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

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1. Introduction

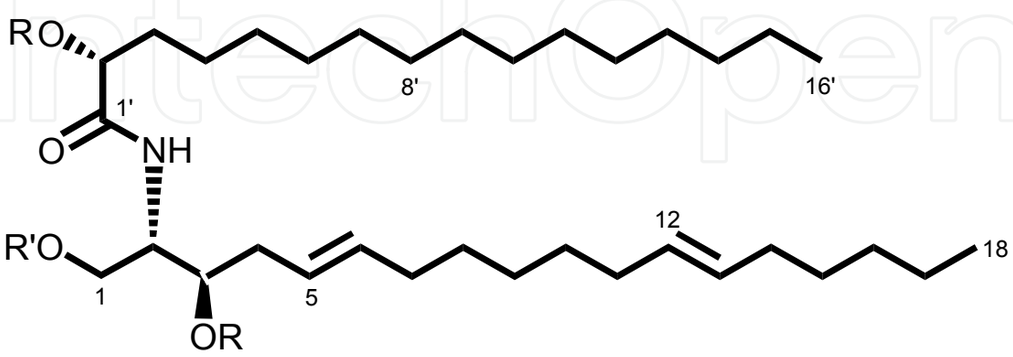
The Moraceae family consists of about 50 genera and nearly 1400 species including important group such as *Artocarpus*, *Morus* and *Ficus*.[1] The genus *Ficus* consists of trees and shrubs that possess latex-like material within their vasculatures, affording protection and self-healing for physical assaults.[2] A number of *Ficus* species are used as food and for medicinal properties in traditional Chinese medicine especially amongst people where these species grow.[3] *Ficus benjamina* is used as ornamental plant in University of Yaounde I, Cameroon.[4] Previous phytochemical studies on the wood of *Ficus lutea* resulted in the isolation of benjaminamide (**2**), *β*-amyrin, *β*-amyrin acetate, lupeol, betulinic acid, *β*-sitosterol glucoside and lutaoside.[5] The strong antioxidant and antibacterial activities exhibited by this genus[6] in addition to the search for the chemical constituents of Cameroonian medicinal plants[7] 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

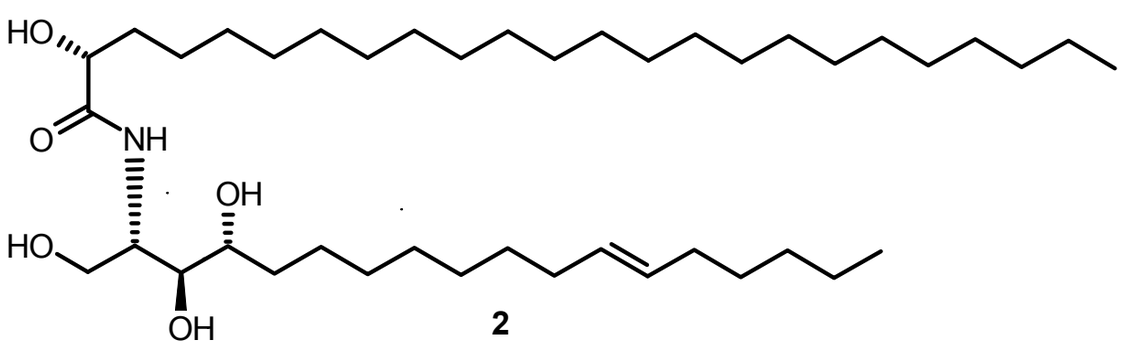
The leaves of *F. lutea* were extracted with MeOH during 30 hours. The extract was submitted to repeated column chromatography to afford benjaminamide (**2**), betulinic acid, 9,19-cycloart-25-ene-3β,24-diol, vitexin as well as one new ceramide (**1a**). The ^{1}H and ^{13}C NMR, and MS of the known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

Lutamide (**1a**) was obtained as an amorphous solid. The molecular formula $C_{34}H_{64}NO_4$ was determined by HRFABMS at *m/z* 550.48348 $[M-H]^-$ (Calcd. 550.48351). The IR spectrum of **1a** indicated absorption bands at ν 3405 cm^{-1} (OH), and strong absorption bands for a secondary amide at ν 1639 and 1590 cm^{-1}. These data were supported by the signals at δ 52.5 and 174.8 in ^{13}C NMR spectrum which confirm the presence of C-N and C=O, respectively. The ^{1}H and ^{13}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.[5] A careful comparison of the spectra data of **1a** and lutaoside (**1c**) let to the conclusion that, the structure of lutamide (**1a**) was (*2R*)-2-hydroxy-*N*-((*2S*,*3R*,*5E*,*12E*)-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_3-C=O; **1c**: R = H, R′ = β-D-glucopyranosyl



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Acetylation of compound **1a** gave **1b** ($C_{40}H_{70}NO_7$; m/z 676.51509 [M-H]$^-$; Calcd. 676.51520).

Position	δ_C	δ_H
1*a*	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$^{\varepsilon}$ (d)	5.50$^{\zeta}$ (dd, 15.4; 5.3)
5	128.1$^{\varepsilon}$ (d)	5.10$^{\zeta}$ (dt, 15.4; 4.7)

Position	δ_C	δ_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$^{\varepsilon}$ (d)	5.50$^{\zeta}$ (dd, 15.0; 4.8)
13	129.0$^{\varepsilon}$ (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 (ε, ζ) in the same column may be interchanged.

Table 1. ^{1}H (400 MHz, C_5D_5N, 30 °C, TMS) and ^{13}C (100 MHz, C_5D_5N) 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 μg of each compound (See Table 2). Compound **1a** and **1b** exhibited *in vitro* good antimicrobial activity against *Mucor miehi* and *Bacillus subtilis* compared to the nystatin as reference.

Micro-organisms tested	**Sample**			
	1a	**1b**	**2**	**Nystatin**
Chlorella vulgaris	10	11	-	-
Scenedesmus subspicatus	13	10	10	-
Chlorella vulgaris	11	9	11	-
Mucor miehei	15	15	13	15
Bacillus subtilis	16	15	14	14
Candida albicans	12	13	13	15
Streptomyces viridochromogenes	-		-	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}H and ^{13}C NMR spectra were acquired with a Jeol EX-400 spectrometer. Chemical shifts are given on a δ (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 H_2SO_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°. 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)[9] and vitexin (11 mg).[10]

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-3β,24-diol (33 mg)[11] while benjaminamide (**2**, 5 mg) was obtained in fractions 33-54 (3 g) eluted with $CHCl_3$/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 (2R)-2-hydroxy-N-((2S,3R,5E,12E)-1,3-dihydroxyoctadeca-5,12-dien-2-yl)hexadecanamide (**1a**)*: Amorphous powder. – R_f = 0.44 (CH_2Cl_2/10% MeOH). – $[\alpha]_D^{25}$ +19 (*c* 0.6, MeOH). – IR (Film): ν = 3405 (OH), 3201 (NH), 2914, 2853, 1639, 1590, 1418, 1217, 1177, 1078, 1057, 1039, 890 cm^{-1}. – ^{1}H NMR (400 MHz, C_5D_5N, 30 °C, TMS) and – ^{13}C NMR (100 MHz, C_5D_5N): see Table 1. - FABMS: *m/z* 550 $[M-H]^-$. - HRFABMS: *m/z* 550.48348 $[M-H]^-$ (Calcd. 550.48351 for $C_{34}H_{64}NO_4$, $[M-H]^-$).

3.4 Acetylation of lutamide (1a)

Lutamide (**1a**, 6.0 mg) was dissolved in pyridine (1.5 mL) and Ac_2O (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 ($CHCl_3$/10% MeOH). – $[\alpha]_D^{25}$ +23 (*c* 0.9, Pyridine). – IR (Film): ν = 3203 (NH), 2905, 2843, 1653, 1579, 1463, 1217, 1100, 886 cm^{-1}. – ^1H NMR (400 MHz, C_5D_5N, 30 °C, TMS): δ = 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_3-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 $[M-H]^-$. – HRFABMS: *m/z* 676.51509 $[M-H]^-$ (Calcd. 676.51520 for $C_{40}H_{70}NO_7$, $[M-H]^-$).

4. Antimicrobial assay

Agar diffusion tests were performed in the usual manner[8] with *Bacillus subtilis* and *Escherichia coli* (on peptone agar), S*taphylococcus 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.

5. References

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