VIETNAM JOURNAL OF CHEMISTRY VOL. 53(2) 243-246 APRIL 2015
DOI: 10.15625/0866-7144.2015-00123

CHEMICAL STUDY OF THE LEAVES OF *LAGERSTROEMIA SPECIOSA* IN VIETNAM

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Received 13 March 2015; Accepted for Publication 20 April 2015

Abstract

The main constituents of the EtOH extract of leaves of *Lagerstroemia speciosa* supplied by Traphaco company have been quantitatively determined by HPLC to be corosolic acid (2.242 %), asiatic acid (1.219 %) and β-sitosterol. Repeated column chromatography of the *n*-hexane, ethyl acetate and dichloromethane extract of these leaves led to the isolation of corosolic acid, asiatic acid, quercetin, β-sitosterol and β-sitosterol-3-O-β-D-glucopyranosyl glucoside. The structures of these compounds have been elucidated by the IR, MS and NMR spectral analysis.

Keywords. *Lagerstroemia speciosa*, leaves, quercetin, corosolic acid, asiatic acid.

1. INTRODUCTION

The species *Lagerstroemia speciosa* (L.) Pers. (Vietnamese name: Bằng lăng nước) belonging to the family Lythraceae is a big tree of 10-15 meters high. This plant is growing wildly in India, Thailand, Laos, Cambodia, Philippine, Vietnam and South China. In the folk medicine of many countries *Lagerstroemia speciosa* is used for treatment of type II diabetes and other diseases like diarrhea, heart, stomach [1]. Studies on the chemical constituents of these plants have shown the presence of tannins, flavonoids [2] fatty acids [3] and terpenoids [4]. In a cooperation with Traphaco Company to develop a functional food product from Vietnamese *L. speciosa* for supporting the treatment of type II diabetes we report here the isolation of the main constituents from the leaves of this plant: corosolic acid, asiatic acid, β-sitosterol, daucosterol and quercetin.

2. EXPERIMENTAL

Plant material: the leaves of *L. speciosa* have been supplied by Traphaco Company, a herbal exemplar. No.BL02 is deposited in the Research Department of this company.

Chemicals and methods: Silica gel (Merck) with different sizes from 0.040-0.063 mm, reverse phase RP18 and Sephadex LH20 were used for column chromatography. TLC used the precoated aluminum sheets with silica gel 60 GF 254, 0.2 mm (Merck). IR: Nicolet Impact 410 (KBr), MS: HP 5989 B MS Engine Agilent, NMR: Bruker Avance 500 MHz. For quantitative determination of the main constituents the HPLC Alliance series was used.

Extraction: Dried leaves of *L. speciosa* (1600 g) were extracted with ethanol-water mixture 70:30 (v/v) at 60 °C three times. Evaporation of solvents under reduced pressure afforded 240 g extract. Quantitative HPLC analysis of this extract gave corosolic acid (2.242 %) and asiatic acid (1.219 %) as the main constituents.

200 g EtOH extract were dissolved in H_2O and extracted with *n*-hexane and ethyl acetate, successively to yield 12 g *n*-hexane and 25 g ethyl acetate extract. Chromatography of 12 g *n*-hexane extract on silica gel eluted by *n*-hexane/EtOAc mixture gradient from 5-20 % EtOAc gave 4 fractions (HE1-HE4). Fraction HE2 was rechromatographed on silica gel with the solvent mixture *n*-hexane/EtOAc 9:1 to furnish 700 mg β–sitosterol **(1).** 25 g EtOAc extract were fractionated on a silica gel column with *n*-hexane/EtOAc mixture gradient from 10-70 % EtOAc to give 5 fractions (EA1-EA5). Repeated chromatography of fraction EA2 on silica gel with a solvent mixture hexane/EtOAc 85:15 (v/v) afforded 250 mg β-sitosterol **(1)** and 30 mg quercetin **(3)**. Fraction EA3 was rechromatographed on a RP 18 column with a mixture MeOH/H_2O 8:1 as eluent to give 650 mg

asiatic acid **(5)**. Fraction EA4 was rechromatographed on silica gel (CH_2Cl_2/MeOH 9:1) to furnish 2500 mg corosolic acid **(4)** and 25 mg β-sitosterol-3-O-β-D-glucopyranoside.

Compound **1** (β-sitosterol):

White crystals, mp 169-171 °C. ^{1}H-NMR (δ, $CDCl_3$): 1.02 (3H, s, H-18), 0.71 (3H, s, H-19), 0.86 (3H, d, *J* = 7.0 Hz, H-26), 0.85 (3H, d, *J* = 7.0 Hz, H-27), 0.93 (3H, d, *J* = 5.5 Hz, H-21), 0.85 (3H,t, *J* = 7.0 Hz, H-29), 5.38 (1H, dd, *J* = 3.0, 2.5 Hz, H-6),3.53 (1H, m, H-3).

^{13}C-NMR spectrum is in full agreement with that of β–sitosterol in [5, 6].

Compound **2** (β-sitosterol-3-*O*-β-D-glucopyranoside, Daucosterol), white powder, ^{1}H-NMR (DMSO-d_6) δ ppm: 0.65 (3H, s), 0.80 (3H, d, *J* = 6.9 Hz), 0.81 (3H, d, *J* = 6.9 Hz), 0.90 (3H, d, *J* = 6.5 Hz), 0.96 (3H, s), 1.00 (3H, d, *J* = 6.7 Hz), 1.23 (3H, s), 3.05-3.08 (1H, m), 3.10-3.14 (3H, m), 3.38-3.48 (2H, m), 3.64 (1H, dd, *J* = 5.5, 10.1 Hz), 4.22 (1H, d, *J* = 7.8 Hz), 4.39 (1H, t, *J* = 5.7 Hz), 4.83 (3H, m), 5.32 (1H, d, *J* = 7.0). ^{13}C-NMR spectrum is in full agreement with that for daucosterol in [7].

Compound **3** (quercetin): yellow needles. IR (KBr, cm^{-1}): 3450 (OH), 1662 (C=O), 1614 (C=C), ^{1}H-NMR (δ, $CDCl_3$): 6.20 (1H, d, *J* = 1.9 Hz, H-6), 6.40 (1H, d, *J* = 1.9 Hz, H-8), 6.90 (1H, d, *J* = 8.5 HZ, H-5'), 7.65 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 7.75 (1H, *J* = 2.0 Hz, H-1'). ^{13}C-NMR spectrum is in full agreement with that for quercetin in [8].

Compound **4** (corosolic acid): white powder, positive ions ESI- MS: *m/z* = 473 $[M+H]^+$, ^{1}H and ^{13}C-NMR spectral data, see table 1.

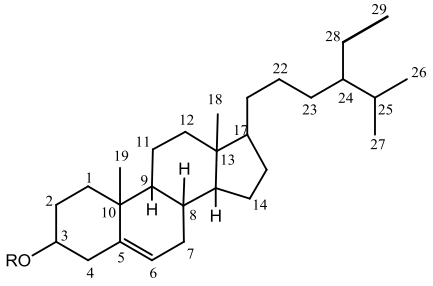
Compound **5** (asiatic acid): white powder, IR (KBr, cm^{-1}): 3144 (OH), 2930, 2866 (CH_2, CH_3), 1690 (COOH), 1459, 1053, 694. ESI-MS (negative ions): *m/z*=487$[M-H]^-$

^{1}H and ^{13}C- NMR spectral data, see table 1.

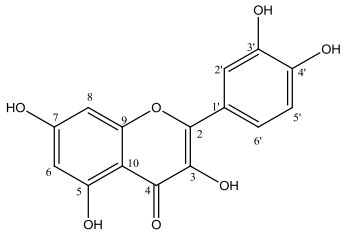
3. RESULTS AND DISCUSSION

It is well known that the leaves extract of *Lagerstroemia speciosa* has a blood sugar content lowering effect and can be used for treatment of diabetes type II. The main active constituent in this extract has been evidenced as corosolic acid **(4)**. Very important is to find out the *Lagerstroemia speciosa* or its varieties in Vietnam, which contain high enough amount of corosolic acid for developing a product used in diabetes type II treatment. In cooperation with Traphaco company we have had screenings of about 10 *Lagerstroemia* samples and found out that the sample BL02 was the best one. However, there were differencies between the amount of corosolic acid and asiatic acid determined by quantitative HPLC (2.242 % and 1.219 % of dry extract, respectively) and the isolated amount by repeated column chromatography (1.25 % and 0.3259 %, respect- tively). That means the contents of corosolic acid and asiatic acid in dry leaves of sample BL02 are 0.35 % and 0.19 %, respectively. So that the sample BL02 can be used as starting material for preparation of a functional food product used in treatment of diabetis type II.

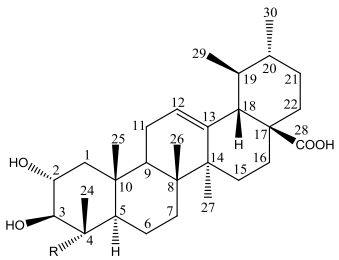
The structures of isolated corosolic acid and asiatic acid have been determined by comparison of ^{1}H- and ^{13}C-NMR spectral data with the published data (table 1).



1: R=H β-sitosterol
2: R = β-D-glucopyranosyl



3: Quercetin



4: R = CH_3 (corosolic acid)
5: R = CH_2OH (asiatic acid)

Table 1: NMR spectral data of **4** and **5**

Pos.	**4** (DMSO-d_6)		Corosolic acid (pyridine –d_5) [9]		**5** ($CDCl_3$ +CD_3OD)		Asiatic acid [10]	
	δ_C	δ_H (J = Hz)	δ_C	δ_H (J = Hz)	δ_C	δ_H (J = Hz)	δ_C	δ_H (J = Hz)
1	47.0		48.1		48.0		48.0	
2	67.1	4.24 (dd)	68.1	4.11 (1H, ddd, J = 4.0, 9.5, 11.0 Hz)	69.3	3.7 (dt,1H)	69.7	3.71 (dt,1H)
3	82.2	3.38 (d, J = 9.5 Hz)	84.3	3.43 (1H, d, J = 9.5 Hz)	78.2	3.38 (d, J = 9.5 Hz, 1H)	78.2	3.38 (d, J = 9.5 Hz, 1H)
4	39.1		40.3		44.1		44.1	
5	54.7		56.1		48.2		48.2	
6	18.0		19.3		19.1		19.1	
7	32.6		34.0		33.6		33.6	
8	39.2		40.5		40.7		40.7	
9	46.9		48.5		48.5		48.5	
10	37.5		38.9		39.0		38.9	
11	22.9		24.2		24.5		24.5	
12	124.5	5.26 (1H, t, J = 3.3 Hz)	126.0	5.48 (1H, br s)	126.4	5.28 (br,1H)	126.6	5.26 (br,1H)
13	138.2		139.8		140.0		139.8	
14	41.7		43.0		43.4		43.4	
15	27.5		29.1		29.2		29.1	
16	23.8		25.4		25.4		25.3	
17	47.0		48.6		48.8		48.7	
18	52.3		54.0		54.4		54.3	
19	38.4	2.12 (1H, d, J = 11.5 Hz)	39.9	2.65 (1H, d, J = 11.0 Hz)	40.5		40.4	
20	38.5	2.74 (1H, dd, J = 4 and 9.5 Hz)	40.0		40.6		40.4	
21	30.1		31.5		31.7		31.7	
22	36.3		37.9		38.2		38.1	
23	28.8		29.8		66.5	3.29 (d, J = 11.0 Hz, 1H) 3.51(d, J = 11.0 Hz, 1H)	66.3	3.29 (d, J = 11.0 Hz, 1H) 3.53(d, J = 11.0 Hz, 1H)
24	17.1		18.2		13.9		13.9	
25	16.4		17.4		17.5		17.6	
26	16.9		17.9		17.5		17.7	
27	23.2		24.4		24.1		24.1	
28	178.2		180.4		181.6		181.6	
29	17.0		18.0		18.0		17.8	
30	21.0		21.9		21.6		21.5	

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