Study on Chemical Constituents of the Vietnamese Medicinal Plant *Fissistigma petelotii*

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A new bisabolene derivative (1*S**, 2*R**, 4*R**, 7*R**, 10*R**)-1,7,10,11-tetrahydroxy-1,7,11-trimethyl-4,10(H)-2-O-cinnamoyl-bisabol-8(9)-ene (**1**), together with cinnamic acid (**2**), methyl cinnamate (**3**), sodium cinnamate (**4**) and methyl elaidate (**5**) have been isolated from the leaves and barks of *Fissistigma petelotii*. Sodium cinnamate (**4**) was isolated for the first time from the nature. The structures of these compounds were elucidated by analysis of their IR, MS, 1D and 2D NMR spectra.

Key words: Fissistigma petelotii, Bisabolene, Cinnamic Acid, Methyl Cinnamate, Sodium Cinnamate, Methyl Elaidate

Introduction

Fissistigma petelotii Merr., (Annonaceae), a climbing branched shrub is distributed throughout China, Vietnam, Thailand, Brazil, and tropical America [1]. Traditionally, its leaves are used to ameliorate malaria and seasonal fever [2]. The chemical constituents of this species have not yet been studied before. As a part of our research project focussing on Vietnamese medicinal plants, we here report the isolation and structural identification of a new bisabolene derivative (**1**) together with four other compounds including cinnamic acid (**2***)*, methyl cinnamate (**3**), sodium cinnamate (**4**) and methyl elaidate (**5**) from the leaves of *Fissistigma petelotii*. Compound **1** is a highly hydroxy-substituted bisabolene derivative and the first bisallylic alcohol oxygenated at C-2 with a cinnamate moiety.

Results and Discussion

Compound **1** was isolated from the ethyl acetate extract of the leaves of *F. petelotii* as a colorless oil. The structure of **1** was deduced from comparison of its NMR spectra with the spectra of similar bisabolene derivatives [3, 4]. Compound **1** produced a molecular ion peak at *m/z* = 441.2250, $[M+Na]^+$, in the positive HR-TOF-ESI-MS, establishing the molecular formula $C_{24}H_{34}O_6$. The UV spectrum of **1** showed absorptions at λ_{max} = 277.7, 216.3, and 201.8 nm indicating the presence of a substitued aromatic ring. Its IR spectrum contained absorptions at ν_{max} = 3449 (OH), 2926 (CH_3, cyclohexyl), 1687 (COO), and 1634 cm^{-1} (C=C). In the ^1H and ^{13}C NMR spectra, a ^1H doublet at δ_H = 7.71 (J = 15.98 Hz), a double doublet at δ_H = 6.47 (J = 15.98, 1.22 Hz) and a carbon signal at δ_C = 165.29 indicated that the compound had an α, β unsaturated ester moiety. The large coupling constant of the protons at the double bond ($J \sim 16$ Hz) suggested a *trans*-configuration. A doublet at δ_H = 5.85 (1H, J = 15.73 Hz) and double doublet at δ_H = 5.74 (1H, J = 15.71, 8.47 Hz) indicated another double bond in the *trans*-configuration. The molecular formula of **1** demands eight double bond equivalents. As three are accounted by an ester carbonyl and two double bonds, the molecule must have an aromatic and another carbocycle. This was confirmed by the presence of five aromatic protons at δ_H = 7.53 (2H) and δ_H = 7.39 (3H). The ^1H NMR spectrum also exhibited signals for oxygenated methines at δ_H = 4.80 – 4.78 (m, 1H) and 3.94 – 3.92 (m, 1H). The ^{13}C NMR and DEPT spectra showed a total of 24 carbons including a carbonyl ester, 4 methyl (δ_C = 26.06 × 2, 25.40, and 23.05), 3 methylenes (δ_C = 36.09, 27.11, and 20.16), 12 methines, and 5 quaternary carbons. The assignment of all carbons and the placement of the methyl and hydroxy groups within the molecule were achieved by 2D experiments. The HMBC spectrum showed correlations between the methyl protons and the quaternary carbons C-7 (δ_C = 73.24), C-11 (δ_C = 71.86), C-1 (δ_C =

Table 1. NMR spectral data of compound **1** in $CDCl_3$ (500 MHz for ^1H and 125 MHz for ^{13}C)[a].

Position	δ_C	δ_H (mult., *J* in Hz)	HMBC correlations (^{13}C No.)	COSY correlations	NOESY correlations
1	69.38	–			
2	76.84	4.80 – 4.78 (m)		H-3, H-4, H-5, H-6	H-15, H-3 (w)
3	36.09	3a: 1.89 (m)	C-4, C-1, C-2	H-4, H-5, H-6	H-4, H-5, H-6, H-15
		3c: 1.42 (m)			
4	45.38	1.58 (m)	C-2, C-1	H-2, H-3, H-5, H-6	H-3, H-5, H-14, H-8
5	27.11	5a: 1.89 (m)	C-4, C-1, C-2	H-3, H-4, H-6	H-4, H-6, H-3
		5b: 1.60 (m)			
6	20.16	6b: 1.63 (m)	C-2, C-1	H-2, H-3, H-4, H-5	H-3, H-5
		6c: 1.47			
7	73.24	–		–	
8	137.99	5.85 (d, 15.73)	C-9, C-7, C-10, C-14	H-9	H-10, H-4, H-14 (w)
9	126.00	5.74 (dd, 15.71, 8.47)	C-8, C-7, C-10	H-8, H-10	H-13 (w)
10	78.14	3.94 – 3.92 (m)	C-11, C-9, C-8, C-13	H-9	H-12, H-8
11	71.86	–		–	
12, 15	26.06	12: 1.22 (s)	C-10, C-11, C-13	–	H-2, H-3
		15: 1.20 (s)	C-1, C-2, C-3	–	H-10, H-13 (w)
13	23.05	1.15 (s)	C-11, C-12, C-10	–	H-12, H-9
14	25.40	1.33 (s)	C-4, C-7, C-8	–	H-4, H-8 (w)
1′	165.29	–		–	
2′	144.39	7.71 (d, 15.98)	C-1′, C-3′, C-4′, C-5′, C-9′	H-3′	H-5′, H-9′
3′	116.79	6.47 (dd, 15.98, 1.22)	C-1′, C-4′	H-2′	H-5′, H-9′
4′	133.26	–			
5′, 9′	127.13	7.53 (m)	C-2′, C-7′, C-8′, C-6′	H-6′, H-7′, H-8′	H-2′, H-3′, H-6′, H-7′, H-8′
6′, 8′	127.93	7.39 (t, 3.27)	C-4′, C-5′, C-9′, C-7′	H-5′, H-9′	H-5′, H-9′
7′	129.45	7.39 (t, 3.27)	C-4′, C-5′, C-9′, C-6′, C-8′	H-5′, H-9′	H-5′, H-9′

[a] Assignments are based on 2D COSY, HSQC, and HMBC experiments; w = weak.

69.38), indicating that the four methyl groups are attached to these carbons. The hydroxyl carbon C-11 exhibited cross peaks with the dimethyl groups δ_{H-12} = 1.22, δ_{H-13} = 1.15 and H-10 (δ_H = 3.93). The hydroxyl carbon C-10 (δ_C = 78.14) was further connected to the double bond proton H-9 (δ_H = 5.74) by a ^2J correlation in the HMBC spectra. The COSY spectra also showed the expected coupling of H-9 with H-8, and H-2′ with H-3′. There are correlations between H-9 (δ_H = 5.74) and H-8 (δ_H = 5.85) with the quaternary carbon C-7 (δ_C = 73.24) in the HMBC experiment. Furthermore, C-7 showed direct coupling with the methyl protons H-14 (δ_H = 1.30). The remaining methyl group was located at C-1 from the HMBC correlation between the signal at δ_C = 69.38 and the proton signals of H-15 (δ_H = 1.20). The chemical shift of the signals of C-7, C-11 and C-1 were compared with those of 7,11-dihydroxy-bisabol-2,9E-diene isolated from *Achillea odorata* [3] and of 1,2,3,6,7-pentahydroxy-2-acetoxy-bisabol-10(11)-ene [4] from *Matricaria aurea*, confirming the placement of the hydroxyl and methyl substituents at these carbons. The presence of a cyclohexane ring was deduced from the ^1H-^1H COSY correlations ($^3J_{HH}$) and the CH long range correlation in the HMBC experiment (Table 1, Fig. 1). The methylene

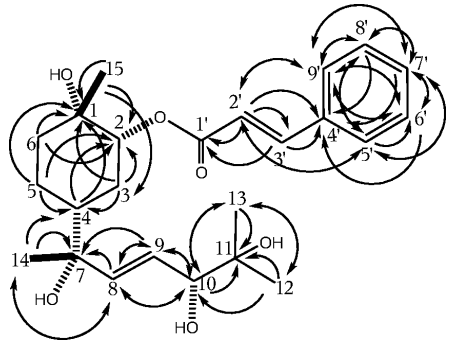


Fig. 1. HMBC correlations in **1**.

protons H-3, H-5, and H-6 were diagonal across from each other. The long range HMBC correlation between the methyl signal at δ_{H-14} = 1.33 (Me-7) and C-4 (δ_C = 45.38) revealed the substitution at position C-4 of the cyclohexane ring. The quaternary C-1 (δ_C = 69.38) was confirmed as a ring member due to the HMBC correlations with protons H-3, H-4, H-5, and H-6. In the HMBC experiment, the double bond proton H-3′ (δ_H = 6.47) showed a ^2J correlation with the quaternary carbon of the aromatic ring C-4′ (δ_C = 137.93), while H-2′ (δ_H = 7.71) showed a ^2J correlation with the ester carbonyl C-1′ (δ_C = 165.29). The downfield

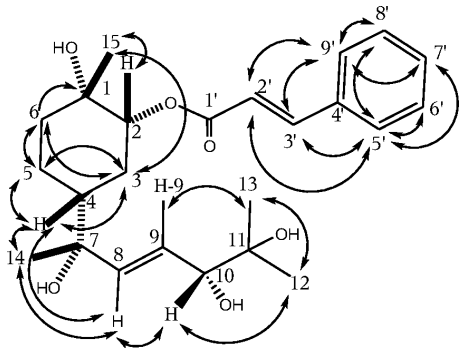


Fig. 2. NOESY correlations observed for **1**.

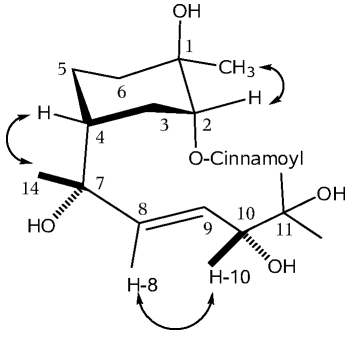
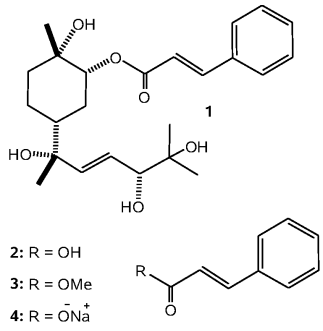
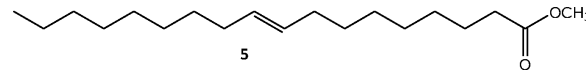


Fig. 3. Important NOESY correlations for **1**.

shift of the double bond proton H-2′ is consistent with a neighboring group effect from the C-1′ ester carbonyl. The *trans* configuration of the double bond and the presence of a cinnamate ion in the ESI-MS spectrum proved that a cinnamate group is a part of the molecule. The joining of a cinnamate moiety with a bisabolene segment could be confirmed by the presence of two fragment peaks at *m/z* = 171, [cinnamic acid + Na]$^+$, and 293, [M+Na–cinnamic acid]$^+$, in the ESI-MS spectrum. Furthermore, the downfield shift of the oxygenated methine proton H-2 (δ_H = 4.80) suggested that the cinnamate moiety was connected to C-2.

The NOESY experiment supported the configuration of the stereogenic centers at C-1, C-2, C-4, C-7, and C-10. The relative stereochemistry of **1** was established as shown in Fig. 2. In the NOESY spectrum, H-10 showed a relatively strong correlation with H-8, whereas its correlation with the neighboring proton H-9 was weak. Furthermore, the large coupling constant between H-9 and H-10 (J = 8.47) revealed that both protons H-10 and H-9 are located in the individual plane of the double bond. This allowed us to assign the orientation to H-10. The





NOEs between the methyl protons H-14 and H-4 of the cyclohexane ring suggested the relative configuration of C-7 and C-4 (see Fig. 3). In addition, the NOESY crosspeaks between the methyl protons H-15 and H-2 established a β-orientation for Me-1 and a β-orientation for H-2, confirming the correct relative configuration (1S^*, 2R^*) of the cyclohexane ring.

The presence of the four hydroxyl groups in compound **1** was confirmed by acetylation of **1** and subsequent LC-MS analysis. At r. t., the monoacetate was detected as the main product of the acetylation mixture. The ESI mass spectra gave an ion peak at *m/z* = 483.2, [M+Na]$^+$, indicating one acetyl group in the molecule. When the acetylation was carried out at 80 °C, the triacetate (*m/z* = 543.4, [M–H]$^-$) and tetraacetate (*m/z* = 585.4, [M–H]$^-$) were also observed.

Cinnamic acid (**2**), methyl cinnamate (**3**), sodium cinnamate (**4**), and methyl elaidate (**5**) have also been isolated from the ethyl acetate extract of the leaves and barks of *F. petelotii*. Their structures were determined by comparison of their spectral data with reference data [5 – 9]. Compound **2** was highly unstable when it was kept in MeOH or DMSO. This is probably due to the attachment of the nucleophilic solvents to the carboxylic-conjugated double bonds of cinnamic acid (**2**), forming an intermediate adduct. This may be the reason why the signal of the COOH group has not been observed in the ^{13}C NMR spectrum of **2** measured in CD_3OD and [D_6]DMSO. By atomic absorption spectroscopy (AAS) measurement, the metal ion in **4** has been determined as sodium. This is the first time, sodium cinnamate was isolated from plant material.

Experimental Section

General

Optical rotation was determined with a POLAX-2L instrument (ATAGO-Japan). IR spectra were measured on an IMPACT 410 (Nicolet, USA) spectrometer as dry film. UV/Vis spectra were recorded on a Cintra 40 (GBC) instrument. AAS was performed on a Perkin-Elmer AAS-3300 (USA) instrument. ^{1}H, ^{13}CNMR, DEPT, and 2D NMR (COSY, NOESY, HSQC, HMBC) spectra were recorded on an Avance 500 Bruker spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C). ESI-MS and LC-MS were recorded on an Agilent LC/MSD Trap 1100 mass spectrometer with a 5 μm, 30 × 150 mm ZORBAX SB-C18. HR-TOF-ESI-MS was obtained on a Bruker 70e FT-ICR instrument (Bruker Daltonic, USA). TLC were performed on silica gel 60 F_{254} (0.2 mm, Merck.Co). Flash column chromatography was carried out on silica gel Merck 60 (230 – 400 mesh). Melting points are uncorrected and were determined using a Electrotherman IA9200 apparatus.

Plant material

The leaves of *F. petelotii* were collected at Sin Ho district, Son La province, Vietnam, in October, 2006 and identified by Mr. Ngo Van Trai (Institute of Materia Medica) and Mr. Nguyen The Anh (Institute of Chemistry, Hanoi). A voucher specimen (No. SHH10) is deposited in the Herbarium of the Institute of Materia Medica Ha Noi, Vietnam.

Extraction and isolation

The dry leaves of *F. petelotii* (1.7 kg) were powdered and extracted three times with aqueous methanol (80 %) at r. t. The organic solvent was evaporated *in vacuo*, and the aq. solution was successively extracted with *n*-hexane, EtOAc and *n*-BuOH. The solvents were concentrated under reduced pressure to afford *n*-hexane (27.6 g), EtOAc (29.2 g), and *n*-BuOH extracts (45.3 g). The EtOAc extract (29.2 g) was chromatographed on a silica gel column [CH_2Cl_2-MeOH (2 % MeOH gradient)] to produce six subfractions (frs. 1 – 6). Subfraction fr. 4 (2.57 g, eluted with 10 % MeOH) was further separated on a RP-18 column [H_2O-MeOH mixture (5 % MeOH gradient)] to give seven subfractions. The fifth subfraction (0.36 g, eluted with 40 % MeOH in H_2O) was further chromatographed on silica gel [*n*-hexane : CH_2Cl_2 : MeOH (1 : 1 : 0.2)] to afford compound **3** (5.2 mg) as a white solid. Repeated column chromatography of the fourth subfraction (0.51 g, eluted with 35 % MeOH in H_2O) using CH_2Cl_2 : MeOH (9.6 : 0.4) gave three subfractions. The first subfraction (0.21 g) was subjected to a Sephadex LH-20 column (100 % MeOH), followed by flash column chromatography on silica gel [*n*-hexane : EtOAc : MeOH (1 : 1 : 0.25)] to yield compound **1** as a colorless oil (22 mg).

Subfraction fr. 3 (10 g, obtained by eluting with 10 % MeOH from the first column) was purified by column chromatography on silica gel [*n*-hexane : CH_2Cl_2 : MeOH (1 : 1 : 0.1)] to produce six subfractions. The fourth subfractions (3.85 g) was subjected to a RP-18 column [MeOH : H_2O (5 % MeOH gradient)], followed by flash column chromatography [CH_2Cl_2 : MeOH (9.7 : 0.3)] to give compound **2** as a white solid (12.8 mg). Compound **4** (15.7 mg) was obtained as a yellow powder from the first subfraction by Sephadex LH20 column chromatography (*n*-hexane : CH_2Cl_2 : MeOH = 2 : 5 : 1), followed by chromatography on an RP-18 column [MeOH : H_2O (1 : 2)] and then a silica gel column [*n*-hexane : CH_2Cl_2 : MeOH (1 : 1 : 0.2)]. Subfraction fr. 2 (2.5 g, obtained by eluting with 6 % MeOH from the first column of the ethyl acetate extract) was chromatographed on a silica gel column [*n*-hexane : CH_2Cl_2 : MeOH (1 : 1 : 0.1)] to give six subfractions. The third subfraction (1.9 g) was submitted to an RP-18 column, eluting with MeOH-H_2O mixtures (6.5 : 3.5, 7.5 : 2.5, 350 mL each) to afford five subfractions. The fifth subfraction (0.29 g) was further purified by chromatography over silica gel [CH_2Cl_2 : MeOH (9 : 1)] to give compound **5** as a colorless oil (8.6 mg).

*Acetylation of compound **1***

To a well-stirred solution of compound **1** (4.5 mg, 0.0107 mmol) in 0.4 mL of pyridine was added slowly acetic anhydride (0.05 mL, 0.15 mmol). The reaction mixture was stirred at r. t. for one day, and pyridine was removed under reduce pressure. Purification by silica gel chromatography [CH_2Cl_2 : MeOH (9.7 : 0.3)] gave 2.4 mg (50.5 % yield) of the monoacetate of **1** as a white solid.

Similarly, a mixture of compound **1** (2 mg, 0.048 mmol) in 0.2 mL of pyridine with acetic anhydride (0.05 mL, 0.15 mmol) and 4-dimethylaminopyridine (3 mg, 0.025 mmol) was heated at 80 °C for 3.5 h. Pyridine was then removed under reduced pressure, and the residue was subjected to LC-MS. A mixture of H_2O/MeOH (85 : 15) was used as mobile phase. The monoacetates appeared at retention times of 19.13, and 19.43 min; diacetates were not detected; triacetates appeared at 19.93, 20.62 and 20.72 min, and tetraacetates at 21.21, and 21.38 min. Based on LC-MS quantitative analysis, the ratio of monoacetate : triacetates : tetraacetates in the acetylation mixture was 1.1 : 2.5 : 1.

[(1S, 2R*, 4R*, 7R*, 10R*)-1,7,10,11-Tetrahydroxy-1,7,11-trimethyl-4,10(H)-2-O-cinnamoyl-bisabol-8(9)-ene] (**1**)*

Colorless oil. – UV/Vis (EtOH): λ_{max} ($\lg \varepsilon_{max}$) = 277.7 (2.21). – $[\alpha]_D^{20} = -77.8°$ (c = 0.19, MeOH). – IR (KBr): ν = 3449 (OH), 2926, 2855 (CH_3, cyclohexyl), 1687 (COOR), 1634 (C=C), 1456, 1377, 1163, 1016, 768 cm^{-1}. – MS ((+)-ESI): *m/z* (%) = 441.2 (91.6) $[M + Na]^+$, 293.0

(12.1) $[M+Na–C_6H_5–CH=CH–COOH]^+$, 171 (6) $[C_6H_5–CH=CH–COOH + Na]^+$. – HRMS-TOF ((+)-ESI): *m/z* = 441.2250 (calcd. 441.2246 for $C_{24}H_{34}O_6Na$, $[M + Na]^+$). – NMR data: see Table 1.

*The monoacetate of compound **1***

White solid. – MS (EI, 70 eV): *m/z* (%) = 140 (57.8), 131 (90.5), 98 (100). – MS ((+)-ESI): *m/z* (%) = 483.2 (70.3) $[M + Na]^+$, 495.6 (85.5) $[M – H + 2H_2O]^+$. – HRMS-TOF ((+)-ESI): *m/z* = 483.2359 (calcd. 483.2353 for $C_{26}H_{36}O_7Na$, $[M + Na]^+$). – ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.72 (d, *J* = 15.99, 1H), 7.55 – 7.52 (m, 2H), 7.40 – 7.30 (m, 3H), 6.47 (d, *J* = 16.00, 1H), 5.97 (d, *J* = 15.75, 1H), 5.74 (dd, *J* = 15.70, 8.15, 1H), 5.30 (d, *J* = 2.81, 1H), 4.77 (dd, *J* = 11.19, 4.45, 1H), 2.10 (s, 3H), 2.02 – 2.00 (m, 1H), 1.90 – 1.86 (m, 1H), 1.81 – 1.69 (m, 3H), 1.48 – 1.47 (m, 2H), 1.33 (s, 3H), 1.25 (s, 3H), 1.21 (s, 3H), 1.08 (s, 3H). – ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 170.09, 166.29, 145.48, 144.66, 134.23, 130.49, 129.04, 128.94, 128.15, 120.76, 120.39, 117.74, 78.49, 77.95, 77.87, 77.68, 75.32, 74.95, 70.62, 70.33, 42.69, 42.31, 37.25, 37.09, 31.93, 30.94, 29.73, 29.70, 29.46, 29.36, 22.70, 21.77, 21.52, 21.33, 19.11.

*Cinnamic acid (**2**)* [5, 6]

White solid. M. p. 132 °C. – IR (KBr): ν = 3433 (OH), 1681 (COOH), 1632 (CH=CH), 1387, 1318, 1219, 983, 771 cm^{-1}. – MS (EI, 70 eV): *m/z* (%) = 148 (72) $[M]^+$, 131 (22.4), 112 (6.4), 103 (65.6) $[M – COOH]^+$, 91 (36), 83 (20), 77 (72) $[M – CH=CH–COOH]^+$, 71 (15.2) $[M – C_6H_5]^+$, 63 (9.6), 57 (26.4), 55 (19.2), 51 (82.4). – MS ((+)-ESI): *m/z* (%) = 149 (93.6) $[M + H]^+$, 144 (30.9), 131 (86.3) $[M – OH]^+$, 113 (33.6), 103 (8.1), 85 (14.5), 74 (50.9), 57 (3.6), 46 (2.7). – ^{1}H NMR (CD_3OD, 500 MHz): δ = 7.63 – 7.57 (m, 3H), 7.42 – 7.38 (m, 3H), 6.51 (d, *J* = 15.9, 1H). – ^{13}C NMR (CD_3OD, 125 MHz): δ = 144.83, 136.28, 130.98, 129.92, 128.97, 121.4.

*Methyl cinnamate (**3**)* [7]

White solid. M. p. 37 °C. – MS (EI, 70 eV): *m*/z = 162 (40) $[M]^+$, 149 (4), 131 (96.8) $[M – OCH_3]^+$, 117 (5.6), 103 (100) $[M – COOCH_3]^+$, 91 (9.6), 85 (17.6), 77 (88), 71 (20.8), 63 (13.6), 57 (36.8), 51 (55.2). – ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.69 (d, *J* = 16.02, 1H), 7.53 – 7.51 (m, 2H), 7.39 – 7.37 (m, 3H), 6.44 (d, *J* = 16.05, 1H), 3.81 (s, 3H).

*Sodium cinnamate (**4**)* [8]

Yellow powder. – IR (KBr): ν = 1640 (CH=CH), 1549, 1416, 970, 771 cm^{-1}. – ^{1}H NMR (D_2O, 500 MHz): δ = 7.58 – 7.57 (m, 2H), 7.42 – 7.37 (m, 3H), 7.34 (d, *J* = 16.07, 1H), 6.47 (d, *J* = 16.06, 1H). – ^{13}C NMR (D_2O, 125 MHz): δ = 176.16, 141.16, 135.64, 130.01, 129.41, 128.1, 124.66.

*Methyl elaidate (**5**)* [9]

Colorless oil. – IR (KBr): ν = 3447, 2924, 2866, 1741 (COO), 1629.73, 1461, 1369, 1172 cm^{-1}. – MS (EI, 70 eV): *m/z* (%) = 297 (22.4) $[M + H]^+$, 272 (14.4), 264 (17.6), 222 (7.2), 199 (4.8), 166 (4.8), 143 (15.2), 111 (17.6), 97 (34.4), 87 (65.6), 74 (100), 55 (96), 53 (11.2). – ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.35 – 5.33 (m, 1H), 3.66 (s, 3H), 2.3 (t, *J* = 7.51, 2H), 2.02 – 1.99 (m, 2H), 1.63 – 1.57 (m, 2H), 1.30 – 1.25 (m, 24H), 0.88 (t, *J* = 6.73, 3H). – ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 174.33, 130.01, 129.76, 51.43, 34.12, 31.93, 29.77, 29.69, 29.53, 29.46, 29.33, 29.26, 29.16, 29.09, 27.22, 27.17, 24.96, 22.69, 14.11.

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