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A New Depside Glucosides from *Salvia miltiorrhiza* f. *alba*

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A new depside named salvinoside (**1**) was isolated from the water-soluble extract of the dried roots of *Salvia miltiorrhiza* f. *alba* together with rosmarinate glucoside (**2**); lithospermate (**3**); lithospermate B (**4**). The structure of compound **1** was elucidated by spectral analysis. Compound **1** showed moderate antioxidation activity against lipid peroxidation *in vitro* with the inhibition of 18.2 % at 1 μg/mL and 26.3 % at 3 μg/mL.

Key Words: *Salvia miltiorrhiza* f. *alba*, Salvianoside, Hydrophilic constituents.

INTRODUCTION

Salvia miltiotiorrhiza f. *alba*, usually called "Bai Hua Dan Shen" in China, has long been used as a traditional folk medicine for the treatment of coronary disease[1]. Previously diterpene quinones and other costituents have been isolated from the non-aqueous extract of this species[2]. As a part of the continued studies on the bioactive hydrophilic constituents of Chinese medicines, the water-soluble constituents of *Salvia miltiorrhiza* f. *alba* were investigated. Herein, the isolation and structural elucidation of the new deposide glucosides named salvinoside (**1**) together with rosmarinate glucoside (**2**), lithospermate (**3**) and lithospermate B (**4**) is reported, which were isolated from this plant for the first time from the acetone-water extract of the dried roots of *Salvia miltiorrhiza* f. *alba*.

EXPERIMENTAL

The UV spectrum was recorded on a Beckman DU-600 spectrophotomer. IR spectra were measured on a Nicolet Magna 750 spectrophotometer. ^{1}H, ^{13}C NMR, HMQC and HMBC spectrum were recorded on a Brucker AM-400(H) or a Brucker AC-100(C) spectrometer. The spectra was taken in D_2O with TMS as internal standard. ESI-MS were performed on a Finnigan LCQ mass spectrometer. Reversed-phase chromatography column: TSK gel Toyopearl HW-40F (30-60 ím, Toso Co., Ltd.), MCI gel CHP 20P (75-

150 ím, Mitsubishi Chemical Industries Co., Ltd.) and Cosmosil 75 C18-OPN (42-105 ím, Nacalai Tesque Inc.) columns. TLC: precoated kiesel gel 60 F254 plates (0.2 mm, Merck).

The material of *Salvial miltiorrhiza* f. *alba* was collected from Zhangqiu city of Shangdong Province, P.R. China in August 2003 and identified by Prof. Shen-Jin Gui. A voucher specimen (SIMMP03516) has been deposited in the Herbarium of Shanghai Institute of Materia Medica.

Extraction and isolation: The root bark of *Salvia miltiorrhiza* f. *alba* (2 kg) was extracted three times with 60 % aqueous aceton at room temperature (3 × 10 L). The solvent was evaporated under reduced pressure to 1 L and filtered with celite. The filtrate was subjected to a reversed-phase chromatography column, such as TSK gel Toyopearl HW-40F, MCI gel CHP 20P and Cosmosil 75 C_{18}-OPN eluting with MeOH-H_2O as gradient eluent. The 10-30 % aqueous MeOH eluate from the MCI column was repeatedly chromatographed on Comosil 75 C_{18}-OPN (eluted with 20 % MeOH) and the 10-30 % aqueous MeOH eluate from the MCI column was repeatedly chromatographed on Cosmosil 75 C_{18}-OPN (eluted with 20 % MeOH), MCI gel CHP 20P (eluted with 10-30 % MeOH) and Toyopearl HW-40F (H_2O) to give salvinoside (**1**) (15 mg), rosmarinate glucoside (**2**), (113 mg), respectively. The 40-60 % aqueous MeOH eluate from the MCI column was repeatedly chromatographed on Cosmosil 75 C_{18}-OPN (eluted with 30 % MeOH), MCI gel CHP 20P (eluted with 40-60 % MeOH) and Toyopearl HW-40F (H_2O) to give lithospermate (**3**) (25 mg) and lithospermate B (**4**) (12 mg), respectively.

Compounds **1** (10 mg) was heated with cellulose (10 mg) in water (10 mL) at 37 ºC for 48 h. The reaction mixture was extracted with ethyl acetate (20 mL × 2) and the water layers were subjected to TLC analysis by comparision with authentic sugars showing the presence of glucose. TLC condition: Kieselgel 60 F_{254} plate (Merck) [eluent: $CHCl_3$-MeOH-H_2O (14:6:1)], R_f = 0.13 (glucose) and cellulose 60F plate [eluent:*n*-BuOH-pyridine-H_2O (6:4:3)], R_f = 0.37 (glucose).

Bioactivity assay: The bioactivity screenings were carried out *in vitro*. Lipid peroxidation was evaluated by measuring the malodialdehyde (MDA) concentration using the TBA test. Liver homogenate was incubated with variable concentration of compounds for 1.5 h at 37 ºC. Then the sample was mixed with an equal volume of 15 % (w/v) TCA and 0.37 % (w/v) TBA, heated for 15 min in boiling water, cooled with flowing water and centrifuged at 4000 rpm for 25 min at room temperature. The supernatant fraction was extracted and determined at 532 nm spectrophotometrically against a sample containing TEP reagent. The standard curve was plotted and the concentration of TBARS was calculated and expressed as nmol of MDA per mg of liver protein.

RESULTS AND DISCUSSION

Compound **1** was obtained as yellow amorphous powder, displayed a molecular ion at m/z 903.1954 (M + Na, HR ESIMS) corresponding to the formula $C_{42}H_{40}O_{21}$. It appeared a dark green colouration with the ferric chloride reagent suggesting its phenolic characteristic. Its UV maxima at 283 and 310 nm revealed the presence of a highly conjugated system. The IR spectrum showed hydroxyl groups (3415 cm^{-1}), carbonyl (1716 cm^{-1}), double bond (1608 cm^{-1}) aromatic (1510, 1446 cm^{-1}) and a glucosidic linkage (1072 cm^{-1}) absorption bands. The ^{1}H NMR spectrum of compound **1** exhibited a pair of separated olefinic proton with an AX pattern at δ 5.55 (1H, d, *J* = 16.2 Hz) and 6.67 (1H, d, *J* = 16.2 Hz), eleven aromatic proton signals, a pair of doublets at δ 3.72 (1H, d, *J* = 5.4 Hz) and a pair of doublets at δ 3.72 (1H, d, *J* = 5.4 Hz) and δ 5.75 (1H, d, *J* = 5.4 Hz) suggesting the presence of a 2,3-disubstituted dihydrobenzo-furanoid skeleton, supported by the ^{13}C NMR signals at δc = 88.7 (C-α''') and δc = 59.1 (C-β''')[3-6]. The ^{1}H NMR spectrum also showed two separate spin systems with ABX pattern: H-α' (2 H, δ 2.93, m, δ 2.38, dd, *J* = 11.5, 14.1 Hz), H-β' (1 H, δ 4.85, dd, *J* = 2.6, 11.5 Hz), H-α'' (2 H, δ 2.77, m), H-β'' (1H, d 4.84, dd, *J* = 3.2, 9.3 Hz), supported by the DEPT, HMQC and HMBC spectra. Yet, the ^{1}H and ^{13}C NMR spectral data (Table-1) displayed the presence of a glucose unit in **1**, linked in the β-configuration (*J* 1a2a = 7.3 Hz and C-1a at δc = 103.6) to a disubstituted benzene ring[7]. The NMR spectral data of **1** were very similar to those of salvianolic acid B[6,8], except for the glucose. Hence, it was deduced that the aglycon of compound **1** had a same structure as that of salvianolic acid B. The position of the glucosidic linkage was established unambiguously by HMBC experiments (Fig. 1). The anomericproton H-1a correlated with C-4''' (δ 147.33) of the aglycon indicated that C-1a of glucose was linked to the oxygen atom of C-4''' in aglycon. Salvianolic acid B was first isolated from *Salvia miltiorrhiza* by Chen *et al.*[8] and its

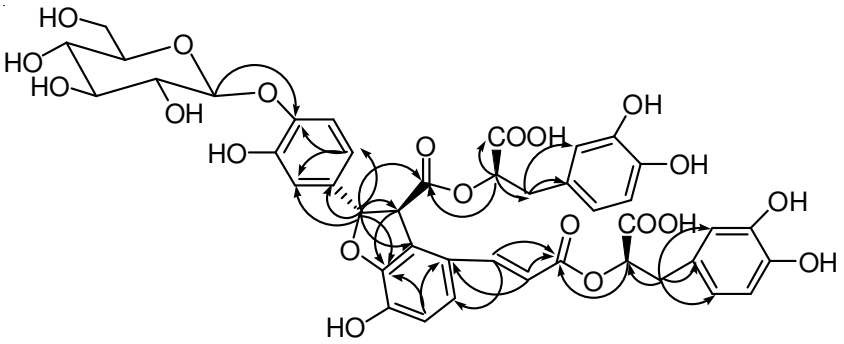

Fig. 1. Selected HMBC correlactions of **1** (H→C)

TABLE-1
^{13}C NMR DATA OF 1 (D_2O, ppm), β-D GLUCOSE AND α-D GLUCOSE

Position	δ^a_C	Position	δ^a_C
COOH	180.1	4'	144.8
	179.9		
COOR	174.5	3"	146.0
	170.6		
α	143.8	4"	144.8
β	118.1	3"'	148.3
α'	39.4	4"'	147.3
β'	80.2	2'	119.0
α"	38.9	5'	118.6
β"	79.1	6'	122.9
α"'	88.7	2"	119.9
β"'	59.1	5"	119.6
5	119.4	6"	124.5
6	119.0	2"'	116.0
2	125.9	5"'	119.0
1	138.0	6"'	120.2
1'	133.0	1[a]	103.6
1"	132.1	2[a]	75.5
1"'	127.1	3[a]	78.6
3	149.5	4[a]	71.9
4	145.3	5[a]	78.0
3'	146.5	6[a]	63.0

[a]100 MHz, $CDCl_3$

plane structure was determined. Later, Lian-Niang Li *et al.*[6] deduced a 2R, 3R configuration and 2β-pseudoequatorial aryl, 3α pseudoaxial carboxyl conformation. The similar spectral data of the aglucone with those of salvianolic acid B revealed a same stereochemical characteristics of the two compounds. Based on the above result and biogenesis consideration, the stereostructure of the aglucone was established to be represented by the Fig. 2.

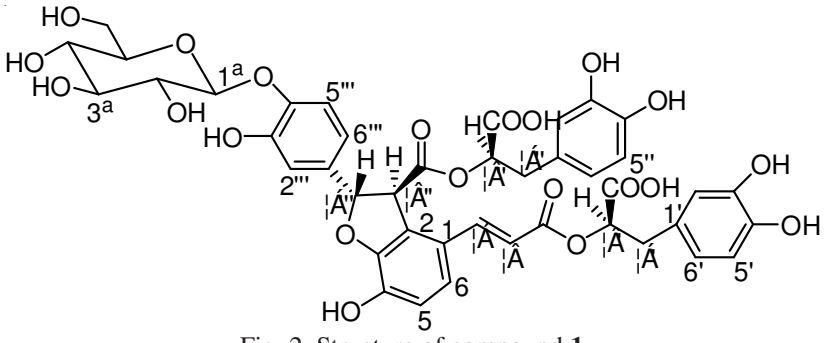

Fig. 2. Structure of compound **1**

Salvinoside (1): A yellow amorphous powder; $C_{42}H_{40}O_{21}$; $[\alpha]_D^{20}$ + 86° (C = 0.44, H_2O); UV (MeOH) λ_{max}: 249, 283 and 310 nm; IR (KBr, ν_{max}, cm^{-1}): 3415, 1716, 1608, 1510, 1446, 1402, 1265, 1182, 1072, 810; ESI MS m/z: 903 $[M+Na]^+$ and 879 $[M-H]^-$, HR ESIMS m/z: 903.1954 $[M+Na]^+$ (calcd. for $C_{42}H_{40}NaO_{21}$ 903.1960); ^1H NMR (D_2O): δ 2.38 (1H, dd, *J* = 2.6, 11.5 Hz, H-α'), 2.77 (2H, m, H-α''), 2.93 (1H, m, H-α'), 3.34-3.50 (4H, m, H-Glc), 3.65 (2H, m, H-6[a]), 3.72 (1H, d, *J* = 5.4 Hz, H-β'''), 4.79 (1H, d, *J* = 7.3 Hz, H-1[a]), 4.84 (1H, dd, *J* = 2.6, 11.5 Hz, H-β''), 4.85 (1H, dd, *J* = 2.6, 11.5 Hz, H-β'), 5.55 (1H, d, *J* = 15.9 Hz, H-β), 5.75 (1H, d, *J* = 5.4 Hz, H-α'''), 5.88 (1H, dd, *J* = 1.6, 8.1 Hz, H-6''), 6.07 (1H, d, *J* = 1.8 Hz, H-2'), 6.24 (1H, d, *J* = 8.1 Hz, H-5'), 6.50-6.53 (3H, m, H-Ar), 6.67 (1H, d, *J* = 16.2 Hz, H-α), 6.73-6.75 (4H, m, H-Ar), 6.82 (1H, d, *J* = 8.5 Hz, H-5); ^{13}C NMR.

Conclusion

A new depside named salvianoside (**1**) was isolated from the water-soluble extract of the dried roots of *S. miltiorrhiza* f. *alba* together with rosmarinate glucoside (**2**); lithospermate (**3**); lithospermate B (**4**). The structures of compound **1** was elucidated by spectral analysis. Compound **1** showed moderate antioxidation avtivity against lipid peroxidation *in vitro* with the inhibition of 18.2 % at 1 μg/mL and 26.3 % at 3 μg/mL.

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