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# 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 Xinjang Uighur Autonomous Region in China, and in the course of our search for the active ingredients of licorice, we examined the constituents of Xinjang 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 Xinjang licorice collected in Xinjang 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 $^1$H-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 $^1$H- and $^{13}$C-NMR signals was made by $^1$H-$^1$H and $^1$H-$^{13}$C COSY, HMBC and NOE experiments. Observations of the $^1$H- and $^{13}$C-NMR signals [$\delta_H$ 1.40 (6H, s), 6.43 (1H, d, *J* = 10.0), 5.49 (1H, d, *J* = 10.0), $\delta_C$ 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 Cβ (δ36.5, t), dimethyl protons (δ 1.40, 6H, s) with C2 (δ77.0, s) and C3 (δ127.5, d), olefinic proton H3 (δ 5.49, 1H, d, *J* = 10.0) with C10 (δ 112.4, s), methylene protons Hα (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)].

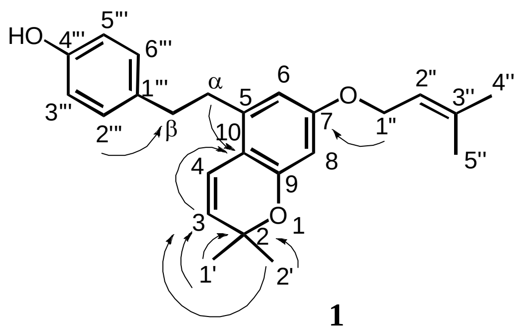
Table 1. $^{1}$H- and $^{13}$C-NMR spectral data of Compounds **1** and **1'**

| | $^{1}$H | | $^{13}$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 |

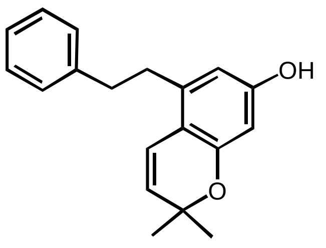
Data are expressed as ppm from TMS in $CDCl_3$.

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

b) These assignments may be interchanged.



**1**



**1'**

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

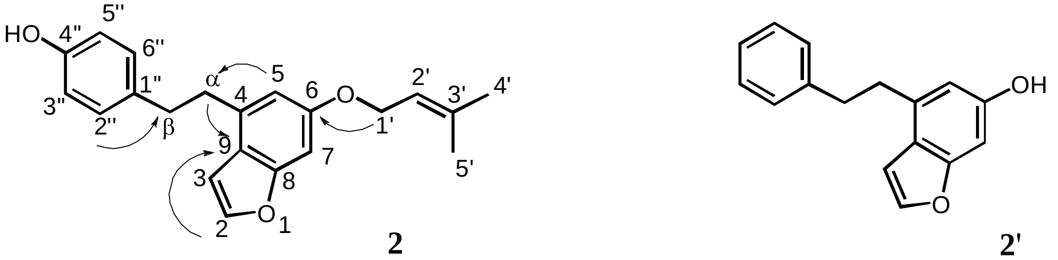
The isopentenyl chain showed signals for two methyl protons at δ 1.76 and 1.80 (each 3H, s), an allylic methylene group at δ 4.52 (2H, d, *J* = 6.8) and a vinyl proton at 5.52 (1H, brt, *J* = 6.8), confirming that, in this case, the methylene group was bonded to an oxygen atom. The $^{13}$C-NMR spectrum and DEPT experiment showed seven quaternary carbons, nine methines, three methylenes and two methyls. The assignment of $^{1}$H- and $^{13}$C-NMR signals was made by $^{1}$H-$^{1}$H and $^{1}$H-$^{13}$C COSY, HMBC and NOE experiments. Observations of the $^{1}$H- and $^{13}$C-NMR signals [$\delta_{H}$ 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), $\delta_{C}$ 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 Cβ (δ35.8, t), olefinic proton H2 (δ 7.50, 1H, d, *J*=2.2) with C9 (δ 119.8, s), aromatic

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

| | $^{1}$H | | $^{13}$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)



**2** **2'**

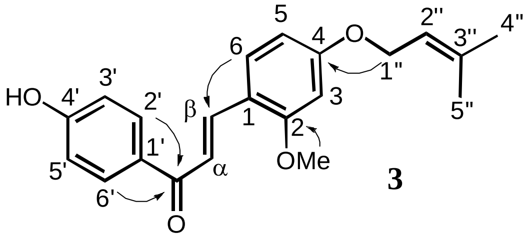
Figure 2. HMBC correlation of compound **2** and structure of compound **2'**

proton H5 (δ 6.70, 1H, d, $J$ = 2.0) with Cα (δ 35.5, t), methylene protons Hα (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. $^1$H- and $^{13}$C-NMR spectral data of Compound **3**

| | $^1$H | $^{13}$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-$d_6$



**3**

Figure 3. HMBC correlation of compound **3**

Compound **3** was isolated as light yellow amorphous solid. The molecular formula $C_{21}H_{22}O_4$ was determined by HR-EIMS spectrum ($m/z$ 338.1517). UV, MS, $^1$H-, and $^{13}$C-NMR spectra of **3** suggested a close similarity in structure with licochalcone C (**6**). The $^1$H-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 $^1$H- and $^{13}$C-NMR signals was made by $^1$H-$^1$H and $^1$H-$^{13}$C COSY and HMBC experiments. HMBC spectrum showed the correlation of methoxyl protons (δ 3.95, 3H, s) with C2 (δ 160.7, s), aromatic protons H6 (δ 7.78, 1H, d, $J$ = 8.5) with Cβ(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, $J$

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

## EXPERIMENTAL

### General Procedure

$^1$H-, $^{13}$C-NMR and 2D NMR spectra were taken with JNM-BM-400 spectrometer (JEOL) in $CDCl_3$ 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

Xinjang 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 ºC. 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 5μm 10 × 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: $C_{24}H_{28}O_3$, 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 $\lambda_{max}^{MeOH}$ nm (log ε): 203 (4.40), 225 (4.27), 278 (3.78), 305 (3.52); $^1$H-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 $\lambda_{max}^{MeOH}$ nm (log ε): 206 (4.46), 224 (4.30), 249 (3.95), 285 (3.66); $^1$H-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 ºC, 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 $\lambda_{max}^{MeOH}$ nm (log ε); 256 (3.77), 298 (3.80), 361 (3.74); $^1$H-NMR and $^{13}$C-NMR spectra are shown in Table 3.

## ACKNOWLEDGEMENTS

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## REFERENCES AND NOTES

1. Part II of the series as “Studies of Traditional Folk Medicines in Xinjiang Uighur Autonomous.” Part I : H. Aishan, M. Baba, and T. Okuyama, The Constituents of *Urtica cannabina* used in Uighur Medicine. *Pharmaceutical Biology*, to be published.
2. H. Hayashi, M. Shibano, G. Kusano, H. Yamamoto, and Y. Ikeshiro, *Natural Medicines*, 1998, **52**, 259.
3. Y. Hano, N. Itoh, A. Hanaoka, Y. Itoh, and T. Nomura, *Heterocycles*, 1995, **41**, 191.
4. T. Fukai, J. Nishizawa, and T. Nomura, *Phytochemistry*, 1994, **35**, 515.
5. H. Nagai, J. X. He, T. Tani, and T. Akao, *J. Pharm. Pharmacol.*, 2007, **59**, 1421.
6. K. Kajiyama, S. Demizu, Y. Hiraga, K. Kinoshita, K. Koyama, K. Takahashi, Y. Tamura, K. Okada, and T. Kinoshita, *Phytochemistry*, 1992, **31**, 3229.
7. H. Achenbach, M. Stocker, and M. A. Constenla, *Phytochemistry*, 1988, **27**, 1835.
8. Q. Liu and Y. Liu, *Acta Pharm. Sinica*, 1989, **24**, 525.
9. Y. Asakawa, K. Kondo, M. Tori, T. Hashimoto, and S. Ogawa, *Phytochemistry*, 1991, **30**, 219.
10. A. Islam and M. A. Hossain, *Indian J. Chem.*, 1993, **32B**, 713.