

HETEROCYCLES, Vol. 78, No. 6, 2009, pp. 1581 - 1587. © The Japan Institute of Heterocyclic Chemistry
Received, 2nd February, 2009, Accepted, 11th March, 2009, Published online, 12th March, 2009
DOI: 10.3987/COM-09-11673

STUDIES OF TRADITIONAL FOLK MEDICINES IN XINJIANG UIGHUR AUTONOMOUS. II.¹ RESEARCH FOR CHEMICAL CONSTITUENTS OF XINJIANG LICORICE

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Abstract – A new chromene, 5-[2-(4-hydroxyphenyl)ethyl]-2,2-dimethyl-7-[(3-methyl-2-butenyl)oxy]chromene, named xinjiastilbene A (**1**), a new benzofuran, 4-[2-(4-hydroxyphenyl)ethyl]-6-[(3-methyl-2-butenyl)oxy]benzofuran, named xinjiastilbene B (**2**), and a chalcone, 4'-hydroxy-2-methoxy-4-prenyloxy-chalcone, named xinjiachalcone A (**3**), were isolated from licorice of Xinjiang origin (botanically assigned to *Glycyrrhiza inflata* Batalin), together with seven known phenolic compounds. The structure was elucidated on the basis of spectrometric evidence.

Licorice (kanzoh in Japanese, gancao in Chinese) is the named applied to the roots and stolons of some *Glycyrrhiza* species (Leguminosae) and is one of the most important crude drugs in the world. Its major triterpene saponin, glycyrrhizin, is a well-known natural sweetener and pharmaceutical.² Extensive chemical studies revealed that *Glycyrrhiza* plants produce not only glycyrrhizin but also many saponins and flavonoids. Furthermore, many species-specific flavonoids have also been reported in the underground portions of respective *Glycyrrhiza* species.

During our study on traditional folk medicines in Xinjiang Uighur Autonomous Region in China, and in the course of our search for the active ingredients of licorice, we examined the constituents of Xinjiang licorice. The material was identified as *G. inflata* by HPLC analysis using the same method as described in a previous report.³ In this paper, we report the isolation and structure elucidation of new chromene, benzofuran and chalcone together with seven known phenolic compounds from Xinjiang licorice collected in Xinjiang Uighur Autonomous Region.

Cut licorice (10.5 kg) was extracted with ethyl acetate and, dried *in vacuo* to give ethyl acetate extract. Ethyl acetate extract was subjected to silica gel column chromatography and followed by normal phase- and reversed phase-HPLC purification to afford a new chromene, xinjiastilbene A (**1**), a new benzofuran, xinjiastilbene B (**2**), a chalcone, xinjiachalcone A (**3**), and seven known compounds. The known compounds were characterized by spectral data comparison with those in the literatures.³⁻⁸

Compound **1** was isolated as pale yellow oil. The molecular formula $C_{24}H_{28}O_3$ was determined by HR-EIMS spectrum (m/z 364.2043). Compound **1** was identified as a bibenzyl derivative with a *p*-hydroxybenzyl group [m/z 107; δ 6.74, 7.03 (each 2H, d, $J = 8.3$)], a 1,2,4-trisubstituted benzyl group [δ 6.27, 6.28 (each 1H, brs)] and two benzylic methylenes [δ 2.77, 2.81 (each 2H, m)]. The 1H -NMR spectrum of **1** showed signals due to dimethyl protons at δ 1.40 ppm (6H, s), and a pair of olefinic protons at δ 6.43 (1H, d, $J = 10.0$) and 5.49 (1H, d, $J = 10.0$). The isopentenyl chain showed signals for two methyl protons at δ 1.72 and 1.79 (each 3H, s), an allylic methylene group at δ 4.43 (2H, d, $J = 6.8$) and a vinyl proton at 5.47 (1H, brt, $J=6.8$), confirming that, in this case, the methylene group was bonded to an oxygen atom. The ^{13}C -NMR spectrum and DEPT experiment showed eight quaternary carbons, nine methines, three methylenes and four methyls. The assignment of 1H - and ^{13}C -NMR signals was made by 1H - 1H and 1H - ^{13}C COSY, HMBC and NOE experiments. Observations of the 1H - and ^{13}C -NMR signals [δ_H 1.40 (6H, s), 6.43 (1H, d, $J = 10.0$), 5.49 (1H, d, $J = 10.0$), δ_C 27.6 (q), 77.0 (s), 118.5 (d), 127.5 (d)] indicated the presence of dimethyl chromene in the molecule; these spectral data are similar to those of 2,2-dimethyl-5-(2-phenylethyl)-7-hydroxychromene (**1'**) isolated from *Radula kojana* by Asakawa *et al.* (see Table 1, Figure 1).⁹ The HMBC spectrum showed the correlation of ortho-coupled aromatic proton H2''',6''' (δ 7.03, 2H, d, $J = 8.3$) with C β (δ 36.5, t), dimethyl protons (δ 1.40, 6H, s) with C2 (δ 77.0, s) and C3 (δ 127.5, d), olefinic proton H3 (δ 5.49, 1H, d, $J = 10.0$) with C10 (δ 112.4, s), methylene protons H α (δ 2.81, 2H, m) with C10 (δ 112.4, s), H1'' (δ 4.43, 2H, d, $J = 6.8$) with C7 (δ 158.9, s). NOE spectrum showed the correlation of H6 (δ 6.27 or 6.28, 1H, brs), H8 (δ 6.27 or 6.28, 1H, brs) with H1'' (δ 4.43, 2H, d, $J = 6.8$). These results led to 5-[2-(4-hydroxyphenyl)ethyl]-2,2-dimethyl-7-[(3-methyl-2-butenyl)oxy]-chromene.

Compound **2** was isolated as pale yellow oil. The molecular formula $C_{21}H_{22}O_3$ was determined by HR-EIMS spectrum (m/z 322.1564). Compound **2** was identified as a bibenzyl derivative with a *p*-hydroxybenzyl group [m/z 107; δ 6.74, 7.05 (each 2H, d, $J = 8.3$)], a 1,2,4-trisubstituted benzyl group [δ 6.70 (1H, d, $J = 2.0$), 6.90 (1H, dd, $J = 2.0, 1.0$)] and two benzylic methylenes [δ 2.91, 3.04 (each 2H, m)].

Table 1. ^1H - and ^{13}C -NMR spectral data of Compounds **1** and **1'**

	^1H		^{13}C	
	1	1' ^a	1	1' ^a
α	2.81 (2H, m)	2.80 (2H, brs)	34.7 t	34.4
β	2.77 (2H, m)	2.80 (2H, brs)	36.5 t	37.4
2			77.0 s	75.6
3	5.49 (1H, d, $J=10.0$)	5.50 (1H, d, $J=10.0$)	127.5 d	127.9
4	6.43 (1H, d, $J=10.0$)	6.44 (1H, d, $J=10.0$)	118.5 d	118.7
5			138.4 s	139.1
6	6.27 ^b (1H, brs)	6.18 (1H, s)	108.4 d	108.7
7			158.9 s	155.9
8	6.28 ^b (1H, brs)	6.19 (1H, s)	100.4 d	102.0
9			154.0 s	154.6
10			112.4 s	112.9
1', 2'	1.40 (6H, s)	1.40 (6H, s)	27.6 q	27.7
1''	4.43 (2H, d, $J=6.8$)		64.5 t	
2''	5.47 (1H, brt, $J=6.8$)		119.3 d	
3''			137.7 s	
4''	1.79 (3H, s)		25.8 q	
5''	1.72 (3H, s)		18.1 q	
1'''			133.6 s	141.6
2''', 6'''	7.03 (2H, d, $J=8.3$)	7.17 (2H, m)	129.1 d	128.4
3''', 5'''	6.74 (2H, d, $J=8.3$)	7.28 (2H, m)	114.9 d	128.4
4'''		7.18 (1H, m)	153.3 s	126.0

Data are expressed as ppm from TMS in CDCl_3 .

a) Data are adopted from Asakawa *et al.* (ref.9)

b) These assignments may be interchanged.

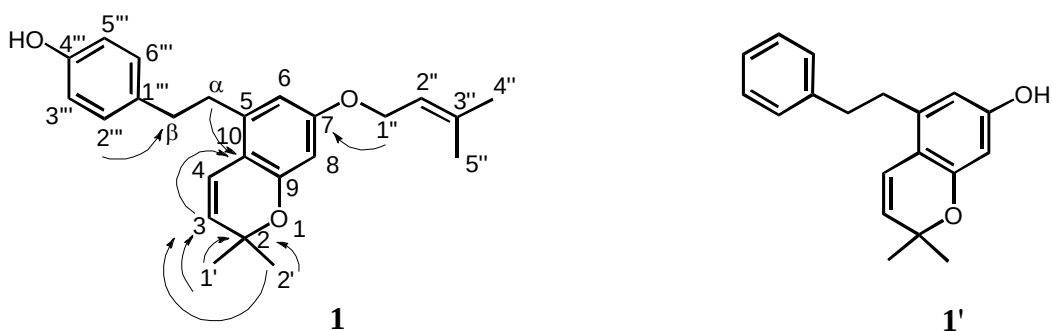


Figure 1. HMBC correlation of compound **1** and structure of compound **1'**

The isopentenyl chain showed signals for two methyl protons at δ 1.76 and 1.80 (each 3H, s), an allylic methylene group at δ 4.52 (2H, d, $J = 6.8$) and a vinyl proton at 5.52 (1H, brt, $J = 6.8$), confirming that, in this case, the methylene group was bonded to an oxygen atom. The ^{13}C -NMR spectrum and DEPT

experiment showed seven quaternary carbons, nine methines, three methylenes and two methyls. The assignment of ^1H - and ^{13}C -NMR signals was made by ^1H - ^1H and ^1H - ^{13}C COSY, HMBC and NOE

Table 2. ^1H - and ^{13}C -NMR spectral data of Compounds **2** and **2'**

	^1H		^{13}C	
	2	2' ^a	2	2' ^a
α	3.04 (2H, m)	3.09 (2H, m)	35.5 t	35.2
β	2.91 (2H, m)	2.99 (2H, m)	35.8 t	36.7
2	7.50 (1H, d, $J=2.2$)	7.47 (1H, d, $J=2.2$)	143.1 d	143.5
3	6.65 (1H, dd, $J=2.2, 1.0$)	6.65 (1H, dd, $J=2.2, 1.0$)	104.5 d	111.5
4			134.9 s	135.2
5	6.70 (1H, d, $J=2.0$)	6.62 (1H, d, $J=2.2$)	111.8 d	104.7
6			156.7 s	154.6
7	6.90 (1H, dd, $J=2.0, 1.0$)	6.83 (1H, dd, $J=2.2, 1.0$)	94.2 d	95.8
8			155.4 s	155.8
9			119.8 s	119.4
1'	4.52 (2H, d, $J=6.8$)		65.1 t	
2'	5.52 (1H, brt, $J=6.8$)		119.3 d	
3'			137.7 s	
4'	1.80 (3H, s)		25.8 q	
5'	1.76 (3H, s)		18.1 q	
1''			133.6 s	141.7
2'',6''	7.05 (2H, d, $J=8.3$)	7.28 (2H, m)	129.1 d	128.3
3'',5''	6.74 (2H, d, $J=8.3$)	7.20 (2H, m)	114.9 d	128.3
4''		7.10 (1H, brs)	153.3 s	125.5

Data are expressed as ppm from TMS in CDCl_3

a) Data are adopted from Asakawa *et al.* (ref. 9)

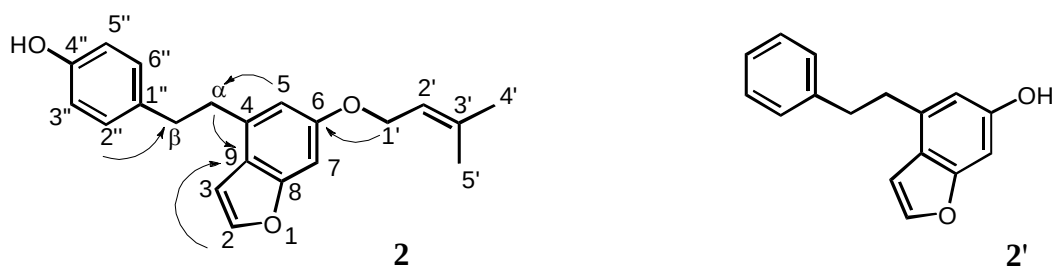


Figure 2. HMBC correlation of compound **2** and structure of compound **2'**

experiments. Observations of the ^1H - and ^{13}C -NMR signals [δ_{H} 6.90 (1H, dd, $J=2.0, 1.0$) 6.70 (1H, d, $J=2.0$), 7.50 (1H, d, $J=2.2$) 6.65 (1H, dd, $J=2.2, 1.0$), δ_{C} 94.2 (d), 111.8 (d), 143.1 (d), 104.5 (d)] indicated the presence of a 4,6-disubstituted benzofuran in the molecule; these spectral data are similar to those of 6-hydroxy-4-(2-phenylethyl)benzofuran (**2'**) isolated from *Radula kojana* by Asakawa *et al.* (see Table 2, Figure 2).⁹ The HMBC spectrum showed the correlation of ortho-coupled aromatic proton H2'',6'' (δ 7.05, 2H, d, $J=8.3$) with C β (δ 35.8, t), olefinic proton H2 (δ 7.50, 1H, d, $J=2.2$) with C9 (δ 119.8, s), aromatic

proton H5 (δ 6.70, 1H, d, J = 2.0) with C α (δ 35.5, t), methylene protons H α (δ 3.04, 2H, m) with C9 (δ 119.8, s), H1' (δ 4.52, 2H, d, J = 6.8) with C6 (δ 156.7, s). The NOE spectrum showed the correlation of H5 (δ 6.70, 1H, d, J = 2.0), H7 (δ 6.90, 1H, dd, J = 2.0, 1.0) with H1' (δ 4.52, 2H, d, J = 6.8). These results led to 4-[2-(4-hydroxyphenyl)ethyl]-6-[(3-methyl-2-butenyl)oxy]benzofuran.

Table 3. ^1H - and ^{13}C -NMR spectral data of Compound 3

	^1H	^{13}C
3		
C=O		187.8 s
α	7.74 (1H, d, J =15.1)	119.9 d
β	8.06 (1H, d, J =15.6)	138.6 d
1		117.3 s
2		160.7 s
3	6.64 (1H, d, J =2.2)	99.4 d
4		162.9 s
5	6.61 (1H, dd, J =8.5, 2.4)	107.4 d
6	7.78 (1H, d, J =8.5)	130.5 d
1'		131.3 s
2',6'	8.04 (2H, d, J =8.8)	131.3 d
3',5'	6.96 (2H, d, J =8.8)	115.8 d
4'		162.0 s
1''	4.65 (2H, d, J =6.6)	65.5 t
2''	5.48 (1H, brt, J =6.6)	120.4 d
3''		138.0 s
4''	1.77 (3H, s)	25.7 q
5''	1.79 (3H, s)	18.2 q
OMe	3.95 (3H, s)	56.0 q

Data are expressed as ppm from TMS in acetone- d_6

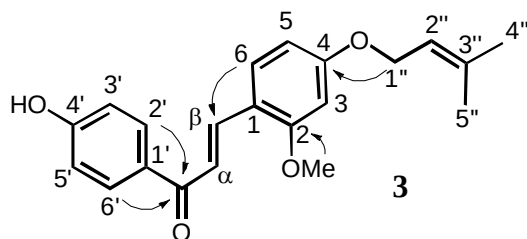


Figure 3. HMBC correlation of compound 3

with C2 (δ 160.7, s), aromatic protons H6 (δ 7.78, 1H, d, J = 8.5) with C β (δ 138.6, d), H2', 6' (δ 8.04, 2H, d, J = 8.5) with carbonyl carbon (δ 187.8, s), methylene proton H1'' (δ 4.65, 2H, d, J = 6.6) with C4 (δ 162.9, s). The NOE spectrum showed the correlation of H3 (δ 6.64, 1H, d, J = 2.2), H5 (δ 6.61, 1H, dd, J

Compound 3 was isolated as light yellow amorphous solid. The molecular formula $\text{C}_{21}\text{H}_{22}\text{O}_4$ was determined by HR-EIMS spectrum (m/z 338.1517). UV, MS, ^1H -, and ^{13}C -NMR spectra of 3 suggested a close similarity in structure with licochalcone C (6). The ^1H -NMR spectrum of 3 showed signals due to methoxyl protons at δ 3.95 ppm (3H, s), a pair of *trans*-olefinic protons at δ 8.06 (1H, d, J =15.6) and 7.74 (1H, d, J = 15.1), a pair of ortho-coupled aromatic protons at δ 8.04 (2H, d, J = 8.5) and 6.96 (2H, d, J = 8.5), ABX-type protons, δ 7.78 (1H, d, J = 8.5), 6.64 (1H, d, J = 2.2), 6.61 (1H, dd, J = 8.5, 2.4). The isopentenyl chain showed signals for two methyl protons at δ 1.79 and 1.77 (each 3H, s), an allylic methylene group at δ 4.65 (2H, d, J = 6.6) and a vinyl proton at 5.48 (1H, brt, J = 6.6), confirming that, in this case, the methylene group was bonded to an oxygen atom. The ^{13}C -NMR spectrum and DEPT experiment showed seven quaternary carbons, ten methines, one methylene and three methyls. The assignment of ^1H - and ^{13}C -NMR signals was made by ^1H - ^1H and ^1H - ^{13}C COSY and HMBC experiments. HMBC spectrum showed the correlation of methoxyl protons (δ 3.95, 3H, s)

= 8.5, 2.4) with H1" (δ 4.65, 2H, d, J = 6.6). These results led to 4'-hydroxy-2-methoxy-4-prenyloxy-chalcone. Although **3** has been previously synthesized, this is the first report of its isolation as a natural product.¹⁰

EXPERIMENTAL

General Procedure

¹H-, ¹³C-NMR and 2D NMR spectra were taken with JNM-BM-400 spectrometer (JEOL) in CDCl₃ or acetone-*d*₆. Chemical shifts are given in δ -values (ppm) with TMS as an internal standard. MS were recorded using JMS-700 Mass spectrometer (JEOL). The UV spectrum was measured in methanol using Shimadzu UV-1700 spectrophotometer

Materials

Xinjiang licorice was purchased in the Khotan district of Xinjiang Uygur Autonomous Region. The materials were analyzed by HPLC under the same condition as described by Hayashi *et al.*³ The characteristic peaks were found to be licochalcone A used as index compounds of *G. inflata*. From these findings, the original plant of the Xinjiang licorice was identified as *G. inflata*.

Extraction and Isolation

Licorice (10.5 kg) was extracted with ethyl acetate (AcOEt 12 L) six times for 6 h at 76 °C. The AcOEt solution was concentrated and then dried *in vacuo* to give dark brown resin (AcOEt extract 750.8 g). AcOEt extract (160 g) was separated into 11 fractions by silica gel column (2.0 kg) chromatography with a gradient mixture of *n*-hexane and AcOEt. Fraction 4 (3.1 g) was carried out using silica gel column chromatography with gradient mixture of *n*-hexane and AcOEt, followed by HPLC using silica gel column (Waters SunFire Silica 5 μ m 10 $\times$ 250 mm) with *n*-hexane and AcOEt (100:3) mixture to give two new compounds (**1**, 1.2 mg), (**2**, 1.4 mg), together with one known compound, paratocarpin B (**10**, 2.0 mg)³, respectively. Fraction 5 (19.0 g) was also separated by silica gel column chromatography, followed by normal-phase HPLC, and gave a new compound, xinjiachalcone A (**3**, 4.9 mg), together with one known compound, kanzonol B (**9**, 11.7 mg),⁴ respectively. Fraction 6 (50.2 g) was also separated by silica gel column chromatography, followed by normal-phase HPLC, and gave licochalcone A (**4**, 72.1 mg)⁵ and licochalcone B (**5**, 15.2 mg),⁶ licochalcone C (**6**, 4.2 mg),⁶ echinatin (**7**, 18.9 mg),⁷ isoliquiritigenin (**8**, 3.6 mg)⁸ respectively.

Xinjiastilbene A (5-[2-(4-hydroxyphenyl)ethyl]-2,2-dimethyl-7-[(3-methyl-2-butenyl)oxy]chromene) (**1**)

pale yellow oil, Formula: C₂₄H₂₈O₃, MW: 364, EI-MS *m/z* (rel. Int. %); 365 [M+1]⁺(3), 364 [M]⁺(11),

349 (13), 296 (14), 281 (100), 174 (22), 107 (9), HR-EI-MS: m/z 364.2043 (calcd for $C_{24}H_{28}O_3$ 364.2038), UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 203 (4.40), 225 (4.27), 278 (3.78), 305 (3.52); ^1H -NMR and ^{13}C -NMR spectra are shown in Table 1.

Xinjiastilbene B (4-[2-(4-hydroxyphenyl)ethyl]-6-[(3-methyl-2-butenyl)oxy]benzofuran) (2)

pale yellow oil, Formula: $C_{21}H_{22}O_3$, MW: 322, EI-MS m/z (rel. Int. %): 323 $[M+1]^+$ (4), 322 $[M]^+$ (16), 254 (65), 215 (12), 147 (32), 107 (100), HR-EI-MS: m/z 322.1564 (calcd for $C_{21}H_{22}O_3$ 322.1569), UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 206 (4.46), 224 (4.30), 249 (3.95), 285 (3.66); ^1H -NMR and ^{13}C -NMR spectra are shown in Table 2.

Xinjiachalcone A (4'-Hydroxy-2-methoxy-4-prenyloxychalcone) (3)

light yellow amorphous, mp: 88.0~91.0 °C, Formula: $C_{21}H_{22}O_4$, MW : 338, EI-MS m/z (rel. Int. %): 339 $[M+1]^+$ (3), 338 $[M]^+$ (12), 307 (19), 270 (20), 239 (100), 121 (17), HR-EI-MS: m/z 338.1517 (calcd for $C_{21}H_{22}O_4$ 338.1518), UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 256 (3.77), 298 (3.80), 361 (3.74); ^1H -NMR and ^{13}C -NMR spectra are shown in Table 3.

ACKNOWLEDGEMENTS

A part of this study was supported by a grant from Japan China Medical Association. Authors are grateful to Assistant Professor Dr. Makio Shibano at Osaka University of Pharmaceutical Sciences in Japan.

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