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Article

Limonoids from *Spiranthera odoratissima* St. Hil

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Onze substâncias foram isoladas das raízes de *Spiranthera odoratissima*: dois novos limonóides, o limonóide já conhecido limonina, três alcalóides furoquinolínicos (dictamina, γ-fagarina e esquimianina), três alcalóides β-indoloquinazolínicos (rutaecarpina, evodiamina e 1-hidroxirutaecarpina), a cumarina aurapteno e β-sitosterol. A elucidação estrutural dessas substâncias foi realizada através de técnicas espectrais como IV e RMN em uma e duas dimensões; as estruturas novas foram confirmadas por difração de raios-X.

Eleven substances have been isolated from the roots of *Spiranthera odoratissima*, two new limonoids, the known limonoid limonin, three furoquinoline alkaloids, dictamnine, γ-fagarine and skimmianine, three β-indoloquinazoline alkaloids, rutaecarpine, evodiamine and 1-hydroxyrutaecarpine, the coumarin aurapten and β-sitosterol. Structure elucidation has been carried out by IR as well as 1D and 2D NMR; the new structures were also confirmed by X-ray crystallographic analyses.

Keywords: *Spiranthera odoratissima*, Rutaceae, limonoids, alkaloids, X-ray diffraction

Introduction

Spiranthera odoratissma St. Hil., is a shrub found in the central Brazilian savannah, as well as in Bolivia.[1] In Mato Grosso state it is known by the vernacular name of "manacá", being used in folk medicine to treat syphilis, rheumatism, kidney infections, urinary retention, abdominal pains, gout, acne and boil.[2] This plant has already been investigated from chemistry viewpoint. From a specimen collected in Bahia were isolated furoquinoline alkaloids; coumarins and terpenes.[3] The Rutaceae family is characterized by the abundance of anthranilic acid derived alkaloids coumarins, limonoids and flavonoids mainly.[4]

We have been interested in the chemistry of Rutaceae,[5-8] and as part of ongoing work on this family, in this study we describe the isolation and the identification of two new limonoids, as well as the known limonin. Also described are the isolation and identification of the furoquinoline alkaloids γ-fagarine dictamnine, skimmianine, the β-indoloquinazoline alkaloids rutaecarpine, evodiamine and 1-hydroxy-rutaecarpine, the coumarin aurapten and β-sitosterol.

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Experimental

General experimental procedures

Melting points were measured in a Mettler FP-80 apparatus and are uncorrected. Specific rotations were determined in a Perkin-Elmer 341 polarimeter. The IR spectra were obtained in a Bomem FT-IR MB100 equipment with the samples in KBr pellets. ^{1}H and ^{13}C NMR spectra were measured in Bruker AC-200 (200 MHz), ARX-400 (400 MHz) and Varian Mercury-300 (300 MHz) apparatus. The chemical shifts (δ) are expressed in ppm and the coupling constants (J) in Hertz; TMS was used as

internal standard, as well as the residual hydrogen from the solvents ($CDCl_3$ and DMSO-d_6). Radial preparative chromatography (RPC) was carried out with a Chromatotron apparatus and were performed with Merck Kiesegel 60 PF254. Column chromatography (CC) was performed with silica gel 60 (Merck, 63-230 μm), and flash column chromatography with silica (Merck, 43-63 μm). Analytical thin layer chromatography (TLC) was carried out with Merck Kiesegel 60 F254 (0.25 mm) plates.

Plant material

The roots from *S. odoratissima* were collected at Cuiabá-Barão de Melgaço road (Km 1) in December 1999. A voucher specimen was deposited at Central Herbarium of Universidade Federal de Mato Grosso (registration # 24246).

Extraction and isolation of compounds

The roots of *S. odoratissima* (3.3 kg) were macerated at room temperature with dichloromethane (3 x 8L), with occasional stirring, during 7 days. The dichloromethane extract-DCE (126 g; 3.82%) was obtained after filtration and solvent removal i*n vacuo*. Subsequent extraction was performed with methanol (3 x 8L) using the same procedure above, affording the methanol extract-ME (120 g; 3.64%).

An aliquot of DCE (20.0 g) was submitted to conventional acid-base extraction (HCl 1%; 4 x 100 mL) and the alkaloid fraction (190 mg) was chromatographed by RPC, in a 4 mm radial plate, with hexane-$CHCl_3$ (8:2; 100 mL); $CHCl_3$ (250 mL) and $CHCl_3$-MeOH (95:5; 100 mL), affording 6 fractions. The furoquinoline alkaloids γ-fagarine (15 mg), dictamnine (8 mg) and skimmianine (30 mg) were isolated after washing fractions 2, 3 and 4 with Et_2O.

Another DCE fraction (42.5 g) was filtered in a silica column (400 g) with petrol ether (0.5 L), CH_2Cl_2 (2 L), $CHCl_3$ (1 L), $CHCl_3$-MeOH (99:1; 2 L), $CHCl_3$-MeOH (9:1; 1 L), MeOH (1 L) and MeOH-0.1%HOAc (0.5 L), affording fractions A to G, respectively.

Column chromatography (CC) was carried out on fraction B (23.0 g) employing the gradient solvent system: hexane-CH_2Cl_2 (1:1; 450 mL), CH_2Cl_2 (200 mL), CH_2Cl_2-MeOH (99:1; 200 mL), (95:5; 200 mL), (9:1; 600 mL) and MeOH (200 mL), affording 8 fractions after TLC analysis. Fraction 2 [from hexane-CH_2Cl_2 (1:1)] was chromatographed by RPC (hexane- CH_2Cl_2; 9:1; 7:3; 1:1), CH_2Cl_2 and $CHCl_3$-MeOH (99:1), affording the coumarin aurapten (50 mg), after washing the crude hexane-CH_2Cl_2 (7:3) fraction with EtOH. Fraction 4 [from hexane-CH_2Cl_2 (99:1)] was submitted to medium pressure chromatography using the gradient solvent system: hexane-CH_2Cl_2 (2:8; 300 mL), (1:9; 70 mL), CH_2Cl_2 (150 mL), CH_2Cl_2-MeOH (9:1; 250 mL). Aurapten (109 mg) was obtained after washing with Et_2O the combined hexane-CH_2Cl_2 (2:8) fractions; β-sitosterol was obtained after washing the combined hexane-CH_2Cl_2 (1:9) fractions with EtOH; **1** (30 mg) was obtained after washing the combined CH_2Cl_2-MeOH (95:5 and 9:1) fractions with EtOH.

Fraction D (10 g) was submitted to medium pressure chromatography using the solvent system: CH_2Cl_2 (300 mL), CH_2Cl_2-CH_3CN (98:2; 200 mL), (95:5; 300 mL), (7:3; 200 mL); (1:1; 300 mL) and MeOH (200 mL). The combined CH_2Cl_2-CH_3CN (98:2 and 95:5) fractions (2.5 g) were re-submitted to medium pressure chromatography employing the gradient hexane-$CHCl_3$ (1:9; 400 mL), $CHCl_3$ (200 mL), $CHCl_3$-MeOH (95:5; 300 mL), (9:1; 300 mL), (1:1; 200 mL) and MeOH (200 mL). Limonin (**3**) (130 mg) precipitated after EtOH addition to the combined hexane-$CHCl_3$ (1:1) and $CHCl_3$ fractions. The supernatant liquid was chromatographed by CC in hexane-$CHCl_3$ (2:8; 270 mL), $CHCl_3$ (50 mL), $CHCl_3$-CH_3CN (8:2; 200 mL), (6:4; 150 mL), (3:7; 100 mL), CH_3CN (50 mL), CH_3CN-EtOH (7:3; 100 mL) and (1:1; 150 mL). Skimmianine (30 mg) was obtained from the combined hexane-$CHCl_3$ (2:8) and $CHCl_3$ fractions after solvent removal and washing the solid with EtOH. The combined $CHCl_3$-MeOH (95:5) fractions were chromatographed by CC using CH_2Cl_2 (50 mL), CH_2Cl_2-EtOAc (8:2; 300 mL), (7:3; 300 mL), (3:7; 200 mL), EtOAc (200 mL), EtOAc-MeOH (8:2; 200 mL) and MeOH (100 mL). The combined CH_2Cl_2-EtOAc (8:2) and (7:3) fractions were submitted to RPC with $CHCl_3$, $CHCl_3$-MeOH (95:5), (9:1) and EtOAc-MeOH (9:1). Limonin (**3**) (60 mg) was obtained after solvent removal from fraction $CHCl_3$-MeOH (9:1).

ME (30 g) was suspended in MeOH-H_2O (7:3; 100 mL) and extracted with CH_2Cl_2 (3 x 100 mL), EtOAc (3 x 100 mL) and n-BuOH (3 x 100 mL). The CH_2Cl_2 fraction (2.7 g) was submitted to CC in CH_2Cl_2 (1.7 L), CH_2Cl_2-MeOH (99:1; 2.2 L), (98:2; 0.8 L), (95:5; 1.5 L); (9:1; 0.7 L), (7:3; 0.6 L), (1:1; 0.8 L) and MeOH (0.9 L). The combined CH_2Cl_2-MeOH (99:1) fractions (550 mg) were submitted to CC in CH_2Cl_2 (300 mL), CH_2Cl_2-MeOH (99:1; 150 mL), (97:3; 50 mL), (95:5; 350 mL), (9:1; 150 mL), (1:1; 50 mL) and MeOH (50 mL). A precipitate (80 mg) was obtained by adding EtOH to the combined CH_2Cl_2 fractions. Flash CC was performed with this precipitate, employing CH_2Cl_2 (150 mL), CH_2Cl_2-MeOH (99:1; 100 mL), (95:5; 100 mL), (1:1; 50 mL) and MeOH (50 mL). Rutaecarpine (**4**) (7 mg) was isolated after solvent removal from the combined CH_2Cl_2 fractions; evodiamine (**5**) (5 mg) was isolated after solvent removal from the combined CH_2Cl_2-MeOH (99:1) fractions. The combined CH_2Cl_2-

MeOH (95:5 and 1:1) fractions were submitted to flash CC in CH_2Cl_2 (40 mL) and CH_2Cl_2-MeOH (99:1; 30 mL). 1-hydroxyrutaecarpine (**6**) (4 mg) and **2** (7 mg) were isolated from the second CH_2Cl_2 fraction and the third CH_2Cl_2-MeOH (99:1), respectively.

*Compound (**1**)*

$[\alpha]^{25}$ ($CHCl_3$, *c*. 0.1): -26 °C. IR (KBr) ν_{max}/ cm^{-1}: 3405, 1767, 1741, 1714 . ^1H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2.

*Compound (**2**)*

$[\alpha]^{25}$ ($CHCl_3$, *c*. 0.05): -20 °C. IR (KBr) ν_{max}/ cm^{-1}: 1766, 1742, 1707. ^1H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2.

Single crystal X-ray analysis

Low temperature X-ray diffraction data collections were performed at 120(2) K, on an Enraf-Nonius Kappa-CCD diffractometer equipped with an Oxford Cryosystem liquid N_2 device, using graphite-monochromated MoK α radiation (0.71073 Å). Data were collected up to 50° in 2θ, with a redundancy of 4 in the phi scans and omega scans with kappa offsets modes. The final unit cell parameters were based on all reflections. Data collections were made using the *COLLECT* program;[9] integration and scaling of the reflections were performed with the HKL Denzo-Scalepack system of programs.[10] No absorption corrections were applied.

The structures were solved by direct methods with *SHELXS 86*[11] and *SHELXS-97*.[12] The models were refined by full-matrix least squares on F^2 with *SHELXL-97*.[13] All the hydrogen atoms were stereochemically positioned and refined with the riding model.[12] Hydrogen atoms of the CH and CH_2 groups were set isotropic with a thermal parameter 20% greater than the equivalent isotropic displacement parameter of the atom to which each one was bonded. This percentage was set to 50% for the hydrogen atoms of the CH_3 groups. Data collections and experimental details for the complexes are summarized in Table 3. The programs *SHELXL-97*,[13] and *ORTEP-3*[14] were used within WinGX[15] to prepare materials for publication. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (see below).

Results and Discussion

Limonoid (**1**) has been isolated from the dichloromethane extract from the roots of *S. odoratissima* as a white amorphous solid (mp 174-177 °C), presenting $[\alpha]_D^{25}$ ($CHCl_3$): – 26°. The IR spectrum has shown absorptions at 3405 (OH), 1767 (lactone), 1741 (α,β-unsaturated ester) and 1714 (ketone).

^1H NMR spectrum presented signals related to the β-substituted furan ring, where H-21 and H-23 appeared as multiplets at δ 7.41 and at δ 7.36, respectively. The β-furan hydrogen H-22 appeared as a double doublet at δ 6.37 (*J*= 1.7, 0.8 Hz). The hydrogen H-17, from the δ-epoxylactone ring, was observed at δ 5.54 (s, 1H).

The ^1H and ^{13}C NMR spectra from (**1**) has shown similarities with the correlated spectra of (**3**), also isolated in this study, as shown in Tables 1 and 2. The absence of two doublets, associated to the geminal hydrogens at C-19, very common in structures related to (**3**), displaying a A,D-*seco* ring,[16] when linked with the low intensity, quaternary carbon signal observed at δ 182.5, from the DEPT experiment, was a good indication for the presence of a carbonyl group at C-19 in **1**. HMBC spectrum has shown long distance coupling (J^3) between H-1, H-9 and C-19, confirming that C-19 in **1** is oxidized.

Table 1. ^1H NMR spectral data for compounds **1-3**

H	**1**[b]	**2**[c]	**3**[c]
1	6.65 d (12.4)	6.64 d (12.5)	4.03 sl
2	6.12 d (12.4)	6.14 d (12.5)	2.66 dd (16.8, 1.8)
2	_	_	2.98 dd (16.8, 3.8)
5	3.09 dd (10.4, 2.5)	3.0 brd (9.8)	2.23 dd (15.8, 3.2)
6ax	3.24 dd (18.4, 10.4)	3.40 dd (18.6, 9.8)	2.85 d (15.8)
6eq	2.74 dd, (18.4, 2.5)	2.73 dd (18.6 e 1.2)	2.46 dd (15.8, 3.2)
9	3.60 s	3.76 s	2.56 m
11	4.51 brd (6.7)	5.66 m	1.81 m
12ax	1.83 d (15.0)	_	_
12eq	1.61 m	1.65 m	1.50 m
15	4.17 s	4.20 s	4.03 s
17	5.54 s	5.41 s	5.47 s
18	1.02 s	1,06 s	*1.17 s
19	_	_	4.46/4.47 d (13.0)
21	7.41 m	7.39 m	7.40 m
22	6.37 dd (1.7, 0.8)	6.34 m	6.35 m
23	7.36 m	7.39 m	7.40 m
28	1.49 s	1.44 s	1.63 s
29	1.44 s	1.36 s	1.17 s
30	1.16 s	1.16 s	*1.07 s
31	3.71 s	3.72 s	_
33	_	2.10 s	_

[a] Chemical shifts are δ values, coupling constants (*J* in parentheses) are given in Hz., in $CDCl_3$; [b] Measured at 400 MHz; [c] Measured at 200 MHz; * Interchangeable

The α,β-unsaturated ester moiety, detected by the IR spectrum, was further confirmed by ^1H NMR (δ 3.71, s, 3H; $-CO_2C\underline{H}_3$), as well as by the signals at δ 165.5 ($-\underline{C}O_2CH_3$) and δ 52.3 ($-CO_2\underline{C}H_3$) in ^{13}C NMR. The olefinic hydrogens H-1 and H-2 were detected as two doublets at δ 6.65 and δ 6.12 respectively; the *cis* configuration of the double bond could be determined by the coupling constant value ($J_{1,2}$ = 12.4 Hz). HSQC spectrum showed correlation between these two protons and the carbons at δ 151.6 (C-1) and δ 123.2 (C-2), respectively.

Table 2. ^{13}C NMR spectral data[a] for compounds **1-3**

C	1	2	3
1	151.6	152.1	79.1
2	123.2	123.1	35.6
3	165.5	165.9	169.1
4	87.6	84.9	80.2
5	49.9	49.8	60.4
6	38.1	38.6	36.3
7	207.3	208.2	206.1
8	48.5	48.6	51.3
9	43.9	42.5	48.1
10	53.6	52.3	45.9
11	66.4	66.5	18.8
12	41.2	40.9	30.1
13	36.5	36.3	37.9
14	65.1	65.6	65.6
15	52.6	52.2	53.8
16	166.9	166.6	167.0
17	77.8	77.6	77.7
18	19.2	19.3	21.3
19	182.5	178.2	65.3
20	120.1	119.8	119.9
21	141.1	141.1	141.1
22	109.9	109.8	109.8
23	142.9	143.1	143.2
28	24.4	24.3	*30.8
29	32.4	32.8	*20.7
30	16.7	16.2	17.6
31	52.3	52.0	_
32	_	170.5	_
33	_	21.2	_

[a] Chemical shifts in δ from TMS taken in $CDCl_3$ measured at 50 MHz; * Interchangeable.

The DEPT ^{13}C NMR spectrum presented two signals related to methylenic carbons at δ 38.1 (C-6) and δ 41.2 (C-12). HMBC experiment has shown H-6 correlation with C-7 (δ 207.3; J^2) and C-4 (δ 87.6; J^3). The methylenic hydrogen H-6 resonated at δ 3.24 (dd, J= 18.4, 10.4 Hz, H-6_{ax}) and δ 2.74 (dd, J= 18.4, 2.5 Hz, H-6_{eq}); COSY spectrum has shown coupling of both hydrogen with H-5 at δ 3.09 (dd, J= 10.4, 2.5 Hz).

Reasonably similar values have been observed when comparing the ^{13}C NMR data for rings B and D in compounds (**1**) and (**3**) (Table 2). DEPT experiment, however, presented three signals related to methylenic carbons in (**3**) and only two signals related to methylenic carbons in (**1**). The hydroxyl group, shown to be present in (**1**) by the IR spectrum, nevertheless, should be located at C-11 or C-12 in ring C. Furthermore, the carbinolic hydrogen appeared as a broad doublet at δ 4.51 (J= 6.7 Hz, 1H), which has been associated to δ 66.4 signal (HSQC), indicating that H-11 couples only with one H-12_{eq}. No coupling was observed between H-11 and H-9 and H-12_{ax}. The HMBC spectrum showed a long distance coupling (J^3) with C-8 (δ 48.5), confirming that the hydroxylated carbon is C-11. The α stereochemistry of the OH group was determined by X-ray crystallography (Figure 2). The H-12 methylenic hydrogens were observed at δ 1.83 (d, J= 15 Hz, H-12_{ax}) and δ 1.61 (m, H-12_{eq}). HMBC showed long range coupling (J^3) between H-12_{ax}

Table 3. Crystal data and structure refinement.

Compound	(**1**)	(**2**)
Empirical formula	$C_{27}H_{30}O_{10}$	$C_{29}H_{32}O_{11}$
Formula weight	514.51	556.55
Temperature	120(2) K	120(2) K
Crystal system	Orthorhombic	Tetragonal
Space group	$P2_12_12_1$	P43
Unit cell dimensions a =	7.7720(2) Å	12.2500(4) Å
b =	13.7450(3) Å	12.2500(4) Å
c =	22.4540(6) Å	17.2440(6) Å
Volume	2398.7(1) $Å^3$	2587.7(2) $Å^3$
Z	4	4
Density (calculated)	1.425 mg/m^3	1.429 mg/m^3
Absorption coefficient	0.109 mm^{-1}	0.110 mm^{-1}
F(000)	1088	1176
Crystal Color	Colorless	Colorless
Crystal size	0.24x0.20x0.04 mm^3	0.20x0.04x 0.03 mm^3
Index ranges	-9,9; -16.16, -26.26	-14;14; -10,10; -17,20
Reflections collected	21468	18726
Independent reflections	4225 [R(int) = 0.051]	4250 [R(int) = 0.069]
Reflections with I>2σ(I)	3778	3709
Completeness to θ = 25.00°	99.7%	99.7%
Data / restraints / parameters	4225 / 0 / 341	4250 / 1 / 368
Goodness-of-fit on F^2	1.134	1.039
Final R indices [I>2σ(I)]	R1=0.0431, wR2=0.1192	R1=0.0463, wR2=0.1163
R indices (all data)	R1=0.0507,wR2=0.1239	R1=0.0570, wR2=0.1246
Extinction coefficient	0.030(2)	0.008(2)
Largest diff. peak and hole	0.364 and -0.201 $e.Å^{-3}$	0.247 and -0.365 $e.Å^{-3}$

with C-9 (δ 43.9), C-14 (δ 65.1), C-17 (δ 77.8) and C-18 (δ 19.2).

Four singlets (3H) relative to the methyl groups, were observed at δ 1.02 (H-18), δ 1.16 (H-30), δ 1.44 (H-29) and δ 1.49 (H-28). The correct attribution of each methyl group position was carried out by using HMBC and HSQC experiments. The long distance correlation of δ 1.02 (Me-18) with C-12 (δ 41.2), C-13 (δ 36.5), C-14 (δ 65.1), C-17 (δ 77.8), and δ 1.16 (C-30) with C-7 (δ 207.3), C-8 (δ 48.5), C-9 (δ 43.9) and C-14 (δ 65.1), could be easily observed in HMBC spectrum.

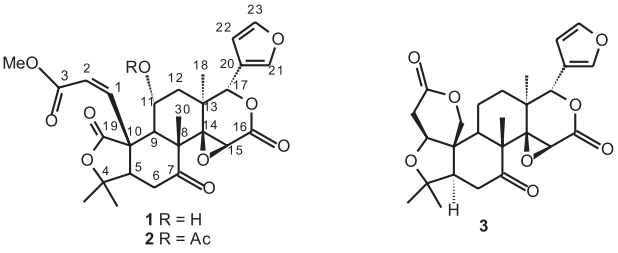

Figure 1. Limonoids isolated from the roots of *S. odoratissima.*

The structure presented for compound (**1**) was definitely confirmed by single crystal X-ray diffraction (Figure 2).

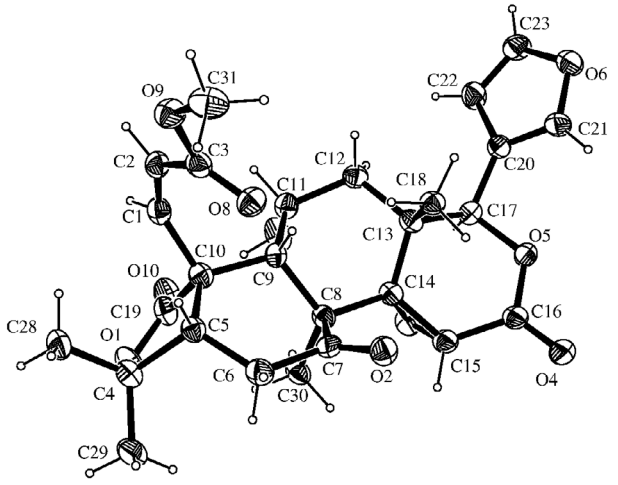

Figure 2. *ORTEP-3*[14] diagram of compound (**1**), showing the atoms labeling and the 50% probability ellipsoids.

The second new limonoid (**2**), was obtained from the methanol extract of the roots of *S. odoratissima*, as a white solid presenting a high mp (> 300 °C) and $[\alpha]_D^{25}$ -20 ($CHCl_3$). The IR spectrum presented absorptions at 1766 (lactone), 1742 (α,β-unsaturated ester) and 1707 (ketone).

Compound (**2**) ^{1}H NMR spectrum presented high similarity with the one from compound (**1**), indicating a limonoid with a β-substituted furan ring, as shown by the two α-furan hydrogen at δ 7.39 (*m*) and the β-furan hydrogen at d 6.34 (*m*). An additional signal was observed at δ 2.10 (*s*, 3H), being attributed to the presence of an extra methyl group in (**2**).

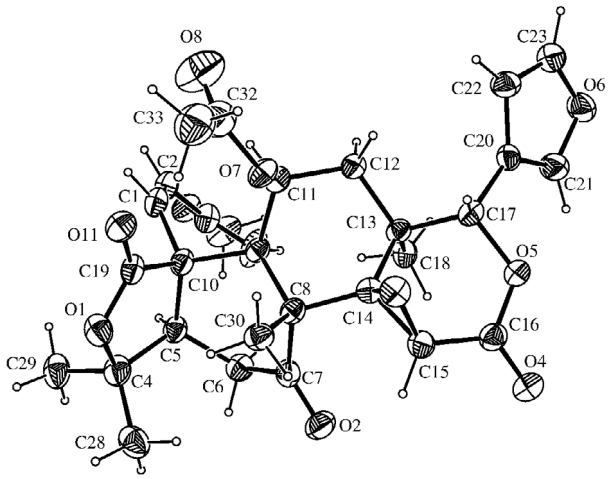

Figure 3. *ORTEP-3*[14] diagram of compound (**2**), showing the atoms labeling and the 50% probability ellipsoids.

^{13}C NMR spectrum presented 29 signals, showing that compound (**2**) should have two more carbons than the analogous (**1**); a quaternary carbon at δ 170.5 and a methyl group at δ 21.2 were observed by the DEPT experiment indicating the presence of an acetyl group in this compound.

These ^{1}H and ^{13}C NMR data, in association with the absence of a hydroxyl absorption in IR spectrum led to the conclusion that the hydroxyl group was esterified in (**2**). Single crystal X-ray diffraction has confirmed the proposed structure for (**2**) (Figure 3).

The known compounds were identified through comparison of their spectral data with the ones in the literature, limonin (**3**),[17] dictamnine, skimmianine;[18] the β-indoloquinazoline alkaloids rutaecarpine (**4**), evodiamine (**5**)[19] and 1-hydroxyrutaecarpine (**6**),[20] and the coumarin aurapten.[21] The isolated alkaloids and limonoids have been described as typical metabolites from the Rutaceae.[22]

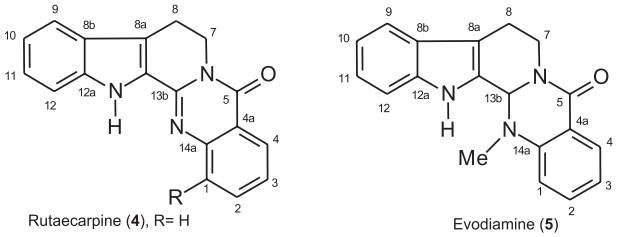

Figure 4. β-Indoloquinazoline alkaloids isolated from the roots of *S. odoratissima.*

Supplementary Information

Crystallographic data (excluding structure factors) for the structures in this paper has been deposited with the

Cambridge Crystallographic Data Centre as supplementary publication no CCDC 238939 and 238940. Copies of the data can be obtained, free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or e-mail: deposit@ccdc.cam.ac.uk).

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