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A SAPONIN CONJUGATED WITH 2,3-DIHYDRO-2,5-DIHYDROXY-6-METHYL-4*H*-PYRAN-4-ONE FROM *VIGNA ANGULARIS*

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Key Word Index—*Vigna angularis*; Leguminosae; adzuki bean; DDMP saponin; triterpenoid.

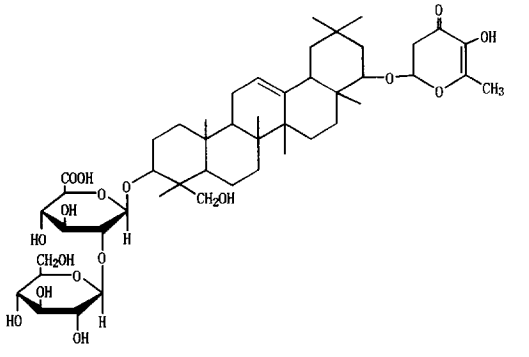
Abstract—A new 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one conjugated saponin, (Az I), was isolated from adzuki beans (*Vigna angularis*). ^{1}H NMR and ^{13}C NMR spectroscopy and chemical techniques showed the structure to be 3-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-(1→)]-22-*O*-[2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one-(2′→)]-3*β*,22*β*,24-trihydroxyolean-12-ene.

INTRODUCTION

Recently, we isolated genuine saponins, soyasaponin αg, βg, βa, γg and γa from soybeans and soyasaponin αa from scarlet runner beans, and demonstrated that these compounds contained 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP), which is attached through an ether linkage to the C-22 hydroxyl group of soyasapogenol B [1,2]. Group B saponins, which are widely distributed in leguminous seeds, might exist as DDMP-conjugated forms, and DDMP-conjugated saponin were also isolated from pea, soybean, alfalfa and hyacinth bean [3–5]. Under harsh experimental conditions or during the isolation process, these fragile molecules might lose the DDMP moiety due to chemical degradation. Therefore, it is necessary to examine the experimental parameters, temperatures and the solvents needed for the isolation of a DDMP saponin. This paper describes a new triterpenoid saponin which is a conjugate of the DDMP moiety from *Vigna angularis* that was obtained under mild experimental conditions.

RESULTS AND DISCUSSION

The result of a screening test for DDMP saponin in 48 kinds of leguminous seeds by the use of a maximum absorbance of 292 nm due to the DDMP moiety revealed some unconfirmed DDMP saponins in an ethanol extract from adzuki beans (*Vigna angularis*). Reversed-phase HPLC analysis of the 70% ethanol extract that contained 0.01% EDTA from adzuki beans gave at least six peaks due to derivatives with a DDMP moiety. One of them (compound **1**) showed a different retention time at 40 min as compared with the known DDMP saponins. The 70% ethanol extract from the whole seed was evaporated and dissolved in water–*n*-butanol (1:1). Compound **1** from the *n*-butanol layer was purified further by Lobar column chromatography. The molecular formula was shown by FAB-mass spectrometry to be $C_{48}H_{74}O_{17}$ (M_r 922). The FAB-mass spectrum (positive-ion mode) gave quasi-molecular ions at *m/z* 923 $[M+H]^+$ 945 $[M+Na]^+$ and 961 $[M+K]^+$. The FAB-mass spectrum (negative-ion mode) gave a quasi-molecular ion at *m/z* 921 $[M-H]^-$, and fragment ions at *m/z* 759 $[M-Glc]^-$ and 583 $[M-Glc-GlcA]^-$. The assignment of the ^{1}H and ^{13}C NMR signals was established by ^{13}C–^{1}H COSY spectra and ^{1}H–^{1}H COSY spectra as compared with the spectral data for soyasaponin βg from soybean. The ^{1}H NMR spectrum of compound **1** indicated the presence of two anomeric protons in the sugar moiety (δ 4.43, 1H, *d*, *J* = 8.0 Hz; 4.60, 1H, *d*, *J* = 7.7 Hz). The C-3 signal (δ 89.7) shifted downfield by δ 11.2 due to the glycosidation shift as in soyasaponin βg [1]. These results suggest that the sugar moiety is linked to the oxygen at C-3 of the



aglycone. TLC analysis of the sugar obtained after hydrolysis indicated the presence of glucose. Compound **1** was indicated to be a 3-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-(1→)]. The ^{13}C NMR spectrum of compound **1** showed six signals at δ_C 185.4, 152.6, 133.0, 96.7, 41.2, 15.1. The ^1H NMR spectrum indicated the presence of one hydroxyl group (δ 7.43, 1H, *br*), one methine group (δ 5.39, 1H, *dd*), one methylene group (δ 2.93, 1H, *dd*; 2.36, 1H, *dd*) and one methyl group (δ 1.90, 3H, *s*). This ^{13}C NMR data suggested the presence of a DDMP moiety in compound **1**. The C-22 signal (δ 81.1) was shifted downfield by δ 7.0 in comparison with that for soyasaponin Bb [1]. The above spectral data identified compound **1** as a 3-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-(1→)]-22-*O*-[2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one-(2′→)]-3*β*,22*β*,24-trihydroxyolean-12-ene. Thus, compound **1** is a new saponin and is assigned the name Az I.

EXPERIMENTAL

Detection and isolation. Saponins were detected by HPLC at a maximum absorbance of 292 nm. HPLC were carried out on a YMC-packed ODS-AM-303 (5 μm, 4.5 × 250 mm) column using EDTA–MeCN–H_2O–HOAc (1:4000:6000:3) mixt. as mobile phase, with a flow rate of 0.9 ml min^{-1}. The whole seed (2 kg) of adzuki beans (*Vigna angularis*) was extracted with 70% EtOH (12 l.), that contained 0.01% EDTA and was filtered with a Buchner funnel. The extract was evapd and was dissolved in H_2O–*n*-BuOH (1:1). The *n*-BuOH layer was evapd. Crude DDMP saponin

Table 1. ^{13}C NMR spectral data for DDMP saponins

	Compound **1**	Soyasaponin *β* g		Compound **1**	Soyasaponin *β* g
Aglycone moiety			3-*O*-*β*-D-Glucuronopyranosyl		
C-1	38.1	38.4	C-1″	103.1	103.9
C-2	25.6	25.8	C-2″	80.0	77.1
C-3	89.7	90.0	C-3″	75.3	74.6
C-4	42.9	43.0	C-4″	71.2	73.6
C-5	55.3	55.1	C-5″	76.6	75.3
C-6	18.0	17.9	C-6″	170.4	171.9
C-7	32.3	32.3			
C-8	39.2	39.2	2″-*O*-*β*-D-Glucopyranosyl		
C-9	46.8	46.8	C-1‴	103.2	
C-10	35.8	35.8	C-2‴	74.2	
C-11	23.2	23.3	C-3‴	76.8	
C-12	121.9	121.8	C-4‴	68.8	
C-13	143.9	143.7	C-5‴	76.5	
C-14	41.2	41.3	C-6‴	60.3	
C-15	25.5	25.5	2″-*O*-*β*-D-Galactopyranosyl		
C-16	26.8	26.8	C-1‴		99.9
C-17	36.4	36.4	C-2‴		75.7
C-18	43.8	43.9	C-3‴		70.6
C-19	45.5	45.5	C-4‴		69.3
C-20	30.0	30.1	C-5‴		74.6
C-21	41.5	41.7	C-6‴		59.8
C-22	81.1	81.0			
C-23	21.8	22.3	2‴-*O*-*α*-L-rhamnopyranosyl		
C-24	62.1	62.4	C-1‴		100.3
C-25	15.1	15.4	C-2‴		70.6
C-26	16.3	16.4	C-3‴		72.4
C-27	25.7	25.5	C-4‴		72.5
C-28	27.3	27.4	C-5‴		68.0
C-29	33.2	33.3	C-6‴		17.9
C-30	20.5	20.5	22-*O*-DDMP*		
			C-2′	96.7	96.6
			C-3′	41.2	41.5
			C-4′	185.4	185.4
			C-5′	152.6	152.5
			C-6′	133.0	132.9
			C-7′	15.1	15.2

* 2,3-Dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one.

was obtained (13.1 g) after freeze-drying. Crude DDMP saponin was loaded onto a Lobar column (25 × 310 mm) using EDTA–MeCN–H_2O–HOAc (1:4000:6000:3) as a mobile phase with a flow rate of 4.0 ml min^{-1}. The eluate was further purified by a Lobar column (10 × 240 mm) and the EDTA was removed by using MeCN–H_2O–HOAc (4000:6000:3) as a mobile phase with a flow rate of 2.0 ml min^{-1} and 40 mg of the new DDMP saponin (Az I) were isolated.

Analyses of sugar. The saponin was dissolved in 1 ml of 2N HCl–50% dioxane and was hydrolysed at 100° for 20 min. After cooling to room temp., the reaction mixt. was evapd to dryness. The hydrolysate was suspended in 1 ml of $CHCl_3$ and extracted with H_2O (1 ml × 2). The aq. layer was evapd until dry and dissolved in 100 μl of 50% MeOH. A solvent system of *n*-PrOH–Me_2CO–H_2O (5:3:1) was used. The spots were made visible by spraying with water satd *n*-BuOH that contained 0.93% aniline and 1.66% phthalic acid and heating at 120° for 10 min. ^1H NMR and ^{13}C NMR spectra were recorded onto a JEOL GSX spectrometer at 300 MHz and 75 MHz, respectively, in DMSO-d_6 with TMS as an int. standard. FAB-mass spectra that use glycerol as a matrix were obtained with a Jeol JMS HX-105.

Soyasaponin βg. UV λ_{max}^{AcCN} nm:292. FAB-mass (+ve) *m/z*: 1069 $[M+H]^+$, 1091 $[M+Na]^+$, 1107 $[M+K]^+$; FAB-mass (−ve) *m/z*: 1067 $[M-H]^-$, 921 $[M-Rha]^-$, 759 $[M-Rha-Gal]^-$. ^1H NMR (DMSO-d_6): 1.90 (3H, *s*, DDMP H-7′), 2.35 (1H, *dd*, J = 14.3 Hz, DDMP H-3b), 2.93 (1H, *dd*, J = 14.3 Hz, DDMP H-3′ a), 4.16 (1H, *dd*, J = 7.6 Hz, GluA H-1), 4.76 (1H, *d*, J = 7.0 Hz, Gal H-1), 4.93 (1 H, *s*, Rha H-1), 5.35 (1H, *dd*, J = 3.3 Hz, DDMP H-2′), 7.45 (1H, *br*, DDMP OH). ^{13}C NMR see Table 1.

Az I. UV λ_{max}^{AcCN} nm:292, $[\alpha]_D^{23}$ −53.0° (pyridine; c = 0.5) FAB-mass (+ve) *m/z*: 923, 945, 961; FAB-mass (−ve) *m/z*: 921, 759, 583. ^1H NMR (DMSO-d_6): 1.90 (3H, *s*, DDMP H-7′), 2.36 (1H, *dd*, J = 13.7 Hz, DDMP H-3′ b), 2.93 (1H, *dd*, J = 12.9 Hz, DDMP H-3′ a), 4.43 (1H, *dd*, J = 8.0 Hz, GluA H-1), 4.60 (1H, *d*, J = 7.7 Hz, Glu H-1), 5.39 (1H, *dd*, J = 3.3 Hz, DDMP H-2′), 7.43 (1H, *br*, DDMP OH). ^{13}C NMR see Table 1 (Az I).

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