FATTY COMPOUNDS FROM THE TRUNK OF COFFEA ROBUSTA

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ABSTRACT

*Robusta coffee, Coffea robusta is the primary coffee contributing significantly to the coffee processing industry in Vietnam. Up to now, the research on coffee trees has mainly focused on coffee beans and leaves. Their chemical composition has been investigated and revealed the presence of caffeine, trigonelline, alkaloids, chlorogenic acids, lipids, steroids, terpenoids, etc. Coffee trees planted for harvesting seeds are often cut off after the aging period. To improve efficiency of coffee trees, the research on chemical compositions of Robusta coffee trunk is proposed. Five fatty compounds were isolated from the dried powdered-trunks of Coffea robusta (Rubiaceae), including stearic acid (**1**), hexacosatrienoic acid (**2**), 2,3-dihydroxypropyl (21Z,24Z) nonacosa-21,24-dienoate (**3**), stigmasterol (**4**), and β-sitosterol 3-O-β-D-glucopyranoside (**5**). Their chemical structures were elucidated by spectroscopic means as well as comparing with data in the literature. To the best of our knowledge, these substances were isolated for the first time from the trunk of Coffea robusta and it is the first time the compounds **2** and **3** were investigated from this genus.*

Keywords: *Coffea; glycerol; lipid; fatty; steroid.*

1. INTRODUCTION

Robusta coffee, *Coffea robusta* [1] is the most primary coffee, contributing significantly to the coffee processing industry in Vietnam. Up to now, the research on coffee trees has mainly focused on coffee beans and leaves. Their chemical composition has been investigated and revealed the presence of caffeine, trigonelline, alkaloids, chlorogenic acids, lipids, steroids, terpenoids, [2] Coffee trees planted for harvesting seeds are often cut off after the aging period. In order to improve the efficiency of coffee trees, research on the chemical composition of coffee trunks is proposed.

This research reported the isolation and structural elucidation of five lipid compounds from the Robusta coffee trunk in Lam Dong province, Vietnam.

2. MATERIALS AND METHOD

2.1 Materials

The trunk of *Coffea robusta* is collected in August 2018 in Lam Dong province. A voucher specimen (UTE–A001) was deposited in the herbarium of the Department of Chemical Technology, Ho Chi Minh City University of Technology and Education.

2.2 Apparatus

NMR spectra were measured on a Bruker Avance III spectrometer, at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C-NMR, using residual solvent signal as internal reference. The ESI-MS spectra were recorded on a MicroOTOF–Q mass spectrometer.

2.3 Method

The dried trunk of *Coffea robusta* (30 kg) were exhaustively extracted with methanol by maceration at room temperature and the methanolic filtrate was concentrated *in vacuo* to yield a residue of 1,400 g. This crude residue was soaked in *n*-hexane and stirred thoroughly. The solvent has been removed from the *n*-hexane-soluble portion under low pressure to obtain *n*-hexane extract (H, 300 g). The same

manner was applied for the *n*-hexane-unsoluble portion using ethyl acetate instead of *n*-hexane to afford ethyl acetate extract (EA, 180 g).

The *n*-hexane extract (H, 300g) was applied on a silica gel chromatographic column eluting with *n*-hexane: ethyl acetate 85:15, 8:2, 7:3, 5:5 to give 7 fractions. The fraction H2 (18.87 g) was separated on a silica gel column eluting with *n*-hexane–ethyl acetate 98:2, 95:5, 9:1, 8:2, 5:5 to obtain 6 subfractions. Subfraction H2.4 (2.4 g) was subjected to silica gel column with the eluent chloroform – methanol 100:0 and 97:3 to yield **2** (6 mg) and **4** (15 mg), respectively. Fraction H3 (4.73 g) was chromatographed through silica gel columns to obtain **1** (20 mg). The same procedure was applied for fraction H6 (5.47 g) with the eluent *n*-hexane - ethyl acetate 7:3 to give 7 fractions. The subfraction H6.5 (1.75 g) was separated into 5 fractions using *n*-hexane - acetone 7:3 as the eluate. Compound **3** (20 mg) was isolated from H6.5.4 (0.27 g) by silica gel chromatography eluting with chloroform-acetone 75:25.

The ethyl acetate (180 g) was fractionated into 9 parts using silica gel chromatography with *n*-hexane-ethyl acetate 5:5, 4:6, 2:8, then 100% ethyl acetate, next ethyl acetate-methanol 9:1, 8:2. Fraction EA9 (20 g) was subjected to silica gel chromatographic column and eluted with ethyl acetate-methanol 1:0, 9:1, 8:2, 5:5, 0:1 to separate into 7 subfractions. The subfraction EA9.3 (1.46 g) was chromatographed three times with eluents of chloroform-methanol 9:1 to yield compound **5** (5 mg).

* Stearic acid (**1**): white wax, HR-ESI-MS *m/z* 283.2635 [M-H]; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm, Hz) 2.34 (2H, t, 7.5, H-2), 1.63 (2H, sept, 7.0, H-3), 1.10-1.35 (brs), 0.88 (3H, t, 7.0, H-18); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C (ppm) 179.3 (C-1), 33.9, 31.4, 29.7, 29.6, 29.4, 29.2, 29.1, 24.7, 22.7, 14.1 (C-18).

*Hexacosatrienoic acid (**2**): white amorphous powder; HR-ESI-MS *m/z* 413.2768 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 5.34 (m), 2.44 (m), 2.04 (m), 1.65 (m), 1.26 (m), and 0.88 (t, 7.0). ^{13}C-NMR (CDCl3, 125 MHz) δ_C(ppm) 178.9 (-COOH), 130.9, 130.0, 129.7, 128.8, 128.1, 127.9 (C=C), 14.0 - 40.0 (saturated carbons).

* 2,3-Dihydroxypropyl (21*Z*,24*Z*) nonacosa-21,24-dienoate (**3**): white amorphous powder; ESI-MS *m/z* 554.4 $[M+2Na]^+$ (554.4 calcd for $C_{32}H_{60}O_4Na_2$); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm, Hz) 5.35 (4H, m), 4.17 (2H, m), 3.92 (1H, m), 3.69 (1H, dd, 11.5, 4.0), 3.59 (1H, dd, 12.0, 5.5), 2.77 (2H, t, 6.5), 2.35 (t, 7.5), 2.05 (4H, q, 7.0), 1.63 (t, 7.0), 1.31 (brs), 0.89 (3H, t, 7.0) and ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C (ppm) 174.3, 130.2, 130.0, 128.1, 127.9, 70.3, 65.2, 63.4, 34.1, 31.5, 25.6, 24.9, 22.6, 14.0.

* Stigmasterol (**4**) : white amorphous powder; ^{1}H and ^{13}C-NMR ($CDCl_3$) showed in Table 1.

* β-Sitosterol 3-*O*- β-D-glucopyranoside (**5**): white amorphous powder; ^{1}H and ^{13}C-NMR ($CDCl_3$) showed in Table 1.

3. RESULTS AND DISCUSSION

The *n*-hexane extract of the dried powdered trunk of *Coffea robusta* was applied on silica gel chromatography columns to afford four compounds, **1**-**4**. Compound **5** was isolated from the ethyl acetate extract.

3.1 Structural elucidation of compound 1

Compound **1** was isolated as white wax. The HR-ESI-MS of **1** showed a pseudo-molecular ion pick at *m/z* 283.2635 $[M-H]^-$ (suitable with the molecular formula of $C_{18}H_{36}O_2$). The ^{1}H-NMR spectrum revealed only aliphatic proton signals in the upfield zone at δ_H 2.34 (2H, t, 7.5), 1.63 (2H, sept, 7.5), 1.10-1.35 (brs), and 0.88 (3H, t, 7.0). The ^{13}C- and DEPT-NMR spectra exhibited a carboxyl signal at δ_C 179.3, 16 methylene carbons at δ_C from 22.0 to 35.0 and a terminal methyl at δ_C 14.1. All these evidence demonstrated a long chain saturated fatty acid. By the above

information and comparison with the report [3], compound **1** was determined as stearic acid.

3.2 Structure elucidation of compound 2

Compound **2** was isolated as white amorphous powder. The HR-ESI-MS of **2** showed a pseudo-molecular ion peak at *m/z* 413.2768 $[M-Na]^+$ (suitable with the molecular formula of $C_{26}H_{46}O_2$). The ^1H NMR spectrum of **2** showed the presence of olefinic protons at δ_H 5.34 (m), saturated protons at δ_H 1.06 – 2.96 and along with a terminal methyl at δ_H 0.88. The ^{13}C-NMR spectrum displayed a carboxyl at δ_C 178.9, olefinic methines at δ_C 127.9, 128.1, 128.8, 129.7, 130.0, 130.9, and saturated carbons at δ_C 14.0 - 40.0. Based on the analyzed spectroscopic data, compound **2** was determined as hexacosatrienoic acid. The position of three double bonds could not be indicated accurately in the long chain by neither 1D nor 2D NMR.

3.3 Structure elucidation of compound 3

Compound **3** was isolated as colorless oil. The ^1H-NMR spectrum revealed a glycerol lipid skeleton. In the ^1H-NMR spectrum, there were four olefinic protons at δ_H 5.35 (4H, m), five oxygenated protons at δ_H 4.17 (2H, m, H-1), 3.92 (1H, m, H-2), 3.69 (1H, dd, 11.5, 4.0, H-3), 3.59 (1H, dd, 12.0, 5.5, H-3), saturated protons near to double bonds at δ_H 2.77 (2H, t, 6.5), 2.05 (4H, q, 7.0), a long chain at δ_H 1.31 (16H, brs) owning a terminal methyl at δ_H 0.89 (3H, t, 7.0). In the COSY spectrum, the correlations from H-1 through H-2 to H-3 were found to support the glycerol moiety. The ^{13}C-NMR spectrum demonstrated the long unsaturated chain belonging to a fatty acid with carboxyl carbon at δ_C 174.3, olefinic methines at δ_C 130.2, 130.0, 128.1, 127.9, and oxygenated carbons at δ_C 70.3, 65.2, 63.4. The attachment of the fatty chain was determined at C-1 of glycerol moiety by the HMBC spectrum (Figure 2). The length of fatty acid was determined by *m/z* 554.4 $[M+2Na]^+$ suitable with the molecular formula of $C_{32}H_{60}O_4$. As the calculation, the long chain was assigned as a 22-carbon fatty acid with 2 double bonds. The location of two double bonds was identified at C-21 and C-24 by HMBC correlations depicted in Figure 2. The configuration of two these double bonds was established *cis*-fashion because of small coupling constants of H-21 and H-24 (J=4.0 Hz). By the above information, the compound **3** was determined as 2,3-dihydroxypropyl (21*Z*,24*Z*) nonacosa-21,24-dienoate.

3.4 Structural elucidation of compound 4

Compound **4** was isolated as white amorphous powder. The ^1H-NMR spectrum exhibited three olefinic proton signals at δ_H 5.35 (1H, d, 5.0, H-6), 5.01 (1H, dd, J = 15.0, 8.5) and 5.15 (1H, dd, J = 15.0, 8.5), a hydroxy methine proton signal at δ_H 3.52 (1H, m, H-3), and saturated proton signals from δ_H 0.60 to 2.60. In the ^{13}C- and DEPT-NMR spectra, an olefinic quaternary carbon signal at δ_C 140.8 and an olefinic tertiary carbon signal at δ_C 121.7 for C-5 and C-6, respectively, two olefinic methines at 138.3 and 129.3 for C-22 and C-23, the oxygenated carbon at δ_C 71.8 for C-3 confirmed the steroid skeleton of **4**. Comparing with the published report [4], compound **4** was identified as stigmasterol.

3.5 Structural elucidation of compound 5

Compound **5** was isolated as white amorphous powder. Its ^1H-NMR spectrum exhibited an olefinic proton signal at δ_H 5.38 (1H, d, 5.0, H-6), a hydroxy methine proton signal at δ_H 3.58 (1H, m, H-3), and saturated proton signals from 0.60 to 2.60 ppm for a steroid skeleton. Moreover, there was an anomeric proton signal at δ_H 4.42 (1H, d, 8.0, H-1') and the oxygenated proton signals from δ_H 3.00 to 5.00 for a sugar unit. In the ^{13}C and DEPT NMR spectra, an olefinic quaternary carbon signal at δ_C 140.3 and an olefinic tertiary carbon signal at δ_C 122.3 for C-5 and C-6, respectively, the oxygenated carbon at δ_C 70.1 for C-3 confirmed the β-sitosterol skeleton of **5**. The anomeric proton signal at δ_H 4.42 (1H, d, 8.0, H-1') assigning to a carbon at δ_C 101.2, the oxygenated

proton signals from δ_H 3.00 to 5.00 ppm along with four oxygenated methines and a methylene from δ_C 60.0 to 80.0 demonstrated the presence of a β-glucopyranosyl moiety. Based on this information and the comparison of the published data [5], compound **5** was identified as β-sitosterol 3-*O*-β-glucopyranoside.

4. CONCLUSION

From the trunk of *Coffea robusta* collected in August 2018 in Lam Dong province, five fatty compounds were isolated and elucidated as stearic acid (**1**), hexacosatrienoic acid (**2**), 2,3-dihydroxypropyl (21*Z*,24*Z*) nonacosa-21,24-dienoate (**3**)**,** stigmasterol (**4**), and β-sitosterol 3-*O*-β-D-glucopyranoside (**5**). These substances were isolated for the first time from the trunk of *Coffea robusta.* It is the first time, the compounds **2** and **3** were investigated from this genus.

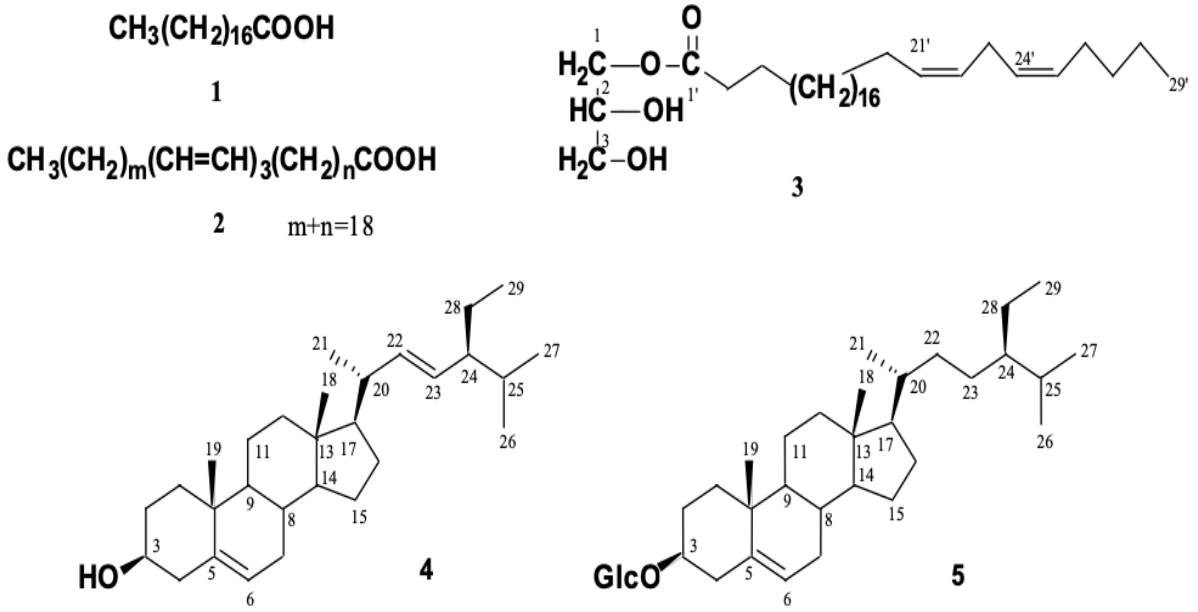

***Figure 1**. Chemical structures of the isolated compounds*

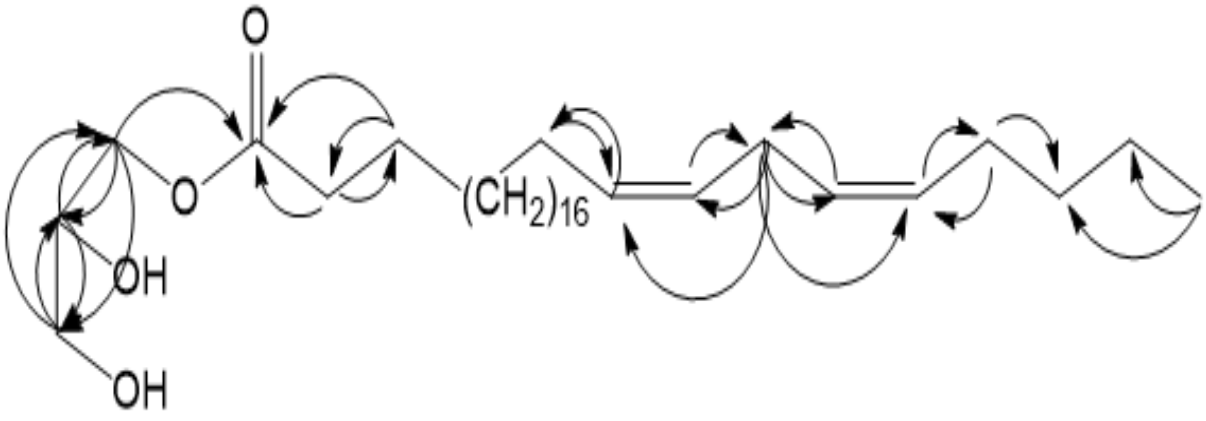

Figure 2**. Some key HMBC correlations in compound **3

Table 1. *The ^{1}H and ^{13}C-NMR data of compounds **4** and **5***

Position	**Compound 4** ($CDCl_3$)		**Compound 5** ($CDCl_3$)	
	δ_H, ppm (J, Hz)	δ_C, ppm	δ_H, ppm (J, Hz)	δ_C, ppm
1	-	37.3	-	37.3
2	-	31.7	-	32.0
3	3.52 (1H, m)	71.8	3.58 (1H, m)	70.1
4	-	45.9	-	42.4
5	-	140.8	-	140.3
6	5.35 (1H, d, 5.0)	121.7	5.38 (1H, d, 5.0)	122.3
7	-	31.8	-	32.0
8	-	31.9	-	31.9
9	-	50.2	-	50.3
10	-	36.5	-	36.8
11	-	21.1	-	21.1
12	-	39.8	-	39.8
13	-	42.3	-	42.4
14	-	56.8	-	56.8
15	-	25.4	-	24.4
16	-	28.9	-	29.0
17	-	55.9	-	56.2
18	1.01 (3H, s)	12.2	1.02 (3H, s)	11.9
19	0.68 (3H, s)	19.3	0.71 (3H, s)	19.4
20	-	40.4	-	36.2
21	1.03 (3H, d, 6.5)	18.9	0.92 (3H, d, 6.5)	18.8
22	5.01 (1H, dd, 15.0, 8.5)	138.3	-	34.0
23	5.15 (1H, dd, 15.0, 8.5)	129.3	-	26.2
24	-	51.2	-	46.0
25	-	31.6	-	29.3
26	0.81 (3H, d, 6.5)	20.2	0.81 (3H, d, 6.5)	19.8
27	0.84 (3H, d, 6.5)	19.1	0.82 (3H, d, 6.5)	19.1
28	-	21.2	-	22.4
29	0.86 (3H, t, 7.5)	11.9	0.85 (3H, t, 7.5)	12.0
1'			4.42 (1H, d, 8.0)	101.2
2'			3.49 (1H, m)	73.6
3'			3.48 (1H, m)	76.9
4'			3.41 (1H, m)	70.1
5'			3.60 (1H, m)	76.9
6'			3.86 (1H, brd, 11.0) 3.78 (1H, dd, 11.0, 4.0)	68.4

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