New Cytotoxic Alkaloids from the Wood of *Vepris punctata* from the Madagascar Rainforest[1]

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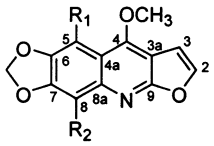
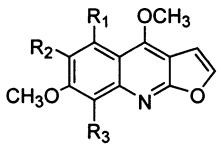
Bioassay-guided fractionation of a CH_2Cl_2/MeOH extract of the wood of *Vepris punctata* resulted in the isolation of three new furoquinoline alkaloids, 5-methoxymaculine (**1**), 5,8-dimethoxymaculine (**2**), and 4,5,6,7,8-pentamethoxyfuroquinoline (**3**), in addition to the four known alkaloids flindersiamine (**4**), kokusaginine (**5**), maculine (**6**), and skimmianine (**7**). The structures of the new alkaloids **1**−**3** were established on the basis of extensive 1D and 2D NMR spectroscopic data interpretation. All the isolated compounds were tested against the A2780 human ovarian cancer cell line, and all seven alkaloids showed weak cytotoxic activity.

In our continuing search for bioactive molecules from the Suriname and Madagascar rainforests as part of an International Cooperative Biodiversity Group (ICBG) program,[2] we obtained a sample of a cytotoxic extract of the wood of the plant *Vepris punctata* (I. Verd.) W. Mziray (Rutaceae) collected in Madagascar.

The genus *Vepris* Comm. ex A. Juss. (which includes the genus formerly known as *Teclea*[3,4]) comprises about 80 species that occur in tropical Africa, Zanzibar (now part of Tanzania), Madagascar, the Mascarenes, tropical Arabia, and southwest India. It is a rich source of alkaloids[5−10] and also of limonoids.[11,12] The extract of *V. punctata* was selected for bioassay-guided fractionation based on its cytotoxicity, with an IC_{50} of 25.5 μg/mL against the A2780 cell line, and because of the absence of any reported phytochemistry of the species. The crude extract, after extensive chromatography over Sephadex LH-20 and MCI gel, followed by reversed-phase HPLC, furnished the three new alkaloids **1**−**3**, in addition to the four known alkaloids **4**−**7**.



1 $R_1 = OCH_3$; $R_2 = H$
2 $R_1 = R_2 = OCH_3$
4 $R_1 = H$; $R_2 = OCH_3$
6 $R_1 = R_2 = H$



3 $R_1 = R_2 = R_3 = OCH_3$
5 $R_1 = R_3 = H$; $R_2 = OCH_3$
7 $R_1 = R_2 = H$; $R_3 = OCH_3$

Table 1. NMR Data for Compounds **1**−**4** ($CDCl_3$)[a]

	1		**2**		**3**		**4**
position	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^{13}C
2	7.62 d, 2.7	143.1	7.57 d, 2.7	143.4	7.60 d, 2.7	143.6	143.1
3	7.04 d, 2.7	103.8	7.01 d, 2.7	103.9	7.05 d, 2.7	104.2	104.4
3a		113.8		114.6		112.8	115.0
4		156.8		160.4		162.7	156.2
4a		104.3		106.2		108.6	103.0
5		146.8		145.4		147.3	92.5
6		137.2		139.6		140.2	138.2
7		142.6		138.6		139.2	137.9
8	7.15 s	102.9		130.4		129.2	136.0
8a		148.2		146.6		145.6	146.8
9		162.6		162.6		162.8	162.8
OCH_3-4	4.40 s	61.6	4.39 s	61.1	4.44 s	61.4	60.7
OCH_3-5	4.17 s	59.5	4.18 s	60.3	4.15 s	60.2	
OCH_3-6					3.99 s	59.5	
OCH_3-7					3.91 s	59.5	
OCH_3-8			4.25 s	57.6	4.26 s	58.6	59.0
OCH_2O	6.05 s	101.4	6.05 s	101.8			101.6

[a] Assignments made on the basis of COSY, HMQC, and HMBC and comparison with the literature data.[5−10,13−15]

Initial liquid−liquid extraction of the crude extract indicated that the bioactivity was concentrated in the *n*-hexane- and $CHCl_3$-soluble portions of the *n*-hexane−aqueous MeOH and $CHCl_3$−aqueous MeOH partitions. The *n*-hexane- and $CHCl_3$-soluble portions were combined on the basis of their similar nature on TLC and almost identical cytotoxicity values. These combined fractions were purified by column chromatography over LH-20, followed by chromatography over MCI gel, and finally by reversed-phase HPLC. This process furnished the three new alkaloids **1**−**3**, in addition to the four known alkaloids **4**−**7**. The structures of the four known alkaloids were identified as flindersiamine (**4**), kokusaginine (**5**), maculine (**6**), and skimmianine (**7**).[5,13−15] Since the ^{13}C NMR spectral data of flindersiamine (**4**) are not reported in the literature, the carbon values were assigned on the basis of HMBC and HMQC spectral data and are given in Table 1.

Compound **1** was isolated as a colorless viscous liquid, and its molecular formula was deduced as $C_{14}H_{11}NO_5$ from HRFABMS, ^{13}C NMR, and DEPT spectra. Its UV absorption at 248, 262, 332, and 355 nm suggested a furoquinoline alkaloid skeleton.[5] The ^1H NMR spectrum showed the

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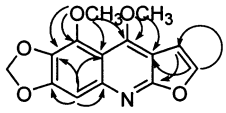

Figure 1. Selected HMBC correlations for **1**.

presence of two aromatic protons represented by a pair of AB doublets (J = 2.7 Hz) centered at δ 7.04 and 7.62, a singlet at δ 6.05 corresponding to a methylenedioxy group, an olefinic proton at δ 7.15, and two methoxy groups at δ 4.40 and 4.17, almost identical to that of the known alkaloid flindersiamine (**4**).[5] The ^{13}C NMR values of **1** were assigned on the basis of DEPT, HMQC, and HMBC spectra and are given in Table 1, which showed the presence of 14 carbons as for **4**. A close comparison of the ^{13}C NMR values of **1** with those of **4** suggested that compound **1** was also a furoquinoline alkaloid like **4**. The HMBC correlations of **1** (Figure 1) showed that there is no change in the position of the methylenedioxy group, while the second methoxy group is located at C-5 as compared with C-8 in **4**. Thus, the structure of **1** was established as 5-methoxymaculine.

The molecular formula of compound **2** was deduced as $C_{15}H_{13}NO_6$ from HRFABMS and ^{13}C NMR spectral data. Its UV and IR spectra were almost identical to those of **1**, suggesting its furoquinoline alkaloid nature. The ^{1}H NMR spectrum of **2** showed the presence of a pair of AB doublets characteristic for the H_α and H_β of the furan ring, a methylenedioxy group, and three methoxy groups, suggesting the presence of an additional methoxy group in **2** compared to **1**. The absence of any other aromatic protons indicated the possible placement of the additional methoxy group in **2** at the C-8 position. The presence of the additional methoxy group at this position was further supported by HMBC (H-3/C-2, C-3a, C-9; OCH_3-4/C-3a, C-4, C-4a; OCH_3-5/C-4a, C-5, C-6; OCH_3-8/C-7, C-8, C-8a) correlations. The ^{13}C NMR values for all the carbons were assigned on the basis of the HMQC and HMBC spectra and were in good agreement with the proposed structure. On the basis of the above spectral data, the structure of **2** was assigned as 5,8-dimethoxymaculine.

The molecular formula of compound **3** was determined to be $C_{16}H_{17}NO_6$ by HRFABMS. The UV absorptions of **3** suggested that it also belonged to the class of furoquinoline alkaloids similar to **1** and **2**. The ^{1}H NMR spectrum showed the presence of two doublets at δ 7.60 (J = 2.7 Hz) and 7.05 (J = 2.7 Hz), for the H_α and H_β of the furan ring, respectively, in addition to five methoxy singlets at δ 4.44, 4.26, 4.15, 3.99, and 3.91. The presence of two additional singlets at δ 3.99 and 3.91 and the absence of the singlet at about δ 6.0 indicated that two methoxy groups in **3** had replaced the methylenedioxy group in **2**. The structure was supported by the ^{13}C NMR values as shown in Table 1. On the basis of the above spectral data, **3** was assigned as 4,5,6,7,8-pentamethoxyfuroquinoline.

All the isolated compounds (**1**−**7**) were tested in duplicate for cytotoxicity against the A2780 human ovarian cancer cell line. All seven alkaloids showed cytotoxic activity, with IC_{50} values in the range 2.8−4.2 μg/mL (Table 2).

Table 2. Cytotoxicities of Compounds **1**−**7**[a]

compound	IC_{50} (μg/mL)
1	2.8
2	3.5
3	3.4
4	3.3
5	3.5
6	4.0
7	4.2

[a] Concentration of each compound that inhibited 50% (IC_{50}) of the growth of the A2780 mammalian cell line according to the procedure described,[2,16] with actinomycin D (IC_{50} 1−3 ng/mL) as the positive control.

Experimental Section

General Experimental Procedures. 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 spectrometer. Mass spectra were obtained on a JEOL HX-110 instrument. The chemical shifts are given in δ (ppm) with TMS (tetramethylsilane) as internal reference. Sephadex LH-20 and MCI gel were used for column chromatography. HPLC was performed on a Shimadzu LC-10AT instrument with an ODS A323 column (250 × 10 mm).

Cytotoxicity Bioassays. The A2780 ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported.[2,16]

Plant Material. The wood sample of *Vepris punctata* was collected in January 2000 1.5 km SE of Ankosy, outside of Zahamena National Park in the province of Toamasina in eastern Madagascar. The collection was made by R. Rakotondrajaona, C. Birkinshaw, S. Randrianasolo, L. M. Randrianjanaka, and L. P. Rakotosoa from a 7 m tall tree with a distinct citrus odor. The voucher specimen is R. Rakotondrajaona et al. 118, and duplicates are deposited at Centre National d'Application et des Recherches Pharmaceutiques in Antananarivo, Madagascar; Missouri Botanical Garden, St. Louis, Missouri; Museum National d'Histoire Naturelle Herbarium in Paris, France; and Parc de Tsimbazaza Herbarium in Antananarivo, Madagascar.

Extract Preparation. The wood of *V. punctata* was dried, ground, and extracted with MeOH to give the dried methanolic extract MG165.

Extraction and Isolation. The crude extract (1.0 g) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 400 mL) and extracted with *n*-hexane (3 × 300 mL). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with $CHCl_3$ (3 × 300 mL). The aqueous layer was concentrated, and the residue obtained was suspended in H_2O (25 mL) and extracted with *n*-BuOH (3 × 25 mL). The *n*-hexane and $CHCl_3$ extracts were found to be cytotoxic and were combined on the basis of their almost identical activities and TLC patterns. The combined fraction (0.65 mg) on column chromatography over Sephadex LH-20 using *n*-hexane/EtOAc (100:0 to 0:100) and then MeOH/H_2O (100:0 to 40:60) furnished eight fractions (A−H), of which fractions D and F were found to be the most active. Fraction D yielded several new and known terpenoids. Fraction F on column chromatography over MCI gel using MeOH/H_2O (80:20) furnished three active fractions F_1, F_2, and F_3. Fractions F_1 and F_2 yielded additional terpenoids, while fraction F_3 on reversed-phase HPLC with the mobile phase CH_3CN−H_2O (85:15) afforded the three new alkaloids **1** (1.2 mg), **2** (1.6 mg), and **3** (1.1 mg), along with the four known compounds **4** (1.3 mg), **5** (1.2 mg), **6** (1.0 mg), and **7** (0.9 mg). The structures of the known compounds **4**−**7** were identified by comparison of their spectral data with literature values.[13−15]

5-Methoxymaculine (1): colorless viscous oil; UV (MeOH) λ_{max} 248 nm (ϵ 21 410), 262 (46 000), 332 (10 000), 355 (4300) nm; IR ν_{max} 3420, 3135, 1670, 1570, 1450, 925 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 274.0735 [M + H]$^+$ (calcd for $C_{14}H_{12}NO_5$, 274.0715).

5,8-Dimethoxymaculine (2): colorless viscous oil; UV (MeOH) λ_{max} 242 (ϵ 20 800), 258 (44 300), 330 (12 400), 350 (6400) nm; IR ν_{max} 3440, 3150, 1650, 1610, 1555, 1450, 935 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 304.0826 [M + H]$^+$ (calcd for $C_{15}H_{14}NO_6$, 304.0818).

4,5,6,7,8-Pentamethoxyfuroquinoline (3): colorless viscous oil; UV (MeOH) λ_{max} 244 (ϵ 24 500), 262 (46 000), 327 (9100), 352 (4200) nm; IR ν_{max} 3430, 3145, 1670, 1560, 1440, 940 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 320.0760 [M + H]$^+$ (calcd for $C_{16}H_{18}NO_6$, 320.0766).

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Supporting Information Available: ^{1}H NMR spectra for compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org

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