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Bisbenzylisoquinoline alkaloids as markers of Atherospermataceae: Tetrandrine and fangchinoline from *Laureliopsis philippiana*

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1. Subject and source

Laureliopsis philippiana (Looser) Schodde [syn. *Laurelia serrata* Phil. and *Laurelia philippiana* Looser] is a slow growing, shade tolerant tree that is native to Chile and Argentina (Veblen et al., 1996; Lusk, 1999). The tree is also known as Tapa, and extracts of the leaves have traditionally been used by the Chilean Mapuche people for the treatment of colds and headaches (Houghton and Manby, 1985). *Laureliopsis* is a monotypic genus that used to be included in Monimiaceae; however based on phylogenetic studies Monimiaceae has been reorganized into Monimiaceae *sensu stricto*, Siparunaceae, and Atherospermataceae, with *L. philippiana* included in the latter (Renner, 1998, 1999, 2004; Renner et al., 2000). In this study, leaves were collected in Chile (coastal area close to Pucomo, Osorno) 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, Osorno, Chile.

2. Previous work

Previous investigations of stem bark resulted in isolation of *S*-(+)-reticuline, *R*-(-)-asimilobine, *R*-(-)-anonaine, *S*-(+)-norcorydine, *S*-(+)-nornantenine, (4*S*,6*aS*)-4-hydroxynornantenine, (4*R*^{*},6*aR*^{*})-4-hydroxyanonaine, liriodenine, atheroline, oxonantenine, laurotetanine, and norisocorydine (Urzúa et al., 1975; Urzúa and Cassels, 1978a, 1978b, 1982), and it was not until recently that laureliopsine A, a 6',7-epoxy analogue of the berbamine subgroup, was identified in a leaf extract (Staerk et al., 2009). Another study investigated the fungistatic activity of essential oils of leaves of *L. philippiana* (Bittner et al., 2009).

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3. Present study

Powdered leaves (197.5 g) of *L. philippiana* were extracted 5 times successively with 600 mL of CH₂Cl₂–MeOH (1:1). The extract was evaporated under reduced pressure below 40 °C to yield 31.6 g of dry crude extract. The residue was dissolved in 1 L of 0.25 N H₂SO₄, filtered and extracted twice with 250 mL heptane and three times with 330 mL EtOAc. The aqueous layer was basified (pH 11) using 25% NH₄OH and extracted four times with 300 mL of EtOAc. The latter ethyl acetate extract was extracted with 300 mL 25% NH₄OH and was evaporated as described above to yield 2.60 g of crude alkaloids.

Semi-preparative HPLC separations of the alkaloid fraction were performed at 40 °C on a Phenomenex C₁₈(2) Luna column (150 × 10 mm i.d., particle size 3 μm) with a flow rate of 2.5 ml/min using a mixture of H₂O/MeOH 95:5 + 0.1% formic acid (eluent A) and MeOH/H₂O 95:5 + 0.1% formic acid (eluent B). Nineteen repeated separations (injection volumes of 100 μl of a 3 mg/mL solution) were performed using the following gradient profile: 0 min, 20% B; 8 min, 20% B; 9 min, 100% B; 16 min, 100% B; 17 min, 20% B; 25 min, 20% B to afford 24.1 mg of **1** and 6.4 mg of **2**.

Compound **1** was obtained as a yellowish gum with specific rotation $[\alpha]_D^{25}$ of +198.8 (c 0.50, CH₂Cl₂), and ¹H and ¹³C NMR data fully assigned by COSY, NOESY, HSQC and HMBC experiments acquired at 800 MHz identified **1** as fangchinoline. Only partly assigned ¹H and ¹³C NMR data have previously been reported for fangchinoline (Philipov and Istatkova, 1997; Lijin et al., 2009), and the fully assigned high field data are therefore given in Table 1.

Compound **2** was obtained as a yellowish gum with specific rotation $[\alpha]_D^{25}$ of +201.5 (c 0.49, CH₂Cl₂), and identified as tetrandrine based on ¹H NMR, ¹³C NMR, COSY, NOESY, HSQC and HMBC experiments acquired at 800 MHz. The ¹H and ¹³C NMR data are in agreement with those reported in the literature (Thevand et al., 2004).

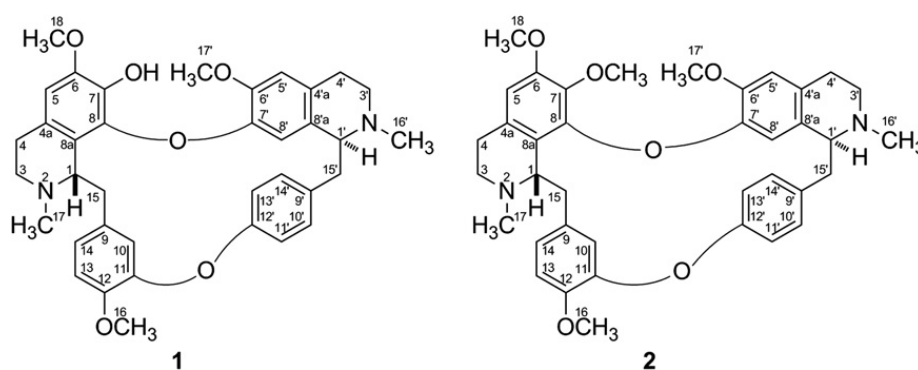


Table 1
¹H and ¹³C NMR data of **1**.

Pos.	¹³ C ^a	¹ H ^{a,b}	Pos.	¹³ C ^a	¹ H ^{a,b}
1	61.8	4.04 br d ($J_{H1-H15B} \approx 10.0$)	1'	63.7	4.14 br dd ($J_{H1-H15B} \approx 11.0$; $J_{H1-H15A} \approx 4.5$)
3	45.3	α : 3.04 m β : 3.60 ddd ($J_{H3\beta-H3\alpha} = 13.0$; $J_{H3\beta-H4\alpha} = 10.9$; $J_{H3\beta-H4\beta} = 15.3$)	3'	44.2	α : 3.16 br dd ($J_{H3\alpha-H3\beta} \approx 12.0$; $J_{H3\alpha-H4\beta} \approx 4.5$) β : 3.67 m
4	23.0	α : 2.66 m β : 2.96 m	4'	23.5	α : 2.94 m β : 3.04 m
4a	122.6		4'a	126.1	
5	105.2	6.33 s	5'	112.9	6.56 s
6	146.6		6'	149.9	
7	135.2		7'	144.3	
8	141.5		8'	120.9	6.06
8a	121.9		8'a	124.7	
9	133.2		9'	133.3	
10	116.0	6.54 d ($J_{H10-H14} = 1.9$)	10'	132.7	6.32 dd ($J_{H10'-H11'} = 8.2$; $J_{H10'-H14'} = 2.0$)
11	149.6		11'	122.3	6.83 dd ($J_{H11'-H13'} = 8.2$; $J_{H11'-H10'} = 2.4$)
12	147.8		12'	154.4	
13	111.9	6.85 d ($J_{H13-H14} = 8.2$)	13'	122.3	7.07 dd ($J_{H13'-H11'} = 8.2$; $J_{H13'-H14'} = 2.4$)
14	123.5	6.94 br d ($J_{H14-H13} = 8.2$)	14'	130.6	7.36 br d ($J_{H14'-H13'} = 8.2$)
15	41.5	A: 2.67 d ($J_{H15A-H15B} = 14.7$) B: 2.85 dd ($J_{H15A-H15B} = 14.7$; $J_{H15A-H1} = 10.1$)	15'	38.6	A: 2.77 t ($J_{H15A-H15B} = J_{H15A-H1} = 11.8$) B: 3.50 m
16	56.3	3.92 s	16'	41.1	2.70 s
17	42.6	2.44 s	17'	56.2	3.40 s
18	56.3	3.78 s			

^a ¹H (800 MHz) and ¹³C (200 MHz) NMR spectral data measured in chloroform-*d*, δ values relative to internal TMS.

^b 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.

4. Chemotaxonomic importance

Atherospermataceae is a small family of trees and shrubs belonging to Laurales. The family is, in its present form, separated from Monimiaceae s.s. and regarded as closely related to Gomortegaceae, a family of trees endemic to Chile (Schodde, 1970; Renner et al., 2000; Renner, 2004). Atherospermataceae comprise 16 species according to Mabberley (Mabberley, 2008) distributed over seven genera, i.e., *Atherosperma* (1), *Laureliopsis* (1), *Laurelia* (2), *Nemuaron* (1), *Dryadodaphne* (3), *Daphnandra* (6), and *Doryphora* (2). However, based on a search in The International Plant Name Index (The International Plant Name Index, 2010) and Tropicos (Tropicos.org, 2010) the number of species may be as high as 25 leaving out dubious taxa. Classification and phylogeny within Atherospermataceae have been intensively studied by Renner and coworkers; however, the results still seem to be ambiguous. Thus, in two separate studies based on molecular data from plastid genome regions, *Laureliopsis* was separated from *Laurelia* and found to form a clade with *Nemuaron* (Renner, 1998) and *Nemuaron* and *Atherosperma* (Renner, 1999), respectively. However, in two other studies (Renner et al., 2000; Renner, 2004), molecular data based on plastid gene regions and correlation with fossil records showed that *Laureliopsis* and *Laurelia* form a clade that was separated from *Atherosperma* and *Nemuaron* at least 83 million years ago. About 79.5 million years ago, *Laurelia sempervirens* separated from *L. philippiana* and *Laurelia novae-zelandiae*, the two latter constituting a sub-clade. Thus, this ambiguity shows that taxonomic classification should be considered a multiparametric discipline, and therefore classification based on information about the genome should preferably be complemented with information about the proteome and the metabolome, i.e., the biological endpoint of the genome and the proteome. The identification of bisbenzylisoquinoline alkaloids (BBIs) reported in this study, i.e., fangchinoline and tetrandrine, covers only a small part of the full metabolome of *L. philippiana*. However, bisbenzylisoquinoline alkaloids are important chemical markers of Atherospermataceae, and they have been subject to intensive investigations. Thus, a compilation of BBIs identified in Atherospermataceae (Schiff, 2000; Staerk et al., 2009) has been added as Supplementary Data. The alkaloids are ordered according to the structural types originally defined by Shamma and Moniot (Shamma and Moniot, 1976) and later given Roman numerals by Guha and coworkers (Guha et al., 1979), and the compilation shows that *Atherosperma*, *Laureliopsis*, and *Laurelia* produce BBIs of structural type VIII in addition to two BBIs of type VI and a single BBI of type XXVIII. These results support the connection between *Laureliopsis* and *Atherosperma* as well as *Laurelia*, as shown by the somewhat contradictory results in the above-mentioned studies (Renner, 1998, 1999, 2004; Renner et al., 2000). It is interesting, however, that whereas fangchinoline and tetrandrine, isolated from leaves of *L. philippiana* in this work, belong to the 1S,1'S-series, previous work with *L. sempervirens* led to isolation of isofangchinoline [syn. thalrugosine] (Cassels and Urzúa, 1985) and isotetrandrine (Bianchi et al., 1962) as well as oxyacanthine and obaberine (Cassels and Urzúa, 1985), all of which have been assigned to the 1R,1'S-series. This supports the classification of *L. philippiana* [syn. *L. serrata* Phil. and *L. philippiana* Looser] into a monotypic genus separated from *Laurelia*.

In conclusion, this work demonstrates the importance of complementing genome-based classification with classical small-molecule metabolome-based classification. The majority of studies of these rather complex BBIs from Atherospermataceae are however rather old, and future in-depth studies utilizing new fast and sensitive hyphenated techniques like HPLC-PDA-MS-SPE-NMR (Pedersen et al., 2009) or utilizing modern multivariate metabolomics approaches (Agnolet et al., 2010) are expected to yield better data for multiparametric chemotaxonomic classification of Atherospermataceae.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: [10.1016/j.bse.2010.03.006](https://doi.org/10.1016/j.bse.2010.03.006).

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