J. Braz. Chem. Soc., Vol. 16, No. 6B, 1382-1386, 2005.
Printed in Brazil - ©2005 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Indole Alkaloid and other Constituents from *Ocotea minarum*

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Dos frutos de *Ocotea minarum* foram isolados um novo alcalóide indólico, triptofol-5-O-β-D-glicopiranosídeo, a cumarina escopoletina e os flavonóides taxifolina, quercetina-7-O-β-D-glicopiranosídeo, eriodictiol-3'-O-β-D-glicopiranosídeo e naringenina-7-O-β-D-glicopiranosídeo. Do cerne foi isolado um novo alquil fenol, 3-(1,4-diidroxipentil)-5-metoxifenol, além de 5-propilresorcinol, *trans*-asarona, lioniresinol, 3-O-β-D-glicopiranosil estigmasterol e estigmasta-4,22-dien-3-ona. Da casca do caule foram obtidos o sesquiterpeno ácido lanceólico (como seu derivado éster metílico após metilação com diazometano) e β-sitosterol. O ácido lanceólico e 5-propilresorcinol estão sendo relatados pela primeira vez como produtos naturais.

From the fruits of *Ocotea minarum* a new indole alkaloid, tryptophol-5-O-β-D-glucopyranoside, was isolated in addition to the coumarin scopoletin and the flavonoids taxifolin, quercetin-7-O-β-D-glucopyranoside, eriodictyol-3'-O-β-D-glucopyranoside and naringenin-7-O-β-D-glucopyranoside. A new alkyl phenol, 3-(1,4-dihydroxypentyl)-5-methoxyphenol, was obtained from the heartwood in addition to 5-propylresorcinol, *trans*-asarone, lyonyresinol, 3-O-β-D-glucopyranosyl stigmasterol and stigmasta-4,22-dien-3-one, whereas from the trunk bark the sesquiterpene lanceolic acid (as its methyl ester derivative after methylation procedures) and β-sitosterol were isolated. 5-propylresorcinol and lanceolic acid are reported for the first time as natural products.

Keywords: *Ocotea minarum*, Lauraceae, indole alkaloid, lanceolic acid, alkyl-phenols

Introduction

In continuation of our program on chemical investigation of Lauraceous plants occurring in the "Pantanal" and "Cerrado" of Mato Grosso do Sul, Brazil, the fruits, heartwood and trunk bark components of *Ocotea minarum* (Meissn.) Mez., an endemic plant which has been found up to now only in the "Cerrado", have been examined.

A previous work on the leaves of a specimen identified as *O. minarum* which was collected in Minas Gerais, Brazil, resulted in the isolation of fourteen aporphinic alkaloids.[1] However, no alkaloids have been detected in the leaves of the specimen investigated in the present work. The great morphological resemblance among the species belonging to the Lauraceae is well known and sometimes this may lead to misidentification of the specimen under investigation.

We describe herein the isolation of an indole alkaloid (**1**), a coumarin (**2**) and four flavonoids (**3** – **6**) from the fruits, a lignan (**7**), two alkyl phenols (**8**, **9**), a aryl propene derivative (**10**) and two steroids (**11**, **12**) from the heartwood and a sesquiterpene (**13**) and a steroid (**14**) from the trunk bark of a specimen of *Ocotea minarum* collected in Campo Grande, MS State.

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Results and Discussion

After a series of partition procedures and a combination of column chromatography on silica gel, gel filtration and reversed phase HPLC separations of the ethanol extract from the fruits, the indole alkaloid tryptophol-5-O-β-D-glucopyranoside (**1**) was isolated, together with the coumarin scopoletin (**2**) and the flavonoids taxifolin (**3**), quercetin-7-O-β-D-glucopyranoside (**4**), eriodictyol-3'-O-β-D-glucopyranoside (**5**) and prunin (naringenin-7-O-β-D-glucopyranoside, **6**).

The positive ESI-mass spectrum of **1** showed a *quasi*-molecular ion at *m/z* 362 $(M + Na)^+$ accordingly to a molecular formula $C_{16}H_{21}NO_7$. Its ^1H NMR spectrum displayed signals attributable to a 1,2,4-trisubstituted aromatic ring (δ 7.21, d, *J* 2.0 Hz; δ 6.85, dd, *J* 8.0 and 2.0 Hz; δ 7.12, d, *J* 8.0 Hz) in addition to a broad singlet at δ 6.96 (1H) (Table 1). In the region of aliphatic hydrogens, a pair of triplets at δ 2.82 (2H, *J* 7.2 Hz) and 3.80 (2H, *J* 7.2

Hz) and signals ascribed to a sugar moiety (δ 4.85 – 3.20) were observed. The sugar moiety was assigned as β-D-glucopyranose on the basis of ^{1}H and ^{13}C NMR data and the *J* value for the anomeric hydrogen at δ 4.85 (d, *J* 7.5 Hz). The presence of a disubstituted indole moiety was suggested by the chemical shifts of the eight sp^2 carbons in the ^{13}C NMR spectrum (Table 1).[2] A downfield signal at δ 152.7 indicated that the O-glucosyl residue was located *para* to the nitrogen atom of the indole nucleus, at the C-5 position. This assumption was also supported by the upfield signals in this spectrum attributed to C-4 and C-6 (δ 106.8 and 114.2, respectively) and the long-range HMBC correlations of H-4, H-6, H-7 and H-1' of the glucosyl moiety with C-5 (Table 1). The presence of a β-hydroxyethyl substituent at C-3 could be inferred by the two methylene carbon signals at δ 29.7 and 62.6 which in turn showed cross-peak correlations with the two aforementioned triplets at δ 2.82 and 3.80, respectively, in the HMQC spectrum. This information was corroborated by the HMBC correlations between the signals of H-10 and that of C-2 and between the signals of H-4 and H-10 and that of C-3. Therefore, compound **1** is thus unambiguously shown to be the 5-O-glucosyl derivative of tryptophol (indole-3-ethanol). Tryptophol has been isolated as a constituent of higher plants, fungi and marine organisms and is considered to be a plant auxin,[3] however its 5-O-glucosyl derivative **1** was hitherto unreported in the literature.

Table 1. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data for **1** (δ, CD_3OD)

C/H	δ H[a]	δ C
	6.96 s	124.7
3	_	112.8
4	7.21 d (2.0)	106.8
5	_	152.7
6	6.85 dd (8.0, 2.0)	114.2
7	7.12 d (8.0)	112.5
8	_	134.5
9	_	124.2
10	2.82 t (7.2)	29.7
11	3.80 t (7.2)	62.6
1'	4.85 d (7.5)	104.2
2'	3.47[b]	75.1
3'	3.45[b]	78.1
4'	3.41[b]	71.6
5'	3.28[b]	78.1
6'	3.49[b]3.64[b]	63.7

[a] Coupling constants (*J* in Hz) are given in parentheses; [b] Overlapped signals, obtained from HMQC data.

Column chromatography on silica gel of a crude ethanol extract obtained from the heartwood of *Ocotea minarum* followed by Sephadex LH-20 separations afforded the lignan lyonyresinol (**7**), the alkyl phenols 5-propylresorcinol (**8**) and 3-(1,4-dihydroxypentyl)-5-methoxyphenol (**9**), the aryl propene derivative *trans*-asarone (**10**) and the steroids 3-O-β-D-glucopyranosyl stigmasterol (**11**) and stigmasta-4,22-dien-3-one (**12**).

The ^{1}H NMR spectrum of **8** showed characteristic signals of a resorcinol derivative (two broad singlets at δ 6.28 and 6.16, the latter integrating for two hydrogens) containing an *n*-propyl group at C-5 (two triplets at δ 0.83, *J* 6.6 Hz and 2.39, *J* 7.5 Hz and one multiplet at δ 1.50). The signal of the phenol hydrogens, which disappeared after addition of D_2O, was observed at δ 5.86. Accordingly, in the ^{13}C NMR spectrum of **8**, four signals at δ 146.1, 107.9, 156.7 and 100.2 accounted for a symmetrically substituted resorcinol ring, while resonances of two methylene and one methyl carbons at δ 32.0, 36.0 and 14.1, respectively, were indicative of an *n*-propyl substituent at C-5. Compound **8** was thus identified as 5-propylresorcinol, which has been previously detected as one of the constituents of the secretion of the ant *Crematogaster deformis* [4] and not reported in higher plants before now.

The molecular formula of **9** was deduced as $C_{12}H_{18}O_4$ on the basis of the *quasi*-molecular ion peak at *m/z* 249 $[M+Na]^+$ observed in its positive ESI-mass spectrum and information provided by its ^{1}H and ^{13}C (including DEPT) NMR spectra. In the ^{1}H NMR spectrum of **9**, the presence of an 1,3,5-trisubstituted aromatic ring was inferred by the three broad singlets at δ 6.41, 6.38 and 6.24 (Table 2). This spectrum also showed signals attributable to one methoxyl (δ 3.73) and an aliphatic chain bearing two carbinolic hydrogens, as revealed by a triplet at δ 4.48 (1H, *J* 7.1 Hz) and a multiplet partly obscured by the OMe singlet (δ 3.73). In the HMQC spectrum, cross-peak correlations between the signals of these carbinolic hydrogens and carbon resonances at δ 75.2 and 68.5, respectively,

Table 2. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data for **9** (δ, $CDCl_3$)

C/H	δ H[a]	δ C
1	-	159.5
2	6.41 br s	101.0
3	-	148.9
4	6.38 br s	104.0
5	-	162.3
6	6.24 br s	106.6
1'	4.48 t (7.1)	75.2
2'	1.72 m	36.30
3'	1.31 m	36.33
4'	3.73 m	68.5
5'	1.22 d (6.2)	23.5
6'	3.73 s	55.6

[a] Coupling constants (*J* in Hz) are given in parentheses.

confirmed these assignments. The ^{13}C NMR spectrum of **9** displayed then signals for twelve carbons, six of which could be attributed to the aromatic carbons, one to the OMe group and the five remaining to the aliphatic carbons of the aromatic ring side-chain (Table 2). The chemical shift values of δ 162.3 and 159.5 were consistent with the presence of a methoxyl and a hydroxyl respectively, in a *meta* orientation with respect to each other in the 1,3,5-trisubstituted aromatic ring, while the signal at δ 148.9 was ascribed to the aromatic carbon bearing the alkyl chain. One of the methine carbons of this side-chain was shown to be directly attached to the aromatic ring on the basis of the correlations observed in the HMBC spectrum between its corresponding carbinol hydrogen (δ 4.48) and the aromatic carbons C-2, C-3 and C-4. Likewise, the location of the second hydroxyl group at C-4' was inferred by the appearance of the C-5' methyl as a doublet at δ 1.22 (J = 6.2 Hz), which in turn showed one-bond ^{1}H-^{13}C connectivity with the carbon signal at δ 23.5 (C-5') and by the long-range correlations of the carbinolic hydrogen H-4' with C-2', C-3' and C-5'. The structure of **9**, which is being described for the first time in the literature, was thus established as 3-(1,4-dihydroxypentyl)-5-methoxyphenol.

Table 3. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data for **13a** (δ, $CDCl_3$)

C/H	δ H[a]	δ C
1	2.04 m	33.5
2	5.35 m	120.5
3	-	134.4
4		31.3
5	1.94 m	30.6
6		39.6
7	-	153.0
8	2.13 m	28.2
8	2.04 m	33.5
9	2.26 m	27.2
10	6.71 m	142.2
11	-	127.5
12	-	168.6
13	1.70 s	12.3
14	4.70 (br s)	107.7
	4.75 (br s)	
15	1.55 s	23.3
OMe	3.68 s	51.6

A crude ethanol extract of the trunk bark of *O. minarum* was partitioned between methanol-H_2O (9:1) and hexane. Sesquiterpene **13** (Lanceolic acid) and β-sitosterol (**14**) were obtained from the hexane soluble fraction after column chromatography on silica gel. Compound **13** was isolated and characterized after methylation with diazomethane as its methyl ester derivative **13a**. The ^{1}H NMR spectrum of **13a** showed, in addition to a singlet at δ 3.68 assignable to a carbomethoxy group, signals for two vinylic hydrogens (two multiplets at δ 5.35 and 6.71), an exocyclic methylene group (two broad singlets at δ 4.70 and 4.75) and two olefinic methyl groups at δ 1.55 and 1.70 (Table 3). The ^{13}C NMR data (including DEPT) of **13a** resembled closely those of the sesquiterpene β-bisabolene,[5] except for the deshielding and shielding shifts of the C-10 and C-11 resonances, respectively, caused by the presence of a carbomethoxy function in **13a** in place of the C-12 methyl group (Table 3). The stereochemical assignments for the 10,11-trisubstituted double bond in **13a** were confirmed as (E) on the basis of the proton and carbon resonances of methyl-13 (δ 1.70 and 12.3, respectively), which were indicative of its *cis* relationship with C-9.[6] Further evidence for the structure **13a** was provided by two- and three-bond correlations discernible in the HMBC spectrum (Table 3). Since the optical rotation of **13a** showed a negative value, the (S) stereochemistry at C-6 is proposed, similarly to other analogous (S)-(E)-bisabolene derivatives.[7,8] Therefore, compound **13a** was characterized as the methyl ester of lanceolic acid **13**. These two compounds were previously obtained by synthesis and only incomplete ^{1}H NMR assignments have been published for **13a**.[7,9] Therefore, **13** is reported for the first time as a genuine natural product.

The structures of the known compounds **2** - **7**, **10** - **12** and **14** were identified by comparison of their ^{1}H and ^{13}C NMR data with those found in the literature and/or with authentic samples.[10]

The isolation of **1** from *Ocotea minarum* is noteworthy for its chemosystematic relevance, since members of the Lauraceae have been reported to contain aporphine and benzyltetrahydroisoquinoline but not indole alkaloids.[11] It is also worth of mention in this work the compartmentalized accumulation of the secondary metabolites present in *O. minarum*: the indole alkaloid and the flavonoid derivatives are accumulated in the fruits, whereas the alkyl phenols, the aryl propene derivative and the lignan were isolated from the heartwood and the sesquiterpene from the trunk bark.

Experimental

General experimental procedures

IR spectra were recorded as KBr pellets on a Bomem-Hartmann & Braun FT IR spectrometer. The uni-dimensional ^{1}H and ^{13}C and the two dimensional ^{1}H-^{1}H COSY, HMQC and HMBC NMR spectra were recorded at 300 MHz (^{1}H) and 75 MHz (^{13}C) on a Bruker DPX-300 spectrometer. Standard pulse sequences were used for

homo- and heteronuclear correlation experiments. ESIMS spectra were obtained using a Micromass Platform II single quadrupole mass spectrometer (Faculdade de Ciências Farmacêuticas, USP, Ribeirão Preto, SP, Brazil). Optical rotations were determined on a Perkin-Elmer 341 polarimeter. Silica gel 60 (70-230 and 230-400 mesh) and Sephadex LH-20 were used for column chromatography. Reversed phase semi-preparative HPLC separations were performed with a Shimadzu LC-6AD pump, using a RP-18, 25x250 mm, 5μm particle size, Shim-Pack PREP-ODS(H) column, with a flow rate of 10 mL min^{-1} and monitoring at 254 nm.

Plant material

Fruits, heartwood and trunk bark of *Ocotea minarum* (Meissn.) Mez. were collected in Campo Grande, Mato Grosso do Sul, Brazil, in December 2001. The plant material was identified by Dr. João Batista Baitello (Horto Florestal – São Paulo, SP, Brazil) and a voucher specimen (No. 11467) was deposited at the CGMS Herbarium, Universidade Federal de Mato Grosso do Sul, MS, Brazil.

Extraction and isolation of chemical constituents

Ground fruits (2.5 kg) were extracted at room temperature with EtOH. The residue obtained from the EtOH extract was partitioned between hexane-CH_3CN-$CHCl_3$-H_2O (20:34:10:10) originating two phases: the organic upper layer and the hydro-organic lower layer. The first contained only fatty material which was not further investigated. The hydro-organic phase was concentrated *in vacuo* and the residue extracted with EtOAc and then with *n*-BuOH. The EtOAc extract (6.5 g) was subjected to CC on silica gel (230-400 mesh, 50 g, $CHCl_3$-MeOH gradient) to afford **2** (3.8 mg) and **3** (16.9 mg). The *n*-BuOH extract (6.4 g) was chromatographed on a Sephadex LH-20 column (30 g, four portions of 1.6 g each, MeOH) to provide fifty fractions of 5 mL each. The fractions showing similar spots by TLC were combined to give eight fractions (A→H). Fraction E was further subjected to CC on Sephadex LH-20 (30 g, MeOH) to yield fifty fractions of 5 mL each. From these, fractions 8-14 consisted of **1** (25.0 mg), fractions 19-27 yielded **6** (4.5 mg) after semi-preparative HPLC (MeOH-H_2O 1:1), fractions 38-42 afforded **4** (9.8 mg) and fractions 47-49 gave **5** (5.3 mg).

Air dried and powdered heartwood (900 g) was extracted at room temperature with EtOH. After concentration *in vacuo*, the residue (29.0 g) was applied to a silica gel CC (70-230 mesh, 100 g, hexane-EtOAc and EtOAc-MeOH gradients) to give nine fractions (A→I) of 100 mL each. Fraction D (683 mg) afforded **10** (6.4 mg) and **11** (8.3 mg) after CC on silica gel (230-400 mesh, 50 g, hexane-EtOAc 7:3), followed by a second CC (silica gel 230-400 mesh, 30 g, $CHCl_3$-CH_2Cl_2 99.5:0.5). Fractions E-G (1.3 g) were subjected to CC on Sephadex LH 20 (50 g, $CHCl_3$-MeOH 4:1) to provide forty-two fractions of 10 mL each. Fractions 4-7 were rechromatographed on a silica gel column (230-400 mesh, 50 g, $CHCl_3$-MeOH 1:1) to yield **8** (7.3 mg) and **9** (5.3 mg). Fraction H (900 mg) was again separated by CC on Sephadex LH-20 (30 g, EtOAc-MeOH 1:1) to give **7** (11.5 mg) and **12** (10.6 mg).

Air-dried and powdered trunk bark (2.7 kg) were extracted at room temperature with EtOH. After concentration *in vacuo*, the residue was partitioned between MeOH-H_2O 9:1 and hexane. The hexane phase (5.0 g) was separated on a silica gel column (230-400 mesh, 45 g) eluted with a gradient of hexane-EtOAc 8:2 and hexane-EtOAc-MeOH 9:1:0.2, 8:2:1 and 7:3:1 yielding one hundred fractions of 10 mL each. Fraction 20 consisted of **14** (465.0 mg). Fraction 8 (600 mg) consisted of a complex mixture from which **13** was isolated as the corresponding methyl ester derivative **13a** (10 mg) after treatment of part of this fraction (300 mg) with an ethereal solution of diazomethane followed by successive CC separations on silica gel (230-400 mesh, 50 g, hexane-EtOAc gradient).

Tryptophol-5-O-β-D-glucopyranoside (***1***). Colorless amorphous solid. [α]: - 29.3° (MeOH; *c* 0.3). IR (KBr) ν_{max}/ cm^{-1}: 3400, 2927, 1628, 1482, 1202, 1074, 1041, 803. UV λ_{max}/nm (log ε) (MeOH): 225 (2.7), 280 (sh). ESIMS *m/z* 362 [M+Na]$^+$. ^{1}H and ^{13}C NMR: see Table 1.

5-propylresorcinol (**8**). Brownish oil. ^{1}H NMR ($CDCl_3$): δ 0.83 (3H, t, *J* 6.6 Hz, H-3'), 1.50 (2H,m, H-2'), 2.39 (2H, t, *J* 7.5 Hz, H-1'), 5.86 (2H, br s, 2 x OH), 6.16 (2H, br s, H-2, H-4), 6.28 (1H, br s, H-2).^{13}C NMR ($CDCl_3$): δ 14.1 (C-3'), 32.0 (C-2'), 36.0 (C-1'), 100.2 (C-4), 107.9 (C-2, 6), 146.1 (C-1), 156.7 (C-3, 5).

3-(1,4-dihydroxypentyl)-5-methoxyphenol (***9***). Colorless amorphous solid. [α]: - 10.0° (MeOH; *c* 0.17). IR (KBr) ν_{max}/ cm^{-1}: 3401, 2926, 2854, 1598, 1456, 1437, 1337, 1301, 1154, 1054, 840, 699. ESIMS *m/z* 249 [M+Na]$^+$. ^{1}H and ^{13}C NMR: see Table 2.

Methyl lanceolate (***13a***). Colorless oil. [a]: - 58.0° (MeOH; *c* 0.25). IR (KBr) ν_{max}/ cm^{-1}: 1720, 1652, 1135, 891. ^{1}H and ^{13}C NMR: see Table 3.

Acknowledgments

The authors are grateful to Fundect-MS, CPq-PROPP-UFMS and PROAP-CAPES for their financial support and to CAPES and CNPq for the scholarships awards. Thanks are also due to Dr. Norberto P. Lopes (Faculdade de Ciências Farmacêuticas, USP, Ribeirão Preto, Brazil) for the ESIMS spectra.

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Received: June 8, 2005
Published on the web: October 11, 2005