Chemical constituents of *Rouea doniana*

**Patrícia V. de Oliveira,**
Rosangela P. L. Lemos,
Lucia M. Conserva*

**1Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, Brazil,**
**2Instituto do Meio Ambiente do Estado de Alagoas, Brazil.**

**Abstract:** The chromatography fractionation of the hexane, chloroform and ethyl acetate extracts from the leaves and stems of *Rouea doniana* Baker, Connaraceae, resulted in the isolation of five triterpenes (lupeol, lupenone, α-amyrenone, β-amyrenone, and taraxerol), a flavonol (7,4'-dimethylkaempferol), a coumarin (scopoletin) and four phytosteroids (β-sitosterol, stigmasterol, β-sitosteryl-3-O-β-D-glucopyranoside and stigmasteryl-3-O-β-D-glucopyranoside). All compounds are being for the first time in this species and all triterpenes and the flavonol are being described for the first time in the family Connaraceae. These compounds were identified on basis of their IR and NMR (1H, 13C, DEPT, HSQC, HMBC, and NOESY) spectral data and by comparison with literature data.

**Keywords:** Connaraceae coumarin flavonol *Rouea doniana* triterpenes

**Introduction**

The family Connaraceae consists twenty genera and about 300 to 350 species distributed in Africa, Southeast Asia and tropical America (Wiart, 2006). In tropical America this family is represented only by five genera. Among them, *Connarus* and *Rouea* are the most representatives (Kalegari, 2009; Groppo et al., 2010).

*Rouea* is a pantropical genus with about 100 species, 48 in the Neotropics (Groppo et al., 2010). The fruits, seeds, or leaves of *R. volubilis* and *R. glabra* are poisonous and are used against wild dogs and coyotes in poisoned baits (Kalegari, 2009); leaves of *R. minor* that are used in Chinese folk medicine to treat minor abrasions and lesions (He et al., 2006), and *R. induta* for treat rheumatism (Kalegari, 2009). Few species have been screened so far for their biological activities. Among them are *R. doniana* Baker, which hexane extract from stems showed significant activity (LD50 12.1 μg/mL) against the fourth instar larvae (Oliveira et al., 2010) and chloroform extract from the stems of *R. minor* showed in vitro activity against *Plasmodium falciparum* (He et al., 2006).

Previous phytochemical investigations on *Rouea* species reported the occurrence of two glycoside derivatives (rourinoside and rouremin) and monoglyceryl derivative [1-(26-hydroxyhexacosanoyl)-glycerol], actives as antimalarial, sphingolipid [1-O-β-D-glucopyranosyl-(2S,3R,4E-8Z)-2-N-(20-hydroxypalmitoyl)-octadecasphinga-4,8-dienine], nor-sesquiterpenes (dihydrovomifoliol-9-β-D-glucopyranoside), besides 95,12S,13S-trihydroxy-10E-octadecenoic acid and β-sitosterol glucoside from *R. minor* (He et al., 2006); quinone (rapanone) and cianidine (leucopelargonidine) from *R. santaloides* (Ramiah et al., 1976a); flavonoids (quercetin, quercetin 3-O-α-L-arabinofuranoside, and quercetin 3-O-β-D-xylopyranoside) from the leaves of *R. induta* (Kalegari, 2009). Thus, this work describes for the first time the occurrence of triterpenes (1-5), flavonol (6), coumarin (9), and four phytosteroids (7-8, 10-11) from the leaves and stems of *R. doniana*.

**Material and Methods**

The melting point was measured using an MQAPF-302 apparatus. NMR experiments were acquired on a Bruker Avance 400, operating for 1H and 13C at 400 and 100 MHz, respectively, in CDCl3 or CD3OD or both solutions with TMS as internal standard.
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Leaves and stems of *Rourea doniana* Baker, Connaraceae, were collected in March 2007, in the Fazenda Lamarão, Pilar, Alagoas State, Brazil. The specimen was identified by Rosangela P. de Lyra Lemos of the Instituto do Meio Ambiente do Estado de Alagoas, Maceió-AL, where a voucher specimen was deposited (MAC-26413).

The air-dried and powdered leaves (770 g) and stems (1700 g) were exhaustively extracted with 90% EtOH at room temperature. After the removal of solvents under vacuum, these extracts (leaves: 38.0 g; stems: 102.7 g) were suspended in MeOH-H$_2$O solution and extracted successively with hexane, CHCl$_3$ and EtOAc [leaves: hexane (5.3 g), CHCl$_3$ (3.7 g), EtOAc (7.3 g) and MeOH-H$_2$O (20.7 g); stems: hexane (36.4 g), CHCl$_3$ (3.0 g), EtOAc (4.0 g), and MeOH-H$_2$O (56.2 g)]. Hexane fraction from leaves (5.3 g) after successive chromatographic fractionations over silica gel column with hexane containing increasing amounts of EtOAc afforded a mixture of 1 and 2 (0.010 g; M1), and 3-5 (0.032 g; M2). The CHCl$_3$ fraction from leaves (3.7 g) after the same experimental procedure, gel filtration (Sephadex LH-20 with MeOH) and successive recrystallizations with MeOH afforded 6 (0.013 g).

The CHCl$_3$ fraction from stems (3.0 g) was also chromatographed on silica gel column with hexane containing increasing amounts of EtOAc for yield a mixture of 7 and 8 (0.065 g; M3). The remaining sub-fraction 130-154 (0.10 g) after gel filtration on Sephadex LH-20 with MeOH afforded 9 (0.010 g). The EtOAc fraction from stems (4.0 g) after successive chromatographic fractionations over silica gel column with CHCl$_3$ containing increasing amounts of MeOH and successive recrystallizations with MeOH afforded a mixture of 10-11 (0.044 g; M4). Structures of isolated compounds were identified on the basis of their IR and NMR spectral data (including DEPT, HSQC, HMBC, and NOESY) and also by comparison of experimental data with those described in the literature.

**Results and Discussion**

The hexane, chloroform and ethyl acetate extracts from leaves and stems of *R. doniana* after chromatography fractionations afforded five triterpenes (1-5), a flavonol (6), a coumarin (9), in addition four phytosteroids (7-8 and 10-11). These compounds were identified by IR and NMR spectral data (including DEPT, HSQC, HMBC, and NOESY) and also by comparison of experimental data with those described in the literature.

The $^1$H NMR spectra of M1 (1 and 2) and M2
In addition several signals for methyl groups, showed signals for oxymethine [1 and 2: δ 3.18 (m, H-3)] and olefinic hydrogens [1: δ 4.56 and 4.67 (sl, H-29), 2: δ 5.52 (dd, J=8.0 and 3.0 Hz, H-15)], 3: δ 5.21 (t, J=3.1 Hz, H-12), 4: δ 5.15 (t, J=3.4 Hz, H-12), and 5: δ 4.69 and 4.57 (sl, H-29)], as well as hydrogens alpha to carbonyl groups [3 and 4: δ 2.48 (m, H-2) and 5: δ 2.40 (m, H-2)]. The 13C NMR spectrum of M1 and M2 showed, in addition several signals for C-sp3, signals for olefinic carbons, compatible with the presence of triterpenes type lup-20(29)-ene (1: δ 109.3 and 150.9), taraxerene (2: δ 116.84 and 158.03), urs-12-ene (3: δ 121.49 and 139.72), olean-12-ene (4: δ 121.49 and 145.27), and lup-20(29)-ene (5: δ 109.31 and 150.89). Also was observed signals at δ 78.98 and δ 79.03 (I and 2, respectively) and at δ 218.33, 217.99, and 217.94 (3, 4 and 5, respectively), characteristics of oxymethine carbon and carbonyl groups at C-3. These data suggested the presence of lupeol (1) and taraxerol (2) for M1 and α-amyrnone (3), β-amyrnone (4) (Mahato & Kundu, 1994) and lupene (5) for M2 (Cursino et al., 2009).

Compound 6 was obtained as a yellow amorphous powder, m.p. 179-182 ºC (lit. 182-184 ºC; Kamaya & Ageta, 1990). The IR spectrum indicated the presence of hydroxyls (1310 cm⁻¹), carbonyl (1694 cm⁻¹), aromatic ring (1593, 1508 cm⁻¹), and saturated carbons (2948, 2849, 1470, and 1366 cm⁻¹). The 1H NMR spectrum showed signals for flavonol substituted at ring A at C-5 and C-7 [δ 6.31 and 6.42 (d, J=2.0 Hz each, H-6 and H-8, respectively)] and ring B at C-4' [δ 6.98 and 8.09 (d, J=8.8 Hz each, H-3'/H-5' and H-2'/H-6', respectively)]. In addition was observed signals for two methoxyl (δ 3.82 and 3.83) and a hydroxyl group in hydrogen bond (δ 11.67). The 13C NMR spectral data displayed signals for fifteen carbon atoms, which chemical shifts are compatible with the presence of a flavonol [δ 135.67 (C, C-3)] substituted at ring A at C-5 and C-7 [δ 92.21 and 97.90 (CH each, C-6 and C-8, respectively)] and ring B at C-4' [δ 114.09 and 129.40 (CH each, C-3'/C-5' and C-2'/C-6', respectively)], as well as signals for one carbonyl (δ 162.59) and two methoxyl (δ 55.43 and 55.85) groups. The upfield shift of C-3'/C-5' (δ 114.09) and the chemical shifts of carbon signals assigned to methoxyl groups, besides comparison with data reported identified 6 as 7,4'-dimethylkaempferol (Silva et al., 2009).

Compound 9 was obtained as a yellow needles, m.p. 208-210 ºC (lit. 207-209 ºC; Duarte & Ferreira, 2007) and and its IR spectrum showed the presence of hydroxyl group (3387 cm⁻¹), carbonyl δ-lactone (1711 cm⁻¹), double bond (1613 cm⁻¹), aromatic ring (1595, 1567 and 1510 cm⁻¹). The 1H NMR spectra of 9 showed signals whose chemical shifts are consistent with a coumarin 6,7-dissubstituted [δ 6.18 (H-3) and δ 7.61 (H-4) (d, J=9.4 Hz each), δ 6.80 (H-5) and δ 6.79 (H-8, s each)] and for a methoxyl group (δ 3.85). The 13C NMR spectral data, including HSQC, allowed us to recognize signals for 10 carbon atoms. Among them, were observed signals for a coumarin at C-6 and C-7 substituted [δ 111.71 (C-3) and 144.21 (C-4)], two aromatic carbons [δ 107.96 (C-5) and 102.98 (C-8)], a γ-lactone carbonyl (δ 162.59), and a methoxyl group (δ 55.90). These data suggested for 9 the structure of scopoletin (Bayoumi et al., 2010). However, the confirmation and complete assignments of this compound were established by the correlations observed in the NOESY, specially by correlations observed between H-5 (δ 6.80) with H-4 (δ 7.61) and methoxyl group (δ 3.85), and HMBC spectra.

The mixtures M3 (7-8) and M4 (10-11) showed NMR data identical to those reported for sitosterol (7), stigmasterol (8), β-sitosterol-3-O-β-D-glucopyranoside (10) and stigmasterol-3-O-β-D-glucopyranoside (11) (Kojima et al., 1990; Chaves et al., 2010).

In the family Connaraceae, only six genera (Agelaea, Byrsocarpus, Cnestis, Connarus, Rourea, and Roureopsis) have been investigated for chemical composition and the major components have been quinolizidine alkaloids (Le et al., 2005), quinones (Aiyar et al., 1964), triterpenes, coumarins, and flavonoids. Triterpenes have been found only in the genus Rourea (Zhang et al., 2008), coumarins in Byrsocarpus (Vickery & Vickery, 1980) and flavonoids in Agelaea (Kuwabara et al., 2003), Byrsocarpus (Ahmadu et al., 2007), Cnestis (Parvez & Rahman, 1992), Connarus (Aiyar et al., 1964; 1965; Ramiah et al., 1976b; Marcano et al., 1984), and Rourea (Jiang et al., 1990; Zhang et al., 2008; Kalegari, 2009). The present study reports for the first time the isolation of five triterpenes, one flavonol, one coumarin and four phytosteroids from R. domiana. Among them, the triterpenes and flavonol are being described for the first time in the Connaraceae.

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References


*Correspondence*

Lucia M. Conserva
Instituto de Quimica e Biotecnologia, Universidade Federal de Alagoas
Campus A. C. Simões, Br 104/km 97, 57072-970 Maceió-AL, Brazil
lmc@qui.ufal.br
Tel. +55 82 3214 1390