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Isoquinoline alkaloids from the leaves of *Xylopia laevigata* (Annonaceae)

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1. Subject and source

Xylopia L. belongs to the Annonaceae family and comprises approximately 157 species of trees and shrubs (Chatrou et al., 2012) with pantropical distribution (Koek-Noorman and Westra, 2012). *Xylopia laevigata* (Mart.) R. E. Fries popularly known as 'meiú' and 'pindaíba' is a small tree endemic of Brazil found in the States of Paraíba, Piauí, Rio de Janeiro, São Paulo, and Sergipe (Pontes et al., 2004; Maas et al., 2001). In Sergipe, it is found in Atlantic forest remaining, sandbank forests and coastal boards. Its leaves are very similar those of certain species of *Oxandra* (Maas et al., 2001). In folk medicine its leaves and flowers are used to the treatment of painful disorders, heart diseases and inflammatory conditions (Quintans et al., 2013).

In the present investigation, the botanical material (leaves) of *X. laevigata* were collected in March 2010 from "Serra de Itabaiana", in the city of Itabaiana [coordinates: 10°44′53″ S and 37°20′21″ W], Sergipe State, Brazil. The identity of the plant was confirmed by Dr. Ana Paula do Nascimento Prata from Department of Biology of Sergipe Federal University (DBI/UFS), Brazil. A voucher specimen (number 15440) was deposited in the ASE herbarium of Sergipe Federal University, Brazil.

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2. Previous work

Previous phytochemical investigations on this species described the isolation and identification of diterpenoids (Silva et al., 2012) and essential oils (Costa et al., 2013; Quintans et al., 2013; Silva et al., 2013).

3. Present study

The dried and powdered leaves of *X. laevigata* (750.0 g) were extracted with hexane (3.0 L, five times), followed by MeOH (3.0 L, five times), yielding of hexane (54.32 g) and MeOH (191.12 g) extracts, after each solvent removal.

TLC analysis indicated a high concentration of alkaloids in the MeOH extract. Therefore, an aliquot of MeOH extract (186.00 g) was initially subjected to an acid-base extraction (Costa et al., 2006) to give alkaloid (1.40 g) and neutral (17.96 g) fractions. An aliquot of alkaloid fraction (1.1 g) was subjected to silica gel CC previously treated with a 10% $NaHCO_3$ solution (Costa et al., 2006), eluted with increasing concentrations of CH_2Cl_2 in hexane (100:0 to 10:90, v/v), followed by EtOAc in CH_2Cl_2 (100:0 to 30:70, v/v), and MeOH in EtOAc (100:0 to 50:50, v/v), giving 150 fractions (30 mL each). These fractions were evaluated and pooled according to TLC analysis yielding fifteen groups (GF1-GF15). Group GF2 (143.0 mg) was submitted to a new silica gel CC previously treated with a 10% $NaHCO_3$ solution, eluted with the same solvent system as described above affording 45 fractions (10 mL each) that were pooled in six groups (GF2.1 to GF2.6), according to TLC analysis. Group GF2.3 (18.5 mg) was subjected to a preparative TLC eluted with $CHCl_3$:MeOH (95:05, v/v, two times), giving a mixture of liriodenine and lanuginosine (**1** and **2**, respectively, 6.6 mg: Wirasathien et al., 2006; Costa et al., 2009, 2011a). Group GF2.4 (7.0 mg) was also subjected to a preparative TLC eluted with $CHCl_3$:MeOH (95:05, v/v, two times), affording lanuginosine (**2**, 3.3 mg; Wirasathien et al., 2006). Group GF3 (31.2 mg) was subjected to a preparative TLC eluted with $CHCl_3$:MeOH (90:10, v/v, three times), resulting in discretine (**3**, 2.3 mg; Hocquemiller et al., 1984; Ohiri et al., 1983), norisolboldine or laurelliptine (**4**, 1.3 mg; Guinaudeau et al., 1975), stepharine (**5**, 1.6 mg; Chang et al., 2000a) and a mixture of laurotetanine and reticuline (**6** and **7** respectively, 2.2 mg; Guinaudeau et al., 1975; Da Cruz et al., 2011). Group GF5 (80.0 mg) was also subjected to a preparative TLC eluted with $CHCl_3$:MeOH (90:10, v/v, three times) giving coreximine (**8**, 4.3 mg; Costa et al., 2009), laurotetanine (**6**, 1.5 mg; Guinaudeau et al., 1975) and norboldine (**9**, 3.1 mg; Chang et al., 2000b; Guinaudeau et al., 1975, 1994) (Fig. 1).

All isolated compounds (Fig. 1) were identified by a series of spectrometric methods, mainly MS and NMR (1D and 2D), as well as comparison with data reported in the literature. Although, discretine (**3**), laurotetanine (**6**) and norboldine (**9**) has been described a long time ago, their NMR data are incomplete and show ambiguities. Therefore, the complete and unequivocal NMR data for these alkaloids were reviewed according to 1D and 2D NMR experiments (Tables 1 and 2).

4. Chemotaxonomic significance

The present work reports the isolation and identification of nine isoquinoline alkaloids; two oxoaporphine, liriodenine (**1**) and lanuginosine (**2**); three aporphine, norisolboldine or laurelliptine (**4**), laurotetanine (**6**), and norboldine or laurolitsine (**9**);

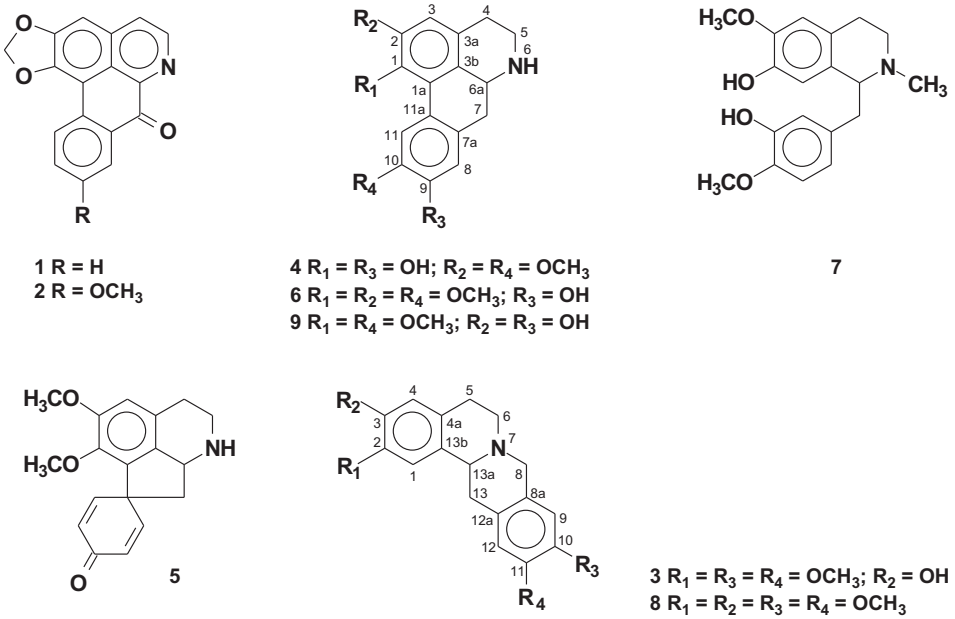

Fig. 1. Isoquinoline alkaloids from the leaves of *Xylopia laevigata*.

Table 1
NMR data (400 MHz) for discretine (**3**).

Position	δ_C mult.[a,b]	δ_H mult. (*J* in Hz)[a]
1	109.8, CH	6.83 *s*
2	147.7, qC	
3	146.2, qC	
4	115.8, CH	6.57 *s*
4a	127.3, qC	
$5_{Pseudoax}$	28.4, CH_2	2.67 *m*
$5_{Pseudoeq}$		3.05 *ddd* (17.4, 12.5, 5.0)
$6_{Pseudoax}$	51.6, CH_2	2.65 *m*
$6_{Pseudoeq}$		3.19 *m*
$8_{Pseudoax}$	58.7, CH_2	3.70 *d* (14.8)
$8_{Pseudoeq}$		3.98 *d* (14.8)
8a	126.5, qC	
9	110.4, CH	6.69 *s*
10	148.9, qC	
11	149.2, qC	
12	112.8, CH	6.78 *s*
12a	127.2, qC	
$13_{Pseudoax}$	36.5, CH_2	2.80 *dd* (16.2, 11.4)
$13_{Pseudoeq}$		3.43 *dd* (16.2, 3.9)
$13a_{Pseudoax}$	61.0, CH	3.66 *dd* (11.4, 3.9)
13b	129.0, qC	
H_3CO-2	56.6, CH_3	3.85 *s*
H_3CO-10	56.4, CH_3	3.81 *s*
H_3CO-11	56.5, CH_3	3.82 *s*

[a] The experiments were obtained in $CDCl_3$ plus drops of CD_3OD at 293 K and the NMR chemical shift are giving in ppm related to TMS signal at 0.00 ppm as internal reference.
[b] Multiplicities determined by DEPT 135 and HSQC experiments.

one benzyltetrahydroisoquinoline, reticuline (**7**); one proaporphine, stepharine (**5**); and two tetrahydroprotoberberine, discretine (**3**) and coreximine (**8**). All isolated alkaloids are been described here for the first time in *X. laevigata*. However, they are very common in Annonaceae species, mainly in genera *Annona*, *Artabotrys*, *Duguetia*, *Guatteria*, *Polyalthia* and *Xylopia* (Guinaudeau et al., 1975, 1979, 1983, 1988, 1994; Leboeuf et al., 1982; Campos et al., 2008; Pinheiro et al., 2009; Costa et al., 2009, 2010, 2011a,b; Da Cruz et al., 2011; Dutra et al., 2012; Zawawi et al., 2012; Vendramin et al., 2013). Therefore, they are considered as chemotaxonomic markers of the Annonaceae family. In *Xylopia* species some of the isolated alkaloids present a

Table 2
NMR data (400 MHz) for the laurotetanine (**6**) and norboldine (**9**).

Position	**6**		**9**	
	δ_C mult.[a,b]	δ_H mult. (*J* in Hz)[a]	δ_C mult.[a,b]	δ_H mult. (*J* in Hz)[a]
1	144.3, qC		143.1, qC	
1a	127.1, qC		126.7, qC	
2	152.2, qC		149.6, qC	
3	110.7, CH	6.61 *s*	114.1, CH	6.63 *s*
3a	128.4, qC		128.0, qC	
3b	126.5, qC		123.7, qC	
$4_{Pseudoax}$	28.2, CH_2	2.75 *m*	27.2, CH_2	2.73 *m*
$4_{Pseudoeq}$		3.04 *m*		3.04 *m*
$5_{Pseudoax}$	42.5, CH_2	3.02 *m*	42.2, CH_2	3.03 *m*
$5_{Pseudoeq}$		3.41 *m*		3.45 *m*
$6a_{Pseudoax}$	53.5, CH	3.84 *dd* (14.5, 4.9)	53.4, CH	3.88 *dd* (13.8, 4.8)
$7_{Pseudoax}$	35.7, CH_2	2.68 *dd* (14.5, 13.8)	34.8, CH_2	2.74 *dd* (13.8, 13.8)
$7_{Pseudoeq}$		2.78 *dd* (13.8, 4.9)		2.84 *dd* (13.8, 4.8)
7a	129.1, qC		128.2, qC	
8	114.4, CH	6.77 *s*	114.7, CH	6.76 *s*
9	145.6, qC		145.7, qC	
10	146.1 qC		146.4, qC	
11	111.8, CH	8.06 *s*	111.3, CH	7.97 *s*
11a	123.6, qC		123.3, qC	
H_3CO-1	60.2, CH_3	3.67 *s*	60.1, CH_3	3.63 *s*
H_3CO-2	56.1, CH_3	3.89 *s*		
H_3CO-10	55.9, CH_3	3.90 *s*	56.1, CH_3	3.89 *s*

[a] The experiments were obtained in $CDCl_3$ plus drops of CD_3OD at 293 K and the NMR chemical shift are giving in ppm related to TMS signal at 0.00 ppm as internal reference.
[b] Multiplicities determined by DEPT 135 and HSQC experiments.

great chemotaxonomic relevance, such as discretine found in *Xylopia discreta* (Leboeuf et al., 1982), *Xylopia championii* (Puvanendran et al., 2008), and *Xylopia parviflora* (Nishiyama et al., 2006; Puvanendran et al., 2010). Liriodenine was found in *Xylopia buxifolia*, *Xylopia brasileinsis*, *Xylopia pancheri*, *Xylopia vielana* (Leboeuf et al., 1982), *Xylopia ferruginea* (Zawawi et al., 2012), and *Xylopia aethiopica* (Harrigan et al., 1994), while lanuginosine was present in *Xylopia lemurica* (Leboeuf et al., 1982) and *Xylopia nigricans* (Puvanendran et al., 2010). Among the aporphine alkaloids, laurotetanine is the most representative, found in *Xylopia amazonica* (Martins et al., 1995), *Xylopia benthamii* (Pimenta and Mendonça, 2012), *Xylopia danguyella* (Leboeuf et al., 1982) and *X. parviflora* (Nishiyama et al., 2006). Norboldine (laurolitsine) has been isolated from *Xylopia papuana* (Leboeuf et al., 1982). Reticuline, the biosynthetic precursor of many alkaloids, including the aporphines (Cordell, 2013) was described in *X. pancheri*, *X. papuana* (Leboeuf et al., 1982), *X. nigricans* (Puvanendran et al., 2010) and *X. parviflora* (Nishiyama et al., 2006). The proaporphine alkaloid stepharine was described only in *X. parviflora* (Nishiyama et al., 2006), being the second report in *Xylopia*. Norisoboldine (laurelliptine) and coreximine are reported for the first time in *Xylopia*. The results obtained in this study, confirm that *X. laevigata* has a strong ability to produce isoquinoline alkaloids, a class of compounds typically found in Annonaceae, and its high chemotaxonomic correlation with other *Xylopia* species.

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