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

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Key Word Index—*Dolichos lablab*; Leguminosae; hyacinth bean; DDMP-saponin; triterpenoids; SOD-like activity.

Abstract—A new 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP)-conjugated saponin, lablab saponin I, was isolated from the hypocotyl of hyacinth bean (*Dolichos lablab*). The structure was elucidated by ^{1}H NMR and ^{13}C NMR spectroscopy and chemical techniques as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl(1 → 2)-β-D-glucuronopyranosyl(1 →)]-22-*O*-[2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one(2′ →)]-3β,22β,24-trihydroxyolean-12-en-28-al. SOD (superoxide dismutase)-like activity depended upon the DDMP group and the aldehyde group (C-28) of the aglycone was observed in the order of lablab saponin I > glutathione > soyasaponin βg > maltol. The soybean saponin Bb, lacking the DDMP moiety was found not to exhibit the SOD-like activity.

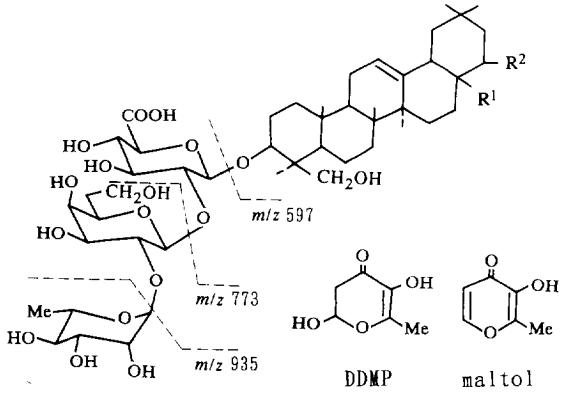
INTRODUCTION

Recently we have isolated genuine saponins, soyasaponin αg, βg, βa, γg and γa from soybean and soyasaponin αa from scarlet runner bean, and showed that the compounds contained an unusual sugar, 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) attached through an acetal linkage to the C-22 hydroxyl group of the soyasapogenol B [1,2]. Therefore group B saponins might be widely distributed in leguminous seeds as DDMP-conjugated forms. A screening test for DDMP-saponin in leguminous seeds (using maximum absorbance at 292 nm due to the DDMP moiety) revealed DDMP-saponins in the ethanol extract of hyacinth bean and azuki bean hypocotyls. This paper describes the structure of a DDMP-saponin which has a new aglycone isolated from hyacinth bean (*Dolichos lablab*) and the superoxide dismutase-like activity of DDMP saponins and related substances.

RESULTS AND DISCUSSION

Reversed-phase HPLC analysis of the 70% ethanol–0.01% EDTA extract from hyacinth bean gave one peak, (R_t 16.7 min) for compound **1**, with maximum absorbance at 292 nm due to the DDMP moiety. The retention time was very different from that of the known DDMP-saponins [2]. Compound **1** was purified further by Lobar column chromatography.

The molecular formula of **1** was found by FAB-mass spectrometry to be $C_{54}H_{82}O_{22}$ (M_r 1082). The FAB-mass spectrum (positive-ion mode) gave quasi-molecular ions at *m/z* 1083 $[M+H]^+$, 1105 $[M+Na]^+$ and 1121 $[M+K]^+$. The FAB-mass spectrum (negative-ion mode) gave a quasi-molecular ion at *m/z* 1081 $[M-H]^-$, and fragment ions at 935 $[M - Rha]^-$, and 773 $[M - Rha - Gal]^-$. MS-MS gave a quasi-molecular ion at



	R1	R2
1 Lablab saponin I	CHO	DDMP
2 Soyasaponin βg	Me	DDMP
3 Soybean saponin Bb	Me	OH

m/z 937 $[M-Rha+2H]^+$, 775 $[M-Rha-Gal+2H]^+$, 599 $[M-Rha-Gal-GlcA+2H]^+$ and 127 [DDMP(2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one)]$^+$ indicating the presence of rhamnose-galactose-glucuronic acid and DDMP. This was confirmed by comparison of the spectral data of **1** with those of soyasaponin *βg* (**2**) from soybean.

The assignment of the ^1H and ^{13}C NMR signals in **1** was established by ^{13}C–^1H COSY spectra and ^1H–^1H COSY spectra. In comparison of the ^{13}C NMR data of **1** with those of *βg* (Table 1), the sugar moiety signals of **1** were almost the same as those of *βg*. The ^1H NMR of **1** and soyasaponin *βg* (**2**) also indicated the presence of three anomeric protons in the sugar, (*δ*4.16 or 4.19, 1H, *d*, $J = 7.6$ Hz; 4.76, 1H, *d*, $J = 7.0$ Hz; 4.93 or 4.94, 1H, *s*). The C-3 signal of **1** (*δ*89.9) was shifted downfield by *δ*11.0 due to the glycosidation shift the same as *βg*. This result suggested that the sugar moiety in **1** linked to the oxygen at C-3 of the aglycone. Compound **1** was indicated to be 3-*O*-[α-L-rhamnopyranosyl(1→2)-*β*-D-galactopyranosyl (1→2)-*β*-D-glucuronopyranosyl(1→)]. The ^{13}C NMR spectra of **1** and **2** showed six signals at *δ*c 185.2 or 184.5, 152.5 or 152.2, 132.9 or 132.7, 96.6 or 95.8, 41.5 or 41.0, 15.2 or 14.8. The ^1H NMR spectrum indicated the presence of one hydroxyl group (*δ*7.45 or 7.40, 1H, *br*), one methine group (*δ*5.35 or 5.31, 1H, *dd*), one unequivalent methylene group (*δ*2.93 or 2.89 1H, *dd*; 2.35 or 2.31 1H, *dd*) and one methyl group (1.90, 3H, *s*). These ^{13}C NMR data suggested the presence of a DDMP moiety in **1** and **2**. The C-22 signal of **1** (*δ*78.4) was shifted downfield by *δ*4.3 in comparison with that of soybean saponin Bb (**3**) [1]. However, this chemical shift was not in agreement with that of **2** (*δ*81.0). Additionally, the chemical shifts of the aglycone moiety of **1** showed a downfield shift (*δ*205.8) due to an aldehyde group. This result suggested that the contradictory chemical shift at C-22 between **1** and **2** was affected by the aldehyde group of a vicinal aglycone moiety. The contradictory chemical shifts were also observed at C-16, 17, 18, 21, 28 and 30 of the aglycone. These were confirmed by comparison of ^{13}C NMR data of 4-methylcyclohexene compared with those of 3-cyclohexanecarboxaldehyde. The α-position signal of the aldehyde group shifted downfield by 17.5 and the *β*-position signal shifted upfield by 8.8 or 10.1 ppm (Fig. 1). This result was in agreement with the chemical shift when an aldehyde group is attached to C-17 of the aglycone. The ^{13}C NMR spectra of three *β*-positions, C-16, C-18 and C-22, showed signals at *δ*21.5, 37.2 and 78.4, and shifted upfield by 5.3, 6.7 and 2.6 ppm, respectively. The α-position, C-17, showed a signal at *δ*51.1 and shifted downfield by 14.7 ppm comparing with those of **2**. The C-21 signal (*δ*78.4) of the aglycone was also shifted upfield by 6.8 ppm. The above spectral data identified **1** as 3-*O*-[α-L-rhamnopyranosyl(1→2)-*β*-D-galactopyranosyl(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-28-al. Thus, compound **1** is a new saponin and is named as lablab saponin I. Although the oleanolic acid(3-hydroxyolean-12-en-28-oic acid) is a typical aglycone of many saponins in plants, the carboxyl group of the aglycone (C-28) might be an aldehyde group like a lablab saponin I.

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

	Compound **1**	Soyasaponin *βg*(**2**)
Aglycone moiety		
C-12	122.6	121.8
C-13	142.1	143.8
C-16	21.5	26.8
C-17	51.1	36.4
C-18	37.2	43.9
C-21	34.9	41.7
C-22	78.4	81.0
C-28	205.3	27.4
C-30	26.5	20.5
3-*O*-*β*-D-Glucuronopyranosyl		
C-1″	103.8	103.9
C-2″	77.1	77.1
C-3″	74.5	74.6
C-4″	73.3	73.6
C-5″	75.3	75.3
C-6″	171.7	171.9
2″-*O*-*β*-D-Galactopyranosyl		
C-1‴	99.9	99.9
C-2‴	75.7	75.7
C-3‴	70.5	70.6
C-4‴	69.2	69.3
C-5‴	74.5	74.6
C-6‴	59.7	59.8
2‴-*O*-α-L-Rhamnopyranosyl		
C-1⁗	100.2	100.3
C-2⁗	70.5	70.6
C-3⁗	72.4	72.4
C-4⁗	72.5	72.5
C-5⁗	67.9	68.0
C-6⁗	17.8	17.9
22-*O*-DDMP*		
C-2′	95.8	96.6
C-3′	41.0	41.5
C-4′	184.5	185.2
C-5′	152.2	152.5
C-6′	132.7	132.9
C-7′	14.8	15.2

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

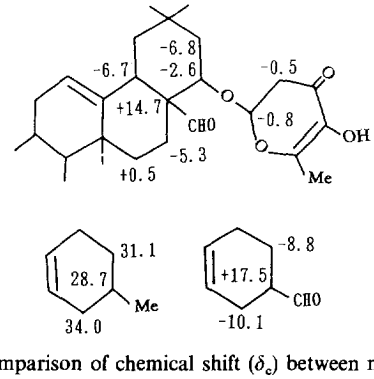


Fig. 1. Comparison of chemical shift (δ_c) between methyl and aldehyde groups.

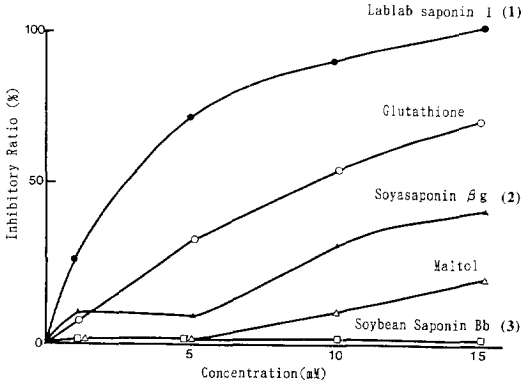


Fig. 2. SOD-like activity of DDMP-saponins and related substances on the hypoxantine-XOD system.

The superoxide dismutase (SOD)-like activity of DDMP-saponins and related substances was investigated (Fig. 2) using XOD–NH_2OH method. The SOD-like activity of the lablab saponin I was greater than that of glutathione. Maltol (a compound related to the DDMP moiety) showed SOD-like activity at over 5 mM concentration. It may be concluded that the SOD-like activity of soyasaponin βg (**2**) was caused by the DDMP moiety, because soybean saponin Bb (**3**) which did not contain a DDMP moiety did not exhibit this activity. The difference in structure between lablab saponin I(**1**) and soyasaponin βg (**2**) was confined to the C-28 moiety of the aglycone, it was an aldehyde group in the former and a methyl group in the latter. The higher SOD-like activity of lablab saponin I suggested the radical reaction occurred not only with the DDMP moiety but that it also involved the vicinal carbon of the aglycone, especially the C-22 grouping. In the case of lablab saponin I, it is hypothesized that the C-28 aldehyde moiety exerted an effect on the C-22 group, thus promoting the radical reaction.

EXPERIMENTAL

Detection and isolation. Saponins were detected by HPLC. HPLC sepns were carried out on a YMC-packed ODS-AM-303 (5 μm, 4.5 × 250 mm) column using EDTA–MeCN–H_2O–HOAc (1:3900:6100:3) mixt. as mobile phase, with a flow rate of 0.9 ml min^{-1}. To isolate the DDMP saponin, the hypocotyls (19.94 g) of hyacinth bean (*Dolichos lablab*) were sepd, extracted with 70% EtOH, containing 0.01% EDTA and centrifuged at 8000 rpm for 15 min. The extract was evapd and dissolved in H_2O–*n*-BuOH (1:1). The *n*-BuOH layer was evapd. The crude DDMP-saponin fr. (1.45 g) was loaded on to a Lobar column (25 × 310 mm) using EDTA–MeCN–H_2O–HOAc (1:3600:6400:3) as mobile phase, flow rate 4 ml min^{-1}. The eluate was further purified by Lobar column (10 × 240 mm) and EDTA was removed using MeCN–H_2O–HOAc (3600:6400:3) as mobile phase. The new DDMP-saponin (**1**) was isolated 145 mg from the crude saponin fr. To isolate the soyasaponin βg (**2**), the hypocotyls of soybean seed were used. The crude DDMP-saponin fr. was isolated according to ref. [2].

^{1}H NMR and ^{13}C NMR spectra were measured at 400 MHz and at 100 MHz, respectively, in DMSO-d_6 with TMS as an int. standard. FAB-mass spectra and MS-MS were measured using glycerol as matrix.

Superoxide dismutase (SOD)-like activity. SOD-like activity of DDMP-saponins and related substance was determined by the XOD–NH_2OH method using hypoxanthing–XOD system and compared with glutathione. Soybean saponin Bb (**3**) was obtained from soyasaponin βg (**2**) by hydrolysis at 100° for 2 hr. The measurements were carried out by absorption spectrophotometry at 550 nm [4]. SOD-like activity was indicated by the inhibitory ratio (100–(ES–EB/EC–EB) × 100, EB, blank, EC, control, ES, sample).

Soyasaponin βg (**2**). UV λ_{max}^{MeOH} nm: 292 FAB-MS(+ ve) *m/z*: 1069 $[M + H]^+$; 1091 $[M + Na]^+$, 1107 $[M + K]^+$; FAB-MS(− ve) *m/z*: 1067 $[M - H]^-$, 921 $[M - Rha]^-$, 759 $[M - Rha - Gal]^-$. ^{1}H 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, *dd*, *J* = 7.6 Hz, GluA 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 (soyasaponin βg).

Lablab saponin I. UV λ_{max}^{MeOH} nm: 292, $[\alpha]_D^{23}$ − 92.2 (MeOH; *c* 0.3). FAB-MS(+ ve) *m/z*: 1083 $[M + H]^+$, 1105 $[M + Na]^+$, 1121 $[M + K]^+$; FAB-MS(− ve) *m/z*: 1081 $[M - H]^-$, 935 $[M - Rha]^-$, 773 $[M - Rha - Gal]^-$. MS-MS *m/z* 937 $[M - Rha + 2H]^+$, 775 $[M - Rha - Gal + 2H]^+$, 599 $[M - Rha - Gal - GlcA + 2H]^+$, 127 [DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one)]$^+$. ^{1}H NMR (DMSO-d_6): δ1.90 (3H, *s*, DDMP H-7′), 2.31 (1H, *dd*, *J* = 14.3 Hz, DDMP H-3′b), 2.89 (1H, *dd*, *J* = 14.3 Hz, DDMP H-3′a), 4.19 (1H, *dd*, *J* = 7.6 Hz, GluA H-1), 4.76 (1H, *d*, *J* = 7.0 Hz, Gal H-1), 4.94 (1H, *s*, Rha H-1), 5.31 (1H, *dd*, *J* = 3.3 Hz, DDMP H-2′), 7.40 (1H, *br*, DDMP OH), 9.21 (1H, *s*, CHO). ^{13}C NMR see Table 1.

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