostemma* saponins (see "Experimental"), showed the same base peak at *m/e* 127 represented by the ion A[2b] in Chart 1 in their mass spectra (MS). Furthermore, they showed close similarity in their fragmentation patterns in the mass number region from *m/e* 60 to 237, suggesting that II has the same carbon skeleton as I.

The aglycone (II) afforded a diacetate (V), whose proton nuclear magnetic resonance (^{1}H NMR) spectrum was comparable with that of panaxadiol 3-monoacetate (VI).[2a] V showed three one-proton signals at δ_H 3.54 (m), 4.72 (d) and 5.12 (d-t) ppm in addition to two three-proton signals due to the acetoxyl groups. The multiplet at δ_H 3.54 ppm was ascribable to 12-H because the signal has a splitting pattern identical with that in the case of VI. The double triplet at δ_H 5.12 ppm was coupled with the doublet at δ_H 4.72 ppm with a coupling constant of 10.2 Hz and with two other protons with coupling constants of 10.2 and 4.4 Hz. Taking into consideration that most triterpenoids have a hydroxyl group at C-3, we presumed the aglycone (II) to be a 2,3-dihydroxylated 12β-hydroxy-20,25-epoxydammarane. Table I shows the coupling constants of 3-H with 2-H of methyl 2,3-dihydroxyolean-12-en-28-oates and the molecular rotation differences at 589 nm between methyl olean-12-en-28-oate (VII) and its 2,3-dihydroxy derivatives.[4] The proton at C-3 of the aglycone (II) must have α-orientation and must be coupled with 2β-H, otherwise the large coupling constant of 10.2 Hz observed between the two protons cannot be explained in terms of the ring A of dammarane triterpenol. This is well supported by the data in Table I [the coupling constant of 10 Hz in methyl 2α, 3β,23-trihydroxyolean-12-en-28-oate (=methyl arjunolate)]. On the other hand, the molecular rotation difference of −38° calculated as the molecular rotation of II minus that of 3-desoxy-panaxadiol (VIII)[2d] agrees fairly well with the rotation difference of −44° between VII and its 2α, 3β-dihydroxy derivative (=methyl crataegolate). In the carbon 13 NMR (^{13}C NMR) spectra of 2α-hydroxypanaxadiol (II) and panaxadiol (I), the carbons at C-20 and -25 resonated at δ_C 72.9 and 76.8 ppm. Referring to the extensive ^{13}C NMR studies by Tanaka and his co-workers[5] on dammarane-type triterpenoids and their glycosides (ginsenosides)[6] isolated from Korean ginseng, we assigned the carbon signals at δ_C 39.4 (t), 28.1 (t), 77.9 (d), and 70.1 (d) ppm to C-1, -2, -3, and -12 of panaxadiol (I), respectively. Taking into consideration



I: R_1=H, R_2=OH
II: R_1=R_2=OH
V: R_1=R_2=CH_3COO
VI: R_1=H, R_2=CH_3COO
VIII: R_1=R_2=H

Chart 1



III: R=β-D-glucopyranosyl
IV: R=α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl

Chart 2

Table I. Coupling Constants (^{1}H NMR) of 3-H with 2-H of Methyl 2,3-Dihydroxyolean-12-en-28-oates and Molecular Rotation Differences at 589 nm between Methyl Olean-12-en-28-oate and Its Derivatives

Compd.	Coupling constant (J in Hz)	Molecular rotation difference ($\Delta[M]_D$)
2α,3α-Dihydroxy	2—3[4a]	−54°[4a]
2α,3β-Dihydroxy	10[4b]	−44°[4c]
2β,3α-Dihydroxy	—	+141°[4d]
2β,3β-Dihydroxy	4—5[4a]	+116°[4e]

The data were taken from reference 4. $\Delta[M]_D$=$[M]_D$ of 2,3-dihydroxy derivative minus $[M]_D$ of methyl olean-12-en-28-oate.[4f]

the substituent effects of a hydroxyl group on α and β carbon signals reported by Djerassi *et al.*,[7] δ_C 48.1 (t), 68.6 (d), 83.5 (d), and 70.0 (d) ppm in the spectrum of 2α-hydroxypanaxadiol (II) can reasonably be assigned to C-1, -2, -3, and -12, respectively.

In the ^{13}C NMR spectra of gynosaponin TN-1 (III) and TN-2 (IV), a pair of double bond carbons was observed at δ_C 125.8 (d) and 130.7 (s) ppm for III and at δ_C 125.8 (d) and 130.9 (s) ppm for IV, and these were assigned to the double bond carbons of dammar-24-ene. Moreover, a singlet near δ_C 83.1 ppm and a doublet at δ_C 98.0 ppm indicated the presence of C-20 bearing the glycosyloxy group and the anomeric carbon atom of the β-D-glucopyranosyl residue linked to the tertiary alcohol at C-20, respectively.[5] We propose the (20*S*) structure for the stereochemistry of C-20 of the gynosaponins, since signals observed at δ_C 51.5 (d), 22.2 (q) and 36.0 (t) ppm of gynosaponin TN-1 (III) are similar to the δ_C values due to C-17, -21, and -22 of ginsenoside F_1, which has (20*S*) configuration.[8] Gynosaponin TN-2 (IV) also showed three similar signals.[9]

Chemical transformation by acids of dammar-24-ene-12β, 20-diols into dammaranes with the tetrahydropyranyl side chain was reported by Shibata and his collaborators.[6a,10]

On the basis of all the above results, we propose the structures (20*S*)-dammar-24-ene-2α, 3β, 12β, 20-tetraol 20-*O*-β-D-glucopyranoside for gynosaponin TN-1 (III), and (20*S*)-dammar-24-ene-2α, 3β, 12β, 20-tetraol 20-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside for gynosaponin TN-2 (IV). These gynosaponins are potentially interesting from the standpoint of biological activity, because their chemical structures resemble those of some ginsenosides from Korean ginseng.

Experimental

All melting points were taken on a Shimadzu micro melting point determination apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-181 automatic polarimeter in a dm tube. NMR spectra were recorded on a JEOL JNM FX-100 spectrometer with tetramethylsilane as the internal standard and pyridine-d_5 as the solvent unless otherwise stated. Chemical shifts are given on the δ scale (ppm) and coupling constants (J values) are expressed in Hz. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. MS were recorded with a JEOL JMS-D 300 machine. IR spectra were obtained with a Shimadzu IR-400 spectrometer. Kiesel gel 60 (230—400 mesh) (Merck) was used as silica gel for column chromatography. Thin-layer chromatography (TLC) was performed on Kiesel gel 60 F_{254} pre-coated plates (Merck) unless otherwise stated, and detection was carried out by spraying 10% H_2SO_4 followed by heating. Analytical samples were dried *in vacuo* overnight at room temperature.

Extraction and Separation——Plant materials were collected in several different places in Japan in May to November. A typical extraction and separation procedure was as follows. Aerial parts of *Gynostemma pentaphyllum* (Amacha-zuru in Japanese) (fresh weight 2.4 kg) collected in Saitama prefecture, Japan, in May, 1980, were cut into small pieces, and dried in air for 10 days. The dried plant material (320 g) was powdered and extracted three times with MeOH for 4 hr each under reflux. The total MeOH solution was concentrated under reduced pressure as far as possible. The dark-green residue was heated with EtOAc (500 ml), and the EtOAc solution was removed by decantation. The insoluble residue (65.5 g) was dissolved in water (400 ml) and washed twice with EtOAc (80 ml each) in a separatory funnel. The aqueous layer was carefully concentrated under reduced pressure in order to evaporate off the EtOAc dissolved in it, and allowed to stand for a week at room temperature. Yellow crystalline matter (1.5 g) precipitated, and was removed by filtration. The filtrate was applied to a column of polyamide (500 g) (Polyamide C-200 from Wako Pure Chemical Industries, Ltd.), and the column was washed with water (4 l). The total eluate was then applied to a column of Amberlite XAD-2 (900 g) and the column was washed with water (4 l).

i) The polyamide column was eluted with MeOH (3 l). The eluate was concentrated *in vacuo*, diluted with 1.5% aq. NaOH (300 ml), and then extracted three times with BuOH (total 550 ml). The combined BuOH layer was washed with water, and concentrated under reduced pressure, affording a wettish residue (3.5 g) which is tentatively called here a "fraction of less polar saponins." The fraction of less polar saponins (3.0 g) was chromatographed on silica gel with $CHCl_3$–EtOH–EtOAc–H_2O (80: 80: 160: 23) (solvent A). Rechromatography of the chromatographic fractions which contained a substance with *Rf* 0.17 on TLC (solvent A), provided gynosaponin TN-2 (72 mg). Colorless prisms from MeOH–$CHCl_3$–EtOAc (1: 1: 4), mp 236—240°, $[\alpha]^{24}_D$ +11.6° (c=1.0, MeOH). *Anal.* Calcd for $C_{42}H_{72}O_{13} \cdot 1/2H_2O$: C, 63.53; H, 9.27. Found: C, 63.84; H, 9.40. IR ν^{KBr}_{max} cm^{-1}: 3370, 1035 (OH). NMR δ_c: 18.6 (two carbons, one of which was ascribable to the methyl carbon (C-6′) of the rhamnosyl residue (R-6′)), 67.1 (t, C-6′ of the glucosyl residue (G-6′)), 69.3

(d, R-5′), 71.4 (d, G-4′), 72.2 (d, R-2′), 72.8 (d, R-3′), 74.2 (d, R-4′), 74.9 (d, G-2′), 76.6 (d, G-5′), 79.2 (d, G-3′), 98.0 (d, G-1′), 101.6 (d, R-1′). See the text for details of some other signals in the ^{13}C NMR spectrum of IV. Repeated chromatography on silica gel (solvent A) of another lot of the fraction of less polar saponins obtained from the plant (dried weight 1.2 kg, collected in October and November in Saitama prefecture) afforded gynosaponin TN-1 (63 mg). Colorless needles from EtOH–H_2O (1: 1), mp 168—173°, $[\alpha]_D^{23}$ +34.5° (c=0.9, MeOH), *Rf* value 0.31 on TLC (solvent A). *Anal.* Calcd for $C_{36}H_{62}O_9 \cdot 3/2H_2O$: C, 64.93; H, 9.84. Found: C, 64.81; H, 9.91. IR ν_{max}^{KBr} cm^{-1}: 3380, 1035 (OH). NMR δ_C: 62.7 (t, G-6′), 71.4 (d, G-4′), 74.8 (d, G-2′), 77.9 (d, G-5′), 79.0 (d, G-3′), 98.0 (d, G-1′). See the text for details of some other signals in the ^{13}C NMR spectrum of III.

ii) The Amberlite XAD-2 column was eluted with MeOH (3 l). The total eluate was concentrated *in vacuo* to afford a brown powder (19.2 g) which is tentatively called here a "fraction of polar saponins."

Hydrolysis of Saponins——i) The fraction of polar saponins (2.0 g) was dissolved in a mixture of 5% aq. H_2SO_4 (100 ml) and MeOH (100 ml), and heated for 4 hr under reflux. The reaction mixture was concentrated *in vacuo* to about a half of its original volume, and extracted with EtOAc. The EtOAc layer was washed with water, and concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$–EtOAc–MeOH (80: 40: 3) (solvent B), and divided into 25 fractions (25 ml per fraction). Rechromatography of fraction No. 5 afforded panaxadiol (I), colorless needles (6.5 mg) from EtOAc, mp 248—251°, which was found to be identical with an authentic sample of I by mixed mp, TLC, IR, and MS. See the text for ^{13}C NMR data of I. Rechromatography of fractions No. 9—12 afforded 2α-hydroxypanaxadiol (II), colorless needles (3.2 mg) from EtOAc, mp 287—289°, $[\alpha]_D^{28}$ −6.0° (c=0.9, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{52}O_4$: C, 75.58; H, 11.00. Found: C, 75.48; H, 10.86. IR ν_{max}^{KBr} cm^{-1}: 3340, 3230 (OH). MS *m/e*: 476 (M^+), 461, 443, 425, 407, 393, 357, 339, 237, 219, 205, 203, 201, 191, 175, 163, 127 (base peak), 123, 121, 119, 109, 81, 69. See the text for ^{13}C NMR data of II.

ii) A mixture of gynosaponin TN-1 (III) (10 mg), EtOH (1.5 ml) and 10% aq. H_2SO_4 (1.5 ml) was heated for 6 hr under reflux. The reaction mixture was concentrated *in vacuo* to a half of the original volume, and extracted with Et_2O. The aqueous layer was approximately neutralized with $Ba(OH)_2 \cdot 8H_2O$. After filtration of white precipitates, the filtrate was passed through a column of Amberlite MB-3, and concentrated to a small volume. Glucose was detected on cellulose TLC (see iii).

The Et_2O extract was washed with water, dried over anhydrous Na_2SO_4, and concentrated to dryness to give a filmy residue. A prominent spot of 2α-hydroxypanaxadiol (II) was detected on TLC (solvent B) and there was no trace of panaxadiol (I).

iii) A mixture of gynosaponin TN-2 (IV) (40 mg), EtOH (5 ml), water (8.5 ml) and 10% aq. H_2SO_4 (1.5 ml) was heated under reflux for an hour. The reaction mixture was concentrated under reduced pressure to about two-thirds of the original volume, and extracted twice with EtOAc (30 ml each) after addition of water (20 ml) to it. The aqueous layer was treated in the same way as in the case of the hydrolysis of III. TLC (Cellulose F_{254} (Merck)) (coloring with aniline–H_3PO_4 after double runs with developing solvent): *Rf* 0.29 (rutinose), 0.39 (glucose), 0.65 (rhamnose) (solvent: BuOH–AcOH–H_2O (6: 1: 2)); *Rf* 0.52 (rutinose), 0.53 (glucose), 0.77 (rhamnose) (solvent: phenol–H_2O (5: 1)).

The extract with EtOAc was washed with water, and concentrated to dryness to give a crystalline powder (21 mg). A mixture of the powder (6 mg) and *p*-toluenesulfonic acid monohydrate (8 mg) in $CHCl_3$ saturated with water was allowed to stand for a day at 45°, then the reaction mixture was chromatographed on silica gel (solvent B), affording 2α-hydroxypanaxadiol (II) (1.5 mg), mp 285—288° (from EtOAc), which was identical with II described in i) above on the basis of mixed mp, TLC and IR.

Diacetate (V) of 2α-Hydroxypanaxadiol (II)——A mixture of II (30 mg), Ac_2O (1.5 ml) and pyridine (3 ml) was allowed to stand overnight at room temperature. After addition of ice and water to it, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 0.1 N aq. HCl and then with water, and dried over anhydrous Na_2SO_4. The product obtained after concentration of the $CHCl_3$ solution was applied to a column of silica gel and eluted with $CHCl_3$, providing the diacetate (V) (25 mg) as an amorphous filmy material. NMR (in $CDCl_3$) δ_H: 0.88 (3H×2, s), 0.90 (3H, s), 0.98 (3H, s), 1.02 (3H, s), 1.18 (3H, s), 1.21 (3H, s), 1.26 (3H, s), 1.97 (3H, s), 2.05 (3H, s), 3.54 (1H, m, 12-H), 4.72 (1H, d, J=10.2, 3-H), 5.12 (1H, d-t, J_1=4.4, J_2=J_3=10.2, 2-H).

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9) *a*) Dammar-24-ene-3β,12β,20-triol 20-glycoside with (20*R*) configuration (=20(*R*)-protopanaxadiol 20-glycoside) has never been found, but the signal at δ_C 43.2 ppm of 20(*R*)-protopanaxadiol in pyridine was assigned to C-22 (see ref. 5*a*). If gynosaponin TN-1 (III) has (20*R*) configuration, its C-22 might resonate at δ_c from 38.2 to 43.7 ppm as a triplet, since the shift value of β-carbons caused by β-D-glucopyranosidation of an alcohol was reported to range from +0.5 to −5 ppm (see ref. 5*b*, 9*b*). We observed no doublet, triplet, or quartet signal at δ_c from 36.1 to 48.1 ppm in its ^{13}C NMR spectrum but only three singlets at δ_c 38.4, 39.8, and 40.0 ppm ascribable to C-10, -4, and -8. This result also supports the (20*S*) stereochemistry proposed for gynosaponins TN-1 (III) and TN-2 (IV); *b*) K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Lett.*, **1977**, 179.

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