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New Triterpenoid Saponins, Cowpeasaponins I and II, from Cowpea Seeds (*Vigna sinensis* K.)

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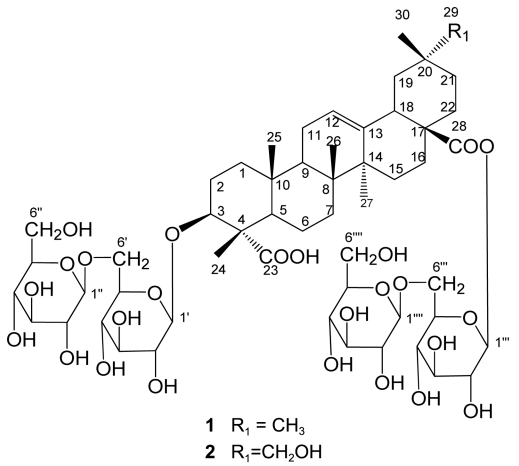
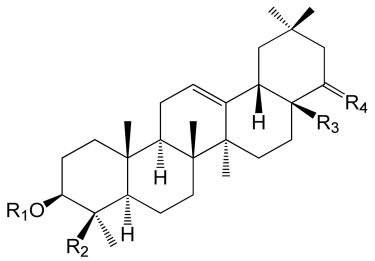
The cowpea (*Vigna sinensis* K., Leguminosae), which is commonly known as the black-eyed pea, southern pea, and crowder pea, is one of the most important legume crops for both human and animal consumption. Cowpea originated in Africa and is now widely grown in Africa, Latin America, Southeast Asia, and the southern United States.[1] Cowpea has therapeutic and protective effects in hypercholesterolemia, cardiovascular diseases, and cancers.[2,3] Steroid glycosides and flavonoid glycosides, which promote heme oxygenase activity and an antioxidant effect have been reported in cowpea seeds.[4-7] In our effort to identify new bioactive compounds from cowpea seeds, we isolated two new triterpenoid saponins, cowpeasaponin I (**1**) and cowpeasaponin II (**2**), along with five known compounds **3**-**7** (Fig. 1). All compounds were isolated from cowpea seeds for the first time. Herein, we describe the isolation and structure elucidation of the new saponins.

Compound **1** was obtained as a pale yellow amorphous powder. The IR spectrum showed the absorbance bands of hydroxyl (3365 cm^{-1}), carbonyl (1739 cm^{-1}), and double bond (1555 cm^{-1}). The molecular formula was determined to be $C_{54}H_{86}O_{25}$ from the $[M-H]^-$ ion peak at *m/z* 1133.5389 (calculated for $C_{54}H_{85}O_{25}$, 1133.5380) in the negative HR-FAB-MS. The spectroscopic features and physicochemical properties suggest **1** to be a triterpenoid saponin. The ^{1}H-NMR spectrum exhibited signals for six tertiary methyls at δ_H 1.01 (H-24), 1.08 (H-27), 0.87 (H-25,29,30), and 0.68 (H-26), a broad singlet for an olefinic proton at δ_H 5.17 (H-12), and an oxygenated methine proton at δ_H 3.64 (H-3). Also, the proton signals due to four sugars, four hemiacetal protons at δ_H 5.24, 4.22, 4.20 and 4.15, along with several oxygenated methine and methylene protons from δ_H 3.99 to δ_H 2.73 were observed. The ^{13}C-NMR spectrum showed signals of a carboxyl (δ_C 179.8, C-23), an ester (δ_C 175.2, C-28), an olefinic quaternary (δ_C 143.5, C-13), an olefinic methine (δ_C 121.6, C-12), an oxygenated methine (δ_C 83.2, C-3), and six methyl carbons (δ_C 32.8, 25.0, 23.5, 16.7, 15.5, 12.0) for the aglycone moiety. These data indicated that **1** is an olean-12-ene-28-oic skeleton. The locations of the carboxyl group at C-23 and the ester at C-28 were determined by the HMBC correlations between δ_H 1.01 (H-24)/δ_C 179.8 (C-23) and δ_H 1.01 (H-24)/δ_C 51.9(C-4), between δ_H 2.75 (H-18)/δ_C 175.2 (C-28) and δ_H 2.75 (H-18)/δ_C 47.3 (C-17). And the double bond position at C-12 was determined by the observation of cross-peaks between δ_H 5.17 (H-12)/δ_C 40.8 (C-18) and δ_H 5.17 (H-12)/δ_C 41.4 (C-14) (Fig. 2). The ^{13}C-NMR signals of four sugars, that is, four hemiacetal carbons at δ_C 103.4, 103.2, 103.1, 94.1, 20 oxygenated methine carbons from δ_C 76.8 to δ_C 69.4, and two oxy-





	R_1	R_2	R_3	R_4	
3	$COCH_3$	H	COOH	H_2	R_4
4	H	H	COOH	H_2	R_4
5	gluA(1→2)-gal(1→2)-rha	CH_2OH	CH_3	O	
6	gluA(1→2)-gal(1→2)-rha	CH_2OH	CH_3	β-OH, α-H	
7	glc	CH_2OH	COO-glc	H_2	

Figure 1. Chemical structures of compounds **1**-**7**. gluA: β-D-glucuronic acid; gal: β-D-galatopyranose; glc: β-D-glucopyranose; rha: α-L-rhamopyranose.

genated methylene carbons at δ_C 68.0, 61.3, 68.5 and 61.2, indicated the presence of two glucopyranosyl (1→6)-glucopyranoside moieties. The coupling constants of four anomer proton signals (J = 8.0, 7.2, 7.6, 7.6 Hz) confirmed all anomeric hydroxyl groups were in a β-configuration. The FAB-MS spectrum showed a quasi-molecular ion at *m/z* 1133 $[M-H]^-$, confirming a molecular weight of 1134. An ion at *m/z* 809 $[M-H-324]^-$ suggested the presence of a disaccharide unit including two hexoses at C-28 of the aglycone, which was confirmed from the chemical shifts of the anomer carbon C-1''' (δ_C 94.1).[8] In addition, the intense ion peaks at *m/z* 647 $[M-H-324-162]^-$ and 485 $[M-H-324-162-162]^-$ also indicated the presence of a disaccharide unit at C-3, which was confirmed by the downfield shift of C-3 owing to the glycosidation effect.[8] So the 3-hydroxy and 28-carbonyl groups possessed the same type of disaccharide chain. Consequently, compound **1** should be a bisdesmosidic saponin in which the disaccharide chain of two glucoses is bound to the aglycone by a glycosidic linkage at C-3, while a disaccharide chain of two glucoses is bound by a glycosidic ester linkage at C-28. The sugar arrangements were determined by the HMBC which showed the correlations between an anomeric proton signal at δ_H 4.15 (glc-1') and an oxygenated methine carbon signal at δ_C 83.2 (C-3), between another anomeric proton at δ_H 5.24 (glc-1''') and the ester at δ_C 175.2 (C-28). Additionally, the cross peak between an anomer proton signal at δ_H 4.20 (glc-1'') and an oxygenated methylene carbon signal at δ_C 68.0 (3-glc-6'), between the other anomer proton signal at δ_H 4.22 (glc-1'''') and the other oxygenated methylene carbon signal at δ_C 68.5 (28-glc-6''') in the HMBC spectrum suggested a 1→6 connection between each of the two sugar units (Fig. 2). This was confirmed by the downfield shifts of C-6' (δ_C 68.0) and C-6''' (δ_C 68.5).[9] On the basis of these data, compound **1** was determined to be a new compound, named cowpeasaponin I, which was established as 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyloleane-12-en-23,28-dioic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Compound **2** was obtained as a pale yellow amorphous powder. The IR spectrum showed the absorbance bands due to hydroxyl (3335 cm^{-1}), carbonyl (1742 cm^{-1}), and double bond (1559 cm^{-1}). The molecular formula was determined to be $C_{54}H_{86}O_{26}$ from the $[M-H]^-$ peak at *m/z* 1149.5326 (calculated for $C_{54}H_{85}O_{26}$, 1149.5329) in the negative HR-FAB-MS. The ^{1}H-NMR and ^{13}C-NMR spectra of **2** were very similar to those of compound **1**, with the exception of an oxygenated methylene signal (δ_H 3.34, δ_C 64.2) instead of a methyl moiety. Compared to C-30 (δ_C 23.5) and C-20 (δ_C 30.3) of compound **1**, the corresponding carbon signals of compound **2** were shifted downfield to δ_C 28.5 and δ_C 35.8, indicating the position of the oxygenated methylene was C-29. It was also confirmed from the cross peaks between H-29 (δ_H 3.34)/C-30 (δ_C 28.5) and those between C-29 (δ_C 64.2)/H-30 (δ_H 0.80) in the HMBC spectrum. On the basis of the above observations, compound **2** was also found to be a new compound, named cowpeasaponin II, which was established as 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl oleane29-hydroxy-12-en-23,28-dioic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

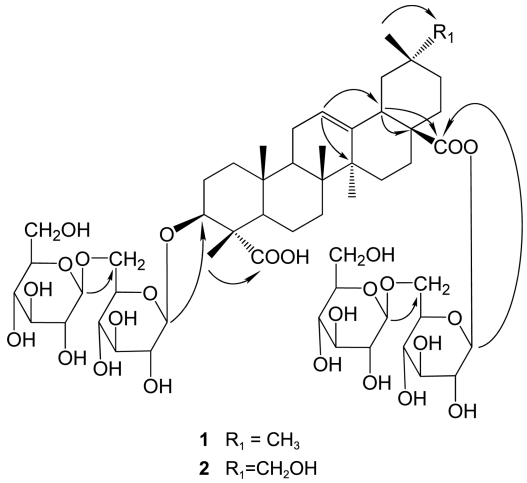


Figure 2. Key HMBC correlations (H→C) of compound **1** and **2**.

Compounds **3-7** were identified as oleanolic acetate (**3**), oleanolic acid (**4**), dehydrosoyasaponin I (**5**), soyasaponin I (**6**), and lucynoside E (**7**), respectively, by comparison of the spectroscopic data with those in the literatures.[10-14] This is the first report on the isolation of these compounds from cowpea seeds.

Experimental

Plant Materials. Cowpea seeds were collected at Yeoncheon-gun, Gyeonggi-do in December, 2008 and identified by Dr. Tae Jung Ha, National Institute of Crop Science, Rural Development Administration, Miryang, Korea. A voucher specimen (KHU081230) is reserved at Natural Products Chemistry Laboratory, Kyung Hee University, Yongin, Korea.

Extraction and Isolation. Cowpea seeds (9 kg) were extracted with 80% MeOH (20 L × 3) three times at room temperature. The concentrated MeOH extract (452 g) were suspended in H_2O (3 L), and then extracted successively with EtOAc (3 L × 3) and *n*-BuOH (2.8 L × 3), and concentrated to afford the residues of EtOAc fraction (18 g, VSE), *n*-BuOH fraction (62 g, VSB), and H_2O fraction (372 g, VSH), respectively.

The EtOAc extracts (18 g) were applied to a SiO_2 (70-230 mesh) column (10 × 16 cm) chromatography (c.c.) and eluted with *n*-hexane-EtOAc (2:1 → 1:1 → 1:3 → $CHCl_3$-MeOH = 15:1 → 10:1 → 7:1 → 5:1 → 3:1) to afford 15 fractions (VSE-1 to VSE-15). VSE-1 (276 mg) was subjected to the SiO_2 c.c. and eluted with *n*-hexane-EtOAc (1:1) to give nine fractions (VSE-1-1 to VSE-1-9) including a purified compound **3** [VSE-1-4, 60 mg, TLC (SiO_2 F_{254}) R_f 0.75, *n*-hexane-EtOAc = 2:1]. VSE-2 (176 mg) was subjected to the SiO_2 c.c. and eluted with *n*-hexane-EtOAc (6:1) to yield 14 fractions. VSE-2-8 (31 mg) was subjected to an ODS c.c. and eluted with MeOH-H_2O (7:1) to give six fractions (VSE-2-1 to VSE-2-6) including a purified compound **4** [VSE-2-8-5, 13 mg, TLC (ODS F_{254S}) R_f 0.20, MeOH-H_2O = 10:1].

The *n*-BuOH extracts (60 g) were chromatographed on SiO_2 (10 × 12 cm) with $CHCl_3$-MeOH-H_2O (20:3:1 → 13:3:1 → 8:3:1 → 6:4:1) to afford 20 fractions (VSB-1 to VSB-20). VSB-17 (1.5 g) was subjected to the SiO_2 c.c. and eluted with $CHCl_3$-MeOH-H_2O (8:3:1) to afford 17 fractions (VSB-17-1 to VSB-17-17). VSB-17-15 (300 mg) was subjected to the ODS c.c. and eluted with MeOH-H_2O (3:2) to yield seven fractions (VSB-17-15-1 to VSB-17-15-7). VSB-17-15-4 (110 mg) was subjected to the ODS c.c. and eluted with MeOH-H_2O (1:1) to give three fractions (VSB-17-15-4-1 to VSB-17-15-4-3) including a purified compound **5** [VSB-17-15-4-3, 12 mg, TLC (ODS F_{254}s) R_f 0.48, MeOH-H_2O = 3:1]. VSB-18 (3.9 g) was subjected to the flash c.c. (SNAP Cartrige KP-Sil 100 g, Biotage®, Uppsala, Sweden) eluting with $CHCl_3$-MeOH (3:1) to produce three fractions (VSB-18-1 to VSB-18-3). VSB-18-3 (2.8 g) was purified by the ODS c.c. eluting with MeOH-H_2O (3:2) to give four fractions (VSB-18-3-1 to VSB-18-3-4) including a purified compound **6** [VSB-18-3-2, 60 mg, TLC (ODS F_{254}s) R_f 0.45, MeOH-H_2O = 3:1]. The H_2O extracts (372 g) were applied to Diaion HP-20 c.c. and eluted with H_2O and MeOH to afford H_2O fraction (VSH-H) and MeOH fraction (VSH-M). VSH-M fraction (17 g) were chromatographed on the SiO_2 (10 × 12 cm) and eluted with $CHCl_3$-MeOH-H_2O (7:3:1 → 65:35:10 → 6:4:1) to provide 18 fractions (VSH-M-1 to VSH-M-18). VSH-M-8 (2.6 g) was subjected to the SiO_2 c.c. and eluted with $CHCl_3$-MeOH-H_2O (7:3:1) to afford seven fractions (VSH-M-8-1 to VSH-M-8-7). VSH-M-8-4 (363 mg) was subjected to the ODS c.c. and eluted with MeOH-H_2O (1:3) to give ten fractions (VSH-M-8-4-1 to VSH-M-8-4-10) including a purified compound **7** [VSH-M-8-4-5, 12 mg, TLC (ODS F_{254}s) R_f 0.65, MeOH-H_2O = 2:1]. VSH-M-12 (1.8 g) was subjected to the SiO_2 c.c. and eluted with $CHCl_3$-MeOH-H_2O (65:35:10) to afford nine fractions (VSH-M-12-1 to VSH-M-12-9). VSH-M-12-6 (857 mg) was purified by the ODS c.c. to give nine fractions (VSH-M-12-6-1 to VSH-M-12-6-9). VSH-M-12-6-9 (224 mg) was subjected to the ODS c.c. and eluted with MeOH-H_2O (1:2) to give nine fractions (VSH-M-12-6-9-1 to VSH-M-12-6-9-9) including a purified compound **1** [VSH-M-12-6-9-5, 37 mg, TLC (ODS F_{254}s) R_f 0.48, MeOH-H_2O = 2:1]. VSH-M-15 (899 mg) was subjected to the ODS c.c. and eluted with MeOH-H_2O (2:3) to afford seven fractions (VSH-M-15-1 to VSH-M-15-7). VSH-M-15-3 (490 mg) was subjected to the ODS c.c. and eluted with MeOH-H_2O (1:2) to afford five fractions (VSH-M-15-3-1 to VSH-M-15-3-5). VSH-M-15-3-2 (321 mg) was subjected to the ODS c.c. and eluted with MeOH-H_2O (1:3) to give three fractions (VSH-M-15-3-2-1 to VSH-M-15-3-2-3) including a purified compound **2** [VSH-M-15-3-2-3, 52 mg, TLC (ODS F_{254}s) R_f 0.20, MeOH-H_2O = 1:1].

Cowpeasaponin I (1): Pale yellow amorphous powder; mp 268-270 °C; $[\alpha]_D$ +4.2° (*c* = 0.07, 20% MeOH); IR (KBr, ν) 3365, 1739, 1555 cm^{-1}; negative HR-FAB/MS *m/z*: 1133.5389 $[M-H]^-$ (calcd for $C_{54}H_{85}O_{25}$, 1133.5380); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.24 (1H, d, *J* = 8.0 Hz, glc-1'''), 5.17 (1H, br. s, H-12), 4.22 (1H, d, *J* = 7.2 Hz, glc-1''''), 4.20 (1H, d, *J* = 7.6 Hz, glc-1''), 4.15 (1H, d, *J* = 7.6 Hz, glc-1'), 3.64 (1H, m, H-3), 1.08 (3H, s, H-27), 1.01 (3H, s, H-24), 0.87 (9H, s, H-25, 29, 30), 0.68 (3H, s, H-26); ^{13}C-NMR (100 MHz, DMSO-d_6): see Table 1.

Table 1. ^{13}C-NMR chemical shifts (100 MHz) of **1** and **2** in DMSO-d_6

NO.	Aglycone		NO.	Sugar	
	1	2		1	2
1	38.2	38.5	C-3		
2	25.5	25.1	Glucose		
3	83.2	84.9	1'	103.2	103.4
4	51.9	50.5	2'	72.4	72.4
5	51.0	52.1	3'	76.6	75.7
6	20.2	20.4	4'	69.4	69.3
7	32.1	32.2	5'	76.7	76.4
8	39.9	41.5	6'	68.0	68.1
9	48.6	47.3	Glucose		
10	35.9	35.8	1"	103.1	103.2
11	23.0	23.0	2"	74.0	73.9
12	121.6	121.7	3"	76.6	76.1
13	143.5	143.4	4"	70.3	70.6
14	41.4	40.1	5"	76.7	76.6
15	27.3	27.1	6"	61.3	61.5
16	23.6	23.0	C-28		
17	47.3	45.9	Glucose		
18	40.8	39.7	1'''	94.1	94.2
19	46.0	40.3	2'''	73.5	73.5
20	30.3	35.8	3'''	76.8	76.7
21	33.3	34.7	4'''	70.0	70.0
22	31.7	28.5	5'''	76.8	77.4
23	179.8	181.4	6'''	68.5	68.5
24	12.0	12.7	Glucose		
25	15.5	15.6	1''''	103.4	103.5
26	16.7	16.8	2''''	73.6	73.6
27	25.0	25.6	3''''	76.7	76.7
28	175.2	175.3	4''''	70.2	70.2
29	32.8	64.2	5''''	76.8	76.8
30	23.5	28.5	6''''	61.2	61.2

Cowpeasaponin II (2): Pale yellow amorphous powder; mp 255-260 °C; $[\alpha]_D$ +19.6° (*c* = 0.04, 20% MeOH); IR (KBr, ν) 3335, 1742, 1559 cm^{-1}; negative HR-FAB/MS *m/z*: 1149.5326 $[M-H]^-$ (calcd for $C_{54}H_{85}O_{26}$, 1149.5329); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 5.24 (1H, d, *J* = 8.0 Hz, glc-1'''), 5.18 (1H, br. s, H-12), 4.20 (1H, d, *J* = 8.0 Hz, glc-1''''), 4.19 (1H, d, *J* = 7.6 Hz, glc-1''), 4.1 (1H, d, *J* = 7.6 Hz, glc-1'), 3.76 (1H, m, H-3), 3.34 (1H, m, H-29), 1.10 (3H, s, H-27), 0.94 (3H, s, H-24), 0.85 (3H, s, H-25), 0.80 (3H, s, H-30), 0.67 (3H, s, H-26); ^{13}C-NMR (100 MHz, DMSO-d_6): see Table 1.

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Supporting Information. The general experimental pro-

cedures and the spectroscopic data of **1** and **2** are available on request from the corresponding author.

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