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SCORZONEROSIDES A, B, AND C, NOVEL TRITERPENE OLIGOGLYCOSIDES WITH HEPATOPROTECTIVE EFFECT FROM CHINESE BUPLEURI RADIX, THE ROOTS OF *Bupleurum scorzonerifolium* WILLD.

Masayuki Yoshikawa,* Toshiyuki Murakami, Kazuhiro Hirano, Masahiro Inadzuki, Kiyofumi Ninomiya, and Hisashi Matsuda

Kyoto Pharmaceutical University, 5, Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan

Abstract: Triterpene glycoside adonitol esters, scorzonerosides A, B, and C, were isolated from Chinese Bupleuri Radix, the roots of *Bupleurum scorzonerifolium*. Their absolute stereostructures were elucidated on the physicochemical and chemical evidence, which included the synthesis of the adonitol moiety from D-ribose. Scorzonerosides A, B, and C were found to show hepatoprotective effect on liver injury induced by D-galactosamine and lipopolysaccharide in mice.
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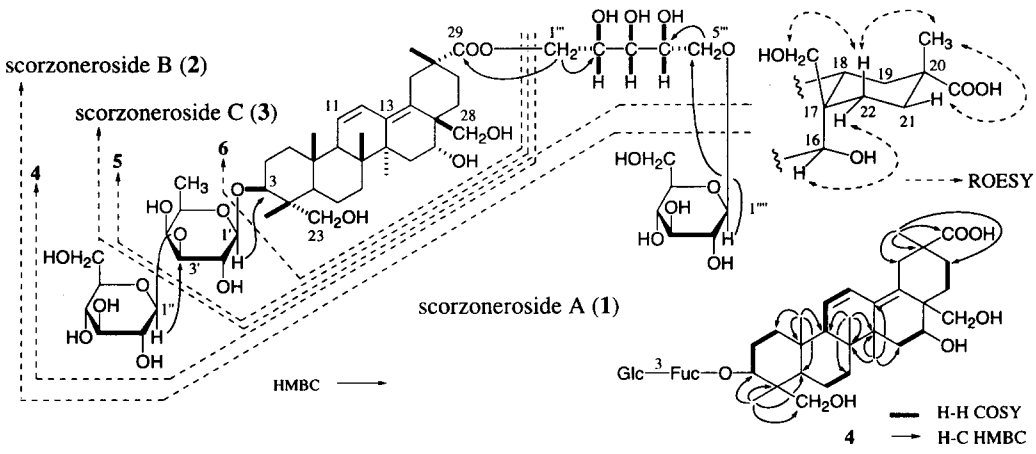
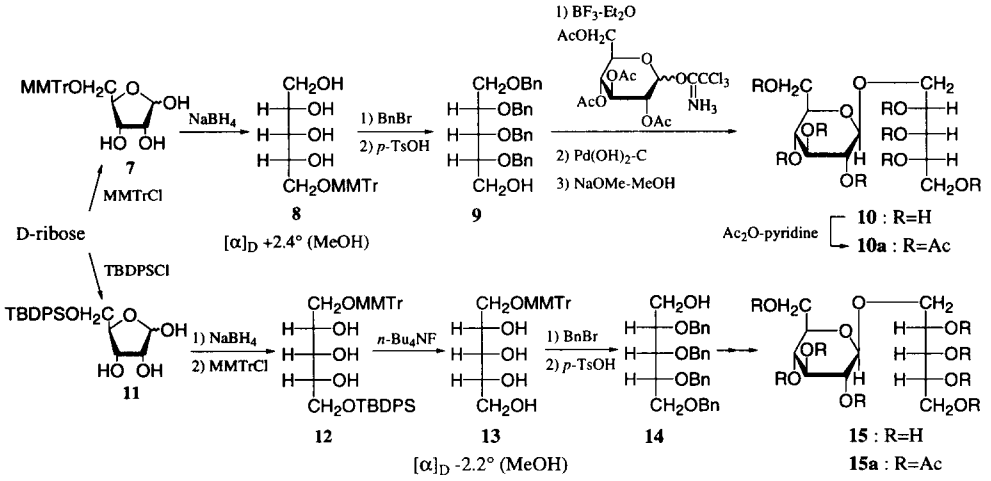
Bupleuri Radix ("柴胡" in Chinese), one of most important natural drugs in Chinese traditional medicine, is prescribed as an anti-inflammatory, antipyretic, and anti-hepatitis agent in many traditional preparations. Previous investigations of this natural medicine led to the isolation of many oleanene-type triterpene monodesmosides[1] and various less polar compounds.[2] In the course of our studies in search of bioactive principles from Chinese natural medicine[3] and medicinal foodstuffs,[4] the glycosidic fraction from a Chinese Bupleuri Radix , the roots of *Bupleurum scorzonerifolium* WILLD. (Umbelliferae), was found to show potent protective effect on liver injury induced by D-galactosamine (D-GalN) and lipopolysaccharide (LPS) in mice. From the active glycosidic fraction, novel triterpene glycosides having an adonitol ester moiety called scorzonerosides A (**1**), B (**2**), and C (**3**) were isolated together with common triterpene monodesmosides saikosaponins and bupleurosides. This communication deals with the structure elucidation of **1**—**3** and their hepatoprotective effects.

The MeOH extract from the roots of *B. scorzonerifolium* was partitioned into an AcOEt-water mixture and the water-soluble portion was extracted with *n*-BuOH. The *n*-BuOH-soluble portion (so-called glycosidic fraction) with hepatoprotective effect was subjected to ordinary- (BW-200, $CHCl_3$-MeOH-H_2O) and reversed-phase (ODS DM1020T, MeOH-H_2O) silica-gel column chromatography and finally HPLC (YMC-Pack D-ODS-5, MeOH-H_2O) to give **1** (0.006% from the natural medicine), **2** (0.004%), and **3** (0.0003%).

Scorzoneroside A (**1**), mp 215—217 °C, $[\alpha]_D^{25}$ -47.3° (*c*=0.1, MeOH), CD (MeOH) : $[\theta]_{230}$ -1500 (neg. max.), $C_{53}H_{83}O_{24}$, showed absorption bands at 3422, 1719, 1655, and 1073 cm^{-1} due to hydroxyl, ester, and olefin functions in the IR spectrum, while its UV spectrum indicated the presence of a hetero-annular diene chromophore by a characteristic triplet with absorption maxima at 243 (log ε 4.30), 252 (4.34), and 260 (4.16) nm. Alkaline hydrolysis of **1** with 5% aq. NaOH liberated prosapogenol **4**[5] and adonitol glucoside (**10**). Acid hydrolysis of **4** with 5% aq. H_2SO_4-dioxane (1 : 1) furnished a new sapogenol bupleurogenin-a (**6**)[6] together with D-fucose and D-glucose, which were identified by GLC analysis of their TMS thiazolidine derivatives.[7] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table I) spectra of **4** showed signals due to the β-D-fucopyranosyl [δ 1.47 (d, *J*=6.4 Hz, 6'-H_3), 5.34 (d, *J*=7.6 Hz, 1'-H)], β-D-glucopyranosyl [δ 5.00 (d, *J*=7.9 Hz, 1''-H)], and bupleurogenin-a moieties [δ 3.72, 4.40 (both m, 23-H_2), 3.84, 4.40 (both m, 28-H_2), 4.31 (dd, *J*=4.9, 12.6 Hz, 3-H), 4.85 (br s, 16-H), 5.74 (d, *J*=10.7 Hz, 12-H), 6.76 (d-like, 11-H)]. The olean-11,13-diene skeleton and the positions of the hydroxyl groups of **4** and **6** were characterized on the basis of ^{1}H-^{1}H COSY and H-C HMBC experimental results as shown in the Chart. Furthermore, in the ROESY experiment of **1**, ROE correlations were observed between the following protons : 16-H





and 22α-H, 21β-H and 30-H_3, 22β-H and 28-H_2, 22β-H and 30-H_3. Finally, by comparison of the ^{1}H-NMR, ^{13}C-NMR, and CD data for **4** and **6** with those for saikosaponin b_2[8] and saikogenin D,[9] the structures of prosapogenol (**4**) and bupleurogenin-a (**6**) were characterized as shown.

Acid hydrolysis of **10** liberated adonitol and D-glucose, while acetylation of **10** with Ac_2O-pyridine furnished the octaacetate (**10a**).[10] In order to elucidate the stereostructure of **10**, we carried out the chemical synthesis of **10** and its isomer **15** from D-ribose and D-glucose. Namely, 5-monomethoxytrityl D-ribose (**7**), which was obtained by tritylation of D-ribose with monomethoxytrityl chloride (MMTrCl)-pyridine, was treated with $NaBH_4$ to give **8** {$[\alpha]_D^{25}$ +2.4° (c=2.0, MeOH)} in 89% yield. Benzylation of **8** followed by detritylation with p-TsOH furnished **9** in 79% yield, which was subjected to glycosidation with O-(2,3,4,6-tetra-O-acetyl-D-glucopyranosyl)trichloroacetimidate in dry CH_2Cl_2 in the presence of BF_3-etherate followed by deprotection and then acetylation to provide **10a** in 52% yield from **9**. On the other hand, 6-t-butyldiphenylsilyl (TBDPS) D-ribose (**11**) was subjected to $NaBH_4$ reduction followed by monomethoxytritylation to yield **12**, which was treated with n-Bu_4NF to give **13** {$[\alpha]_D^{25}$ -2.2° (c=1.2, MeOH)}. By the same procedure to **10** and **10a** from **8**, their isomers **15** and **15a**[11] were synthesized from **13** *via* **14** for comparison of their the physical data with those of **10** and **10a**.

Table I. ^{13}C-NMR Data of Scorzonerosides A (**1**), B (**2**), and C (**3**) and Prosapogenol (**4**)

	1	**2**	**3**	**4**		**1**	**2**	**3**	**4**
C-1	38.4	38.4	38.5	38.5	C-28	65.0	64.8	64.9	65.1
C-2	26.1	26.1	26.0	26.1	C-29	178.6	179.0	179.0	181.2
C-3	81.7	81.7	81.8	81.6	C-30	21.0	21.0	21.1	21.5
C-4	43.6	43.7	43.7	43.7	Fuc-1'	106.5	106.0	106.5	106.0
C-5	47.3	47.3	47.5	47.4	2'	71.5	71.5	72.4	71.6
C-6	18.2	18.2	18.3	18.3	3'	85.2	85.2	75.5	85.7
C-7	32.3	32.2	32.3	32.3	4'	72.1	72.1	73.0	72.2
C-8	41.1	41.1	41.2	41.1	5'	71.0	71.0	71.3	71.0
C-9	54.0	54.0	54.0	54.0	6'	17.2	17.3	17.5	17.2
C-10	36.5	36.5	36.6	36.5	Glc-1"	106.6	106.7		106.7
C-11	126.0	126.0	126.0	126.1	2"	75.8	75.8		75.8
C-12	127.0	126.9	127.0	126.9	3"	78.3	78.4		78.4
C-13	137.6	137.6	137.6	137.4	4"	71.8	71.8		71.8
C-14	42.0	42.0	42.1	42.1	5"	78.7	78.7		78.7
C-15	31.8	31.8	31.9	31.9	6"	62.7	62.7		62.7
C-16	67.6	67.5	67.9	67.8	Ado-1'''	64.9	67.5	67.6	
C-17	45.3	45.3	45.4	45.5	2'''	75.4	72.3	72.4	
C-18	130.6	130.7	130.7	131.3	3'''	72.8	74.1	74.2	
C-19	33.4	33.5	33.6	33.8	4'''	74.1	74.4	74.4	
C-20	44.2	44.2	44.3	44.0	5'''	72.8	64.8	64.9	
C-21	30.8	30.7	30.8	31.2	Glc-1''''	105.3			
C-22	23.9	23.8	23.9	24.2	2''''	75.1			
C-23	64.1	64.0	64.4	64.1	3''''	74.2			
C-24	13.1	13.1	13.1	13.1	4''''	71.8			
C-25	18.9	18.9	18.9	18.9	5''''	78.4			
C-26	17.2	17.3	17.3	17.2	6''''	64.9			
C-27	21.9	21.8	21.8	21.9					

125MHz, pyridine-d_5

The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table I) spectra of **1** indicated the presence of the prosapogenol (**4**) moiety [δ 3.71, 4.37 (both m, 23-H_2), 3.75, 4.27 (both m, 28-H_2), 4.32 (m, 3-H), 4.78 (br s, 16-H), 4.99 (d, *J*=7.9 Hz, 1'-H), 5.34 (d, *J*=7.9 Hz, 1"-H), 5.73 (d, *J*=11.0 Hz, 12-H), 6.65 (dd-like, 11-H)] and β-D-glucopyranosyl adonitol moiety [δ 4.04 (m, 2'''-H), 4.37, 4.85 (both m, 5'''-H_2), 4.50 (m. 4'''-H), 4.76, 5.04 (both dd-like, 1'''-H_2), 4.95 (d, *J*=7.9 Hz, 1''''-H)]. The HMBC experiment of **1** showed long-range correlations between the following protons and carbons : 1"-H and 3'-C, 1'-H and 3-C, 1''''-H and 5'''-C, 5'''-H_2 and 4'''-C, 1'''-H_2 and 29, 2'''-C. The above evidence led us to confirm the total structure of scorzoneroside A (**1**) including the absolute structure of the adonitol glucoside moiety.

Scorzonerosides B (**2**)[12] and C (**3**)[13] furnished **4** and a new prosapogenol **5**, respectively, together with the common adonitol by the alkaline hydrolysis. The structure of prosapogenol **5** was clarified on the basis of the ^{1}H-NMR and ^{13}C-NMR data and the chemical evidence including the acid hydrolysis of **5**. The carbon signals in the ^{13}C-NMR (Table I) spectrum of **2** were found to be superimposable on those of **1**, except for the signals due to the 29-adonitol moiety, whereas the carbon signals in the ^{13}C-NMR (Table I) of **3** were very similar to those of **2**, except for the signals due to the 3-terminal glucopyranosyl moiety of **2**. The H-C HMBC experiments of **2** and **3** showed long-range correlations between the 1'''-methylene protons of the adonitol moiety and the 29-carboxyl carbon. Finally, monotritylation of **2** and **3** followed by alkaline hydrolysis liberated **8**, which was an optical active intermediate in previous adonitol β-D-glucoside (**10**) synthesis. Consequently, the total structures of scorzonerosides B (**2**) and C (**3**) including the absolute stereostructure of the adonitol moiety were clarified as shown.

Protective effects of scorzonerosides (**1**—**3**) on liver injury induced by D-GalN and LPS in mice[14] are summarized in Table II. Since all scorzonerosides (**1**—**3**) were found to exhibit protective effect on the liver injury, these compounds may be related to the traditional effects of this natural medicine.

Table II. Inhibitory Effects of Scorzonerosides A (**1**), B (**2**), and C (**3**) on D-GalN/LPS Induced Liver Injury

	Dose (mg/kg, *i.p.*)	N	s-GPT (K.U.)	s-GOT (K.U.)
control	-	15	6878±879	6349±765
scorzoneroside A (**1**)	10	15	4088±928*	3940±1029
	20	9	2638±375**	2707±412*
scorzoneroside B (**2**)	10	15	3206±655**	3091±622**
scorzoneroside C (**3**)	10	10	1560±509**	1427±446**
normal (saline)	-	10	16±1**	51±4**

Male ddy mice weighing about 25—27 g were used. After 20 h of fasting, a mixture of D-GalN and LPS from *Salmonella enteritidis* was injected intraperitoneally (*i.p.*) at a dose of 350 mg/kg and 10 μg/kg to produce liver injury. Each test sample was administered *i.p.* 1 h before D-GalN/LPS injection. Blood samples were collected 10 h after D-GalN/LPS injection, and serum GPT and GOT levels determined by Reitman and Frankel's method. Each value represents the mean ±S.E. (**p<0.01).

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5. **4** : mp 243—246 °C, $[\alpha]_D^{25}$ -11.9° (c=0.1, MeOH), CD (MeOH) : $[\theta]_{230}$ -2000 (neg. max.), $C_{42}H_{70}O_{14}$, IR (KBr) : 3453, 1702, 1655, 1074 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ : 4.32 (m, 3-H), 4.85 (br s, 16-H), 5.00 (d, J=7.9 Hz, 1'-H), 5.34 (d, J=7.6 Hz, 1''-H), 5.75 (d, J=10.7 Hz, 12-H), 6.76 (d-like, 11-H). Positive-ion FAB-MS : m/z 833 $(M+Na)^+$.
6. **6** : mp 202—205 °C, $[\alpha]_D^{25}$ +19.9 °C (c=0.1, MeOH), $C_{30}H_{46}O_6$, IR (KBr) : 3425, 1702, 1046 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ : 3.72, 4.18 (both m, 28-H_2), 3.82, 4.35 (both m, 23-H_2), 4.24 (dd-like, 3-H), 4.83 (br s, 16-H), 5.76 (d, J=10.6 Hz, 12-H), 6.78 (dd-like, 11-H). Positive-ion FAB-MS : m/z 525 $(M+Na)^+$.
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10. **10a** : $[\alpha]_D^{25}$ -18.0° (c=0.1, MeOH), $C_{27}H_{38}O_{18}$, IR (KBr) 1752, 1225, 1042 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ : 3.61 (dd, J=6.4, 11.3 Hz), 4.02 (dd, J=3.6, 11.3 Hz) (5'''-H_2), 3.68 (ddd, J=2.4, 4.5, 10.0 Hz, 5''''-H), 4.14 (m), 4.25 (dd, J=4.6, 12.2 Hz) (6''''-H_2), 4.15 (m), 4.33 (dd, J=3.0, 12.3 Hz) (1'''-H_2), 4.51 (d, J=7.9 Hz, 1''''-H), 4.97 (dd, J=7.9, 9.5 Hz, 2''''-H), 5.08 (dd-like, 4''''-H), 5.17 (dd-like, 3''''-H), 5.20—5.30 (m, 2''', 3''', 4'''-H). Positive-ion FAB-MS : m/z 651 $(M+H)^+$.
11. **15a** : $[\alpha]_D^{25}$ +2.5° (c=0.1, MeOH), $C_{27}H_{38}O_{18}$, IR (KBr) 1752, 1225, 1042 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ : 3.70 (ddd, J=2.4, 4.5, 10.0 Hz, 5''''-H), 3.80 (dd, J=3.0, 11.6 Hz), 4.02 (dd, J=6.1, 11.6 Hz) (5'''-H_2), 4.13 (m), 4.25 (dd, J=5.2, 12.8 Hz) (6''''-H_2), 4.15 (m), 4.32 (dd, J=2.8, 11.9 Hz) (1'''-H_2), 4.54 (d, J=7.9 Hz, 1''''-H), 4.94 (dd, J=7.9, 10.1 Hz, 2''''-H), 5.08 (dd-like, 4''''-H), 5.19 (dd-like, 3''''-H), 5.20—5.26 (m, 2''', 3''', 4'''-H). Positive-ion FAB-MS : m/z 651 $(M+H)^+$.
12. **2** : mp 226-228 °C, $[\alpha]_D^{25}$ -36.0° (c=0.1, MeOH), $C_{47}H_{76}O_{19}$, UV (MeOH, log ε) : 243 (4.30), 252 (4.34), 260 (4.16) nm. IR (KBr) : 3420, 1707, 1655, 1073 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ : 4.32 (m, 3-H), 4.41, 4.58 (both m, 5'''-H_2), 4.80 (br s, 16-H), 4.96, 5.10 (both dd-like, 1'''-H_2), 5.01 (d, J=7.9 Hz, 1'-H), 5.36 (d, J=7.9 Hz, 1''-H), 5.68 (d, J=10.3 Hz, 12-H), 6.61 (dd-like, 11-H). Positive-ion FAB-MS : m/z 967 $(M+Na)^+$.
13. **3** : mp 208-211 °C, $[\alpha]_D^{25}$ +12.2° (c=0.1, MeOH), $C_{41}H_{66}O_{14}$, UV (MeOH, log ε) : 243 (4.25), 252 (4.29), 260 (4.11) nm. IR (KBr) : 3419, 1706, 1655, 1071 cm^{-1}. ^{1}H-NMR (pyridine-d_5) δ : 4.30 (m, 3-H), 4.40, 4.57 (both m, 5''-H_2), 4.81 (br s, 16-H), 4.96, 4.98 (both dd-like, 1''-H_2), 5.01 (d, J=7.9 Hz, 1'-H), 5.69 (d, J=10.4, 12-H), 6.62 (dd, J=2.8, 10.4 Hz, 11-H). Positive-ion FAB-MS : m/z 805 $(M+Na)^+$.
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