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Short communication

Acridone alkaloids from *Vepris verdoorniana* (Excell & Mendonça) Mziray (Rutaceae)

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ABSTRACT

Two new acridone alkaloids, verdoocridone A (**1**) and B (**4**), together with fifteen known compounds were isolated from methanol extracts of the roots and leaves of *Vepris verdoorniana*. The structures of all compounds were determined by comprehensive spectroscopic analyses (1D and 2D NMR, EI- and ESI–MS). The ^{13}C NMR values of 1,2,3,5-tetramethoxy-*N*-methylacridone (**2**) and 5-methoxyaborinine (**3**) are also reported. The crude extracts and compounds (**1**-**6**) were tested for their antimicrobial activity. The test delivered moderate activities for crude extracts and compounds **1**, **5** and **6** against the bacterium *Staphylococcus aureus* and the fungi *Mucor meihei* and *Candida albicans* with MIC values between 115 and 180 μg/mL for extracts and between 21.3 and 29.4 μM for compounds, compared to gentamycin with 0.2 μM and nystatin with 5.2 μM against both fungi. The determination of the radical scanvenging activity using 1,1-dephenyl-2-picrylhydrazyl (DPPH) assay gave moderate antioxidant values for all tested compounds, with IC_{50} between 0.29 and 0.41 μM, compared to the standard 3-t-butyl-4-hydroxyanisole (BHA) displaying 0.03 μM.

1. Introduction

Vepris verdoorniana (Excell & Mendonça) Mziray (Rutaceae), synonym *Teclea verdoorniana* Excell & Mendonça, is a shrub or small tree up to 22 m tall, the trunk is up to 1.2 m in girth. The species is endemic to Western and Central Africa, streching along the coast line from Liberia over Nigeria to Cameroon and Gabon and then westwards to the Central African Republic, Eastern Democratic Republic of Congo and Western *Uganda*. Various plant parts are used in traditional African medicine to treat fever, malaria, colds, conjunctivitis, arterial hypertension, tape-worm and cough (Nordeng et al., 2013; Rasoanaivo et al., 1999; Chhabra et al., 1991; Gurib-Fakim et al., 1993). In Uganda, the wood is used to make walking sticks, spear shafts and bark-cloth mallets. The leafy twigs, containing an inflammable resin, are bundled to serve as torches. To the best of our knowledge, no phytochemical studies had yet been carried out on this species, while other more than fifteen species of the genus revealed the presence of acridones, furoquinolines, quinolones, amides, azole, coumarins, fatty acids, flavonoids, indoloquinazolines, limonoids, lignans, phenolic compounds and terpenoids (Ayafor et al., 1980, 1982a, 1982b, 1982c; Khalid and Waterman, 1982; Brader et al., 1996; Rasoanaivo et al., 1999; Chaturvedula et al., 2003; Cheplogoi et al., 2008; Kiplimo et al., 2012; Kiplimo and Koorbanally, 2012). Interestingly, some compounds isolated from above chemical classes exhibit potent antibacterial, antioxidant, antimalarial and cytotoxic activities (Rasoanaivo et al., 1999; Chaturvedula et al., 2003; Cheplogoi et al., 2008; Kiplimo et al., 2012; Kiplimo and Koorbanally, 2012). In this article, we report the isolation and structural elucidation of two new acridone alkaloids, verdoocridone A (**1**) and B (**4**), together with the ^{13}C NMR values of 1,2,3,5-tetramethoxy-*N*-methylacridone (**2**) and 5-methoxyaborinine (**3**).

2. Results and discussion

The roots and the leaves of *V. verdoorniana* were separately extracted with MeOH and subjected to vacuum liquid

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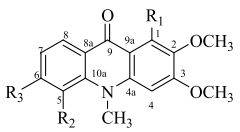
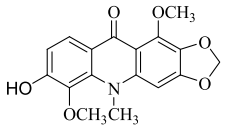


1. $R_1 = R_2 = R_3 = OCH_3$

2. $R_1 = R_2 = OCH_3$; $R_3 = H$

3. $R_1 = OH$; $R_2 = OCH_3$; $R_3 = H$



4.

Fig. 1. Structures of some isolated compounds.

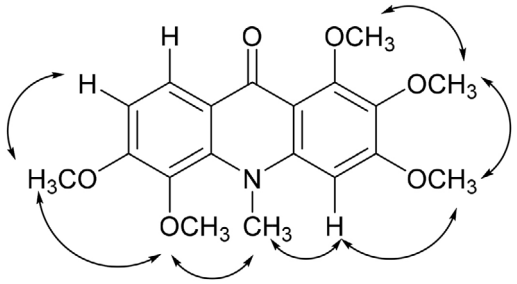


Fig. 2. Selected NOESY correlations of compound 1.

chromatography (VLC), column chromatography carried out on silica gel and preparative thin layer chromatography (pTLC) to afford two new acridone alkaloids and fifteen known compound (Fig. 1). By comparison with the reported data, the known compounds were identified as 1,2,3,5-tetramethoxy-*N*-methylacridone (**2**), 1-hydroxy-2,3,5-trimethoxy-*N*-methylacridone (**3**), 6-methoxytecleanthine (**5**), tecleanthine (**6**), citracridone II (**7**), 5-methoxynoracronycine (**8**), citropsine A (**9**), scopoletin (**10**), limonin (**11**), 24R-ethyl-5α-cholestane-3*β*,6α-diol (**12**), *β*-sitosterol, stigmasterol, stigmasterol-3-*O*-*β*-D-glucopyranoside, *β*-sitosterol-3-*O*-*β*-D-glucopyranoside and lupeol (Rasoanaivo et al., 1999; Wijeratne et al., 1992; Bowen et al., 1980; Mbaze et al., 2010; Happi et al., 2011; Fomani et al., 2016; Chaurasia and Wichti, 1987), while the new acridone alkaloids were identified by comprehensive spectroscopic analyses.

Compound **1** was obtained as a yellow amorphous powder. The molecular ion was found to be $C_{19}H_{21}O_6N$ by HR-EIMS ($[M]^+$ at *m/z* 359.1360, calcd. 359.1369). The IR spectrum exhibited vibration bands due to a conjugated carbonyl group (υ_{max} 1633 cm^{-1}). The UV absorptions at 222, 265, 338 and 409 nm indicated **1** to be a 9-acridone derivative (Wansi et al., 2006).

The ^1H NMR spectrum (Table 1) exhibited five methoxy groups at δ_H 4.01 (*s*, OCH_3-1), 4.00 (*s*, OCH_3-3), 3.98 (*s*, OCH_3-6), 3.89 (*s*, OCH_3-2), and 3.78 (*s*, OCH_3-5), and a *N*-methyl group at δ_H 3.94 (*s*). In the aromatic region, a singlet at δ_H 6.59 (H-4) and an AB system of two aromatic protons at δ_H 8.18 (d, *J* = 7.2 Hz); 6.93 (d, *J* = 7.2 Hz) could be attributed to H-8 and H-7, respectively. These assignments were determined through the lower-field signal of H-8, which was deshielded by the adjacent carbonyl group of the acridone moiety (Wansi et al., 2006). This inference was confirmed by the ^{13}C NMR data (Table 1) and DEPT, displaying five methoxy groups at δ_C 61.9 (C-1), 61.3 (C-5), 61.2 (C-2), 56.4 (C-6) and 56.1 (C-1), an *N*-methyl group at δ_C 41.5 and a carbonyl at δ_C 176.6 (Naidoo et al., 2005). The positions of the five methoxy groups were determined by HMBC and NOESY spectra. The HMBC spectrum showed correlation between *N*-CH_3 (δ_H 3.94) and carbon signals at C-10a (δ_C 138.6); C-4a (δ_C 144.9) and in addition an important ^4J correlation with C-4 (δ_C 94.0) confirming the singlet proton at H-4 (δ_H 6.59). Furthermore, the proton H-4 (δ_H 6.59) displayed correlation with the carbon signals at C-9a (δ_C 112.4), C-2 (δ_C 138.0), C-4a (δ_C 144.9) and C-3 (δ_C 157.8) affirming the position C-2 and C-3 for two methoxy groups. The HMBC correlations between H-8 (δ_H 8.18) and carbon signals at C-7 (δ_C 107.4), C-8a (δ_C 121.4), C-10a (δ_C 138.6) and C-6 (δ_C 158.6) as well as between H-7 (δ_C 6.93) and carbon signals at C-8a (δ_C 121.4), C-8 (δ_C 123.6), C-5 (δ_C 137.1) and C-6 (δ_C 158.6) verified the position C-5 and C-6 for further two methoxy groups. The position of MeO-1 was reassured by the NOESY correlations indicating cross-peaks between *N*-CH_3 and H-4, H-4 and MeO-3, MeO-3 and MeO-2 as well as MeO-2 and MeO-1 and between *N*-CH_3 and MeO-5 and in addition between MeO-5 and MeO-6, as shown in Fig. 2. In addition, cross-peaks confirming the position C-5 and C-6 were observed as well between H-7 and MeO-6 and in addition between MeO-6 and MeO-5. From the above spectroscopic data, the structure of compound **1** was determined as 1,2,3,5,6-pentamethoxy-*N*-methylacridone and was named verdoocridone A.

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR assignments for (**1**-**4**) in $CDCl_3$.

Attribution	**1**		**2**	**3**	**4**	
	^1H	^{13}C	^{13}C	^{13}C	^1H	^{13}C
1	–	154.3	153.9	155.1	–	143.2
2	–	138.0	138.0	138.0	–	134.3
3	–	157.8	157.9	157.8	–	153.6
4	6.59 (s)	94.0	93.9	93.2	6.65 (s)	90.9
4a	–	144.9	145.0	145.2	–	144.9
5	–	137.1	149.7	149.5	–	134.3
6	–	156.8	114.8	114.9	–	153.6
7	6.93 (d, *J* = 7.2)	107.4	122.1	122.5	6.93 (d, *J* = 7.0)	111.2
8	8.18 (d, *J* = 7.2)	123.6	119.0	120.0	8.13 (d, *J* = 7.0)	124.5
8a	–	121.4	127.4	127.5	–	121.1
9	–	176.6	176.9	176.7	–	176.7
9a	–	112.4	112.7	112.3	–	112.5
10a	–	138.6	135.0	135.2	–	137.4
OCH_3-1	4.01 (s)	61.9	61.9	–	4.15 (s)	61.1
OCH_3-2	3.89 (s)	61.2	61.6	61.5	–	–
OCH_3-3	4.00 (s)	56.1	56.1	55.9	–	–
OCH_3-5	3.78 (s)	61.3	56.5	56.4	3.76 (s)	61.2
OCH_3-6	3.98 (s)	56.4	–	–	–	–
N-CH_3	3.94 (s)	41.5	42.4	42.1	3.91 (s)	40.5
O-CH_2-O	–	–	–	–	6.03 (s)	101.9

Assignments were based on HMQC, HMBC and NOESY experiments.

Compound **4** was obtained as a yellow amorphous powder. The molecular composition was found to be $C_{17}H_{15}NO_6$ by HR-EIMS ($[M]^+$, *m/z* 329.0890, calcd. 329.0899). This value was 30 mass units less than that of compound **1** suggesting the absence of two CH_3 in compound **4**. The UV absorption bands (220, 267, 340 and 425 nm) and the IR spectrum (1220, 1596, 1635, 1720, 3310, 3390 cm^{-1}) also suggested an acridone skeleton for compound **4** as well (Wansi et al., 2006).

The ^1H NMR, ^{13}C NMR, DEPT, COSY, HMQC and HMBC spectra showed the presence of the same aromatic spin systems as in compound **1** (Table 1). While the ^1H NMR spectrum revealed the presence of the free hydroxyl function at δ_H 6.28 (brs), which was exchangeable with D_2O and a singlet at δ_H 6.03 corresponding to a methylenedioxy group, the ^{13}C NMR and DEPT spectra confirmed the presence of the free hydroxy group at δ_C 153.6, and methylenedioxy at δ_C 101.9. The methylenedioxy revealed to be fixed to C-2 and C-3 by the HMBC correlation from H-4 (δ_H 6.65) to C-9a (δ_C 112.5), C-2 (δ_C 134.3), C-4a (δ_C 144.7) and to C-3 (δ_C 153.6), while the free hydroxy group was shown to be at C-6 position by the HMBC correlations between H-8 (δ_H 8.13) and carbon signals at C-7 (δ_C 111.2), C-8a (δ_C 121.1), C-10a (δ_C 137.4) and C-6 (δ_C 153.6) and further correlations between H-7 (δ_C 6.93) and carbon signals at C-8a (δ_C 121.1), C-8 (δ_C 124.5), C-5 (δ_C 134.3) and C-6 (δ_C 153.6). The position of MeO-1 was confirmed by the NOESY cross-peaks between *N*-CH_3 and H-4, H-4 and methylenedioxy as well as between methylenedioxy and MeO-1. Furthermore, cross-peaks confirming the position C-5 for the methoxy and C-6 for the