A New Indole Alkaloid from *Cleome droserifolia*

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The phytochemical investigations on *Cleome droserifolia* resulted in the isolation and characterization of a new indole alkaloid, 5-hydroxy-2-methoxy-1-methyl-1*H*-indole-3-carbaldehyde (**1**). The structure elucidation was carried out on the basis of 1D- and 2D-NMR techniques. In addition to **1**, two known aromatic derivatives, veratrol (**2**) and 2-methoxy-4-methylacetophenone (**3**), were also obtained. All these compounds were purified by repeated column chromatography of the CH_2Cl_2 fraction obtained from MeOH extract of *Cleome droserifolia*. The structure of the new compound **1** was finally confirmed by the combined 1D (^{1}H- and ^{13}C-) and 2D (H–C correlations; HMBC and HSQC) NMR and IR spectroscopy, mass spectrometry (MS), and UV absorption spectroscopy techniques. The comparison of the physical and spectroscopic data with those in the literature provided evidence for the structure confirmation of known compounds. All the purified compounds were subjected to urease and α-glucosidase enzymes inhibition. The results showed that compound **1** was more potent with an IC_{50} value 11.97 ± 2.067 μg/ml towards urease inhibition, while the activity of α-glucosidase enzyme was marginal.

Introduction. – Indole-3-carbaldehyde and its derivatives are considered to play an important role in the pathogenic defence system of the cruciferous plants [1]. Therefore, they have been the subject of intensive investigations during the last few decades [1][2]. *Cleome droserifolia* Forssk. belongs to the family Cleomaceae which contains 33 genera and *ca.* 700 species [3]. Various species of the genus are used in folk medicine against stomachache, scabies, rheumatic fever, and inflammation [3]. They are also reported to have antimicrobial [4], analgesic, antipyretic, anti-inflammatory, and anticancer activities [5]. During a search for biologically active natural products, we were interested to investigate the chemical constituents of the title plant, and thus the structure elucidation of a new alkaloid, **1**, along with two known constituents (**2** and **3**; *Fig. 1*), is reported in this article.

Results and Discussion. – The phytochemical investigations were carried out on the MeOH extract of *C. droserifolia* which afforded three compounds (*Fig. 1*). The known compounds, **2** and **3**, [6] were purified from the CH_2Cl_2 fraction, and structure

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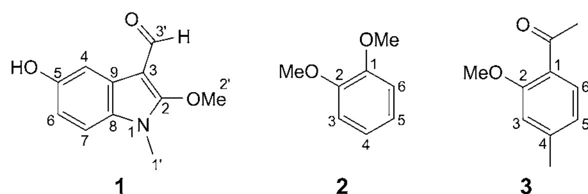

Fig. 1. *Structures of Compounds* **1**–**3**

elucidation was carried out by comparison of the spectroscopic data with those reported in the literature [6].

Compound **1** was isolated from the CH_2Cl_2 fraction of the crude MeOH extract and gave positive test for alkaloids with *Dragendorff*'s reagent [7]. The IR spectrum of **1** showed absorptions for a OH (3500 cm^{-1}) and a aldehyde C=O group (1715 cm^{-1}). The UV maxima at 270, 281, 295, and 320 nm indicated the presence of an aromatic functionality with an extended heterocyclic ring (indole system) [8]. The molecular formula, $C_{11}H_{11}NO_3$, was deduced from HR-EI-MS (*m*/*z* 205.0735 ($C_{11}H_{11}NO_3^{+}$; calc. 205.0739). This molecular formula was further supported by ^{1}H- and ^{13}C-NMR data. The EI-MS indicated the presence of fragment-ion peaks at *m*/*z* 176, 174, 188, and 190, corresponding to the loss of CHO, MeO, OH, and Me groups, respectively (*Fig. 2*), from the molecular ion of **1**.

The detailed analysis of the ^{1}H- and ^{13}C-NMR data (*Table*) revealed the presence of two Me groups, three sp^2 olefinic CH C-atoms, five sp^2 quaternary C-atoms, and one C=O C-atom of an aldehyde group. The ^{1}H-NMR spectrum of **1** exhibited signals for one Me group (δ(H) 3.02 (*s*)), one MeO group (δ(H) 3.87 (*s*)), and three aromatic CH H-atoms (δ(H) 7.87 (*d*, $J = 9.0$), 7.01 (*dd*, $J = 9.0$, 2.4), and 6.96 (*d*, $J = 2.4$)). The coupling constants of these aromatic H-atoms indicated the *ortho*- and *meta*-substitution in benzene ring, and hence the presence of an indole skeleton [9]. A

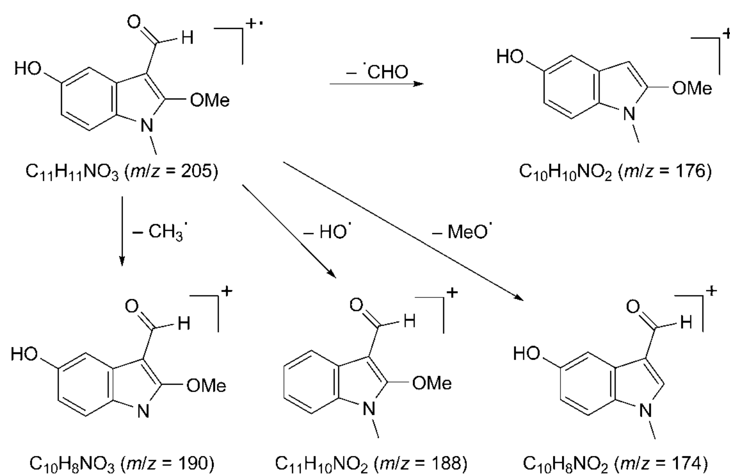

Fig. 2. *Key mass fragmentation pattern for compound* **1**

Table. *^{13}C- and ^{1}H-NMR Data* (150 and 600 MHz; $CDCl_3$)*, and HMBCs of Compound* **1**. δ in ppm, J in Hz.

	δ(C)	δ(H)	HMBC
C(2)	143.7	–	–
C(3)	114.7	–	–
H–C(4)	95.2	6.96 (*d*, $J = 2.4$)	C(5), C(9)
C(5)	158.2	–	–
H–C(6)	114.2	7.01 (*dd*, $J = 2.4$, 9.0)	C(5), C(7)
H–C(7)	120.1	7.87 (*d*, $J = 9.0$)	C(8)
C(8)	136.7	–	–
C(9)	121.5	–	–
Me(1′)	42.0	3.02 (*s*)	C(2)
MeO(2′)	55.7	3.87 (*s*)	C(2), C(3)
H–C(3′)	183.9	10.20 (*s*)	C(3), C(3′)

downfield signal at δ(H) 10.20 (*s*) with HMQC with C=O C-atom (H–C(3′)) was assigned to aldehydic H-atom. These functions were thus combined together by the HMBC experiments (*Fig. 3*) for the overall assembly of the structure for compound **1**. The HMBCs show cross-peaks between Me H-atoms (Me(1′) at δ(H) 3.02 (*s*)) and the quaternary C-atoms (C(8) at δ(C) 136.7) and C(2) at δ(C) 143.7), and MeO H-atoms (Me(2′) at δ(H) 3.87 (*s*)) and the quaternary C-atoms C(2) (δ(C) 143.7) and C(3) (δ(C) 114.7). Similarly, the HMBCs between aldehydic H-atoms (H–C(3′) at δ(H) 10.20) and the C=O C-atom (C(3′) at δ(C) 183.9), and the quaternary C-atoms C(2) (δ(C) 143.7) and C(3) (δ(C) 114.7). Assignment of ^{13}C-NMR chemical shifts of compound **1** was completed with the help of HMQC and DEPT experiments, which further supported the assigned substitution at the indole skeleton.

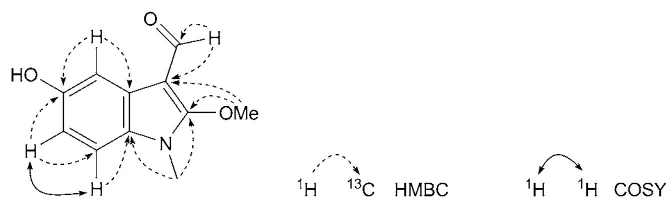

Fig. 3. *Important HMBCs and key COSY correlations in compound* **1**

The proposed structure of **1** was further confirmed by comparison of the ^{1}H- and ^{13}C-NMR spectra obtained with those of a known indole alkaloid [1] with a difference in the substitution pattern at the benzene and the heterocyclic rings. Thus, all of the above evidences led to the elucidation of structure as 5-hydroxy-2-methoxy-1-methyl-1*H*-indole-3-carbaldehyde for compound **1**.

The urease and α-glucosidase enzyme inhibition activities were evaluated for compounds **1**–**3**. Compound **1** was more potent with an IC_{50} value of 11.97 ± 2.067 µg/ml towards urease inhibition, while the activity of α-glucosidase enzyme was marginal (*Fig. 4*). The enzyme inhibition pattern of compound **1** was found to be dose-dependent. Other compounds, **2** and **3**, exhibited low enzyme-inhibition activities.

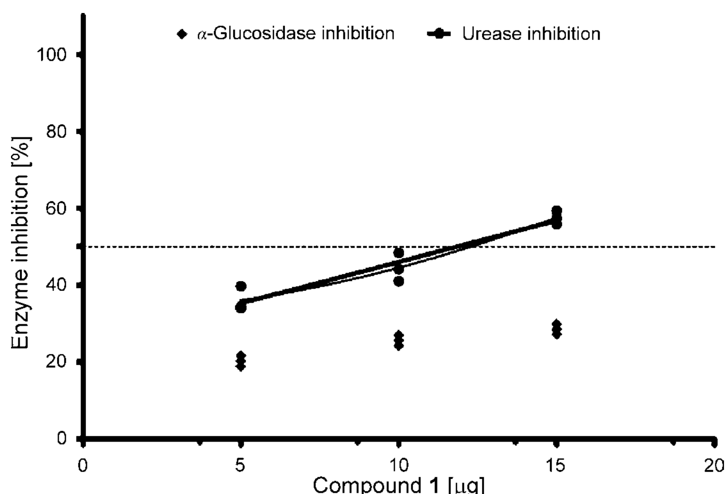

Fig. 4. *Enzymes-inhibition activities of compound* **1**

Experimental Part

General. TLC: Precoated silica-gel plates (*Merck*; *G60 F-254* and *UV-254*). Column chromatography: commercial silica gel (*Merck*, 0.040 – 0.062 mm). IR Spectra: *Bruker-ATR* spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker Avance 600* (at 600 and 150 MHz, resp.) spectrometer; chemical shifts, δ, in ppm, with residual $CDCl_3$ (δ(H) 7.24, δ(C) 77.0) as internal standard; coupling constants *J* in Hz. EI- and HR-EI-MS: *JEOL JMS HX 110* mass spectrometers, in *m/z*.

Plant Material. The aerial parts of *C. droserifolia* FORSSK. was collected from Al-Jabel Al-Akhdar, Al-Dakhliya Region, Oman, in 2012, and was identified by plant taxonomist at the Department of Biological Sciences and Chemistry, University of Nizwa, Nizwa, Oman. The voucher specimen has been deposited with the Herbarium of the University.

Extraction and Isolation. The dried and powdered aerial parts of *C. droserifolia* (7.5 kg) were extracted with 85% MeOH at r.t. The crude MeOH extract (118 g) was then fractionated with hexane (50 g), CH_2Cl_2 (7 g), AcOEt (12 g), and BuOH (20 g). The CH_2Cl_2 fraction (7 g) was subjected to gradual CC (SiO_2; hexane/CH_2Cl_2 and CH_2Cl_2/MeOH). Eight subfractions, $NCD_1 – NCD_8$, were obtained. The subfractions $NCD_1 – NCD_3$ were compiled and again subjected to repeated CC (SiO_2; hexane/CH_2Cl_2 and CH_2Cl_2/MeOH) to afford compound **1** (12 mg) as gummy solid along with some semi-pure compounds, which were then purified to yield compounds **2** (7 mg) and **3** (9 mg) (with hexane/CH_2Cl_2 5 : 95 and hexane/CH_2Cl_2 2 : 98, resp.).

*5-Hydroxy-2-methoxy-1-methyl-1*H*-indole-3-carbaldehyde* (**1**). Gummy solid. IR (MeOH): 3500, 1715. ^{1}H-NMR (600 MHz, $CDCl_3$): see the *Table.* ^{13}C-NMR (150 MHz, $CDCl_3$): see the *Table.* HR-EI-MS: 205.0735 (M^+, $C_{11}H_{11}NO_3^+$; calc. 205.0739).

α-Glucosidase Enzyme Inhibition. *p*-Nitrophenyl α-D-glucopyranoside (PNPG), yeast aglucosidase (EC 3.2.1.20), and sodium phosphate salts were purchased from *Sigma* (Germany). α-Glucosidase enzyme-inhibition assay was performed as described by *Everette et al.* [10] with slight modification. The assay mixture comprised of 50 mM sodium phosphate buffer containing 100 mM NaCl (pH 6.8), 0.7 mM PNPG and 200 m units/ml enzyme incubated at 37° with compounds (5, 10, and 15 μg/ml) in 96-well plates. Acrabose was used as a positive control. The increment in absorption at 400 nm due to the hydrolysis of PNPG by α-glucosidase was monitored continuously with ELISA microplate reader (*xMark*, *Biorad*, USA). The percentage of enzyme inhibition by the sample was calculated by the formula: % Inhibition = {[(*AC* – *AS*)/*AC*] × 100}, where *AC* is the absorbance of the control and *AS* is the absorbance of the tested sample. The experiment was repeated three times, and graphical analyses were performed on *GraphPad Prisom* (Santiago, USA).

Urease Enzyme Inhibition. The urease enzyme inhibition assay was performed as described by *Ahmed et al.* [11] with a slight modification. Briefly, the reaction soln. comprising *Jack* bean urease (EC 3.5.1.5; 25 µl), buffer (100 mM $K_2HPO_4 \cdot 3\,H_2O$, 1.0 mM EDTA, and 0.01M LiCl; pH 8.2; 55 µl) and urea (100 mM; 20 µl) was incubated with compounds (5, 10, and 15 µg/ml) at 30° for 20 min in 96-well plates. The production of NH_3 was measured by indophenol method which was used to assess the urease inhibition. The phenol reagent (0.005% (*w*/*v*) sodium nitroprusside and 1% (*w*/*v*) phenol; 50 µl) and alkali reagent (0.5% (*w*/*v*) NaOH and 0.1% NaOCl; 70 µl) were added to each well. The absorbance was read at 630 nm after 50 min of incubation (30° for 20 min) with an ELISA microplate reader (*xMark*, *Biorad*, USA). Thiourea was used as the standard inhibitor. All the tests were performed in triplicate.

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