

Iridoids from *Hymenodictyon floribundum*

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Das cascas do tronco e das folhas do *Hymenodictyon floribundum* B. L. Rob foram isolados dois novos iridoides, floribundano A (1) e floribundano B (2), e três compostos conhecidos, lupenona (3), escopoletina (4) e 4,5-di-hidroblumenol A (5). As estruturas de todos os compostos foram estabelecidas com base nos seus dados espectroscópicos.

Two new iridoids floribundane A (1) and floribundane B (2) and the known compounds lupenone (3), scopoletin (4) and 4,5-dihydroblumenol A (5) were isolated from trunk bark and leaves of *Hymenodictyon floribundum* B.L. Rob. The structures of all compounds were established from their spectroscopic data.

Keywords: *Hymenodictyon floribundum*, monoterpenes, secoiridoids, nitrogen-containing iridoid, C-9 iridoid

Introduction

The *Hymenodictyon* genus comprises 22 species. Of these, 11 are endemic to Madagascar, 4 to Asia and 7 to Tropical Africa.¹ *Hymenodictyon floribundum* B.L. Rob. (Rubiaceae), endemic to Tropical Africa, is a small tree that grows in the mountains of the Huíla province and its traditional name is NDambi Yov`olwi, (omu)Lia-tyimeme. Its trunk bark is used in Angola folk medicine to treat fever.²

A previous study has shown that the trunk bark of this tree contains scopoletin, hymeselsin, scopolin and 3-*O*-β-D-glucopyranosyl-β-sitosterol.³ An exhaustive literature review revealed that, with the exception of an ongoing study examining the trunk bark and dried leaves of *Hymenodictyon floribundum* (in the context of other medicinal plants of Angola),⁴ other parts of the tree have not yet to be studied.

In the aforementioned study, powdered trunk barks and dried leaves were extracted by maceration with methanol. The methanol extract was partitioned with hexane and chloroform. The hexane extract from trunk barks yielded lupenone (3)⁵ and the chloroform extract yielded scopoletin (4).⁶ The chloroform extract from dried leaves yielded floribundane A (1), 4,5-dihydroblumenol A (5)^{7,8} and floribundane B (2).

Results and Discussion

Floribundane A (1) was obtained as a colourless oil with $[\alpha]_D^{20} = +25.31^\circ$. The HR-TOF- EI-MS showed a molecular ion peak at m/z 223.0843 $[M]^+$ (in agreement with the molecular formula $C_{11}H_{13}NO_4$) implying 6 degrees of unsaturation. The UV maximum at 234 nm and the IR absorptions at 1724, 1633 and 1268 cm^{-1} suggested the presence of the chromophore $-OOC=CHO$.⁹ The IR also showed the presence of a lactam ring: NH band (3403 cm^{-1}), and the amide band I (1690 cm^{-1}) with an absence of the amide II band.¹⁰ The ¹H NMR spectrum of compound (1) (Table 1) displayed signals of two vinylic protons (H-3 δ_H 7.50, s and H-8 δ_H 6.85, q, J 7.2), a ABX system [H-6 α , β δ_H 2.11, dd, J 13.1 and 2.0; δ_H 2.06, dd, J 13.1 and 2.6 (AB part) and H-5 δ_H 3.94, m (X part)] and a vinyl methyl group at δ_H 2.01 (d, J 7.2); typical signals of a secoiridoid nucleus.¹¹ A few iridoid alkaloids have been isolated from natural sources¹²⁻¹⁵ (gardenamide class), and some were obtained as metabolites produced by human¹⁶ and rat intestinal bacteria.¹⁷ Comparison with the published data of known iridoid alkaloids, such as (6) (Figure 1), and hemiacetal-secoiridoids^{11,18} like (7) (Figure 1) and compound (1), showed that the H-3 chemical shift (δ_H 7.50 ppm), despite the presence of a nitrogen atom in

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the molecule, was more compatible with the hemiacetal-secoiridoids (δ_{H} ca. 7.5 ppm) than with the gardenamide class and mor-1¹⁷ (**8**) (Figure 1) (δ_{H} ca. 7.3 ppm). Further, the upfield shift of H-1 (δ_{H} 5.46) relative to the corresponding signal of mor-1 (δ_{H} 5.11 ppm),¹⁷ and a downfield shift (δ_{H} 5.94 ppm) to the corresponding hemiacetals,^{11,18} showed that (**1**) it was neither a hemiacetal nor a nitrogen-containing iridoid (N-2) but that it was compatible with a C-1(N)O-2 substitution. This was also supported by the presence of the ¹H-¹H COSY correlation between H-1/NH, rather than the H-1/NH, H-3/NH correlations expected in the iridoid alkaloids,¹⁷ and by UV and IR data indicating the presence of the chromophore -OOC=CHO.⁹ The ¹³C NMR spectrum (Table 1) showed 11 carbons which were analysed as two methyls (one vinylic and a methyl ester), one methylene, four methines two of them vinylic, and four quaternary carbons (two vinylic and two carbonylic) from its DEPT spectrum. The ¹³C NMR chemical signals at δ_{C} 152.13 (C-3), 136.11 (C-8), 130.25 (C-9), the carbonyl group at δ_{C} 166.56, the methoxy signal at δ_{C} 51.31 and the vinyl methyl group at δ_{C} 13.96, were found to be consistent with a secoiridoid skeleton.¹¹ The unusual downfield shift of C-1 to δ_{C} 76.97 was in agreement with the C-1(O) (N) substitution as observed on mor-1,¹⁷ but only the presence of O in the position 2 of the iridoid skeleton could explain the upfield shift of C-3 and C-4. The HMBC correlation between H-1/C-7 established the presence of the 1,7- δ -lactam ring in the molecule. With the aid of ¹H - ¹H COSY, HSQC and HMBC data (Table 1,

Figure 2) it was possible to establish the structure of (**1**) as a 7,8-secoiridoid, with C-11 as a methyl ester, a C-1(N) O-2 substitution and a 1,7- δ -lactam ring. The NOESY correlations H _{α} -5/H _{α} -6 and H _{β} -6/H _{β} -1 indicated that the 1,7- δ -lactam ring is *trans*-fused. To our best knowledge, this is the first time that a secoiridoid has presented as a 1,7-*trans*- δ -lactam ring. A similar structure, isosweroside, as a hemiacetal, was found in the roots of *Sambucus ebulus* L.¹⁹ Compound (**1**) was named floribundane A.

Floribundane B (**2**) was obtained as a colourless oil with $[\alpha]_{\text{D}}^{20} = +41.66^{\circ}$. The molecular formula C₉H₁₄O₃ was established by HR-TOF-MS-EI which showed a molecular ion peak at m/z 170.0942 [M]⁺ (calculated for 170.2270), implying 3 degrees of unsaturation. The IR showed OH bands (3384 cm⁻¹), an α,β -unsaturated lactone group (1692 cm⁻¹, 1620 cm⁻¹); the UV spectrum a α,β -unsaturated lactone system (230 nm). The ¹H RMN spectrum (Table 1) showed signals of one diastereotopic oxymethylene (H-3) δ_{H} 4.23 (ddd, J_1 11.3, J_2 9.1, J_3 4.4 Hz) and δ_{H} 4.42 (ddd, J_1 11.3, J_2 9.5, J_3 3.6 Hz) that presented ¹H - ¹H COSY correlation with a diastereotopic methylene (H-4) δ_{H} 1.83 (m) and δ_{H} 2.12 (m); a oxymethylene multiplet δ_{H} 3.71 (H-7) that presented an ¹H - ¹H COSY correlation with another methylene δ_{H} 1.74 (m, H-6); a methine δ_{H} 3.18 (m, H-5) that showed cross peaks with H-4 and H-6 and a vinyl methine δ_{H} 7.02 (qd, J_1 7.4, J_2 1.2 Hz, H-8) coupled with a methyl δ_{H} 1.86 (d, J 7.4 Hz, Me-10). This spectrum presents some similarities to that of compound (**1**): the presence of the vinyl double bond and the ¹H - ¹H COSY correlations between H-5, H-6 and H-7. Thus, it appears

Table 1. ¹H NMR and ¹³C NMR data (J in Hz) and HMBC correlations of compounds **1** and **2** (600 MHz, CDCl₃)

C	1				2			
	δ_{C}	δ_{H}	COSY	HMBC	δ_{C}	δ_{H}	COSY	HMBC
1	76.97	5.46, br s	H-5, NH	3, 5, 7	167.38			
3	152.13	7.50, s		1, 4, 5, 11	65.35	4.23, ddd (11.3, 9.1, 4.4) a 4.42, ddd (11.3, 9.5, 3.6) b	H-3, H-4a, H-4b	1, 4, 5
4	109.36				27.51	1.83, m a 2.12, m b	H-3a, H-3b, H-4a, H-4b, H-5	3, 5, 6, 9
5	24.67	3.94, m	H-1, H-6	1, 3, 4, 6, 7, 8, 9	28.93	3.18, m	H4a, H-4b, H-5, H-6	1, 3, 4, 6, 8, 9
6	26.76	2.06, dd (13.1, 2.6) 2.11, dd (13.1, 2.0)	H-5, H-6	4, 5, 9	36.11	1.74, m	H-5, H-7	4, 5, 9
7	166.36				59.97	3.71, m	H-6	5, 6
8	136.11	6.85, q (7.2)	H-10	5, 9, 10	140.67	7.02, qd (7.4, 1.2)	H-5, H-10	1, 5, 9, 10
9	130.25				131.16			
10	13.96	2.01, d (7.2)	H-8	8, 9	14.18	1.86, d (7.4)	H-8	8, 9
11	166.56							
11-OCH ₃	51.31	3.69, s		11				
NH	-	6.93, br s	H-1					

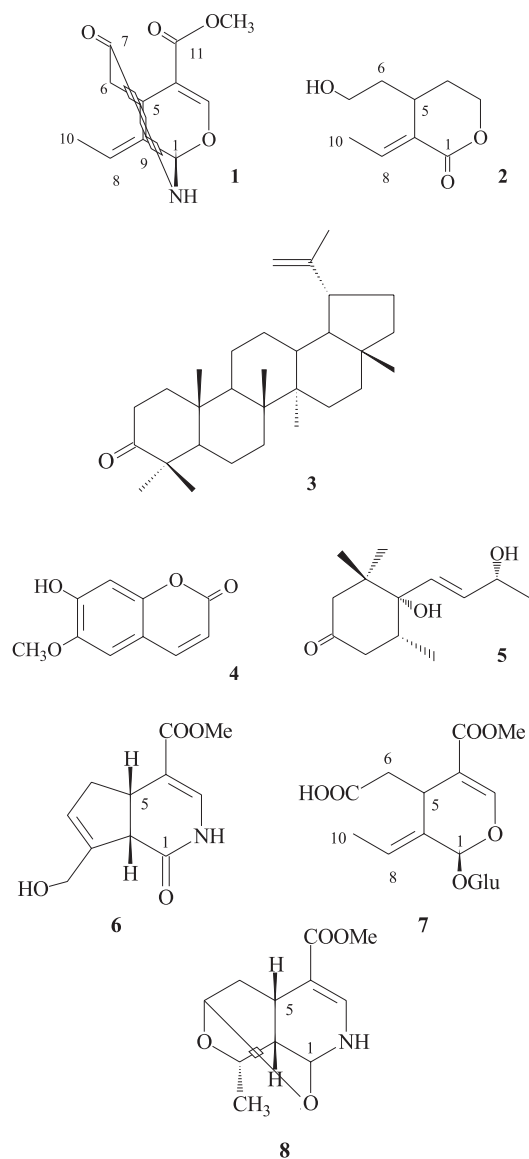


Figure 1. Structures of selected examples of iridoid alkaloids¹³ (**6**), hemiacetal-secoiridoids¹¹ (**7**) and mor-1 (**8**).¹⁷

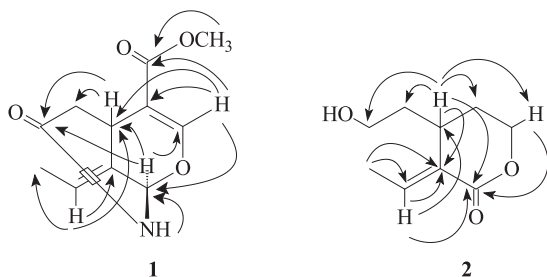


Figure 2. Key HMBC correlations of compounds **1** and **2**.

that the A ring, as in (**1**), is open as a 7,8-secoiridoid with a hydroxyl moiety at C-7. The ¹³C NMR spectrum (Table 1) showed 9 carbons which were analysed, from its DEPT spectrum, as one vinylic methyl (δ_c 14.18), four

methylene (two of them oxymethylenes, δ_c 27.51, 36.11, 59.97, 65.35), two methines (one vinylic, δ_c 28.93, 140.67), and two quaternary carbons (one vinylic, δ_c 131.16, and the other carbonylic, δ_c 167.38). The lack of the two quaternary sp^2 carbons belonging to the C-3 - C-4 double bond of (**1**) and the methine C-1, and the presence of signals characteristic of a methylene (δ_c 27.51), an oxymethylene (δ_c 65.35) and a sp^2 carbon (δ_c 167.38) belonging to the α,β -unsaturated lactone group detected in the IR and UV spectra, is indicative of a major transformation in the B ring of (**2**). However, these new features of the B ring are in good agreement with those of the δ -lactone ring of C-9 iridoid derivatives viteoid II,^{20,21} 7-hydroxyviteoid II²² and ovatolactone.²³ The vinylic double bond can unambiguously be assigned to C-8 and C-9 and the lactone carbonyl to C-1, by means of ¹H - ¹H COSY, HSQC and HMBC data (Table 1, Figure 2). Several C-9 iridoid and secoiridoid (lactone ring opening) derivatives are known²⁰⁻²⁵ but to our best knowledge this is the first time that a C-9 7,8-secoiridoid derivative has been isolated. Thus, (**2**) was identified as a new natural product for which we propose the name floribundane B.

Conclusions

Although trunk bark components of *H. floribundum* have been well studied, there has as yet been no study which focuses solely on the leaves of this medicinal tree. The major compound trunk bark extract is scopoletin. The occurrence of iridoids has been observed for the first time in this genus. A new secoiridoid alkaloid, floribundane A, and a C-9 7,8-secoiridoid, floribundane B was isolated from the leaves of this tree.

Experimental

General experimental procedures

Optical rotations were obtained with a Bellingham+Stanley Ltd ADP 220 polarimeter. HREIMS measurements were carried out on a VG Autospec M and recorded at 70 eV. FTIR and UV spectra were measured in a Unicam Mattson 5000 FTIR and Unicam He λ ios α respectively. NMR spectra were recorded in a Bruker Avance II, 600 MHz (¹H NMR) and 150.9 MHz (¹³C NMR), in CDCl₃. Chemical shifts are given in δ ppm and are referenced to the residual CHCl₃, 7.26 ppm for the ¹H and 77.0 ppm for ¹³C. Two-dimensional experiments were performed with standard Bruker software. Column chromatography was carried out on silica gel (silica gel 60 (70-230 mesh), Merck, Darmstadt, Germany).

Plant material

Leaves and bark of *Hymenodictyon floribundum* were collected in the waterfall at the Comuna da Huíla, Huíla province, Angola in July 2001. A voucher specimen (3668) has been deposit at the Lubango Herbarium, Angola.

Extraction and isolation

Powdered trunk bark (3 kg) was extracted with methanol for a week at room temperature. The methanol extract was partitioned between MeOH-H₂O (5:1) and hexane yielding 16.6 g of the hexane extract. The aq. methanolic fraction was concentrated, H₂O added and extracted with chloroform to obtain the chloroform extract (36.8 g). A sample of the hexane extract (4.7 g) was fractionated in silica gel column with a hexane/EtOAc, EtOAc and EtOAc/MeOH gradients. The fraction eluted with hexane/EtOAc (95:5) was separated in silica gel column with a hexane/EtOAc gradient to yield lupenone (**3**) (20.3 mg). From the chloroform extract and by crystallization scopoletin (**4**) (30 g) was isolated.

Dried leaves (0.9 kg) were extracted with methanol for a week at room temperature. The methanol extract was partitioned between MeOH-H₂O (5:1) and hexane yielding 34.02 g of the hexane extract. The aq. methanolic fraction was concentrated, H₂O added and extracted with chloroform to obtain the chloroform extract (5.2 g). A sample of the chloroform extract (1.6 g) was fractionated in silica gel column with a hexane/EtOAc, EtOAc/CHCl₃ and EtOAc/MeOH gradients. Two fractions were eluted with EtOAc/CHCl₃ (7:3) (fraction I and II). Fraction I was separated in silica gel column with CHCl₃ and a CHCl₃/MeOH gradient to yield floribundane A (**1**) (10.2 mg) and 4,5-dihydroblumenol A (**5**) (5.6 mg). Fraction II was separated in silica gel column with CHCl₃ and a CHCl₃/MeOH gradient to yield floribundane B (**2**) (7.8 mg).

Floribundane A (**1**)

Oil; $[\alpha]_D^{20} = +25.31^\circ$ (CHCl₃, *c* 0.079). FTIR (film) $\nu_{\max}/\text{cm}^{-1}$: 3403, 2959, 2930, 1724, 1690, 1633, 1379, 1268, 1196, 1092, 943, 756; UV (MeOH) λ_{\max}/nm (log ϵ): 234 (4.00), 210.0 (4.15) ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150.9 MHz): see Table 1; TOF-MS-EI pos: *m/z* 223 [M]⁺ (15), 206 (77), 190 (100), 164 (62), 163 (12), 162 (11), 149 (28), 146 (22), 134 (11), 122 (23), 121 (82), 120 (13), 93 (13), 83 (13), 79 (10); HREIMS: *m/z* 223.0843 [M]⁺ (calc. for C₁₁H₁₃NO₄, 223.0845).

Floribundane B (**2**)

Oil; $[\alpha]_D^{20} = +41.66^\circ$ (CHCl₃, *c* 0.048). FTIR (film) $\nu_{\max}/\text{cm}^{-1}$: 3384, 2962, 2926, 2866, 1692, 1620, 1262, 1154,

1094, 1060, 1026, 800, 760; UV (MeOH) λ_{\max}/nm (log ϵ): 230 (3.16), 219 (3.34). ¹H NMR (CDCl₃, 600 MHz) and ¹³C NMR (CDCl₃, 150.9 MHz) see Table 1; TOF-MS-EI pos: *m/z* 226 [M]⁺ (1), 168 (25), 137 (11), 135 (12), 128 (19), 126 (39), 125 (31), 124 (34), 123 (38), 122 (11), 121 (24), 111 (100), 110 (29), 109 (73), 108 (12), 107 (22), 99 (15), 98 (17), 97 (33), 96 (17), 95 (72), 93 (17), 91 (14), 85 (81), 84 (11), 83 (11), 83 (32), 82 (19), 81 (40), 79 (17), 77 (12), 71 (39), 69 (46), 67 (20); HREIMS: *m/z* 170.0942 [M]⁺ (calc. for C₉H₁₄O₃, 170.2270).

Supplementary Information

¹H NMR, ¹³C NMR, ¹H-¹H COSY, HSQC, HMBC, and NOESY NMR spectra of compounds **1** and **2** are available free of charge at <http://jbcbs.s bq.org.br>, as PDF file.

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