ISSN 1070-3632, Russian Journal of General Chemistry, 2014, Vol. 84, No. 7, pp. 1434–1441. © Pleiades Publishing, Ltd., 2014.

Antitumor Activity of Stilbenoids and Flavonoids Isolated from *Acanthopanax Brachypus*[1]

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Received April 1, 2014

Abstract—This report is the first to detail the antitumor activity of *Acanthopanax brachypus*, a species well recognized by eastern folk medicine. Four stilbeniods and nine flavonoids have been isolated from stem bark of the plant. The isolates have been tested for their antitumor activity against selected human cancer cell lines (HgpG-2, MCF-7, K-562, and A-549). Even though three of the compounds have shown high to moderate cytotoxicity against certain cell lines (IC_{50} of 4.5 to 6.5 μg/mL), in most of the experiments little or no anticancer activity has been revealed.

Keywords: araliaceae, *Acanthopanax brachypus*, stilbenoid, flavonoid, antitumor activity

DOI: 10.1134/S1070363214070329

Acanthopanax (*Eleutherococcus*) genus belonging to the Araliaceae family includes 37 species (plus varieties) that are widely ranged in temperate regions of the Northern Hemisphere, mainly in the countries of Eastern Asia. China and Korea are natural habitats of the most of the *Acanthopanax* representatives: about 26 species and 18 varieties grow in mainland China, especially in its northwest [1, 2]. Root and stem bark of some *Acanthopanax* species have been clinically used for long as tonic and sedative drugs, as well as for treatment of rheumatism arthritis, diabetes mellitus, chronic bronchitis, hypertension, ischemic heart disease, and gastric ulcer [3, 4], and are popular as anti-stress drugs and health supplements in China and Korea [5]. Isolation and biological activity of some compounds from the *Acanthopanax* genus have been well established [6].

In vitro and in vivo experimental studies have demonstrated that many *Acanthopanax* species exhibit multiple biological effects against obesity, oxidative stress, inflammation, and even cancer. For example, the extract of *A. senticosus* has been shown to inhibit proliferation of mice sarcoma S180 and human chronic myelogenous leukemia K562 cells [7] as well as of some other cancer cells. The extract of *A. giraldii* var. *hispidus* inhibits proliferation, colony formation, and viability of human gastric cancer SGC-7901 cells [6]. Triterpenoid saponin from *Acanthopanax koreamum* exhibits anticancer effect against several cell lines including MCF-7, A-549, and HL-60 [8].

Acanthopanax brachypus, an endemic endangered shrub found mostly in the loess plateau of northwestern China [9], belongs to the same group of plants. It is a peculiar traditional medicinal plant; nowadays, different parts of *A. brachypus* are applied for various therapeutic purposes in China [10]. The rhizome extract of *A. brachypus* is also believed to function as cancer-preventing and anti-cancer drug.

The above-listed medicinal properties are further backed by studies showing that *A. brachypus* is rich in stilbenoids and flavonoids. We have previously reported on chemistry of *A. brachypus* constituents [11]; however, its potentially antitumor components have not been investigated up to now. In continuation of the study, in this paper we report on isolation and characterization of four stilbenoids and nine flavonoids from the alcoholic extract of the stem bark. In particular, we tested the isolated compounds in vitro to determine their antitumor activity against human cancer cell lines (HgpG-2, MCF-7, K-562, and A-549).

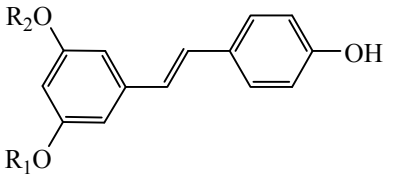
The four stilbenoids were isolated from stem bark of *A. brachypus*, and their structures were elucidated by means of FAB-MS, IR, and NMR studies. In particular, **I** was identified as (*E*)-resveratrol 3-*O*-α-D-

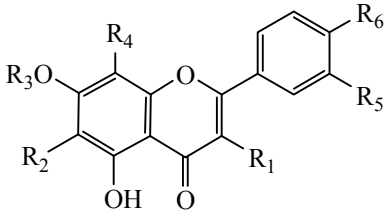
[1] The text was submitted by the authors in English.

glucopyranoside (**I**) previously isolated from red wine [12] and *Streptococcus mutans* [13]; the other three compounds were 3-*O*-methyl-(*E*)-resveratrol 5-*O*-β-D-apiofuranosyl-(1→6)-β-D-[2''-vanilloyl]-glucopyranoside (**II**), 3-*O*-methyl-(*E*)- resveratrol 5-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-[2''-(*E*)-feruloyl]-glucopyranoside (**III**), and 3-*O*-methyl-(*E*)-resveratrol 5-*O*-β-D-apiofuranosyl-(1→6)-β-D-[2''-(*E*)-cinnamoyl]-glucopyranoside (**IV**) [14, 15].

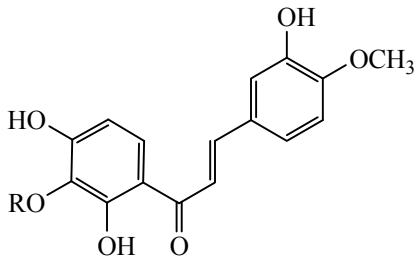
The nine flavonoids isolated from stem bark of *A. brachypus* were identified as wogonin (**V**) [16], pachypodol (**VI**) [17], kaempferol-7-*O*-β-D-glucopyranoside (**VII**) [18, 19], baicalin (**VIII**) [20], hyperin (**IX**) [7], vitexin (**X**) [21, 22], orientin (**XI**) [23], 4-*O*-methylokanin 3'-*O*-β-D-glucopyranoside (**XII**) [24, 25], and (–)-epicatechin (**XIII**) [26, 27]. All the isolated flavonoids were reported to be found in the Araliaceae for the first time, except for compound **IX** that was previously isolated from *A. senticosus* and *A. sessiliflorus* [7, 28].

Summary of the isolated compounds structures is as follows.

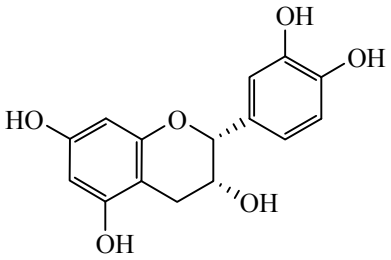




Comp. no.	R_1	R_2	Comp. no.	R_1	R_2	R_3	R_4	R_5	R_6
I	β-D-Glc	H	**V**	H	H	H	OCH_3	H	H
II	CH_3	β-D-[2-vanilloyl]-Glc-(6←1)-β-D-Api	**VI**	OCH_3	H	CH_3	H	OCH_3	OH
III	CH_3	β-D-[2-(E)-feruloyl]-Glc-(6←1)-α-L-Rha	**VII**	OH	H	β-D-Glc	H	H	OH
IV	CH_3	β-D-[2-(E)-cinnamoyl]-Glc-(6←1)-β-D-Api	**VIII**	H	OH	β-D-GlcA	H	H	H
			IX	O-β-D-Gal	H	H	H	OH	OH
			X	H	H	H	β-D-Glc	H	OH
			XI	H	H	H	β-D-Glc	OH	OH



XII, R = β-D-Glc



XIII

The isolated compounds were tested for cytotoxicity against certain cancer cell lines, the determined values of IC_{50} are collected in Table 1. In particular, compound **VI** showed excellent activity against HgpG-2 with IC_{50} of 4.5 μg/mL; compounds **VIII** and **XII** were moderately active against A-549 with IC_{50} of 5.6 and 6.5 μg/mL, respectively. Other isolates were weakly active (IC_{50} of 10–20 μg/mL) or inactive (IC_{50} > 20 μg/mL).

To conclude, in this report we detailed antitumor activity of *A. brachypus* for the first time. It was evident that most of stilbenoids and flavonoids from *A. brachypus* had little or no antitumor activity.

Table 1. Cytotoxicity (IC_{50}, μg/mL) of the studied compounds[a]

Cell line	Compound									
	I	**II**	**III**	**IV**	**V**	**VI**	**VIII**	**IX**	**XII**	**XIII**
K-562	> 20	> 20	> 20	> 20	[b]	11.4	[b]	> 20	11.4	18.5
HgpG-2	12.5	18.7	> 20	> 20	15.4	4.5	> 20	[b]	> 20	> 20
MCF-7	16.8	10.6	> 20	> 20	> 20	[b]	13.6	[b]	> 20	[b]
A-549	> 20	> 20	> 20	> 20	12.8	> 20	5.6	15.2	6.5	15.6

[a] Compounds **VII**, **X**, and **XI** showed no activity. [b] Stands for not tested.

EXPERIMENTAL

Melting points were determined with the X-4 digital micro-melting point apparatus and are reported uncorrected. Optical rotation was measured with the Perkin-Elmer 341 digital polarimeter. UV spectra were recorded using the Shimadzu UV-2401 spectrophotometer. IR spectra (KBr) were recorded with the Perkin-Elmer-577 spectrometer. ^1H (400 MHz) and ^{13}C (100 MHz) spectra were registered with the Bruker DRX-400 spectrometer using TMS as internal standard; ^1H MMR and ^{13}C NMR results are collected in Tables 2 and 3, respectively. FAB-MS spectra were obtained with the VG Auto Spec-3000 mass spectrometer using glycerol as matrix. EI-MS studies were performed using the Finnigan MAT 95 mass spectrometer. Column chromatography was carried using silica gel (100–200 or 200–300 mesh, Qingdao Haiyang Chemical Plant, China) or Sephadex LH-20 gel (25–100 μm, Amersham Pharmacia Biotech, Sweden) as stationary phase. The precoated silica gel plates (GF_{254}, Qingdao Haiyang Chemical Plant, China) were used for thin-layer chromatography experiments, the spots were visualized under UV light (254 or 365 nm) by spraying with a suitable colored reagent. All the solvents used were of analytical grade (Xi'an Chemical Plant, China).

Stem bark of *A. brachypus* was collected from Qingyang of Gansu Province in August, 2009 and authenticated by Prof. Xiao-qiang Guo (College of Life-Sciences and Technology, Longdong University). The voucher specimen (20090810732) was deposited in the herbarium of College of Life-Sciences and Technology, Longdong University, Qingyang, People's Republic of China. The stem barks were washed, dried in shade, and powdered using grinder.

The air-dried and pulverized stem bark (15.0 kg) of *A. brachypus* was extracted with 95% EtOH three times (7 h each) at room temperature. The extracts were combined and concentrated under reduced pressure at 60°C to yield 648 g of brown viscous residue. The residue was suspended in water and partitioned sequentially with *n*-hexane (5 L), EtOAc (10 L), and *n*-BuOH (8 L). The EtOAc-soluble extract (188 g) was subject to silica gel column chromatography eluting with *n*-hexane, $CHCl_3$–Me_2CO (5 : 1 to 1 : 5, v/v), and Me_2CO–MeOH (10 : 1 to 1 : 10, v/v) linear gradient; the eluted fractions were examined by analytical TLC, and alike fractions were pooled together to give 5 major fractions (E_1-E_5). Those fractions were repeatedly subject to column chromatography on silica gel, Sephadex LH-20 and RP-18 as well as to preparative TLC. From fraction E_1, compounds **V** (19 mg), **VI** (23 mg), and **XIII** (63 mg) were obtained. From fraction E_3, compounds **VII** (36 mg), **VIII** (21 mg), **IX** (9 mg), and **XII** (9 mg) were isolated. Compounds **I** (16 mg), **X** (25 mg), and **XI** (52 mg) were obtained from fraction E_4, and compounds **II** (19 mg), **III** (23 mg), and **IV** (31 mg) were found in fraction E_5.

The in vitro cytotoxicity test of the isolates was performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method [29]. The human leukemia K-562, hepatoma HgpG-2, breast carcinoma MCF-7, and lung carcinoma A-549 cells were obtained from China Centre for Typical Culture Collection, Wuhan University. The freshly trypsinized cells suspensions were seeded in 96-well microtiter plates (5000–10000 cells per well) containing the DMSO-diluted stocks of the tested compounds. After 3 days in culture, the attached cells were incubated with MTT (0.5 mg/mL, 1 h) and subsequently dissolved in DMSO. Absorbance at 550 nm was then measured using the microplate reader. Cytotoxicity was ex-pressed as IC_{50}, the tested compound concentration to reduce the cell growth by 50% under the experimental conditions.

Table 2. [1]H NMR chemical shifts of the studied compounds, ppm (*J*, Hz)[a]

Position	I	II	III	IV	V	VI	VII	VIII	IX	X	X	XII	XIII
2	6.71 br.s	6.70 br.s	6.72 br.s	6.70 br.s								7.18 d (1.6)	4.75 d (4.5)
3					6.83 s			7.04 s		6.81 s	6.65 s		4.07 d (3.5)
4	6.39 br.s	6.40 br.s	6.41 br.s	6.40 br.s									2.85 d. d (17.0, 4.5) 2.72 d. d (17.0, 3.5)
5												6.96 d (7.6)	
6	6.57 br.s	6.63 br.s	6.65 br.s	6.63 br.s	6.33 s	6.38 d (2.0)	6.40 d (2.0)		6.41 d (1.8)	6.37 s	6.25 s	7.15 d. d (7.6, 1.8)	5.87 d (2.0)
7	6.85 d (16.1)	6.85 d (16.0)	6.84 d (16.1)	6.85 d (16.0)								7.26 d (16.2)	
8	6.99 d (16.1)	7.01 d (16.0)	6.99 d (16.1)	7.01 d (16.0)		6.45 d (2.0)	6.78 d (2.0)	6.98 s	6.58 d (1.8)			7.64 d (16.2)	6.07 d (2.0)
2'	7.38 d (8.6)	7.43 d (8.5)	7.45 d (8.5)	7.43 d (8.5)	7.68 m	7.69 d (2.0)	8.01 d (9.0)	7.63 m	7.54 d (2.1)	7.76 d (8.7)	7.44 d (2.1)		6.97 d (2.0)
3'	6.78 d (8.6)	6.88 d (8.5)	6.86 d (8.5)	6.88 d (8.5)	7.31 m		6.97 d (9.0)	7.39 m		6.98 d (8.7)			
4'					7.31 m			7.39 m					
5'	6.78 d (8.6)	6.88 d (8.5)	6.86 d (8.5)	6.88 d (8.5)	7.31 m	7.06 d (8.0)	6.97 d (9.0)	7.39 m	6.89 d (8.4)	6.98 d (8.7)	6.90 d (8.2)	6.48 d (7.1)	6.80 d (8.0)
6'	7.38 d (8.6)	7.43 d (8.5)	7.45 d (8.5)	7.43 d (8.5)	7.68 m	7.67 d. d (2.0, 8.0)	8.01 d (9.0)	7.63 m	7.68 d. d (2.1, 8.4)	7.76 d (8.7)	7.48 d. d (2.1, 8.0)	7.85 d (7.1)	6.76 d. d (8.0, 2.0),
1"	5.33 d (3.5)	4.91 d (7.5)	4.89 d (7.5)	4.91 d (7.5)			5.08 d (7.2)	5.12 d (7.5)	5.37 d (7.8)	4.89 d (9.8)	4.78 d (9.0)	4.82 d (7.5),	
1"'		5.10 d (2.4)	5.08 d (1.7)	5.10 d (2.4)									
2""		7.18 d (2.6)	7.04 d (2.0)	7.48 d (7.6)									
3""				7.31 d. d (7.6, 7.0)									

Table 2. (Contd.)

4""				7.35 t (7.0)									
5""		6.76 d (8.0)	6.95 d (8.0)	7.31 d. d (7.6, 7.0)									
6""		7.25 d. d (8.0, 2.6)	7.08 d. d (2.0, 8.0)	7.48 d (7.6)									
7""			7.48 d (15.9)	7.23 d (16.0)									
8""		3.75 s	6.25 d (15.9)	6.30 d (16.0)									
10""			3.82 s										
CH_3O-5		3.81 s	3.79 s	3.81 s		3.90 s							
CH_3O-7						3.85 s							
CH_3O-8					3.91 s								
CH_3O-3'						3.87 s							
CH_3O-4'												3.89 s	

[a] Compounds **V**, **VI**, and **XIII** were tested in $CDCl_3$; compounds **I–IV** and **VII-XII** were tested in CD_3OD.

Table 3. ^{13}C NMR chemical shifts of the studied compounds, ppm[a]

Position	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	**XIII**
1	140.6	141.5	141.8	141.5								130.1	
2	103.4	107.3	106.9	107.3	163.0	155.8	154.9	163.3	158.0	164.0	164.5	116.1	79.4
3	159.4	162.1	160.4	160.1	105.0	138.2	136.1	106.2	135.6	102.5	103.2	148.5	66.5
4	105.2	104.0	103.8	104.0	181.9	178.7	176.2	181.5	178.9	182.2	182.3	152.7	28.3
5	158.8	159.3	159.6	159.3	156.2	156.5	160.9	145.8	162.8	162.7	163.2	112.9	157.0
6	107.5	107.8	108.1	107.8	99.1	97.5	99.3	130.7	99.9	98.2	98.5	123.2	95.6
7	126.1	126.8	126.8	126.8	157.3	165.2	162.9	151.3	164.8	160.5	162.9	145.6	156.1
8	128.8	129.8	129.8	129.8	137.8	92.4	94.8	93.8	94.6	104.7	104.6	121.4	96.2
9					149.5	161.9	156.3	149.1	157.3	156.1	156.7	194.2	157.6
10					103.7	106.2	105.1	104.6	104.8	103.8	103.9		100.2
1'	128.5	131.6	131.8	131.6	130.8	122.1	121.9	133.8	122.4	121.7	122.3		
2'	127.9	128.7	128.5	128.7	126.2	110.8	130.1	126.4	116.8	129.0	118.0		

Table 3. ^{13}C NMR chemical shifts of the studied compounds, ppm[a]

Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
3'	115.5	115.4	115.1	115.4	129.1	145.9	115.8	128.9	144.8	115.9	146.9	132.7	145.7
4'	158.3	158.7	160.9	158.7	131.9	148.1	160.0	131.8	149.8	161.2	150.9	158.6	144.5
5'	115.5	115.4	115.1	115.4	129.1	114.2	115.8	128.9	115.9	115.9	117.3	110.2	119.5
6'	127.9	128.7	128.5	128.7	126.2	122.8	130.1	126.4	121.8	129.0	115.8	128.3	114.8
1"	98.3	101.3	101.5	101.3			101.5	105.2	105.6	78.7	78.9	104.8	
2"	72.4	76.8	76.3	76.8			74.3	72.7	73.5	73.5	73.2	74.1	
3"	73.3	74.5	75.1	74.5			77.3	75.1	76.5	74.1	74.8	77.9	
4"	70.3	71.3	71.5	71.3			70.2	71.5	69.9	70.6	71.8	70.1	
5"	73.8	76.9	76.8	76.9			75.9	75.6	77.7	81.3	81.9	76.5	
6"	61.2	68.2	67.9	68.2			60.9	170.2	61.8	62.8	62.8	60.9	
CH_3O-5		55.9	56.1	55.9		60.2							
CH_3O-7						55.8							
CH_3O-8					60.7								
CH_3O-3'						56.3							
CH_3O-4'												55.9	
1'''		109.5	102.1	109.5									
2'''		78.2	72.3	78.2									
3'''		80.7	71.6	80.7									
4'''		74.8	74.1	74.8									
5'''		65.1	69.8	65.1									
6'''			18.2										
1''''		122.8	126.8	133.8									
2''''		110.2	109.8	130.2									
3''''		147.1	148.5	128.1									
4''''		150.2	149.1	129.2									
5''''		116.9	114.5	128.1									
6''''		123.2	123.8	130.2									
7''''		164.5	144.8	144.5									
8''''		55.6	115.7	117.4									
9''''			165.5	164.8									
10''''			55.7										

[a] Compounds **V**, **VI**, and **XIII** were tested in $CDCl_3$; compounds **I–IV** and **VII-XII** were tested in CD_3OD.

(*E*)-Resveratrol 3-*O*-α-D-glucopyranoside (I). White acicular crystals (MeOH), mp 202–205ºC. UV spectrum (MeOH), λ_{max}, nm: 216, 304. IR spectrum, ν, cm^{-1}: 3378 (OH), 1612 (C=C), 1601, 1512, 1464, 1380, 1246, 1078, 831, 735. HRFAB-MS *m/z*: 391.1384 $[M + H]^+$ ($C_{20}H_{23}O_8^+$ requires 391.1393); FAB-MS *m/z*: 391 $[M + H]^+$, 229 $[M + H - 162]^+$.

3-*O*-Methyl-(*E*)-resveratrol 5-*O*-β-D-apiofuranosyl-(1→6)-β-D-[2''-vanilloyl]-glucopyranoside (II). Pale yellowish amorphous powder (MeOH), $[\alpha]_D^{25}$ –72.4º (*c* = 0.12, MeOH). UV spectrum (MeOH), λ_{max}, nm: 326, 308, 258. IR spectrum, ν, cm^{-1}: 3429, 1715, 1600, 1508, 1456, 1280, 1145, 1054, 854, 812. HRFAB-MS *m/z*: 687.2296 $[M + H]^+$ ($C_{34}H_{39}O_{15}^+$ requires 687.2289); FAB-MS *m/z*: 687 $[M + H]^+$, 555 $[M + H - 132]^+$, 537 $[M + H - 150]^+$, 405 $[M + H - 150 - 132]^+$, 243 $[M + H - 162 - 150 - 132]^+$, 151 [vanilloyl]$^+$.

3-*O*-Methyl-(*E*)-resveratrol 5-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-[2''-(*E*)-feruloyl]-glucopyranoside (III). Pale yellowish amorphous powder (MeOH), $[\alpha]_D^{25}$ –78.7º (*c* = 0.25, MeOH). UV spectrum (MeOH), λ_{max}, nm: 324, 306, 218. IR spectrum, ν, cm^{-1}: 3411, 1701, 1632, 1600, 1512, 1455, 1276, 1141, 1055, 972. HRFAB-MS *m/z*: 727.2610 $[M + H]^+$ ($C_{37}H_{43}O_{15}^+$ requires 727.2602); FAB-MS *m/z*: 727 $[M + H]^+$, 581 $[M + H - 146]^+$, 551 $[M + H–176]^+$, 405 $[M + H - 176 - 146]^+$, 243 $[M + H - 162 - 176 - 146]^+$, 177 [feruloyl]$^+$.

3-*O*-Methyl-(*E*)-resveratrol 5-*O*-β-D-apiofuranosyl-(1→6)-β-D-[2''-(*E*)-cinnamoyl]-glucopyranoside (IV). Pale yellowish powder (MeOH), mp 232–233ºC, $[\alpha]_D^{25}$ –32.9º (*c* = 0.14, MeOH). UV spectrum (MeOH), λ_{max}, nm: 324, 308, 220. IR spectrum, ν, cm^{-1}: 3429, 1698, 1632, 1600, 1512, 1456, 1278, 1140, 1058, 970. HRFAB-MS *m/z*: 667.2381 $[M + H]^+$ ($C_{35}H_{39}O_{13}^+$ requires 667.2391); FAB-MS *m/z*: 667 $[M + H]^+$, 537 $[M + H - 130]^+$, 535 $[M + H–132]^+$, 405 $[M + H - 130 - 132]^+$, 243 $[M + H–130–132–162]^+$, 131 [cinnamoyl]$^+$.

Wogonin (**V**). Yellow acicular crystals (acetone), mp 202–205ºC. UV spectrum (MeOH), λ_{max}, nm: 365, 308, 252. IR spectrum, ν, cm^{-1}: 3324 br, 1658, 1604, 1585, 1495, 1435, 1046, 887, 831, 738, 706. EI-MS *m/z*: 284 $[M]^+$, 269 (100), 255, 241, 167, 153, 139, 110, 103, 77, 69.

Pachypodol (VI). Yellow acicular crystals (acetone), mp 172–174ºC. IR spectrum, ν, cm^{-1}: 3308, 1687, 1656, 1600, 1562, 1475, 1348, 1160, 1046, 856, 813. FAB-MS *m/z*: 345 $[M + H]^+$.

Kaempferol-7-*O*-β-D-glucopyranoside (VII). Yellow acicular crystals (MeOH), mp 267–269°C. IR spectrum, ν, cm^{-1}: 3413, 2938, 1687, 1656, 1600, 1562, 1475, 1348, 1160, 1046, 856, 813. FAB-MS *m/z*: 449 $[M + H]^+$, 287 $[M + H - 162]^+$.

Baicalin (VIII). Yellow acicular crystals (MeOH), mp 222–224ºC. UV spectrum (MeOH), λ_{max}, nm: 316, 268. FAB-MS *m/z*: 447 $[M + H]^+$, 271 $[M + H - 176]^+$.

Hyperin (IX). Yellow crystals (MeOH), mp 232–234ºC (238–239ºC [7]). $[\alpha]_D^{25}$ –124º (*c* = 0.16, MeOH); $[\alpha]_D^{25}$ –250º (*c* = 0.28, MeOH) [7]. UV spectrum (MeOH), λ_{max}, nm: 362, 301, 269, 258. IR spectrum, ν, cm^{-1}: ~3426–3200 (–OH), 1656 (>C=O), 1608, 1600, 1508, 1462, 1370, 1088, 997, 860, 728. FAB-MS *m/z*: 465 $[M + H]^+$, 303 $[M + H - 162]^+$.

Vitexin (X). Pale yellowish powder (MeOH), mp 265–267ºC. UV spectrum (MeOH), λ_{max}, nm: 346, 268, 253. IR spectrum, ν, cm^{-1}: 3358, 2981, 1687, 1655, 1603, 1516, 1468, 1056, 843. HRFAB-MS *m/z*: 433.1141 $[M + H]^+$ (requires 433.1135).

Orientin (XI). Pale yellowish powder (EtOH), mp 260–262ºC. UV spectrum (MeOH), λ_{max}, nm: 345, 270, 255. IR spectrum, ν, cm^{-1}: 3348, 2985, 1685, 1654, 1601, 1518, 1469, 1047, 837, 739. HRFAB-MS *m/z*: 449.1068 $[M + H]^+$ (requires 449.1084).

4-*O*-Methylokanin 3'-*O*-β-D-glucopyranoside (XII). Yellow powder (acetone), UV spectrum (MeOH), λ_{max}, nm: 365, 278. IR spectrum, ν, cm^{-1}: 3402, 2978, 1682, 1658, 1600, 1516, 1467, 1047, 829, 726. FAB-MS *m/z*: 465 $[M + H]^+$, 303 $[M + H - 162]^+$.

(–)-Epicatechin (XIII). White powder (MeOH), mp 246–247ºC (236–239ºC [26, 27]). $[\alpha]_D^{25}$ –109º (*c* = 0.08, MeOH); $[\alpha]_D^{25}$ –114º (*c* = 0.05, EtOH) [26, 27]. UV spectrum (MeOH), λ_{max}, nm: 284, 280, 205. IR spectrum, ν, cm^{-1}: 3453, 1680, 1615, 1527, 1469, 1389, 1349, 1314, 1291, 1223, 1185, 1045, 978, 810. EI-MS *m/z*: 290 $[M]^+$, 152, 139, 123.

ACKNOWLEDGMENTS

This work was financially supported by the Applied Chemistry Key Subject of Gansu Province (no. GSKS201304AC), P.R. China.

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