VIETNAM JOURNAL OF CHEMISTRY 53(5) 652-656 OCTOBER 2015
DOI: 10.15625/0866-7144.2015-00197

CHEMICAL CONSTITUENTS OF THE BARKS OF *LITSEA GLUTINOSA* COLLECTED IN THAI NGUYEN PROVINCE, VIETNAM

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Received 27 October 2015; Accepted for Publication 30 October 2015

Abstract

From the ethyl acetate extract of the barks of *Litsea glutinosa* four compounds including *cis*-5,8,11,14,17-eicosapentaenoic acid methyl ester (**1)**, spatozoate (**2)**, betasitosterol (**3)** and daucosterol (**4)** have been isolated. Compound (**5**) 1-heptadecanol, compound (**6**) 1-eicosanol and compound (**7**) glycerol 1,3-di-(9Z,12Z-octadecadienoate) 2-hexadecanoate were isolated from the *n*-hexane extract. Their structures were elucidated by the analysis of the IR, MS and NMR spectra. Compounds (**1**), (**2**), (**5**), (**6**), (**7**) were isolated for the first time from *Litsea glutinosa*.

Keywords. *Litsea glutinosa*, *cis*-5,8,11,14,17-eicosapentaenoic acid methyl ester, spatozoate, betasitosterol, daucosterol, 1-heptadecanol, 1-eicosanol, glycerol 1,3-di-(9Z,12Z-octadecadienoate) 2-hexadecanoate.

1. INTRODUCTION

Litsea glutinosa (Lour.) C.B. Rob. belongs to the family Lauraceae. It is well-known as an evergreen medium size tree, distributed widely throughout tropical and subtropical Asia: south and southwest of China, India, Malaysia, Indonesia, and Cambodia. In Vietnam, it is found along the country, from Lang Son to An Giang. This plant is up to 20 m in height [1]. Its leaves are used as a demulcent and mild astringent for diarrhea and dysentery. The roots are used for poulticing sprains and bruises, and the oil extracted from the seed is used for the treatment of rheumatism [2]. The barks of *Litsea glutinosa*, according to Kirtikar & Basu, "are one of the best known and most popular of native drugs", and are considered to be capable of relieving pain, arousing sexual power and also producing a soothing effect on the body [3]. Recently, the phytochemical studies on this plant have been reported. It was known that arabinoxylan [4], abscisic acid derivative and lignans [5], aporphine alkaloids [6], flavones glycosides [7] and megastimane diglycoside [8] were isolated from *L. glutinosa*. However, there are no reports on chemical constituents from *L. glutinosa* in Vietnam, except the only report on the chemical composition of the the essential oil of leaves collected in Ha Tinh province [9].

This paper describes the isolation and structural elucidation of the constituents of the *n*-hexane and ethyl acetate extract of the barks of *L. glutinosa* collected in Thai Nguyen province, Vietnam.

2. EXPERIMENTAL

2.1. Equipments and methods

IR: Impact 410, Nicolet, Germany; ESI-MS: LC-MSD-Trap-SL, Varian, USA, NMR: Bruker Avance 500, Germany with TMS as internal reference (for ^{1}H) and solvent signal (for ^{13}C). CC used silica gel 60G, size 0.043-0.063 mm (Merck), TLC: precoated silica gel G60F254 plates (Merck); spots were detected by spraying with vanillin 1 % in conc. H_2SO_4 and heating at 110 ^{o}C.

2.2. Plant material

Fresh barks of *L. glutinosa* were collected in Thai Nguyen province, Vietnam in October 2014. A voucher specimen (LG01) is deposited in Institute of Chemistry, VAST, Hanoi, Vietnam. Botanical identification was made by Mr. Ngo Van Trai. The barks were shaded, dried, ground into powder and stored at room temperature.

2.3. Extraction and isolation of the compounds

The air dried & powdered stem barks (5 kg) of *L. glutinosa* was extracted successively with *n*-hexane, ethyl acetate and methanol. The organic solvents were evaporated in reduced pressure to furnish the *n*-hexane, EtOAc, MeOH and MeOH/H_2O (1:1) extracts (21.0, 13, 650 and 450 g, respectively). The ethyl acetate extract (13 g) was chromatographed on silica gel, eluted with *n*-hexan/EtOAc from 2 % EtOAc gradient to 50 % (v/v) to afford 6 fractions. Fraction 2 (1.08 g) was subjected to a silica gel column, eluted with *n*-hexane/acetone (98:2 to 50:50) gradient to obtain compound **1**. Fraction 4 (2.3 g) was chromatographed on a silica gel column, eluted with *n*-hexane/EtOAC (7/3) to obtain 6 subfractions (Fr.4.1-4.6). From fraction Fr.4.2 white needles appeared, which were washed with cold MeOH then cold *n*-hexane to give compound **3** (60 mg). Fraction 4.5 was further chromatographed on a silica gel column using a gradient of CH_2Cl_2/MeOH (9.8:0.2-1:1) to afford five sub-fractions (Fr.4.5.1-4.5.5). From fraction Fr.4.5.3 a white solid crystallized, which was washed with cold *n*-hexane, then with CH_2Cl_2 to give 40 mg compound **4**. Fraction 6 (4.5 g) was separated by column chromatography on silica gel eluting with *n*-hexane/EtOAc (98:2 to 1:1) gradient to afford 6 sub-fractions (Fr.6.1-6.6). Fraction 6.5 was further purified over a Sephadex LH-20 column equilibrated with methanol to yield compound **2**.

The *n*-hexane extract (21 g) was separated by silica gel column chromatography using a mixture of *n*-hexane/EtOAc (100:0-0:100) gradient, resulting in 10 subfraction. Fraction 3 (3 g) was subjected to a silica gel column, eluted with *n*-hexane/CH_2Cl_2 (98:2-1:1) gradient to give 5 further sub-fraction (Fr.3.1-3.5). From sub-fraction Fr.3.2 a white solid crystallized, which was washed with cold MeOH to yield compound **5** (56 mg). From fraction 5 (0.5 g) a white solid appeared, which was then recrystallized with MeOH and further purified by silica gel column chromatography, using mixture of solvent (*n*-hexane/EtOAc 9.8:0.2) to afford compound **7**. Fraction 6 (0.1 g) was subjected to a silica gel column eluted with *n*-hexane/CH_2Cl_2 gradient (9.8:2-1:1) to give 4 sub-fractions (Fr.6.1-Fr.6.4). From fraction Fr.6.3 a white solid precipitated, which was then recrystallized from CH_2Cl_2 to give compound **6**.

***cis*-5,8,11,14,17-eicosapentaenoic acid methyl ester (1) ($C_{21}H_{32}O_2$)**: yellow oil. IR (KBr, cm-1): 2923.11 (CH), 1740.14 (COO). ESI-MS m/z: 317.0 (25 %, $[M+H]^+$).

^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm), *J* (Hz): 0.98 (3H, t, *J* = 7.5), 1.60-1.62 (2H, m), 2.03-2.09 (4H, m), 2.30 (2H, t, *J* = 7.5), 2.75-2.81 (8H, m), 3.66 (3H, s), 5.32-5.37 (10H, m).

^{13}C-NMR ($CDCl_3$, 125 MHz), δ_C (ppm), *J* (Hz): 14.27 (CH_3), 20.56 (CH_2), 22.58 (CH_2), 24.96, 25.55, 25.65, 27.22, 29.10-29.71 (CH_2), 31.54 (CH_2), 34.12, 51.43 (OCH_3), 127.14, 127.75, 127.93, 128.07, 128.28, 128.31, 130.06, 130.23, 130.28, 131.98, 174.31.

Spatozoate (2) ($C_{19}H_{20}O_4$): yellow oil. IR (KBr, cm-^{1}): 2964 (C-H), 1724 (COOR- ester), 1283 (C-O). ESI-MS (m/z, %): 313.0 (98 %) $[M+H]^+$.

^{1}H- and ^{13}C-NMR: see Tab. 1.

β-sitosterol (3) ($C_{29}H_{50}O$): white solid.

^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm), *J* (Hz): 0.70 (3H, s), 0.83 (3H, d, *J* = 7.0), 0.85 (3H, d, *J* = 7.0), 0.87 (3H, t, *J* = 7.1), 1.00 (3H, d, *J* = 6.7 Hz), 1.07 (3H, s), 1.11-1.54 (24H, m), 1.65-1.70 (2H, m), 1.82-1.89 (3H, m), 1.97-2.05 (2H, m), 2.25-2.33 (2H, m), 3.52-3.56 (1H, m), 5.36-5.38 (1H, m).

^{13}C-NMR: identical with [14].

β-sitosterol 3-O-β-D-glycopyranoside (4) ($C_{35}H_{60}O_6$)**:** white solid.

^{1}H-NMR (DMSO-d_6, 500 MHz), δ_H (ppm), *J* (Hz): 0.65 (3H, s), 0.96 (3H, s), 0.80 (3H, d, *J* = 6.9 Hz), 0.81 (3H, d, *J* = 6.8), 0.90 (3H, *t*, *J* = 6.5), 1.00 (3H, d, *J* = 6.7), 3.42 (1H, m), 5.32 (1H, brs); glucopyranosyl: 3.05-3.08 (1H, m), 3.14-3.20 (3H, m), 3.64 (1H, dd, *J* = 5.5, 10.1), 3.46 (1H, m), 4.22 (1H, d, *J* =7.8, H-1'), 4.39 (1H, t, *J* = 5.7 Hz), 4.83 (3H, m).

^{13}C-NMR (DMSO-d_6, 125 MHz), δ_C (ppm), *J* (Hz) identical with data in [15]: 35 carbon signals including 121.13 and 140.12 (olefine), 11.6, 11.7, 18.5, 18.9, 19.0, 19.6 (all methyl groups), 70.09 (C-3), glucopyranosyl: 100.7 (C-1'), 73.4, 76.6, 76.7 and 76.9 (oxymethine).

1-heptadecanol (5) ($C_{17}H_{36}O$): white solid. ESI-MS m/z: 256.1 (98 %), $[M]^+$.

^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm), *J* (Hz): 0.88 (3H, t, *J* = 6.5), 1.25 (28H, brs), 1.55-1.62 (2H, m) 4.05 (2H, t, *J* = 7.0 Hz).

^{13}C-NMR ($CDCl_3$, 125 MHz), δ_C (ppm), *J* (Hz): 14.12 (CH_3), 22.70 (CH_2), 25.06 (CH_2), 25.96 (CH_2), 28.68-31.94 (CH_2, very strong), 31.94 (CH_2), 34.44 (CH_2), 64.41 ($\underline{CH_2}$OH).

1-eicosanol (6) ($C_{20}H_{42}O$): white solid. ESI-MS m/z: 298.2 (15 %) $[M]^+$, 338.2 (80 %) $[M+K+H]^+$.

^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm), *J* (Hz): 0.88 (3H, t, *J* = 7.0), 1.25 (36H, brs), 1.59-1.53 (2H, m), 3.63 (2H, t, *J* = 6.5).

^{13}C-NMR ($CDCl_3$, 125 MHz), δ_C (ppm), *J* (Hz): 14.10 (CH_3), 22.69, 25.74 (CH_2), 29.36-32.83 (CH_2), 63.12 ($\underline{CH_2}$OH).

Glycerol 1,3-di-(9Z,12Z-octadecadienoate) 2-hexadecanoate (7) ($C_{55}H_{98}O_6$): yellow oil. ESI-MS m/z: 875.7 (98 %) $[M+H_2O+3H]^+$, 595.4 (10 %) $[M+3H-CO(CH_2)_{14}CH_3)]^+$.

^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm), *J* (Hz): 0.86-0.92 (6H, m), 0.97 (3H, t, *J* = 7.5), 1.34-1.06 (52H, brs), 1.59-1.67 (6H, m), 2.04-2.09 (8H, m), 2.29-2.32 (6H, m), 2.76-2.81 (4H, m), 4.28 (2H, dd, *J* = 11.5, 4.0), 4.15 (2H, dd, *J* = 12.0, 6.0), 5.15-5.18 (1H, m), 5.32-5.39 (8H, m).

3. RESULTS AND DISCUSSION

Compound **1** was isolated as yellow oil. The IR spectrum exhibited the absorptions of an ester group at υ 1740.14 (COO) and ankyl 2923.11 (CH). The ^{1}H- and ^{13}C-NMR spectrum of compound **1** showed the characteristic of an unsaturated methyl ester, corresponding to 5 double bonds at δ_H 5.32-5.37 (10H, m)/δ_C 127.14-131.98, a cacbonyl ester at δ_C 174.31, a methyl group at δ_H 0.98 (3H, t, *J* = 7.5)/δ_C 14.27, a methoxy group at δ_H 3.66 (3H, s)/δ_C 51.43, together with the signals of methylene groups at δ_H 1.60-1.62 (2H, m, $\underline{CH_2}CH_2COOCH_3$), 2.03-2.09 (4H, m), 2.30 (2H, t, *J* = 7.5, $CH_2\underline{CH_2}COOCH_3$), 2.75-2.81 (8H, m, 4xCH=CH$\underline{CH_2}$CH=CH). The ESI-MS spectrum showed a pseudomolecular ion peak at m/z 317.0 (25 %, $[M+H]^+$). On the basis of the above mentioned data and comparison with those of literature data [10], the structure of compound **1** was elucidated as glycerol 1,3-di-(9Z,12Z-octadecadienoate) 2-hexadecanoate. This compound is a minor component of sunflower and some vegetable oils.

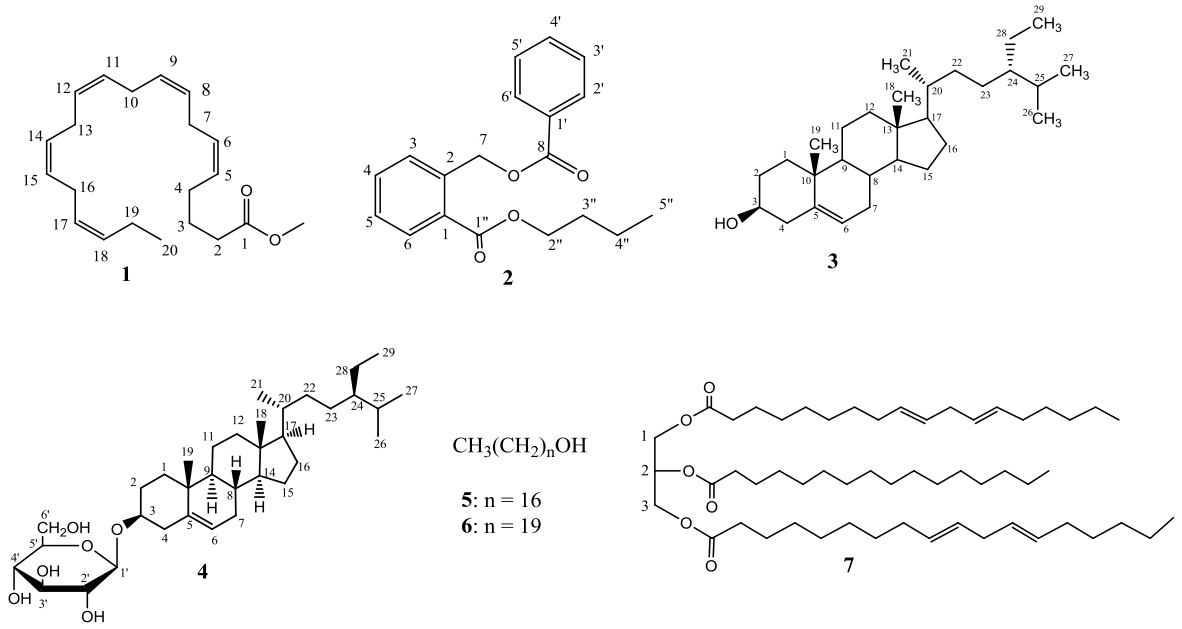

Figure 1: Compounds isolated from the ethyl acetate (**1**-**4**) and *n*-hexane (**5**-**7**) extracts of the barks of *Litsea glutinosa*

Compound **2** was obtained as colour less oil. The IR spectrum of **2** exhibited absorption at 2964 (C-H), 1724 (COOR- ester), 1283 (C-O). The ESI-MS spectrum showed a pseudomolecular ion peak at 313.0 (98 %) $[M+H]^+$. The ^{1}H-NMR of **2** (table 1) indicated the presence of nine aromatic protons corresponding to the two aromatic six-member ring with mono- and disubstitution.The aromatic protons resonated at δ_H 7.75-7.74 (1H, m, H-6), 7.71-7.70 (1H, m, H-3), 7.53-7.51 (2H, m, H-5 and H-4), 7.42-7.41 (2H, m, H-2',6'), 7.37-7.32 (3H, m, H- 3', 4', 5'). The ^{1}H-NMR also displayed four sets of methylene protons including one oxygenated methylene proton singlet at δ_H 5.33 (2H, s), one oxygenated methylene proton triplet and δ_H 4.19 (2H, t, *J* = 7.0 Hz) and other two sets of methylene multiplets at δ_H 1.66-1.60 (2H,m) and 1.40-1.36 (2H, m). The methyl protons signal appeared at δ_H 0.92 (3H, t, *J* = 7.0 Hz).

The ^{13}C-NMR of compound **2** (Table 1) revealed 19 carbon signals including one methyl (δ_C 13.70), four methylene (δ_C 67.46, 65.57, 30.51, 19.57), two carbonyl ester (δ_C 167,65 and 167,40) and twelve aromatic carbons (δ_C 128.36-135.52). On the basis of the spectroscopy study (IR, MS and NMR), it was concluded that compound **2** is an aromatic ester containing a side chain bearing a hydroxymethylene functionality. The molecular formula of **2** was

established as $C_{19}H_{20}O_4$. By comparison of the spectral data of compound **2** with those of reported data [11], it was determined that **2** was spatozoate. This compound was isolated for the first time from the brown alga *Spatoglossum variable*.

Compounds **3** (ß-sitosterol) and (**4**) (β-sitosterol 3-O-β-D-glycopyranoside or daucosterol) were isolated as white needles. Their structures were determined by comparison of their ^{1}H- and ^{13}C-NMR spectral data with those in [12] and [13], respectively.

Table 1: Comparison of the spectral data of compound **2** with spatozoate in the literature [11]

Nr.	Compound **2** ($CDCl_3$)		Spatozoate ($CDCl_3$, [11])	
	δ_H (500 MHz) *J* (Hz)	δ_C (125 MHz)	δ_H (500 MHz) *J* (Hz)	δ_C (125 MHz)
1	-	132.52	-	132.6
2	-	135.52	-	132.0
3	7.71-7.70 (m)	130.92	7.70 (m)	130.8
4	7.53-7.51 (m)	128.86	7.51 (td, 7.5, 1.5)	128.3
5	7.53-7.51 (m)	129.03	7.53 (td, 7.5, 1.5)	129.1
6	7.75-7.74 (m)	131.12	7.74 (dd, 7.5, 1.5)	131.1
7	5.33 (s)	67.46	5.32 (s)	67.5
8	-	167.65	-	167.6
1'	-	131.83	-	131.9
2'	7.42-7.41 (m)	128.58	7.47 (dd, 8.3, 1.8)	128,6
3'	7.37-7.32 (m)	128.40	7.30 (td, 8.3, 1.8)	128.4
4'	7.37-7.32 (m)	128.30	7.12 (dd, 8.5, 1,8)	128.0
5'	7.37-7.32 (m)	128.40	7.30 ((td, 8.3, 1.8)	128.4
6'	7.42-7.41 (m)	128.58	7.47 (dd, 8.3, 1.8)	128.6
1"	-	167.40	-	167.4
2"	4.19 (t, 7.0)	65.57	4.23 (t, 7.0)	65.6
3"	1.66-1.60 (m)	30.51	1.75 (m)	30.5
4"	1.40-1.36 (m)	19.14	1.42 (m)	19.2
5"	0.92 (t, 7.0)	13.70	0.90 (t, 7.4)	13.7

Compound **5** (1-heptadecanol) and **6** (1-eicosanol) were isolated as white powder. The ^{1}H-NMR of these compounds demonstrated the signals of saturated primary alcohols for compound **5** at δ_H 0.88 (3H, t, *J* = 6.5), 1.25 (28H, brs), 1.62-1.55 (2H, m) 4.05 (2H, t, *J* = 7.0 Hz) and 0.88 (3H, t, *J* = 7.0), 1.25 (36H, brs), 1.59-1.53 (2H, m), 3.63 (2H, t, *J* = 6.5) for compound **6,** respectively. The molecular formula of compound **5** ($C_{17}H_{36}O$) and compound **6** ($C_{20}H_{42}O$) were deduced from their ESI-MS mass spectra. Their structures were determined by comparison NMR and MS spectra with those of literature data [14, 15]. These compounds are presented in many conifer waxes.

Compound **7** (glycerol 1,3-di-(9Z,12Z-octadecadienoate) 2-hexadecanoate) was isolated as yellow oil. The ^{1}H-NMR spectrum of this compound showed the signals characterized of a triacylglycerol, which were assignable to four double bonds protons at δ_H 5.32-5.39 (8H, m), an oxygenated methine at δ_H 5.15-5.18 (1H, m, H-2), two oxygenated methilene protons at δ_H 4.28 (2H, dd, *J* = 11.5, 4.0, H-1) and 4.15 (2H, dd, *J* = 12.0, 6.0, H-3), three methyl proton groups at 0.86-0.92 (6H, m), 0.97 (3H, t, *J* = 7.5) and the signals of methilene protons of the saturated and unsaturated glycerol ester at C-1, C-2 and C-3. The molecular formula of this compound was determined as $C_{55}H_{98}O_6$, based on the analysis

of mass spectrum. The ESI-MS spectrum showed an ion peak at 875.7 (98 %) $[M+H_2O+3H]^+$ and a fragment ion peak at 595.4 (10 %) $[M+3H-CO(CH_2)_{14}CH_3)]^+$, indicating the presence of an hexadecanoyl group attached at C-2 of glycerolester. The structure of compound **7** was identified by comparison of its spectral data with those reported in the literature [16].

4. CONCLUSION

In conclusion, the study of chemical constitution of the barks of *Litsea glutinosa* led to the isolation of seven compounds, among them compounds (**1**), (**2**), (**5**), (**6**), (**7**) were isolated for the first time from this plant. This is also the first report of the phytochemistry of *Litsea glutinosa* in Vietnam.

Acknowledgment. *The authors sincerely thank the foundation for the seven priority research fields of science and technology-Vietnam Academy of Science and Technology (Project Nr. VAST04.03/1516).*

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