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Triterpenoid saponins from *Vigna angularis*

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Abstract

Three new 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one conjugated saponins, named AzII, AzIII and AzIV, were isolated from hypocotyls of adzuki beans (*Vigna angularis*). On the basis of chemical and spectral evidence, the structures of AzII, AzII and AzIV were established as 3-*O*-[*β*-D-glucopyranosyl(1 → 2)-*β*-D-glucuronopyranosyl(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, 3-*O*-[*α*-L-rhamnopyranosyl(1 → 2)-*β*-D-glucuronopyranosyl(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, and 3-*O*-[*β*-D-glucopyranosyl(1 → 2)-*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, respectively.

Keywords: *Vigna angularis*; *Leguminosae*; Adzuki bean; Triterpenoid; DDMP saponin

1. Introduction

Recently, we isolated soyasaponins *α*g, *β*g, *β*a, *γ*g and *γ*a from soybeans (Kudou et al., 1993). These saponins possess a 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) moiety attached to the C-22 hydroxyl group of soyasapogenol B through an ether linkage. DDMP saponins have some physiological activities such as hydrogen peroxide and superoxide scavenging activities, antioxidant activity and metal ion chelating activity (Yoshiki et al., 1995, 1996, 1997; Yoshiki & Okubo, 1995). The DDMP moiety contributes to antiradical activity. However, DDMP saponins convert easily into group B or E saponins due to chemical degradation under high temperature and alkaline conditions, and metal ion contamination during the isolation process. Therefore, it is necessary to isolate the saponin with DDMP moiety under mild experimental conditions. Previously we identified some DDMP saponins from scarlet runner beans, American groundnuts, hyacinth beans and adzuki beans (Yoshiki et al., 1994, Okubo et al., 1994, Iida et al., 1997), these also being widely distributed in leguminous seeds. In this paper, three new triterpenoid saponins isolated from hypocotyls of *Vigna angularis* are described.

2. Results and discussion

By the analysis of reversed-phase HPLC with detection at 292 nm, at least four peaks were obtained in the 70% ethanol extracts from hypocotyls and two from cotyledons of adzuki beans. The retention times of four peaks from hypocotyls were 20 min, 24 min, 33 min and 50 min, respectively. Isolated DDMP saponins showed retention times at 24 min (1), 33 min (2) and 50 min (3). The 70% ethanol extract from hypocotyls was purified using an ODS column. The molecular formulae of *1, 2* and *3* were shown by FAB-mass spectrometry to be $C_{54}H_{82}O_{23}$ (*Mr* 1098), $C_{54}H_{82}O_{22}$ (*Mr* 1082) and $C_{54}H_{84}O_{22}$ (*Mr* 1084), respectively. FAB-

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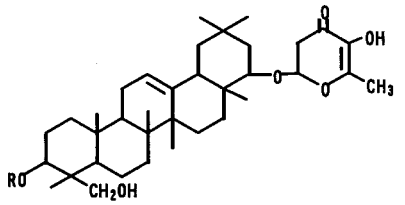
Table 1
^{13}C-NMR spectral data for DDMP saponins

	1	2	3	4			1	2	3	4
C-1	38.1	38.1	38.1	38.4	3-*O*-Glc A	C-1″	104.9	104.4	104.5	103.9
2	25.6	25.6	25.5	25.8		2″	75.1	76.0	79.2	77.1
3	89.7	88.4	89.5	90.0		3″	75.1	74.8	75.9	74.6
4	43.3	43.2	42.9	43.0		4″	71.8	72.4	71.3	73.6
5	55.4	55.3	55.2	55.1		5″	75.1	75.0	75.5	75.3
6	18.3	18.0	17.9	17.9		6″	167.0	167.0	171.0	171.9
7	32.5	32.3	32.2	32.3	2″-*O*-Glc A	C-1‴	99.9	100.1		
8	39.2	39.2	39.2	39.2		2‴	83.7	77.2		
9	46.9	46.8	46.8	46.8		3‴	76.0	76.8		
10	36.0	35.8	35.8	35.8		4‴	75.4	74.8		
11	23.3	23.2	23.2	23.3		5‴	76.2	76.8		
12	121.9	121.8	121.9	121.8		6‴	167.0	167.0		
13	143.7	143.7	143.8	143.7	2″-*O*-Glc	C-1‴			100.8	
14	41.3	41.3	41.2	41.3		2‴			83.8	
15	25.6	25.6	25.5	25.5		3‴			75.0	
16	26.8	26.8	26.7	26.8		4‴			68.7	
17	36.4	36.5	36.4	36.4		5‴			76.4	
18	43.9	43.8	43.8	43.9		6‴			60.1	
19	45.5	45.5	45.5	45.5	2″-*O*-Gal	C-1‴				99.9
20	29.8	29.8	30.0	30.1		2‴				75.7
21	41.5	41.5	41.5	41.7		3‴				70.6
22	81.1	81.1	81.1	81.0		4‴				69.3
23	22.4	22.4	21.9	22.3		5‴				74.6
24	61.8	61.8	62.1	62.4		6‴				59.8
25	15.2	15.2	15.1	15.4	2‴-*O*-Glc	C-1⁗	103.2		103.2	
26	16.4	16.4	16.3	16.4		2⁗	77.4		77.5	
27	25.8	25.8	25.7	25.5		3⁗	71.5		75.0	
28	27.4	27.4	27.3	27.4		4⁗	70.0		69.8	
29	33.3	33.3	33.2	33.3		5⁗	77.4		76.2	
30	20.6	20.6	20.5	20.5		6⁗	61.2		61.0	
22-*O*-DDMP[a]					2‴-*O*-Rha	C-1⁗		100.2		100.3
C-2′	96.7	96.6	96.7	96.6		2⁗		70.6		70.6
3′	41.3	41.3	41.3	41.5		3⁗		71.3		72.4
4′	185.2	185.2	185.4	185.2		4⁗		72.0		72.5
5′	152.5	152.5	152.6	152.5		5⁗		68.1		68.0
6′	133.0	132.8	133.0	132.9		6⁗		18.0		17.9
7′	15.2	15.2	15.1	15.2						

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

mass spectra (positive-ion mode) gave quasi-molecular ions at m/z 1099 $[M+H]^+$ and 1121 $[M+Na]^+$ for 1; m/z 1083 $[M+H]^+$ and 1105 $[M+Na]^+$ for 2; m/z 1085 $[M+H]^+$ and 1107 $[M+Na]^+$ for 3. FAB-mass spectra (negative-ion mode) gave quasi-molecular ions at m/z 1097 $[M-H]^-$, and fragment ions at 935 $[M-Glc]^-$, and 759 $[M-Glc-GlcA]^-$ for 1; m/z 1081 $[M-H]^-$, and fragment ions at 935 $[M-Rha]^-$, and 759 $[M-Rha-GlcA]^-$ for 2; m/z 1083 $[M-H]^-$, and fragment ions at 921 $[M-Glc]^-$ and 759 $[M-Glc-Glc]^-$ for 3. The assignments of ^{1}H and ^{13}C NMR spectral signals were established by ^{13}C–^{1}H COSY spectra and ^{1}H–^{1}H COSY spectra compared with the spectral data of soyasaponin βg (4) from soybeans. The ^{1}H NMR spectrum indicated the presence of three anomeric protons in the sugar moiety at δ 4.32 (1H, *d*, $J=6.8$ Hz), 5.01 (1H, *d*, $J=7.6$ Hz) and 4.42 (1H, *d*, $J=6.3$ Hz) for 1; δ 4.25 (1H, *d*, $J=7.1$ Hz), 4.84 (1H, *d*, $J=6.3$ Hz) and 4.99 (1H, *s*) for 2; δ 4.40 (1H, *d*, $J=6.6$ Hz), 4.84 (1H, *d*, $J=7.7$ Hz) and 4.38 (1H, *d*, $J=7.4$ Hz) for 3. The C-3 signals (δ 89.7, 88.4, 89.5) from its ^{13}C NMR spectrum shifted downfield due to the glycosylation shift as in 4 (Kudou et al., 1993). These results suggested that the sugar moiety was linked to the oxygen at C-3 of the aglycone. TLC analysis of sugars after hydrolysis indicated the presence of glucuronolactone and glucose for 1; glucuronolactone and rhamnose for 2; glucuronolactone and glucose for 3. The absolute configurations of these sugars were chosen in keeping with those mostly encountered among plant glycosides. The inter-glycosidic linkages for the three sugar units were deduced by comparing ^{13}C NMR spectral data with that of soyasaponin αg and βg (4) (Kudou et al., 1993) and by the ^{1}H–^{1}H COSY spectra and ^{13}C–^{1}H COSY spectra. ^{1}H NMR spectra of *1, 2* and *3* indicated the presence of

one hydroxyl group (δ 7.42 or 7.44, 1H, *br*), one methine group (δ 5.37 or 5.39, 1H, *dd*), one methylene group (δ 2.93 or 2.92, 1H, *dd*; 2.34 or 2.35 or 2.36, 1H, *dd*) and one methyl group (δ 1.89 or 1.90, 3H, *s*) derived from DDMP moiety. The ^{13}C NMR spectra of *1, 2* and *3* also showed six signals derived from DDMP moiety (Table 1). These NMR data suggested the presence of DDMP moiety in *1, 2* and *3*. The C-22 signal (δ 81.1) was shifted downfield by δ 7.0 in comparison with that of soyasaponin I (Kudou et al., 1993). The above data suggested that *1, 2* and *3* were new saponins, these being named AzII, AzIII and AzIV.



1:R=GlcA(2→1)-GlcA-(2→1)-Glc
2:R=GlcA(2→1)-GlcA-(2→1)-Rha
3:R=GlcA(2→1)-Glc-(2→1)-Glc

Fig. 1

3. Experimental

Detection and isolation: Adzuki beans separated into hypocotyls and cotyledons were extracted with 70% ethanol which contained 0.01% EDTA. DDMP saponins were detected by HPLC at a maximum absorbance of 292 nm being characteristic of DDMP moiety. HPLC analysis was carried out on an ODS column (ODS-AM-303, 5 μm, 4.5 × 250 mm, YMC Co.) using a EDTA–MeCN–H_2O–HOAc (1:3800:6200:3) mixture as a mobile phase, with a flow rate of 0.9 ml min^{-1}. The dry hypocotyls (46 g) of adzuki beans (*Vigna angularis*) were extracted with 70% EtOH, that contained 0.01% EDTA and filtered with a Buchner funnel. The extract was evaporated and 14 g of Crude DDMP saponin fraction was obtained after freeze drying. Crude DDMP saponin fraction was loaded on to an ODS column (ODS-AM-323, 7 μm, 10 × 250 mm, YMC Co.) using EDTA–MeCN–H_2O–HOAc (1:3700:6300:3) as a mobile phase with a flow rate of 2.0 ml min^{-1} to give fractions containing AzII, AzIII and AzIV, respectively. Each fraction was further purified by an ODS column (Lobar column, 10 × 240 mm, Merck Co.) and EDTA was removed by using MeCN–H_2O–HOAc (3900:6100:3) as a mobile phase with a flow rate of 2.0 ml min^{-1}. The new DDMP saponins, AzII, AzIII and AzIV, were isolated at 300 mg (0.652% in hypocotyl), 55 mg (0.120% in hypocotyl) and 40 mg (0.087% in hypocotyl), respectively.

Analyses of sugar: Saponins were dissolved in 1 ml of 2 N HCl–50% dioxane and were hydrolyzed at 100° for 2 h. After being concentrated to dryness, the hydrolysates were suspended in 1.5 ml of $CHCl_3$ and extracted with H_2O (1 ml × 3). The aqueous layers were subjected to TLC analysis (*n*-PrOH–acetone-H_2O (5:3:1) and $CHCl_3$-MeOH-H_2O (6:4:1), reagent: water-saturated *n*-BuOH that contained 0.93% aniline and 1.66% phthalic acid). ^1H and ^{13}C NMR spectra were recorded on 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-110.

Soyasaponin βg (4).UV λ_{max}^{AcCN} nm: 292, $[\alpha]_D^{17}$−82.6 (90% MeOH; c 0.5) (Tsurumi et al., 1992). 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-3′b), 2.93 (1H, *dd, J*=14.3 Hz, DDMP H-3′a), 4.16 (1H, *d, J*=7.6 Hz, GlcA H-1″), 4.76 (1H, *d, J*=7.0 Hz, Gal H-1‴), 4.93 (1H, *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 (4).

AzII(1). UV λ_{max}^{AcCN} nm: 292, $[\alpha]_D^{23}$−37.4 (MeOH; c 0.43). FAB-mass (+ve) m/z: 1099, 1121; FAB-mass (−ve) m/z: 1097, 935, 759. ^1H NMR (DMSO-d_6): 1.89 (3H, *s*, DDMP H-7′), 2.34 (1H, *dd, J*=15.1 Hz, DDMP H-3′b), 2.93 (1H, *dd, J*=19.5 Hz, DDMP H-3′a), 4.32 (1H, *d, J*=6.8 Hz, GlcA H-1″), 5.01 (1H, *d, J*=7.6 Hz, GlcA H-1‴), 4.42 (1H, *d, J*=6.3 Hz, Glc H-1⁗), 5.37 (1H, *dd, J*=3.3 Hz, DDMP H-2′), 7.42 (1H, *br*, DDMP OH). ^{13}C NMR see Table 1 (1) .

AzIII(2). UV λ_{max}^{AcCN} nm: 292, $[\alpha]_D^{23}$−68.25 (85% MeOH; c 0.4). FAB-mass (+ve) m/z: 1083, 1105; FAB-mass (−ve) m/z: 1081, 935, 759. ^1H NMR (DMSO-*d*6): 1.89 (3H, *s*, DDMP H-7′), 2.35 (1H, *dd, J*=14.3 Hz, DDMP H-3′b), 2.93 (1H, *dd, J*=13.4 Hz, DDMP H-3′a), 4.25 (1H, *d, J*=7.1 Hz, GlcA H-1″), 4.84 (1H, *d, J*=6.3 Hz, GlcA H-1‴), 4.99 (1H, *s*, Rha H-1⁗), 5.37 (1H, *dd, J*=3.3 Hz, DDMP H-2′), 7.42 (1H, *br*, DDMP OH). ^{13}C NMR see Table 1 (2).

AzIV(3). UV λ_{max}^{AcCN} nm: 292, $[\alpha]_D^{23}$−80.75 (77% MeOH; c 0.4). FAB-mass (+ve) m/z: 1085, 1107; FAB-mass (−ve) m/z: 1083, 921, 759. ^1H NMR (DMSO-d_6): 1.90 (3H, *s*, DDMP H-7′), 2.36 (1H, *dd, J*=14.0 Hz, DDMP H-3′b), 2.93 (1H, *dd, J*=16.5 Hz, DDMP H-3′a), 4.40 (1H, *d, J*=6.6 Hz, GlcA H-1″), 4.84 (1H, *d, J*=7.7 Hz, Glc H-1‴), 4.38 (1H, *d, J*=7.4 Hz, Glc H-1⁗), 5.39 (1H, *dd, J*=3.3 Hz, DDMP H-2′), 7.44 (1H, *br*, DDMP OH). ^{13}C NMR see Table 1 (3).Fig. 1

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