Fagraldehyde, a Secoiridoid Isolated from *Fagraea fragrans*

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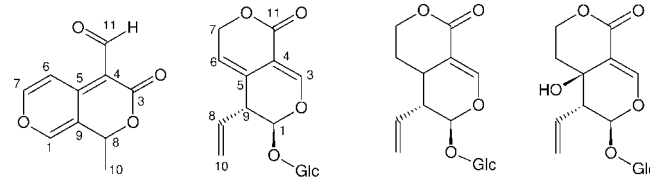
A secoiridoid aglycone with an atypical skeleton, named fagraldehyde (**1**), together with several known secoiridoids (gentiopicroside (**2**), sweroside (**3**), and swertiamarin (**4**)) were isolated from the bark and leaves of *Fagraea fragrans* collected in Cambodia. The conformations of **1** were evaluated on the basis of molecular modeling and NOESY correlations. A hypothetical biogenesis of fagraldehyde was proposed to explain the unusual skeleton. Compound **1** was weakly active *in vitro* against *Plasmodium falciparum*.

Fagraea fragrans Roxb. (Gentianaceae) is an evergreen tree that grows up to 25 m in open and swampy lowland forests and is distributed in an area that stretches from Birmania to Indomalaysia. The timber is a heavy, durable hardwood that is often used locally in construction and furniture. Its trade name is Tembusu. Medicinal uses of this plant have been reported in various regions where it grows. A decoction of the bark is used to treat malaria in India, Cambodia, and Malaysia and as a febrifuge in the Philippines. The decoction of twigs and leaves is used to control dysentery in Malaysia and Cambodia.[1−4]

In the course of our investigations on new antiplasmodial agents, we have selected the plant on the basis of an ethnobotanical survey in Cambodia and an *in vitro* antiplasmodial screening.[5] Secoiridoids are common compounds in Gentianales. Previously, only the presence of gentianine has been described in *F. fragrans*.[6] Gentianine is known as an artifact resulting from the extraction of gentiopicroside using ammonia.[7] The present report describes the isolation and characterization in *F. fragrans* of a new aldehydic natural compound (**1**, hence the name *fagraldehyde*) that could probably be classified as a new secoiridoid aglycone, accompanied by several known secoiridoids.

The methanol extract of the bark of *F. fragrans* contains two common secoiridoids, gentiopicroside (**2**) and sweroside (**3**). In addition, swertiamarin (**4**), previously unknown in the genus *Fagraea*,[8] was isolated from the leaf extract. The identity of **2**, **3**, and **4** has been confirmed after chromatographic, UV, MS, and NMR comparison with reference samples and literature data.[9] The DCM extract of the bark was fractionated by silica gel column chromatography. After a second separation, a bright pink spot appeared on the TLC control after spraying with H_2SO_4. This compound, fagraldehyde (**1**), was then isolated and characterized using different physical methods.

The UV absorption spectrum of **1**, showing maxima at 205, 241, and 368 nm in MeOH, indicated a highly conjugated chromophore. The IR spectrum revealed C═O vibrations at 1682 and 1718 cm^{-1}. These vibrations were assignable to an unsaturated aldehyde group linked to an unsaturated group and to an unsaturated δ-lactone moiety, respectively. The mass spectrum, analyzed by HRESIMS,



Fagraldehyde (**1**) Gentiopicroside (**2**) Sweroside (**3**) Swertiamarin (**4**)

displayed an $[M + H]^+$ ion at *m/z* 193.0499, corresponding to the elemental composition $C_{10}H_9O_4$ (theoretical value 193.0495). The MS/MS analysis showed the presence of several typical fragments: *m/z* 175 (− H_2O); *m/z* 147 (− HCOOH); *m/z* 119 (− HCOOH − CO); *m/z* 91. More detailed understanding of the structure was gained by examination of the 1D and 2D NMR data that are listed in Table 1. The broadband decoupled ^{13}C NMR spectrum exhibited 10 carbon signals and the ^1H NMR spectrum revealed six signals, representing eight protons. The carbon signals were sorted by edited HSQC and proton integration as one methyl, five methine, and four quaternary carbons. Among these, an ester carbonyl was identified at δ_C 165.6 ppm, an aldehyde group at δ_H 10.2/δ_C 190.8, and a methyl at δ_H 1.7/δ_C 19.8 coupled in the COSY spectrum to a CH at δ_H 5.27/δ_C 70.5. Starting from the aldehyde function, it was possible to reconstitute the skeleton using the HMBC correlations (Table 1) and particularly to solve unambiguously the position of the lactone and the aldehyde moieties. Correlations between C-3, C-4, and C-5 and H-11 were detected but not between C-7 or C-6 and H-11. This supported that the aldehyde group was not bound to C-6 but to C-4. The HMBC correlations confirm the methyl substitution at C-8, and the C-7/H-1 and C-1/H-7 correlations confirmed the linkage between C-1 and C-7. The relatively deshielded proton chemical shift of H-6 (δ_H 8.4), compared to the oxymethine H-1 and H-7, in contrast to the nondeshielded carbon chemical shift of C-6 (δ_C 109), compared to C-1 and C-7, could be explained by the carbonyl anisotropy caused by the spatial proximity of the aldehyde carbonyl group. This is confirmed by the absence of NOESY correlation between H-6 and H-11, indicating an orientation of the carbonyl group toward H-6. These data were different from those of related secoiridoid aglycones.[10−12]

As fagraldehyde was optically inactive, we deduced that **1** was a racemic product. Nevertheless, in order to analyze the constrained conformational space of this molecule, different geometry optimizations have been performed depending on the *R* or *S* configuration at C-8, the rotation of the aldehydic group, and the conformation of the lactone ring. For each conformation, all the degrees of freedom describing the geometry have been fully optimized by

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Table 1. ^{1}H and ^{13}C NMR Data for Fagraldehyde (**1**) (500/125 MHz in $CDCl_3$)

position	^{1}H[a]	COSY H/H corr	NOESY H/H corr	^{13}C	HMBC[b] C→H corr
1	7.61 (d, 1.4)	8	8, 10	146.9	7, 8
3				165.6	8, 10, 11
4				104.0	11, 6
5				145.0	7, 11, 1, 8, 10
6	8.37 (d, 5.7)	7	7	109.2	7
7	7.63 (d, 5.7)	6	6	153.4	1, 6
8	5.27 (qd,6.5; 1.4)	10,1	1, 10	70.5	10, 1
9				121.2	1, 8, 6, 10, 11
10	1.70 (d, 6.5)	8	8, 1,11	19.8	8
11	10.22 (s)		10	190.8	

[a] Chemical shifts (δ) in ppm from TMS. Multiplicities and coupling constants in Hz are in parentheses. [b] Correlations (corr) from C to the indicated hydrogens.

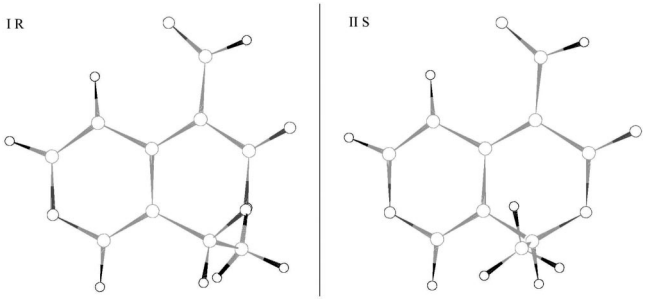


Figure 1. Enantiomers of fagraldehyde based on the B3LYP geometry and the NOESY correlations

energy minimization at the RHF level with the MINI-1′ basis set[13] and with the B3LYP functional[14] using the double-ζ basis set 6-31G(d). The more stable conformer is stabilized by a H bond between the H of aldehyde and the carbonyl of the lactone (2.40 Å). At the B3LYP geometry, a good agreement can be found between the IR vibrations of the carbonyl and the unsaturated lactone. Their calculated values, using the correction factor of 0.9613, were respectively 1692 and 1740 cm^{-1}. Because the lactone ring is not planar, two conformations were obtained: the one presenting oxygen above the plane was named I, and the one having the oxygen below the plane was named II. Examination of NOESY correlations, particularly between H-11 and H-10, in comparison with molecular modeling, elicited us to propose the enantiomers (I R and II S) depicted in Figure 1.

Fagraldehyde appears to be the first naturally occurring monoterpene product possessing this kind of bicyclic skeleton. Scheme 1 represents a possible biogenetic pathway, showing that fagraldehyde should derive directly from the cleavage of iridotrial rather than from loganin, which is considered to be the common precursor of other known secoiridoids.[15,16] Here, the usual secoiridoid numbering has been adopted for ready biosynthetic comparison. The C_7-C_8 oxidative cleavage, in fact, appears in the cyclopentane ring for both fagraldehyde putative biosynthesis and the known secoiridoid biogenesis. Nevertheless, for most of the iridoids, glycosylation and oxidation of iridodial lead to loganoside formation, with cleavage following this formation. The putative biosynthesis process should involve direct C_7-C_8 cleavage of iridotrial, allowing rotation about the C_5-C_9 bond. Sequential cyclization, oxidation, and dehydration reactions should lead to the lactone and pyran rings of fagraldehyde. Of course, the use of labeled precursors ought to be considered to clearly confirm our hypothesis.

In our continuing search of original antiplasmodial agents in plants, compounds **1**−**4** were tested *in vitro* against *Plasmodium falciparum*. Compounds **2**−**4** were inactive, displaying IC_{50} values higher than 50 μg/mL. Compound **1** was weakly active, exhibiting an IC_{50} value of W2 = 22.4 ± 1.8 μg/mL.

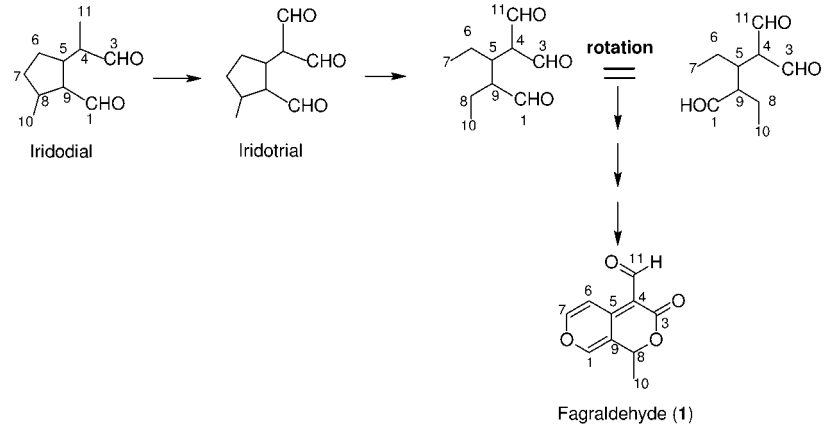
Experimental Section

General Experimental Procedures. Optical rotation was determined on a Bruhat monochromator (A. Jobin & G. Yvon, Paris-Arcueil). The UV spectrum was recorded on a Kontron Uvikon spectrophotometer in a MeOH solution. The FT-IR spectrum was measured on a Perkin-Elmer Spectrum GX (Perkin-Elmer Limited, Beaconsfield, England), equipped with an MIRTGS detector. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker DRX 500 spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz), with TMS as an internal reference. 2D experiments were performed using standard Bruker microprograms. HRESIMS was carried out with a Qstar Elite (Applied Biosystems SCIEX) apparatus, and ESIMS/MS was obtained with a 3200 QTRAP (Applied Biosystems SCIEX) apparatus. Silica gel 60 (0.040−0.063 mm, Merck) was used for CC. Analytical TLC was performed on precoated Si gel 60 F_{254} (Merck) aluminum plates. After development (CH_2Cl_2−MeOH, 80:4), the dried plates were examined under short- (254 nm) and long-wavelength (366 nm) UV light, sprayed with 20% H_2SO_4 in MeOH, and heated for 10 min at 110 °C. All solvents used were analytical grade (Merck, Carlo Erba). All calculations to determine the geometry of the molecule have been performed with the Gaussian 03 program.[17]

Plant Material. *F. fragrans* barks and leaves were collected in Koh Kong Province, Cambodia, in December 2005. A voucher specimen (no. FFKK05) was deposited in the herbarium of the Faculty of Pharmacy (Marseilles, France) and identified by Dr. Hul (Museum National d'Histoire Naturelle de Paris, France). The different parts of the plant were air-dried at room temperature, with no direct sunlight.

Extraction and Isolation. Dried bark and leaf powders were extracted successively with CH_2Cl_2 and MeOH by percolation after a night of maceration and evaporated *in vacuo*. MeOH bark extract (1.5 g, 11.3%) was chromatographed on a Si gel column (Merck 60) eluted with EtOAc−MeOH (80:15), affording compounds **2** (47 mg) and **3** (81 mg). Five grams of leaf powder was extracted with 150 mL of boiling distilled H_2O for 5 min. The solution was filtered and freeze-dried, yielding 1.4 g of aqueous extract. Then 100 mg of aqueous leaf

Scheme 1. Hypothesis for the Conversion of Iridodial into Fagraldehyde (**1**)



extract was subjected to CC on Si gel Merck 60 using an EtOAc−MeOH (80:20) mixture. A second CC was performed on Si gel using EtOAc−MeOH (90:10) to yield **4** (22 mg). CH_2Cl_2 bark extract (6.15 g: 1.2%) was chromatographed over a Si gel column using an EtOAc−MeOH gradient (0%, 2%, 10%, 20%, 50% MeOH). The fraction (MeOH 2→10%, 530 mg), containing a bright pink compound when the TLC control (CH_2Cl_2−MeOH, 80:4) was sprayed with H_2SO_4 (20% in MeOH, heated 10 min at 110 °C), was subjected to Si gel CC using EtOAc−MeOH (80:5). Finally, the pink compound, **1** (15 mg), was purified on Si gel CC using CH_2Cl_2−MeOH (80:4).

Fagraldehyde (1): light yellow, amorphous powder; $[\alpha]^{25}_D = 0$; UV (MeOH) λ_{max} (log ε) 368 (3.45), 241 (3.27), 205 (3.20) nm; FT-IR (C_2Cl_4) ν_{max} 2928, 1718, 1682, 1640, 1564, 1533, 1458, 1322, 1232, 1124, 970, 833 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), Table 1; ESIMS/MS *m/z* (rel int) 193 (82), 175 (32), 147 (27), 119 (100), 91 (15); HRESIMS *m/z* [MH^+] 193.0499 (calcd. for $C_{10}H_9O_4$, 193.0495).

Antiplasmodial Test. Cultures of chloroquine-sensitive (3D7) *Plasmodium falciparum* strain was assessed following the procedure described by Frédérich et al.[18] The strain was obtained from Prof. Grellier (Museum d'Histoire Naturelle in Paris, France). Parasite growth was estimated by determination of lactate dehydrogenase activity as described previously by Kenmogne et al.[19] Antiplasmodial assays were also performed in Marseilles. *P. falciparum* W2 chloroquine-resistant strain growth was calculated by flow cytometry using hydroethidine as described by Hout et al.[5]

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