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Oliveramine: An Isolated Alkaloid from *Fagraea fragrans* (Tembesu) Bark

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Abstract

This paper described the progress of phytochemical, especially alkaloids, work on *Fagraea fragrans* species (tembesu), Loganiaceae. A dimeric-pyridine alkaloid with molecular formula $C_{20}H_{20}O_4N_2$ has been successfully isolated from diethyl ether extracts from this plant's barks. The alkaloid was explored from diethyl ether extracts using sulphuric acid (2%), then basified with ammonium hydroxide (25%) until the solution had a pH of 10.5. The later solution was extracted again with chloroform. The alkaloid residue was subjected to column chromatography and eluted with 10% acetone in chloroform. The chemical structure of the isolated dimeric-pyridine alkaloid was determined by LCMS/MS, ^{13}C-NMR, and ^{1}H-NMR spectroscopies. As a result, the isolated dimeric-pyridine alkaloid was closely similar to the oliveramine alkaloid, and this is also the first report about oliveramine from *Fagraea fragrans* (*F. Fragrans*).

1. Introduction

Selecting and exploring alkaloids from a species abundance, such as *Fagraea fragrans* Roxb. species, is a core determinant for developing a natural product into medicine in the future. *Fagraea fragrans*, the Loganiaceae (Gentianaceae) family called Tembesu plant, is a potential plant on the east coast of Sumatera island, mainly in the lowland forest of South Sumatera. Loganiaceae, the family of flowering plants in the order of Gentianales, contains 13 genera with more than 4000 species of woody vines, shrubs, or trees native primarily to tropical areas. Their fruits vary from capsules to fleshy drupes. Many members of the Loganiaceae are highly poisonous, causing death by convulsion. The toxic properties are primarily brought on by alkaloids, such as those in *Strychnos electri*. The secondary metabolite of the *Fagraea fragrans* (*F. fragrans*) plant is reported to have antimycobacterial activity [1, 2]. Our phytochemical research on its fruits has discovered three alkaloids. Those alkaloids are isaindigotone, gentianine, and gentioflavine [3, 4]. Meanwhile, this paper described our work on the bark of this plant and new lignin from this species was also reported [5], and a new secoiridoid glycoside and other constituents from the roots and flowers of *F. fragrans* Roxb. (Gentianaceae) [6] besides alkaloids [7, 8, 9]. Most of Gentiana, a cosmopolitan and essential genus of the Gentianaceae family, has alkaloids (gentianine, gentianidine) with antibacterial, antifungal, and hypotensive activities [10].

Alkaloids are organic bases (alkali-like), nitrogen-containing natural products, often with a rather complicated chemical structure. They are produced by plants, fungi, bacteria, and rarely by animals and usually show substantial toxicological and pharmaceutical effects. About 10% to 25% of higher plants contain alkaloids, including *Fagraea fragrans* [11]. Alkaloids are often localized in specific organs: in some plants, roots, fruits (seeds) or latex, bark, or leaves.

The seeds of *F. fragrans* reach a physiologically mature phase when the fruits are red, increasing their alkaloid content [9]. The fruits are required to develop for approximately 18 to 19 weeks. Based on the study by Prastyono and Haryjanto [12] and Rustam and Pramono [13], the ideal time to harvest can be predicted as early as the trees begin to generate flower buds. The last information is essential for phytochemical researchers working on those fruits. In addition, the need for the *F.*

fragrans wood for building constructions and household furniture in Palembang city reaches 1650–1700 m^3 per year. In summary, the bark of the *F. fragrans* plant is widely available in or around South Sumatra, including Malaysia Peninsula [12, 14]; see Figure 1 of the *F. fragrans* (Tembesu) plant during the fruiting season. A species with alkaloids shows a higher abundance than a non-alkaloid species, often linked to cultivation [12].

Figure 1. Fagraea fragrans (Tembesu) plant

2. Methodology

2.1. Materials

F. fragrans barks, ammonium hydroxide, diethyl ether, ethyl acetate, *n*-hexane, acetone, chloroform, hydrochloric acid, sodium sulfate anhydrous, silica gel G 60 F_{254} Merck, TLC plate 20 × 20 cm Merck, methanol, potassium hydroxide, distilled water, sodium agar, sodium borate, dragendorff's reagent.

2.2. Instruments

UNIFI-LC-MS Spectrometer, Xevo G2-XS QTOF [Channel: TOF MS^E (50-1200) 6eV ESI+ - Low CE (BPI)], Gamax UV, NMR spectrometer JNM-ECZ500R/S1500 MHz (Germany).

2.3. Plant Collection

F. fragrans barks (5 kg) were collected from lowland forest, Muaro Penimbung, OganIlir in October 2021, dried over one month, and then ground to be dried powder.

2.4. Extraction

The *F. fragrans* bark powder (1 kg) was extracted with diethyl ether (4 × 3 L) in two dark bottles (2.5 L) for 4 × 24 hours at room temperature. The filtrates (16 L) were then evaporated under reduced pressure to a volume of 350 mL. The concentrated diethyl ether extracts were extracted with 2% sulphuric acid (400 mL) using a separating funnel. The mixtures were shaken for 2 hours and kept overnight.

The sulphuric acid fraction was separated from diethyl ether and basified with ammonium hydroxide (25%) until the solution had a pH of 10.5. The later solution was extracted with chloroform (3 × 300 mL). The concentrated chloroform (250 mL) was dried with sodium sulfate anhydrous, filtered by Whatman paper, and then dried under reduced pressure using a rotary evaporator. This dried residue was prepared for the purification of alkaloids by chromatography.

The dried residue (500 mg) was dissolved with a small amount of methanol. The later solution was then preadsorbed on 2 g silica gel 60 (70–230 mesh), moved to column chromatography containing 20 g stationer phase of gel 60 $F_{254,}$ and eluted with 10% *n*-hexane in ethyl acetate. Vials 25 to 100 of 103 (10 mL each) of collected vials produced positive alkaloids. The separating process was continued using another column chromatography with 10% acetone in chloroform as solvent. Fifty vials were collected, and vials with numbers 11 to 17 gave positive alkaloids, white crystals, and oliveramine. In addition, vials numbered 18 to 25 did not provide solid crystals even though the isolated sample spots were positive for alkaloids under dragendorff's reagent and glowed under UV light at λ 254 nm. The chemical structure of the isolated alkaloid was determined using LCMS/MS and NMR 500 Mhz spectrometers.

3. Results and Discussion

3.1. Results

Isolated alkaloids as white crystals gave a single spot with R_f of 0.45, 0.40, and 0.23 in the conditional solvent ratio of 20% *n*-hexane in ethyl acetate, 30% acetone in *n*-hexane, and 10% acetone in chloroform respectively. The TLC performances under UV light at λ 254 nm were given in Figure 2a and with dragendorff's reagent in Figure 2b.

The LCMS/MS data of isolated alkaloid showed respectively the LC chromatogram with a single peak at retention time (r.t.) of 3.54 minutes and mass spectral with several peaks at m/z (for low energy) = 705 (10%), 375 (5%), 353 (100%), 335 (15%), 177 (13%), as shown in Figure 7. The several peaks of isolated alkaloid were at m/z (for high energy) =705 (10%), 354 (80%), 335 (90%), 317 (100%), 291 (87%), 265 (35%) and 132 (45%), as displayed in Figure 4. Double bond equivalent (DBE) for isolated alkaloid with molecular formula $C_{20}H_{20}O_4N_2$ can be calculated using the following equation.

$$DBE = C - \left(\frac{H}{2}\right) + \left(\frac{N}{2}\right) + 1 \quad (1)$$

where C is the number of carbon atoms, H is the number of hydrogen atoms, and N is the number of nitrogen atoms.

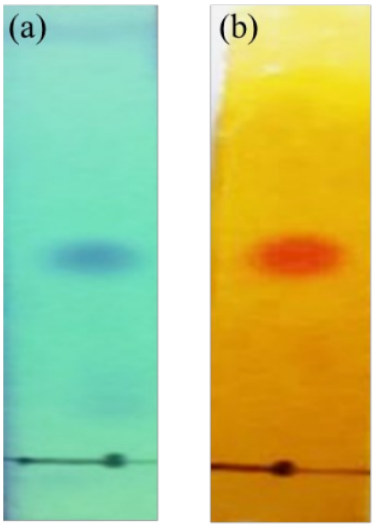

Figure 2. Chromatogram of the isolated alkaloid (a) under UV light at λ 254 nm and (b) dragendorff's reagent

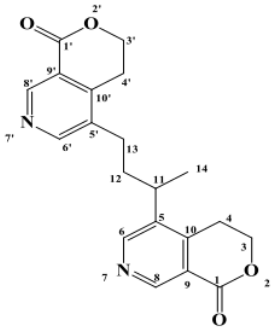

Figure 3. Chemical structure of isolated alkaloid with MW of 352

3.2. Discussion

An isolated alkaloid with retention time of 3.54 minutes, see also Figure 2a and 2b for TLC, exhibited its mass spectral peaks (*low energy*) at m/z: 705 (10%) as $[2M + H]^+$, 375.13182 (5%) as precursor ion of $[M + Na]^+$. It was synchronized to precursor ion data at m/z 375.12127 in reference [15] for oliveramine, 353 (100%) as $[M + H]^+$ so that the $[M]^+$ or MW was 352.335 (15%) as $[M-OH]^+$, 177 (13%), see Figure 8 and 9 for its fragmentation reaction. In addition this alkaloid showed its mass spectral peaks (*high energy*) at m/z 705 (10%) as $[2M + H]^+$, 354 (80%) as $[M + 2H]^+$ so that the $[M]^+$ or molecular weight (MW) was accepted to be 352, m/z 335 (90%) as $[M-OH]^+$, m/z 317 (100%) as [M–OH radical – H_2O]$^+$, m/z 291 (87%) as [M–OH radical –H_2O – HCN]$^+$, m/z 265 (35%) as [M – OH radical – H_2O – HCN – C_3H_2O]$^+$, and m/z 132 (45%) as [M – OH radical – H_2O – HCN – C_3H_2O – $C_{12}H_{11}NO$]$^+$ respectively. The fragmentation reaction of these alkaloids is based on high-energy mass spectral data, presented in Figure 4.

The ^{1}H-NMR, ^{13}C-NMR, and DEPT 135 spectra data of the isolated alkaloid were given in Table 1, while ^{1}H-NMR, ^{13}C-NMR, and DEPT 135 spectrums of this alkaloid were also clearly shown in Figure 5, 6, and 7. It was proven that isolated alkaloid was closed to dimeric pyridine alkaloid with molecular formula $C_{20}H_{20}N_2O_4$ and double bond equivalents (DBE) of 12, which consist of four rings, six double bonds in aromatic rings, and two carbonyls. The chemical structure of this base is presented in Figure 3. As a result, the isolated alkaloid from the *F. Fragrans* bark was oliveramine with an MW of 352 [15]. Oliveramine alkaloid was reported as the treatment of rheumatic arthritis [15, 16], and this alkaloid was firstly found in *Gentiana olivieri* and *Gentiana straminea* [17, 18].

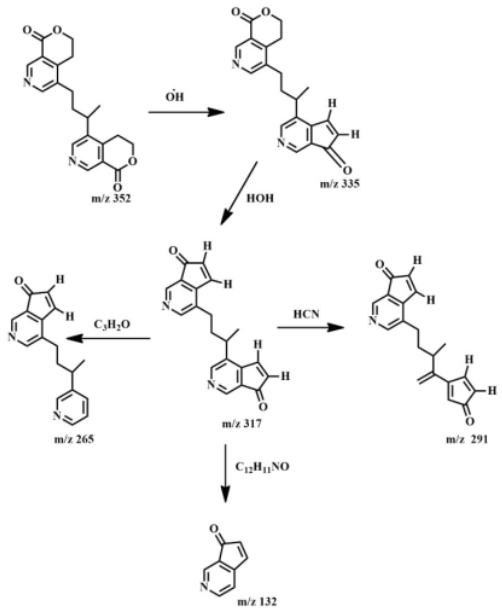

Figure 4. Fragmentation reaction of isolated alkaloid with MW of 352

The four rings of isolated alkaloids consisted of two aromatic rings, two lactone rings, and four carbons as the aliphatic bridge. The carbon and proton chemical shifts of the alkaloid are given in Table 1, and their carbon numbers are also clearly shown in Figure 3. Their carbon and proton spectrums are also provided in Figures 5, 6, and 7. They were one methyl, six methylene, and five methine groups. The eight remaining carbons were quaternary. The four aliphatic carbons as the bridge were resonance at δ_C = 32.9, 37.0, 27.8, and 21.7 (J = 7 Hz).

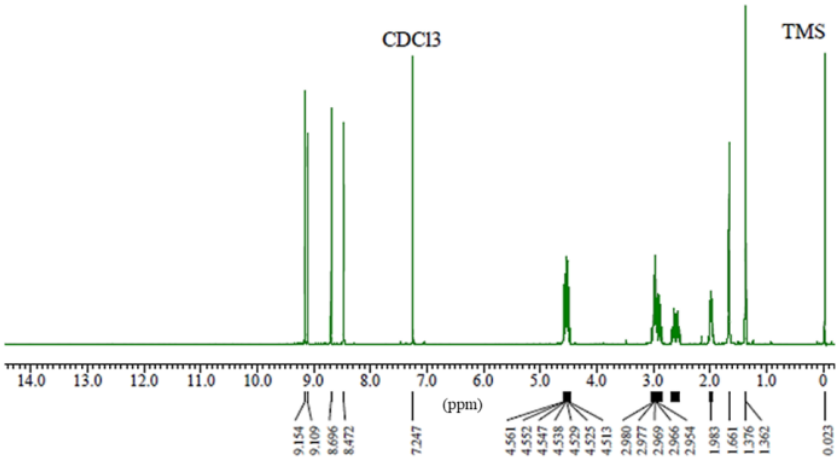

Figure 5. ^{1}H-NMR (500 Mhz) of isolated alkaloid with MW of 352 in $CDCl_3$

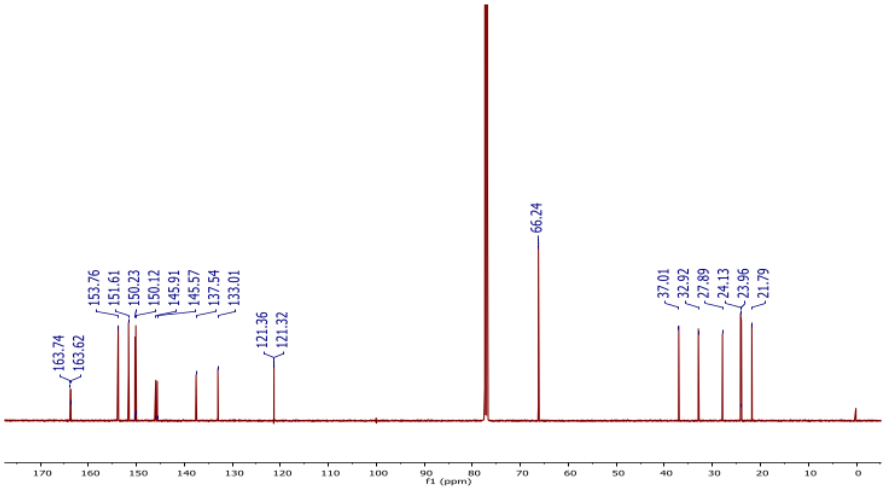

Figure 6. ^{13}C-NMR (125 MHz) of isolated alkaloid with MW of 352 in $CDCl_3$

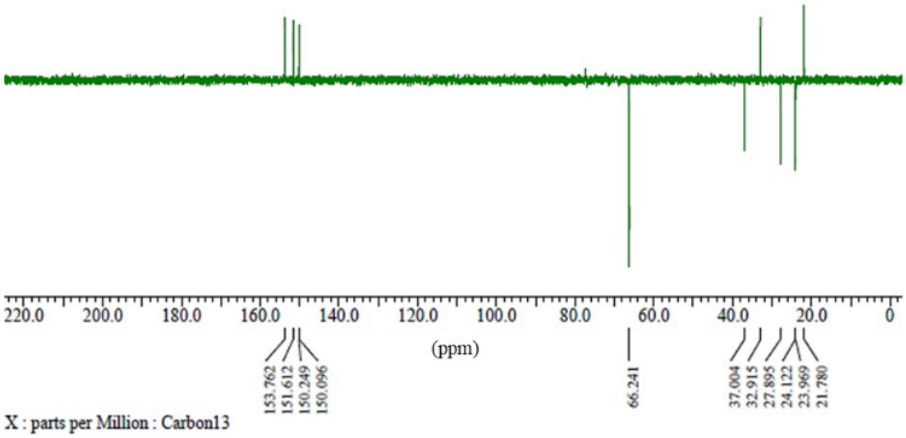

Figure 7. ^{13}C-NMR (125 MHz) of isolated alkaloid with MW of 352 in $CDCl_3$, (DEPT 135)

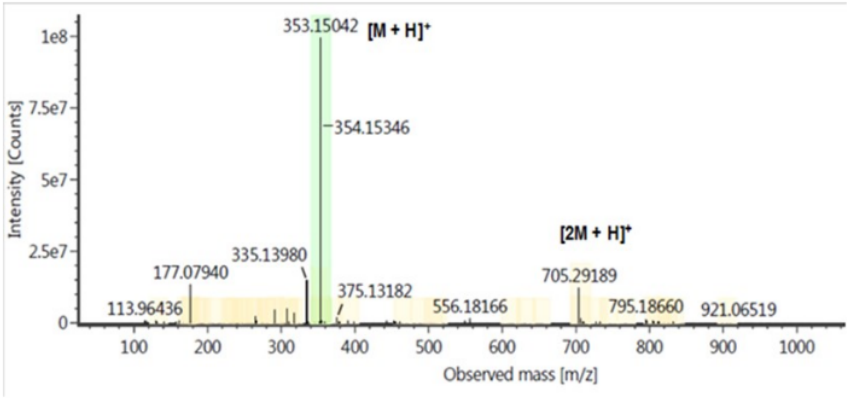

Figure 8. Mass spectral [low energy] of isolated alkaloid with MW of 352

Table 1. The chemical shift of isolated alkaloid with MW of 352 in $CDCl_3$

C	$\delta^{13}C$	δ ^1H, J Hz	DEPT 135
1	163	-	-C-
1'	163	-	-C-
2	-	-	-
2'	-	-	-
3	66.5	4.5 (2H, *m*), J = 6Hz	CH_2
3'	66.5	4.5 (2H, *m*), J = 6Hz	CH_2
4	23.9	2.9 (2H, *m*), J = 6 Hz	CH_2
4'	24.1	2.8 (2H, *m*), J = 6Hz	CH_2
5	133	-	-C-
5'	137	-	-C-
6	150	8.4 (1H, *s*)	CH
6'	150	8.6 (1H, *s*)	CH
7	-	-	-
7'	-	-	-
8	153	9.1 (1H, *s*)	CH
8'	151	9.1 (1H, *s*)	CH
9	121	-	-C-
9'	121	-	-C-
10	145	-	-C-
10'	145	-	-C-
11	32.9	3 (1H, *m*)	CH
12	37.0	1.9 (2H, *m*)	CH_2
13	27.8	2.6 (2H, *m*)	CH_2
14	21.7	1.3 (3H, *m*, J=7Hz)	CH_3

The other eight quaternary carbons were two carbon for carbonyl and six carbons for aromatic. As a result, the spectroscopy data of the isolated alkaloid $C_{20}H_{20}N_2O_4$ compound was subjected to oliveramine. In additional work on similar alkaloids, separating the chloroform fraction of the combined alkaloids of *Gentiana olivieri* according to basicities (pH 6.0 and 4.0) has also yielded gentioflavine, gentianine, and the new base oliveramine, $C_{20}H_{20}N_2O_4$ [17]. This alkaloid, oliveramine, had been found in *Gentiana olivieri* that was reported to biological activities as an antioxidant (metal-chelation capacity) and antihyperglycaemic (glucose-hyperglycaemic model, STZ-diabetic rats model) [17, 19]. The oliveramine alkaloid was also reported to be useful as a potential neuroprotectant in treating or preventing depression [18], including as traditional herbal medicine [16, 20]. In addition, the alkaloid provided unique lead compounds to be drugs [21]. It must have basic properties: water-soluble under acidic conditions and lipid soluble under neutral and basic conditions. This was especially important for dissolution in protonated form and membrane permeation in the deprotonated form [22].

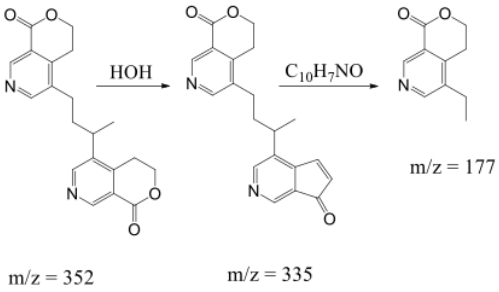

Figure 9. Fragmentation reaction of isolated alkaloid with MW of 352, based on low energy mass spectral data

4. Conclusion

A dimeric pyridine alkaloid found in *Fagraea fragrans* Roxb bark, the chemical structure of this base has been determined from LCMS/MS, ^{13}C-NMR, and ^1H-NMR spectral data. This alkaloid structure was closed to oliveramine alkaloid with molecular formula $C_{20}H_{20}O_4N_2$ with the IUPAC name was 5,5'-(1-methyl-1,3-propanediyl)bis[3,4-dihydro-1H-pyrano[3,4-c]pyridin-1-one].

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