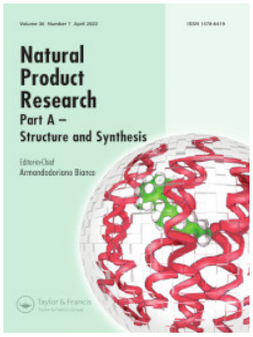


Natural Product Research

Formerly Natural Product Letters

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

A new lignan from the stem bark of *Fagraea fragrans* Roxb

Suthida Rattanaburi, Kaodeeya Kaikaew, Ramida Watanapokasin, Souwalak Phongpaichit & Wilawan Mahabusarakamb

To cite this article: Suthida Rattanaburi, Kaodeeya Kaikaew, Ramida Watanapokasin, Souwalak Phongpaichit & Wilawan Mahabusarakamb (2022) A new lignan from the stem bark of *Fagraea fragrans* Roxb, Natural Product Research, 36:7, 1851-1856, DOI: 10.1080/14786419.2020.1821020

To link to this article: https://doi.org/10.1080/14786419.2020.1821020

NATURAL PRODUCT RESEARCH
2022, VOL. 36, NO. 7, 1851–1856
https://doi.org/10.1080/14786419.2020.1821020

Taylor & Francis
Taylor & Francis Group

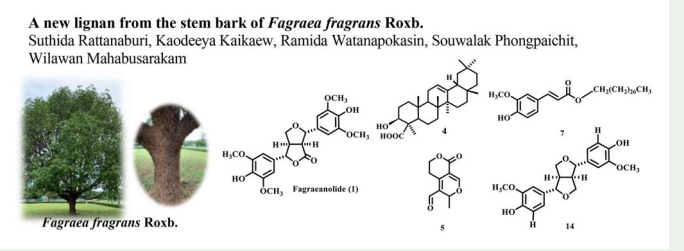
A new lignan from the stem bark of *Fagraea fragrans* Roxb

Suthida Rattanaburi[a], Kaodeeya Kaikaew[b], Ramida Watanapokasin[c], Souwalak Phongpaichit[d] and Wilawan Mahabusarakamb[e]*

[a]Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Muang, Phuket 83000, Thailand; [b]Division of Physical Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand; [c]Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, Sukhumvit 23, Bangkok 10110, Thailand; [d]Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand; [e]Natural Product Research Center of Excellence, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.

ABSTRACT

A new lignan, named fagraeanolide (**1**), and 14 known compounds were isolated from the stem bark of *Fagraea fragrans* Roxb. Their structures were determined by spectroscopic methods. Fagraeanolide is the first identified oxofurofuran lignan from the genus *Fagraea*, while *β*-boswelic acid (**4**), gentiogenol (**5**), 3-(4-hydroxy-3-methoxyphenyl)-acrylic acid octacosyl ester (**7**) and pinoresinol (**14**) were isolated from this plant for the first time. The crude extract of *F. fragrans* was not toxic to cell lines. The isolated compounds showed no antibacterial activity.



ARTICLE HISTORY
Received 10 June 2020
Accepted 25 August 2020

KEYWORDS
Gentianaceae; *Fagraea fragrans*; oxofurofuran lignan; antibacterial activity; cytotoxic activity

1. Introduction

Fagraea fragrans (Gentianaceae) has been used in herbal treatments of conditions such as malaria, asthma, coughing, breathing difficulties, anemia, disability and hemorrhoids. It has been used to treat skin blisters, to maintain spleen function, as a blood tonic and as an expectorant (Latip et al. 1999). Alkaloids, isocoumarins, iridoids, secoiridoids, secoiridoid glycoside, and terpenes were reported as secondary metabolites in

CONTACT Wilawan Mahabusarakam wilawan.m@psu.ac.th
Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1821020.

the bark (Madmanang et al. 2016), roots, flowers (Sangsopha et al. 2020), leaves and fruit (Wan and Chow 1964) of the plant. Antiplasmodial (Jonville et al. 2008), anti-inflammatory and anti-tumor (Jonville et al. 2010), and cytotoxicity activities (Sangsopha et al. 2020) of the isolated compounds have also been evaluated. In this article, the isolation and structure determination of a new lignan from the stem bark of *F. fragrans*, and the antimicrobial and cytotoxic activities of isolated compounds are described.

2. Results and discussion

A CH_2Cl_2 extract of the stem bark of *F. fragrans* was fractionated and purified by column chromatography, resulting in the isolation of a new oxofurofuran lignan named fagraeanolide (**1**) and 14 previously reported compounds (Figure 1). The known compounds were identified as α-boswelic acid (**2**) (Culioli et al. 2003), fraganal (**3**) (Madmanang et al. 2016), β-boswelic acid (**4**) (Culioli et al. 2003), gentiogenol (**5**) (Van der Sluis et al. 1983), syringaldehyde (**6**) (Okuyama et al. 1995), 3-(4-hydroxy-3-methoxyphenyl)-acrylic acid octacosyl ester (**7**) (Gong et al. 2006), 3-(4-hydroxy-3-methoxyphenyl)-acrylic acid 30-oxo-triacontyl ester (**8**) (Madmanang et al. 2016), fagraldehyde (**9**) (Jonville et al. 2008), angelone (**10**) (Mulholland et al. 2006), eudesmin (**11**) (Latip et al. 1999), monomethylpinoresinol (**12**) (Miyauchi and Ozawa 1998), medioresinol (**13**) (Ando et al. 2007), pinoresinol (**14**) (Latip et al. 1999), and syringaresinol (**15**) (Lin-Gen et al. 1982). Compounds **2**, **4**, **5**, **7** and **14** were isolated for the first time from this plant species.

Fagraeanolide **(1)** was obtained as yellow viscous liquid. $[\alpha]_D^{26} = +12^\circ$ ($c = 0.1$, $CHCl_3$). A molecular ion at *m/z* 432.1415 in the HREI-MS spectrum corresponded to a molecular formula of $C_{22}H_{24}O_9$. The UV spectrum exhibited maximum absorptions at 222 (4.4) and 272 (3.8) nm. The ^{13}C NMR spectrum (Table S1) showed signals for four methoxy (δ 56.7, 3′/5′/3′′/5′′-OCH_3), two methine (δ 53.4, C-1; δ 50.2, C-5), two benzylic oxymethine (δ 83.4, C-2; δ 84.6, C-6), one oxygenated methylene (δ 72.9, C-4), one carbonyl (δ 176.8, C-8,), four aromatic methine (δ 102.0, C-2′/C-6′; δ 102.2, C-2′′/C-6′′), and eight aromatic quaternary carbons (δ 130.3, C-1′; δ 131.6, C-1′′; δ 147.6, C-3′/C-5′; δ 147.5, C-3′′/C-5′′; δ 135.2, C-4′; δ 134.4, C-4′′). The ^1H NMR spectrum showed the resonances of *meta*-aromatic protons at δ 6.50 (2H, *s*, H-2′/H-6′) and δ 6.64 (2H, *s*, H-2′′/H-6′′), two hydroxy protons at δ 5.57 (1H, *s*, 4′-OH) and δ 5.50 (1H, *s*, 4′′-OH), and four signals of methoxy protons at δ 3.90 (12H, *s*, 3′/5′/3′′/5′′-OCH_3). The HMBC correlations (Figure S5) of H-2′/H-6′ to C-4′ (δ 135.2), 4′-OH and -OCH_3 to C-3′/C-5′ (δ 147.6), H-2′′/H-6′′ to C-4′′ (δ 134.4), and 4′′-OH and -OCH_3 to C-3′′/5′′ (δ 147.5) suggested that the signals belonged to two moieties of 4-hydroxy-3,5-dimethoxy phenyl. The remaining resonances were assigned to two methines at δ 3.44 (1H, *dd*, $J = 9.3$, 4.2 Hz, H-1) and δ 3.25 (1H, *m*, H-5), two benzylic oxy-methines at δ 5.33 (1H, *d*, $J = 4.2$ Hz, H-2) and δ 5.32 (1H, *d*, 3.6 Hz, H-6), and non-equivalent oxygenated methylene protons at δ 4.07 (1H, *dd*, $J = 9.6$, 5.4 Hz, H-4) and δ 4.40 (1H, *dd*, $J = 9.6$, 6.2 Hz, H-4). The protons were also confirmed by the COSY correlations of H-1 to H-2 and H-5, and H-5 to H-4 and H-6. The HMBC correlations of H-2 to C-4, C-5, C-8 and C-2′′/C-6′′, and H-6 to C-1, C-4, C-8 and C-2′/C-6′ allowed the assignation of a furofuran lignan with a carbonyl carbon

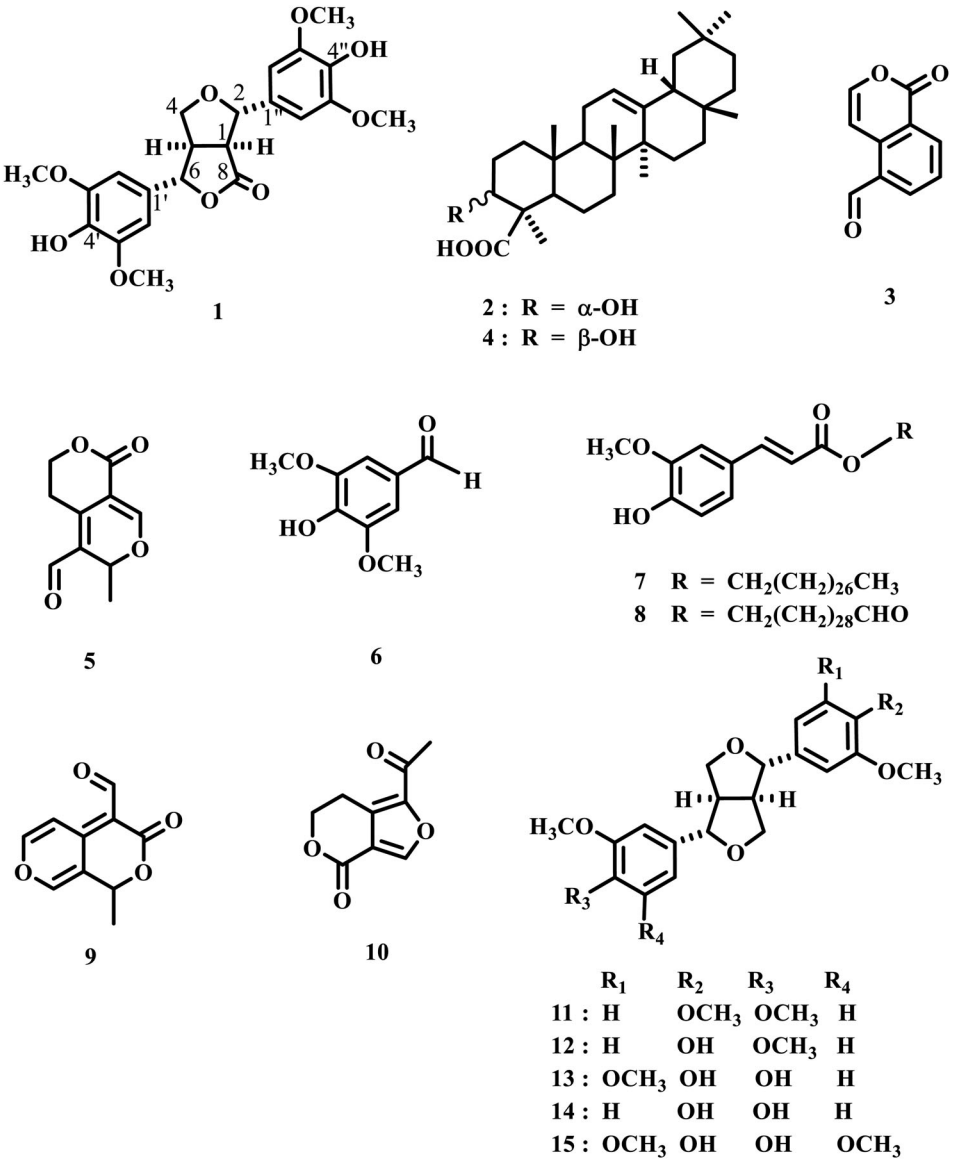


Figure 1. Structures of compounds isolated from the stem bark of *Fagraea fragrans*.

(δ 176.8) at C-8 and with aryl groups at C-2 and C-6. The small coupling constants of H-2/H-1 ($J = 4.2$ Hz) and H-6/H-5 ($J = 3.6$ Hz) indicated trans orientation of both sets of protons in the bipentacyclic (Min et al. 2004, Muhit et al. 2016). The lignan **1** could then be determined as 3,6-bis-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-3,7-dioxabicyclo [3.3.0]octane.

Crude extracts of *F. fragrans* at a concentration of 30 μg/ml were non-toxic to human colorectal cancer cells (COLO 205), human epidermoid carcinoma cells (A431) and canine osteosarcoma cells (D-17). Compounds **2**-**8** at a concentration of 200 μg/ml showed no effect on *Staphylococcus aureus* ATCC25923, *Methicillin-resistant*

Staphylococcus aureus SK1, *Pseudomonas aeruginosa, Eschericia coli*, *Candida albicans* NCPF3153, *Cryptococcus neoformans* ATCC90113, and *Microsporum gypseum*.

3. Experimental

3.1. General experimental procedures

UV spectra were produced using the UV-160A spectrophotometer (SHIMADZU). ^{1}H and ^{13}C-nuclear magnetic resonance spectra were recorded in $CDCl_3$ using FT-NMR (Bruker Avance 300 MHz). The EI-MS and HREIMS mass spectra were obtained using a MAT 95 XL mass spectrometer (Thermo Finnigan). Optical rotation $[\alpha]_D$ was measured in chloroform with Sodium D line (590 nm) on a JASCO P-1020 digital polarimeter. Thin layer chromatography (TLC) was carried out using pre-coated TLC sheets of silica gel 60 GF254. Quick column chromatography was carried out on silica gel 60H (Merck). Column chromatography (CC) was performed with silica gel 100 (Merck) and Sephadex LH-20.

3.2. Plant material

The bark of *F. fragrans* was collected in Nakhon Sri Thammarat Province in the South of Thailand, in May 2008. Identification was made by Mr. Ponlawat Pattarakulpisutti, Division of Biological Science, Faculty of Science, Prince of Songkla University. A specimen of the bark (K. kaikaew 01) has been deposited in the Herbarium of the Division of Biological Science, Faculty of Science, Prince of Songkla University, Thailand.

3.3. Extraction and isolation

Chopped, dried bark of *F. fragrans* (4.0 kg) was immersed in CH_2Cl_2 (12 liters) at room temperature for 4 days. After removal of the solvent, a dark brown gum of CH_2Cl_2 extract (38.9 g) was obtained. The extract (35.5 g) was separated by quick column chromatography using gradient elution with solvent systems of hexane, CH_2Cl_2 and Me_2CO to give fractions 1-20 according to their TLC characteristics. Fraction 2 (0.83 g) was further purified by CC, eluted with hexane-Me_2CO (9:1 to 8:2), to give **2** (80 mg) and **3** (5.5 mg) as white solids. A white solid **4** (2.5 mg) was obtained from fraction 3 (0.59 g). Crystallization of fraction 4 (0.56 g) in hexane-Me_2CO gave a yellow solid **5** (90.0 mg). Fraction 6 (0.55 g) was purified by CC, eluted with hexane-Me_2CO (9:1), to give a white solid **6** (11.8 mg). Fraction 7 (2.16 g) was fractionated by CC, using hexane-CH_2Cl_2-Me_2CO (7:2:1), to yield a white solid **7** (7.8 mg). Fraction 9 (2.27 g) was separated by CC, eluted with CH_2Cl_2-MeOH (9:1), to give a white solid **8** (4.9 mg) and a yellow solid **9** (8.8 mg). Fraction 12 (2.136 g) was separated by CC, eluted with hexane-CH_2Cl_2-Me_2CO (7:2:1), to produce a yellow solid **10** (12.6 mg), a white solid **11** (16.2 mg) and a brown gum **12** (8.8 mg). Fraction 13 (1.642 g) was separated on Sephadex LH-20, eluted with CH_2Cl_2-MeOH (9:1), and by CC, eluted with hexane-CH_2Cl_2-Me_2CO (3:1:1), to give a white solid **13** (5.6 mg), a brown gum **14** (12.6 mg) and **15** (18.8 mg). Fraction 17 (1.002 g) was isolated on Sephadex LH20, eluted with CH_2Cl_2-MeOH (7:3),

and by CC, using hexane-CH_2Cl_2-Me_2CO (2:2:1) as an eluent, to give a yellow viscous liquid **1** (3.3 mg).

Fagraeanolide (1)

A yellow viscous liquid, $[\alpha]_D^{26} = +12^\circ$ ($c = 0.1$, $CHCl_3$); UV (CH_3OH) λ_{max} nm (log ε): 222 (4.4), 272 (3.8); ^{1}H-NMR 300 MHz ($CDCl_3$) δ (ppm): **δ** 6.64, *s*, 2H, H-2′′/H-6′′; **δ** 6.50, *s*, 2H, H-2′/H-6′; **δ** 5.57, *s*, 1H, 4′-OH; **δ** 5.50, *s*, 4′′-OH; **δ** 5.33, *d*, $J = 4.2$ Hz, 1H, H-2; **δ** 5.32, *d*, $J = 3.6$ Hz, 1H, H-6; **δ** 4.40, *dd*, $J = 9.6$, 6.2 Hz, 1H, H-4; **δ** 4.07, *dd*, $J = 9.6$, 5.4 Hz, 1H, H-4; **δ** 3.90, *s*, 12H, 3′/5′/3′′/5′′-OCH_3; **δ** 3.44, *dd*, $J = 9.3$, 4.2 Hz, 1H, H-1; **δ** 3.25, *m*, 1H, H-5; ^{13}C-NMR 75 MHz ($CDCl_3$) δ (ppm): δ 176.8, C-8; δ 148.7, C-3′/C-5′; δ 147.5, C-3′′/C-5′′; 135.2, C-4′; δ 134.4, C-4′′; δ 131.6, C-1′′; δ 130.3, C-1′; δ 102.2, C-2′′/C-6′′; δ 102.0, C-2′/C-6′; δ 84.6, C-6; δ 83.4, C-2; δ 72.9, C-4; δ 56.7, 3′/5′/3′′/5′′-OCH_3, δ 53.4, C-1; δ 50.2, C-5.

3.4. Cytotoxic activity

Cancer cell lines used in this experiment included the normal human colorectal cancer cell (COLO 205), the human epidermoid carcinoma cell (A431) and the canine osteosarcoma cell (D-17). The cytotoxicity of the compounds on cancer cells was measured by standard 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, carried out according to the previously reported procedure of Rattanaburi et al. (2014).

3.5. Antibacterial activity

The antibacterial activity study followed the procedures of Rattanaburi et al. (2012).

To conclude, in this study, along with previously reported compounds, five other compounds were isolated from this plant for the first time. These compounds were α-boswelic acid (**2**), β-boswelic acid (**4**), gentiogenol (**5**), 3-(4-hydroxy-3-methoxy-phenyl)-acrylic acid octacosyl ester (**7**) and pinoresinol (**14**). In addition, a new oxofurofuran lignan, named fagraeanolide (**1**), was reported for the first time from the genus *Fagraea*.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Ando H, Hirai Y, Fujii M, Hori Y, Fukumura M, Niiho Y, Nakajima Y, Shibata T, Toriizuka K, Ida Y. 2007. The chemical constituents of fresh Gentian root. J Nat Med. 61(3):269–279.

Culioli G, Mathe C, Archier P, Vieillescazes C. 2003. A lupane triterpene from frankincense (Boswellia sp., Burseraceae). Phytochemistry. 62 (4):537–541.

Gong YQ, Yang H, Liu Y, Liang AQ, Wang ZT, Xu LS, Hu ZB. 2006. Studies on chemical constituents in stem of *Dendrobium chrysotoxum*. Zhongguo Zhong Yao Za Zhi. 31(4):304–306.

Jonville M, Baghdikian B, Ollivier E, Angenot L, Frédérich M, Legault J. 2010. Anti- inflammatory potency of the traditionally used antimalarial plant *Fagraea fragrans*. Planta Med. 76(12): WSI_6.

Jonville MC, Capel M, Frédérich M, Angenot L, Dive G, Faure R, Azas N, Ollivier E. 2008. Fagraldehyde, a secoiridoid isolated from *Fagraea fragrans*. J Nat Prod. 71(12):2038–2040.

Latip J, Hartley TG, Waterman PG. 1999. Lignans and coumarins metabolites from *Melicope hayesii*. Phytochemistry. 51(1):107–110.

Lin-Gen Z, Seligmann O, Jurcic K, Wagner H. 1982. Inhaltsstoffe von *Daphne tangutica*. Planta Med. 45(07):172–176.

Madmanang S, Cheyeng N, Heembenmad S, Mahabusarakam W, Saising J, Seeger M, Chusri S, Chakthong S. 2016. Constituents of *Fagraea fragrans* with Antimycobacterial Activity in Combination with Erythromycin. J Nat Prod. 79(4):767–774.

Min BS, Na MK, Oh SR, Ahn KS, Jeong GS, Li G, Lee SK, Joung H, Lee HK. 2004. New furofuran and butyrolactone lignans with antioxidant activity from the stem bark of *Styrax japonica*. J Nat Prod. 67(12):1980–1984.

Miyauchi T, Ozawa S. 1998. Formation of (+)-eudesmin in *Magnolia kobus* DC. var. Borealis sarg. Phytochemistry. 47(4):665–670.

Muhit MA, Umehara K, Noguchi H. 2016. Five furofuranone lignan glucosides from *Terminalia citrina* inhibit in vitro E2-enhanced breast cancer cell proliferation. Fitoterapia. 113(6):74–79.

Mulholland DA, Langlois A, Randrianarivelojosia M, Derat E, Nuzillard JM. 2006. The structural elucidation of a novel iridoid derivative from *Tachiadenus longiflorus* (Gentianaceae) using the LSD programme and quantum chemical computations. Phytochem Anal. 17(2):87–90.

Okuyama E, Suzumura K, Yamazaki M. 1995. Pharmacologically active components of Todopon Puok (*Fagraea racemosa*), a medicinal plant from Borneo. Chem Pharm Bull. 43(12):2200–2204.

Rattanaburi S, Mahabusarakam W, Phongpaichit S, Carroll A R. 2012. Neolignans from Callistemon lanceolatus. Phytochemistry Letters 5:18–21.

Rattanaburi S, Daus M, Watanapokasin R, Mahabusarakam W. 2014. A new bisanthraquinone and cytotoxic xanthones from Cratoxylum cochinchinense. Natural Product Research. 28(9): 606–610.

Sangsopha W, Lekphrom R, Schevenels FT, Byatt B, Pyne SG, Sridadom N, Sawanyawisuth K. 2020. A new secoiridoid glycoside and other constituents from the roots and flowers of *Fagraea fragrans* Roxb. (Gentianaceae). Natural Products Research. DOI:10.1080/14786419.2020.1749611.

Van der Sluis WG, Van der Nat JM, Spek AL, Ikeshiro Y, Labadie RP. 1983. Gentiogenal, a conversion product of gentiopicrin (gentiopicroside)*). Planta Med. 49(12):211–215.

Wan ASC, Chow YL. 1964. Alkaloids of *Fagraea fragrans* Roxb. J Pharm Pharmacol. 16(7): 484–486.