*Asian Journal of Chemistry; Vol. 24, No. 10 (2012), 4619-4622*

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

# Isolation and Characterization of Chemical Constituents from the Petals of *Nelumbo nucifera*

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(*Received*: 16 August 2011; *Accepted*: 9 May 2012) AJC-11451

In the present study, eight compounds were isolated and characterized from ethanolic extract of the petals of *Nelumbo nucifera*. These compounds were elucidated as myricetin-3-O-β-D-glucopyranoside (**I**), quercetin-3-O-β-D-glucuronide (**II**), astragalin (**III**), quercetin (**IV**), 3,4-dihydroxybenzoic (**V**), kaempeforol (**VI**), *p*-hydroxybenzoic acid (**VII**) and β-sitosterol (**VIII**). Among these compounds, compounds **I**, **II**, **IV**, **V**, **VI**, **VII** and **VIII** were isolated from the petals of *Nelumbo nucifera* for the first time and compounds **I** and **V** were first reported from the genus of *Nelumbo*.

**Key Words: Petals, *Nelumbo nucifera*, Chemical constituents.**

## INTRODUCTION

*Nelumbo nucifera* Gaertn., known as lotus, is a perennial aquatic herb. It is one of the most important ornamental and economic plants grown widely in Asia, Australia and North America[1]. Almost all parts of the plant were used in Chinese traditional medicine and used as inflammatory, anticancer, antiobesity, antiemetic and antiviral medicine[2]. The flowers of *N. nucifera* also have a long history in Chinese folk medicine and have been used for the therapies of hematemesis, eczema, weak spleen and stomach trouble[3].

In former studies, a large amount of flavonoids and alkaloids have been isolated from the leaves of *N. nucifera*[2]. However, there were few reports on preparative separation and purification of chemical constituents from the petals of *N. nucifera* except the study carried out by Guo *et al.*[2]. In order to clarify its active constituents, an investigation on the ethanol extract of the petals of *N. nucifera* was carried out. Eight compounds were isolated and identified as: myricetin-3-O-β-D-glucopyranoside (**I**), quercetin-3-O-β-D-glucuronide (**II**), astragalin (**III**), quercetin (**IV**), 3,4-dihydroxybenzoic acid (**V**), kaempeforol (**VI**), *p*-hydroxybenzoic acid (**VII**), β-sitosterol (**VIII**) (Fig. 1). Among these compounds, compounds **I**, **II**, **IV**, **V**, **VI**, **VII** and **VIII** (Fig. 1) were isolated from the petals of *N. nucifera* for the first time. Compounds **I** and **V** were first reported from the genus of *Nelumbo*.

## EXPERIMENTAL

The HPLC used throughout in this study consisted of a Waters Empower system (Milford, MA, USA), a model 600 pump, a model 600 system controller, a model 600 multi-solvent delivery system, a model 996 photodiode array detector (PAD), a sample injector with a 20 μL loop and an Empower workstation. The $^{1}H$ and $^{13}C$ NMR spectra were obtained on a 600 MHz NMR spectrometer instrument with TMS as internal reference (Varian, INOVA USA). The preparative HSCCC instrument employed in the present study was a model GS10A-2 (Beijing Institute of New Technology Application, Beijing, China).

Organic solvents including ethanol, petroleum ether, ethyl acetate, chloroform and methanol were all of analytical grade (Guangcheng Chemical Factory, Tianjin, China). Methanol used for HPLC analysis was of chromatographic grade (Tedia Company Inc, Fairfield, USA). Reverse osmosis Milli-Q water (Millipore, Bille-rica, USA) was used for all solutions and dilutions. Silica gel (Qingdao Marine Chemical Factory, 200-300 mesh), Sephadex LH-20 (GE Healthcare).

Fresh flowers of *N. nucifera* were collected from Jinan, China, in July 2010 and identified by Dr. Li Jia, Shandong University of Traditional Chinese Medicine. After dried in the shade at room temperature, the flowers were ground into powder.

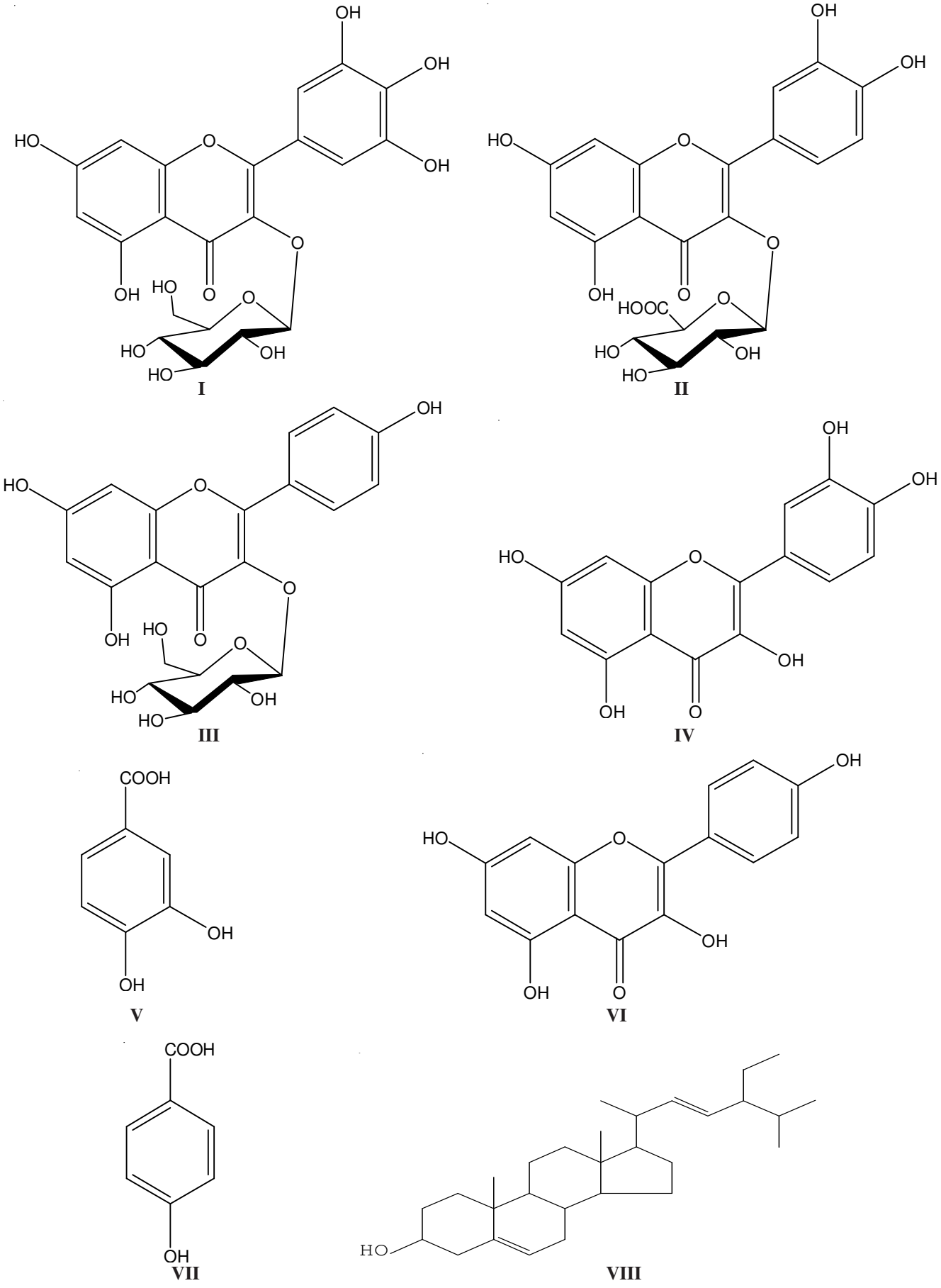


Fig. 1. Structures of eight compounds: myricetin-3-O-β-D-glucopyranoside (**I** ); quercetin-3-O-β-D-glucuronide (**II**); astragalin (**III**); quercetin (**IV**); 3,4-dihydroxybenzoic (**V**); kaempeforol (**VI**); *p*-hydroxybenzoic acid (**VII**); β-sitosterol (**VIII**)

**Extraction and isolation:** The powder (10 kg) of the lotus flowers were extracted with 95 % ethanol three times under reflux. After removing the solvent under reduced pressure at 50 ºC, the ethanol extract was suspended in distilled water and. extracted with petroleum ether, ethyl acetate, consecutively. The ethyl acetate extract was chromatographed over a silica gel column and eluted with a solvent system composed of $CHCl_3$-$CH_3OH$ (100:0-1:1). Fraction 1-8 were obtained according to TLC analysis. Fraction 7 was repeatedly subjected to silica gel column with a solvent system $CHCl_3$-$CH_3OH$ (10:1-1:1) and the fraction eluted by $CHCl_3$-$CH_3OH$ (3:1) were further purified by HSCCC with the solvent system composed of ethyl acetate-ethanol-water-acetic acid (4:1:5:0.25, v/v) to yield compounds **I** (80 mg) and compound **II** (15 mg). Fraction 6 was repeatedly subjected to silica gel column with a solvent system composed of $CHCl_3$-$CH_3OH$ (10:1-3:1) and then purified on Sephadex LH-20 column to afford compound **III** (90 mg). Fraction 5 was first subjected to silica gel column eluted with a solvent system $CHCl_3$-$CH_3OH$ (15:1-5:1) and then purified on Sephadex LH-20 column to afford compound **IV** (100 mg). Fraction 4 was purified on Sephadex LH-20 column and then subjected to preparative HPLC to afford compound **V** (18 mg). Fraction 3 was repeatedly subjected to silica gel with a solvent system composed of $CHCl_3$-$CH_3OH$ (100:1-20:1) and recrystallized (ethyl acetate) to afford compounds **VI** (25 mg) and **VII** (80 mg). Fraction 2 was repeatedly subjected to silica gel with a solvent system composed of petroleum ether-ethyl acetate (6:1-1:1) to afford compound **VIII** (70 mg).

**Compound I (myricetin-3-O-β-D-glucopyranoside):** Yellow amorphous powder, ESI-MS (m/z): 481 $[M + H]^+$, $^1H$ NMR (DMSO-$d_6$, 600 MHz) δ: 6.19 (1H, d, *J* = 1.2 Hz, H-6), 6.37 (1H, d, *J* = 1.2 Hz, H-8), 7.19 (2H, s, H-2', H-6'), 5.46 (1H, d, *J* = 7.8 Hz, H-1"). $^{13}C$ NMR (DMSO-$d_6$, 150 MHz) δ: 156.7 (C-2), 133.8 (C-3), 177.8 (C-4), 161.7 (C-5), 99.0 (C-6), 164.5 (C-7), 93.7 (C-8), 156.6 (C-9), 104.3 (C-10), 120.4 (C-1'), 108.9 (C-2'), 145.8 (C-3'), 137.0 (C-4'), 145.8 (C-5'), 108.9 (C-6'), 101.2 (C-1"), 71.3 (C-2"), 76.9 (C-3"), 70.3 (C-4"), 78.0 (C-5"), 60.9 (C-6").

**Compound II (quercetin-3-O-β-D-glucuronide):** Yellow amorphous powder, ESI-MS (m/z): 479 $[M + H]^+$, $^1H$ NMR (DMSO-$d_6$, 600 MHz) δ: 7.60 (1H, s, H-2'), 7.59 (1H, d, *J* = 7.2 Hz, H-6'), 6.84 (1H, d, *J* = 7.2 Hz, H-5'), 6.42 (1H, s, H-8), 6.22 (1H, s, H-6), 5.49 (1H, d, *J* = 5.4 Hz, H-1"). 13C NMR (DMSO-$d_6$, 150 MHz) δ: 156.3 (C-2), 133.2 (C-3), 177.2 (C-4), 161.2 (C-5), 98.8 (C-6), 164.3 (C-7), 93.6 (C-8), 156.4 (C-9), 103.9 (C-10), 120.8 (C-1'), 115.3 (C-2'), 144.9 (C-3'), 148.6 (C-4'), 116.2 (C-5'), 121.6 (C-6'), 101.2 (C-1"), 75.8 (C-2"), 75.9 (C-3"), 71.4 (C-4"), 73.8 (C-5"), 170.0 (C-6").

**Compound III (astragalin):** Yellow amorphous powder, ESI-MS (m/z): 449 $[M + H]^+$, $^1H$ NMR (DMSO-$d_6$, 600 MHz) δ: 6.44 (1H, d, *J* = 1.2 Hz, H-8), 6.22 (1H, d, *J* = 1.2 Hz, H-6), 8.05 (2H, d, *J* = 9.0 Hz, H-2' , H-6'), 6.89 (2H, d, *J* = 9.0 Hz, H-3', H-5'), 12.62 (1H, s, 5-OH), 5.48 (1H, d, *J* = 7.8 Hz, H-1"), 3.08-3.58 (6H, m, H-2"-6"). $^{13}C$ NMR (DMSO-$d_6$, 150 MHz) δ: 156.7 (C-2), 133.6 (C-3), 177.9 (C-4), 161.7 (C-5), 99.1 (C-6), 164.6 (C-7), 94.1 (C-8), 156.7 (C-9), 104.4 (C-10), 121.3 (C-1'), 131.3 (C-2', C-6'), 115.5 (C-3', C-5'), 160.3 (C-4'), 101.2 (C-1"), 74.6 (C-2"), 77.9 (C-3"), 70.3 (C-4"), 76.8 (C-5"), 61.2 (C-6").

**Compound IV (quercetin):** Yellow amorphous powder, ESI-MS (m/z): 303 $[M + H]^+$, $^1H$ NMR (DMSO-$d_6$, 600 MHz) δ: 7.68 (1H, d, *J* = 1.2 Hz, H-2'), 7.54 (1H, dd, *J* = 1.2, 9.0 Hz, H-6'), 6. 88 (1H, d, *J* = 9.0 Hz, H-5'), 6.40 (1H, d, *J* = 1.8 Hz, H-8), 6.19 (1H, d, *J* = 1.2 Hz, H-6). $^{13}C$ NMR (DMSO-$d_6$, 150 MHz) δ: 149.5 (C-2), 137.0(C-3), 176.9 (C-4), 162.0 (C-5), 99.5 (C-6), 166.3 (C-7), 94.6 (C-8), 157.9 (C-9), 104.8.0 (C-10), 123.6. (C-1') , 117.3 (C-2') , 146.7 (C-3'), 149.1 (C-4'), 116.0 (C-5'), 123.5 (C-6').

**Compound V (3, 4-dihydroxybenzoic):** White needle crystal, ESI-MS (m/z): 155 $[M + H]^+$, $^1H$ NMR (DMSO-$d_6$, 600 MHz) δ: 6.80 (1H, d, *J* = 7.8 Hz, H-5), 7.35 (1H, s, H-2), 7.30 (1H, d, *J* = 7.8 Hz, H-6). $^{13}C$ NMR (DMSO-$d_6$, 150 MHz) δ: 115.6 (C-5), 116.9 (C-2), 122.0 (C-1), 122.3 (C-6), 145.3 (C-3), 150.4 (C-4), 167.8 (C-7).

**Compound VI (kaempeforol):** Yellow amorphous powder, ESI-MS (m/z): 287 $[M + H]^+$, $^1H$ NMR (DMSO-$d_6$, 600 MHz) δ: 6.19 (1H, d, *J* = 1.8 Hz, H-6), 6.44 (1H, d, *J* = 1.8 Hz, H-8), 6.93 (2H, d, *J* = 9.0 Hz, H-3', H-5'), 8.05 (2H, d, *J* = 9.0 Hz, H-2', H-6'). $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) δ: 147.0 (C-2), 135.6 (C-3), 175.9 (C-4), 164.2 (C-5), 98.3 (C-6), 164.2 (C-7), 93.9 (C-8), 159.3 (C-9), 103.9 (C-10), 121.8 (C-1'), 129.7 (C-2',C-6'), 160.3 (C-4'), 115.6 (C-3', C-5').

**Compound VII (*p*-hydroxybenzoic acid):** White needle crystal, ESI-MS (m/z): 139 $[M + H]^+$, $^1H$ NMR (DMSO-$d_6$, 600 MHz) δ: 7.80 (2H, d, *J* = 7.8 Hz, H-2, H-6), 6.83 (2H, d, *J* = 7.8 Hz, H-3, H-5). $^{13}C$ NMR (DMSO-$d_6$, 150 MHz) δ: 115.1 (C-3, C-5), 121.4 (C-1), 131.6 (C-2, C-6), 161.6 (C-4), 174.6 (C-7).

**Compound VIII (β-sitosterol):** White needle crystal, ESI-MS (m/z): 415 $[M + H]^+$, $^1H$ NMR (DMSO-$d_6$, 600 MHz) δ: 5.36 (1H, m, H-6), 3.49 (1H, m, H-3), 1.03 (3H, s, 19-$CH_3$), 0.69 (3H, s, 18-$CH_3$), 0.93 (3H, d, *J* = 6.6 Hz, 21-$CH_3$). $^{13}C$ NMR (DMSO-$d_6$, 150 MHz) δ: 36.7 (C-1), 29.7 (C-2), 72.5 (C-3), 40,7 (C-4), 140.9 (C-5), 122.4 (C-6), 32.3 (C-7), 32.3 (C-8), 50.7 (C-9), 36.8 (C-10), 20.4 (C-11), 37.8 (C-12), 42.9 (C-13), 58.0 (C-14), 23.6 (C-15), 26.7 (C-16), 56.7 (C-17), 12.8 (C-18), 19.4 (C-19), 34.5 (C-20), 19.3 (C-21), 32.5 (C-22), 24.9 (C-23), 46.2 (C-24), 28.8 (C-25), 19.8 (C-26), 19.8 (C-27), 21.7 (C-28), 12.6 (C-29).

## RESULTS AND DISCUSSION

Compound **I** was isolated as yellow amorphous powder. The ESI-MS (m/z) of compound I showed a molecular ion at m/z 480 corresponding to a molecular formula $C_{21}H_{20}O_{13}$. $^1H$ NMR data of δ 6.19 and 6.37 could be ascribed to H-6 and H-8. It was unambiguously identified as myricetin-3-O-β-D-glucopyranoside on the basis of its $^1H$ and $^{13}C$ NMR spectral data[4].

Compound **II** was isolated as yellow amorphous powder. The ESI-MS (m/z) of compound **II** showed a molecular ion at m/z 478 corresponding to a molecular formula $C_{21}H_{18}O_{13}$. The UV shifts spectra were in agreement with quercetin skeletal pattern[5]. The $^1H$ NMR spectrum of compound **II** displayed the characteristic signals of quercetin nucleus[5] and the presence of glucuronide in the molecule. It was unambiguously identified as quercetin-3-O-β-D-glucuronide on the basis of its $^1H$ and $^{13}C$ NMR spectral data[6].

Compound **III** was isolated as yellow amorphous powder. The ESI-MS (m/z) of compound **III** showed a molecular ion at m/z 448 corresponding to a molecular formula $C_{21}H_{20}O_{11}$. It was unambiguously identified as astragalin on the basis of its $^1H$ and $^{13}C$ NMR spectral data[2].

Compound **IV** was isolated as yellow amorphous powder. The ESI-MS (m/z) of compound **IV** showed a molecular ion at m/z 302 corresponding to a molecular formula $C_{15}H_{10}O_7$. It was unambiguously identified as quercetin on the basis of its $^1H$ and $^{13}C$ NMR spectral data[6].

Compound **V** was isolated as white needle crystal. The ESI-MS (m/z) of compound **V** showed a molecular ion at m/z 154 corresponding to a molecular formula $C_7H_6O_4$. It was unambiguously identified as 3,4-dihydroxybenzoic on the basis of its $^1H$ and $^{13}C$ NMR spectral data[7].

Compound **VI** was isolated as yellow amorphous powder. The ESI-MS (m/z) of compound VI showed a molecular ion at m/z 286 corresponding to a molecular formula $C_{15}H_{10}O_6$. Two-proton double doublet at δ 6.93 (2H, $J$ = 9.0 Hz) and at δ 8.05 (2H, $J$ = 9.0 Hz) were accounted to H-3', H-5' and H-2', H-6'. One-proton double doublet at δ 6.19 (1H, $J$ = 1.8 Hz) and δ 6.44 (1H, $J$ = 1.8 Hz) were accounted to H-6 and H-8. It was identified as kaempeforol on the basis of its $^1H$ NMR spectral data and the data was further conformed by $^{13}C$ NMR[8].

Compound **VII** was isolated as white needle crystal. The ESI-MS (m/z) of compound **VII** showed a molecular ion at m/z 138 corresponding to a molecular formula $C_7H_6O_3$. $^1H$ NMR spectrum of compound **VII** showed four hydrogen of benzene ring at δ 7.80 (2H, d, H-2, H-6) and δ 6.83 (2H, d, H-3, H-5). It was unambiguously identified as *p*-hydroxybenzoic acid on the basis of its $^1H$ and $^{13}C$ NMR spectral data[9].

Compound **VIII** was isolated as white needle crystal. The ESI-MS (m/z) of compound **VIII** showed a molecular ion at m/z 414 corresponding to a molecular formula $C_{29}H_{50}O$. It was unambiguously identified as β-sitosterol on the basis of its $^1H$ and $^{13}C$ NMR spectral data[10].

## ACKNOWLEDGEMENTS

Financial supports from the Natural Science Foundation of China (20872083), the Major National S&T Program (2010ZX09401-302-5-12) and the Key Science and Technology Program of Shandong Province are gratefully acknowledged.

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