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Pseudo-ginsenoside-RP$_1$ and Zingibroside-R$_1$ from Seeds of *Strongylodon macrobotrys* A. GRAY

AKIRA INADA,* HIROKO MURATA, MARI KOBAYASHI, and TSUTOMU NAKANISHI

Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-01, Japan

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From seeds of *Strongylodon macrobotrys* A. GRAY (Leguminosae), two saponins were isolated as their dimethyl esters, and their structures were shown to be identical with the dimethyl esters of pseudo-ginsenoside-RP$_1$ and zingibroside-R$_1$ on the basis of chemical and spectral evidence. This report gives the second example of the natural occurrence of the two saponins, and the first example from Leguminosae. Both dimethyl esters are new compounds and their physical and spectral data are reported here for the first time.

Keywords——*Strongylodon macrobotrys*; Leguminosae; seed; saponin; pseudo-ginsenoside-RP$_1$; zingibroside-R$_1$

Strongylodon macrobotrys A. GRAY (Family: Leguminosae, subfamily: Papilionoideae) is a climber mainly distributed in the Philippine Islands.[1)] This plant is usually called a "Jade Vine" in English, due to the bluish-green color of its flowers, and bears large indehiscent fruits, each of which contains 3–10 large seeds.[1b)] Up to date, no phytochemical study of this plant as well as the other members of the genus *Strongylodon* has appeared. In our search for medicinal plants in Leguminosae, we have studied the chemical constituents of *S. macrobotrys*.

From the seeds of this plant, we isolated two saponins as their dimethyl esters (**1**, mp 182–184°C, $[\alpha]_D^{20}$ +9.0°, and **2**, mp 196–197°C, $[\alpha]_D^{20}$ +11.7°). On complete acid hydrolysis with 10% aq. H_2SO_4–MeOH (1:2), both **1** and **2** gave oleanolic acid methyl ester (**4**) as their aglycone [identified by mixed mp and by comparison of infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR), and thin layer chromatography (TLC)]. As sugar moiety, **1** gave xylose and methyl glucuronate, and **2** gave glucose and methyl glucuronate (identified by paper chromatography). The sugar moieties of **1** and **2** were also identified by an alternative route: i. $NaBH_4$ reduction, ii. methanolysis, iii. trimethylsilylation, and iv. gas liquid chromatography (GLC) analysis (xylose and glucose from **1**, and 2 moles of glucose from **2**). On partial acid hydrolysis with 4% aq. H_2SO_4–MeOH (1:2), both **1** and **2** gave the prosapogenol, 3-O-(6-O-methyl-β-D-glucuronopyranosyl) oleanolic acid methyl ester (**3**).[2)]

On the basis of this chemical evidence and additional spectral information about **1**, **2** and their hydrolysates **3** and **4**, [IR, field desorption mass spectrometry (FD-MS), ^{1}H-NMR, and carbon-13 nuclear magnetic resonance (^{13}C-NMR) (TABLE I)], the structure of the two dimethyl esters were determined as **1** and **2**, which are identical with the dimethyl esters of pseudo-ginsenoside-RP$_1$ (=PG-RP$_1$)[3)] and zingibroside-R$_1$ (=Z-R$_1$),[4)] respectively. PG-RP$_1$ has so far been isolated from *Panax pseudo-ginseng* WALL. subsp. *himalaicus* HARA (Araliaceae) and Z-R$_1$ from *P. zingiberensis* C. Y. WU *et* K. M. FENG.

This report presents the second example of the natural occurrence of these saponins and the first instance of co-occurrence of the two saponins. Many pharmacologically active saponins have been isolated from plants of the genus *Panax* such as *P. ginseng* C. A. MEYER, *P. japonicus* C. A. MEYER, and *P. notoginseng* (BURK.) F. H. CHEN, which are well-known important medicinal plants in the Orient.[5)] Consequently, the isolation of PG-RP$_1$ and Z-R$_1$ from the Leguminosae as well as the genus *Panax* (Araliaceae) is of interest from both chemotaxonomical and pharmacological viewpoints.

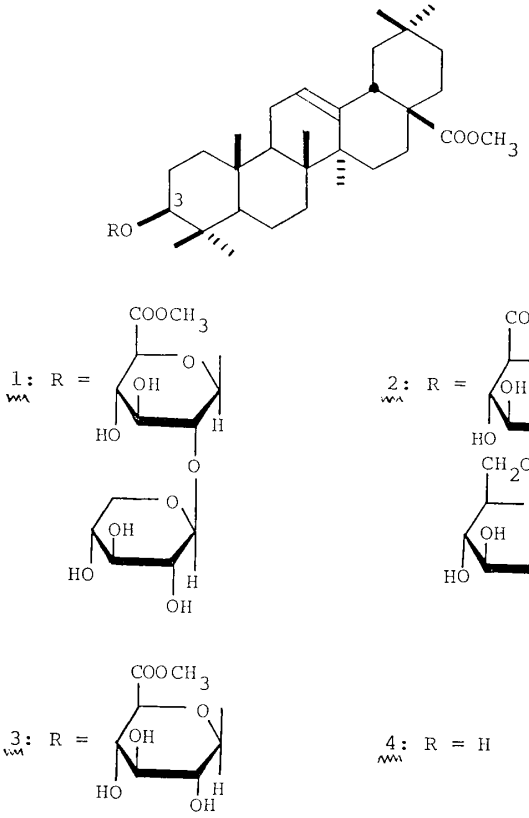

Chart 1.

TABLE I. ^{13}C-NMR Chemical Shifts of **1**, **2**, **3**, and **4** (100.5 MHz, in pyridine-d_5, δ_C)

		4	**3**	**1**	**2**
Aglycone	C-2	28.11	26.61	26.63	26.57
	C-3	78.11	89.14	89.36	89.32
	C-12	122.91	122.86	122.86	122.84
	C-13	144.18	144.16	144.16	144.16
	C-28	177.97	178.00	178.00	178.00
	$COO\underline{C}H_3$	51.58	51.59	51.61	51.61
Methyl glucuronate	C-1		107.33	105.30	105.36
	C-2		75.44	83.34	82.61
	C-3		77.94	77.58[a]	77.54[a]
	C-4		73.21	72.84	72.86
	C-5		77.25	76.81	76.79
	C-6		170.85	170.46	170.48
	$COO\underline{C}H_3$		52.03	52.08	52.08
Xylose	C-1			106.95	
	C-2			76.57	
	C-3			78.22[a]	
	C-4			71.10	
	C-5			67.56	
Glucose	C-1				105.94
	C-2				77.03
	C-3				78.33[a]
	C-4				71.69
	C-5				77.98[a]
	C-6				62.75

[a] Assignments may be reversed in each vertical column.

Furthermore, as a results of these findings, the discovery of possible medicinal uses of *S. macrobotrys* may be expected.

Finally, dimethyl esters **1** and **2** are both new compounds, and their physical data (melting points and optical rotations) and spectral data (IR, FD-MS, ^{1}H- and ^{13}C-NMR) are reported here for the first time.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured with a JASCO A-302 instrument. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100.5 MHz) spectra were measured with a JEOL GX-400 spectrometer in pyridine-d_5 as a solvent and with tetramethylsilane (TMS) as an internal standard. FD-MS spectra were taken with a JEOL DX-300 spectrometer using carbon emitters under the following conditions: accelerating voltage, 3 kV; emitter current, 15–29 mA; chamber temperature, room temperature. Optical rotations were determined in MeOH on a JASCO DIP-140 digital polarimeter. GLC was carried out on a Shimadzu GC-7AG gas chromatograph under the following operating conditions: 1.5% SE-52 on Chromosorb W (AW-DMCS) (2 m×3 mm i.d.); detector, FID; injection temperature, 250°C; column temperature, 180°C; carrier N_2 gas, 30 ml/min. For column chromatography and TLC, Merck Kieselgel 60 (230–400 mesh) and precoated silica gel plates (Merck HF-254) were used, respectively.

Plant material——Mature fruit of *Strongylodon macrobotrys* cultivated in the hothouse of the Botanical Gardens of the University of Tokyo (Faculty of Science, Hakusan, Tokyo, Japan) were harvested in August, 1984.

Isolation of 1 and 2——Twenty-two seeds (797 g) obtained from the fresh fruits were cut and extracted four times with MeOH at room temperature for 10 d and the solvent was evaporated under reduced pressure. The extracts (48 g) were treated with hot MeOH (500 ml) to give a saponin mixture as a precipitate. The saponin mixture (27.0 g) was collected by filtration, and a part (5 g) of it was dissolved in H_2O (300 ml) and treated with Dowex 50 W× 8 (50 g).[6] Evaporation of the solvent gave a residue which was dissolved in MeOH and reacted with a solution of diazomethane in Et_2O. After the usual work-up, the product (5.1 g) was chromatographed on silica gel (400 g), with a lower phase of $CHCl_3$–MeOH–H_2O (7:3:1). Dimethyl esters **1** (1.10 g) and **2** (0.88 g) were eluted in that order and showed the following physical and spectral data; ester **1**: colorless crystals from MeOH–Et_2O, mp 182–184°C, $[\alpha]_D^{20}$ +9.0° (c=0.60). IR ν_{max}^{KBr} cm^{-1}: 1720 (COOMe), 1160, 1070, 1035. FD-MS m/z (%): 793 (M^++H, 81), 792 (M^+, 100), 660 (M^+−132, 22), 471 (33). ^{1}H-NMR δ: 0.82, 0.87, 0.93, 0.94, 1.11, 1.24, 1.30 (3H each, all s. 7×*tert*. Me), 3.28 (1H, dd, J=11.8, 4.3 Hz, H-3α), 3.72 (6H, s, 2×COOMe), 4.99 (1H, d, J=7.5 Hz, anomeric H of 6-O-Me-glucuronoside), 5.29 (1H, d, J=7.2 Hz, anomeric H of xyloside), 5.37 (1H, m, H-12). ^{13}C-NMR: given in TABLE I. *Anal.* Calcd. for $C_{43}H_{68}O_{13}\cdot 2H_2O$: C, 62.37; H, 8.70. Found: C, 62.19; H, 8.87. ester **2**: colorless needles from MeOH, mp 196–197°C, $[\alpha]_D^{20}$ +11.7° (c=0.44). IR ν_{max}^{KBr} cm^{-1}: 1720 (COOMe), 1160, 1070, 1040. FD-MS m/z (%): 823 (M^++H, 83), 822 (M^+, 34), 661 (M^++H−162, 3), 471 (100). ^{1}H-NMR δ: 0.82, 0.85, 0.93, 0.94, 1.12, 1.23, 1.30 (3H each, all s. 7×*tert*. Me), 3.27 (1H, dd, J=11.8, 4.3 Hz, H-3α), 3.72, 3.74 (3H each, all s. 2×COOMe), 4.99 (1H, d, J=7.5 Hz, anomeric H of 6-O-Me-glucuronoside), 5.37 (1H, m, H-12), 5.43 (1H, d, J=7.6 Hz, anomeric H of glucoside). ^{13}C-NMR: given in TABLE I. *Anal.* Calcd for $C_{44}H_{70}O_{14}\cdot 3H_2O$: C, 60.32; H, 8.67. Found C, 60.84; H, 8.26.

Acidic hydrolysis of 1 and 2——Dimethyl ester (**1**) (35 mg) in 10% aq. H_2SO_4–MeOH (1:2) (6 ml) was refluxed for 7 h. The reaction mixture was poured into ice-water and extracted with Et_2O. The Et_2O solution was washed with H_2O, dried over $MgSO_4$, and concentrated to give **4** (22 mg) as colorless needles, which was shown to be identical with authentic oleanolic acid methyl ester by mixed mp and by comparison of IR, ^{1}H-NMR, and TLC. The aqueous layer was neutralized with Amberlite IRA-45 (OH^- form). Evaporation of the solvent gave a residue, which was, after treatment with diazomethane in Et_2O, subjected to paper chromatography (iso-PrOH-*n*-BuOH–H_2O=7:1:2 was used for development and aniline hydrogen phthalate for detection) to demonstrate the presence of xylose and methyl glucuronate. Acidic hydrolysis of **2** in a similar manner demonstrated that **2** is composed of **4**, glucose, and methyl glucuronate.

$NaBH_4$ reduction of 1 and 2 followed by methanolysis——$NaBH_4$ (20 mg) was added to **1** (20 mg) in MeOH (3 ml), and the reaction mixture was stirred overnight. The product was poured into H_2O and extracted with *n*-BuOH. Evaporation of the solvent gave a residue (23 mg), a part (*ca.* 5 mg) of which was dissolved in 5% dry HCl–MeOH (2 ml) and refluxed for 3 h. The reaction mixture was neutralized with Ag_2CO_3, filtered, and evaporated to dryness. The residue was trimethylsilylated with N,O-bis(trimethylsilyl)trifluoroacetamide-pyridine and subjected to GLC to demonstrate the presence of Me xyloside and Me glucoside in a molar ratio of 1:1. When treated in a similar manner as in the case of **1**, **2** gave two moles of Me glucosides.

Partial hydrolysis of 1 and 2——A solution of **1** (200 mg) in 4% aq. H_2SO_4–MeOH (1:2) (60 ml) was refluxed for 4 h. The reaction mixture was poured into H_2O and extracted with *n*-BuOH. Evaporation of the solvent gave a residue which was, after treatment with diazomethane in Et_2O, chromatographed on silica gel. Successive elution with $CHCl_3$, $CHCl_3$–MeOH (20:1), and (4:1) gave **4** (51 mg), 3-O-[6-O-Me-β-D-glucuronopyranosyl]

oleanolic acid methyl ester (**3**) (12 mg), and unreacted **1** (20 mg) in that order. The physical and spectral properties of **3** are as follows; colorless crystals from EtOH, mp 238–241°C [ref. 2a, mp 202–205°C; ref. 2b, mp 239–242°C], $[\alpha]_D^{20}$ +14.6° (c=0.24) [ref. 2a +18.0° (EtOH); ref. 2b, +15.0° (MeOH)]. IR ν_{max}^{KBr} cm^{-1}: 1735 (COOMe), 1165, 1060, 1020. FD-MS m/z (%): 660 (M^+, 100). ^{1}H-NMR δ: 0.82, 0.85, 0.93, 0.94, 1.00, 1.25, 1.33 (3H each, all s. 7×*tert*. Me), 3.38 (1H, dd, J=11.6, 4.6 Hz, H-3α), 3.71 3.75 (3H each, all s, 2×COOMe), 5.02 (1H, d, J=7.6 Hz, anomeric H of 6-O-Me-glucuronoside), 5.38 (1H, m, H-12). ^{13}C-NMR: given in TABLE I. **2** (270 mg) was hydrolyzed in a similar manner as in **1** to give **4** (73 mg), **3** (27 mg), and unreacted **2** (34 mg).

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References and Notes

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