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Furoquinoline Alkaloids of *Ertela (Monnieria) trifolia* (L.) Kuntze from the Suriname Rainforest

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Abstract

7-(2'-Hydroxy-3'-chloroprenyloxy)-4,8-dimethoxyfuroquinoline (**1**) and 6-(2'-hydroxy-3'-chloroprenyloxy)-4,7-dimethoxyfuroquinoline (**2**), together with ten known compounds have been isolated from the aerial parts of *Ertela (Monnieria) trifolia* (L.) Kuntze. All the isolates were tested for antiproliferative activity against the A2780 human ovarian cancer cell line.

Keywords

Ertela trifolia; *Monnieria trifolia*; Rutaceae; Furoquinoline alkaloids; Acridone alkaloids; Furofuran lignans; NMR; Cytotoxicity

1. Introduction

In our continuing search for bioactive molecules from the Suriname and Madagascar rainforests as part of an International Cooperative Biodiversity Group (ICBG) program (Cao et al, 2007) we obtained a methanol extract of the aerial parts of *Ertela trifolia* (L.) Kuntze (Rutaceae). This extract (BGVS 320017) showed antiproliferative activity against the A2780 human ovarian cancer cell line, with an IC₅₀ value of 20 µg/mL. *Ertela trifolia* is also known as *Monnieria trifolia*, and it has been the subject of several previous phytochemical studies (Bhattacharyya et al., 1981, 1983, 1984; Bulhoses et al., 1976; Cave et al., 1971; Fouraste et al., 1973; Keita et al., 1985; Moulis et al., 1981; Rouffiac et al., 1969). To the best of our knowledge, none of the *Monnieria* sp. have been investigated for their bioactivities. In the present communication, we describe the A2780 guided-separation of twelve alkaloids, the structure elucidation of the two new alkaloids 7-(2'-hydroxy-3'-chloroprenyloxy)-4,8-dimethoxyfuroquinoline (**1**) and 6-(2'-hydroxy-3'-chloroprenyloxy)-4,7-dimethoxyfuroquinoline (**2**), and the reassignment of the structure of a previously isolated compound as **13**.

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2. Results and discussion

Compound **1** was obtained as pale yellow powder. The EIMS of **1** showed the presence of a molecular ion peak at m/z 366 $[M+1]^+$ corresponding to the molecular formula $C_{18}H_{20}ClNO_5$, together with a peak at m/z 368 (34% relative to the molecular ion peak) due to ^{37}Cl isotope, which confirmed the presence of a chlorine atom. The UV spectrum exhibited absorptions at λ_{max} 249 nm and a broad band in the region 300–345 nm, typical of a furoquinoline alkaloid. The 1H NMR spectrum of **1** showed signals for two olefinic doublets, two methoxy groups, two methyl groups, one oxygenated methine, one oxygenated methylene, and two aromatic doublets. The 1H and ^{13}C NMR spectral data (Table 1) of **1** were similar to those of **3**, except for the C_5 side chain at C-7. The HMBC correlations from H-5 (δ 7.95, d, J = 9.3 Hz) and 4-OMe (δ 4.43, s) to C-4 (δ 156.7), and 8-OMe (δ 3.94, s) to C-8 (δ 141.8), together with the ROESY correlations between H-2 (δ 8.00, d, J = 2.8 Hz) and H-3 (δ 7.45, d, J = 2.8 Hz), H-3 and 4-OMe, H-5 and H-6 (δ 7.44, d, J = 9.3 Hz), and H-6 and H₂-1' (δ 4.49, dd, J = 2.8, 10.2 Hz; 4.14, dd, J = 7.5, 10.2 Hz) demonstrated that **1** was a 4,8-dimethoxyfuroquinoline alkaloid substituted at C-7 (Figure 1). HMBC correlations were observed from 2'-OH (δ 5.77, d, J = 6.1 Hz) to C-1' (δ 71.3), C-2' (δ 75.9), and C-3' (δ 73.0), and from H₃-5' (δ 1.64, s) to C-2', C-3', and C-4' (δ 27.9). Therefore, based on the EIMS spectrum of **1**, the functional group at C-3' of the side chain at C-7 must be -Cl, and the substituent at C-7 was then established as 2'-hydroxy-3'-chloro-3'-methyl butoxy. Hence, the structure of **1** was determined as shown.

The EIMS of **2** also showed a molecular ion peak at m/z 366 $[M+1]^+$ corresponding to the molecular formula $C_{18}H_{20}ClNO_5$, together with a peak at m/z 368 (36% relative to the molecular ion peak) due to ^{37}Cl isotope, which demonstrated that **2** and **1** were isomers. The 1H NMR spectrum (Table 1) of **2** exhibited peaks for two olefinic doublets, two methoxy groups, two methyl groups, one oxygenated methine, one oxygenated methylene, and two aromatic singlets. In the HMBC spectrum, 3J correlations from 4-OMe (δ 4.43, s) to C-4 (δ 155.0), 7-OMe (δ 3.93, s) to C-7 (δ 152.4), and H-5 (δ 7.49, s) to C-4 were observed, and in the ROESY spectrum, correlations between H-2 (δ 7.95, d, J = 2.8 Hz) and 4-OMe to H-3 (δ 7.42, d, J = 2.8 Hz), and 7-OMe and H-8 (δ 7.29, s) were exhibited. The ROESY correlation between H-5 and H₂-1' (δ 4.40, dd, J = 2.2, 10.2 Hz; 4.06, dd, J = 7.5, 10.2 Hz) indicated that 2'-hydroxy-3'-chloro-3'-methyl butoxy was located at C-6. Hence, the structure of **2** was determined as 6-(2'-hydroxy-3'-chloroprenyloxy)-4,7-dimethoxyfuroquinoline.

Neither compound **1** nor **2** showed any optical rotation, suggesting that both were racemic. This observation, coupled with the fact that the isolation of chlorine-containing plant products is unusual, made it important to demonstrate that compounds **1** and **2** were not artefacts caused by ring opening of an epoxide with HCl. The crude extract was thus examined by LC-MS. A peak with the same retention time and molecular ion as compounds **1** and **2** was detectable in this crude extract, demonstrating that these compounds were present in the crude extract and were not an artifact of isolation. Since the extract was prepared by simple room temperature extraction of the plant material with methanol, and had never been treated with HCl, it is unlikely that hydrochlorination could have occurred at this stage, and the compounds are thus presumably genuine natural products. Although chlorinated prenyl groups are rare, they are not unknown. As one example *Toddalia asiatica*, also a member of the Rutaceae family, yielded the chlorinated prenyl derivative 5,7-dimethoxy-6-(3'-chloro-2'-hydroxy-3'-methylbutyl) coumarin (Sharma et al., 1981).

The structure of 6-(2'-hydroxy-3'-chloroprenyloxy)-4,7-dimethoxyfuroquinoline (**2**) was previously published under the name chlorodesnkolbisine (compound **AR5**, Al-Rehaily et al., 2003). A comparison of the NMR data of **2** with those of **AR5** indicated that the two compounds were different. A sample of **AR5** was then supplied by Dr. Al-Rehaily and investigated by MS

and NMR spectroscopy. These data indicated that it was in fact nkolbisine (**13**) (Ayafor et al., 1982).

The structures of the ten known compounds isolated in this study were determined to be chaplophytin-B (**3**) (Ali et al., 2001), 7-(2'-hydroxy-3'-methoxy-prenyloxy)-4,8-dimethoxyfuroquinoline (**4**) (Akhmedzhanova et al., 1975), 7-isopentenylloxy- γ -fagarine (**5**) (Dreyer, 1969), skimmianine (**6**) (Chakravarty et al., 1999), 4,8-dimethoxyfuro[2,3-*b*]quinoline (**7**) (Ito et al., 2004), tecleanatalesine B (**8**) (Tarus et al., 2005), methylnkolbisine (**9**) (Al-Rehaily et al., 2003), 1-hydroxy-2,3-dimethoxy-10-methylacridin-9(10*H*)-one(**10**) (Bergenthal et al., 1979), sesamin (**11**) (Hsieh et al., 2005), and fargesin (**12**) (Brown et al., 2001) by comparison of their spectroscopic data with literature data.

All the isolates were tested against A2780 human ovarian cancer cell line. Compounds **1**, **2**, **5**, **6**, **8**, **9**, and **10** showed weak antiproliferative activity with IC₅₀ values of 13, 9, 13, 13, 16, 14, 12 μ g/mL, respectively.

3. Experimental

3.1. General

Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 and an Inova 400 spectrometer. The chemical shifts are given in δ (ppm), and constants are reported in Hz. Mass spectra were obtained on a JEOL JMS-HX-110 instrument, in the positive ion mode. HPLC was performed on a Shimadzu LC-10AT instrument with a semi-preparative phenyl Varian Dynamax column (5 μ m, 250 $\times$ 10 mm) and a preparative C₁₈Varian Dynamax column (8 μ m, 250 $\times$ 21.4 mm). Finnigan LTQ LC/MS was also used for EIMS analysis of compounds **1**, **2**, and **13**.

3.2. Plant material

A collection of stems, twigs, leaves, and flowers of *Ertela trifolia* (L.) Kuntze (also known as *Monnieria trifolia*) was made in late 2000 at Solan; its local name is: Kofimaka. Voucher specimens have been deposited at the National Herbarium of the University of Suriname, Paramaribo, Suriname.

3.3. Extraction and isolation

The plant samples were dried, ground, and extracted with MeOH to give extracts BGVS 320017. Extract BGVS 320017 (1.9 g, IC₅₀ 20 μ g/mL) was suspended in aqueous MeOH (MeOH-H₂O, 9:1, 100 mL) and extracted with hexanes (3 $\times$ 100 mL portions). The aqueous layer was then diluted to 70% MeOH with H₂O and extracted with CH₂Cl₂ (3 $\times$ 100 mL portions). The CH₂Cl₂ extract (896 mg) was active with an IC₅₀ of 15 μ g/mL, while both the hexane and aqueous MeOH extracts were inactive. The CH₂Cl₂ extract was chromatographed on an open C₁₈ column (130 $\times$ 22 mm) using H₂O-MeOH-CH₂Cl₂ (80:20:0 to 0:100:0, then 0:80:20) to yield three fractions, I [80 mg, inactive], II [700 mg, IC₅₀ 12 μ g/mL], and III [100 mg, inactive]. Fraction II furnished 12 subfractions (A-L) after HPLC separation on a C₁₈ column (60% MeOH/H₂O in 30 min, then to 100% MeOH/H₂O in 10 min, 10 mL/min). Further separation of subfractions B-F and H-K was carried out by preparative Si gel TLC to afford 12 compounds. Subfraction B yielded compound **3** [1.2 mg, CHCl₃-MeOH (100:2), *R*_f 0.2]; subfraction C, **7** [1.2 mg, CHCl₃-MeOH (100:2), *R*_f 0.4] and **12** [0.6 mg, CHCl₃-MeOH (100:2), *R*_f 0.3]; subfraction D, **6** [0.6 mg, CHCl₃-MeOH (100:2), *R*_f 0.3]; subfraction E, **9** [0.8 mg, CHCl₃-MeOH (100:2), *R*_f 0.5] and **10** [1.1 mg, CHCl₃-MeOH (100:2), *R*_f 0.7]; subfraction F, **4** [1.1 mg, CHCl₃ (with 0.5% MeOH), *R*_f 0.15]; subfraction H, **2** [0.6 mg, CHCl₃-MeOH

(100:2), R_f 0.5] and **11** [0.6 mg, CHCl₃-MeOH (100:2), R_f 0.2]; subfraction I, **1** [1 mg, CHCl₃-MeOH (100:1), R_f 0.3]; and subfractions J and K, **5** [1 mg, CHCl₃, R_f 0.1] and **8** [1 mg, CHCl₃, R_f 0.15].

3.3.1. 7-(2'-Hydroxy-3'-chloroprenyloxy)-4,8-dimethoxyfuroquinoline (1)—Light yellow powder. $[\alpha]_D^{20}$ 0° (c; 0.10 in MeOH); UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 249 (4.68), broad band in region 300–345 nm [320 (3.97)]; IR (film) $\nu_{\max}$ cm⁻¹: 2934, 1590, 1474, 1363, 1251, 1084, 1016; ¹H NMR (500 MHz, CDCl₃): 1.68 (3H, s, H₃-4'), 1.69 (3H, s, H₃-5'), 3.31 (1H, d, J = 4.0 Hz, 2'-OH), 4.02 (1H, m, H-2'), 4.14 (3H, s, 8-OMe), 4.26 (1H, dd, J = 8, 10.2 Hz, H-1'), 4.45 (3H, s, 4-OMe), 4.56 (1H, dd, J = 3.2, 10.2 Hz, H-1'), 7.07 (1H, d, J = 2.8 Hz, H-3), 7.25 (1H, d, J = 9.2 Hz, H-6), 7.61 (1H, d, J = 2.8 Hz, H-2), 8.03 (1H, d, J = 9.2 Hz, H-5); ¹H (500 MHz, DMSO-*d*₆): and ¹³C (125 MHz, DMSO-*d*₆) NMR: see Table 1; (+)-EIMS m/z (rel. int. %): 368 (34), 366 (100); HRFABMS m/z 366.1077 (calcd for C₁₈H₂₁ClNO₅, 366.1108).

3.3.2. 6-(2'-Hydroxy-3'-chloroprenyloxy)-4,7-dimethoxyfuroquinoline (2)—Light yellow powder. $[\alpha]_D^{20}$ 0° (c; 0.06 in MeOH); UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 247 (4.57), broad band in region 300–345 nm [320 (4.03)]; IR (film) $\nu_{\max}$ cm⁻¹: 2935, 1617, 1499, 1375, 1255, 1093; ¹H NMR (500 MHz, CDCl₃): 1.72 (3H, s, H₃-4'), 1.73 (3H, s, H₃-5'), 3.00 (1H, d, J = 4.4 Hz, 2'-OH), 4.00 (3H, s, 7-OMe), 4.14 (1H, m, H-2'), 4.20 (1H, dd, J = 7.6, 10.2 Hz, H-1'), 4.45 (3H, s, 4-OMe), 4.49 (1H, dd, J = 2.4, 10.2 Hz, H-1'), 7.05 (1H, d, J = 2.8 Hz, H-3), 7.34 (1H, s, H-8), 7.57 (1H, s, H-5), 7.58 (1H, d, J = 2.8 Hz, H-2); ¹H (500 MHz, DMSO-*d*₆): and ¹³C (125 MHz, DMSO-*d*₆) NMR: see Table 1; (+)-EIMS m/z (rel. int. %): 368 (36), 366 (100); HRFABMS m/z 366.1077 (calcd for C₁₈H₂₁ClNO₅, 366.1108).

3.4. Antiproliferative Bioassays

The A2780 ovarian cancer cell line assay was performed on compounds **1–4** at Virginia Polytechnic Institute and State University as previously described (Cao et al., 2007). The A2780 cell line is a drug - sensitive human ovarian cancer cell line (Louie et al., 1985).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

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Figure 1.
Key HMBC and ROESY correlations for **1**

Figure 2.
Key HMBC and ROESY correlations for 2

Figure 3.

Table 1

^1H and ^{13}C NMR spectral data for compounds **1**, **2**, and **13** ($\text{DMSO}-d_6$)

no.	$^1\text{H}^a$			no.	$^{13}\text{C}^b$		
	1	2	13		1	2	13
2	8.00 d (2.8)	7.95 d (2.8)	7.90 d (2.6)	2	143.8	143.0	143.6
3	7.45 d (2.8)	7.42 d (2.8)	7.38 d (2.8)	3	105.4	105.2	105.9
3a				3a	114.2	101.8	102.5
4				4	156.7	155.0	155.7
4a				4a	101.8	112.0	112.8
5	7.95 d (9.3)	7.49 s	7.44 s	5	117.7	101.1	101.4
6	7.44 d (9.3)			6	113.8	146.6	147.7
7				7	151.4	152.4	153.1
8		7.29 s	7.26 s	8	141.8	106.6	107.0
8a				8a	140.8	141.8	142.3
9a	4.43 s	4.43 s	4.40 s	9a	163.8	162.5	163.1
4-OMe				4-OMe	59.2	59.2	59.8
7-OMe	3.94 s	3.93 s	3.90 s	7-OMe	60.8	55.5	56.2
8-OMe	4.49 dd (2.8, 10.2)	4.40 dd (2.2, 10.2)	4.30 dd (2.2, 10.2)	8-OMe	71.3	70.2	71.6
1'	4.14 dd (7.5, 10.2)	4.06 dd (7.5, 10.2)	4.06 dd (7.5, 10.2)	1'			
2'	3.91 m	3.92 m	3.92 m	2'	75.9	75.4	76.3
3'				3'	73.0	73.0	71.0
4'	1.59 s	1.60 s	1.12 s	4'	27.9	27.8	24.8
5'	1.64 s	1.64 s	1.16 s	5'	29.6	29.5	27.9
2'-OH	5.77 d (6.1)	5.78 d (6.3)	5.16 d (5.5)	2'-OH			
3'-OH			4.59 s				

^a δ (ppm) 500 MHz; multiplicities: *J* values (Hz) in parentheses.

^b δ (ppm) 125 MHz.