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 Huila 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 *Hymendictyon 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), scopoletin (4) and 4,5-dihydroblumenol A (5) were isolated from trunk bark and leaves of *Hymendictyon floribundum* B.L. Rob. The structures of all compounds were established from their spectroscopic data.

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 $-OOCC=CHO$.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 $^1$H NMR spectrum of compound (1) (Table 1) displayed signals of two vinylic protons (H-3 $\delta_H$ 7.50, s and H-8 $\delta_H$ 6.85, q, $J = 7.2$), a ABX system [H-6 $\alpha$, $\beta$ $\delta_H$ 2.11, dd, $J = 13.1$ and 2.0; $\delta_H$ 2.06, dd, $J = 13.1$ and 2.1 (AB part) and H-5 $\delta_H$ 3.94, m (X part)] and a vinyl methyl group at $\delta_H$ 2.01 (d, $J = 7.2$); typical signals of a secoiridoid nucleus.¹¹ A few iridoid alkaloids have been isolated from natural sorcees¹²⁻¹⁵ (gardenamidie 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-secoiridoids¹¹,¹⁸ like (7) (Figure 1) and compound (1), showed that the H-3 chemical shift ($\delta_H$ 7.50 ppm), despite the presence of a nitrogen atom in
the molecule, was more compatible with the hemiacetal-secoiridoids (δ_H ca. 7.5 ppm) than with the gardenamide class and mor-1^{17} (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 hemicetals,^{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=C=CHO.^{9} The 13C NMR spectrum (Table 1) showed 11 carbons which were analysed as two methyls (one vinylc and a methyl ester), one methylene, four methines two of them vinylc, and four quaternary carbons (two vinylc 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} 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 oxymethene (H-3) δ_H 4.23 (ddd, J1.1 13.1, J1.9 1.2 Hz) and δ_H 4.42 (ddd, J1.1 11.3, J1.9 5.4 Hz) that presented a 1H – 1H COSY correlation with a diastereotopic methylene (H-4) δ_H 1.83 (m) and δ_H 2.12 (m); a oxyethylene 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, J7.4 1.2, J1.2 7.4 Hz) coupled with a methyl δ_H 1.86 (d, J7.4 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)

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<td>166.36</td>
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<td>11</td>
<td>166.56</td>
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methylenes (two of them oxymethylenes, \( \delta_c 27.51, 36.11, 59.97, 65.35 \)), two methines (one vinylic, \( \delta_c 28.93, 140.67 \)), and two quaternary carbons (one vinylic, \( \delta_c 131.16 \), and the other carbonylic, \( \delta_c 167.38 \)). 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 (\( \delta_c 27.51 \)), an oxymethylene (\( \delta_c 65.35 \)) and a sp\(^2\) carbon (\( \delta_c 167.38 \)) belonging to the \( \alpha,\beta \)-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 \( \delta \)-lactone ring of C-9 iridoid derivatives viteoid II, \(^{20,21}\) 7-hydroxyviteoid II\(^{12}\) 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 \(^1\)H – \(^1\)H COSY, HSQC and HMBC data (Table 1, Figure 2). Several C-9 iridoid and secoiridoid (lactone ring opening) derivatives are known\(^{20-25}\) 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 Unican Helios \( \alpha \) respectively. NMR spectra were recorded in a Bruker Avance II, 600 MHz (\(^1\)H NMR) and 150.9 MHz (\(^13\)C NMR), in CDCl\(_3\). Chemical shifts are given in \( \delta \) ppm and are referenced to the residual CHCl\(_3\), 7.26 ppm for the \(^1\)H and 77.0 ppm for \(^13\)C. 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 deposited 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-H$_2$O (5:1) and hexane yielding 16.6 g of the hexane extract. The aq. methanolic fraction was concentrated, H$_2$O 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-H$_2$O (5:1) and hexane yielding 34.02 g of the hexane extract. The aq. methanolic fraction was concentrated, H$_2$O 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/CHCl$_3$ and EtOAc/MeOH gradients. Two fractions were eluted with EtOAc/CHCl$_3$ (7:3) (fraction I and II). Fraction I was separated in silica gel column with CHCl$_3$ and a CHCl$_3$/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 CHCl$_3$ and a CHCl$_3$/MeOH gradient to yield floribundane B (2) (7.8 mg).

**Floribundane A (1)**

Oil; [α]$_D^{20}$ = +25.31° (CHCl$_3$, c 0.079), FTIR (film) $v_{max}$/cm$^{-1}$: 3403, 2959, 2930, 1724, 1690, 1633, 1379, 1268, 1196, 1092, 943, 756; UV (MeOH) $\lambda_{max}$/nm (log ε): 230 (3.16), 219 (3.34). $^1$H NMR (CDCl$_3$, 600 MHz) and $^{13}$C NMR (CDCl$_3$, 150.9 MHz) see Table 1; TOF-MS-EI pos: m/z 226 [M]+ (1), 162 (25), 150 (11), 138 (12), 136 (12), 135 (11), 123 (23), 121 (23), 120 (13), 119 (3), 118 (3), 108 (13), 80 (10); HREIMS: m/z 223.0843 [M]+ (calc. for C$_7$H$_{14}$O$_2$, 223.0845).

**Floribundane B (2)**

Oil; [α]$_D^{20}$ = +41.66° (CHCl$_3$, c 0.048), FTIR (film) $v_{max}$/cm$^{-1}$: 3384, 2962, 2926, 2866, 1692, 1620, 1262, 1154, 1094, 1060, 1026, 800, 760; UV (MeOH) $\lambda_{max}$/nm (log ε): 230 (3.16), 219 (3.34). $^1$H NMR (CDCl$_3$, 600 MHz) and $^{13}$C NMR (CDCl$_3$, 150.9 MHz) see Table 1; TOF-MS-EI pos: m/z 226 [M]+ (1), 162 (25), 150 (11), 138 (12), 136 (12), 135 (11), 123 (23), 121 (23), 120 (13), 119 (3), 118 (3), 108 (13), 80 (10); HREIMS: m/z 223.0843 [M]+ (calc. for C$_7$H$_{14}$O$_2$, 223.0845).

**Supplementary Information**

$^1$H NMR, $^{13}$C NMR, $^1$H-1H COSY, HSQC, HMBC, and NOESY NMR spectra of compounds 1 and 2 are available free of charge at http://jbcs.sbq.org.br, as PDF file.

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**References**


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