Flavonoid Glycosides from *Terminalia catappa* L.

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Under the inhibition of Cu^{+2}-induced LDL oxidation-guided fractionation, two new flavone glycosides with galloyl substitution were isolated from the dried fallen leaves of *Terminalia catappa* L. Their structures were established as apigenin 6-*C*-(2″-*O*-galloyl)-β-*D*-glucopyranoside (**1**) and apigenin 8-*C*-(2″-*O*-galloyl)-β--*D*-glucopyranoside (**2**), together with four known flavone glycosides, isovitexin, vitexin, isoorientin, and rutin, on the basis of spectroscopic method. Compounds **1** and **2** showed significant antioxidative effects. Their IC_{50} were 2.1 and 4.5 μM, respectively.

INTRODUCTION

The dried fallen leaves of *Terminalia catappa* L. (Combretaceae) are a commonly used folk medicine in Taiwan. It has been claimed to have therapeutic effects for liver related diseases.[1] Hydrolyzable ellagitannins and other tannin related compounds have been isolated from the leaves and the bark of *T.catappa*.[2,3] Several tannins were shown inhibiting HIV replication in infected H9 lymphocytes with little cytotoxicity.[4] Liu et al. have shown the water extract of the leaves of *T. catappa* suppressed mitomycin C-induced micronuclei in CHO-K1 cells. It was suggested that its *in vitro* and *in vivo* anticlastogenic effects may be attributed to its antioxidative potential.[5] And many studies have provided supporting evidence that Chinese medicine used on blood stasis and hepatic injury contains antioxidants to prevent LDL lipid peroxidation.[6-8] Therefore, we investigated the chemical constituents under the antioxidation-guided fractionation of its leaves. This paper deals with the isolation of two new flavone *C*-glycosides with galloyl substitution and their antioxidant activities.

RESULTS AND DISCUSSION

The ethanol extract of the dried fallen leaves of *T. catappa* suspended in water was successively partitioned with ethyl acetate and *n*-butanol. The *n*-butanol soluble fraction was subjected to separation and purification on Diaion HP-20 and Sephadex LH-20 column chromatography, and led to the isolation of two new glucosides **1** and **2** together with four known flavonoid glycosides, isovitexin (**3**),[9,10] vitexin (**4**),[9,10] isoorientin (**5**),[11] and rutin (**6**),[12] gallic acid and ellagic acid.

Compound **1** was isolated as yellow amorphous powder. The molecular formula was established as $C_{28}H_{24}O_{14}$ from FABMS [*m/z* 585 $(M + H)^+$] and ^{13}C NMR data. The IR spectrum showed hydroxyl (3350 cm^{-1}), ester (1705, 1220 cm^{-1}), conjugated carbonyl (1650 cm^{-1}), and aromatic (1610, 1500 cm^{-1}) absorption bands. The UV absorptions at 270, 336 nm, showed that compound **1** was a flavone derivative.[13] The ^1H and ^{13}C NMR (Table 1) spectrum contained signals due to a galloyl moiety: δ_H 6.79 (s, 2H); δ_C 108.6, 108.6, 119.8, 137.9, 145.2, 145.2 and 165.3. Without the galloyl moiety, it was similar to that of apigenin 6-*C*-glucoside [isovitexin (**3**)].[9,10] A para-substituted phenol was characterized by the A_2X_2 pattern [δ 6.89 and 7.86 (each 2H, d, J = 8.7 Hz)] for four aromatic protons. Two singlet protons [(δ 6.41 and 6.68 (each 1H, s)] were assigned as H-8 and H-3 from HMQC and HMBC correlations, and an anomeric proton with larger coupling constant (δ 4.89, d, J = 9.9 Hz) had a long-range correlation with C-6. A methine proton at δ 5.65 (dd, J = 9.9, 8.5 Hz) had a C-H correlation with glucosyl C-2 and a long range correlation with the carbonyl of galloyl. These indicated that the galloyl was connected with C-2 hydroxyl of the glucosyl. Therefore, compound **1** was determined as apigenin 6-*C*-(2″-*O*-galloyl)-β--*D*-glucopyranoside.

The NMR spectral data together with the molecular ion at *m/z* 585 $(M + H)^+$ in FABMS indicated that **2** was closely related to **1** except that one of the singlet phenyl protons in **2** was higher field at δ 6.09. This suggested that the glucosylated po-

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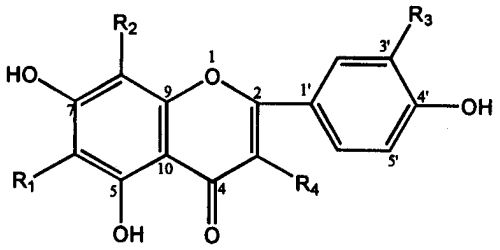

1 R_1=*C*-β-*D*-glucosyl(2"-galloyl), R_2=R_3=R_4=H

2 R_1=R_3=R_4=H, R_2=*C*-β-*D*-glucosyl(2"-galloyl)

3 R_1=*C*-β-*D*-glucosyl, R_2=R_3=R_4=H

4 R_1=R_3=R_4=H, R_2=*C*-β-*D*-glucosyl

5 R_1=*C*-β-*D*-glucosyl, R_2=R_4=H, R_3=OH

6 R_1=R_2=H, R_3=OH, R_4=*O*-α-*L*-rhamno(1‴→6")glucosyl

sition was changed from C-6 to C-8 in **2**. That was further supported by a correlation between the proton at δ 6.09 and C-6 (δ 98.1) in HMQC spectrum and a correlation between strong hydrogen bonding of the C-5 hydroxyl proton and C-6 in the HMBC spectrum.

The known compounds **3**, **4**, and **5** were confirmed by direct comparison of their spectral data with values reported in the literature,[9-11] and compound **6**, gallic acid and ellagic acid were identified by direct comparison with authentic samples.

The isolated compounds were tested for antioxidative activity on Cu^{+2}/O_2-induced low density lipoprotein (LDL) lipid peroxidation with probucol (IC_{50} 4.0 μM)as positive control. The inhibitory effects of a more potent antioxidant showed dose response curves. The IC_{50} of compounds **1** and **2** are 2.1 and 4.5 μM, respectively.

EXPERIMENTAL

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. NMR spectra were run on a Bruker AC-300 in DMSO-d_6. UV spectra were taken on a Hitachi U-3200 spectrophotometer. MS were measured on a FINNIGAN TSQ-46C MS spectrometer. Pobucol was obtained from Merrell Dow. Cupric sulfate

Table 1. ^{1}H NMR Spectral Data for Flavonoids **1-6** from *T. Catappa* (in DMSO-d_6, δ)

H-position	**1**	**2**	**3**	**4**	**5**	**6**
3	6.68s	6.73s	6.75s	6.62s	6.63s	
6		6.09s		6.27s		6.18d (*J*=2.0)
8	6.41s		6.51s		6.46s	6.37d (*J*=2.0)
2′	7.86d (*J*=8.7)	8.09d (*J*=8.7)	7.91d (*J*=8.4)	8.01d (*J*=8.4)	7.38d (*J*=2.0)	7.52d (*J*=2.0)
3′	6.89d (*J*=8.7)	6.93d (*J*=8.7)	6.93d (*J*=8.4)	6.89d (*J*=8.4)		
5′	6.89d (*J*=8.7)	6.93d (*J*=8.7)	6.93d (*J*=8.4)	6.89d (*J*=8.4)	6.88d (*J*=7.8)	6.83d (*J*=9.0)
6′	7.86d (*J*=8.7)	8.09d (*J*=8.7)	7.91d (*J*=8.4)	8.01d (*J*=8.4)	7.44dd (*J*=7.8, 2.0)	7.53dd (*J*=9.0, 2.0)
1″	4.89d (*J*=9.9)	4.94d (*J*=10.2)	4.61d (*J*=9.9)	4.68d (*J*=9.9)	4.59d (*J*=9.6)	5.33d (*J*=6.9)
1‴						4.39br s
2″	5.65dd (*J*=9.9, 8.5)	5.48dd (*J*=10.2, 9.3)	4.03dd (*J*=9.9, 8.4)		4.02dd (*J*=9.6, 8.4)	
CH_3						0.99d (*J*=6.0)
galloyl-H	6.79s (2H)	6.67s (2H)				
OH	9.43br s 10.25br s 10.25 br s 12.62br s	9.21br s 10.42br s 10.98br s 13.08brs	9.58br s 10.25br s 13.53br s	10.26br s 10.73br s 13.14br s	9.31br s 9.811br s 10.43br s 13.53br s	9.51br s 9.87br s 10.25brs 12.56br s

Table 2 ^{13}C NMR Spectral Data for Flavonoids **1-6** from *T. catappa* (in DMSO-d_6, δ)

^{13}C-position	**1**	**2**	**3**	**4**	**5**	**6**
2	164.5	165.2	163.5	163.9	163.6	165.8
3	102.8	102.8	102.8	102.4	102.8	133.6
4	181.7	182.2	181.9	182.0	181.8	177.3
5	161.1	162.5	161.2	161.0	160.6	156.9
6	107.0	98.1	108.8	98.1	108.8	99.4
7	163.6	164.4	163.2	162.5	163.2	161.1
8	93.7	104.1	93.7	104.6	93.5	94.3
9	156.3	156.7	156.2	155.9	156.2	156.8
10	105.7	104.1	103.4	104.0	103.4	103.7
1′	121.5	122.0	121.1	121.5	121.4	122.0
2′	130.3	129.3	128.4	128.8	113.3	116.4
3′	115.9	116.2	116.0	115.7	145.7	144.9
4′	160.8	161.3	160.6	160.3	149.7	148.7
5′	115.9	116.2	116.0	115.7	116.0	115.9
6′	130.3	129.3	128.4	128.8	118.9	130.3
glc-1	70.7	71.4	73.1	73.3	73.1	101.8
glc-2	72.0	72.5	70.6	70.8	70.6	74.3
glc-3	76.4	76.2	78.9	78.8	78.9	76.6
glc-4	70.7	71.0	70.3	70.5	70.3	70.7
glc-5	81.7	82.1	81.4	81.7	81.4	76.1
glc-6	61.3	61.3	61.4	61.3	61.4	67.3
rha-1						101.1
rha-2						70.2
rha-3						70.7
rha-4						72.1
rha-5						68.5
rha-6						17.9
galloyl						
1	119.8	119.8				
2,6	108.6	108.9				
3,5	145.2	145.4				
4	137.9	138.4				
C=O	165.3	165.2				

($CuSO_4$), and EDTA were purchased from Sigma Chemical Co. Cholesterol kit (Merkotest, 14366 cholesterol enzymatic) was obtained from Merck. Ultracentrifugation were taken on a Beckman L8-80M; R50 rotor.

Plant Materials

The dried fallen leaves of *Terminalia catappa* were collected in Taitung. and identified by comparison with the specimens which have been deposited at the Herbarium of the Department of Botany, National Taiwan Unversity, Taipei, Taiwan.

Extraction and Isolation

The dried fallen leaves of *T. catappa* (6 Kg) were extracted exhaustively with ethanol. Under the inhibition of Cu^{+2}-induced LDL lipid peroxidation-guided fractionation, the ethanol extract suspended in water was partitioned with ethyl acetate (EtOAc) and *n*-butanol (BuOH), successively. The BuOH soluble fractions (131 g) were subjected to separation and purification by Diaion HP-20 (H_2O-MeOH gradient). The 50% MeOH, 75% MeOH and MeOH eluates were further purified by Sephadex LH-20 (MeOH or 80% MeOH/H_2O) column to afford **1** (275 mg), **2** (65 mg), isovitexin (46 mg), vitexin (256 mg), isoorientin (43 mg), rutin (36 mg), gallic acid (156 mg) and ellagic acid (452 mg).

Apigenin 6-*C*-(2″-galloyl)-β-*D*-glucoside (1)

Yellow amorphous powder; FABMS *m/z*: 585 $(M+1)^+$; IRν_{max} (KBr) cm^{-1}: 3350, 1705, 1650, 1610, 1500, 1220, 1180, 1060, 1030, 830; UV λ_{max} (MeOH)(log ε) 270 (3.97), 336 (3.80) nm; λ_{max} (MeOH+$AlCl_3$) (log ε) 279 (3.95), 302 (3.96), 337 (3.87), 382 (3.78) nm; λ_{max} (MeOH + $AlCl_3$ + HCl) (log ε) 279 (4.00), 293 (3.94), 345 (3.90), 371(3.87) nm; λ_{max} (MeOH + NaOAc) (log ε) 273 (3.97), 297 (3.88), 349 (3.91) nm; λ_{max}(MeOH + NaOAc+H_3BO_3) (log ε) 272 (3.96), 297 (3.95), 348 (3.95) nm; ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2.

Apigenin 8-*C*-(2″-galloyl)-β-*D*-glucoside (2)

Yellow amorphous powder; FABMS *m/z*: 585 $(M+1)^+$; IRν_{max} (KBr) cm^{-1}: 3400, 1705, 1650, 1605, 1510, 1220, 1180, 1050, 1035, 835; UV λ_{max} (MeOH) (log ε) 271 (3.95), 293sh (3.86), 313 (3.85) nm; λ_{max} (MeOH+$AlCl_3$) (log ε) 278 (4.03), 302 (4.02), 322 (3.89), 383 (3.88) nm; λ_{max} (MeOH + $AlCl_3$ + HCl) (log ε) 277 (4.08), 301 (4.02), 344 (3.90), 378 (3.89) nm; λ_{max} (MeOH + NaOAc) (log ε) 273 (4.20), 304 (4.06), 335 (3.91)nm; λ_{max} (MeOH + NaOAc + H_3BO_3) (log ε) 272 (4.15), 301 (4.17), 348 (3.92) nm; ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2.

Isovitexin (3) [9,10]

Yellow amorphous powder; FABMS *m/z*: 433 $(M+1)^+$; IRν_{max} (KBr) cm^{-1}: 3300, 1655, 1610, 1590, 1515, 1500, 1085, 1020, 835; UV λ_{max} (MeOH) (log ε) 272 (3.86), 331 (3.71) nm; λ_{max} (MeOH + $AlCl_3$) (log ε) 280 (3.87), 294 (3.84), 344 (3.67), 380 (3.65) nm; λ_{max} (MeOH + $AlCl_3$ + HCl) (log ε) 278 (3.92), 297 (3.75), 347 (3.73), 383 (3.70) nm; ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2.

Vitexin (4) [9,10]

Yellow amorphous powder; FABMS *m/z*: 433 $(M+1)^+$; IRν_{max} (KBr) cm^{-1}: 3400, 1660, 1605, 1590, 1510, 1500, 1080, 1030, 830; UV λ_{max} (MeOH) (log ε) 270 (3.89), 334 (3.89) nm; λ_{max} (MeOH + $AlCl_3$) (log ε) 276 (3.88), 304 (3.76), 345 (3.88) , 386 (3.76) nm; λ_{max} (MeOH + $AlCl_3$ + HCl) (log ε) 277 (3.88), 303 (3.76), 343 (3.88), 384 (3.76) nm; ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2.

Isoorientin (5)[11]

Yellow amorphous powder; FABMS *m/z*: 449 $(M+1)^+$; IRν_{max} (KBr) cm^{-1}: 3400, 1660, 1630, 1580, 1510, 1090, 1035, 840; UV λ_{max} (MeOH) (log ε) 257 (3.99), 271 (4.02), 349 (4.03) nm; λ_{max} (MeOH + $AlCl_3$) (log ε) 276 (4.13), 300sh (3.80), 333 (3.74), 424 (4.15) nm; λ_{max} (MeOH + $AlCl_3$ + HCl) (log ε) 263sh (3.96), 278 (4.00), 294 (3.90), 361 (3.99), 386 (3.99) nm; ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2.

Rutin (6)[12]

Yellow crystals, mp 241-243 °C; FABMS *m/z*: 611$(M + 1)^+$; IRν_{max} (KBr) cm^{-1}: 3400, 1660, 1600, 1500, 1060, 1015, 805; ^{1}H NMR (DMSO-d_6): Table 1; ^{13}C NMR (DMSO-d_6): Table 2.

LDL Lipid Peroxidation and Screening for Antioxidants

Fasting plasma samples were collected from healthy male adults not using vitamin supplements. LDL was obtained by following a previously reported method.[14] LDL assay was carried out in a 96 well microtiter plate as described.[15] Probucol (5-20 μM, 10% ethanolic solution, 10 μl) was used as a positive control. The conjugated diene formation in the supernatant was determined by UV absorption at 232 nm.

ACKNOWLEDGEMENTS

This work was supported in part by the National Health Research Institutes of the Republic of China (NHRI 86-HR-515).

Received October 27, 1999

Key Words

Terminalia catappa L.; Combretaceae; Dried fallen leaves; Flavone glucopyranosides; Antioxidant.

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