Chemical Constituents from the Stem Bark of *Fagraea fragrans* Roxb.

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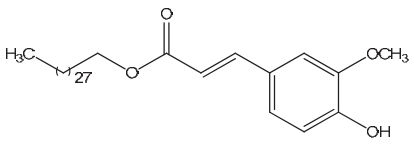
ABSTRACT

Fagraea fragrans Roxb. is a plant in the Gentainaceae family. This plant grows abundantly in Southeast Asia. It is known locally as Tammusu or Temmusu. F. *fragrans* has been used in the folk medicine. The isolated compounds from this plant were reported to show antimalarial, anti-HSV-1 and mild antimycobacterial activities. In the present work we investigated the chemical constituents of the crude methylene chloride extract from stem bark of F. *fragrans*. Four aromatic derivatives, (2*E*)-nonacosyl ester (1), cinnamaldehyde (2), vanillin (3) and syringaldehyde (4), were isolated. Their structures were elucidated by analysis of spectroscopic data, including comparison of data with those previously reported.



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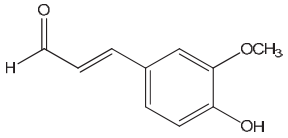

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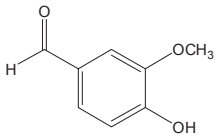

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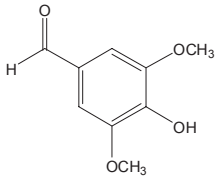

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Keywords: *Fagraea fragrans* Roxb., (2*E*)-Nonacosyl ester, Cinnamaldehyde, Vanillin, Syringaldehyde

1. INTRODUCTION

Fagraea fragrans Roxb. is a tall tree, 8 - 30 meter in height. This plant grows sparsely in southern and northeastern parts of Thailand, where it is called Kankrao (central), Tamsao or Thamsao (southern) and Man Pla (northern and northeastern). The medicinal uses of this plant have been reported in various regions where it grows. In Malay folk medicine, a decoction of the leaves and twigs of *F. fragrans* is used for the treatment of dysentery. The bark is believed to have medicinal value for malaria. The decoction of the bark is used to treat malaria in India, Cambodia, and Malaysia and as a febrifuge in Philippines. In Thai traditional medicine, it is believed that leaves contain antimalarial, element tonic, and antiasthmatic agents, and are externally used for mild infectious skin diseases, while an aqueous extract of the stems is used as a remedy for coughs [1-2]. In this report, we investigated the constituents of *F. fragrans* Roxb. and described the isolation and characterization of four aromatic derivatives.

2. MATERIALS AND METHODS

Plant material

The stem bark of *F. fragrans* Roxb. was collected from Satun province, in the southern of Thailand, in May 2012.

General Experimental Procedure

Quick column chromatography and column chromatography were carried out on silica gel 60 (230-400 Mesh ASTM, Merck) and silica gel 100 (70-230 Mesh ASTM, Merck), respectively. Aluminum Sheets of silica gel 60 F_{254} (Merck) were used for thin layer chromatography (TLC) and preparative thin-layer chromatography (PTLC), glass plates of silica gel 60 F_{254} (20×20 cm, 0.2 mm, Merck) were used for analytical purposes and the compounds were visualized under the ultraviolet light. Solvents for extraction and chromatography were distilled at their boiling ranges prior to use. The IR spectra were measured with a Perkin-Elmer FT-IR spectrophotometer. The ^{1}H- and ^{13}C-Nuclear magnetic resonance spectra were recorded on a FT-NMR Bruker Ultra Shield^{TM} 300 MHz spectrometer. Spectra were recorded in deuterochloroform ($CDCl_3$) and recorded as δ values in ppm downfield from TMS (internal standard δ 0.00). The Ultraviolet spectrums were measured with a Hewlett-Packard 8453. Melting point was recorded in °C on a digital Electrothermal 9100 Melting point apparatus. The EIMS mass spectra was recorded using Mass Spectrometer, MAT 95 XL, Thermo Finnigan, Germany at the Scientific Equipment Center, Prince of Songkla University.

Extraction and Isolation

The dried stem bark of *F. fragrans* (4.10 kg) was extracted successively three times with methylene chloride over a period of 3 days at room temperature. After evaporation, a dark brown gum of the methylene chloride extract (49.0 g) was separated by quick column chromatography using gradient solvent systems of hexane, hexane-methylene chloride, methylene chloride-methanol and finally with pure methanol. On the basis of their TLC characteristics, the fractions which contained the same major components were combined to give thirteen fractions (T1-T13). Fraction T3 (3.32 g) was separated by column chromatography on silica gel 100 and eluted with methylene chloride:hexane (4:6 v/v) to obtain fractions T3A-T3I. Fraction T3E was further purified by column chromatography on silica gel 100 and eluted with ethyl acetate:hexane (1:9 v/v) as an eluent to give a white solid of **1** (57.1 mg). Fraction T4 (2.81 g) was separated by column chromatography on silica gel 100 and eluted with ethyl acetate:hexane (1:9 v/v) to obtain fractions T4A-T4K. Fraction T4I (80.6 mg) was further purified by column chromatography on silica gel 100 and eluted with methylene chloride:hexane (9:1 v/v) as an eluent to give a yellow crystal of **3** (3.0 mg). Fraction T4J (102.5 mg) was further purified by column chromatography on silica gel 100 and eluted with acetone:methylene chloride (1:9 v/v) to obtain fractions T4J1-T4J5. Fraction T4J3 (21.1 mg) was further purified on preparative TLC and eluted with acetone:methylene chloride:hexane (1:4:5 v/v) to give a yellow powder of **2** (2.5 mg). Fraction T5 (4.90 g) was separated by column chromatography on silica gel 100 and eluted with methylene chloride:hexane (8:2 v/v) to obtain fractions T5A-T5L. Fraction T5F (275.6 mg) was further purified by column chromatography on silica gel 100 and eluted with acetone:hexane (1:9 v/v) as an eluent to give a yellow crystal of **4** (54.6 mg).

Compound 1 a white solid; m.p. 76.0-78.0ºC; EIMS (*m/z*) = 600; UV λ_{max} (MeOH) (log ε): 218 (3.16), 227 (3.10) and 324 (2.94) nm; IR (neat): 3518, 1701 and 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 7.61 (1H, *d*, *J* = 15.9 Hz, H-1′), 7.08 (1H, *dd*, *J* = 8.1, 1.9 Hz, H-6), 7.04 (1H, *d, J* = 1.9 Hz, H-2), 6.91 (1H, *d, J* = 8.1 Hz, H-5), 6.29 (1H, *d, J* = 15.9 Hz, H-2′), 6.01 (H, *brs*, 4-OH), 4.19 (2H, *t*, *J* = 6.7 Hz, H-1″), 3.91 (3H, *s*, 3-OCH_3), 1.69 (2H, *t*, *J* = 6.7 Hz, H-2″), 1.25-1.47 (52H, *m*, H-3″-H-28″), 0.90 (3H, *t*, *J* = 6.7 Hz, H-29″); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 14.1

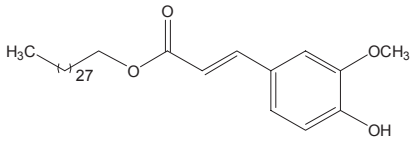
(C-29″), 22.7-32.8 (C-3″-C-28″), 26.0 (C-2″), 55.9 (3-OCH_3), 64.6 (C-1″), 109.3 (C-2), 114.7 (C-5), 115.6 (C-2′), 123.0 (C-6), 127.0 (C-1), 144.7 (C-1′), 146.8 (C-3), 147.9 (C-4), 167.4 (C-3′).

Compound 2 a yellow powder; m.p. 65.0-66.0ºC; UV-Vis (MeOH) λ_{max} (nm) (log ε) 201 (3.02), 221 (2.98) and 339 (3.09) nm; IR (neat): 3431, 1653 and 1628 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 9.65 (1H, *d*, *J* = 7.7 Hz, H-3′), 7.41 (1H, *d*, *J* = 15.8 Hz, H-1′), 7.13 (1H, *dd*, *J* = 8.2, 1.9 Hz, H-6), 7.07 (1H, *d*, *J* = 1.9 Hz, H-2), 6.96 (1H, *d*, *J* = 8.2 Hz, H-5), 6.60 (1H, *dd*, *J* = 15.8, 7.7 Hz, H-2′), 6.00 (1H, *brs*, 4-OH), 3.95 (3H, *s*, 3-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 55.9 (3-OCH_3), 109.5 (C-2), 114.9 (C-5), 124.0 (C-6), 126.5 (C-2′), 126.7 (C-1), 147.0 (C-3), 149.0 (C-4), 152.9 (C-1′), 193.5 (C-3′).

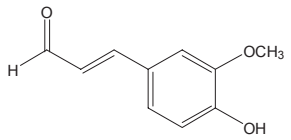
Compound 3 a yellow crystal; m.p. 78.0-79.0ºC; UV λ_{max} (MeOH) (log ε): 206 (3.38), 230 (3.39) and 307 (3.20) nm; IR (neat) : 3420, 1652 and 1540 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 9.83 (1H, *s*, H-7), 7.44 (1H, *d*, *J* = 1.8 Hz, H-2), 7.42 (1H, *dd*, *J* = 8.5, 1.8 Hz, H-6), 7.04 (1H, *d*, *J* = 8.5 Hz, H-5), 3.97 (3H, *s*, 3-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 56.1 (3-OCH_3), 108.8 (C-2), 114.4 (C-5), 127.6 (C-6), 129.9 (C-1), 147.2 (C-3), 151.7 (C-4), 191.0 (C-7).

Compound 4 a yellow crystal; m.p. 117.0-118.0 ºC; UV-Vis (MeOH) λ_{max} (nm) (log ε) 215 (3.36), 230 (3.31) and 307 (3.21) nm; IR (neat): 3425, 1689 and 1538 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 9.82 (1H, *s*, H-7), 7.16 (2H, *s*, H-2, H-6), 3.98 (6H, *s*, 3, 5-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 56.5 (3, 5-OCH_3), 106.7 (C-2, C-6), 128.4 (C-1), 140.9 (C-4), 147.4 (C-3, C-5), 190.7 (C-7).

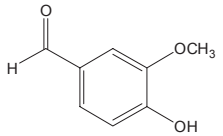
3. RESULTS



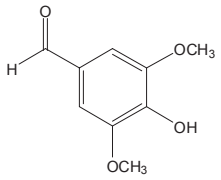
Compound 1 was obtained as a white solid. The UV spectrum exhibited the absorption bands characteristic of aromatic and carbonyl at 218, 227 and 324 nm. The IR spectrum showed absorption bands at 3518 (O-H stretching), 1701 (C=O stretching), and 1630 (C=C stretching) cm^{-1}. The EIMS mass spectrum afforded a signal for at *m/z* 600, consistent with the molecular formula $C_{39}H_{68}O_4$. In the ^{1}H NMR spectrum of compound **1** exhibited the signal of aromatic protons of a ferulic acid moiety at δ_H 7.08 (1H, *dd*, *J* = 8.1, 1.9 Hz, H-6), 7.04 (1H, *d*, *J* = 1.9 Hz, H-2) and 6.91 (1H, *d*, *J* = 8.1 Hz, H-5) which indicated the presence of a 1,3,4-trisubstituted benzene ring. A methoxyl singlet signal at δ_H 3.91 (3-OCH_3) and a hydroxy proton signal at δ_H 6.01 (*brs*, 4-OH) were proposed to be substituted at C-3 and C-4 position, respectively. The spectrum further showed the two *trans*-olefinic protons at δ_H 7.61 (1H, *d*, *J* = 15.9 Hz, H-1′) and 6.29 (1H, *d*, *J* = 15.9 Hz, H-2′). The large coupling constant of 15.9 Hz indicated *E*-configuration of H-1′ and H-2′, together with 29 carbon side-chain signals seen as signal of oxygenated methylene proton at δ_H 4.19 (2H, *t*, *J* = 6.7 Hz, H_2-1″), methylene proton at δ_H 1.69 (2H, *m*, H_2-2″) and 1.25-1.47 (52H, *m*, H_2-3″-H_2-28″) and one methyl proton at δ_H 0.90 (3H, *t*, *J* = 6.7 Hz, H_3-29″). These data corresponded to the feruloyl moiety with long chain hydrocarbon of 29 carbons. whose HMBC correlations of H-2 at δ_H 7.04 with the carbons at δ_C 147.9 (C-4), 123.0 (C-6) and 144.7 (C-1′). The proton signal at δ_H 6.91 (H-5) showed correlation with the carbons at δ_C 127.0 (C-1), 109.3 (C-2), 146.8 (C-3), and 147.9 (C-4) while the proton signal at δ_H 7.08 (H-6) showed correlation with the carbons at δ_C 147.9 (C-4), 144.7 (C-1′) and 109.3 (C-2). A methoxy proton signal at δ_H 3.91 showed correlation with the carbon at δ_C 146.8 (C-3) suggested this methoxyl group was located at C-3, and the hydroxyl group at δ_H 6.01 showed correlation with the carbons at δ_C 147.9 (C-4) and 114.7 (C-5) suggested this hydroxyl group at C-4 of benzene ring. The olefinic proton at δ_H 7.61 (H-1′) showed correlation with the carbons at δ_C 127.0 (C-1), 109.3 (C-2), 123.0 (C-6), 115.6 (C-2′) and 167.4 (C-3′). The olefinic proton at δ_H 6.29 (H-2′) showed correlation with the carbons at δ_C 127.0 (C-1), 144.7 (C-1′) and 167.4 (C-3′). The ester linkage was confirmed by the HMBC correlations of H_2-1″ (δ_H 4.19) and H-2′(δ_H 6.29) to the ester carbonyl carbon at δ_C 167.4 (C-3′). Thus, the structure of compound **1** was identified as (2*E*)-nonacosyl ester [3].



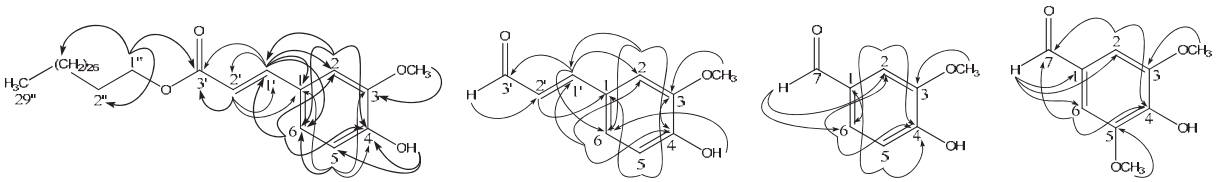
Compound 2 was obtained as a yellow powder. The UV spectrum exhibited the absorption bands characteristic of aromatic and carbonyl at 201, 221 and 339 nm. The IR spectrum showed absorption band at 3431 (O-H stretching) and 1653 (C=O stretching) and 1628 (C=C stretching) cm^{-1}. The ^{1}H NMR spectrum of compound **2** showed a signal pattern similar to those of compound **1**. The differences were shown as the absence of signals (*E*) nonacosyl as in compound **1** but the presence of a singlet signal of aldehyde group at δ_H 9.65. In the HMBC correlation spectrum, the olefinic proton at δ_H 6.60 (H-2′) showed correlation with the carbon at δ_C 126.7 (C-1) and another olefinic proton at δ_H 7.41 (H-1′) showed correlation with the carbons at δ_C 109.5 (C-2), 124.0 (C-6) and aldehyde carbon at δ_C 193.5 (C-3′), indicating the attachment of a propenal group at C-1. Thus on the basis of its spectroscopic data and comparison with the previously reported therefore compound **2** was identified as cinnamaldehyde [4].



Compound 3 was obtained as a yellow crystal. The UV spectrum exhibited the absorption bands characteristic of aromatic and carbonyl at 206, 230 and 307 nm. The IR spectrum showed absorption band at 3420 (O-H stretching), 1652 (C=O stretching) and 1540 (C=C stretching) cm^{-1}. The ^{1}H NMR spectrum of compound **3** showed a signal pattern similar to those of compound **2**. The differences were the absence of propenal signals but the presence of the aldehydic proton at δ_H 9.83, δ_C 191.0. The formyl group (δ_H 9.83) was assigned to be at C-1 (δ_C 129.9) due to HMBC correlations between the carbons at δ_C 108.8 (C-2) and 127.6 (C-6). Therefore compound **3** was identified as vanillin [4].



Compound 4 was obtained as a yellow crystal. The UV spectrum exhibited the absorption bands characteristic of aromatic and carbonyl at 215, 230 and 307 nm. The IR spectrum showed absorption band at 3425 (O-H stretching), 1689 (C=O stretching) and 1538 (C=C stretching) cm^{-1}. The ^{1}H and ^{13}C NMR spectral data of compound **4** were similar to those of compound **3**. The differences were the absence of signals for aromatic ABX system but the presence of a singlet signal of two aromatic protons at δ_H 7.16, suggesting the presence of a symmetrical 3,4,5-trisubstituted benzaldehyde. The formyl group at δ_H 9.82 was assigned to be at C-1 due to HMBC correlations between the carbons at δ_C 106.7 (C-2, C-6) and 128.4 (C-1). The proton signal at δ_H 7.16 (H-2, H-6) showed correlations with the carbons at δ_C 128.4 (C-1), 147.4 (C-3, C-5), 140.9 (C-4) and 190.7 (C-7). The two methoxyl groups at δ_H 3.98 (6H) showed HMBC correlations with the carbon at δ_C 147.4 (C-3 and C-5), indicating the location of these two methoxyl groups at C-3 and C-5, respectively, leaving the hydroxyl group at C-4. Therefore compound **4** was identified as syringaldehyde [5].



1 **2** **3** **4**

Figure 2. Major HMBC correlations of compounds **1-4** (H➡C).

4. CONCLUSIONS

The methylene chloride extract from the dried stem bark of *Fagraea fragrans* Roxb. was purified by chromatographic technique led to the isolation of four aromatic derivatives: (2*E*)-nonacosyl ester (**1**), cinnamaldehyde (**2**), vanillin (**3**) and syringaldehyde (**4**). Their structural elucidations were determined by spectroscopic data.

ACKNOWLEDGEMENTS

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