

Obscurine: a New Cyclostachine Acid Derivative from *Beilschmiedia obscura*

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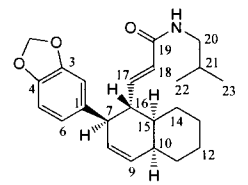
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From the methylene chloride extract of the stem bark of *Beilschmiedia obscura*, a new cyclostachine derivative, obscurine (**1**), has been isolated, together with six known compounds. The structure of compound **1** was established by spectroscopic methods, including 1- and 2-dimensional NMR techniques.

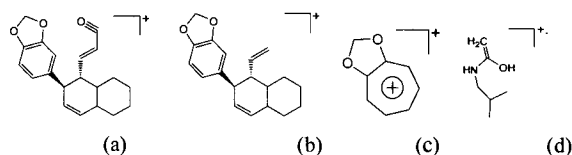
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Beilschmiedia (Lauraceae) is a pantropical genus of about 200 species, distributed in tropical Asia and Africa. The bark and leaves of some *Beilschmiedia* species are used in traditional medicine [1a-1c]. Alkaloids, arylpropanoids, benzopyrans, endiandric acids, and flavonoids have been isolated from some *Beilschmiedia* species [1b-1g, 2a-2c]. Herein we describe the isolation and structural elucidation of a new compound, obscurine (**1**) from *B. obscura*.

Compound **1** was obtained as white amorphous powder with the molecular formula C₂₄H₃₂NO₃, as deduced from the (+)-HRESIMS. Its IR spectrum showed strong absorptions at 3329 (NH), 1669 (C=O), 1632, and 1536 (C=C) cm⁻¹. The ¹H NMR spectrum showed signals of an ABX system of aromatic protons at δ 6.58 (1H, d, *J* = 1.2 Hz, H-2), 6.68 (1H, d, *J* = 8.1 Hz, H-5) and 6.55 (1H, dd, *J* = 7.5, 1.2 Hz, H-6), *cis* olefinic protons at δ 5.86 (1H, ddd, *J* = 8.1, 5.6, 3.1 Hz, H-9) and 5.48 (1H, m, H-8) and *trans* olefinic protons at δ 6.48 (1H, dd, *J* = 15.7, 10.0 Hz, H-17) and 5.50 (1H, m, H-18). Signals of methylenedioxy protons at δ 5.88 (2H, brs), an isobutyl unit at δ 0.89 (6H, d, *J* = 6.9 Hz, H-22/23), 1.78 (1H, m, H-21), and 3.00 (2H, m, H-20), four methine groups at δ 3.19 (1H, dd, *J* = 9.4, 1.8 Hz, H-7), 2.59 (1H, q, *J* = 10.0 Hz, H-16), 2.20 (1H, brs, H-10), and 2.02 (1H, m, H-15), and four methylene groups in the region δ 1.9-1.2 (8H, m, H-11-14), were also observed. The ¹³C NMR spectrum of **1** showed 24 carbon signals (4C, 12CH, 6CH₂, 2CH₃). The HMBC spectrum showed correlations between the olefinic protons at δ 6.48 (H-17) and 5.50 (H-18), and C-19, and from protons at



δ 3.00 (H-20), to C-19 (δ_C 166.8), C-21 (δ_C 28.2), C-22 (δ_C 19.0) and C-23 (δ_C 19.0). All these findings clearly indicated the presence of an *N*-isobutylacrylamide unit. The HMBC spectrum of **1** also showed correlations between H-16 and C-7, C-10; H-8 and C-10, C-16; H-9 and C-7, C-11; H-7 and C-6, C-9 and C-16, which suggested the presence of an octahydronaphthalene moiety substituted at C-7 by a methylenedioxyphenyl group. The *N*-isobutylacrylamide fragment was attached to C-16 (δ_C 44.5), according to HMBC correlations from H-7, H-8, H-17 and H-18 to C-16. This was confirmed by ¹H-¹H COSY correlations between H-7/H-16, H-16/H-15, H-16/H-17, H-10/H-15 and H-9/H-10. The relative configuration of asymmetric carbons was attributed according to structural similarities with cyclostachine A [2d] and the NOESY spectrum, where cross peak were observed between H-7/H-15 and H-10/H-15 and no cross peak between H-7/H-16 and H-15/H-16. The EIMS exhibited the molecular ion peak at *m/z* 381 and prominent ions at *m/z* 309, 281, 135 and 115 due to ion fragments (a)-(d) (Scheme 1). Thus **1** was a new compound for which the trivial name obscurine (**1**) was given. Cyclostachine derivatives are common in the Rubiaceae [2d].



Scheme 1: Selected fragments from EIMS.

Experimental

General experimental procedures: Melting points, Büchi-540 melting point apparatus; optical rotations, Jasco digital polarimeter (model DIP-3600); IR, Jasco Fourier Transform IR-420 spectrometer; NMR, Bruker 500 NMR spectrometer equipped with 5 mm probes. Silica gel 230-400 mesh (Merck) and silica gel 70-230 mesh (Merck) were used for CC, while precoated aluminum silica gel 60 F254 sheets were used for TLC, with different mixtures of CH₂Cl₂/MeOH as eluents.

Plant material: The stem bark of *Beilschmiedia obscura* Engl. ex Stapf was collected in June 2008 at Mount Kalla, Yaoundé/Cameroon. The plant (a voucher specimen (No 1004HNC) was identified by Mr Nana Victor, a botanist at the National Herbarium of Cameroon.

Extraction and isolation: Air-dried stem bark of *B. obscura* (4.20 kg) was macerated with methanol at room temperature for 72 h. The methanol extract was concentrated under reduced pressure to give a residue of 72 g, which was selectively extracted with CH₂Cl₂ at room temperature to afford 15 g of CH₂Cl₂ soluble residue. This extract was subjected to column chromatography (CC) over silica gel (0.023-0.20 mesh, Merck) and eluted with a gradient system of *n*-hexane/CH₂Cl₂ (from 5 to 100%) and CH₂Cl₂/MeOH (from 1 to 5%). Fractions (189) of ca 150 mL each were collected. Frs.18-19 (*n*-hexane/CH₂Cl₂, 8/2) afforded 3-β-acetylsitosterol (8 mg). Frs. 59-65 (*n*-hexane/CH₂Cl₂, 4/6) afforded sitosterol (36 mg). Frs.124-129 (CH₂Cl₂/MeOH, 99/1), after a second CC with an isocratic gradient system of CH₂Cl₂ afforded compound **1** (17 mg).

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Frs.135-136 (CH₂Cl₂/MeOH, 98/2) after a second CC with isocratic gradient afforded 2,3-dihydroxypropyl-heptacosanoate (43 mg). Frs.153-155 (CH₂Cl₂/MeOH, 98/2) yielded 1-(26-ferulyloxyhexacosanoyl)-glycerol (32 mg). Frs. 170-173 (CH₂Cl₂/MeOH, 97/3) afforded 1-(26-hydroxyhexacosanoyl)-glycerol (128 mg) [2e], and Frs.183-186 (CH₂Cl₂/MeOH, 95/5) β-sitosterol-3-*O*-D-glucopyranoside (29 mg) [2f].

Obscurine (1)

White amorphous solid.

[α]_D²⁵: +20.3 (c 0.6, CHCl₃).

IR (neat): 3329, 1669, 1632, 1536 cm⁻¹.

¹H NMR (500 MHz, MeOH-*d*₄) δ: 6.68 (1H, d, *J* = 8.1 Hz, H-5), 6.58 (1H, d, *J* = 1.2 Hz, H-2), 6.55 (1H, dd, *J* = 7.5, 1.2 Hz, H-6), 6.48 (1H, dd, *J* = 15.7, 10.0 Hz, H-17), 5.86 (1H, ddd, *J* = 8.1, 5.6, 3.1 Hz, H-9), 5.48 (1H, m, H-8), 5.50 (1H, m, H-18), 3.19 (1H, dd, *J* = 9.4, 1.8 Hz, H-7), 3.00 (2H, m, H-20), 2.59 (1H, q, *J* = 10.0 Hz, H-16), 2.20 (1H, m, H-10), 2.02 (1H, m, H-15), 1.9-1.2 (8H, m, H-11-14), 1.78 (1H, m, H-21), 0.89 (6H, d, *J* = 6.9 Hz, H-22/23).

¹³C NMR (125 MHz, MeOH-*d*₄) δ_C: 166.8 (C, C-19), 147.4 (C, C-3), 145.9 (C, C-4), 145.5 (CH, C-17), 138.3 (C, C-1), 132.4 (CH, C-9), 128.4 (CH, C-8), 124.6 (CH, C-18), 121.2 (CH, C-6), 108.0 (CH, C-2), 107.2 (CH, C-5), 100.6 (O-CH₂-O), 48.9 (CH, C-7), 46.4 (CH₂, C-20), 44.5 (CH, C-16), 37.0 (CH, C-15), 36.6 (CH, C-10), 29.9 (CH₂, C-11), 28.5 (CH₂, C-14), 28.2 (CH, C-21), 26.0 (CH₂, C-13), 20.0 (CH₂, C-12), 19.0 (CH₃, C-22), 19.0 (CH₃, C-23). EIMS *m/z* (%): 381 [M⁺] (100), 309 (8), 281 (19), 246 (42), 135 (81), 115 (16); (+)-HRESIMS: *m/z* 382.23851, calcd. for C₂₄H₃₂NO₃, 382.23767.

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