Secoiridoid Sulfonates from the Sulfiting-Processed Buds of *Lonicera japonica*

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The new secoiridoid sulfonates **1**–**3** were isolated from the 50% EtOH/H_2O extract of the sulfiting-processed *Lonicera japonica Flos* (*LJF*) by semi-prep. HPLC, and their structures were identified on the basis of mass spectrometry and NMR spectroscopy. HPLC and LC-DAD-MS/MS analyses of the different samples of *LJF* obtained by various process techniques suggested that the sulfur fumigation led to the decrease of secologanic acid (**4**) and the formation of secologanic acid-derived sulfonate **1** and its derivatives **2**, **2a**, and **3** in the crude materials, which revealed that sulfur fumigation, the traditional process technique, could alter the phytochemical profiles of some Chinese herbal medicines.

Introduction. – The sulfiting process is widely used in foods, beverages, and drugs [1] for a variety of important technical purposes, including the control of enzymatic and nonenzymatic browning and antimicrobial actions [2]. Sulfiting agents usually consist of sodium or potassium metabisulfite, bisulfite, and sulfite [3], and sometimes, Chinese herb medicines are also treated with sulfur dioxide gas by burning sulfur. In addition to the residue content of free and conjugated sulfites, also much attention is paid to the influence of the sulfiting process on the active constituents in the treated herbal materials. For example, several recent reports [4–9] indicate that sulfur fumigation can alter the phytochemical profiles of white peony root, due to bisulfite addition to the hemiketal group of paeoniflorin.

Lonicera japonica Flos (*LJF*), one of the most important dietary sources and traditional Chinese medicines (TCM) in China, was widely used for the treatment of various diseases including arthritis, diabetes mellitus, fever, infection, sore, and swelling [10]. Numerous compounds such as alkaloids, cerebrosides, flavonoids, iridoids, polyphenols, and triterpenoid saponins have been reported from various parts of *Lonicera japonica* THUNB. [11–13]. The traditional process methods for *LJF* consist of roasting, sun drying and shade drying. In some main production areas such as in the Shandong Province, however, sulfur fumigation is used before drying during the preparation of *LJF*.

Upon HPLC analyses of the different samples obtained by various process techniques, such as sun drying, shade drying, and sulfur fumigation, new chromatographic peaks were found in the HPLC profiles of the sulfur-fumigated samples of *LJF*

[14]. In the present investigation, LC-DAD-MS/MS analysis was conducted for the identification of the derived compounds **1**, **2**, **2a**, and **3** as well as the naturally occurring known compound **4** in the sulfur-fumigated samples. Compounds **1**–**3** were further isolated by semi-prep. HPLC, and their structures were elucidated on the basis of mass and NMR spectroscopy (*Fig. 1*).

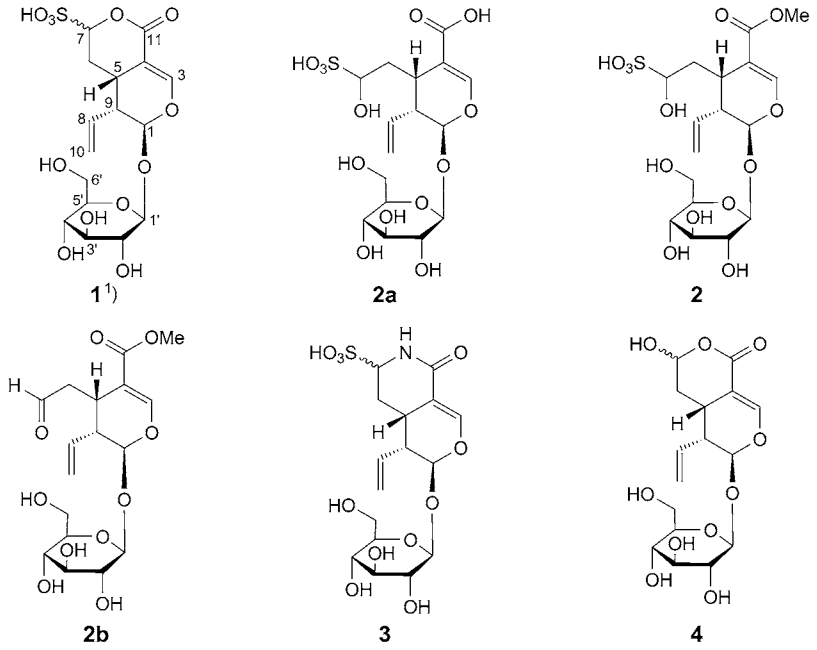

Fig. 1. *Secologanic acid* (**4**) *and its derivatives from sulfur-fumigated samples of* LJF

Results and Discussion. – Compound **1** showed a λ_{max} at 247 nm in the on-line UV spectrum, and its molecular formula was established as $C_{16}H_{22}O_{12}S$ by a negative-mode HR-ESI-MS experiment (*m*/*z* 437.0750 ($[M-H]^-$)). Compound **1** was poorly ionized in the positive ESI mode, however, the negative mode ESI-MS gave more information. A prominent loss of 64 Da from the deprotonated molecular ion at *m*/*z* 437.1 ($[M-H]^-$), corresponding to the loss of neutral SO_2, was observed in the MS^2. The MS^3 of the $[M-SO_2-H]^-$ ion at *m*/*z* 373 showed fragment ions identical to those of secologanic acid (**4**), including the characteristic loss of 180 Da (neutral $C_6H_{12}O_6$) due to the elimination of the glucose unit. The above evidences suggested that compound **1** could be a secologanic acid-derived sulfonate. The ^{1}H-NMR spectrum of **1** (*Table 1*) exhibited the typical H–C(3)[1]) of a secoiridoid at δ(H) 7.66, and the ^{13}C-NMR spectrum (*Table 1*) displayed sixteen C-atom signals. The comparison of the ^{13}C- and ^{1}H-NMR data of **1** with those reported in the literature [15] indicated the structural similarity of **1** and **4**, with no additional H- or C-atom. The chemical shifts of **1** were identical with those of secologanic acid (**4**), except for the signals of H–C(7) and C(7),

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

which were shifted upfield by 0.6 and 11.3 ppm, respectively. These chemical-shift differences were consistent with those described in the literature for a prodcut resulting from the 'SO_2' addition into a hemiketal group [4]. Accordingly, **1** was deduced as the addition product of secologanic acid, with the sulfonate substitution at C(7). The HMQC and HMBC experiments (*Table 1*, *Fig. 2*), also confirmed that the sulfonate group was located at C(7) in **1**, and that compound **1** possessed a C-atom skeleton identical to that of **4**. The 'SO_2' addition into a hemiketal group was solely related to the position C(7) in the lactone ring, and therefore, the configuration at C(1), C(5), and C(9) in the structure of **1** was the same as that of the parent compound, secologanic acid (**4**). The key NOESY correlations between H–C(7) at δ(H) 5.19 (*dd*, $J = 12.0$, 2.4 Hz) and H_β–C(6) at δ(H) 2.27 (*dd*, $J = 12.8$, 2.4 Hz) as well as H–C(5) at δ(H) 3.12,

Table 1. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp., D_2O) *of* **1**[1]). δ in ppm, J in Hz.

Position	δ(H)	δ(C) (DEPT)	HMBC	NOESY
H–C(1)	5.56 (br. *s*)	97.6 (CH)	C(3), C(5), C(1′)	H–C(9), H–C(1′)
H–C(3	7.66 (*s*)	154.4 (CH)	C(1), C(4), C(5), C(11)	
C(4)		103.2 (C)		
H–C(5)	3.12 (*m*)	25.0 (CH)	C(4), C(8)	H–C(9)
CH_2(6)	1.72 (*dd*, $J = 12.8$, 12.0, H_α),	25.1 (CH_2)	C(4), C(5), C(7), C(9)	
	2.27 (*dd*, $J = 12.8$, 2.4, H_β)		C(4), C(5)	
H–C(7)	5.19 (*dd*, $J = 12.0$, 2.4)	87.4 (CH)		H–C(5), H_β–C(6)
H–C(8)	5.49 (*m*)	130.7 (CH)	C(9)	H_α–C(6), H–C(9), H–C(10)
H–C(9)	2.82 (*m*)	41.5 (CH)	C(4), C(6), C(8), C(10)	
CH_2(10)	5.28 (*d*, $J = 10.2$, H_a),	121.3 (CH_2)	C(9)	H–C(9)
	5.33 (*d*, $J = 17.4$, H_b)			
C(11)		167.2 (C=O)		
H–C(1′)	4.80 (*d*, $J = 7.8$)	98.4 (CH)	C(1)	
H–C(2′)	3.24 (*t*, $J = 9.0$)	72.6 (CH)	C(1′), C(3′)	
H–C(3′)	3.47 (overlapped)	76.3 (CH)		
H–C(4′)	3.35 (*t*, $J = 9.0$)	69.6 (CH)	C(6′)	
H–C(5′)	3.45 (overlapped)	75.5 (CH)		
CH_2(6′)	3.68 (*dd*, $J = 12.6$, 6.0, H_a),	60.7 (CH_2)	C(5′)	H_b–C(6′)
	3.86 (*d*, $J = 12.6$, H_b)		C(4′)	

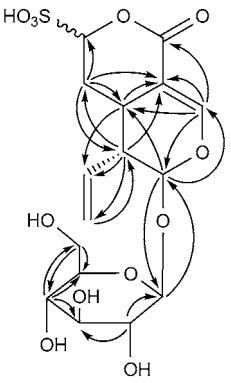

Fig. 2. *Key HMBC features* (H → C) *of compound* **1**

indicated that the H-atoms of H–C(7), H_β–C(6) and H–C(5) were on the same side of the ring.

Compound **2** showed a λ_{max} at 242 nm in the on-line UV spectrum and a relative molecular mass of 470 (*m/z* 469 for the $[M-H]^-$ ion). The MS^2 of the $[M-H]^-$ ion of **2** was dominated by the loss of 18 Da (H_2O), which was also observed in the MS^1 with high abundance due to in-source decay. Similarly, a prominent loss of 64 Da from the $[M-H-H_2O]^-$ ion at *m/z* 451 of **2**, corresponding to the loss of neutral SO_2, was observed in the MS^2. The other signals at *m/z* 355 for the $[387-MeOH]^-$ and 255 for the $[387-glucose]^-$ ions further indicated fragmentation pathways identical to those of compound **1**. This information suggested that **2** could be a derivative of **1**. Although the purity of collected compound **2** looked well (Fig. S1E[2]), no NMR data of **2** could be obtained due to its degradation on removal of the solvent after the preparative isolation. Instead, secologanin (**2b**), the iridoid building block of a majority of the terpenoid indole alkaloids [16–18], was obtained when the collected fraction containing compound **2** was rapidly lyophilized (Fig. S1F[2]). According to the on-line UV and MS information as well as the derived product secologanin, the structure of compound **2** was tentatively identified.

Compound **2a** could not be detected in the UV chromatogram due to its low amount in the sulfiting-processed *LJF* sample (Fig. S2C[2]); however, it was easily detected by negative-mode LC/MS with high sensitivity (Fig. S3C[2]). Compound **2a** had the relative molecular mass of 456 according to the $[M-H]^-$ ion at *m/z* 455, which was 18 mass units more than **1** and 14 mass units less than **2**. Moreover, compound **2a** displayed a similar fragmentation pattern to that of **1** and **2**, e.g., the ion at *m/z* 373 was explained as $[M-H_2O-SO_2-H]^-$ for the loss of neutral SO_2 and H_2O, and the other ions at *m/z* 193 ($[373-glucose-H]^-$) and 149 ($[373-glucose-CO_2-H]^-$) were observed in the MS^2. Compound **2a** was deduced as the lactone-ring-opened derivative of compound **1**, solely based on the above MS information.

Compound **3** exhibited a λ_{max} at 243 nm in the on-line UV spectrum, and its molecular formula was established as $C_{16}H_{23}NO_{11}S$ by the positive-mode HR-ESI-MS (*m/z* 438.1057 ($[M+H]^+$)) and negative-mode ESI-MS (*m/z* 436 ($[M-H]^-$)). The relative molecular mass of **3** was only 1 mass unit less than that of **1**, and the MS^2 of the $[M-H]^-$ ion showed the same characteristic fragmentations as secoiridoid sulfonates **1**, **2** and **2a**, indicating the loss of neutral SO_2 and H_2O. The ^{1}H-NMR spectrum of **3** (*Table 2*) exhibited the typical H–C(3)[1]) of a secoiridoid at δ(H) 7.22. The ^{13}C- and ^{1}H-NMR data of **3** (*Table 2*) were similar to those of secologanic acid-derived sulfonate **1**, with the exception of the signals for H–C(7) and C(7), which moved upfield from δ(H) 5.19 to 3.95 and from δ(C) 87.4 to 66.3, respectively. Comprehensive analyses on the NMR and MS data indicated that compound **3** was the lactam analog of lactone **1**, namely, the O-atom attached to C(11)=O was replaced by an NH group. In the HMBC experiment (*Table 2*), the key correlation δ(H) 1.42 (H_α–C(6))/δ(C) 66.3 (C(7)) provided another evidence, supporting the change from a lactone to lactam ring. So, the structure of **3** was determined and shown in *Fig. 1*.

Compound **4** exhibited a λ_{max} at 245 nm in the on-line UV spectrum, and its molecular formula was determined as $C_{16}H_{22}O_{10}$ based on the positive-mode ESI-MS

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[2]) These supplementary electronic data are available upon request from the authors.

Table 2. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp., (D_6)DMSO) *of* **3**[1]). δ in ppm, J in Hz.

Position	δ(H)	δ(C) (DEPT)	HMBC
H–C(1)	5.33 (br. *s*)	95.6 (CH)	C(3), C(5), C(1′)
H–C(3)	7.22 (*s*)	146.7 (CH)	C(1), C(4), C(5), C(11)
C(4)		107.9 (C)	
H–C(5)	2.90 (*m*)	26.1 (CH)	
CH_2(6)	1.42 (*dd*, J = 13.2, 12.0, H_α), 1.97 (*dd*, J = 13.2, 3.6, H_β)	26.6 (CH_2)	C(7)
H–C(7)	3.95 (*dd*, J = 12.0, 3.3)	66.3 (CH)	
H–C(8)	5.46 (*m*)	133.5 (CH)	C(9)
H–C(9)	2.65 (*m*)	42.9 (CH)	C(1), C(4), C(6), C(8), C(10)
CH_2(10)	5.24(*d*, J = 10.2, H_a), 5.33 (*d*, J = 16.8, H_b)	120.4 (CH_2)	C(9)
C(11)		164.3 (C=O)	
H–C(1′)	4.48 (*d*, J = 7.8)	98.5 (CH)	C(1)
H–C(2′)	3.04 (*t*, J = 9.0)	73.5 (CH)	
H–C(3′)	3.14 (overlapped)	77.0 (CH)	
H–C(4′)	2.98(*t*, J = 9.0)	70.5 (CH)	
H–C(5′)	3.14(overlapped)	77.7 (CH)	
CH_2(6′)	3.44 (*dd*, J = 11.6, 4.2, H_a), 3.68 (*d*, J = 11.6, H_b)	61.5 (CH_2)	

(m/z 397 ($[M+Na]^+$)), negative-mode ESI-MS (m/z 373 ($[M-H]^-$)), and negative-mode HR-ESI-MS (m/z 373.1151 ($[M-H]^-$)). The MS^2 of the $[M-H]^-$ ion at m/z 373 displayed the characteristic loss of 180 Da (neutral $C_6H_{12}O_6$) due to the elimination of the glucose unit attached to position C(1)[1]). The base peak in the MS^2, namely the fragmentation ion at m/z 193 ($[M-H-Glc]^-$), further gave the ion at m/z 149 in the MS^3, resulting from the loss of CO_2. The above-described fragmentation pathways were in accordance with those of secoiridoid glycosides [19]. Compound **4** was identified as secologanic acid, and it was previously isolated from *L. japonica* [20], *L. coerulea* [21], and *L. ruprechtiana* [22] of Caprifoliaceae.

According to the HPLC and total-ion-current (TIC) profiles of different *FLJ* samples recorded under the same conditions (Figs. S2 and S3[2])), compounds **1**, **2**, **2a**, and **3** were only found to occur in the sulfur-fumigated sample and were not detected in the sun-dried or shade-dried samples. Considering their structures and the process procedures of the crude samples, it could be deduced that the major compound **1** was derived from secologanic acid (**4**) due to the sulfur fumigation. As shown in Fig. S2, the relative ratio of the chromatographic peak representing compound **4** in the sun-dried or shade-dried *LJF* samples was much higher than that in the sulfur-fumigated sample. The chromatographic peak **4** in the HPLC profile of each crude *LJF* sample was identified by comparison of the on-line UV spectra, the deprotonated molecular ion, and the MS fragmentation pathways. The present interesting findings suggest that the sulfur fumigation led to the decrease of compound **4** and the formation of compound **1** and its related derivatives in the crude materials.

A number of diverse compounds, such as phenolic acid (= hydroxybenzoic acid), a flavonoid, a triterpenoid saponin, as well as an iridoid glycoside, have been isolated from *LJF* samples [11], and most of them are highly reactive; however, only

secologanic acid (**4**) with a hemiketal group reacted to give the corresponding hydroxysulfonate product **2a** when *LJF* was sulfur-fumigated. A similar transformation was reported during the sulfur fumigation of *Paeonia lactiflora* root, and the proposed reaction mechanism was stimulated by converting paeoniflorin, α-D-glucose, and β-D-glucose to their sulfonates [4], respectively. These previous and present investigations revealed that sulfur fumigation, the traditional technique, could alter the phytochemical profiles of processed herbs, and that even such an alteration had a high structural selectivity for the chemical constituents naturally occurring in the crude herbs.

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Experimental Part

General. Macroporous resin *HPD$_{100}$* was purchased from *Hebei Baoen Chemical Corporation* (Hebei, P. R. China). HPLC grade MeOH (*Tedia*, USA), MeCN (*Fisher*, USA) and ultra-purity H_2O were used for HPLC analysis. All other reagents and solvents were of anal. grade and obtained from *Beijing Chemical Company* (Beijing, P. R. China). CC = Column chromatography. HPLC: *Alltech* system (*Alltech Associates, Inc.*, USA), equipped with a binary *426* solvent delivery pump and a UV *2000* detector. NMR Spectra: *Bruker-Avance-600* spectrometer; at 600 (^{1}H) and 150 (^{13}C) MHz; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Bruker-Apex-IV* FT-MS spectrometer; in *m*/*z*.

Plant Material. The fresh *LJF* samples were collected in Linyi County, Shandong Province, P. R. China, in May 2007 and identified by Prof. *Zhi-Min Wang* as the buds of *Lonicera japonica* THUNB. The fresh samples were processed by the following procedures: the sun-dried sample was obtained by drying in the sun, the shade-dried sample was obtained by drying gradually in the shade, and the sulfur-fumigated sample was obtained by fumigating with SO_2 gas and then drying. The voucher specimens (No. LJF 20070514-83) were deposited with our laboratory.

Semi-preparative Isolation of Compounds **1**–**3**. The sulfur fumigated *LJF* samples (dry weight, 1.0 kg) were extracted by a percolation process with EtOH/H_2O 1 : 1. The percolate was concentrated and then fractioned by CC (macroporous resin *HPD$_{100}$*, H_2O). For the semi-prep. HPLC purification, 0.72 g of the H_2O fraction was dissolved with ultra-purity H_2O to give a soln. of 120 mg · ml^{-1}.

Semi-prep. separation was performed by HPLC (*Xtimate-C$_{18}$* column (10.0 × 250 mm, 5 μm; *Welch Materials Inc.*, USA), flow rate 3.0 ml min^{-1}; linear gradient with *A* = H_2O (adjusted to pH 2.0 with CF_3COOH) and *B* = MeCN, *i.e.*, 8% *B* → 13% *B* in 12 min; injected volume per run, 200 μl; detection at 254 nm). The fractions containing peak **1**, **2**, and **3** were manually collected and combined: **1** (138 mg), **2b**, and **3** (54 mg). The purity of the compounds was determined by anal. HPLC (*Xtimate-C$_{18}$* column (4.6 × 250 mm, 5 μm; *Welch Materials Inc.*, USA); flow rate 1.0 ml min^{-1}).

*(4a*S*,5*R*,6*S*)-5-Ethenyl-6-(β-*D*-glucopyranosyloxy)-4,4a,5,6-tetrahydro-1-oxo-1*H*,3*H*-pyrano[3,4-*c*]pyran-3-sulfonic Acid* (**1**): White amorphous powder. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (neg.): 875 ($[2M-H]^-$), 437 ($[M-H]^-$), 393 ($[M-H-CO_2]^-$), 373 ($[M-H-SO_2]^-$), 355 ($[M-H-SO_2-H_2O]^-$), 303, 193 ($[M-H-SO_2-Glc]^-$), 149 ($[373-Glc-CO_2]^-$). HR-ESI-MS (neg.): 437.0750 ($[M-H]^-$; calc. 437.0759).

*(2*S*,3*R*,4*S*)-3-Ethenyl-2-(β-*D*-glucopyranosyloxy)-3,4-dihydro-4-(2-hydroxy-2-sulfoethyl)-2*H*-pyran-5-carboxylic Acid 5-Methyl Ester* (**2**): ESI-MS (neg.): 469 ($[M-H]^-$), 451 ($[M-H-H_2O]^-$), 387 ($[M-H-H_2O-SO_2]^-$), 355 ($[M-H-H_2O-SO_2-MeOH]^-$), 255 $[M-H-H_2O-SO_2-Glc]^-$).

*(2*S*,3*R*,4*S*)-3-Ethenyl-2-(β-*D*-glucopyranosyloxy)-3,4-dihydro-4-(2-oxoethyl)-2*H*-pyran-5-carboxylic Acid Methyl Ester* (= *Secologanin*; **2b**): White powder. ^{1}H-NMR ((D$_6$)DMSO, 600 MHz)[1]): 9.63 (*s*, CHO); 7.49 (*s*, H–C(3)); 5.41 (*d*, *J* = 4.8, H–C(1)); 4.50 (*d*, *J* = 7.8, H–C(1′)); 3.60 (*s*, MeO). ^{13}C-NMR ((D$_6$)DMSO, 150 MHz)[1]): 95.9 (C(1)); 152.8 (C(3)); 108.7 (C(4)); 26.5 (C(5)); 44.1 (C(6)); 202.0 (C(7)); 134.2 (C(8)); 43.9 (C(9)); 120.4 (C(10)); 167.0 (C(11)); 51.5 (MeO); 99.1 (C(1′)); 73.5 (C(2′)); 77.8

(C(3′)); 70.5 (C(4′)); 77.1 (C(5′)); 61.6 (C(6′)). ESI-MS (pos.): 389 ($[M+H]^+$). HR-ESI-MS (pos.): 389.1451 ($[M+H]^+$; calc. 389.1442).

*(3*S*,4*R*,5*S*)-4-Ethenyl-3-(β-*D*-glucopyranosyloxy)-4,4a,5,6,7,8-hexahydro-8-oxo-3*H*-pyrano[3,4-*c*]-pyridine-6-sulfonic Acid* (**3**): White amorphous powder. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS (neg.): 873 ($[2M-H]^-$), 436 ($[M-H]^-$), 418 ($[M-H-H_2O]^-$), 354 ($[M-H-H_2O-SO_2]^-$), 192 ($[M-H-SO_2-Glc]^-$), 174 ($[M-H-H_2O-SO_2-Glc]^-$). HR-ESI-MS (pos.): 438.1057 ($[M+H]^+$; calc. 438.1065).

*Preparation of (4a*S*,5*R*,6*S*)-5-Ethenyl-6-(β-*D*-glucopyranosyloxy)-4,4a,5,6-tetrahydro-3-hydroxy-1*H*,3*H*-pyrano[3,4-*c*]pyran-1-one* (= *Secologanic Acid*; **4**) *for MS*^{n} *and HR-ESI-MS Analyses.* The shade-dried *LJF* sample (dry weight, 0.4 g) was extracted with MeOH/H_2O 1:1 (50 ml) by ultrasonication for 20 min at r.t., and the extract was subjected to HPLC (linear gradient with $A = H_2O$ (adjusted to pH 2.0 with HCOOH) and B = MeCN, *i.e.*, 3% $B \rightarrow 15\%$ B in the first 20 min, then 15% $B \rightarrow 24\%$ B in the next 25 min; injected volume per run, 20 µl; detection at 254 nm). The fraction containing **4** was manually collected. LC/MS^n and HR-ESI-MS Analyses were carried out. ESI-MS (pos.): 397 ($[M+Na]^+$). ESI-MS (neg.): 373 ($[M-H]^-$), 193 ($[M-H-Glc]^-$), 149 ($[M-H-Glc-CO_2]^-$). HR-ESI-MS (neg.): 373.1151 ($[M-H]^-$; calc. 373.1140).

Sample Preparation for HPLC and LC/MS^n *Analyses.* Each of the powdered sample (0.4 g, 60 mesh), obtained by different process techniques, was extracted with MeOH/H_2O 1:1 (50 ml) by ultrasonication for 20 min at r.t. The extract was filtered through a 0.22-µm micropore membrane (*Jinteng Corp.*, Tianjin, P. R. China) and used for HPLC and LC/MS^n analyses.

HPLC Analysis of the LJF *Samples Obtained by Different Process Techniques.* The analyses were performed at r.t. by HPLC (*Alltech-C*$_{18}$ column (4.6 × 250 mm, 5 µm), linear gradient with $A = H_2O$ (adjusted to pH 2.0 with HCOOH) and B = MeCN, *i.e.*, 3% $B \rightarrow 15\%$ B in the first 20 min, then 15% $B \rightarrow 24\%$ B in the next 25 min, and then 24% $B \rightarrow 40\%$ B in the last 10 min; flow rate 1.0 ml min^{-1}; detection at 254 nm; A injected volume, 10 µl).

LC/ESI-MS^n *Analysis of the* LJF *Samples Obtained by Different Process Techniques.* LC/MS Analyses were carried out with an *Agilent-6320* ion trap mass spectrometer connected to an *Agilent-1200* HPLC system (*Agilent Technologies*, Waldbronn, Germany). The HPLC conditions were the same as those used for the HPLC analysis. The LC effluent was introduced into an electrospray ionization source after a post-column split ratio of 1:4. The optimized parameters for LC/MS analysis in the negative-ion mode were as follows: dry temp. 350°, nebulizer 50.0 psi, and dry gas 12.0 l min^{-1}. Full-scan mass spectra were acquired in the range m/z 100–1000. The $[M-H]^-$ ions were selected and subjected to MS^n analysis.

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