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ISOLATION AND STRUCTURE ELUCIDATION OF A NEW COUMARIN GLYCOSIDE FROM *XEROMPHIS NILOTICA*.

Ibrahim Abdurrahman[1,2], Yang Cai- Xia[1]* and Hu Yu-Lai[1]*

[1]College of Chemistry and Chemical Engineering, Northwest Normal University, Lanzhou 730070, People's Republic of China.

[2]Faculty of Agriculture, University of Zalingei, Zalingei, Sudan.

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***Correspondence for Author**
Yang Cai- Xia and Hu Yu-Lai
College of Chemistry and Chemical Engineering, Northwest Normal University, Lanzhou 730070, People's Republic of China.

ABSRACT

Beside three known coumarin derivatives, scopoletin **1**, 7-[O-β-D-glucopyranosyl]-6-methoxy coumarin **2** and 7-[O-β-D-aboifuranosyl-(1→6)-β-glucopyranosyl] -6-methoxy coumarin **3**, A new coumarin glycoside, 7-[O-α-L-arabinopyranosyl - (2→1)-β-D-glucopyranosyl] -6-methoxy coumarin **4** were isolated from stem barks of *Xeromphis nilotica* (Rubiaceae), on the bases of chromatographic and spectroscopic [1D, 2D-NMR and HR-ESI-MS] and chemicals methods, as well as basic and acid hydrolysis we identified their structures.

KEYWORDS: Xeromphis *nilotica*, Rubiaceae, Coumarin derivatives, Coumarin glycosides.

INTRODUCTION

Xeromphis nilotica is an important Sudanese medicinal plant, belonging to the (Rubiaceae) family[1], it is a lowland shrub that grows wild in tropical areas of Africa and Asia.[2] It is widespread in central and east Africa, as well as in Cameroon and Nigeria, locally in (Sudan), commonly known as Shagarat Almarfaein, and as 'barbaji' (in Nigeria).[1,2] In Sudan it is found in cetral darfur State (Gabal Marra aria) and also reported to found in Rashad area in the Eastern Nuba Mountains.[3] The plant's extract is used for the treatment of various diseases in folk medicine including stomach pain related ailments, anti-epileptic agent, dropsy, fever, abdominal pain, asthma, and to induce labour.[4,5] The fruits of this plant show a very strong molluscicide activity against the schistosomiasis transmitting snail Biomphalaria glabrata and also well known for their antispasmodic, antifertility and

antidysentric properties.[6] The bark is not only used as an anthelmintic and in connection with the treatment of jaundice and rabies but also utilized as a fish poison.[7]

The constituents of barks, leaves and fruits of *X. Nilotica* were studied and variety of coumarins, alkaloids, flavonoids, terpenes saponins, Iridoids and other compounds were reported in the literature.[3-10] We now report on the isolation and structure elucidation of a new coumarin identified as 7-[O-α-L-arabinopyranosyl - (2→1)-β-D-glucopyranosyl] -6-methoxycoumarin.

2. EXPERMENTAL

2-1. General experimental procedure

All melting points were determined on micro-melting point apparatus and are uncorrected. NMR were recorded on a Bruker Avance DRX-500- spectrometer (^{1}H at 400Hz and ^{13}C at 100Hz) and chemical shift values are given on a δ (ppm) scale with TMS as internal standard, 2D-NMR experiment were performed using standard Bruker micro-program (XWIN-NMR version 2.6 softwere, HR-ESI-MS experiment was performed using micro-mass –QTOF micro instrument, with an electro-spray ionization source (eV=35V, 80°C). Column chromatography was carried out on silica gel (Merck kiesel gel 300-400 mech), TLC were carried out on silica gel GF_{254} (Merck), all the chemicals and solvents were commercial grade and used after further purification.

2-2. Plant material

The stem barks of *Xermophis Nilotica* were collected in august 2014 from Zalingei area, central Darfur state –Sudan, the plant was authenticated by prof: G, A, Yagoub, department of botany, faculty of agriculture, University of Zalingei. Voucher specimens have been deposited in the herbarium of author's laboratory.

2-3. Extraction and isolation

The stem barks of *Xermophis Nilotica* were air-dried for four weeks and grinded in to a powder. The stem barks powder (1.5Kg) was extracted three times with 95% EtOH at room temperature (each 7days × 3L). Then, the filtrates were combined and concentrated in vacuum (Rotary evaporation) for removal of the organic solvent and dried a total of 355g of ethanolic extracts were dissolved in 2L of hot distilled water and then prepared by successive partition with petroleum ether (40-60 C) (fraction I), chloroform (fraction II), ethyl acetate (fraction III) and n-butanol (fraction V). Each partition step was repeated three times to

ensure complete extraction in each case. (Fraction I) was rejected since it was rich on fatty substances, (fraction II) was evaporated to yield 40g, which were chromatographed on 800g of silica gel (Merck kiesel gel 300-400 mech) used ($CHCl_3$: MeOH) gradual, first eluted with $CHCl_3$ pure then with ($CHCl_3$: MeOH) (10:1- 1:1) and in the last eluted with MeOH pure. thirty three fractions were collected, then checked by Thin Layer Chromatography (TLC) used the $CHCl_3$: MeOH 10:1, 10:2, 10:3 as mobile phases, fractions showing similar on TLC were combined together to provided tow fractions, the fractions were further purified on column chromatography to afford tow pure compounds **1** and **2**.

Fraction III was loaded on a silica gel column. Elution was carried out with EtOAc by increasing polarity with addition of MeOH, yielding subfractions A to N.

Subfraction D was further subjected to column chromatography (CC) using again EtOAc and MeOH mixtures with increasing polarities to give two major fractions D1 and D2, Fraction D1 was re-chromatographied over silica to obtain compound **3**. The n-butanol fraction V was subjected to Polyamide and was eluted with increasing polarities of a MeOH: H_2O mixture to obtain subfractions A to F. re-Chromatography of subfraction F over polyamide afforded compound **4** as white powder.

2-4. Acid Hydrolysis of compound 4

Compound **4** (25mg) was heated with 5% H_2SO_4 on an H_2O bath for 2 h. the mixture was cooled and diluted with water then filtered, the residue was crystallized from MeOH to afford yellow needles, mp 202-206°C which was identified as scopoletin by the comparative 1H and ^{13}C-NMR with compound **1**. The filtrate was neutralized and evaporated; D-glucose and L-Arabinose were detected by PC with comparison by ref.[20]

2-5. Partial Hydrolysis of compound 4

A mixture of **4** (25mg) and 5ml of 10% acetic acid was refluxed on water bath. After 6h, the reaction mixture was neutralized with 10% $NaHCO_3$ solution and was evaporated in vacuum, and L- arabinose was detected by PC method, the residue was chromatographed on a column of silica gel in the $CHCl_3$/EtOH (4:1) system. It was obtained white crystal, which was identified as **2** by direct comparison.

2-6. Basic Hydrolysis of compound 4

A solution of **4** (13mg) in 5ml of 0.5% KOH solution was refluxed in the boiling water bath for 4h. Then the mixture was neutralized with 2% HCl and was evaporated in vacuum. Checked by TLC system $CHCl_3$/MeOH (4:1), it was found that the initial substance had not changed.

3. RESULTS AND DISCUSSION

The chloroform extract from stems barks of *Xeromphis Nilotica* after chromatography fractionations afforded three Compounds (**1** and **2**).

Compound **1,** obtained by repeated column chromatography on silica gel of fraction (1) with $CHCl_3$\ MeOH followed by re-crystallization using MeOH afforded compound **1** (75mg) as yellow crystal, mp 202-204. By using ^{1}H and ^{13}C-NMR data Table (**1**) compared with published data.[11,12] We identified compound **1** as scopoletin.

Compounds **2,** the ^{1}H-NMR spectra of compound **2** showed two doublets with coupling constant of 9.6 and 9.5Hz at δ 7.29 and 7.88 ppm, which were characteristic of coumarin and assigned as H-3 and H-4, respectively.[13] The spectrum showed singlet peak at δ 3.92ppm which correspond to the methoxy group, and two singlets at δ 7.17 and 7.19ppm which were explain by disubstitution, indicating for scopoletin formwork.[14] The spectrum also showed multiplets peacks at δ 3.40- 3.71ppm which were attributed to glucose proton, the sugar anomeric proton was also appeared as doublet peak (J=7.5Hz) at δ 5.06ppm, ^{13}C-NMR spectrum Table (**1**) were in good agreement with published.[15] It was found by acid hydrolysis that compound **2** contained a glycone identical with **1** and glucose in equimolar proportions. Study of spectral characteristics and the result of acid hydrolysis analysis showed that **2** was scopoletin 7-O- β-D-glucopyranoside (scopolin).[15]

Compound **3**, was isolated as white crystal, the ^{1}H and ^{13}C-NMR showed the characteristic coumarin, C-3 and C-4 proton doublet pair centered at δ 7.89ppm and δ 6.30ppm (1H, d, J = 9.5 Hz each) and δ114.76(C-3), δ145.54 ppm (C-4), the two singlet signals at δ7.22 ppm (1H,s, H-8), δ 7.19 ppm (1H,s,H-5). Anomeric glycosidic protons appeared doublet pair at δ 4.98(1H, d, J= 7.7Hz, Api, H-1") and 4.93(1H, d, J= 2.6Hz, Glc, H-1"). The chemical shifts in the 13C-NMR spectrum of compound **2** were compared with 7-O-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-6-methoxy-coumarin which has been isolated from *Xeromphis Spinosa*[16], their ^{13}C-NMR data were in good agreement Table (**1**).

Compound **4**, investigation of ^{1}H-NMR spectrum showed the aromatic part was similar to that of compound **1** (scopoletin) suggesting a glycosides. Two signal doublets at δ 7.96ppm (1H,d,J = 9.5 Hz) and δ 6.33 (1H,d,J = 9.5 Hz) of H-3 and H-4 respectively which were typical for the pyran ring in coumarin nucleus.[17] A three protons singlet in the spectrum at δ 3.82ppm, revealed the presence of a methoxyl group, the presence of two aromatic protons singlet at 7.15 and 7.30ppm were attribute to H-8 and H-5 respectively. Comparison of the chemical shifts of methoxy group allowed placing this substituent at C-6. The multiplet peaks at δ 3.23ppm to 4.93 were attributed to glucose and Arabinose protons, the sugars anomeric protons were also resonated at δ 5.07and 5.36ppm appeared as doublet peaks, respectively, this shows that C-1 conformation of the glucopyranose ring and the C-1 conformation of the arabinopyranose ring and consequently, the β- configuration of the glycosidic center of the D-glucose and the α-configuration of the L-arabinose.[18]

The ^{13}C-NMR spectral data indicated 21 carbon signals Table (**2**). Nine signals appeared in the region 103.49- 161.00ppm represents carbon atoms of coumarin nucleus and 11 signals in the region 63.89-109.01ppm represents carbon atoms of sugar moiety and one signal at δ56.46ppm represents carbon atom of methoxygroup. The high resolution electrospry ionization mass spectrometry (HR-ESI-MS), showed peaks at m/z 509$[M+Na]^{+}$ which were correspond to the molecular formula $C_{21}H_{26}O_{13}$.

The interpretation of ^{1}H-^{1}H COSY –NMR was confirmed the glycone part of this molecule table (**2**). In the key HMBC spectrum of compound **4**, the anomeric proton H-1" of arabinose at δ 5.36ppm has cross beaks with C-2' of glucose at δC-77.08 ppm and C-2"of arbinose at δ 73.44ppm. Another anomeric proton H-1' of glucose at 5.07ppm showed cross peak with C-7 of aglycoe at δ C-150ppm. Thus, glucose linked with C-7, and arabinose linked with C-2' of glucose. Consequently, Arabinose is the terminal sugar and the glucose is attached directly to the aglycone.

By acid hydrolysis, compound **4** formed scopoletin, glucose and arabinose identified by PC. On partial hydrolysis of the compound **4** with 10% acetic acid let the formation of Arabinose and coumarin glycoside identical with compound **2**. The resistance of the glycoside to basic hydrolysis give grounds for assuming that the arabinose was attached to the glucose by a (1-2) bond.[19] According to above information, the structure of compound **4** can be represented as 7-[O-α-L-arabinopyranosyl - (2→1)-β-D-glucopyranosyl] -6-methoxycoumarin.

Table (1): Characteristic ^{13}C-NMR data of compound 1, 2 and 3(100 MHz, $CdCl_3$/MeOH-d_4 for 1, 2 and DMSO-d_6 for 3).

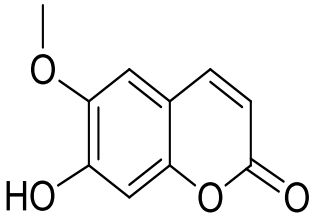
No of Carbon	Compound 1	Compound 2	Compound 3
C-2	163.09	162.40	161.94
C-3	112.07	113.45	114.76
C-4	144.71	144.47	145.54
C-5	108.43	109.54	110.71
C-6	111.34	113.43	113.74
C-7	151.43	150.51	151.29
C-8	109.40	104.16	104.47
C-9	145.78	147.03	147.37
C-10	150.24	149.50	150.29
O-Me	56.30	56.02	57.34
Sugar			
Glc C-1		100.97	101.00
C-2		73.45	74.20
C-3		77.19	78.04
C-4		70.00	71.13
C-5		76.58	76.83
C-6		63.18	68.84
Api C-1			111.16
C-2			77.38
C-3			80.13
C-4			74.60
C-5			64.84

Table (2): the complete NMR spectroscopic data (100 MHz, DMSO-d_6) for compound 4.

Position	type	δ_C	δ_H(J in Hz)	HMBC	COSY
2	C	161.00			
3	CH	113.81	6.31(d, *J* = 9.5 Hz, 1H)	2,4,10	H-3,H-4
4	CH	144.60	7.94(d, *J* = 9.4 Hz, 1H)	2,3,5,9	
5	CH	110.16	7.13(s, 1H)	4,6,9,10	H-4,H-5
6	C	149			
7	C	150.24			
8	CH	103.49	7.27(s, 1H)	7,9,10	
9	C	146.40			
10	C	112.77			
11	OCH_3	56.46	3.80(s, 3H)	5	
Glc					
1'	CH	100.01	5.07 (d, *J* = 6.5 Hz, 1H),	7,3'	H-1',H-2'
2'	CH	77.08	3.29 (d, *J* = 17.4 Hz, 2H)	1',1", 2'	
3'	CH	76.42			
4'	CH	70.16			
5'	CH	73.85			
6'	CH_2	63.89			
1"	CH	109.01	5.36(d, *J* = 3.6 Hz, 1H)	1', 2", 3", 5"	H-1",H-2"
2"	CH	73.42	3.43 (dd, *J* = 10.8, 6.7		

			Hz, 1H)		
3"	CH	79.18	4.77(s, 1H)	1", 5"	
4"	CH	75.87			
5"	CH_2	67.89	3.59 (dd, *J* = 19.8, 9.1 Hz, 2H)	1", 3"	

*****HMBC correlations are from proton (s) state to the indicated carbon and ^{1}H-^{1}H COSY.



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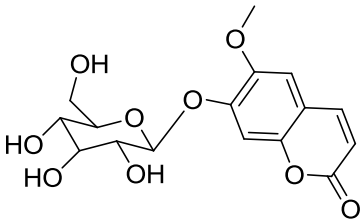

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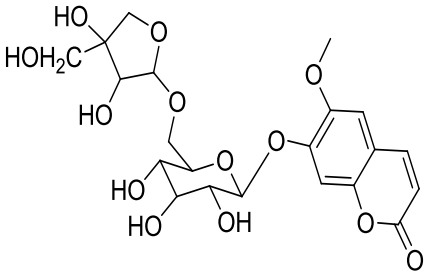

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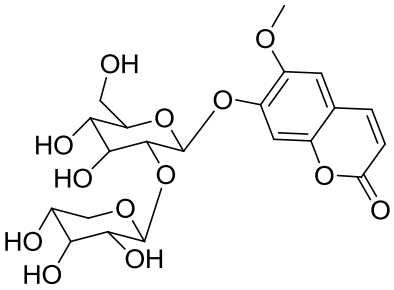

4

Scopoletin **1**, (75 mg) isolated as yellow crystal, mp 202-204°C, UV 365 nm showed blue florescence color, ^{1}H NMR (400 MHz, cdcl$_3$) δ 7.59 (d, *J* = 9.2 Hz, 1H), 6.91 (s, 1H), 6.84 (s, 1H), 6.26 (d, *J* = 9.3 Hz, 1H), 3.95 (s, 3H). ^{13}C NMR, see Table **1**.

Scopolin **2,** obtained as white crystal, mp 213-214°C, UV 365 nm showed blue color, yellow color after spraying 7% H_2SO_4 reagent, ^{1}H NMR (400 MHz, MeOH-d4) δ 7.88 (d, *J* = 9.6 Hz, H-4), 7.19 (s,H-4),7.17(s,H-8) 6.29 (d, *J* = 9.5 Hz, H-3), 5.06 (d, *J* = 7.5 Hz, H-1'), 3.92 (3H,s, 6-OCH_3), 3.71 (1H,m, H-6'a), 3.60 (2H, m,H-6'b and H-3'), 3.55 (1H, m, H-2'), 3.50 (1H, m, H-5'), 3.46 (1H, m, H-4'), ^{13}C NMR, see table **1.**

7-O-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-6-methoxy-coumarin **3,** 84 mg, isolated as white crystal, mp 238-240°C, UV 365 nm showed blue color, deep yellow color after spraying 7% H_2SO_4 reagent, ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.89 (d, *J* = 9.0 Hz, H-4), 7.22 (s, H-4), 7.20 (s, H-8), 6.30 (d, *J* = 9.5 Hz, H-3), 4.98 (d, *J* = 7.7 Hz,H-1'), 4.93 (d, *J* = 2.6 Hz, H-1"), 4.04 (dd, *J* = 15.5, 5.8 Hz, Ha-6'), 3.97 (d, *J* = 2.6 Hz, H-2"), 3.90 (3H ,s, 6-OMe),

3.76 (d, *J* = 9.7 Hz, H-4"), 3.69 (dd, *J* = 7.6, 2.0 Hz, H-3'), 3.57 (m, H-5'), 3.48 (d, *J* = 8.9 Hz, H-3"), 3.46 (s, H-5"), 3.31 (m, H-2'), 3.30 (dd, *J* = 4.8, 3.3 Hz, H-4'), ^{13}C NMR, see table **1**.

7-[O-α-L-arabinopyranosyl - (2→1)-β-D-glucopyranosyl] -6methoxy-coumarin **4**, 98mg ,obtained as white powder, mp over 300°C, UV 365 nm showed blue color, deep yellow color after spraying 7% H_2SO_4 reagent, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (d, *J* = 9.4 Hz, 1H), 7.27 (s, 1H), 7.13 (s, 1H), 6.31 (d, *J* = 9.5 Hz, 1H), 5.36 (d, *J* = 3.6 Hz, 1H), 5.15 (m, 2H), 5.07 (d, *J* = 6.5 Hz, 1H), 4.92 (d, *J* = 6.1 Hz, 1H), 4.92 (d, *J* = 6.1 Hz, 1H), 4.77 (s, 1H), 4.65 (t, *J* = 5.2 Hz, 1H), 4.46 (s, 1H), 3.86 (m, 2H), 3.83 (t, *J* = 9.1 Hz, 1H), 3.80 (s, 3H), 3.69 (d, *J* = 16.6 Hz, 1H), 3.59 (dd, *J* = 19.8, 9.1 Hz, 2H), 3.43 (dd, *J* = 10.8, 6.7 Hz, 1H), 3.32 (m, 10H), 3.29 (d, *J* = 17.4 Hz, 2H), 3.13 (m, 1H), ^{13}C NMR and HMBC, see table **2**.

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