PERGAMON

Phytochemistry 52 (1999) 1145–1148

PHYTOCHEMISTRY

A phenolic glycoside and *N*-(*p*-coumaroyl)-tryptamine from *Ravensara anisata*

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Received 7 May 1999; accepted 23 June 1999

Abstract

A new phenolic glycoside, 1-(α-L-rhamnosyl(1-6)-β-D-glucopyranosyloxy)-3,4,5-trimethoxybenzene, together with the alkaloid *N*-(*p*-coumaroyl)-tryptamine and four known flavonoids were isolated from the methanolic bark extract of *Ravensara anisata* Danguy (Lauraceae). Their structures have been established by NMR spectroscopy, and chemical methods. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Ravensara anisata; Lauraceae; Phenolic glycoside; Tryptamine derivative; NMR spectroscopy

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1. Introduction

Ravensara anisata Danguy (Lauraceae) is an endemic species in Madagascar and is used as a spasmolytic and neurosedative drug (Kostermans, 1950) and for its antimicrobial properties (de Medeci, Pieretti, Salvatore, Nicoletti & Rasoanaivo, 1992; Raharivelomanana, 1989). Recently, the isolation of two new antifungal α-pyrones from lipophilic extracts of the leaves and the bark of *R. anisata* was reported (Andrianaivoravelona, Sahpaz, Terreaux, Hostettmann, Stoeckli-Evans & Rasolondramanitra, 1999).

The present paper describes the isolation of a new phenolic glycoside 1-(α-L-rhamnosyl(1-6)-β-D-glucopyranosyloxy)-3,4,5-trimethoxybenzene and a known tryptamine derivative (*N*-(*p*-coumaroyl)-tryptamine), together with four known flavonoids. The structures have been established by 1D- and 2D-NMR spectroscopy, including COSY, HSQC, HMBC experiments and chemical methods.

2. Results and discussion

Dried and powdered bark of *R. anisata* was successively extracted at room temperature with CH_2Cl_2 and MeOH and after several chromatographic steps, compound **1** was obtained as a white powder. The UV spectrum showed maxima at 220 and 275 nm. ^1H NMR data indicated the presence of three methoxyl groups (δ 3.80 ppm, $2 \times OCH_3$ and 3.70 ppm, $1 \times OCH_3$), two anomeric protons at 4.80 ppm (*d*, $J = 7$ Hz) and 4.70 ppm (*d*, $J = 2$ Hz), two aromatic protons at 6.43 ppm (*s*, 2H), and multiplets corresponding to sugar moieties overlapping in the range of 3.3–4.1 ppm and one methyl group at 1.21 ppm (*d*, $J = 6.4$ Hz). Signals of three methoxyl groups at 61.2 ppm (1 *ortho*-disubstituted OCH_3) and 56.7 ppm (2 OCH_3) were also detected in the ^{13}C NMR spectrum, together with signals characteristic of glucose and rhamnose (see Table 1) (Markham, 1972). 2D long range correlations (HMBC) clearly indicated the attachement of the anomeric proton of β-glucose ($J = 7$ Hz) to C-1 of a trisubstituted phenol and a (6-1)-linkage between glucose and rhamnose (Fig. 1).

The EI-MS spectrum of **1** displayed a peak at m/z 184 corresponding to the aglycone ion. This infor-

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PII: S0031-9422(99)00377-5

Table 1
^{1}H-,^{13}C-NMR chemical shifts (ppm, *J* in Hz) of compound **1**[a].

	^{1}H-NMR	^{13}C-NMR
Aglycone		
1		155.8
2; 6	6.43, *s*	96.2
3; 5		154.7
4		134.6
3-OMe; 5-OMe	3.80, *s*	56.7
4-OMe	3.70, *s*	61.2
Glucose		
1′	4.80, *d*, 7	102.9
2′	3.42	74.8
3′	3.46	77.8
4′	3.34	71.4
5′	3.58	76.8
6′	3.55–4.04	67.8
Rhamnose		
1″	4.70, *d*, 2	101.9
2″	3.81	72.1
3″	3.63	72.3
4″	3.35	73.9
5″	3.61	69.75
6″	1.21, *d*, 6.4	17.8

[a] In CD_3OD.

mation was confirmed by the D/CI-MS spectrum in which peaks at *m/z* 185 $[Aglyc+H]^+$ and *m/z* 510 $[M+NH_4]^+$ were observed.

Acid hydrolysis of **1** afforded glucose and rhamnose which were identified by TLC comparison with authentic samples. Comparison of NMR data of the aglycone with literature data indicated the presence of 3,4,5-trimethoxyphenol (Shimomura, Sashida, Oohara & Tenma, 1988).

Therefore, the structure of **1** was established as the new phenolic glycoside 1-(α-L-rhamnosyl(1-6)-β-D-glucopyranosyloxy)-3,4,5-trimethoxybenzene.

Compound **2** was obtained as a yellow powder. A molecular formula of $C_{19}H_{18}O_2N_2$ was determined on the basis of NMR, D/CI and EI-MS data. Molecular ion signals were recorded at *m/z* 306 $[M]^+$ (EI-MS). ^{1}H- and ^{13}C-NMR studies including H-H COSY, HSQC and HMBC of compound **2** suggested the presence of two aromatic moieties. One part was identified as a *p*-hydroxy-*trans*-cinnamoyl (*p*-coumaroyl) with a *trans* double bond, considering the characteristic coupling constant (15.6 Hz) between the two olefinic protons at δ 7.47 and 6.46 ppm. These data were

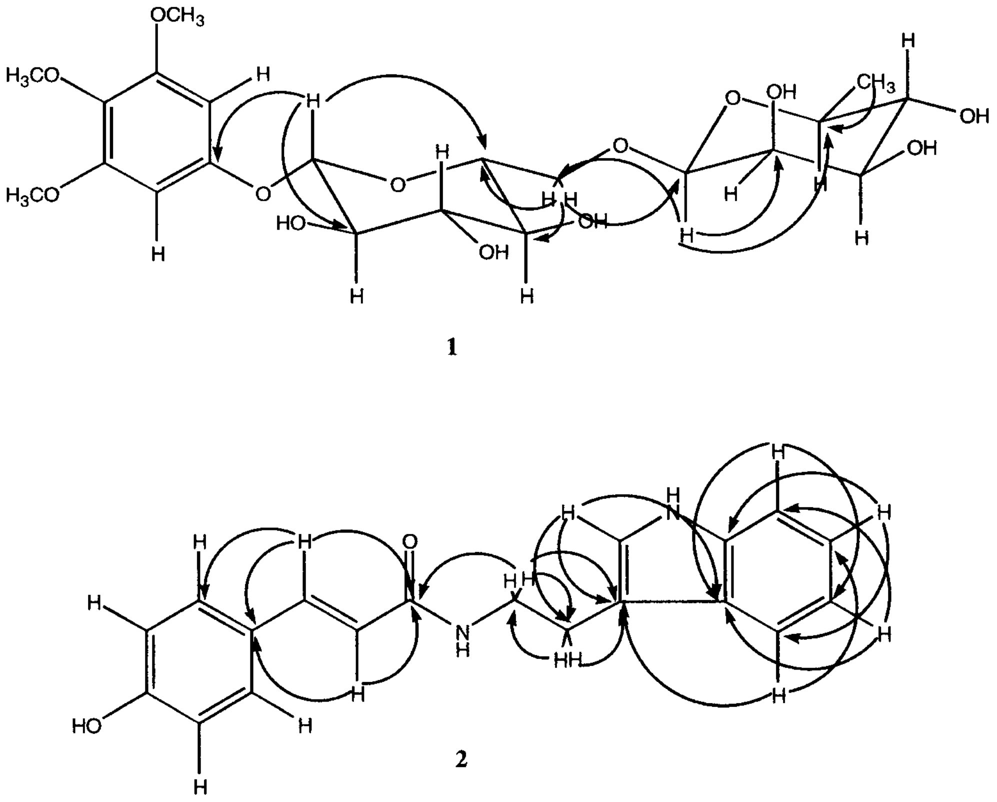


Fig. 1. HMBC correlations observed in compounds **1** and **2**.

Table 2
^{1}H-,^{13}C-NMR shifts (ppm, *J* in Hz) of compound **2**[a]

	^{1}H-NMR	^{13}C-NMR
Coumaroyl part		
1′		169.2
2′	6.46, *d*, 15.6	118.5
3′	7.47, *d*, 15.6	141.6
1″		127.7
2″; 6″	6.79, *dd*, 6.4, 2.0	116.7
3″; 5”	7.39, *dd*, 6.4, 2.0	130.5
4″		160.4
Tryptamine part		
2	7.09, *s*	123.4
3		113.3
3a		128.8
4	7.58, *d*, 7.8	119.3
5	7.01, *dd*, 7.8, 7.7	119.5
6	7.09, *dd*, 7.8, 8.3	122.3
7	7.33, *d*, 8.3	112.2
7a		138.1
8	3.00, *t*, 7.3	26.4
9	3.60, *t*, 7.3	41.6

[a] In CD_3OD.

in good agreement with literature data (Kisiel & Jakupowic, 1995; Li, Ahmed, Del Carmen Arias & Mabry, 1997). ^{1}H- and ^{13}C-NMR data (Table 2) of the second part were in good concordance with those reported for *N*-acyl derivatives of tryptamine (Maeda, Hara, Fujimoto, Srivastava, Gupta & Sahai, 1993; Thomas, Zawilska & Iuvone, 1990). The absorption maxima observed at 222 nm and 291 nm in the UV spectrum and the presence of a peak at *m*/*z* 164 [M–142]$^{+}$, arising from the C-N bond scission commonly observed in EI-MS spectra of *N*-acyl derivatives of tryptamine (Thomas et al., 1990) further supported the structural assignment of **2** as *N*-(*p*-coumaroyl)-tryptamine.

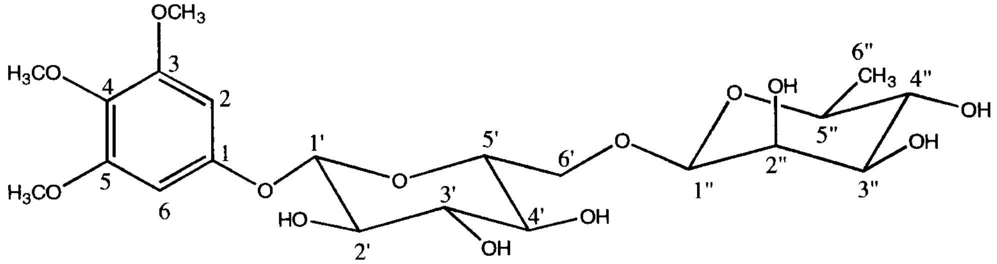
Whereas **1** represents a new structure, **2** has been isolated and identified by GC-MS from kernels of *Zea mays* (Ehmann, 1974) and from safflower seeds (*Carthamus tinctorius* L.) (Sato et al., 1985). Full NMR data of compound **2** are reported for the first time.

In addition, four known flavonoids were isolated: kaempferol-3-*O*-sophoroside-7-*O*-rhamnoside (Dembinska-Migas, 1990; Harborne & Boardley, 1984), kaempferol-3,7-*O*-dirhamnoside (Inigo, de Iglesias & Catalan, 1988), isorhamnetin-3-*O*-sophoroside-7-*O*-rhamnoside (Krolikowska, 1972) and isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside (Hörhammer, Wagner, Krämer & Farkas, 1967).

3. Experimental

3.1. General

Mps: Mettler FP-80/82 Hot stage apparatus, uncorr. $[\alpha]_D$: Perkin–Elmer 241 polarimeter. UV: Perkin–Elmer Lambda 20 spectrophotometer. TLC: Si 60 F_{254} Al sheets (Merck). CC: Sephadex LH-20 (Pharmacia), Si 60 (40–63 and 70–200 μm) (Merck), Lobar RP18 (40–60 μm, Merck). Anal. HPLC: Hewlett–Packard 1090 series, Novapak C18 column (250 × 3.9 mm id, 5 μm; Waters). ^{1}H- and ^{13}C-NMR: Varian Unity Inova 500 (500 and 125 MHz, respectively), chemical



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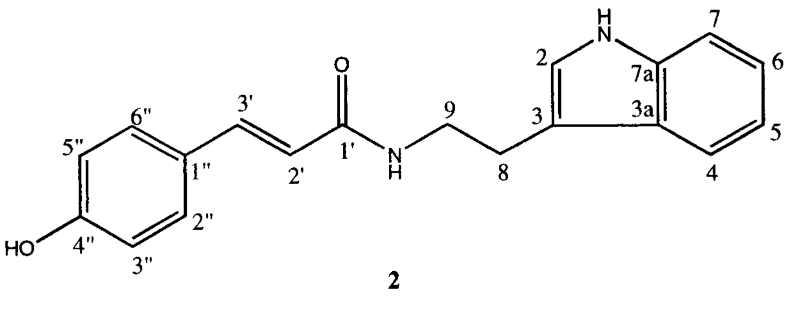

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shifts (δ) in ppm (TMS as int. stand., J in Hz). Complete attribution was performed on the basis of 2D experiments (COSY, HMBC, HSQC and selective INEPT). MS: Finnigan MAT TSQ-700 triple stage quadripole instrument.

3.2. Plant material

Bark of *R. anisata* Danguy was collected in the forest of Mandraka (Madagascar) in September 1996. A voucher specimen has been deposited at the Institute of Pharmacognosy and Phytochemistry (University of Lausanne) under the No. 96201.

3.3. Extraction and isolation

Dried and powdered bark, 459 g, were successively extracted with CH_2Cl_2 (3×2500 ml) and MeOH (3×2500 ml) to afford 15.8 and 27.7 g of extract, respectively. The MeOH extract (25 g) was fractionated by Si 60 CC (70–200 μm) with a step gradient ($CHCl_3$–MeOH 9:1 to pure MeOH) into 6 fractions (I–VI). Gel filtration of fraction III on Sephadex LH-20 ($CHCl_3$–MeOH 1:1) and further purification on Lobar RP-18 (MeOH–H_2O 3:7) afforded compound **1** (120 mg). Compound **2** (48 mg) was obtained from fraction II after repetitive gel filtration on Sephadex LH-20 ($CHCl_3$–MeOH 1:1, and EtOAc).

3.4. Hydrolysis

Acid hydrolysis of compound **1** (5 mg) was carried out by refluxing in 5 ml HCl 3% for 2 h. Sugars were identified on TLC by comparison with authentic glucose and rhamnose. TLC: SiO_2 F_{254} Al sheet (Merck), EtOAc–MeOH–H_2O–HOAc (65:15:15:20), detection with the anisidine phthalate reagent. The aglycone was identified by comparison with ^{1}H-NMR spectroscopy (Shimomura et al., 1988).

3.4.1. 1-(α-L-rhamnosyl(1-6)-β-D-glucopyranosyloxy)-3,4,5-trimethoxybenzene ((3,4,5-methoxyphenol)-rutinoside)

White amorphous powder Mp 117–120°C. $[\alpha]_D = -98°$ (MeOH, c 0.05). UV λ_{max} (MeOH) (log ϵ): 220 (3.6), 275 (2.7). D/CI-MS (NH_3, pos.) m/z: 510 $[M+NH_4]^+$, 202 $[Aglyc+NH_4]^+$; 185 $[Aglyc+H]^+$, EI-MS (70 eV) m/z (rel. int.): 184 (100), 169 (48). ^{1}H- and ^{13}C-NMR: Table 1.

3.4.2. N-(p-coumaroyl) tryptamine

Yellow powder UV λ_{max} (MeOH) (log ϵ): 222 (3.6), 291(3.2). D/CI-MS (NH_3, pos.) m/z: 324 $[M+NH_4]^+$, 307 $[M+H]^+$; EI-MS (70 eV) m/z (rel. int): 306 (100), 164 (48). ^{1}H- and ^{13}C-NMR: Table 2.

Acknowledgements

The Swiss Confederation is gratefully acknowledged for a scholarship to one author (J.O.A.) and the Swiss National Science Foundation for supporting this work.

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