Two new anthraquinones from *Gladiolus psittascinus*

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1. Subject and source

Gladiolus psittascinus HOOK, an herbaceous plant, is propagated by its bulb. This onion-looking plant occurs in rocky places in western province of Cameroon. The bulbs are used to treat asthma, gonorrhoea, diabetes and intestinal parasites (Adjanohoun et al., 1990). The bulbs of *G. psittascinus* were collected in Dschang (West Province of Cameroon) in July 2005 and identified by Mr Francois Nana, a botanist at the National Herbarium, Yaounde where a voucher specimen (55925 HCN) is deposited.

We report here the isolation and structural elucidation from the chloroform extract of two new anthraquinones namely: 1,6,7-trihydroxy-3-methoxy-8-methyl-anthraquinone (**1**) and 1-hydroxy-3,6,7-trimethoxy-8-methyl-anthraquinone (**2**) along with four known compounds. The structures of the compounds were elucidated by spectroscopic analysis, mainly, 1D and 2D NMR and by comparing their physical (mp) and spectroscopic (Table 1) data with those reported in the literature.

2. Previous work

Phytochemical reports on the genus *Gladiolus* have revealed the occurrence of anthraquinones (Wang et al., 2003a,b; Chen et al., 2005), alkaloids (Viladomat et al., 1986), anthocyanidins (Seilleur, 1977) and flavonols (Salehian, 1973). To the best of our knowledge, there is no phytochemical report on *G. psittascinus*.

3. Present study

3.1. General procedure

Melting points (uncorr.) were recorded on a Reichter microscope. The IR and UV spectra were recorded on FT-IR Shimadzu Hyper 1.51 spectrophotometer and a Shimadzu UV-3101 PC spectrophotometer, respectively. ^{1}H NMR

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Table 1
^{1}H (coupling constant J in Hz in parentheses) and ^{13}C NMR data of 1,3,6-trihydroxy-8-methyl-anthraquinone (**3**); 1-hydroxy-3,6-dimethoxy-8-methyl-anthraquinone (**4**); 1,6-dihydroxy-3-methoxy-8-methyl-anthraquinone (**5**) and 1-hydroxy-3,6-dimethoxy-8-methyl-anthraquinone-7-carboxylic acid (**6**)

Position	**3**		**4**		**5**		**6**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	164.9		165.1		165.3		165.6	
2	108.2	6.64 (d, 2.4)	104.8	6.65 (d, 2.0)	107.7	6.66 (d, 2.5)	106.1	6.78 (d, 2.4)
3	163.9		165.5		165.7		165.5	
4	106.9	7.19 (d, 2.4)	106.4	7.20 (d, 2.0)	106.8	7.11 (d, 2.5)	106.7	7.21 (d, 2.4)
4a	134.9		137.2		135.0		132.7	
5	112.2	7.57 (d, 2.5)	110.3	7.54 (d, 2.2)	113.1	7.42 (d, 2.3)	111.6	7.72 (s)
6	161.7		158.2		162.8		160.8	
7	124.5	7.09 (d, 2.4)	123.4	7.08 (d, 2.2)	125.7	7.06 (d, 2.3)	134.4	
8	145.4		136.9		146.0		140.2	
8a	123.6		126.7		123.3		116.8	
9	188.6		186.0		189.0		185.4	
9a	110.7		111.8		111.7		114.4	
10	182.4		181.7		183.0		181.8	
10a	134.9		137.2		137.6		143.6	
1-OH		13.30		12.97		13.29		12.80
3-OMe			55.9	3.97 (s)	56.7	3.91 (s)	51.8	3.98 (s)
6-OMe			55.3	3.90 (s)			55.7	3.95 (s)
7- COOH							171.8	
8-Me	23.2	2.80 (s)	22.3	2.75 (s)	24.4	2.71 (s)	19.4	2.74 (s)

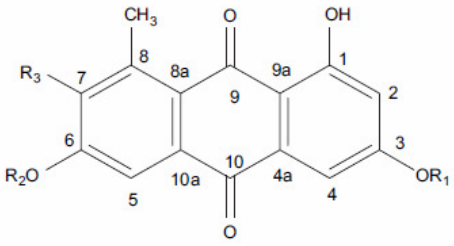
(300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded at room temperature in CD_3COCD_3 on a Bruker DMX300 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. Chemical shifts (δ) are reported in parts per million (ppm) with the residual solvent signals (δ_H 2.05 and δ_C 29.8 and 206.0), as internal reference. Coupling constants (J values) are given in Hertz. HMQC and HMBC experiments were recorded with gradient enhancements using sine shape gradient pulses. CIMS, HRCIMS spectra (direct inlet at 70 eV) were recorded on a JEOL JMS-700 spectrometer. Column chromatography and gel permeation were run on Merck silica gel 60 and Sephadex LH-20, respectively. Preparative TLC was done on 0.5 mm thick layer of silica gel (Merk 60 PF254). Analytical TLC was carried out on 0.25 mm thick layer of silica gel precoated on aluminium foil (Merk GF_{254}). Spots on chromatograms were detected by observing under UV light (254 nm) and were further visualized by spraying with 5% KOH in methanol.

3.2. Extraction, isolation and structural elucidation

Air dried and powdered bulbs of *G. psittascinus* (2 kg) were percolated (10 L × 3) (3 days) with methanol. Removal of the solvent in vacuo resulted in 300 g of extract. The extract was dissolved in a mixture of methanol/chloroform/water (2:2:1). The chloroform soluble portion (50 g) was subjected to column chromatography, eluted with a mixture of petroleum ether (60–80°) and acetone in increasing polarity to yield five fractions: I (15 g, petroleum ether–acetone 100:0 and 18:2), II (6 g, petroleum ether–acetone 4:1), III (4 g, petroleum ether–acetone 7:3), IV (7 g, petroleum ether–acetone 1:1) and V (6 g, acetone). TLC examination showed that fraction I did not contain compounds of interest and was not worked on further. Fractions II, III and IV were separately subjected to silica gel column chromatography (hexane–EtOAc gradient elution of increasing polarity). The resulting fractions were further purified on preparative TLC to yield pure compounds as follows. Fraction II gave 1,6-dihydroxy-3-methoxy-8-methyl-anthraquinone **5** (30 mg, Wang et al., 2003a). Fraction III yielded 1-hydroxy-3,6-dimethoxy-8-methyl-anthraquinone **4** (10 mg, Abdelfattah, 2004). Fraction IV afforded 1-hydroxy-3,6,7-trimethoxy-8-methyl-anthraquinone **2** (9 mg) and 1,6-dihydroxy-3-methoxy-8-methyl-anthraquinone-7-carboxylic acid **6** (20 mg, Ali et al., 1989). 1,6,7-Trihydroxy-3-methoxy-8-methyl-anthraquinone **1** (8 mg) and 1,3,6-trihydroxy-8-methyl-anthraquinone **3** (5 mg, Yagi et al., 1974) were obtained from fraction V by column chromatography eluted with $CHCl_3$–acetone–MeOH (20:1:1).

Compound **1** was obtained as yellow powder. The HR-EIMS of **1** showed a molecular ion peak at *m/z*: 300.0643 (calcd. for 300.0634) corresponding to the molecular formula $C_{16}H_{12}O_6$. The IR spectrum of **1** exhibited the presence of the following functionalities: hydroxyl (3386 cm^{-1}), conjugated unchelated carbonyl (1622 cm^{-1}), chelated quinone carbonyl (1578 cm^{-1}) and aromatic ring (1601, 1566 cm^{-1}). Along with the IR data, UV–Vis absorption maxima at 214, 289, 320, 401 nm, two carbonyl carbons resonating at δ 185.8 and 182.4 in the ^{13}C NMR spectrum and the generation of a pink colour on TLC when sprayed with methanolic potassium hydroxide indicated that **1** could be an anthraquinone (Huang and Yu, 2000). The ^{1}H NMR spectrum reveals signals for a pair of meta coupled aromatic protons at δ 7.40 and 6.70 (each d, $J = 2.4$ Hz), and signal from an isolated aromatic proton at δ 7.51 that could be assigned to H-4, H-2 and H-5, respectively. These assignments were in agreement with the HMBC correlations observed between H-2 and C-1 and C-3; H-4 and C-3 and 4a; H-5 and C-6, C-9a and C-8a. The HMBC correlation between the chelated hydroxyl group signal at δ 13.20 and C-9a (δ 113.8) was used to place the hydroxyl group on C-1. The signal at δ 2.56 was assigned to a methyl group on C-8 that is downfield shifted due to the deshielding effect of the neighbouring carbonyl group. This assignment was further confirmed from the HMBC correlations between the methyl signal and C-7 (δ 160.6), C-8 (δ 120.7) and C-8a (δ 130.8). The protons H-2, H-4 and a methoxyl group (δ 3.96) correlated with C-3 (δ 166.1) in the HMBC experiment, thus the methoxyl group could only be located on C-3. From the foregoing discussion compound **1** is established to be 1,6,7-trihydroxy-3-methoxy-8-methyl-anthraquinone which is reported here for the first time.

Compound **2** (Fig. 1) was obtained as orange powder and gave a molecular ion peak at *m/z* 328 in EIMS. Its molecular formula $C_{18}H_{16}H_6$ is established from the quasi-molecular ion peak at *m/z* 329.0955 $[M + H]^+$ in the positive HR-CIMS. The IR absorptions at ν_{max} 3380 (hydroxyl), 1620 and 1595 cm^{-1} (free and chelated quinone ketones), the UV–Vis absorptions at λ_{max} 218, 290, 318 and 400 nm and the visualization of **2** as pink spot on TLC after spraying with methanolic potassium hydroxide suggested an anthraquinone skeleton for compound **2**. General similarities were observed between the NMR data of compound **2** and compound **1**. Thus the ^{1}H NMR spectrum of **2** displayed signals for chelated hydroxyl group at δ 13.18, a pair of meta coupled aromatic protons at δ 6.78 (d, $J = 2.5$ Hz, H-2) and 7.18 (d, $J = 2.5$ Hz, H-4), an isolated aromatic proton at δ 7.63 (s, H-5) a deshielded aromatic methyl at δ 2.55 (C8-CH_3) and 3 methoxyls at δ 3.85, 3.81 and 3.80. The major difference between the NMR spectra of **1** and **2** is that **2** displays signals for two additional groups of methoxy protons. The molecular formula of **2** is higher by 48 amu than that of **1** indicating that **2** is *O*-methyl ether of **1**. The methoxy group resonating at δ 3.80 is associated with a carbon signal at δ 60.3 in the HMQC spectrum and showed HMBC cross peaks with C-8 and C-7, thus could only be located on C-7. HMBC correlations between signals at δ 3.81 and C-6, C-5 and C-7 were used to place the second methoxy on C-6.



	R_1	R_2	R_3
1	CH_3	H	OH
2	CH_3	CH_3	OCH_3
3	H	H	H
4	CH_3	CH_3	H
5	CH_3	H	H
6	CH_3	CH_3	COOH

Fig. 1. Anthraquinones from *G. psittascinus*.

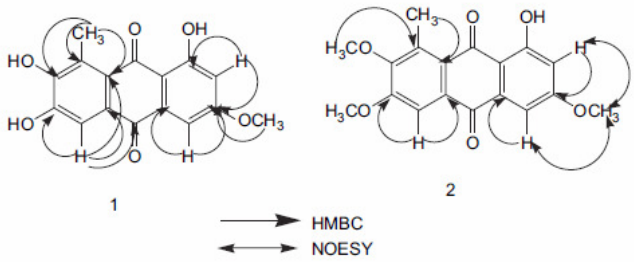


Fig. 2. Important HMBC and NOESY correlations.

The third methoxy group resonating at δ 3.85 was located on C-3 by virtue of its HMBC correlations with C-3, C-2 and C-4. Further confirmation for the assignment of the last methoxyl group was derived from NOE experiments. Thus, NOE irradiation of the signal at δ 3.85 resulted in inhacements of the signals due to H-2 and H-4 (Fig. 2). On the basis of the above evidences the new compound **2** was established to be 1-hydroxy-3,6,7-trimethoxy-8-methyl-anthraquinone.

*3.2.1. 1,6,7-Trihydroxy-3-methoxy-8-methyl-anthraquinone (**1**)*

Yellow powder (ethyl acetate); mp 236–238 °C. IR (KBr, cm^{-1}): 3386, 2900, 2850, 1620, 1601, 1578, 1566, 1350, 1271, 1223, 1161, 1104, 1037, 765, 760. UV–Vis λ_{max}^{MeOH} nm (log ε): 214 (4.01), 289 (3.97), 320 (3.61) and 401 (3.55). ^{1}H NMR (300 MHZ, CD_3COCD_3) δ: 13.20 (s, 1-OH), 6.70 (d, 1H, $J = 2.4$ Hz, H-2), 7.40 (d, 1H, $J = 2.4$ Hz, H-4), 7.51 (s, 1H, H-5), 2.56 (s, 3H, 8-Me), 3.96 (s, 3H, 3-OMe). ^{13}C NMR: see Table 2. EIMS *m/z* (rel. int. %): 300 $[M]^+$ (100), 244 (26), 241 (18), 226 (18). HREIMS *m/z*: 300.0643 (calcd. for $C_{16}H_{12}O_6$, 300.0634).

*3.2.2. 1-Hydroxy-3,6,7-trimethoxy-8-methyl-anthraquinone (**2**)*

Orange powder from diethyl ether; mp 275–276 °C. IR (KBr, cm^{-1}): 3380, 2900, 2850, 1660, 1620, 1601, 1578, 1566, 1353, 1270, 1223, 1161, 1104, 1037, 765, 760. UV–Vis λ_{max}^{MeOH} nm (log ε): 218 (3.98), 290 (3.95), 318 (3.58) and 400 (3.56). ^{1}H NMR (300 MHZ, CD_3COCD_3) δ: 13.18 (s, 1-OH), 6.78 (d, 1H, $J = 2.5$ Hz, H-2), 7.18 (d, 1H, $J = 2.5$ Hz, H-4), 7.63 (s, 1H, H-5), 2.55 (s, 3H, 8-Me), 3.85 (s, 3H, 3-MeO), 3.81 (s, 3H, 6-MeO), 3.80 (s, 3H,

Table 2
^{13}C NMR (75 MHz, CD_3COCD_3) data of **1** and **2**

Position	**1**	**2**
1	165.0	163.2
2	105.1	106.8
3	166.1	165.5
4	107.3	107.4
4a	136.1	136.3
5	110.3	108.8
6	150.6	156.0
7	160.6	154.5
8	120.7	120.2
8a	130.8	128.0
9	185.8	185.2
9a	113.8	113.7
10	182.4	183.3
10a	131.6	133.3
3-OMe	56.0	55.3
6-OMe		55.6
7-OMe		60.3
8-Me	15.2	15.1

7-MeO). ^{13}C NMR: see Table 2. EIMS *m/z* (rel. int. %): 328 $[M]^+$ (84), 300 (34), 272 (26), 269 (17), 254 (21), 223 (10). HREIMS *m/z*: 328.0955 (calcd. for $C_{18}H_{16}O_5$, 328. 328.0947).

4. Chemotaxonomic significance

The genus *Gladiolus* (Iridaceae) consists of 250 species. It is native to tropical Africa, coastlands along the Mediterranean Sea and South West Asia. 8-Methyl anthraquinones have been obtained from four families of plants: *Rhamnus fallax* (Rhamnaceae), *Aloe saponaria* (Liliaceae), *Aloe ferrox* (Liliaceae), *Asphodelus fistulosus* var. *teniufolus* (Liliaceae), *Eleutherine americana* (Iriadaceae), *Rheum* sp. (Polgonaceae), *Gladiolus segetum* (Iridaceae), *Crocus sativus* (Iridaceae), *Araliorhamnus vaginata* (Rhamnaceae) (Thomson, 1987, 1997), and *Gladiolus gandavensis* (Wang et al., 2003b; Chen et al., 2005). Many of the 8-methyl anthraquinones from plant sources are isolated from the family Iridaceae the majority of which were reported from the genus *Gladiolus*. The isolation of two new and four known 8-methyl anthraquinones, from *G. psittascinus* therefore, suggests that these compounds have chemotaxonomic value at the genus level.

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