Obscurine: a New Cyclostachine Acid Derivative from 
Beilschmiedia obscura

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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.

Keywords: Beilschmiedia obscura, Lauraceae, cyclostachine acid derivative, amide.

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

Compound 1 was obtained as white amorphous powder with the molecular formula C\textsubscript{24}H\textsubscript{32}NO\textsubscript{3}, as deduced from the (+)-HRESIMS. Its IR spectrum showed strong absorptions at 3329 (NH), 1669 (C=O), 1632, and 1536 (C=C) cm\textsuperscript{-1}. The \textsuperscript{1}H 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-13), 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 \textsuperscript{13}C NMR spectrum of 1 showed 24 carbon signals (4C, 12CH, 6CH\textsubscript{2}, 2CH\textsubscript{3}). 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\textsubscript{C} 166.8), C-21 (δ\textsubscript{C} 28.2), C-22 (δ\textsubscript{C} 19.0) and C-23 (δ\textsubscript{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\textsubscript{C} 44.5), according to HMBC correlations from H-7, H-8, H-17 and H-18 to C-16. This was confirmed by \textsuperscript{1}H-\textsuperscript{1}H 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].
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 (4.20 kg) was macerated with methanol at room temperature to afford 15 g of CH₂Cl₂ soluble residue. This was concentrated under reduced pressure to give a residue of 1000 mg.

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 mm) and eluted with a gradient system of n-hexane/CH₂Cl₂ (from 5 to 100%) and 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. 183-186 (CH₂Cl₂/MeOH, 95/5) afforded sitosterol-3-O-D-glucopyanoside (29 mg) [2f].

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-glucopyanoside (29 mg) [2f].

Obscure (1)
White amorphous solid. [α]D25: + 20.3 (c 0.6, CHCl₃).

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References