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New bisbenzylisoquinoline alkaloid from *Laureliopsis philippiana*

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ABSTRACT

Phytochemical investigation of *Laureliopsis philippiana* resulted in isolation of a new bisbenzylisoquinoline alkaloid (1) named laureliopsine A. The structure was established by spectroscopic methods, including 2D homo- and heteronuclear NMR experiments. This finding of a bisbenzylisoquinoline alkaloid in *Laureliopsis* supports its close relationship to *Atherosperma* and its taxonomic segregation from *Laurelia*.

1. Introduction

Laureliopsis philippiana (Looser) Shodde is a slow growing, shade tolerant tree found in Chile and Argentina [1,2]. Extracts of the leaves have traditionally been used for the treatment of colds and headaches by the Mapuche people [3]. *Laureliopsis* is a monotypic genus belonging to the Atherospermataceae [4], and previous investigations of stem bark constituents revealed the presence of the monomeric aporphinoids asimilobine, anonaine, norcorydine, nornantenine, 4-hydroxynornantenine, and (+)-reticuline [5–7]. In this study, a new 6',7-epoxy analogue (1) of the berbamine subgroup of the bisbenzylisoquinolines was identified in the extract of leaves from *Laureliopsis philippiana*.

2. Experimental

2.1. General

Optical rotation was measured using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a Bruker Avance NMR spectrometer (proton frequency 600.13 MHz) at 25 °C, using TMS as internal standard. Gradient selected HMBC and HSQC spectra were optimized for $^{n}J_{C,H}$ = 7.7 Hz and $^{1}J_{C,H}$ = 145 Hz, respectively, and the NOESY experiment was acquired with a mixing time of 600 ms. High-resolution mass measurements for exact mass determination were performed on a Micromass QTOF spectrometer equipped with an ESI ion source, operating in positive-ion mode. Samples were dissolved in MeOH and polyethylene glycol (PEG) was added for calibration. Column chromatography was performed on Matrex Silica gel 60A (particle size 70–200 μm). Alkaloid-containing fractions were monitored using pre-coated silica gel 60 F_{254} TLC plates using Dragendorff's reagent for visualization.

2.2. Plant material

Leaves of *Laureliopsis philippiana* (Looser) Shodde [Atherospermataceae] were collected in Chile (coastal area close to Pucomo, Ozorno) in October 2006 by Alfonso Guzmán. A voucher specimen (accession number: PM2001/5) has been deposited in Herbarium C (Botanical Museum, University of Copenhagen, Copenhagen, Denmark) as well as at Universidad de los Lagos, Ozorno, Chile.

2.3. Extraction and isolation

Powdered leaves (325 g) of *L. philippiana* were extracted with CH_2Cl_2–MeOH (1:1) for 6 h using a Soxhlet extraction

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system. The extract was evaporated under reduced pressure below 40° C to yield 67 g of dry crude extract. The residue was dissolved in 1.5 L of 0.25 N H_2SO_4 and extracted twice with 1.5 L of $CHCl_3$. The aqueous layer was basified (pH 11) using 2 N NH_4OH and extracted twice with 1.5 L of $CHCl_3$. The latter chloroform extract was evaporated as described above to yield 5.95 g of crude alkaloids. This fraction was subjected to vacuum liquid chromatography (silica gel 60H (Merck), 8×12 cm i.d.) and eluted with heptane containing 33–75% of CH_2Cl_2–MeOH (9:1). Fractions of 100 mL were assessed by TLC and visualized with Dragendorff's reagent, and fractions showing similar TLC-profiles were pooled into four fractions A–D. Fraction B (655 mg) was further purified by column chromatography (silica gel 60) using a 46×2 cm i.d. column eluted with 300 mL of heptane-EtOAc (1:1) followed by 300 mL of heptane-EtOAc (2:3) to give 24 mg of 1.

Laureliopsine A (1), yellowish gum, $[\alpha]^{25}_D = 115.6$ (*c* 0.45, $CHCl_3$); ^1H and ^{13}C NMR data: see Table 1. HR-ESI-TOF-MS *m/z* 575.2500 $[MH]^+$. Calc. for $[C_{36}H_{35}N_2O_5]^+$ 575.2541.

3. Results and discussion

Repeated chromatographic separation of the leaf extract resulted in isolation of 24 mg of 1. Combined use of the ^1H, ^{13}C, and DEPT NMR experiments showed the presence of two methoxy groups, two *N*-methyl groups, five methylenes, 12

Table 1
^1H (600 MHz) and ^{13}C (150 MHz) NMR Data of 1

Position	^{13}C [a]	^1H [a, b]	COSY [a, c]	NOESY [a, c, d]	HMBC [a, c, e]
1	60.3	3.62 br d ($J_{H1\text{-}H15B}$ = 10.7)	H-15A, H-15B	H-8', H-10, H-15A, H-17	H-3α, H-3β, H-5, H-15A, H-15B, H-17
3	44.1	α: 2.82 m	H-3β, H-4α, H-4β	H-3β, H-4β, H-5, H-17	H-1, H-4α, H-17
		β: 3.40 m	H-3α, H-4α, H-4β	H-3A, H-4β, H-5, H-15B	
4	22.6	α: 2.82 m	H-3α, H-3β, H-4β	H-4β,	H-3α, H-3β, H-5
		β: 2.39 br dd ($J_{H4\beta\text{-}H4\alpha}$ = 15.9; $J_{H4\beta\text{-}H3\beta}$ = 5.7)	H-3α, H-3β, H-4α	H-3α, H-3β, H-4α	
4a	128.5				H-1, H-3α, H-3β, H-4α
5	106.5	6.26 br s	H-4α, H-4β	H-4α, H-4β, H-18	H-3α, H-4α, H-4β, H-18
6	145.9				H-5, H-18
7	129.5				H-5
8	139.6				H-1, H-5
8a	119.1				H-1, H-5, H-3α, H-4α, H-15A
9	134.2				H-1, H-10, H-13, H-15A, H-15B
10	114.6	6.64 d ($J_{H10\text{-}H14}$ = 1.9)	H-14	H-1, H-11', H-13', H-15A	H-13, H-14, H-15A, H-15B
11	149.6				H-10, H-13, H-15B
12	146.6				H-10, H-13, H-14, H-16
13	111.5	6.85 d ($J_{H13\text{-}H14}$ = 8.1)	H-14	H-14, H-16	H-10, H-16
14	122.6	6.77 d ($J_{H14\text{-}H13}$ = 8.1; $J_{H14\text{-}10}$ = 1.9)	H-10, H-13	H-13, H-15B, H-17(weak)	H-10, H-13, H-15A, H-15B
15	41.1	A: 2.53 br d ($J_{H15A\text{-}H15B}$ = 14.0)	H-1, H-15B	H-1, H-8', H-10, H-15B	H-1, H-4, H-10, H-13
		B: 2.64 dd ($J_{H15A\text{-}H15B}$ = 14.0; $J_{H15A\text{-}H1}$ = 10.7)	H-1, H-15A	H-3β, H-14, H-15A	
16	56.1	3.96 s		H-13	
17	42.8	2.22 s		H-1, H-3α, H-14(weak)	H-3α, H-3β
18	56.2	3.83 s		H-5, H-5'(weak)	
1'	141.6				H-3'A, H-3'B, H-8', H-15', H-16'
3'	50.5	A: 3.16 dt ($J_{H3'A\text{-}H3'B}$ = 11.2; $J_{H3'A\text{-}H4'A}$ = $J_{H3'A\text{-}H4'B}$ = 5.2)	H-3'B, H-4'A, H-4'B	H-3'B, H-4'B, H-16'	H-4'A, H-4'B, H-16'
		B: 3.21 ddd ($J_{H3'B\text{-}H3'A}$ = 11.2; $J_{H3'B\text{-}H4'B}$ = 9.2; $J_{H3'B\text{-}H4'A}$ = 4.2)	H-3'A, H-4'A, H-4'B	H-3'A, H-4'A, H-4'B, H-16'	
4'	30.4	A: 2.76 ddd ($J_{H4'A\text{-}H4'B}$ = 15.4; $J_{H4'A\text{-}H3'A}$ = 5.2; $J_{H4'A\text{-}H3'B}$ = 4.2)	H-3'A, H-3'B, H-4'B	H-3'B, H-4'B, H-5'	H-3'A, H-3'B, H-5', H-16'
		B: 2.94 dddd ($J_{H4'B\text{-}H4'A}$ = 15.4; $J_{H4'B\text{-}H3'B}$ = 9.2; $J_{H4'B\text{-}H3'A}$ = 5.2; $J_{H4'B\text{-}H5'}$ = 0.8)	H-3'A, H-3'B, H-4'A	H-3'A, H-3'B, H-4'A, H-5'	
4'a	133.1				H-3'A, H-3'B, H-4'A, H-4'B, H-5', H-8'
5'	115.8	6.68 t ($J_{H5'\text{-}H4'A}$ = $J_{H5'\text{-}H4'B}$ = 0.8)	H-4'A, H-4'B	H-4'A, H-4'B, H-18	H-4'A, H-4'B, H-8'
6'	140.9				H-5', H-8'
7'	138.9				H-5', H-8'
8'	116.6	6.68 s		H-1, H-10', H-14', H-15A(weak),	H-5', H-15'
8'a	126.8				H-4'A, H-4'B, H-5', H-8', H-15'
9'	137.8				H-11', H-13', H-15'
10'	131.2	7.37 dd ($J_{H10'\text{-}H11'}$ = 8.1; $J_{H10'\text{-}H14'}$ = 2.1)	H-11', H-15'	H-8', H-11', H-15', H-16'	H-11', H-13', H-14'
11'	123.1	7.26 m (overlapping with H-14')	H-10'	H-10, H-10'	H-13'
12'	153.0				H-10', H-11', H-13', H-15'
13'	123.7	7.09 dd ($J_{H13'\text{-}H14'}$ = 8.4; $J_{H13'\text{-}H11'}$ = 2.4)	H-11', H-14'	H-10, H-14'	H-11'
14'	131.2	7.26 m (overlapping with H-11')	H-13', H-15'	H-8', H-13', H-15', H-16(weak)	H-10'
15'	100.7	5.71 br s	H-10', H-14'	H-10', H-14', H-16'	H-3'A, H-3'B, H-8', H-10', H-14', H-16'
16'	41.4	2.97 s		H-3'A, H-3'B, H-10'(weak), H-14'(weak), H-15'	H-3'A, H-3'B, H-15'

[a] ^1H (600 mHz) and ^{13}C (150 mHz) NMR spectral data measured in $CDCl_3$, δ values relative to internal TMS.
[b] The multiplicity of signals is given in parentheses: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Coupling constants (apparent splittings) are reported as numerical values in Hz.
[c] Signal correlating with ^1H resonance.
[d] Mixing time 600 ms.
[e] Optimized for $^nJ_{C,H}$ = 7.7 Hz.

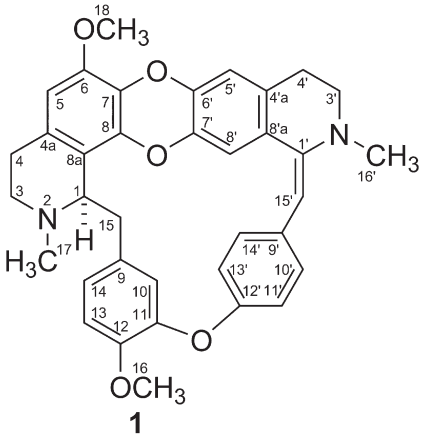

Fig. 1. Structure of laureliopsine A (1).

methines and 15 quaternary carbon atoms, which together with information obtained from MS ($[M+H]^+$ 575.2100) led to the molecular formula $C_{36}H_{34}N_2O_5$. The structure of 1 (Fig. 1) was established by combined use of COSY, NOESY, HSQC and HMBC experiments. Thus, the spin systems H-3–H-4, H-1–H-15 and H-10–H-13–H-14 were identified by analysis of the COSY spectrum, and they were correlated with the benzylisoquinoline moiety containing H-5 by correlation pathways found in the NOESY spectrum (Fig. 2). Correlations observed in the NOESY spectrum also established the position of the methoxy groups at C-6 and C-12, respectively, and the *N*-methyl group at position 2. Similarly, analysis of spectral data starting with H-15' as anchoring point allows assignment of all ^1H and ^{13}C resonances of the second benzylisoquinoline moiety. Thus, the NOESY correlation pathways H-15'→H-16'→H-3'→H-4'→H-5' and H-15'→H-10' and H-14' together with correlations found in the HMBC spectrum (see Fig. 2) established the structure of the second benzylisoquinoline moiety, with the unusual exocylic double bond at C-1'. The head-to-head connection of the two moieties was established by NOESY correlations observed between H-13' and H-10, between H-8' and H-15A and H-1, and between H-5' and H-18 (see Fig. 2). Compound 1 is a new compound for which the name laureliopsine A is suggested.

This is the first report of a bisbenzylisoquinoline alkaloid from *Laureliopsis philippiana* and this finding is very important from a chemotaxonomic point of view. One of the first alkaloids from this group, berbamine, was isolated from *Atherosperma moschatum* from Australia more than 100 years ago [8]. The presence of these alkaloids further supports the connection between *Laureliopsis* and *Atherosperma*. Together with the genus *Nemuaron*, these two genera form a well defined clade within the Atherospermataceae, which separated from the genus *Laurelia* 83 million years ago as estimated in the most recent phylogenetic investigations of the Laurales [9,10]. Further investigations of other parts of the plant would be of great interest as well as correlation of future chemotaxonomic information with DNA-based phylogenetic investigations.

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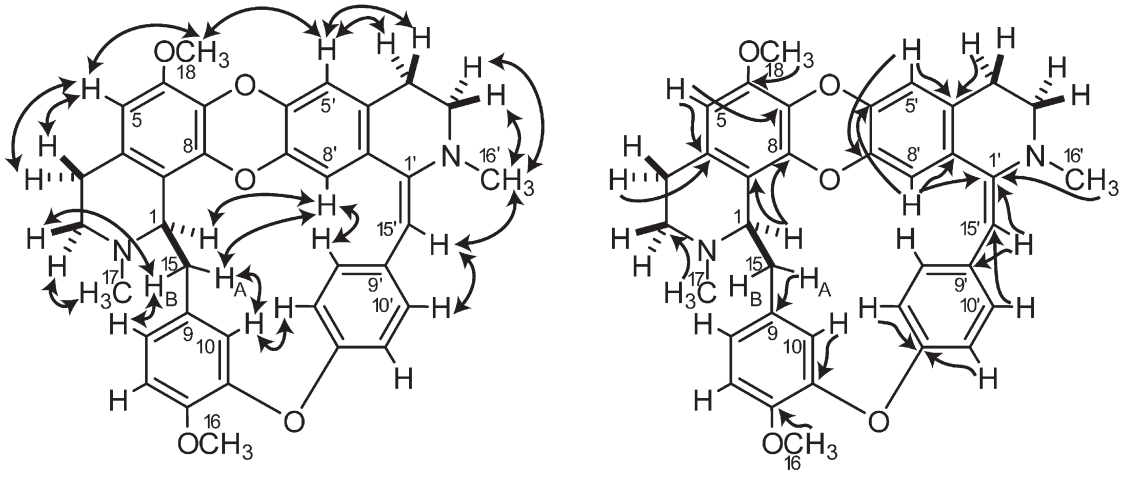

Fig. 2. Selected correlations found in the NOESY experiment (left) and the gradient-selected HMBC experiment (right, arrows indicate correlations from hydrogen to carbon).