Antimicrobial Arylcoumarins from *Asphodelus microcarpus*[⊥]

Hesham R. El-Seedi*

Division of Pharmacognosy, Department of Medicinal Chemistry, Biomedical Centre, Uppsala University, Box 574, S-751 23 Uppsala, Sweden, and Department of Chemistry, Faculty of Science, El-Menoufia University, Shebin El-Kom, 32512, Egypt

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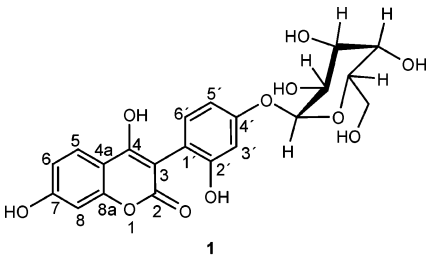
A new aryl coumarin glucoside, asphodelin A 4′-*O*-*β*-D-glucoside (**1**), and its aglycon, asphodelin A (**2**), were isolated from *Asphodelus microcarpus*. The structures were determined by detailed spectroscopic analysis and chemical transformation as 3-(2′-hydroxy-*p*-*O*-*β*-D-glucopyranosyloxyphenyl)-4,7-dihydroxy-2*H*-1-benzopyran-2-one (**1**) and 3-(2′,4′-dihydroxyphenyl)-4,7-dihydroxy-2*H*-1-benzopyran-2-one (**2**), respectively. These compounds were isolated following bioactivity-directed fractionation, using antimicrobial activity, in which **1** and **2** exhibited moderate and potent activities, respectively. This is the first report of a 3-arylcoumarin derivative, a rare class of isoflavonoids, from a plant in the family Liliaceae.

The genus *Asphodelus* Reichb. (Asphodelaceae or Liliaceae s.l.) is a circum-Mediterranean genus, which includes five sections and is represented by 16 species.[1] A new class of anthraquinone-anthrone-*C*-glycosides has been isolated from *A. ramosus*.[2] Sesquiterpene lactones, flavonoids, and anthraquinones have been reported from *A. globifera*, *A. anatolica*, and *A. damascene*,[3] whereas anthraquinones and glycosides have been isolated from *A. microcarpus*.[4] The bulbs and roots of *A. microcarpus* Salzm. & Viv. are used to treat ectodermal parasites, jaundice, and psoriasis, also *A. microcarpus* is used by Bedouins as an antimicrobial agent.[5] In the present work, this species was collected in Egypt and investigated as part of an ongoing study of plants used in folk medicine, with emphasis on antimicrobial activity.[6a,b] The aim of the present investigation was to find novel chemical compounds having antimicrobial activity.

Guided by an assay for inhibition of microbial activity, an EtOAc extract of the bulbs and roots of *A. microcarpus* was subjected to a succession of chromatographic procedures, which resulted in the isolation and structure elucidation of a new aryl coumarin glucoside (**1**) and its aglycon (**2**).



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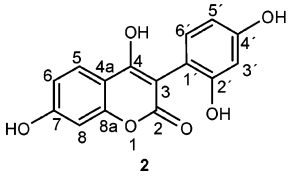

2

Compound **2** gave an intense blue fluorescence under UV light. Its UV spectrum gave peaks at λ_{max} 352, 285, 257, and 222 nm and resembled that of 3-arylcoumarin.[7] The IR spectrum presented strong absorption bands for hydroxyl (ca. 3350, 1090 cm^{-1}), carbonyl (1671 cm^{-1}), −C═C−H (1635, 1540 cm^{-1}), and C−O−C (1180−1165 cm^{-1}) functionalities. These properties suggested this compound to be a 3-arylcoumarin derivative, a rare structural class of isoflavonoids.[8] The HREIMS showed a molecular ion peak at *m*/*z* 286.0485, corresponding to $C_{15}H_{10}O_6$. The ^{1}H NMR spectrum exhibited two ABX systems, which correlated with one another in the COSY NMR spectrum. The assignment to H-8 of the signal at *δ* 6.85 was established by means of the HMBC spectrum (Figure 1) due to clear correlations with C-8a at *δ* 156.4 and C-7 at 162.7 ppm. Also, correlations of H-5 at *δ* 7.82, C4a at *δ* 106.1, and C-6 at *δ* 114.9 were observed. Similarly, the HMBC spectrum showed additional correlations of the H-3′ signal at *δ* 7.08 with C-4′ at *δ* 158.4 and C-5′ at 114.9 ppm, confirming the ABX system in ring B. Analysis of all of the data obtained and comparison with data of closely related structures[9,10] were used to establish the structure of **2** as 3-(2′,4′-dihydroxyphenyl)-4,7-dihydroxy-2*H*-1-benzopyran-2-one, which has been given the trivial name asphodelin A.

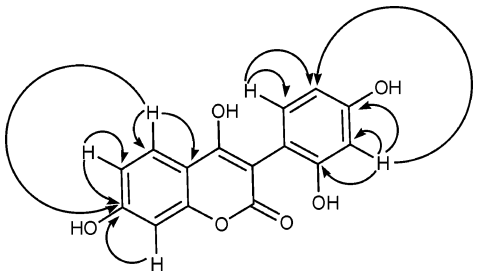

Figure 1. Long-range correlations in the HMBC NMR spectrum of **2**.

The ^{1}H and ^{13}C NMR data of **1** were very similar to those of **2**, but showed resonances for a glucose unit. The coupling constant, $J = 7.3$ Hz, of the signal resulting from the anomeric proton at *δ* 4.99 indicated the glucosidic linkage to have a *β*-configuration. The ^{13}C NMR spectrum of **1** showed 21 carbon signals, six from the glucopyranosyl group and 15 from the aryl coumarin moiety. The location of the glucoside linkage at C-4′ was confirmed by the HMBC spectrum (Figure 2), which showed a correlation of the H-1″ signal at *δ* 4.99 with the C-4′ resonance at *δ* 157.5. Furthermore, a downfield shift of C-4′ and upfield shifts of C-3′ and C-5′ were observed. The COSY, ^{13}C NMR (DEPT sequence), TOCSY, HMQC, and HMBC spectra were used to assign **1** as 3-(2′-hydroxy-*p*-*O*-*β*-D-glucopyranosyloxyphenyl)-4,7-dihydroxy-2*H*-1-benzopyran-2-one.

Only a few examples of natural coumarins with a substituted 3-phenyl ring have been reported in the literature, and this is the first report of this compound class from a plant in the family Liliaceae.[11]

[⊥] Dedicated to Dr. Jan G. Bruhn on the occasion of his 60th birthday.

* Corresponding author. Tel: +46-18-4714496. Fax: +46-18-509101. E-mail: hesham.el-seedi@fkog.uu.se.

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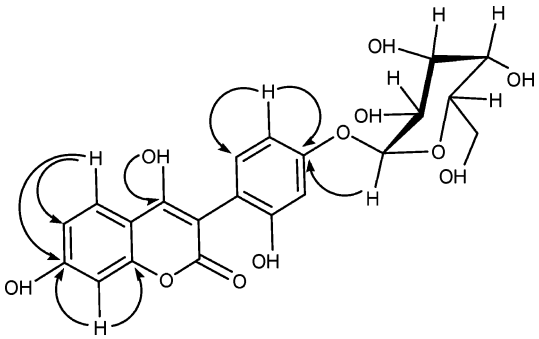

Figure 2. Long-range correlations in the HMBC NMR spectrum of **1**.

Table 1. Antibacterial Activity of Compounds **1** and **2**

	minimum inhibitive concentration (μg/mL)				
compound	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*B. cinerea*
1	128	128	256	512	1024
2	16	4	8	64	128
ampicillin[a]	<0.5	1.0			
penicillin G[a]			1.0		
nystatin[a]				<0.5	<0.5

[a] Positive control substances used.

The results of antimicrobial and antifungal activity assays for **1** and **2** are summarized in Table 1. At a concentration of 5 μg per disk, compound **1** exhibited medium antibacterial activity against *Staphylococcus aureus* (IAM1011) (Gram-positive), *Escherichia coli* (IAM1257) and *Pseudomonas aeruginosa* (IAM1058) (Gram-negative) and low antifungal activity against *Candida albicans* (IAM1099) and *Botrytis cinerea* (IAM1069), while compound **2** showed more potent activity. These results support the folk medicinal use of *A. microcarpus* as an antimicrobial.[5]

Experimental Section

General Experimental Procedures. Melting points were determined using a digital melting apparatus (Model 1A 8103, Electrothermal Engineering Ltd., Southend-on-Sea, Essex, U.K.) and are uncorrected. UV spectra were recorded with a Perkin-Elmer Lambda 2 UV/vis spectrophotometer. IR spectra were recorded with a Nicolet MX-S spectrophotometer. NMR spectra were recorded on a Varian VXR-400 at 25 °C using CD_3OD as solvent, and all chemical shifts are expressed with reference to TMS. TLC was performed on precoated aluminum sheets [silica 60 F_{254}, 0.25 mm (Merck, Darmstadt, Germany)] and preparative TLC on precoated glass sheets [silica 60 F_{254}, 0.5 mm (Merck)], with the detection provided by UV light (254 and 366 nm) and by spraying with vanillin−sulfuric acid reagent followed by heating (120 °C).

Accelerating gradient chromatography (AGC) was performed using variable-length MPLC glass columns (Baeckström Separo AB, Lidingö, Sweden) with inner diameters of 4.0, 2.5, and 1.5 cm, packed with silica gel 60, 40−63 μm (Merck), and an FMI Lab pump, model QD (Fluid Metering Inc., Oyster Bay, NY), delivering a flow rate of 15−18 mL/min. Fractions of 20 mL were collected manually. Analytes were eluted from the columns by continuous gradient elution running from hexane, through CH_2Cl_2, EtOAc, and MeOH, to H_2O generated by a Separo constant-volume mixing chamber combined with an open reservoir. The mixing chamber initially contained 100 mL of hexane and the reservoir contained the first of 15−20 premixed binary gradient mixtures of gradually increasing polarity, each of 15−30 mL, which were successively fed to the reservoir during the separation.[12]

Plant Material. Bulbs and roots of *A. microcarpus* were collected in June 2003 from Al-Halal Mountain, Sinai, Egypt. The species were identified by both Prof. Ahmed M. Ahmed and Dr. Zaki Turki. A voucher specimen, HRE 2, is monitored in the Herbarium, Department of Botany, Faculty of Science, El-Menoufia University, Shebin El-Kom, Egypt.

Extraction and Isolation. The powdered plant material (13.6 kg) was extracted with Me_2CO at room temperature three times with occasional stirring and filtered to give 265.1 g of a Me_2CO extract. The macerate was then extracted three times with MeOH for 7 days each to give 46.5 g of a MeOH extract. Both extracts were combined and evaporated in vacuo to give 311.6 g. The extract was partitioned between EtOAc and H_2O to give 165.6 g of an EtOAc extract and 121.2 g of a H_2O extract after freeze-drying. An interfacial residue, 24.8 g, was also produced. The EtOAc extract (60 g) was adsorbed onto silica gel (120 g) and chromatographed on a silica gel (280 g) column eluted with continuous hexane−CH_2Cl_2, CH_2Cl_2−MeOH, to MeOH gradients. The eluted tubes from AGC columns were evaluated by TLC to give 22 main fractions. Fraction 7 (3.3 g) was purified over repeated AGC eluted with $CHCl_3$−MeOH (21:1), which afforded 15 mg of compound **2**. Fraction 17 (4.1 g) was chromatographed over Sephadex LH-20 eluted with $CHCl_3$−MeOH (1:1) and further separated by preparative TLC using $CHCl_3$−MeOH−H_2O (7:3:0.5) as eluent to yield 32 mg of compound **1**.

3-(2′-Hydroxy-*p*-*O*-β-D-glucopyranosyloxyphenyl)-4,7-dihydroxy-2*H*-1-benzopyran-2-one (Asphodelin A 4′-*O*-β-D-glucoside) (1): yellowish-white solid; mp 143−146 °C; $[\alpha]_D^{22}$ +5.7 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.39), 242 (3.58), 325 (3.71) nm; IR (KBr) ν_{max} 1616, 1540 (aromatic), 1696 (C=O), 3425 (OH), 1635 (−C=C−H) cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.83 (1H, d, J = 8.3 Hz, H-5), 6.89 (1H, dd, J = 2.2, 8.3 Hz, H-6), 6.85 (1H, d, J = 2.2 Hz, H-8), 7.14 (1H, d, J = 2.2 Hz, H-3′), 7.01 (1H, dd, J = 2.2, 8.3 Hz, H-5′), 7.77 (1H, d, J = 8.3 Hz, H-6′), 4.99 (1H, d, J = 7.3 Hz, H-1″), 3.46−3.57 (1H, m, H-2″), 3.80 (1H, dd, H-3″), 3.46−3.57 (1H, m, H-4″) 3.46−3.57 (1H, m, H-5″), 3.67 (1H, dd, J = 2.5, 2.8, 11.0 Hz, H-6a″), 3.80 (1H, dd, J = 5.3, 5.5, 11.0 Hz, H-6b″); ^{13}C NMR (CD_3OD, 100 MHz) δ 160.5 (C-2), 103.8 (C-3), 161.8 (C-4), 106.1 (C-4a), 122.1 (C-5), 114.9 (C-6), 162.7 (C-7), 104.2 (C-8), 156.4 (C-8a), 118.4 (C-1′), 158.0 (C-2′), 100.4 (C-3′), 157.5 (C-4′), 115.6 (C-5′), 125.4 (C-6′), 102.3 (C-1″), 73.5 (C-2″), 77.7 (C-3″), 70.7 (C-4″), 77.5 (C-5″), 61.4 (C-6″); HREIMS *m*/*z* 448.1071 $[M]^+$ (calcd for $C_{21}H_{20}O_{11}$, 448.1066).

3-(2′,4′-Dihydroxyphenyl)-4,7-dihydroxy-2*H*-1-benzopyran-2-one (Asphodelin A) (2): yellow oil; UV (MeOH) λ_{max} (log ϵ) 210 (4.40), 245 (3.47), 328 (3.91) nm; IR (KBr) ν_{max} 1619, 1539 (aromatic), 1671 (C=O), 3431 (OH), 1630 (−C=C−H) cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.82 (1H, d, J = 8.3 Hz, H-5), 6.90 (1H, dd, J = 2.2, 8.3 Hz, H-6), 6.85 (1H, d, J = 2.2 Hz, H-8), 7.08 (1H, d, J = 2.2 Hz, H-3′), 6.91 (1H, dd, J = 2.2, 8.3 Hz, H-5′), 7.74 (1H, d, J = 8.3 Hz, H-6′); ^{13}C NMR (CD_3OD, 100 MHz) δ 160.6 (C-2), 103.7 (C-3), 161.8 (C-4), 106.1 (C-4a), 122.1 (C-5), 114.9 (C-6), 162.7 (C-7), 104.2 (C-8), 156.4 (C-8a), 116.5 (C-1′), 158.0 (C-2′), 99.5 (C-3′), 158.4 (C-4′), 114.9 (C-5′), 125.7 (C-6′); HREIMS *m*/*z* 286.0485 $[M]^+$ (calcd for $C_{15}H_{10}O_6$, 286.0477).

Acid Hydrolysis of 1. Compound **1** (14 mg) was refluxed for 75 min in 4 M HCl−dioxane (20 mL). The acid hydrolysate was concentrated, extracted with $CHCl_3$, and purified by preparative TLC using EtOAc−hexane (60:40) as eluent, yielding the liberated aglycon, compound **2**. The acidic mother liquor was neutralized with Na_2CO_3, filtered, and evaporated to dryness for examination of the sugar moiety. This proved to be β-D-glucose by detection on TLC [EtOAc−propanol−H_2O (65:23:12)], sprayed with freshly prepared anisaldehyde−H_2SO_4 reagent, followed by heating and measuring the specific rotation: $[\alpha]_D^{22}$ +19.7 (*c* 0.45, H_2O).

Antimicrobial Evaluation. *In vitro* antimicrobial studies were carried out against five microorganisms by determination of minimum inhibitory concentrations (MIC), as described previously, with the reference antibiotics ampicillin used as a positive control for *S. aureus* and *E. coli*, penicillin G for *P. aeruginosa*, and nystatin for the fungal microorganisms *C. albicans* and *B. cinerea*.[13] MIC values were determined by a microdilution method, as follows. Bacterial cultures grown on BHI agar plates for 16 h were suspended in MH broth. The bacterial cell numbers were adjusted to approximately (3−6) × 10^6 cfu (colony forming units)/mL. Test materials were applied at concentrations of between 20 and 100 mg/mL, respectively, and subjected to serial 2-fold dilutions. An 80 μL portion of each bacterial suspension was added to 20 μL of each of the serial 2-fold dilutions of each test material and mixed. The bacteria were incubated at 37 °C for 24 h, and the MIC values were then determined. Results were obtained from three independent experiments performed in duplicate. MIC values ≥ 1024 μg/mL were considered to indicate inactivity, while values < 64

μg/mL and 128−512 μg/mL indicated high and moderate activity, respectively.[14]

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