CHEMICAL STUDY FOR SECONDARY METABOLITES IN THE ROOTS OF POLYGONUM STRINDBERGII SCHUST. IN BULL. HERB. BOISS. SER.

Hong-yan Ma[a], Li-rong Nie[b] and Shun Yao[b]*

Keywords: Polygonum Strindbergii Schust. in Bull. Herb. Boiss. ser., secondary metabolites, Ionic liquids, separation and identification.

To study the secondary metabolites in *Polygonum Strindbergii Schust*. in Bull. Herb. Boiss. ser. in Yunnan province of China, the ground roots were extracted with IL (ionic liquid) aqueous solution, then partitioned by organic solvents and isolated by column chromatography repeatedly. Eight compounds were obtained and their structures were identified by spectral methods, which were found from the plant for the first time. Moreover, the key conditions of ILs application were investigated, which are wished to be helpful for related researchers. The plant is supposed to be further utilized and developed as a meaningful natural resource in the genus.

* Corresponding Authors
Fax & Tel: +86-028-85405221
E-Mail: cusack@scu.edu.cn
[a] School of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou, P. R. China
[b] Department of Pharmaceutical and Biological Engineering, Sichuan University, Chengdu 610065, P. R. China

Introduction

Because of the existence of various meaningful secondary metabolites in natural resources, systematic chemical research for them, discovery of bioactive constituents and the relationship between chemistry and biodiversity are always attractive tasks for chemists in related fields. Polygonum Strindbergii Schust. in Bull. Herb. Boiss. ser. (abbreviated as 'PSS' in the following content) is a relatively rare perennial herb distributed in the southwest regions of China, which mainly include Yunnan, Guizhou and Tibet areas (see Fig.1). It is usually found in brae, forest, valley at an altitude of over 2000~3000 meters.[1] Although many plants of Polygonaceae have been studied, there is no report about the secondary metabolites in PSS up to now.

Figure 1. The plant of PSS and its distribution in China

Ionic liquids (ILs), also known as molten salts, are composed of organic cations and inorganic or organic anions in liquid status near room temperature. In recent years, ILs have attracted extensive attention in modern chemistry and have been successfully applied in extraction process as green solvents,[2-4] which have great application potential for natural products with a wide range of polarity. So it is much anticipated for the combination of green chemistry and natural product chemistry in daily laboratory work.

In order to explore symbolic secondary metabolites in this rare plant, the present paper introduced the isolation and structural elucidation of eight compounds for the first time. Moreover, ILs are applied in the study and the key conditions were investigated, which are wished to be meaningful and helpful for related researchers.

Materials and Methods

Instruments and reagents

The melting point was measured with an XRC-1 microscope melting-point apparatus and was not corrected. IR (KBr-discs) spectra were recorded by Thermo-Nicolet AVATAR 360-FTIR spectrometer. NMR spectra were recorded at 300K on Bruker ACF-500 NMR instrument (^{1}H: 300 MHz, ^{13}C: 125 MHz), with TMS as internal standard. Mass spectra were obtained on ~~MS~~ Agilent 1100 Series with LC/MSD Trap Mass spectrometer. HPLC analysis was performed with an LC-20AT pump, an SPD-M20A PDA detector (Shimadzu, Kyoto, Japan), a Waters symmetry C_{18} column, 5 μm, 3.9×150 mm i.d. (Waters, Massachusetts, USA), and an HCT-360 LC column cooler/heater (Hengao Tech & Dev, Tianjin, China). A Class-VP workstation (Shimadzu, Kyoto, Japan) was used for data acquisition. Silica gel H (200-300 mesh, Ocean Chemical Co. Ltd, Qingdao, China) and Sephadex LH-20 (Pharmacia) were used for column chromatography. Other reagents were of analytical purity.

Plant material

The roots of PSS were purchased from Yunnan Province, China, and the roots were dried, ground and controlled in 0.45~0.90 mm by passed through a stainless steel sieve.

Synthesis of ILs

Seven kinds of 1-alkyl-3-methylimidazolium ILs ($[C_4MIM][BF_4]$, $[C_4MIM][CH_3SO_3]$, $[C_4MIM][Br]$, $[C_4MIM][PF_6]$, $[C_4MIM][Cl]$, $[HMIM][BF_4]$ and $[C_2MIM][BF_4]$) were synthesized according to the literature procedures.[5-9] All ILs were dried for 4 h under vacuum at 90°C and stored in closed desiccators before use. The purity of ILs was determined by HPLC and their purities were greater than 97%.

Extraction and isolation

The ground roots (5 kg) of PSS were extracted with 0.6 M IL aqueous solution (5 L × 1 h × 3) under reflux. After removal of the residue by filtration and appropriate concentration, the extracting solution was partitioned with the equal volume of petroleum ether and ethyl acetate for 3 times successively. The ethyl acetate soluble portion (535 g) was fractionated by a silica gel column (200~300 mesh), which was eluted with $CHCl_3$/MeOH (100:10→100:30) to give ten fractions (fractions 1~10); each fraction was further subjected to repeated column chromatography with petroleum ether/ethyl acetate or petroleum ether/acetone (9:1→3:7) as eluents. Then subfractions were further purified by Sephadex LH-20 column and recrystallization to obtain eight pure compounds. The whole process is shown in Fig. 2.

The structure of ILs has significant influence on their physicochemical properties and their distinct multiple interactions with target constituents, which could greatly affect the extraction efficiency of active substances.[10]

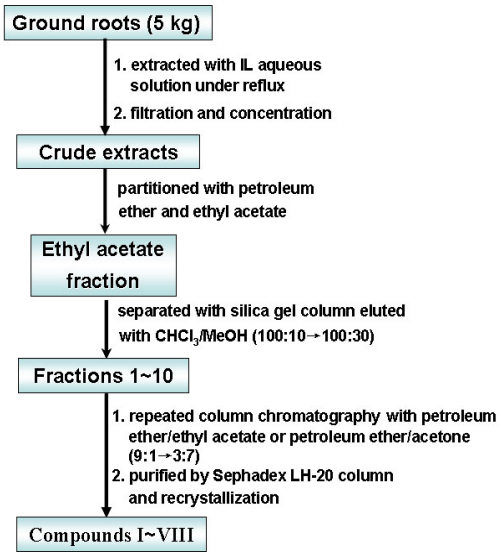

Figure 2. Extraction and separation process of PSS roots

Results and Discussion

Selection of IL-based extraction phase

Therefore the effect of the structure of ILs on the extraction efficiency should be investigated. In the following study, the extraction efficiency of 7 kinds of ILs aqueous solution (0.4 M) was compared with each other and 95% ethanol (as shown Fig.3, the extraction efficiency is expressed as the relative values of the yield of crude extracts and the maximum value is taken to be 100% among them). The yield of crude extracts (%) is the percentage of dry weight of crude extracts divided by the weight of roots.

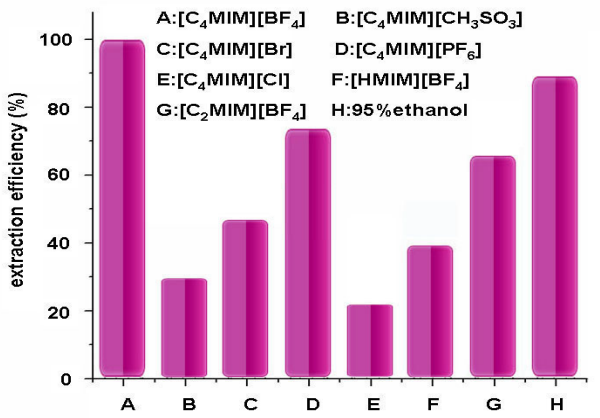

Figure 3. Effect of ILs structure on extraction efficiency

From Fig.3, it is found the extraction efficiency of anion in ILs ~~was~~ decreases as $[BF_4]^- > [PF_6]^- > [Br]^- > [CH_3SO_3]^- > [Br]^-$, meanwhile the extraction efficiency of cation in ILs ~~was~~ decreases as $[C_4MIM]^+ > [C_2MIM]^+ > [HMIM]^+$. The latter result accords with the order of hydrophobicity of the three cations. But the former should be the result of joint action from solubility and polarity. 95% ethanol is usually used as conventional extraction solvent and its performance is also good. Considering the above results, $[C_4MIM][BF_4]$ was selected as the extraction phase in this work.

Effect of ILs concentration

The high viscosity of ionic liquids is a limiting factor for mass transfer in extraction process, and it has been proved that IL concentration has significant influence on the extraction performance of target constituents. Therefore, the concentration of IL on the yield of crude extracts was studied and the result was shown in Fig.4. It can been seen that the extraction efficiency is gradually improved with the increase of $[C_4MIM][BF_4]$ concentration in the range from 0.2 to 0.6 M, and at the end the curve tends to be flat. However, if the molar concentration is above 0.6, higher viscosity would result in poorer diffusivity and mass transfer capacity, so the yield of crude extracts declines at some extent. Considering the above results, 0.6 M of $[C_4MIM][BF_4]$ aqueous solution was selected in the following experiments.

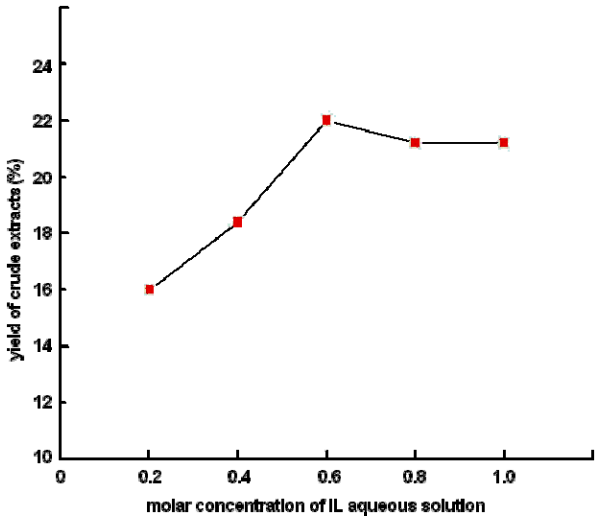

Figure 4. Effect of ILs concentration on extraction efficiency

Structure identification

Compound **I** was isolated as a yellowish crystal (methanol) with the molecular formula of $C_{15}H_{10}O_6$, as determined from data of the negative EI-MS showing ion peaks at *m/z* 285 $[M-H]^-$. Mp.276-278 °C, both the reaction of HCl-Mg and ferric chloride were positive. IR (KBr) υ (cm^{-1}): 3411, 1660, 1608, 1506. ^{1}H-NMR (300 MHz, DMSO-d_6) δppm: 12.4 (1H, s), 10.1 (1H, brs), 7.44 (2H, d, *J*=8.2 Hz), 6.9 (2H, d, *J*=8.2 Hz), 6.43 (1H, d, *J*=1.8 Hz), 6.28 (1H, d, *J*=1.8 Hz). Compared with the relative spectral data of the literature,[11] the structure of **I** was determined to be kaempferol (robigenin).

Compound **II** was obtained as a yellow crystal (methanol) with the molecular formula of $C_{15}H_{10}O_7$, as determined from data of the positive ESI-MS showing ion peaks at *m/z* 303$[M+H]^+$. Mp.313-314 °C, both the reaction of HCl-Mg and ferric chloride were positive. IR (KBr) υ (cm^{-1}): 3405, 1644, 1602, 1500. ^{1}H-NMR (300 MHz, CD_3OD) δppm: 6.19 (1H, d, *J* = 1.6 Hz), 6.41 (1H, d, *J* = 1.6 Hz), 7.68 (1H, s), 7.52 (1H, d, *J* = 8.5 Hz), 6.89 (1H, d, *J* = 8.5 Hz). Compared with the relative spectral data of the literature,[12] the structure of **II** was determined to be quercetin (meletin).

Compound **III** was obtained as amorphous yellow powder with the molecular formula of $C_{27}H_{36}O_{16}$, as determined from data of the negative ESI-MS showing ion peaks at *m/z* 609 $[M-H]^-$, 463 $[M-H-Rha]^-$, 301 $[M-H-Rha-Glc]^-$. Mp.218-220 °C, both the reaction of HCl-Mg and molish were positive. UV λ_{max} (nm): 259, 359; IR (KBr) υ (cm^{-1}): 3421, 1654, 1603, 1505, 1452. ^{1}H-NMR (300 MHz, DMSO-d_6) δppm: 12.51(1H, s), 7.51 (2H, m), 6.81 (1H, d, *J* = 8.1 Hz), 5.33 (1H, d, *J* = 6.6 Hz), 4.38(1H, s), 3.06-3.79 (m), 0.99 (3H, d, *J* = 6.0 Hz). Compared with the relative spectral data of the literature,[13] the structure of **III** was determined to be rutin (Vitamin P).

Compound **IV** was isolated as orange-red needle crystal (methanol) with the molecular formula of $C_{15}H_{10}O_4$, as determined from data of the negative ESI-MS showing ion peaks at *m/z* 255 $[M-H]^-$. Mp.195-197 °C, and Borntrager reaction was positive. IR (KBr) υ (cm^{-1}): 3411, 1680, 1630; ^{1}H-NMR (300 MHz, CD_3OD) δppm: 7.87 (1H, d, *J* = 7.2 Hz), 7.69 (1H, dd, *J* = 7.2 and 8.4 Hz), 7.64 (1H, s), 7.32 (1H, d, *J* = 8.4 Hz), 7.13 (1H, s), 2.50 (3H, s). Compared with the relative spectral data of the literature,[14] the structure of **IV** was determined to be chrysophanol (chrysophanic acid).

Compound **V** was isolated as a orange-red needle crystal (methanol) with the molecular formula of $C_{15}H_{10}O_5$, as determined from data of the negative ESI-MS showing ion peaks at *m/z* 269 $[M-H]^-$. Mp.233-234 °C, and Borntrager reaction was positive. IR (KBr) υ (cm^{-1}): 3390, 1678, 1625. ^{1}H-NMR (300 MHz, CD_3OD) δppm: 7.64 (1H, s), 7.30 (1H, d, *J* = 2.4 Hz), 7.09 (1H, s), 6.70 (1H, d, *J*= 2.4 Hz), 2.45 (3H, s). Compared with the relative spectral data of the literature,[15] the structure of **V** was determined to be emodin.

Compound **VI** was obtained as orange-yellow needle crystal (methanol) with the molecular formula of $C_{16}H_{12}O_5$, as determined from data of the negative ESI-MS showing ion peaks at *m/z* 283 ($[M-H]^-$. Mp.196-197 °C, and Borntrager reaction was positive. IR (KBr) υ (cm^{-1}): 3432, 1683, 1624. ^{1}H-NMR (300 MHz, DMSO-d_6) δppm: 12.25 (1H, s), 12.12 (1H, s), 7.61 (1H, d, *J* =2.4 Hz), 7.35 (1H, d, *J*=1.2 Hz), 7.07 (1H, d, *J*=2.4 Hz), 7.68 (1H, d, *J*=1.2 Hz), 2.47 (3H, s), 3.94 (3H, s). Compared with the relative spectral data of the literature,[15] the structure of **VI** was determined to be physcion (rheochrysidin).

Compound **VII** was obtained as white powder with the molecular formula of $C_{30}H_{48}O_3$, as determined from data of the negative ESI-MS showing ion peaks at *m/z* 455 $[M-H]^-$. Mp.304-306 °C, and Liebermann-Burchard reaction was positive. IR (KBr) υ (cm^{-1}): 3540, 2946, 2883, 1699. ^{1}H-NMR (300MHz, MeOD) δppm: 0.74 (3H, s), 0.79 (3H, s), 0.86 (3H, s), 0.94 (3H×2, s), 0.99 (3H, s), 1.13 (3H, s), 2.28 (1H, br t, *J*=14.0Hz), 2.89 (1H, dd, *J*=4.4, 14.0Hz), 3.15 (1H, dd, *J*=4.3, 10.5Hz), 5.24 (1H, t, *J*=3.5Hz). ^{13}C-NMR (125MHz, MeOD) δppm: 39.3, 28.3, 77.6, 37.1, 55.6, 18.7, 33.5, 41.2, 47.8, 39.6, 24.1, 125.3, 138.9, 42.4, 30.9, 23.6, 47.6, 53.2, 39.1, 39.6, 29.6, 37.3, 29.0, 16.7, 15.9, 17.7, 24.6, 179.0, 17.6, 21.7. Compared with the relative spectral data of the literature,[16] the structure of **VII** was determined to be ursolic acid.

Compound **VIII** was obtained as yellowish powder with the molecular formula of $C_9H_8O_4$, as determined from data of the negative ESI-MS showing ion peaks at *m/z* 179 $[M-H]^-$. Mp.196-197 °C, and $FeCl_3$-K_3 [Fe (CN) $_6$] reaction was positive. UV λ_{max} (nm): 215, 326; IR (KBr) υ (cm^{-1}): 3432, 1683, 1624. ^{1}H-NMR (300 MHz, CD_3OD) δppm: 7.52 (1H, d, *J*=15.9Hz), 7.02 (1H, d, *J*=2.1Hz), 6.93 (1H, dd, *J*=8.2 and 2.1Hz), 6.77 (1H, d, *J*=8.2Hz), 6.20 (1H, d, *J*=15.9Hz). Compared with the relative spectral data of the literature,[17] the structure of **VIII** was determined to be caffeic acid.

Conclusions

As major symbolic secondary metabolites, kaempferol (robigenin), quercetin (meletin), rutin (Vitamin P), chrysophanol (chrysophanic acid), emodin, physcion (rheochrysidin), ursolic acid, caffeic acid were isolated from Polygonum Strindbergii Schust. in Bull. Herb. Boiss. ser. for the first time. Moreover, 0.6 M of $[C_4MIM][BF_4]$ aqueous

solution has been successfully applied in extraction process as green solvents, and the effects of ILs structure and molar concentration were investigated. The plant was supposed to be further utilized and developed as a meaningful natural resource in the genus, and above results and methods are hoped to be helpful for related chemists.

Acknowledgement

Preparation of this paper was supported by ~~the~~ National Scientific Foundation of China (No.81102344).

References

[1]Flora of China Editorial Committee of Chinese Academy of Sciences, *Flora of China*, *Science Press*, **1998**.

[2]Zeng, H., Wang, Y., Kong, J., Nie, C., Yuan, Y., *Talanta*, **2010**, *15*, 582-590.

[3]Xia, E. Q., Wang, B. W., Xu, X. R., Zhu, L., Song, Y., Li, H. B., *Int. J. Mol. Sci*., **2011**, *12*, 5319-5329.

[4]Cao, Y., Xing, H., Yang, Q., Bao, Z., Su, B., Yang, Y., Ren, Q., *J. Agric. Food Chem*., **2012**, *60*, 3432-3440.

[5]Wang, Y.Y., Li, W., Dai, L.Y., *Chem. Papers*., **2008**, *62*, 313-317.

[6]Bonhote, P., Dias, A. P., Papageorgiou, N., Kalyanasundaram, K., Gratzel, M., *Inorg. Chem.,* **1996**, *35*, 1168-1178.

[7]Chu, Y., Deng, H., Cheng, J. P., *J. Org. Chem.,* **2007**, *72*, 7790-7793.

[8]Fang, D.; Cheng, H.; Gong, K., Shi, Q. R.; Zhou, X. L.; Liu, Z. L. *J. Fluorine Chem.,* **2008**, 129,108-111.

[9]Li, L., Yu, S. T., Xie, C. X., Liu, F. S., Li, H. J., *J. Chem. Technol. Biot.,* **2009**, *84*, 1649-1652.

[10]Lu, Y. B., Ma, W. Y., Hu, R. L., Dai, X. J., Pan, Y. J., *J. Chromy. A,* **2008**, *1208*, 42-46.

[11]Farzin, H., Naaman, K., Hossein, H., Randa, K. A., *Iran. J. Pharm. Res*., 2**003**, *2*, 251-252

[12]Güvenalp, Z., Demirezer, L. O., *Turk. J. Chem*., **2005**, *29*, 163-169.

[13] Francois, N. M., Rachid, S., Amadou, D., *J. Nat. Prod.*, **2011**, *4*, 100-107.

[14]Rani, M. S., Emmanuel, S., Sreekanth, M. R., Ignacimuthu, S., *Int. J. Pharm. Pharm. Sci*., **2010**, *2*, 67-70.

[15] Chu, X., Sun, A. L., Liu, R.M., *J. Chromy. A*, **2005**, *1097*, 33-39.

[16] Silva, M. G. V., Vieira, Í. G. P., Mendes, F. N. P., Albuquerque, I. L., Santos, R. N. D., Silva, F. O., Morais, S. M., *Molecules*, **2008,** *13*, 2482-2487.

[17] Wei,Y., Gao,Y. L., Zhang, K., Ito, Y., *J. Liq. Chromy. Relat. Technol*., **2010,** 1; *33*, 837-845.

Received: 27.01.2013.

Accepted: 10.02.2013