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Chemical constituents of *Rourea doniana*

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Abstract: The chromatography fractionation of the hexane, chloroform and ethyl acetate extracts from the leaves and stems of *Rourea 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 (^{1}H, ^{13}C, DEPT, HSQC, HMBC, and NOESY) spectral data and by comparison with literature data.

Short Communication

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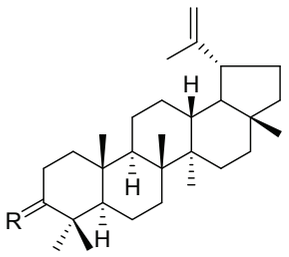
Introduction

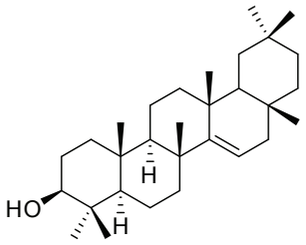
The family Connaraceae consists twenty genera and about 300 to 350 species distributed in Africa, Southeast Asia and tropical America (Wiart, 2006). In tropical America this family is represented only by five genera. Among them, *Connarus* and *Rourea* are the most representatives (Kalegari, 2009; Groppo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

Rourea 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 chlroform extract from the stems of *R. minor* showed *in vitro* activity against *Plasmodium falciparum* (He et al., 2006).

Previous phytochemical investigations on *Rourea* 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-(2*S*,3*R*,4*E*-8*Z*)-2-*N*-(20-hydroxypalmitoyl)-octadecasphinga-4,8-dienine], nor-sesquiterpene (dihydrovomifoliol-9-β-D-glucopyranoside), besides 9*S*,12*S*,13*S*-trihydroxy-10*E*-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*-β-L-rhamnopyranoside, hyperin, astilbin, kaempferol, and rutin), anthraquinone (physcion and erythroglaucin), triterpenes (23-hydroxybetulinic acid, ursolic acid and hederagenin), coumarin (daphnetin), phenylpropanoid derivative [nonacosyl (*E*)-ferulate], phytosteroids (β-sitosterol, β-sitosteryl-β-D-glucopyranoside), besides fatty acids, alkane, alcohol, and glyceryl derivative from *R. microphylla* (Jiang et al., 1990; Zhang et al., 2008), and flavonoids (quercetin, hyperin,



1 R=β-OH
5 R=O



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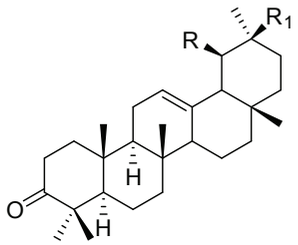
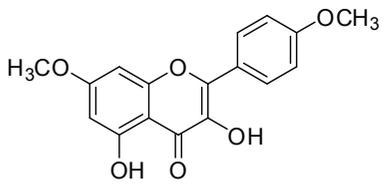
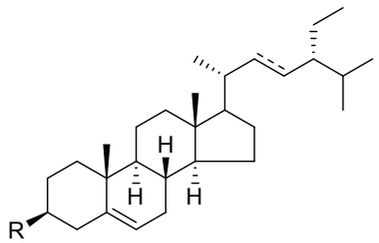


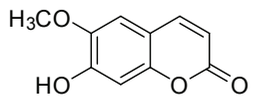
3 R=Me; R_1=H
4 R=H; R_1=Me



6



7 R=H, $\Delta^{22\ dihydro}$, H-24α
8 R=H, Δ^{22}
10 R=Glc, $\Delta^{22\ dihydro}$
11 R=Glc; Δ^{22}



9

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 ^{1}H and ^{13}C at 400 and 100 MHz, respectively, in $CDCl_3$ or CD_3OD or both solutions with TMS as internal reference. IR spectra were obtained on a FT-IR 1750 Perkin-Elmer spectrometer. Silica gel (70-230 and 230-400 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used for column chromatographic separations and silica gel 60 PF_{254} (Merck) was used for analytical (0.25 mm) TLC.

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_2O 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_2O (20.7 g); stems: hexane (36.4 g), $CHCl_3$ (3.0 g), EtOAc (4.0 g), and MeOH-H_2O (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 two mixtures containing **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 and by comparison with other

physical data with those reported 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 (**3**-**5**), 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 ^{13}C NMR spectra of M1 and M2 showed, in addition several signals for C-sp^3, 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 124.19 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 (**1** 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-amyrenone (**3**), β-amyrenone (**4**) (Mahato & Kundu, 1994) and lupenone (**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 (3417 cm^{-1}), carbonyl (1694 cm^{-1}), aromatic ring (1593, 1508 cm^{-1}), and saturated carbons (2948, 2849, 1470, and 1366 cm^{-1}). The ^{1}H 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 ^{13}C 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 175.70) 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 ^{1}H 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 ^{13}C 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-sitosteryl-3-*O*-β-D-glucopyranoside (**10**) and stigmasteryl-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., 1965; Ramiah et al., 1976a), 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. doniana*. Among them, the triterpenes and flavonol are being described for the first time in the Connaraceae.

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