Coumarins from the Malagasy *Cedrelopsis rakotozafyi* Cheek and Lescot (Rutaceae)

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Keywords: *Cedrelopsis rakotozafyi,* coumarins, 8-hydroxy-7-methoxy-6-(2*R*-hydroxy-3-methylbut-3-enoxy)-2H-1-benzopyran-2-one, 7-hydroxy-6-(2*R*-hydroxy-3-methylbut-3-enoxy)-2H-1-benzopyran-2-one, 5,6,7-trimethoxy-2H-1-benzopyran-2-one.

Abstract: A phytochemical analysis of the bark of the Malagasy *Cedrelopsis rakotozafyi* Cheek and Lescot (Rutaceae) yielded the novel 8-hydroxy-7-methoxy-6-(2*R*-hydroxy-3-methylbut-3-enoxy)-2H-1-benzopyran-2-one, and 7-hydroxy-6-(2*R*-hydroxy-3-methylbut-3-enoxy)-2H-1-benzopyran-2-one, 5,6,7-trimethoxy-2H-1-benzopyran-2-one, scoparone, scopoletin, lupeol and β-amyrin. The placement of *Cedrelopsis* within the Rutaceae is supported phytochemically by the typically Rutaceous coumarins isolated.

1. Subject and source

Cedrelopsis rakotozafyi Cheek and Lescot *(*Rutaceae), locally known as Hazondranta, was collected from Ampitsinjovagna, Irodo in the northern part of Madagascar during the dry season in July 2006 by Dr. Milijaona Randrianarivelojosia. Plant identification was confirmed at the Botany Department of the University of Antananarivo. Voucher specimen (CCL 040 – TAN) was retained. The plant collection site was 12° 38.195 S; 49° 31.854 E.

The genus *Cedrelopsis* is endemic to Madagascar and comprises eight species, four of which, *C. gracilis* (Mulholland et al., 2004), C. *microfoliata* (Koorbanally et al., 2002), *C. grevei* (Cavalli et al., 2004; Mulholland et al., 1999; Mulholland et al., 2002; Mulholland et al., 2003; Randrianarivelojosia et al., 2005; Um et al., 2003) and *C. longibracteata* (Randrianarivelojosia et al., 2005) have been previously examined. Plants belonging to the genus *Cedrelopsis* are widely used as folk remedies as febrifuges, fortifying agents, relaxants and as postnatal medications

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(Gauvin et al., 2004; Um et al., 2003). They are also used to treat rheumatism, cardiovascular diseases and sexually transmitted infections (Mulholland et al., 2004; Ranaivo et al., 2004). We report the phytochemical investigation of the bark of *Cedrelopsis rakotozafyi* which has yielded seven compounds including a new coumarin, 8-hydroxy-7-methoxy-6-(2*R*-hydroxy-3-methylbut-3-enoxy)-2H-1-benzopyran-2-one **1** along with four known coumarin derivatives and two known triterpenoids. No limonoids were isolated.

2. Previous work

There is no previous work reported on *Cedrelopsis rakotozafyi*. Other *Cedrelopsis* species have yielded either coumarins or limonoids.

3. Present study

The air-dried, ground stem bark (452 g) was extracted twice using a MARSXpress™ microwave extraction system (voltage: 1600W, power: 100%, time: 10 min hold: 20 min, temp 110°C) with CH_2Cl_2, EtOAc and MeOH successively, to yield 11.3, 6.4 and 33.8 g, respectively, of each extract. Gravity column chromatography over silica gel (Merck 9385) using hexane:CH_2Cl_2:EtOAc:MeOH mixtures of increasing polarity were undertaken separately for the three extracts. The CH_2Cl_2 extract afforded the novel 8-hydroxy-7-methoxy-6-(2*R*-hydroxy-3-methylbut-3-enoxy)-2H-1-benzopyran-2-one **1** (5.1 mg), 7-hydroxy-6-(2*R*-hydroxy-3-methylbut-3-enoxy)-2H-1-benzopyran-2-one **2** (6.0 mg) (Chen et al., 2006), 5,6,7-trimethoxy-2H-1-benzopyran-2-one **3** (8.1 mg) (Kayser and Kolodziej, 1995), scoparone **4** (5.2 mg), scopoletin **5** (10.3 mg) (Razdan et al., 1987), lupeol (7.9 mg) and β-amyrin (8.3 mg) (Ahmad and Rahman, 1994), the EtOAc extract yielded compound **2** (2 mg), **4** (6 mg), **5** (10 mg), lupeol (7 mg) and β-amyrin (8 mg) and the MeOH extract yielded lupeol (4.0 mg) and β-amyrin (3.8 mg). Structures of the isolated constituents were established by analysis of their NMR and MS data and by comparison against reported data. The ^{1}H NMR spectrum of compound **1** showed typical resonances for a methoxylated prenylated coumarin. The H-3 and H-2 resonances appeared as a pair of doublets (δ 6.28 *J*= 9.5 Hz, δ 7.55, *J*= 9.5 Hz respectively), and the NOESY spectrum showed a correlation between the H-4 resonance and a singlet at δ 6.78 which could be ascribed to H-5. The H-5 resonance showed a correlation with two H-1' resonances of a 2-hydroxy-3-methylbut-3-enoxy group present at δ 4.46 (dd, *J*=11.4, 2.3 Hz) and δ 4.05 (dd, *J*=8.3, 2.3 Hz). The COSY spectrum showed coupling between the two H-1' resonances and an oxymethine

resonance at δ 4.54 (H-2', d, *J*=8.3 Hz) which, in turn, showed vinylic coupling with the two H-4' proton resonances at δ 5.19 (br s) and δ 5.14 (br s). The COSY spectrum showed coupling between the two H-4' resonances and the H-5 methyl group proton resonance at δ 1.86. Correlations seen between the 2H-1' resonances and a methoxy group proton resonance (δ 4.03, s) in the NOESY spectrum established the presence of a methoxy group at C-7, and the remaining hydroxyl group was placed at C-8. We present for the first time the NMR and MS, IR data for compound **1** and the determination of the absolute configuration at C-2' of compounds **1** and **2**.

*3.1. 8-Hydroxy-7-methoxy-6-(2R-hydroxy-3-methylbut-3-enoxy)-2H-1-benzopyran-2-one **(1)***, brown-yellow amorphous material; $[\alpha]_{Na}^{26}$= +33.5° (c = 0.0026 g/ml, $CHCl_3$); HRMS: 297.0733 [[M+Na^+]-18], ($C_{15}H_{16}O_6$+Na^+-H_2O; calc. 297.0733), IR_{Vmax}3454, 2924, 2852, 1727 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.55 (1H, d, *J*= 9.5 Hz, H-4), δ 6.78 (1H, s, H-5), δ 6.28 (1H, d, *J*= 9.5 Hz, H-3), δ 5.19 (1H, br s, H-4'a), δ 5.14 (1H, br s, H-4'b), δ 4.54 (1H, br d, *J*=8.3 Hz, H-2'), δ 4.46 (1H, dd, *J*=11.4, 2.3 Hz, H-1'b), δ 4.05 (1H, dd, *J*=8.3, 2.3 Hz, H-1'a), δ 4.03 (3H, s, OMe), δ 1.86 (1H, br s, H-5'); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.0 (C-2), δ 114.9 (C-3), δ 143.8 *(C-4),* δ 113.1 (C-4a), δ 109.2 (C-5), δ 140.6 (C-6), δ 136.0 (C-7), δ 140.7 (C-8), δ 142.8 (C-8a), δ 67.8 (C-1'), δ 76.0 (C-2'), δ 139.6 (C-3'), δ 115.2 (C-4'), δ 19.0 (C-5'), δ 62.0 (C-7, OMe).

The absolute configuration at C-2' of compounds **1** and **2** was established using Horeau's method (Horeau, 1961; Horeau and Kagan, 1964; Hou et al., 2008). The secondary alcohol **1** (4 mg) was treated with an excess of racemic 2-phenylbutyric acid anhydride (Sigma Aldrich, 12.7 µl) in dry pyridine (0.5 mL). The mixture was stirred for 20 h at room temperature, 10% $NaHCO_3$ (1 mL) was added and the mixture was stirred for a further 1 h. After adding distilled water (10 mL) the reaction mixture was extracted with ether (5 x 8 mL). The ether phases were combined and dried over Mg_2SO_4, and the solvent was evaporated *in vacuo* yielding the Horeau's ester. The aqueous phase was acidified with 2M HCl (2.2 mL) and extracted with chloroform (4 x 8 mL). The chloroform phase was washed with water to neutral, dried over Mg_2SO_4 and the solvent was removed to give unreacted 2-phenylbutyric acid. The optical rotation of the unreacted 2-phenylbutyric acid was positive, indicating that (-)-2-phenylbutyric acid had reacted preferentially with **1**. This indicated a 2*R*-configuration for compound **1.** The experiment was repeated for compound **2** and the same result was obtained (**Fig. 1.**).

(Insert Figure 1)

4. Chemotaxonomic significance

Cedrelopsis has been placed in several plant families over the years. Chase et al. (1999) recommended a broad circumscription of the Rutaceae that included the subfamily Spathelioideae, containing seven genera, *Bottegoa*, *Cedrelopsis*, *Cneorum*, *Dictyoloma*, *Harrisonia*, *Ptaeroxylon* and *Spathelia*. This concept has subsequently been adopted by Groppo et al. (2008) and Razafimandimbison et al. (2010). Recent investigations into anatomical characteristics and a species-level phylogenetic analysis of five plastid DNA regions (rbcL, atpB, trnL–trnF, rps16 and psbA–trnH) of the *Spathelia–Ptaeroxylon* clade (which includes *Cedrelopsis*), shows it is reasonable to unite the genera into the Spathelioideae subfamily within the Rutaceae (Appelhans et al., 2011). The placement of *Cedrelopsis* within the Rutaceae is moreover supported phytochemically by the typically Rutaceous coumarins isolated in our work.

Acknowledgments

We are grateful to Claude Christian, Riri Guito and Estephin Williams Jaomamy for their assistance in collecting the plant material.

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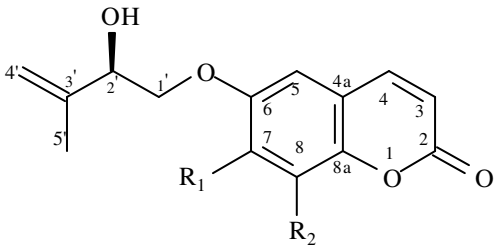
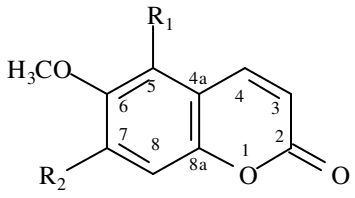


	R_1	R_2
1	OCH_3	OH
2	OH	H



	R_1	R_2
3	OCH_3	OCH_3
4	H	OCH_3
5	H	OH

Fig. 1. Coumarins isolated from *C. rakotozafyi*