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ANTHRAQUINONES FROM THE POLAR FRACTIONS OF *GALIUM SINAICUM*

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Key Word Index—*Galium sinaicum*; Rubiaceae; roots; anthraquinones; anthraquinone glycosides; spectroscopic analysis.

Abstract—The new anthraquinones, 6,7-dimethoxy xanthopurpurin, 6-hydroxy-7-methoxy rubiadin, 5-hydroxy-6-hydroxymethyl anthragallol 1,3-dimethyl ether, 7-carboxy anthragallol 1,3-dimethyl ether, anthragallol 1-methyl ether 3-*O*-β-D-glucopyranoside, anthragallol 1-methyl ether 3-*O*-rutinoside, anthragallol 3-*O*-rutinoside and alizarin 1-methyl ether 2-*O*-primeveroside were isolated from the CH_2Cl_2 and n-BuOH extracts of *Galium sinaicum* roots and their structures were established by various spectroscopic techniques. In addition, two known anthraquinones were also isolated and fully characterized.

INTRODUCTION

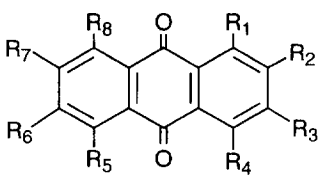
In previous studies [1, 2], the new anthraquinones, 6-hydroxy anthragallol 1,3-dimethyl ether, 7-hydroxymethyl anthragallol 1,3-dimethyl ether, 7-methyl anthragallol 1,3-dimethyl ether, 7-methyl anthragallol 2-methyl ether, 6-methyl anthragallol 3-methyl ether, 8-hydroxy anthragallol 2,3-dimethyl ether, 7-formyl anthragallol 1,3-dimethyl ether, copareolatin 5,7-dimethyl ether, copareolatin, 6,7-dimethyl ether, 6-methoxy lucidin ω-ethyl ether, 6-hydroxy xanthopurpurin and the novel bianthraquinone, bisinaiquinone (10,2′-bis(9-hydroxy-3-methyl-1,4-anthraquinone)) were isolated from the *n*-hexane and ether extracts of *Galium sinaicum* Boiss roots. In addition, the known compounds alizarin 1-methyl ether, alizarin 2-methyl ether, anthragallol 2-methyl ether, anthragallol 1,3-dimethyl ether, 8-hydroxy-7-methyl anthragallol 1,3-dimethyl ether, copareolatin 6-methyl ether, lucidin ω-ethyl ether, 2-methyl quinizarin, quinizarin and soranjidiol were also isolated and fully characterized.

In the present study, the polar fractions of *G. sinaicum* roots were investigated for their anthraquinone content and the structure elucidation of eight new compounds (**1**–**8**) carried out.

RESULTS AND DISCUSSION

From the slightly cytotoxic CH_2Cl_2 and *n*-BuOH extracts (P388 cell line, IC_{50}, 16 and 70 μg ml^{-1}, respectively) of *G. sinaicum* roots, eight new anthraquinones were isolated and identified as 6,7-dimethoxy xanthopurpurin (**1**), 6-hydroxy-7-methoxy rubiadin (**2**), 5-hydroxy-6-hydroxymethyl anthragallol 1,3-dimethyl ether (**3**), 7-carboxy anthragallol 1,3-dimethyl ether (**4**), anthragallol 1-methyl ether 3-*O*-β-D-glucopyranoside (**5**), anthragallol 1-methyl ether 3-*O*-rutinoside (**6**), anthragallol 3-*O*-rutinoside (**7**) and alizarin 1-methyl ether 2-*O*-primeveroside (ruberythric acid 1-methyl ether) (**8**). The known compounds 6-hydroxy anthragallol 1,3-dimethyl ether (**9**) [1] and 7-hydroxy-methyl anthragallol 1,3-dimethyl ether (**10**) [1, 2] were also characterized.

Compound **1** showed IR bands at 3450, 1662 and 1630 cm^{-1} for a free hydroxyl and unchelated and chelated carbonyl groups, respectively. The mass spec-



(**1**) R_1=R_3=OH, R_6=R_7=OCH_3, others=H
(**2**) R_1=R_3=R_6=OH, R_2=CH_3, R_7=OCH_3, others=H
(**3**) R_1=R_3=OCH_3, R_2=R_5=OH, R_6=CH_2OH, others=H
(**4**) R_1=R_3=OCH_3, R_2=OH, R_7=COOH, others=H
(**5**) R_1=OCH_3, R_2=OH, R_3=O-glc, others=H
(**6**) R_1=OCH_3, R_2=OH, R_3=O-glc(6→1)rham, others=H
(**7**) R_1=R_2=OH, R_3=O-glc(6→1)rham, others=H
(**8**) R_1=OCH_3, R_2=O-glc(6→1)xyl, others=H
(**9**) R_1=R_3=OCH_3, R_2=R_6=OH, others=H
(**10**) R_1=R_3=OCH_3, R_2=OH, R_7=CH_2OH, others=H

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†Deceased.

tra EI-MS displayed the $[M]^+$ peak at *m/z* 300, calculated for $C_{16}H_{12}O_6$. The ^1H NMR data (Table 1) showed, like 6-hydroxy xanthopurpurin [2], a peri-hydroxyl at δ 12.95 and two *m*-coupled protons (2.2 Hz) at δ 6.55 (H-2) and 7.17 (H-4). However, singlets for two OMe at δ 3.98 and 4.01 and also two downfield shifted *p*-protons at δ 7.59 (H-5) and δ 7.64 (H-8) were observed. The ^{13}C NMR data (Table 2) indicated the presence of two equivalent methoxyl, and unchelated and chelated carbonyl carbons (δ 56.1, 181.2 and 184.5). Two-dimensional NOESY cross-peak correlations were observed between the two methoxyl protons at δ 3.98 and δ 4.01 and the two, one-proton singlets at δ 7.59 (H-5) and 7.64 (H-8), respectively, confirming the location of the two methoxyl groups at C-6 and C-7. These spectral findings confirmed the identity of compound **1** as 6,7-dimethoxy xanthopurpurin.

Compound **2** showed IR bands at 3425, 1660 and 1630 cm^{-1} for a free hydroxyl and unchelated and chelated carbonyl groups, respectively. The FAB-mass spectrum displayed the $[M + H]^+$ peak at *m/z* 301 and $[M]^+$ at *m/z* 300 consistent with the molecular formula $C_{16}H_{12}O_6$. The ^1H NMR data (Table 1) showed signals for one methyl at δ 2.06, one methoxyl at δ 3.96 and a peri-hydroxyl at δ 13.28. A trisubstituted ring C was apparent from the aromatic proton singlet at δ 7.18 (H-4) and the β,β-disubstitution pattern in ring A by the two, one-proton singlets at δ 7.46 (H-5) and δ 7.59 (H-8). The ^{13}C NMR data (Table 2) confirmed the presence of one methyl, one methoxyl and unchelated and chelated carbonyl carbons δ 8.0, 55.9, 181.2 and 185.6, respectively). Two-dimensional NOESY experiments revealed a cross-peak correlation between the methoxyl protons at δ 3.96 and H-8 at δ 7.59. The HMQC and HMBC data (Fig. 1) confirmed the location of the methoxyl group at C-7 through two and three bond correlations from the methoxyl protons to C-7, from H-5 to C-7, C-10, C-11 and C-12 and from H-8 to C-6, C-7, C-9, C-11 and C-12. The remaining correlations, together with the above-mentioned spectral finding support the structure for compound **2** as 6-hydroxy-7-methoxy rubiadin.

Compound **3** showed IR bands at 3400, 1660 and 1627 cm^{-1} of a free hydroxyl and unchelated and chelated carbonyl groups, respectively. The FAB-mass spectrum displayed the $[M + H]^+$ peak at *m/z* 331 and $[M]^-$ at *m/z* 330, calculated for $C_{17}H_{14}O_7$. The ^1H NMR data (Table 1) showed the splitting pattern of an α,β-disubstituted ring A by two *o*-coupled aromatic protons (*d*, 7.8 Hz) detected at δ 7.82 (H-7) and δ 7.64 (H-8) in addition to a trisubstituted ring C by a proton singlet at δ 7.57 (H-4). It also showed signals for two methoxyl groups at δ 3.80 and δ 3.98, one CH_2OH at δ 4.62 and a peri-hydroxyl at δ 12.94. The ^{13}C NMR data (Table 2) and DEPT experiments confirmed the presence of two methyl, one methylene, three methine and 11 carbons of which two carbonyl ones were observed at δ 180.3 and 187.4. Two-dimensional NOESY experiments revealed three cross-peak correlations between the methoxyl protons at δ 3.98 and H-4, the CH_2OH and H-7 and the two *o*-coupled protons

Table 1. ^1H NMR (400 MHz, DMSO-d_6) spectral of compounds **1–8**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1-OH	12.95 *brs*	13.28 *brs*	–	–	–	–	13.1 *brs*	–
1-OCH_3	–	–	3.80 *s*	3.81 *s*	3.80 *s*	3.82 *s*	–	3.90 *s*
2-CH_3	–	2.06 *s*	–	–	–	–	–	–
2-H	6.55 *d* (2.2)	–	–	–	–	–	–	–
3-H	–	–	–	–	–	–	–	7.72 *d* (8.8)
3-OCH_3	–	–	3.98 *s*	3.98 *s*	–	–	–	–
4-H	7.17 *d* (2.2)	7.18 *s*	7.57 *s*	7.55 *s*	7.58 *s*	7.69 *s*	7.48 *s*	8.02 *d* (8.8)
5-OH	–	–	12.94 *brs*	–	–	–	–	–
5-H	7.59 *s*	7.46 *s*	–	8.13 *brd* (7.0)	8.07 *m*	8.10 *m*	8.24 *m*	8.15 *m*
6-H	–	–	–	8.27 *brd* (7.0)	7.78 *m*	7.81 *m*	7.94 *m*	7.89 *m*
6-CH_2OH	–	–	4.62 *s*	–	–	–	–	–
6-OCH_3	3.98 *s*	–	–	–	–	–	–	–
7-OCH_3	4.01 *s*	3.96 *s*	–	–	–	–	–	–
7-H	–	–	7.82 *d* (7.8)	–	7.78 *m*	7.81 *m*	7.94 *m*	7.89 *m*
8-H	7.64 *s*	7.59 *s*	7.64 *d* (7.8)	8.59 *brs*	8.07 *m*	8.10 *m*	8.24 *m*	8.15 *m*
H-1′	–	–		–	4.56 *d* (6.9)	4.90 *brs*	5.10 *d* (7.0)	5.03 *d* (7.5)
H-1″	–	–	–	–	–	4.56 *brs*	4.53 *brs*	4.17 *d* (7.4)
CH_3-6″	–	–	–	–	–	1.09 *d* (6.1)	1.07 *d* (6.2)	–

Table 2. ^{13}C NMR (100 MHz, DMSO-d_6) spectral data of compounds **1–8**

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	166.9	162.1	148.3 *s*	148.2 *s*	151.2	149.3 *s*	161.8	149.2
2	105.2	116.7	147.8 *s*	147.0 *s*	153.0	150.4 *s*	161.8	156.9
3	164.7	162.1	152.4 *s*	152.8 *s*	151.6	152.0 *s*	161.9	124.5
4	107.3	107.3	105.8 *d*	106.3 *d*	116.7	111.8 *d*	106.3	121.0
5	111.3	112.6	157.8 *s*	126.2 *d*	125.9	126.3 *d*	127.1	126.6
6	153.3†	162.5	114.2 *s*	134.0 *d*	132.9	133.6 *d*	134.7	134.6
7	153.6†	152.7	133.2 *d*	134.3 *s*	132.4	133.4 *d*	134.6	134.3
8	108.3	108.5	118.2 *d*	127.5 *d*	125.4	126.0 *d*	126.4	126.6
9	184.5	185.6	180.3 *s*	181.0 *s*	180.7	180.7 *s*	187.1	181.7
10	181.2	181.2	187.4 *s*	181.7 *s*	181.1	181.7 *s*	181.8	181.6
11	127.8	128.0	136.7 *s*	134.3 *s*	133.7	132.8 *s*	133.0	132.3
12	127.5	125.9	132.6 *s*	139.0 *s*	134.6	134.4 *s*	133.8	133.8
13	108.2	108.5	121.0 *s*	121.0 *s*	123.4	122.2 *s*	123.5	124.5
14	134.8	131.8	124.7 *s*	126.7 *s*	134.6	126.1 *s*	132.8	127.8
1-OCH_3	–	–	60.5 *q*	61.0 *q*	60.2	60.0 *q*	–	60.8
2-CH_3	–	8.0	–	–	–	–	–	–
3-OCH_3	–	–	56.1 *q*	56.5 *q*	–	–	–	–
6-CH_2–OH	–	–	57.3 *t*	–	–	–	–	–
6-OCH_3	56.1	–	–	–	–	–	–	–
7-OCH_3	56.1	55.9	–	–	–	–	–	–
7-COOH	–	–	–	167.0 *s*	–	–	–	–
C-1′	–	–	–	–	104.8	102.0 *d*	100.7	100.4
C-2′	–	–	–	–	73.7	73.2 *d*	73.2	73.7
C-3′	–	–	–	–	76.9	76.0 d	75.9	76.1
C-4′	–	–	–	–	69.5	69.7 *d*	69.7	69.7
C-5′	–	–	–	–	77.4	75.9 *d*	75.9	76.6
C-6′	–	–	–	–	60.7	66.2 *t*	65.9	68.4
C-1″	–	–	–	–	–	100.6 *d*	100.6	104.0
C-2″	–	–	–	–	–	70.2 *d*	70.1	73.4
C-3″	–	–	–	–	–	70.7 *d*	70.6	76.4
C-4″	–	–	–	–	–	72.1 *d*	72.1	69.6
C-5″	–	–	–	–	–	68.3 *d*	68.2	65.5
CH_3-6″	–	–	–	–	–	17.8 *q*	17.8	–

†Exchangeable values.

*^{13}C Multiplicities were determined by DEPT pulse sequence.

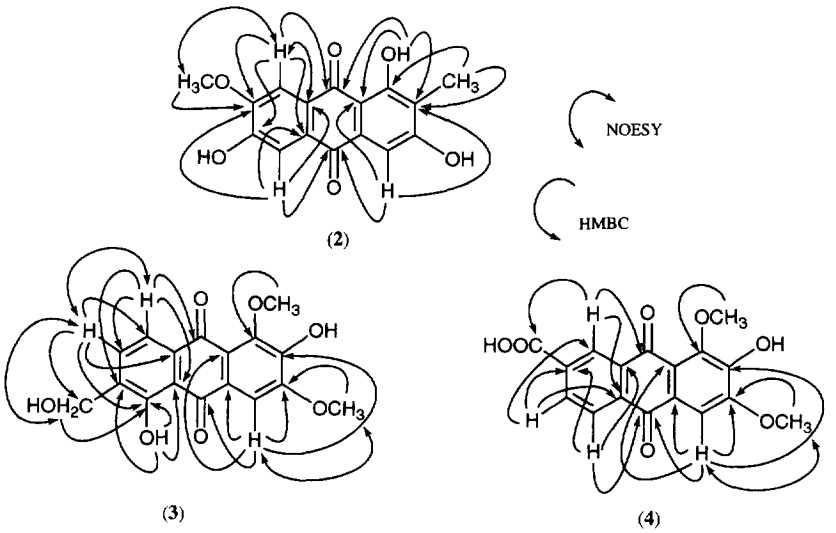

Fig. 1. Two-dimensional NOESY and HMBC correlations of compounds **2–4**.

H-7 and H-8. The HMQC and HMBC spectra confirmed the location of the CH_2OH group at C-6 through three bond correlations with C-5 and C-7 as shown in Fig. 1. The remaining correlations together with the above-mentioned spectral finding, confirmed the identity of compound **3** as 5-hydroxy-6-hydroxymethyl anthragallol 1,3-dimethyl ether.

Compound **4** showed IR bands at 3370, 1700 and 1664 cm^{-1} of a free hydroxyl and two unchelated carboxyl groups, respectively. The EI-mass spectrum displayed the $[M]^+$ peak at *m/z* 328, calculated for $C_{17}H_{12}O_7$. The ^1H NMR data (Table 1) showed the splitting pattern of a β-substituted ring A by three coupled protons at δ 8.13 (*brd*, 7.0, H-5), 8.27 (*brd*, 7.0, H-6) and 8.59 (*brs*, H-8) and also a trisubstituted ring C by a proton singlet at δ 7.55 (H-4). Signals for two methoxyl protons were also observed at δ 3.81 and δ 3.98 of which the latter displayed a NOESY correlation with H-4 at δ 7.55. The ^{13}C NMR data (Table 2) and DEPT experiments indicated the presence of two methoxyl and three carbonyl carbons at δ 56.5, 61.1, 167.0, 181.0 and 181.7. The carbonyl resonance at δ 167.0 together with the broad absorption IR band at 3370 cm^{-1} (hydroxyl stretch) and the other one at 1700 cm^{-1} (C=O stretch) suggested a COOH substituent in ring A. The HMQC and HMBC spectra confirmed the location of the COOH group at C-7 through three bond correlations from H-8 to the carbonyl resonance at δ 167.0 (COOH), C-6, C-9 and C-11 and from H-5 to C-7, C-10 and C-12 as shown in Fig. 1. The remaining correlations, together with the above-mentioned spectral finding, confirmed compound **4** as 7-carboxyl anthragallol 1,3-dimethyl ether.

Compounds **5–8** were generally characterized by strong and broad IR bands at *ca* 3400 and 1060 cm^{-1} and the strong positive colour reaction they gave with Molisch's test. The corresponding sugar and aglycone residues were readily liberated on acid hydrolysis with 2M HCl–MeOH (2:1) suggesting that all are *O*-glycosides. The ^1H NMR data (Table 1) showed, for the aglycone moieties, two sets of 2-proton multiplets at *ca* δ 7.8 (H-6, H-7) and δ 8.1 (H-5, H-8) of an unsubstituted ring A. However, compounds **5–7** contained a trisubstituted ring C as shown by a 1-proton singlet detected at *ca* δ 7.6, whereas compound **8** had an α,β-disubstitution patterns as shown by two *o*-coupled protons (*d*, J = 8.8 Hz) at δ 7.72 (H-3) and 8.02 (H-4).

Compound **5** showed δ_H signals (Table 1) for one methoxyl at δ 3.80(*s*) and one anomeric proton with diaxial coupling at δ 4.56 (*d*, J = 6.9 Hz). Glucose was the monosaccharide detected in its acidic hydrolysate and the liberated aglycone was identified as anthragallol 1-methyl ether by comparison with a reference sample. The FAB-mass spectrum displayed a $[M + Na + 2H]^+$ peak at *m/z* 457 calcd for $C_{21}H_{20}O_{10}$ and significant fragment ions at *m/z* 295 $[M - glc + Na + 2H]^+$ and 271 $[M - glc + H]^+$ corresponding to the loss of the glucosyl moiety. The ^{13}C NMR data (Table 2) indicated the presence of one methoxyl, and one anomeric and two unchelated carbonyl carbons (δ 60.2, 104.8, 180.7 and 181.1, respectively). The glycosylation site at the C-3 hydroxyl was confirmed through NOE experiments where irradiation of the anomeric proton doublet at δ 4.56 enhanced the aromatic proton singlet at δ 7.58 (H-4). A similar effect was also observed on the anomeric proton upon irradiation of H-4. Therefore, compound **5** was established as anthragallol 1-methyl ether 3-*O*-β-D-glucopyranoside.

Compound **6** in terms of its ^1H NMR data (Table 1) showed the same splitting pattern of the aglycone part as compound **5**, while the sugar moiety demonstrated signals for two anomeric protons at δ 4.90 (unresolved doublet) and 5.56 (*brs*) indicating that it was a bioside in nature. A three-proton doublet was also observed at δ 1.09 (J = 6.1 Hz) indicating that rhamnose was one of the constitutent sugars. Acid hydrolysis yielded anthragallol 1-methyl ether, glucose and rhamnose and the FAB-mass spectrum displayed $[M + Na]^+$ and $[M + H]^+$ peaks at *m/z* 601 and 579, respectively, calculated for $C_{27}H_{30}O_{14}$, which were 146 amu higher than those of compound **5**. The ^{13}C NMR data (Table 2) and DEPT experiments indicated the presence of 27 carbons of which one methyl, one methoxyl, two anomeric and two unchelated carbonyl carbons were observed at δ 17.8, 60.6, 100.6., 102.0, 180.7 and 181.7, respectively. Two-dimensional NOESY (Fig. 2) presented a cross-peak correlation between the anomeric proton at δ 4.90 (H-1′) and the aromatic proton singlet at δ 7.69 (H-4) confirming the glycosylation site at C-3. The HMQC and HMBC data (Fig. 2) unambiguously identified the biose as rutinose through a three bond correlation from H-1″ to C-6′. The remaining correlations together with the above mentioned data confirmed the identity of compound **6** as anthragallol 1-methyl ether 3-*O*-rutinoside.

Compound **7** showed close resemblance to compound **6** by demonstrating the same splitting pattern of the aromatic protons (Table 1) and the presence of glucose and rhamnose in its acidic hydrolysate. The constitutent sugars were confirmed by the signals for two anomeric protons at δ 5.10 (*d*, J = 7.0 Hz, H-1′) and 4.53 (*brs*, H-1″) and a three proton doublet at δ 1.07 (*d*, J = 6.2 Hz, Me-6″) accompanied by their corresponding carbon signals (δ_{13} 100.7, 100.6 and 17.8, respectively) (Table 2). However, compound **7** was significantly different from **6** in terms of its aglycone part. The NMR data of the latter was characterized by a peri-hydroxyl at δ 13.1 (*brs*, 1-OH), a chelated carbonyl at δ 187.1 (1630 cm^{-1}, C-9) and the absence of the methoxyl resonance. The FAB-mass spectrum displayed the $[M + glycerol]^+$ peak at *m/z* 656 calculated for $C_{26}H_{28}O_{14}$, which is 14 amu less than that of compound **6**. The two-dimensional NOESY, HMQC and HMBC data (Fig. 2) confirmed, in similar ways to those mentioned above, the glycosylation site at C-3 and identified the biose as rutinose to finally establish the structure of compound **7** as anthragallol 3-*O*-rutinoside.

Compound **8** showed δ_H signals (Table 1) for one methoxyl at δ 3.90 and two anomeric protons with

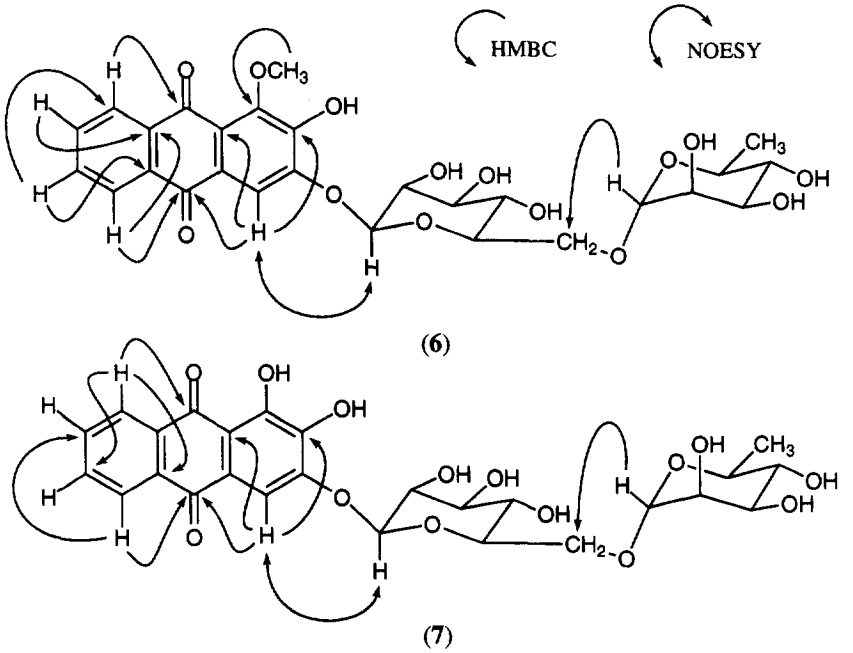

Fig. 2. Two-dimensional NOESY and HMBC correlations of compounds **6** and **7**.

diaxial coupling at δ 5.03 (*d*, $J = 7.5$ Hz, H-1′) and 4.17 (*d*, $J = 7.4$ Hz, H-1″). Glucose and xylose were the monosaccharides detected in its acidic hydrolysate and the liberated aglycone was identified as alizarin 1-methyl ether by comparison with a reference sample. The FAB-mass spectrum displayed the $[M + \text{glycerol} + Na + H]^+$ peak at *m/z* 664, calculated for $C_{26}H_{28}O_{13}$, and significant fragment ions at *m/z* 531 $[M - \text{xyl} + \text{glycerol} + Na]^+$ and 255 $[M - \text{xyl} - \text{glc} + H]^+$, corresponding to the successive loss of a xylosyl and a glucosyl moieties, proved that xylose was the terminal sugar. The ^{13}C NMR data (Table 2) confirmed the biose nature of the sugar residue by the presence of two anomeric carbon signals at δ 100.4 and δ 104.0. It also revealed the presence of 26 carbons of which one methoxyl and two unchelated carbonyl carbons were observed at δ 60.8, 181.6 and 181.7, respectively, thus confirming the glycosylation site at C-2. Comparison of the sugar carbon resonances with published data revealed that the signal at δ 68.4 assigned to C-6′ of glucose was downfield shifted by *ca* 7 ppm from that of methyl-β-D-glucose [3]. Thus, the terminal xylosyl moiety should be attached to the glucosyl part at C-6′ through $(1 \rightarrow 6)$-β-linkage. Moreover, all the biose carbon resonances were in full agreement with those published for the sugar parts of lucidin 3-*O*-primeveroside and alizarin 2-*O*-primeveroside (ruberythric acid) [4]. Accordingly, compound **8** was identified as alizarin 1-methyl ether 2-*O*-primeveroside (ruberythric acid 1-methyl ether).

It is noteworthy that a total of 23 anthraquinones, four anthraquinone glycosides and one bianthraquinone have been isolated from the roots of *G. sinaicum*, of which 18 components are hitherto unreported. Anthragallol-derived anthraquinones are apparently the dominant secondary metabolites, as they constitute half of the isolated compounds. Although the genus *Galium* has long been known to contain substantial amounts of anthraquinones of diverse types [5, 6], *G. sinaicum* is characterized, in the present study, as the only *Galium* species from which anthragallol-, copareolatin-, quinizarin- and soranjidiol-derived anthraquinones as well as a bianthraquinone have been isolated. Moreover, the anthragallol-derived anthraquinone glycosides are reported here for the first time in the Rubiaceae. However, purpurin-, pseudopurpurin-, and munjistin-derived anthraquinones, previously reported from other *Galium* species, were not detected. The present isolation of bisinaiquinone and the anthragallol-derived secondary metabolites from the roots of *G. sinaicum* provide valuable markers for its identification and characterization within the genus and the family.

Anthraquinones **1–4** and **6–8** isolated from *G. sinaicum* were subjected to the cytotoxic bioassay against P388 leukemic cells. As can be seen from Table 3, the free anthraquinones **1–4** showed stronger activity than the anthraquinone glycosides **6–8**.

EXPERIMENTAL

General. Mps: uncorr; ^1H and ^{13}C NMR: DMSO-d_6 with TMS as int. standard, Bruker AM400. ^{13}C Multiplicities were determined by the DEPT pulse sequence. 2D-NMR (NOESY, HMQC, HMBC): Bruker AM500; EI-MS (70 eV) and FAB-MS (8 kV, glycerol): Hitachi M-80 or VG 70-SE Autospec; IR: KBr; UV: CH_3OH; Prep. HPC: 10 μm ODS column; MPLC: 20 μm ODS column, Kusano Scientific Co; CC: Kieselgel 60 and

Table 3. Cytotoxic activity of anthraquinones **1–8** against P388 cells

Compound	IC_{50} values (μg ml)
1	1.0
2	10.4
3	10.2
4	3.4
6	>100.0
7	>100.0
8	48.0

Compound **5** could not be bioassayed owing to the lack of sufficient material.

Diaion HP-20; Anal. TLC: silica gel $60F_{254}$ and RP-18 F_{254} (0.25 mm) precoated plates. Spot detection: UV light at 254 nm, exposure to NH_3 vapour and/or spraying with 10% H_2SO_4 or 5% KOH in MeOH. Prep. TLC: silica gel $60F_{254}$ (0.5 mm).

Plant material. Roots of *G. sinaicum* Boiss. were collected, in May 1991, from the mountains of the Saint Katherine district, Sinai Peninsula. The plant's identity was kindly verified by Professor Dr N. El-Hadidi, Faculty of Science, Cairo University. A voucher specimen, documenting this collection, has been deposited at the Pharmacognosy Department, Faculty of Pharmacy, University of Mansoura.

Extraction and isolation. The CH_2Cl_2 and *n*-BuOH extracts of *G. sinaicum* roots, obtained after extraction with *n*-hexane [2], were subjected to the following fractionation and purification procedures. The CH_2Cl_2 extract (40 g) was fractionated over a silica gel column eluted with *n*-hexane–EtOAc and $CHCl_3$–MeOH gradient, respectively, and similar fractions were pooled together. Some of the fractions were partially purified by partitioning with 2N Na_2CO_3. The *n*-BuOH extract (11 g) was coarsely fractionated over a Diaion HP-20 column eluted with H_2O, H_2O–MeOH (3:2 and 2:3), MeOH and Me_2CO, respectively. The frs obtained, in each case, were subjected either to direct crystallization or to further purification on RP-18 columns using various chromatographic techniques (HPLC, MPLC, PTLC) to yield the anthraquinones **1–10**.

6,7-*Dimethoxy xanthopurpurin* (**1**). Yellow powder (4 mg) from the CH_2Cl_2 extract (Na_2CO_3 fr); mp 177–180°. UV λ_{max} (MeOH) nm: 206, 286, 306sh, 380sh; IR ν^{KBr} cm^{-1}: 3450 (OH), 2925, 1662 (CO), 1630 (chelated CO), 1580, 1517, 1460, 1320, 1240, 1220, 1180, 1105, 1060, 982, 880, 772, 620; EI-MS *m/z* (rel. int.): 300 $[M]^+$ (100), calcd for $C_{16}H_{12}O_6$, 281 (12), 257 (13), 229 (19), 212 (5), 186 (18), 155 (7), 136 (14), 109 (5), 91 (12), 69 (19); ^1H NMR (400 MHz, DMSO-d_6): Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

6-*Hydroxy*-7-*methoxy rubiadin* (**2**). Yellow crystals (5 mg) from the CH_2Cl_2 extract (Na_2CO_3 fr.); mp 270–273°. UV λ_{max} (MeOH) nm: 205, 288, 328, 400; IR ν^{KBr} cm^{-1}: 3425 (OH), 2920, 2310, 1660 (CO), 1630 (chelated CO), 1595, 1517, 1450, 1320, 1220, 1160, 1117, 1060, 1000, 895, 817, 760, 605; FAB-MS *m/z* (rel. int.): 301 $[M + H]^+$ (30), 300 $[M]^+$ (6) calcd for $C_{16}H_{12}O_6$, 243 $[M - 2CHO + H]^+$ (6), 223 (31), 207 (30), 185 (48), 153 (10), 131 (32), 115 (100); ^1H NMR (400 HMz, DMSO-d_6): Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

5-*Hydroxy*-6-*hydroxymethyl anthragallol* 1,3-*dimethyl ether* (**3**). Yellow needles (6 mg) from the CH_2Cl_2 extract (Na_2CO_3 fr); mp 237–239°. UV λ_{max} (MeOH) nm: 216, 284, 406; IR ν^{KBr} cm^{-1}: 3400 (OH), 2945, 1660 (CO), 1627 (chelated CO), 1588, 1495, 1465, 1430, 1365, 1315, 1297, 1275, 1235, 1200, 1130, 1100, 1070, 1025, 1002, 917, 860, 740, 620; FAB-MS *m/z* (rel. int.): 331 $[M + H]^+$ (78), 330 $[M]^+$ (22) calcd for $C_{17}H_{14}O_7$, 315 (100), 299 (65), 285 (33), 264 (28), 235 (18), 223 (43), 205 (31), 193 (24), 181 (15), 164 (22), 151 (56), 137 (55), 115 (79); ^1H NMR (400 MHz, DMSO-d_6): Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

7-*Carboxy anthragallol* 1,3-*dimethyl ether* (**4**). Yellow needles (15 mg) from the *n*-BuOH extract (aq. MeOH, 60% fr.); mp 244–246°. UV λ_{max} (MeOH) nm: 209, 245sh, 284; IR ν^{KBr} cm^{-1}: 3370 (OH), 2925, 1700 (CO), 1664 (CO), 1592, 1560, 1498, 1381, 1342, 1283, 1140, 1085, 983, 880, 800, 720; EI-MS *m/z* (rel. int.): 328 $[M]^+$ (47), calcd for $C_{17}H_{12}O_7$, 310 $[M - H_2O]^+$ (34), 281 $[M - CHO - H_2O]^+$ (28), 252 $[M - 2CHO - H_2O]^+$ (13), 214 (25), 181 (42), 152 (12), 123 (56), 91 (32), 69 (100); ^1H NMR (400 MHz, DMSO-d_6): Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

Anthragallol 1-*methyl ether* 3-*O*-β-D-*glucopyranoside* (**5**). Red powder (5 mg) from the *n*-BuOH extract (aq. MeOH, 60% fr.); mp 107–109°. UV λ_{max} (MeOH) nm: 206, 242, 278, 316sh, 354sh; IR ν^{KBr} cm^{-1}: 3400 (OH), 2935, 1660 (CO), 1590, 1545, 1465, 1342, 1265, 1075, 800, 717; FAB-MS *m/z* (rel. int.): 457 $[M + Na + 2H]^+$ (25) calcd for $C_{21}H_{20}O_{10}$, 432 (15), 421 (27), 399 (91), 385 (21), 371 (19), 343 (21), 315 (25), 295 $[M - glc + Na + 2H]^+$ (13), 271 $[M - glc + H]^+$ (15), 253 (13), 223 (100), 207 (76), 185 (83); ^1H NMR (400 MHz, DMSO-d_6): Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

Anthragallol 1-*methyl ether* 3-*O*-*rutinoside* (**6**). Red powder (15 mg) from the *n*-BuOH extract (aq. MeOH, 60% fr.); mp 154–156°. UV λ_{max} (MeOH) nm: 206, 240, 277, 316sh; IR ν^{KBr} cm^{-1}: 3360 (OH), 2920, 1663 (CO), 1585, 1450, 1340, 1050, 797, 765, 724; FAB-MS *m/z* (rel. int.): 601 $[M + Na]^+$ (22), 579 $[M + H]^+$ (13), 533 (14), 493 (12), 469 (14), 416 (28), 281 (61), 271 $[M - glc - rha + H]^+$ (58), 220 (49), 207 (69), 185 (81); ^1H NMR (400 MHz, DMSO-d_6): Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

Anthragallol 3-*O*-*rutinoside* (**7**). Orange crystals (10 mg) from the *n*-BuOH extract (aq. MeOH, 60% fr.); mp 187–190°. UV λ_{max} (MeOH) nm: 204, 228sh, 244, 266, 323sh, 404; IR ν^{KBr} cm^{-1}: 3400 (OH), 2915, 1668 (CO), 1630 (chelated CO), 1592, 1483, 1368, 1330, 1290, 1223, 1060, 1000, 715; FAB-MS *m/z* (rel. int.): 656 $[M + glycerol]^+$ (13), 642 $[M + 2K]^+$, 623 (15), 603 $[M + K]^+$ (9), 601 (64), 557 (13), 537 (17),

490 (13), 467 (18), 430 (25), 421 (41), 411 (37), 399 (64), 390 (31), 371 (19), 329 (33), 275 (42), 256 (72), 253 (99), 199 (23), 192 (34), 177 (71), 149 (100), 133 (64), 119 (50); ^{1}H NMR (400 MHz, DMSO-d_6): Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

Alizarin 1-methyl ether 2-O-primeveroside (*ruberythric acid 1-methyl ether*) (**8**). Yellow powder (5 mg) from the *n*-BuOH extract (aq. MeOH, 60% fr.); mp 150–153°. UV λ_{max} (MeOH) nm: 208, 224sh, 256, 328sh, 368sh; IR ν^{KBr} cm^{-1}: 3390 (OH), 2920, 1672 (CO), 1575, 1480, 1410, 1335, 1270, 1060, 990, 900, 812, 760, 718; FAB-MS *m/z*: 664 [M + glycerol + Na + H]$^+$ (42), 619 (32), 549 [M + H]$^+$ (9), 531 [M − xyl + glycerol + Na]$^+$ (27), 421 (29), 387 [M − xyl − glc + glycerol + K + 2H]$^+$ (33), 295 (24), 255 [M − xyl − glc + H]$^+$ (29), 207 (34), 181 (60), 172 (100); ^{1}H NMR (400 MHz, DMSO-d_6): Table 1; ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

Cytotoxicity. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was performed in a 96-well plate [7, 8]. The assay is dependent on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. Mouse P388 leukaemia cells (2×10^4 cells ml^{-1}) were inoculated in each well with 100 μl ml^{-1} of RPMI-1640 medium (Nissui Pharm. Co., Ltd.) supplemented with 5% fetal calf serum (Mitsubishi Chemical Industry Co., Ltd) and kanamycin (100 μg ml^{-1}) at 37°C in a humidified atmosphere of 5% CO_2. Various drug concentrations (10 μl) were added to the cultures at day 1 after the transplantation. At day 3, 20 μl of MTT soln (5 mg ml^{-1}) per well was added to each cultured medium. After a further 4 hr incubation, 100 μl of 10% SDS–0.01 N HCl soln was added to each well and the formazan crystals in each well were dissolved by stirring with a pipette. The optical density measurements were made using a microplate reader (Tohso MPR-A4i) with a two-wavelength system (550 and 700 nm). In all these experiments, three replicate wells were used to determine each point.

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