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Phytochemical Constituents from the Leaves of Soybean [*Glycine max* (L.) Merr.]

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Abstract Phytochemicals study from the leaves of soybean [*Glycine max* (L.) Merr.], one of Korean edible plant materials were investigated through various chromatographic procedures. The methanolic leaves extracts of soybean yielded 16 phytochemicals, including 5 isoflavones **1-5**, 3 flavones **6-8**, 1 flavonol **9**, 2 pterocarpans **10** and **11**, 2 phenolic compounds **12** and **13**, 2 phytosterols **14** and **15**, and 1 sugar alcohol **16**. The structures were fully characterized by analysis of physical and spectral data and were defined clearly as 4,5,7-trihydroxyisoflavone (**1**), 4,5,7-trihydroxyisoflavone-7-*O*-β-D-glucopyranoside (**2**), 4,7-dihydroxy-6-methoxyisoflavone (**3**), 4,7-dihydroxyisoflavone (**4**), 4,7-dihydroxyisoflavone-7-*O*-β-D-glucopyranoside (**5**), 5,7,4'-trihydroxyflavone (**6**), 3',4',5,7-tetrahydroxyflavone (**7**), 3',4',5-trihydroxyflavone-7-*O*-β-D-glucopyranoside (**8**), 3,4',5,7-tetrahydroxyflavonol (**9**), coumestrol (**10**), glyceofuran (**11**), 4-hydroxybenzoic acid (**12**), methyl-4-hydroxybenzoate (**13**), soyasapogenol B (**14**), stigmasterol (**15**), and D-mannitol (**16**), respectively. Among them, phytochemicals **7-16** were reported for the first time on the isolation and confirmation from the leaves of this species. These results suggest that the leaves extracts of soybean may possess possible health related benefits to human due to the isolated phytochemicals **1-16** which have been well known potential effects on various chronic diseases.

Keywords: soybean, leave, phytochemical, isolation, structure elucidation

Introduction

Soybean [*Glycine max* (L.) Merr.] belongs to the Leguminosae family has been used as a food and dietary supplement for a long time because of the importance of its nutritional properties and the functional characteristics (1,2). It is well established that isoflavones found in high concentrations in soybeans have received great attention due to their potential beneficial effects on human health including prevention of breast and prostate cancers (3,4), cardiovascular disease (5), reduced symptoms of diabetes (6), and lower blood pressures (7). Recently, in our previous studies demonstrated that pterocarpans of soybean showed potent low density lipoprotein (LDL) oxidative and human acyl-CoA: cholesterol acyltransferase (hACAT) inhibitory activities (8,9). Among different parts of this species, especially, leaves have been used in Korean folk medicine as the detoxifying agent for snake poison and particularly as food in southern province. Although studies of the phytochemicals and their biological activities in soybean seeds are well documented (10-14), the elucidation of phytochemicals and biologically activities from leaves part have been few studied extensively (15). Therefore, it is necessary to investigate resources of functional ingredients such as dietary fiber, bioactive substances, and dietary supplements from soybean leaves.

In our continuing search for phytochemicals and biological activities of natural plants, we found that 16 phytochemicals were isolated through various chromatographic procedures from the leaves of soybean. It has been well established that isolated phytochemicals **1-16** are beneficial to health owing to reduce risk of cancer and cardiovascular diseases. At first, isoflavone derivatives **1-5** are essential for preventing menopausal symptoms and the incidences of osteoporosis (16,17) as well as lowing of serum cholesterol level (18,19). At second, flavone **6-8** and flavonol **9** have been shown to be highly effective against several diseases involved in oxidative stress (20). At third, our studies have reported that pterocarpan compounds **10** and **11** have a potent LDL oxidation inhibitory activities regarding antiatherosclerosis (8). Moreover, their derivatives are very attractive target for the prevention and treatment of cardiovascular diseases owing to hACAT inhibitory activities (9). At fourth, phenolic compounds **12** and **13** are widely distributed in the plants and food products as secondary metabolic products and nutraceutical importance (21-24), which possess potent antioxidant properties and free radical scavenging capabilities (22,25). At fifth, triterpenoids **14** and **15** and sugar alcohol **16** are widely distributed in natural plants have been reported during the last decades due to their medicinal properties such as cytotoxic activities, anticancer, and antiviral (26). Thus, soybean leaves not only form the basis of the human food

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chain but they are also important sources to improve sources to improve human health and well-being. In this study, The investigation of the chemical constituents in the methanol extracts of soybean leaves to further elucidate various bioactive phytochemicals having beneficial health effects was carried out. All isolated compounds were identified their structures through spectroscopic methods and these structures were 5 isoflavones **1-5**, 3 flavones **6-8**, 1 flavonol **9**, 2 pterocarpans **10** and **11**, 2 phenolic compounds **12** and **13**, 2 phytosterols **14** and **15**, and 1 sugar alcohol **16**, respectively. Although phytochemicals **7-16** were naturally present in many plants (26-32), which were the first report on the isolation and confirmation from the leaves of this species.

Materials and Methods

Plant material The leaves of cv. Taekwang one of Korea soybeans was selected in this study. This cultivar was grown at the experimental field of Yeongnam Agricultural Research Institute, National Institute of Crop Science, Rural Development Administration, Miryang, Korea, in 2005. The leaves were collected at the end of September 2005 and subsequently dried at room temperature for 7 days. The dried leaves were stored at 4°C until they were used.

General apparatus and chemicals All purifications were monitored by thin layer chromatography (TLC) (Merck Co., Darmstadt, Germany), using commercially available glass-backed plates sprayed with phosphomolybdic acid (PMA), *p*-anisaldehyde, and 10% H_2SO_4 solution. Column chromatography was carried out using 230-400 mesh silica gel (Kieselgel 60, Merck). Melting points were measured on a Thomas Scientific capillary melting point apparatus (Electrothermal 9300; Manchester, UK) and are uncorrected. Infrared (IR) spectra were recorded on a Bruker IFS66 (Bruker, Karlsruhe, Germany) IR Fourier transform spectrophotometer (KBr) and ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) along with 2D-NMR data were obtained on a Bruker AM 500 (^{1}H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz) spectrometer (Bruker) in $CDCl_3$, acetone-d_6, DMSO-d_6, CD_3OD, and D_2O. Electron impact mass spectrometry (EIMS) was obtained on a Jeol JMS-700 mass spectrometer (Tokyo, Japan). All reagent-grade chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Extraction and isolation of phytochemicals The leaves (1.5 kg) of soybean (*G. max* cv. Taekwang) were air-dried, chopped, and extracted 2 times with methanol (10 L×2) for 10 days at room temperature and filtered to remove the precipitate. The combined methanol extracts was concentrated *in vacuo* to yield a dark green gum (240 g). The methanol extracts was dissolved in 1.5 L of a mixture of water and methanol (3:1) and successively partitioned with *n*-hexane, EtOAc, and *n*-BuOH (each 3×1.0 L), yielding *n*-hexane (18.1 g), EtOAc (85.4 g), *n*-BuOH (55.9 g), and H_2O extracts (48.6 g). The EtOAc phase was chromatographed on silica gel (9×50 cm, 230-400 mesh, 980 g) using hexane/acetone solvent system under gradient conditions [20:1 (1.5 L), 15:1 (1.5 L), 10:1 (1.5 L), 6:1 (1.5 L), 3:1 (1.5 L), and 1:1 (1.5 L)] and $CHCl_3$/acetone [10:1 (1.2 L), 6:1 (1.2 L), 3:1 (1.2 L), and 1:1 (1.2 L)] mixtures to give 11 fractions (A-J). The fraction C (9.1 g) was subjected to silica gel column (6×50 cm, 230-400 mesh, 280 g) chromatography with *n*-hexane/EtOAc under gradient conditions [18:1 (450 mL), 14:1 (450 mL), 10:1 (450 mL), 7:1 (450 mL), 4:1 (450 mL), and 1:1 (450 mL)] to give 25 subfractions (C1-C25) and subfractions C13 (590 mg) was precipitated with hexane/EtOAc to give compound **15** (250 mg) as a white powder. The subfraction C20 (740 mg) was further purified in small chromatographic column containing silica gel (2.5×50 cm, 230-400 mesh, 100 g), eluting with *n*-hexane/EtOAc (1:1) to afford the pure compound **14** (180 mg). The fraction H (7.4 g) was submitted to a silica gel column (5.5×50 cm, 230-400 mesh, 230 g) chromatography eluted with a *n*-hexane/acetone solvent system (15:11:2) resulting in 56 subfractions (H1-H56). Among them, subfractions H20-H24 (850 mg) was applied to silica gel column (2.5×50 cm, 230-400 mesh, 110 g) chromatography with *n*-hexane/acetone (10:1→1:1), and then purified by a second flash silica gel column (2.5×50 cm, 230-400 mesh, 100 g) using a gradient of *n*-hexane/acetone [8:1 (300 mL), 5:1 (250 mL), 3:1 (250 mL), and 1:1 (250 mL)] to yield compound **11** (45 mg). The subfractions H29-H33 (680 mg) were combined and chromatographed using a stepwise gradient of $CHCl_3$/acetone [12:1 (200 mL), 8:1 (200 mL), 6:1 (200 mL), 4:1 (200 mL), 2:1 (200 mL), and 1:1 (200 mL)], then purified by second flash silica gel column (2.0×50 cm, 230-400 mesh, 100 g) using gradient of $CHCl_3$/acetone (8:1→1:1) to yield compounds **1** (25 mg) and **10** (31 mg). The subfractions H39-H46 (140 mg) were combined on the basis of their comparative TLC profiles and submitted to preparative TLC ($CHCl_3$/acetone 4:1) to give compounds **2** (9.4 mg) and **3** (7.9 mg). The *n*-BuOH phase (55.9 g) was chromatographed over a silica gel column (7.0×50 cm, 230-400 mesh, 780 g) using $CHCl_3$/acetone (15:1→1:2), resulting in the production of 10 fractions (B1-B12). The fraction B4 (1.4 g) was then applied to silica gel column (2.0×50 cm, 230-400 mesh, 160 g) with $CHCl_3$/MeOH (20:1→4:1) to afford 43 subfractions. The subfractions 20-26 (340 mg) was applied to the silica gel column (1.0×60 cm, 230-400 mesh, 60 g) eluted with $CHCl_3$/acetone (2:1) to ultimately produce compound **6** (69 mg). The subfractions 34-40 (230 mg) were subjected to the silica gel column chromatography eluting with $CHCl_3$/EtOH (4:1) to afford crude compound **7** and was further purified by chromatography on silica gel eluting $CHCl_3$/CH_3CN (8:1→4:1) to afford compound **7** (42 mg). The fraction B6 (820 mg) was subjected to Sephadex LH-20 column chromatography using a stepwise procedure, with increasing acetone in MeOH yielded 9 subfractions (B6-1-B6-9). The subfractions B6-3-B6-4 (59 mg) after purification through small column [silica gel, $CHCl_3$/CH_3CN (8:1)] were obtained compound **13** (13 mg). The subfractions B6-8 (125 mg) were subjected to the silica gel column chromatography with $CHCl_3$/MeOH (16:1→2:1) and was subsequently purified by recrystallization ($CHCl_3$/CH_3CN) to yield compound **9** (21 mg). The fraction B8 (2.3 g) was fractionated to 29 subfractions (B8-1-B8-29) by silica gel column chromatography with $CHCl_3$/MeOH as eluents. The subfractions B8-6-B8-10 were subjected to silica gel chromatography using a solvent gradient from

$CHCl_3$/MeOH (15:1) to $CHCl_3$/MeOH (2:1) to obtain compounds **4** (39 mg) and **8** (16 mg). The subfractions B8-13-B8-14 were separately subjected to silica gel column chromatography with the same solvent [$CHCl_3$/MeOH (8:1→1:1)] and further purified through Sephadex LH-20 using methanol to yield compound **5** (52 mg). Also, the subfraction B8-2 was evaporated and submitted to preparative TLC [$CHCl_3$/MeOH (10:1)] to give compound **12** (11.2 mg). The H_2O phase (48.6 g) was filtered, and then washed with MeOH. The washed MeOH extracts was purified by RP-18 open column chromatography and recrystallized from $CHCl_3$/MeOH mixture to afford compound **16** (125 mg).

Results and Discussion

During our search for phytochemicals and their biological activities derived from natural plants, phytochemicals **1-16** were isolated from the leaves of soybean and analyzed by NMR to determine their structures (Fig. 1 and 2). Among them, the structures of phytochemicals **1-5** were confirmed by spectroscopic analysis and comparison with values previously reported (33,34).

Compound 6: Yellow needles; mp 343-345°C; EIMS *m/z* (relative intensity) 270 (M^+); IR (KBr) ν_{max} 3,600, 1,655, 1,600, 1,436/cm; UV λ_{max} 335, 267 nm (MeOH); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 6.19 (1H, d, *J*=2.1 Hz, H-6), 6.48 (1H, d, *J*=2.1 Hz, H-8), 6.76 (1H, s, H-3), 6.93 (2H, d, *J* =8.8 Hz, H-3' and 5'), 7.92 (2H, d, *J*=8.8 Hz, H-2' and 6'), and 12.95 (1H, s, 5-OH). ^{13}C NMR (125 MHz, DMSO-d_6): see Table 1.

Compound 7: Yellowish amorphous powder; mp 317-320°C; EIMS *m/z* (relative intensity) 286 (M^+, 100), 153, (30), 134 (15); IR (KBr) ν_{max} 3,412, 1,645/cm; UV λ_{max} 242, 254 nm (MeOH); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 6.20 (1H, d, *J*=2.1 Hz, H-6), 6.46 (1H, d, *J*=2.1 Hz, H-8), 6.67 (1H, s, H-3), 6.91 (1H, d, *J*=8.2 Hz, H-5'), 7.41 (1H, dd, *J* =2.4 and 3.6 Hz, H-2'), 7.43 (1H, d, *J*=2.3 Hz, H-6'), 9.39 (1H, br, 4'-OH), 9.90 (1H, br, 3'-OH), 10.82 (1H, br, 7-OH), and 12.97 (1H, br, 5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6): see Table 1.

Compound 8: Yellow powder; mp 227-228°C; EIMS *m/z* (relative intensity) 448 (M^+); $[\alpha]_D^{20}$ -24.5 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3,642, 1,640/cm; UV λ_{max} 263, 317 nm (MeOH); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 3.21 (1H, t, *J*=9.1 Hz, H-4"), 3.30 (1H, dd, *J*=13.5, 6.1 Hz, H-2"), 3.35 (1H, d, *J* =8.9 Hz, H-3"), 3.45-3.48 (1H, m, H-5"), 3.52 (1H, dd, *J* =11.6, 5.8 Hz, H-6β"), 3.75 (1H, dd, *J*=11.6, 1.6 Hz, H-

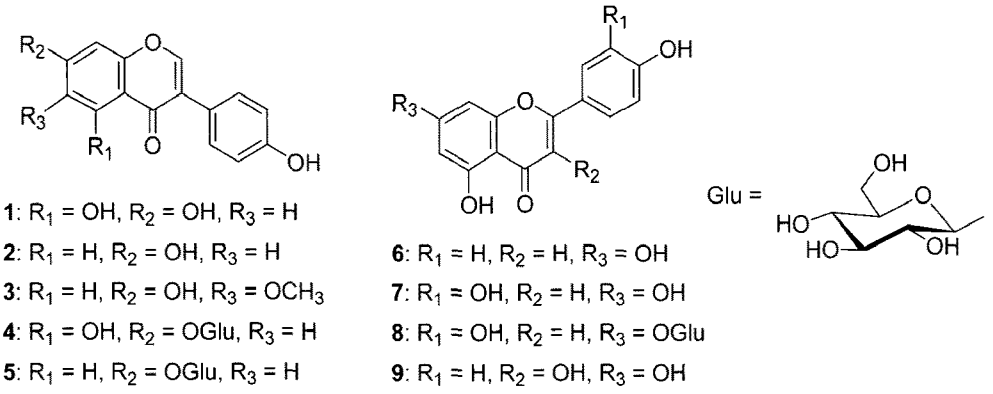

Fig. 1. Structures of isoflavones 1-5, flavones 6-8, and flavonol 9 from soybean leaves.

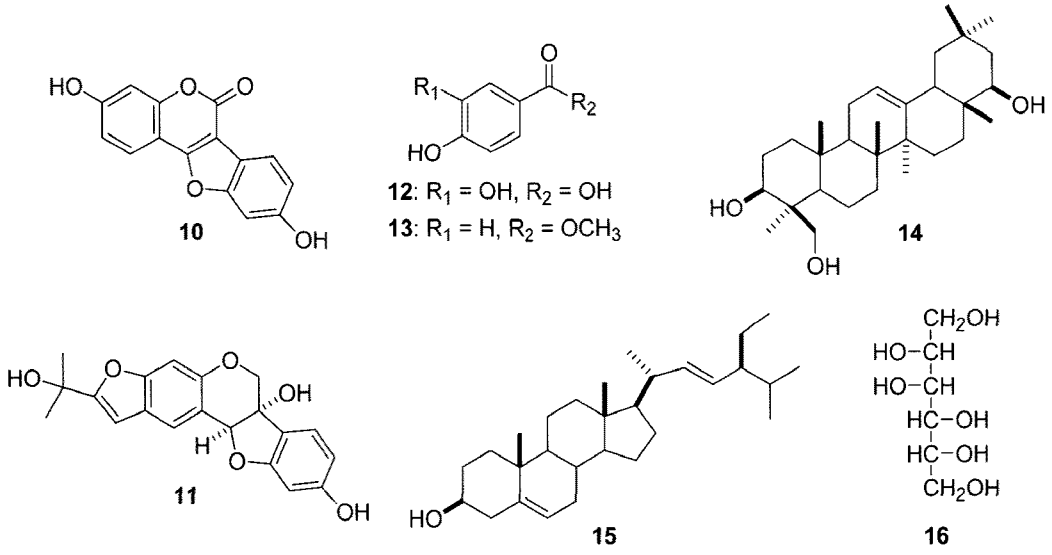

Fig. 2. Structures of pterocarpans 10 and 11, phenolic compounds 12 and 13, phytosterols 14 and 15, and sugar alcohol 16 from soybean leaves.

Table 1. ^{13}C-NMR of compounds 6-9 at 125 MHz (ppm, m)[1)]

Position	Compound			
	6	7	8	9
1				
2	164.1 (s)[2)]	164.3 (s)	164.9 (s)	147.2 (s)
3	103.2 (d)	103.2 (d)	103.6 (d)	136.0 (s)
4	182.1 (s)	182.0 (s)	182.2 (s)	176.3 (s)
5	161.5 (s)	161.9 (s)	161.5 (s)	161.1 (s)
6	99.2 (d)	99.2 (d)	100.0 (d)	98.6 (d)
7	164.5 (s)	164.5 (s)	163.4 (s)	164.3 (s)
8	94.3 (d)	94.2 (d)	95.2 (d)	93.9 (d)
9	157.7 (s)	157.7 (s)	157.3 (s)	156.6 (s)
10	104.1 (s)	104.1 (s)	105.8 (s)	103.4 (s)
1'	121.6 (s)	121.9 (s)	121.8 (s)	122.1 (s)
2'	128.8 (d)	113.8 (d)	114.0 (d)	129.9 (s)
3'	116.3 (d)	146.1 (s)	146.2 (s)	115.8 (d)
4'	161.8 (s)	150.1 (s)	150.3 (s)	159.6 (s)
5'	116.3 (d)	116.4 (d)	116.4 (d)	115.8 (d)
6'	128.8 (d)	119.3 (d)	119.5 (d)	129.9 (s)
1"			100.4 (d)	
2"			73.6 (d)	
3"			76.9 (d)	
4"			70.1 (d)	
5"			77.6 (d)	
6"			61.1 (t)	

[1)]The chemical shifts of compounds **6-9** were determined in DMSO-d_6.
[2)]Multiplicity was established from DEPT data.

6α"), 5.09 (1H, d, *J*=7.4 Hz, H-1"), 6.46 (1H, d, *J*=2.1 Hz, H-6), 6.73 (1H, s, H-3), 6.79 (1H, d, *J*=2.1 Hz, H-8), 6.92 (1H, d, *J*=8.3 Hz, H-5'), 7.44 (1H, dd, *J*=5.8, 2.3 Hz, H-2'), 7.45 (1H, d, *J*=2.3 Hz, H-6'), and 12.96 (5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6): see Table 1.

Compound 9: Yellow powder; mp 228-230°C; EIMS *m/z* (relative intensity) 286 (M^+, 100); IR (KBr) ν_{max} 3,370, 1,661, 1,614, 1,570, 1,510/cm; UV λ_{max} 322, 294, 266 nm (MeOH); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 6.19 (1H, d, *J* =2.0 Hz, H-6), 6.44 (1H, d, *J*=2.0 Hz, H-8), 6.92 (2H, dd, *J*=6.9, 2.0 Hz, H-3' and 5'), 8.05 (2H, dd, *J*=6.9, 2.0 Hz, H-2' and 6'), 9.35 (3-OH), 10.08 (4'-OH), 10.76 (7-OH), and 12.47 (5-OH). ^{13}C-NMR (125 MHz, DMSO-d_6): see Table 1.

Compound 10: Yellow needles; mp 356-359°C; EIMS *m/z* (relative intensity) 268 (M^+, 100); IR (KBr) ν_{max} 3,500, 2,820, 1,710/cm; UV λ_{max} 378, 310, 280, 210 nm (MeOH); ^{1}H-NMR (500 MHz, DMSO-d_6) δ 6.91 (1H, d, *J*=2.2 Hz, H-4), 6.93 (1H, dd, *J*=6.4, 1.2 Hz, H-2), 6.95 (1H, dd, *J*= 7.1, 2.0 Hz, H-8), 7.16 (1H, d, *J*=2.0 Hz, H-10), 7.69 (1H, d, *J*=8.4 Hz, H-7), 7.84 (1H, d, *J*=8.6 Hz, H-1), 10.0 (9-OH), and 10.7 (3-OH). ^{13}C-NMR (125 MHz, DMSO-d_6): see Table 2.

Compound 11: White needles; mp 181-183°C; EIMS *m/z* (relative intensity) 354 (M^+, 28); IR (KBr) ν_{max} 3,414, 1,660, 1,555/cm; UV λ_{max} 306, 293, 287 nm (EtOH); ^{1}H-NMR (500 MHz, acetone-d_6) δ 1.60 (6H, s, H-15 and 16), 4.15 (1H, d, *J*=11.3 Hz, H-6a), 4.19 (1H, d, *J*=11.3 Hz, H-6b), 4.39 (14-OH), 5.02 (6a-OH), 5.48 (1H, s, H-11a), 6.26 (1H, d, *J*=2.1 Hz, H-10), 6.46 (1H, dd, *J*=8.2, 2.1 Hz, H-8), 6.65 (1H, d, *J*=0.9 Hz, H-12), 6.96 (1H, d, *J*=0.4 Hz, H-4), 7.26 (1H, d, *J*=8.2 Hz, H-7), 7.68 (1H, s, H-1), and 8.49 (9-OH). ^{13}C-NMR (125 MHz, acetone-d_6): see Table 2.

Compound 12: Slightly yellow powder; mp 255-257°C; EIMS *m/z* 154; IR (KBr) ν_{max} 3,340, 1,655/cm; UV λ_{max} nm 338, 272 (MeOH); ^{1}H-NMR (500 MHz, CD_3OD) δ 6.89 (1H, d, *J*=11.0 Hz, H-5), and 7.53 (2H, m, H-2 and H-6). ^{13}C-NMR (125 MHz, CD_3OD): see Table 2.

Compound 13: Colorless needle; mp 80-83°C; EIMS *m/z* 168; IR (KBr) ν_{max} 3,312, 3,034, 1,918, 1,679, 1,588/cm; UV λ_{max} nm 206, 255 ($CHCl_3$); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.92 (3H, s, 7-OCH_3), 6.91 (2H, d, *J*=8.7 Hz, H-3 and H-5), 7.98 (2H, d, *J*=8.7 Hz, H-2 and H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz): see Table 2.

Compound 14: White needles; mp 258-259°C; $[\alpha]_D^{20}$ +92.4 (*c* 1.0, CH_3OH); EIMS *m/z* 458; IR (KBr) ν_{max} 3,440, 2,250, 1,360/cm; ^{1}H-NMR (500 MHz, CD_3OD) δ 0.74 (3H, s, H-28), 0.79 (1H, dd, *J*=1.8, 12.3 Hz, H-6), 0.81 (3H, s, H-29), 0.85 (1H, m, H-19α), 0.86 (3H, s, H-25), 0.88 (3H, s, H-26), 0.90 (1H, m, H-1α), 0.91 (3H, s, H-30), 0.95 (1H, m, H-2α), 1.03 (3H, s, H-27), 1.10 (3H, s, H-23), 1.21 (1H, m, H-15α), 1.24 (1H, m, H-21α), 1.29 (2H, m, H-6), 1.33 (1H, m, H-21β), 1.35 (1H, m, H-7α), 1.46 (1H, m, H-7β), 1.51 (1H, m, H-9), 1.56 (1H, m, H-1β), 1.58 (1H, m, H-16α), 1.62 (1H, m, H-16β), 1.63 (1H, m, H-2β), 1.64 (1H, m, H-15β), 1.65 (1H, m, H-19β), 1.80 (2H, m, H-11), 1.96 (1H, d, *J*=12.8 Hz, H-18), 3.25 (1H, dd, *J*=3.9, 12.0 Hz, H-3), 3.28 (1H, d, *J*=11.1 Hz, H-24α), 3.28 (1H, m, H-22), 4.03 (1H, d, *J*=11.1 Hz, H-24β), and 5.16 (1H, t, *J*=3.6 Hz, H-12). ^{13}C-NMR (125 MHz, CD_3OD): see Table 2.

Compound 15: White powder; mp 163-165°C; EIMS *m/z* 412; IR (KBr) ν_{max} 3,450, 2,950, 1,655/cm; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.73 (3H, s, H-18), 0.83 (3H, d, *J*=6.4 Hz, H-26), 0.85 (3H, d, *J*=7.4 Hz, H-29), 0.88 (3H, d, *J*=6.4 Hz, H-27), 0.96 (1H, m, H-12α), 0.97 (1H, m, H-11α), 1.03 (1H, m, H-1α), 1.04 (3H, d, *J*=2.4 Hz, H-21), 1.06 (3H, s, H-19), 1.08 (1H, m, H-17), 1.11 (1H, m, H-28α), 1.19 (1H, m, H-24), 1.21 (1H, m, H-14), 1.20 (1H, m, H-16b), 1.28 (1H, m, H-9), 1.45 (1H, m, H-28β), 1.46 (2H, m, H-15),1.49 (1H, d, *J*=5.3 Hz, H-8), 1.52 (1H, m, H-2α), 1.54 (1H, m, H-20), 1.58 (1H, m, H-25), 1.73 (1H, m, H-16α), 1.75 (1H, m, H-12β), 1.88 (1H, 1β), 1.89 (1H, m, H-11β), 1.98 (1H, m, H-4α), 2.00 (1, m, H-2β), 2.09 (1H, m, H-4β), 2.25-2.31 (2H, m, H-7), 3.51-3.57 (1H, m, H-3), 5.05 (1H, dd, *J*=15.2, 8.6 Hz, H-23), 5.18 (1H, dd, *J*=15.2, 8.6 Hz, H-22), and 5.37 (1H, dd, *J*=3.1, 2.2 Hz, H-6). ^{13}C-NMR (125 MHz, $CDCl_3$): see Table 2.

Compound 16: White powder, ^{1}H-NMR (500 MHz, D_2O) δ 3.66 (2H, dd, *J*=5.6, 11.4 Hz, H-1a and H-6a), 3.70-3.75 (2H, m, H-3 and H-4), 3.79 (2H, m, H-2 and H-5), and 3.85 (2H, dd, *J*=2.4, 11.5 Hz, H-1b and H-6b). ^{13}C-NMR (125 MHz, D_2O): see Table 2.

Structural elucidation of isolated phytochemicals

Compound **6** was obtained as yellow needles and the IR spectrum showed absorption bands for hydroxyl at 3,600/cm and carbonyl at 1,655/cm. The characteristics of compound **6** was essentially the same as those for compound **9** except for the ^{1}H- [**6**: δ 6.76 (1H, s, H-3)] and

Table 2. ^{13}C-NMR of compounds 10-15 at 125 MHz (ppm, m)[1)]

Position	Compound						
	10	11	12	13	14	15	16
1	123.1 (d)[2)]	124.3 (d)	121.7 (s)	122.3 (s)	40.2 (t)	37.7 (t)	63.3 (s)
2	114.1 (d)	125.1 (s)	116.4 (d)	132.0 (d)	27.3 (t)	32.1 (t)	71.0 (s)
3	161.6 (s)	156.9 (s)	144.6 (s)	115.3 (d)	81.7 (d)	72.2 (d)	69.4 (s)
4	103.4 (d)	100.2 (d)	150.1 (s)	160.3 (s)	43.8 (s)	42.7 (t)	69.4 (s)
5			114.5 (d)	115.3 (d)	57.7 (d)	141.2 (s)	71.0 (s)
6	158.0 (s)	71.8 (t)	122.8 (d)	132.0 (d)	20.2 (t)	122.1 (d)	63.3 (s)
7/OCH_3	121.0 (d)	125.7 (d)	169.2 (s)	167.4 (s)/52.1 (q)	35.0 (t)	32.32 (d)	
8	114.4 (d)	109.5 (d)			41.2 (s)	32.31 (d)	
9	156.3 (s)	161.3 (s)			49.6 (d)	50.6 (d)	
10	99.1 (d)	99.0 (d)			39.0 (s)	36.9 (s)	
11					25.2 (t)	21.49 (t)	
12		100.9 (d)			124.0 (d)	40.1 (t)	
13		165.4 (s)			145.7 (s)	42.6 (s)	
14		69.8 (s)			44.0 (s)	57.3 (d)	
15		29.9 (q)			30.3 (t)	24.77 (t)	
16		29.9 (q)			28.8 (t)	29.3 (t)	
1a	104.6 (s)						
4a	155.0 (d)	154.2 (s)					
6a	102.4 (s)	77.7 (s)					
6b	115.0 (s)	121.3 (s)					
10a	157.4 (s)	162.4 (s)					
11a	159.8 (s)	87.3 (d)					
11b		118.5 (s)					
17					38.3 (t)	56.4 (d)	
18					47.9 (d)	12.6 (q)	
19					47.9 (t)	19.8 (q)	
20					32.0 (s)	40.9 (d)	
21					42.7 (t)	21.46 (q)	
22					77.4 (d)	138.7 (d)	
23					23.6 (q)	129.7 (d)	
24					65.7 (t)	51.7 (d)	
25					17.0 (q)	32.27 (d)	
26					18.0 (q)	21.62 (q)	
27					26.0 (q)	19.4 (q)	
28					20.8 (q)	25.8 (t)	
29					33.0 (q)	12.5 (q)	
30					29.5 (q)		

[1)]The chemical shifts of compound **10** was determined in DMSO-d_6, compound **11** was measured in acetone-d_6, compounds **12** and **14** were measured in CD_3OD, compounds **13** and **15** were measured in $CDCl_3$, and compound **16** was measured in D_2O.
[2)]Multiplicity was established from DEPT data.

^{13}C-NMR spectrums [**6**: δ 103.2 (C-3, d) and **9**: δ 136.0 (C-3, s)]. These results suggested that compound **6** are 5,7,4'-trihydroxyflavone (35). Compound **7** was obtained as yellowish amorphous powder and IR spectrum showed absorption bands at 3,412 and 1,645/cm, which indicated the presence of hydroxyl and carbonyl groups. The ^{13}C-NMR spectrum with DEPT experiments showed the presences of 15 carbons as 6 methins [δ 99.2 (C-6), 94.2 (C-8), 103.2 (C-3), 113.8 (C-2'), 116.4 (C-5'), and 119.3 (C-6')], 1 carbonyl 182.0 (C-4) and 8 quaternary carbons [δ 104.1 (C-10), 121.9 (C-1), 146.1 (C-3), 150.1 (C-4), 161.9 (C-5), 157.7 (C-9), 164.3 (C-2), and 164.5 (C-7)], respectively. The ^{1}H-NMR data showed the evidence for 6 aromatic protons [δ 6.20 (1H, H-6), 6.46 (1H, H-8), 6.91 (1H, H-5), 7.41 (1H, H-2), 7.43 (1H, H-6), and 6.67 (1H, H-3)]. Namely, the ^{1}H-NMR spectra of compound **7** showed 2 *meta*-coupled doublets ascribable to H-8 and H-6 of A-ring in the flavonoid skeleton, and a *meta*-coupled doublet-doublet, an *ortho*, *meta*-coupled doublet and an *ortho*-coupled doublet attributable to H-2', H-6', and H-5' of B-ring, respectively. The HMBC spectrum showed correlations of H-3 with C-10, C-1', C-2, and C-4, and H-6 with C-8, C-10, and C-7, and H-8 with C-6, C-7, C-9, and C-10, and H-2'/6' with C-2 (Fig. 3). Thus, on the basis

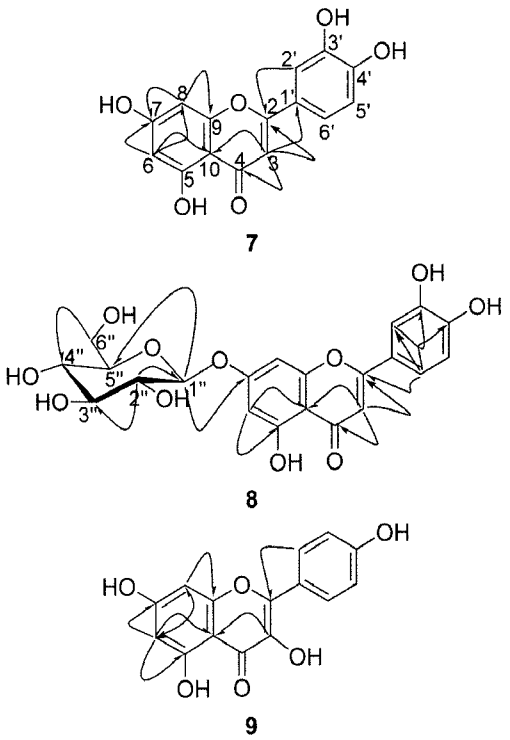

Fig. 3. Important HMBC correlations in phytochemicals 7-9.

of the above obtained data as well as remaining analysis of HMBC and HMQC data, compound **7** was identified as the 3',4',5,7-tetrahydroxyflavone (36). Compound **8** was obtained as yellow powder and had a molecular ion peak at *m/z* 448. The ^{1}H-NMR spectrum of compound **8** was almost the same as those for **7**, except for the sugar moiety. Sugar conformation was assumed to be β-configuration form from the chemical shift and coupling constant value of this anomeric proton signal at 5.09 (1H, d, *J*=7.4 Hz, H-1). The signal at δ 100.4 (C-1), 73.6 (C-2), 76.9 (C-3), 70.1 (C-4), 77.6 (C-5), and 61.1 (C-6) in the ^{13}C-NMR spectrum, and 3.21 (1H, t, *J*=9.1 Hz, H-4), 3.30 (1H, dd, *J*=13.5, 6.1 Hz, H-2), 3.35 (1H, d, *J*=8.9 Hz, H-3), 3.45-3.48 (1H, m, H-5), and 5.09 (1H, d, *J*=7.4 Hz, H-1) in the ^{1}H-NMR spectrum suggested the presence of a β-glucopyranose group. In HMBC spectrum, the cross peak between the anomeric proton signal at δ 5.09 (H-1) and compound **7** carbon signal at δ 163.4 (C-7) confirmed that glucose was substituted for 7 position of compound **7** (Fig. 3). Therefore, on the basis of these assignments, the structure of **8** was 3',4',5-trihydroxyflavone-7-*O*-β-D-glucopyranoside (37). Compound **9** was obtained yellow powder and had a molecular ion peak at *m/z* 286. Ultra violet (UV) spectrum analysis showed an absorption maximum at 322 nm and IR spectrum analysis showed strong hydroxyl and carbonyl group absorption bands at 3,370 and 1,661/cm, respectively. In the ^{1}H-NMR spectrum of **9** showed signals for 2 symmetric protons of the B-ring [δ 8.05 (2H, dd, *J*=6.9, 2.0 Hz, H-2' and 6') and 6.92 (2H, dd, *J*=6.9, 2.0 Hz, H-3' and 5')] and 2 aromatic protons of the A-ring [δ 6.19 (1H, d, *J*=2.0 Hz, H-6) and 6.44 (1H, d, *J*=2.0 Hz, H-8)]. Also, a characteristic hydrogen bonded proton signal of the hydroxyl group was detected at δ 12.47 (1H, s, 5-OH). The HMBC spectrum of **9** showed correlations of H-2'/6' with C-2, H-6 with C-5, C-7, C-8, and C-10, and H-8 with C-6, C-9, and C-10 (Fig. 3). Thus, based on all the above obtained spectral data, compound **9** was identified as 3,4',5,7-tetrahydroxyflavonol (38).

Compound **10** was obtained as yellow needles having the molecular formula of $C_{15}H_8O_5$, and a molecular ion peak at *m/z* 268. The IR spectrum showed the presence of hydroxyl (3,500/cm) and carbonyl (1,710/cm) moieties. The ^{1}H- and ^{13}C-NMR data with DEPT experiments showed the presence of 15 carbon atoms as 1 carbonyl (158.0, C-6), 6 methins [δ 123.1 (C-1), 114.1 (C-2), 103.4 (C-4), 121.0 (C-7), 114.4 (C-8), and 99.1 (C-10)] and 8 quarternary carbons [δ 161.6 (C-3), 155.0 (C-4a), 102.4, (C-6a), 115.0 (C-6b), 156.3 (C-9), 157.4 (C-10a), 159.8 (C-11a), and 104.6 (C-1a)], respectively. In the ^{1}H-NMR spectrum of **10** showed an ABX-type aromatic proton system appearing at δ 7.84 (H-1), 6.93 (H-2), and 6.91 (H-4) due to A ring protons on AB-type aromatic proton signals resonating at δ 7.69 (H-7), 6.95 (H-8), and 7.16 (H-10) due to B ring protons. Exact position of protons and carbons in compound **10** was determined by the COSY spectrums and unassigned connectivities carbonyl and quaternary carbons were determined on the basis of HMBC correlations. The connectivity between H-7 with C-10, C-9, and C-6a, and H-10 with C-8, C-9, and C-6b, and H-1 with C-4, C-3, C-4a, and C-11a, and H-2 with C-4, C-1a, and C-3 were determined on the basis of HMBC correlations (Fig 4). Thus, based on all the above obtained spectral data, compound **10** was identified as coumestrol by pterocarpan structure (39). Compound **11** was obtained as white needles having the molecular formula of $C_{20}H_{18}O_5$, and a molecular ion peak at *m/z* 354. The IR spectrum showed the presence of hydroxyl (3,414/cm) and carbonyl (1,660/cm) moieties. The ^{1}H- and ^{13}C-NMR data with DEPT experiments showed the presence of 20 carbon atoms as 7 methins [δ 87.3 (C-11a), 99.0 (C-10), 100.2 (C-4), 100.9 (C-12), 109.5 (C-8), 124.3 (C-1), and 125.7 (C-7)], 1 methylene 71.8 (C-6), 10 quaternary carbons [δ 69.8 (C-14), 77.7 (C-6a), 118.5 (C-11b), 121.3 (C-6b), 125.1 (C-2), 154.2 (C-4a), 156.9 (C-3), 161.3 (C-9), 162.4 (C-10a), and 165.4 (C-13)], and 2 methyl carbons (29.9, C-15 and C-16), respectively. The ^{1}H-NMR data showed the evidence for 2 methyl protons [δ 1.60 (H-15 and H-16)], 2 furanoid protons [δ 5.48 (H-11a) and 6.65 (H-12)], 5 aromatic protons [δ 6.26 (H-10), 6.46 (H-8), 6.96 (H-4), 7.26 (H-7), and 7.68 (H-1)], and 1 methylene proton [δ 4.15 (H-6β) and 4.19 (H-6α)], respectively. Namely, ^{1}H-NMR data showed a set of proton signals 4.15 (1H, d, *J*= 11.4 Hz, Hax-6), 4.19 (1H, d, *J*=11.4 Hz, Heq-6), 5.48 (1H, s, H-11a) characteristic of pterocarpan, exhibited the presence of an aromatic protons system appearing at 6.96 (H-4) and 7.68 (H-1) due to A ring protons, an AB-type aromatic proton signals resonating at 6.46 (H-8), 6.26 (H-10), and 7.26 (H-7) due to B ring protons. The HMBC spectrum of compound **11** showed correlations of H-1 with C-3, C-4, C-4a, and C-11a, H-11a with C-4a, C-6a, C-10a, and C-11b, H-6α/6β with C-4a, C-6a, C-6b, and C-11a, and H-12 with C-2, C-3, and C-13, respectively (Fig. 4). Additionally, the following long-range correlations appeared the dimethyl group protons at δ 1.60 (H-15 and H-16) with C-14 and C-13 of furanoid carbon (Fig. 4). Thus, based on all the above assignments and remaining analysis of

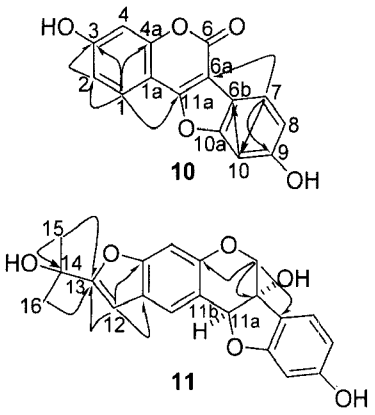

Fig. 4. Important HMBC correlations in phytochemicals 10 and 11.

HMBC spectra allowed the unequivocal assignment of all carbons. All data mentioned above indicate that the structure of compound **11** was glyceofuran (40).

Compounds **12** and **13** were 4-hydroxybenzoic acid and methyl-4-hydroxybenzoate on the basis of ^{1}H- and ^{13}C-NMR spectrums, together with a comparison of the above data with those published in the literatures (27,41). Compound **14** was obtained as white needles. The IR spectrum revealed absorption bands due to hydroxyl (3,440/cm) group. The ^{1}H-NMR data showed the evidence for 7 methyl protons [δ 0.74, 0.81, 0.86, 0.88, 0.91, 1.03, and 1.10 (each 3H, s)], 5 methine protons [δ 3.25 (H-3), 0.79 (H-6), 1.51 (H-9), 1.96 (H-18), and 3.28 (H-22)], 1 olefinic methine proton δ 5.16 (1H, t, *J*=3.6 Hz, H-12), and 10 methylenes protons, respectively. The complex overlapping proton signals in the 0.5-2.1 ppm region of the proton spectrum was analyzed using ^{1}H-^{1}H COSY. Figure 7 showed important HMBC correlation and the positioning of the substituents on the ring system. All data mentioned above indicate that the structure of compound **14** were soysapogenol B (42). Compound **15** was obtained as white powder and had a molecular ion peak at *m/z* 412. The IR spectrum showed absorption bands at 3,450/cm, which indicated the presence of hydroxyl group. The ^{13}C-NMR data with DEPT experiments showed the presence of 29 carbon atoms as 8 methines [δ 72.2 (C-3), 32.31 (C-8), 50.6 (C-9), 57.3 (C-14), 56.4 (C-17), 40.9 (C-20), 51.7 (C-24), and 32.27 (C-25)], 3 olefinic [δ 122.1 (C-6), 138.7 (C-22), and 129.7 (C-23)], 9 methylenes [δ 37.7 (C-1), 32.1 (C-2), 42.7 (C-4), 32.32 (C-7), 21.49 (C-11), 40.1 (C-12), 24.77 (C-15), 29.3 (C-16), and 25.8 (C-28)], 3 quaternary [δ 141.2 (C-5), 36.9 (C-10), and 42.6 (C-13)], and 6 methyl carbons [δ 12.6 (C-18), 19.8 (C-19), 21.46 (C-21), 21.62 (C-26), 19.4 (C-27), and 12.5 (C-29)], respectively. The ^{1}H-NMR data showed the evidence for 6 methyl protons [δ 0.73 (H-18), 1.06 (H-19), 1.04 (H-21), 0.83 (H-26), 0.88 (H-27), and 0.85 (H-29)], 8 methine protons [δ 3.51-3.57 (H-3), 1.49 (H-8), 1.28 (H-9), 1.21 (H-14), 1.08 (H-17), 1.54 (H-20), 1.19 (H-24), and 1.58 (H-25)], 3 olefinic protons [δ 5.37 (H-6), 5.18 (H-22), and 5.05 (H-23)], and 9 methylene protons [δ 1.03 and 1.88 (H-1α/β), 1.52 and 2.00 (H-2α/β), 1.98 and 2.09 (H-4α/β), 2.25-2.31 (2H, H-7), 0.97 and 1.89 (H-11α/β), 0.96 and 1.75 (H-12α/β), 1.46 (2H, H-15), 1.20 and 1.73 (H-16α/β), 1.11 and 1.45 (H-28α/β)], respectively. In fact, almost all of the methine and

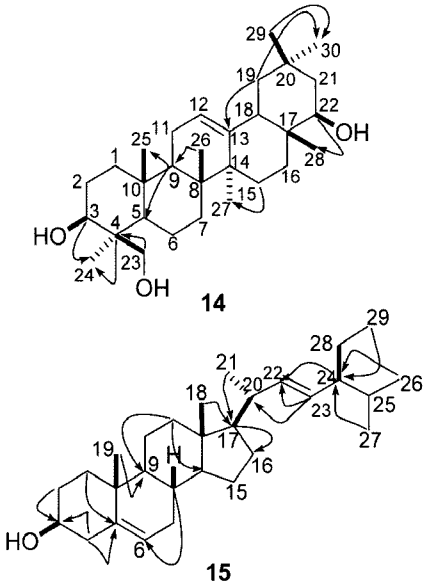

Fig. 5. Important HMBC correlations in phytochemicals 14 and 15.

methylene proton singals were overlapped, like other sterols, and therefore it was ambiguous and difficult to distinguish the HMBC correlations. Especially, the correlations from these methyl proton signals to carbon signals through two and three bonds showed very strong cross peaks in the HMBC, which played an important role in signal assignment. Example of structure elucidation: The methyl proton signal at δ 1.06 (3H, s, H-19) was correlated to the methine carbon signal δ (50.6, C-9) in the HMBC, and the methane proton signal at δ 1.28 (1H, s, H-9) was coupled with the methylene proton signals at δ 0.97 and 1.89 (2H, m, H-11α/β) in the ^{1}H-^{1}H COSY. Both of the methyl proton signals at δ 0.73 (3H, H-18) and 1.04 (3H, H-21) were correlated to the carbon signal at δ (56.4, C-17), and the methane proton signal at δ 1.08 (H-17) which was assigned by the HMQC was coupled with the methylene proton signals at δ 1.20 (1H, m, H-16α), 1.73 (1H, m, H-16β) (Fig. 5). Thus, these assignments and analysis of HMBC spectra allowed the unequivocal assignment of all carbons. All data mentioned above indicate that the structure of compound **15** was stigmasterol (43). Compounds **16** was D-mannitol on the basis of ^{1}H- and ^{13}C-NMR spectrums, together with a comparison of the above data with those published in the literature (29).

In conclusion, we reported that phytochemicals **1-16** were isolated through various chromatographic procedures from the leaves of soybean. Their structures including 5 isoflavones **1-5**, 3 flavones **6-8**, 1 flavonol **9**, 2 pterocarpans **10** and **11**, 2 phenolic compounds **12** and **13**, 2 phytosterols **14** and **15**, and 1 sugar alcohol **16** were identified by spectroscopic methods and physical data. Although isolated phytochemicals were usually present in natural plants (phytochemcals **1-16**) and seeds and roots of soybean (phytochemicals **1-5**, **10**, and **11**), as far we know, the isolation and identification of phytochemicals **7-16** from the leaves were reported for the first time in this study. We are currently examining the various biological activities and in future studies, the various activities of these components should have clarified to better understand the

beneficial effects of soybean leaves. Also, isolated phytochemicals have attracted attention to physiological effects including antioxidant, anticancer, and antiatherosclerotic would contribute to enhance the value of soybean and its leaves as a dietary supplements and functional foods.

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