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## Lutamide, a New Ceramide Isolated from the Leaves of *Ficus lutea*

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### 1. Introduction

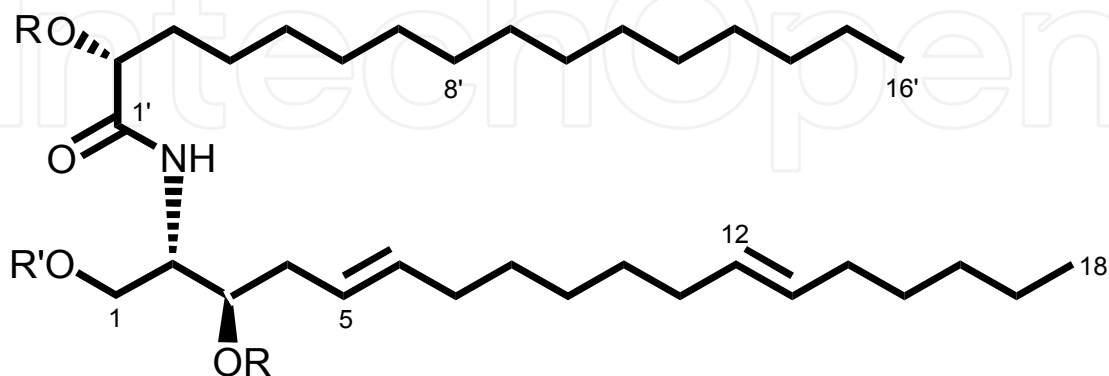
The Moraceae family consists of about 50 genera and nearly 1400 species including important group such as *Artocarpus*, *Morus* and *Ficus*.<sup>1</sup> The genus *Ficus* consists of trees and shrubs that possess latex-like material within their vasculatures, affording protection and self-healing for physical assaults.<sup>2</sup> A number of *Ficus* species are used as food and for medicinal properties in traditional Chinese medicine especially amongst people where these species grow.<sup>3</sup> *Ficus benjamina* is used as ornamental plant in University of Yaounde I, Cameroon.<sup>4</sup> Previous phytochemical studies on the wood of *Ficus lutea* resulted in the isolation of benjaminamide (2),  $\beta$ -amyrin,  $\beta$ -amyrin acetate, lupeol, betulinic acid,  $\beta$ -sitosterol glucoside and lutaoside.<sup>5</sup> The strong antioxidant and antibacterial activities exhibited by this genus<sup>6</sup> in addition to the search for the chemical constituents of Cameroonian medicinal plants<sup>7</sup> justified further attempts to isolate and identify active compounds. The few differences between the secondary metabolites isolated from the wood and the leaves of *F. lutea* are may be related to the real specific differences or more probably to a geographic or environmental influence on biosynthesis.

### 2. Results and discussion

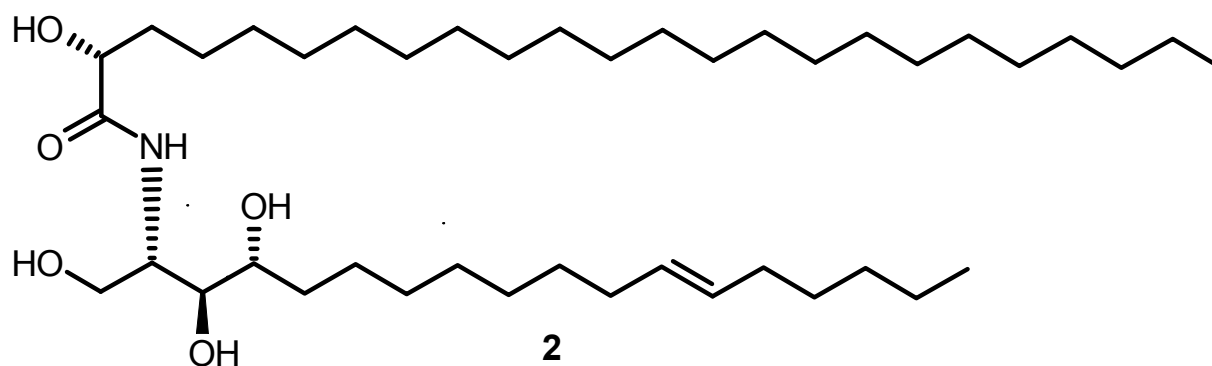
The leaves of *F. lutea* were extracted with MeOH during 30 hours. The extract was submitted to repeated column chromatography to afford benjaminamide (2), betulinic acid, 9,19-cycloart-25-ene-3 $\beta$ ,24-diol, vitexin as well as one new ceramide (1a). The <sup>1</sup>H and <sup>13</sup>C NMR, and MS of the known compounds were consistent with those reported in the literature.

Lutamide (1a) was obtained as an amorphous solid. The molecular formula C<sub>34</sub>H<sub>64</sub>NO<sub>4</sub> was determined by HRFABMS at *m/z* 550.48348 [M-H]<sup>-</sup> (Calcd. 550.48351). The IR spectrum of 1a indicated absorption bands at  $\nu$  3405 cm<sup>-1</sup> (OH), and strong absorption bands for a secondary amide at  $\nu$  1639 and 1590 cm<sup>-1</sup>. These data were supported by the signals at  $\delta$  52.5 and 174.8 in <sup>13</sup>C NMR spectrum which confirm the presence of C-N and C=O, respectively. The <sup>1</sup>H and <sup>13</sup>C NMR spectral data (table 1) of 1a indicated the presence of an amide linkage, two long chain aliphatic moieties, suggesting the sphingolipid (glycolipid) nature of the molecule. 1D and 2D NMR spectral data of 1a were

nearly superimposable to that of lutaoside (**1c**) which was further isolated from the wood extract of this plant.<sup>5</sup> A careful comparison of the spectra data of **1a** and lutaoside (**1c**) led to the conclusion that, the structure of lutamide (**1a**) was (2*R*)-2-hydroxy-*N*-((2*S*,3*R*,5*E*,12*E*)-1,3-dihydroxyoctadeca-5,12-dien-2-yl)hexadecanamide (**1a**), which is reported here for the first time.



**1a:** R = R' = H; **1b:** R = R' = CH<sub>3</sub>-C=O; **1c:** R = H, R' = β-D-glucopyranosyl



Acetylation of compound **1a** gave **1b** (C<sub>40</sub>H<sub>70</sub>NO<sub>7</sub>; m/z 676.51509 [M-H]<sup>+</sup>; Calcd. 676.51520).

Position	δ <sub>C</sub>	δ <sub>H</sub>
1a	68.2 (t)	4.58 (dd, 10.7; 4.9)
1β	68.2 (t)	4.40 (dd, 10.7; 4.3)
2	52.5 (d)	5.18 (m)
3	75.7 (d)	4.39 (m)
4	128.5 <sup>ε</sup> (d)	5.50 <sup>ξ</sup> (dd, 15.4; 5.3)
5	128.1 <sup>ε</sup> (d)	5.10 <sup>ξ</sup> (dt, 15.4; 4.7)

Position	$\delta_C$	$\delta_H$
6	33.5 (t)	2.25 (m)
7-10, 15-17	24.0-26.5 (t each)	1.27 (br s)
11	33.0 (t)	2.11 (m)
12	130.0 $\epsilon$ (d)	5.50 $\zeta$ (dd, 15.0; 4.8)
13	129.0 $\epsilon$ (d)	5.44 $\zeta$ (dd, 15.0; 4.6)
14	32.1 (t)	1.99 (m)
18	13.0 (q)	0.90 (t, 6.4)
NH	-	8.50 (d, 8.0)
1'	174.8 (s)	-
2'	73.1 (d)	4.20 (t, 7.3)
3'	31.0 (t)	1.78 (m)
4'-14'	27.8-29.0 (t each)	1.27 (br s)
15'	21.9 (t)	1.70 (m)
16'	12.7 (q)	0.89 (t, 6.4)

Multiplicities and coupling constants in Hz are given in parentheses  
Resonances with the same superscripts ( $\epsilon$ ,  $\zeta$ ) in the same column may be interchanged.

Table 1.  $^1\text{H}$  (400 MHz,  $\text{C}_5\text{D}_5\text{N}$ , 30 °C, TMS) and  $^{13}\text{C}$  (100 MHz,  $\text{C}_5\text{D}_5\text{N}$ ) NMR data of lutamide (**1a**)

The antifungal and antibacterial activities of compounds **1a**, **1b** and **2** were determined using the agar diffusion method with 8 mm paper disks loaded with 40  $\mu\text{g}$  of each compound (See Table 2). Compound **1a** and **1b** exhibited *in vitro* good antimicrobial activity against *Mucor miehei* and *Bacillus subtilis* compared to the nystatin as reference.

Micro-organisms tested	Sample			
	1a	1b	2	Nystatin
<i>Chlorella vulgaris</i>	10	11	-	-
<i>Scenedesmus subspicatus</i>	13	10	10	-
<i>Chlorella vulgaris</i>	11	9	11	-
<i>Mucor miehei</i>	15	15	13	15
<i>Bacillus subtilis</i>	16	15	14	14
<i>Candida albicans</i>	12	13	13	15
<i>Streptomyces viridochromogenes</i>	-	-	-	14

Diameter of inhibition zone in mm. Nystatin was used as reference and the experiments were repeated 3 times.

Table 2. Antimicrobial activity of compounds **1a**, **1b** and **2**

### 3. Experimental section

#### 3.1 Materials and method

Melting point is uncorrected and was obtained with a micro melting point apparatus (Yanaco, Tokyo-Japan). Optical rotation values were measured with a Horiba SEPA-300 polarimeter, and IR spectra were recorded with JASCO J-20A spectrophotometer.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were acquired with a Jeol EX-400 spectrometer. Chemical shifts are given on a  $\delta$  (ppm) scale with TMS as an internal standard. Mass spectra were obtained with a Jeol JMS-700 instrument. Column chromatography was conducted on silica gel 60 (Kanto Chemical Co., Inc., Japan), Sephadex LH-20 (Pharmacia, Sweden) and ODS (Fuji Silysia, Japan). TLC analysis was carried out by using precoated silica gel plates (Merck), and the spots were detected by spraying with  $\text{H}_2\text{SO}_4$ /10% vanillin and then heating. Flash chromatography was carried out on silica gel (230-400 mesh).  $R_f$  values were measured on Polygram SIL G/UV254 (Macherey-Nagel & Co.).

#### 3.2 Plant material

The leaves of *Ficus lutea* Vahl were collected in July 2008 at Kribi, South Cameroon. A voucher specimen (Ref. N $^{\circ}$ . 3471/SRFK) has been deposited in the National Herbarium, Yaoundé, Cameroon.

#### 3.3 Extraction and isolation

The powdered leaves of *Ficus lutea* (2 Kg) were soaked in 10 l of MeOH during 30 hours at room temperature. Solvent was removed under reduced pressure and 60 g of organic extract were obtained. Part of this dark-green residue (58 g) was subjected to vacuum liquid chromatography (VLC) on silica gel and eluted with pure *n*-hexane (Fraction A), followed by mixture of *n*-hexane/ethyl acetate in incremental steps 50%, 100% (Fractions B, C respectively) and finally 10% of the mixture of ethyl acetate/methanol (Fraction D). Four main fractions (A-D) were obtained and, basis of analytical TLC, fractions C and D were combined.

Fraction A (7 g) gave mainly betulinic acid (53.0 mg)<sup>9</sup> and vitexin (11 mg).<sup>10</sup>

Fractions B (6 g) were chromatographed on silica gel and eluted with a mixture of *n*-hexane/ethyl acetate of increasing polarity to yield 54 fractions (ca. 100 ml each). Fractions 1-32 (2 g), subjected to column chromatography over silica gel, yielded mainly 9,19-cycloart-25-ene-3 $\beta$ ,24-diol (33 mg)<sup>11</sup> while benjaminamide (**2**, 5 mg) was obtained in fractions 33-54 (3 g) eluted with  $\text{CHCl}_3$ /MeOH (6:1).

Fraction C and D (21 g) was passed through a Sephadex LH-20 column and subjected to silica gel column chromatography and preparative TLC to afford benjaminamide (**2**, 19 mg) and lutamide (**1a**, 34 mg).

*Lutamide* or (2*R*)-2-hydroxy-*N*-((2*S*,3*R*,5*E*,12*E*)-1,3-dihydroxyoctadeca-5,12-dien-2-yl)hexadecanamide (**1a**): Amorphous powder. –  $R_f$  = 0.44 ( $\text{CH}_2\text{Cl}_2$ /10% MeOH). –  $[\alpha]_{\text{D}}^{25}$  +19 ( $c$  0.6, MeOH). – IR (Film):  $\nu$  = 3405 (OH), 3201 (NH), 2914, 2853, 1639, 1590, 1418, 1217, 1177, 1078, 1057, 1039, 890  $\text{cm}^{-1}$ . –  $^1\text{H}$  NMR (400 MHz,  $\text{C}_5\text{D}_5\text{N}$ , 30  $^{\circ}\text{C}$ , TMS) and –  $^{13}\text{C}$  NMR (100 MHz,  $\text{C}_5\text{D}_5\text{N}$ ): see Table 1. – FABMS:  $m/z$  550  $[\text{M}-\text{H}]^-$ . – HRFABMS:  $m/z$  550.48348  $[\text{M}-\text{H}]^-$  (Calcd. 550.48351 for  $\text{C}_{34}\text{H}_{64}\text{NO}_4$ ,  $[\text{M}-\text{H}]^-$ ).

### 3.4 Acetylation of lutamide (1a)

Lutamide (**1a**, 6.0 mg) was dissolved in pyridine (1.5 mL) and Ac<sub>2</sub>O (1.2 mL). The solution was stirred for 10 hours at 50 °C. The usual work-up gave three-acetoxylutaoside (**1b**) (4.1 mg, 84 %) as an amorphous solid with  $R_f = 0.94$  (CHCl<sub>3</sub>/10% MeOH). –  $[\alpha]_D^{25} +23$  (c 0.9, Pyridine). – IR (Film):  $\nu = 3203$  (NH), 2905, 2843, 1653, 1579, 1463, 1217, 1100, 886 cm<sup>-1</sup>. – <sup>1</sup>H NMR (400 MHz, C<sub>5</sub>D<sub>5</sub>N, 30 °C, TMS):  $\delta = 0.87$  (t,  $J = 6.0$  Hz, 6 H, H-18, H-16'), 1.20-1.30 (br s); 1.48-1.55 (m, 4 H, H-3', H-15'), 1.90 (m, 8 H, H-4, H-7, H-11, H-14), 2.05, 2.06, 2.12 (s, 3 H each, CH<sub>3</sub>-C=O), 5.17 (dd,  $J = 11.0, 3.5$  Hz, 1 H, H-1a), 5.25 (dd,  $J = 11.0, 5.0$  Hz, 1 H, H-1b), 5.42-5.48 (m, 4 H, H-5, H-6, H-12, H-13), 5.51 (m, 1 H, H-2'), 5.53 (m, 1 H, H-3), 8.49 (d,  $J = 8.0$  Hz, 1 H, NH). – FABMS:  $m/z$  676 [M-H]<sup>-</sup>. – HRFABMS:  $m/z$  676.51509 [M-H]<sup>-</sup> (Calcd. 676.51520 for C<sub>40</sub>H<sub>70</sub>NO<sub>7</sub>, [M-H]<sup>-</sup>).

### 4. Antimicrobial assay

Agar diffusion tests were performed in the usual manner<sup>8</sup> with *Bacillus subtilis* and *Escherichia coli* (on peptone agar), *Staphylococcus aureus* (Bacto nutrient broth), *Streptomyces viridochromogenes* (M Test agar), the fungi *Mucor miehei* and *Candida albicans* (Sabouraud agar), and three microalgae (*Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus*).

Compounds were dissolved in an azeotrope chloroform/MeOH (87:13) and 40 µg pro paper disks (Ø 8 mm) were impregnated with each using a 100 µl syringe, dried for 1 h under sterile conditions and placed on the pre-made agar test plates. Bacteria and fungi plates were kept in an incubator at 37 °C for 12 h, micro algae plates for three days at room temperature in a day light incubator. The diameter of inhibition zones was measured.

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