Pergamon

0031-9422(95)00145-X

Phytochemistry, Vol. 40, No. 1, pp. 245–251, 1995

0031-9422/95 $9.50 + 0.00

ANTHRAQUINONES FROM *GALIUM SINAICUM*

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(*Received in revised form* 18 *January* 1995)

Key Word Index—*Galium sinaicum*; Rubiaceae; roots; anthraquinones; bianthraquinone; cytotoxic activity.

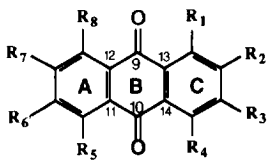
Abstract—The new anthraquinones, 7-methylanthragallol 1,3-dimethyl ether, 7-methyl-anthragallol 2-methyl ether, 6-methylanthragallol 3-methyl ether, 8-hydroxyanthragallol 2,3-dimethyl ether, 7-formylanthragallol 1,3-dimethyl ether, copareolatin 5,7-dimethyl ether, copareolatin 6,7-dimethyl ether, 6-methoxylucidin ω-ethyl ether and 6-hydroxyxanthopurpurin, as well as a novel bianthraquinone, bisinaiquinone [10,2′-bi(9-hydroxy-3-methyl-1,4-anthraquinonyl)] were isolated from the roots of *Galium sinaicum*. Their structures were established by spectroscopic techniques including 2D-NMR. In addition, eight known anthraquinones were also isolated and fully characterized.

INTRODUCTION

The genus *Galium* has long been known to contain substantial amounts of anthraquinones, with the roots being especially rich sources of these secondary metabolites. Alizarin-, anthragallol-, lucidin-, munjistin-, purpurin-, xanthopurpurin-, and rubiadin-derived anthraquinones and others are reported [1–3]. In a previous study [3], the new anthraquinones, 6-hydroxyanthragallol 1,3-dimethyl ether and 6-(or 7-)hydroxymethylanthragallol 1,3-dimethyl ether, were isolated from *G. sinaicum* roots. In addition, alizarin 1-methyl ether, and anthragallol 1,3-dimethyl ether were also isolated and fully characterized. We have now reinvestigated the roots of the title species for their anthraquinone content.

RESULTS AND DISCUSSION

From the cytotoxic *n*-hexane extract (P388 cell line, IC_{50}, 3.8 $\mu g\,ml^{-1}$) of *G. sinaicum* roots, 18 anthraquinones were isolated. In addition to the known ones, quinizarin (**11**) [2, 4], 2-methylquinizarin (**12**) [5], alizarin 2-methyl ether (**13**) [5], lucidin ω-ethyl ether (**14**) [6], soranjidiol (**15**) [7], anthragallol 2-methyl ether (**16**) [8, 9], copareolatin 6-methyl ether (**17**) [10] and 7-methyl-8-hydroxyanthragallol 1,3-dimethyl ether (**18**) [11], 10 new compounds were isolated and spectroscopically characterized as 7-methylanthragallol 1,3-dimethyl ether **1**, 7-methylanthragallol 2-methyl ether **2**, 6-methylanthragallol 3-methyl ether (**3**), 8-hydroxyanthragallol 2,3-dimethyl ether (**4**), 7-formylanthragallol 1,3-dimethyl ether (**5**), copareolatin 5,7-dimethyl ether (**6**), copareolatin 6,7-dimethyl ether (**7**), 6-methoxylucidin ω-ethyl ether (**8**), 6-hydroxyxanthopurpurin (**9**) and 10,2′-bi(9-hydroxy-3-methyl-1,4-anthraquinonyl), named bisinaiquinone (**10**) whose structure elucidation is described herein. On the other hand, the previously reported 6- (or 7-) hydroxymethylanthragallol 1,3-dimethyl



(1) $R_1 = R_3 = OMe$, $R_2 = OH$, $R_7 = Me$, others = H
(2) $R_1 = R_3 = OH$, $R_2 = OMe$, $R_7 = Me$, others = H
(3) $R_1 = R_2 = OH$, $R_3 = OMe$, $R_6 = Me$, others = H
(4) $R_1 = R_8 = OH$, $R_2 = R_3 = OMe$, others = H
(5) $R_1 = R_3 = OMe$, $R_2 = OH$, $R_7 = CHO$, others = H
(6) $R_1 = R_6 = OH$, $R_2 = Me$, $R_5 = R_7 = OMe$, others = H
(7) $R_1 = R_5 = OH$, $R_2 = R_6 = R_7 = OMe$, others = H
(8) $R_1 = R_3 = OH$, $R_2 = CH_2\text{-}O\text{-}CH_2\text{-}Me$, $R_6 = OMe$, others = H
(9) $R_1 = R_3 = R_6 = OH$, others = H
(10) Bianthraquinone, see Fig. 2.
(11) $R_1 = R_4 = OH$, others = H
(12) $R_1 = R_4 = OH$, $R_2 = Me$, others = H
(13) $R_1 = OH$, $R_2 = OMe$, others = H
(14) $R_1 = R_3 = OH$, $R_2 = CH_2\text{-}O\text{-}CH_2\text{-}Me$, others = H
(15) $R_1 = R_6 = OH$, $R_2 = Me$, others = H
(16) $R_1 = R_3 = OH$, $R_2 = OMe$, others = H
(17) $R_1 = R_5 = R_7 = OH$, $R_2 = Me$, $R_6 = OMe$, others = H
(18) $R_1 = R_3 = OMe$, $R_2 = R_8 = OH$, $R_7 = Me$, others = H
(19) $R_1 = R_3 = OMe$, $R_2 = OH$, $R_7 = CH_2OH$, others = H

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ether [3] was confirmed to be the 7-hydroxymethyl compound **19** by the two or three bond correlations from H-4 to C-2, C-10, C-13, from H-5 to C-7, C-10, C-12, from the hydroxymethyl protons to C-6, C-7, C-8, and from H-8 to C-6, C-9, C-11 in the HMBC spectrum.

Compound **1** showed IR bands at 3310, 1167 cm^{-1} of a free hydroxyl and unchelated carbonyl groups, respectively. The EI-mass spectrum displayed a very weak $[M]^+$ at *m/z* 298, confirmed by FAB, consistent with the molecular formula $C_{17}H_{14}O_5$. The ^1H NMR spectrum (Table 1) showed signals for one methyl at δ2.51, two methoxyl at δ4.03 and 4.08 and a phenolic β-hydroxyl group at δ6.3. A trisubstituted ring C was indicated by the aromatic proton singlet at δ7.7 (H-4) and the β-monosubstitution pattern in ring A by the three coupled protons at δ8.04 (*d*, J = 8 Hz, H-5), 7.51 (*dd*, J = 8.0, 1.6 Hz, H-6) and 7.97 (*d*, J = 1.6 Hz, H-8). ^{13}C NMR data (Table 2) and DEPT experiments confirmed the presence of three Me, four CH and 10 quaternary carbons, of which two carbonyl carbons were observed at δ182.1 and 182.3. NOE measurements revealed the presence of a 1,3-dimethoxy-2-hydroxy pattern and a β-Me group. HMQC and HMBC confirmed the location of the methyl group at C-7 through two- and three-bond correlations (Fig. 1) from the methyl protons to C-7, C-6 and C-8, from H-6 to C-8 and C-11, from H-8 to C-6, C-9 and C-11 and from H-5 to C-7, C-10 and C-12. These correlations together with the above spectra support the structure **1** of a 7-methylanthragallol 1,3-dimethyl ether.

Compound **2** showed IR bands at 3392 1633 and 1673 cm^{-1} of a free hydroxyl group and two chelated and unchelated carbonyl absorptions, respectively. The EI-mass spectrum displayed a $[M]^+$ at *m/z* 284, 14 mu less than that of **1**, and calculated for $C_{16}H_{12}O_5$. The ^1H NMR data (Table 1) showed, like **1**, the same splitting pattern of the aromatic protons but a *peri*-hydroxyl and only one methoxyl group were detected at δ13.14 and 4.13, respectively. NOE exhibited 5 and 7% enhancements for the protons at δ7.55 and 8.07, respectively, upon irradiation of the methyl resonance at δ2.52. No effect was observed upon irradiation of the methoxyl protons at δ4.13 and, thus, it must be at C-2. Methylation

Table 1. ^1H NMR spectral data of compounds 1–9 (400 MHz, $CDCl_3$)

H	1	2	3*	4	5	6	7	8	9*
OH-1	—	13.14 *b, s*	—	12.21 *b, s*	—	12.90 s	12.84 *s*	13.21 *s*	—
OMe-1	4.03 *s*	—	—	—	4.05 *s*	—	—	—	—
OH-2	6.30 *b, s*	—	—	—	6.41 *b, s*	—	—	—	—
Me-2	—	—	—	—	—	2.35 *s*	2.31 *s*	—	—
2	—	—	—	—	—	—	—	—	6.62 *d*
OMe-2	—	4.13 *s*	—	4.02 *s*	—	—	—	—	—
CH_2-2 (a)	—	—	—	—	—	—	—	4.96 *s*	—
CH_2-2 (b)	—	—	—	—	—	—	—	3.71 *q*	—
								(7.2)	—
Me-2 (c)	—	—	—	—	—		—	1.32 *t*	—
								(7.2)	—
OH-3	—	6.50 *b, s*	—	—	—	—	—	9.55 *b, s*	—
3	—	—	—	—	—	7.84 *d*	7.49 *d*	—	—
						(7.6)	(7.6)	—	—
OMe-3	4.08 *s*	—	3.99 *s*	4.05 *s*	4.11 *s*	—	—	—	—
4	7.70 *s*	7.47 *s*	7.62 *s*	7.49 *s*	7.73 *s*	7.67 *d*	7.65 *d*	7.29 *s*	7.22 *d*
						(7.6)	(7.6)	—	(2.3)
OH-5	—	—	—	—	—	—	12.89 *s*	—	—
OMe-5	—	—	—	—	—	4.02 *s*	—	—	—
5	8.04 *d*	8.14 *d*	7.59 *d*	7.80 *dd*	8.38 *d*	—	—	7.69 *d*	7.56 *d*
	(8.0)	(7.9)	(0.8)	(7.6, 1.2)	(7.8)			(2.6)	(2.6)
Me-6	—	—	2.28 *s*	—	—	—	—	—	—
OMe-6	—	—	—	—	—	—	3.96 *s*	3.97 *s*	—
6	7.51 *dd*	7.55 *dd*	—	7.63 *t*	8.22 *dd*	—	—	—	—
	(8.0, 1.6)	(7.9, 0.5)		(7.6)	(7.8, 1.6)				
Me-7	2.51 *s*	2.52 *s*	—	—	—	—	—	—	—
CHO-7	—	—	—	—	10.21 *s*	—	—	—	—
OMe-7	—	—	—	—	—	4.08 *s*	3.98 *s*	—	—
7	—	—	7.40 *dd*	7.25 *dd*	—	—	—	7.23 *dd*	7.17 *dd*
			(7.7, 0.8)	(7.6, 1.2)				(8.6, 2.6)	(8.4, 2.4)
OH-8	—	—	—	12.09 *b, s*	—	—	—	—	—
8	7.97 *d*	8.07 *d*	7.61 *d*	—	8.74 *d*	7.71 *s*	7.41 *s*	8.20 *d*	8.14 *d*
	(1.6)	(0.5)	(7.6)		(1.6)			(8.6)	(8.4)

*Measured in $CDCl_3$–CD_3OD.

Table 2. ^{13}C NMR spectral data of some isolated compounds and xanthopurpurin (100 MHz, $CDCl_3$)

C	1	3*	5†	6	8	14	9†	xanthopurpurin† [14]
1	147.4	160.3	148.3	160.5	161.7	161.9	165.0	164.8
2	144.7	152.1	147.2	114.7	114.9	114.7	107.8	107.7
3	151.5	152.8	152.7	137.1	163.7	164.2	164.5	165.3
4	106.3	108.8	106.1	119.0	109.7	109.8	108.2	108.3
5	126.9	118.9	126.9	147.6	110.4	127.4	112.6	126.7
6	134.2	134.4	132.7	145.3	164.4	134.1	163.2	134.6
7	145.1	136.6	139.4	151.4	121.0	134.1	121.4	134.5
8	127.4	112.4	127.9	106.0	129.2	126.7	129.4	126.3
9	182.1	188.4	180.0	188.0	186.4	186.9	185.3	185.9
10	182.3	182.0	181.0	181.1	182.4	182.3	182.1	181.8
11	134.6	131.5	134.8	121.2	134.1	134.1	135.1	133.0
12	130.5	128.4	135.7	127.3	126.9	133.6	124.6	133.0
13	120.8	115.1	120.7	133.9	109.4	109.7	109.0	108.4
14	127.8	127.7	125.6	133.0	135.8	133.6	135.0	135.0
OMe-1	61.8	—	60.6	—	—	—	—	—
Me-2	—	—	—	16.1	—	—	—	—
OMe-3	56.6	56.2	56.1	—	—	—	—	—
OMe-5	—	—	—	61.9	—	—	—	—
Me-6	—	15.8	—	—	—	—	—	—
OMe-6	—	—	—	—	56.0	—	—	—
Me-7	22.0	—	—	—	—	—	—	—
OMe-7	—	—	—	56.6	—	—	—	—
CHO-7	—	—	192.6	—	—	—	—	—
$\underline{CH_2}$-O-CH_2-Me	—	—	—	—	67.6	67.6	—	—
CH_2-O-$\underline{CH_2}$-Me	—	—	—	—	67.0	67.0	—	—
CH_2-O-CH_2-$\underline{Me}$	—	—	—	—	15.0	15.0	—	—

*$CDCl_3$ + CD_3OD.
†$(CD_3O)_2SO_2$.

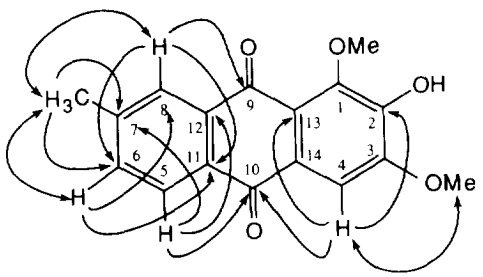

Fig. 1. HMBC (→) and NOESY (↔) correlations of compound **1**.

of both **1** and **2** with methyl iodide gave one and the same methylated product to confirm the identity of **2** as 7-methylanthragallol 2-methyl ether.

Compound **3** showed IR bands at 3353 cm^{-1} of a free hydroxyl group and two chelated and unchelated carbonyl absorptions at 1632 and 1652 cm^{-1}, respectively. The EI-mass spectrum and ^1H NMR data (Table 1) were very similar to those of **2**. However, the methoxyl protons exhibited a significant NOE upon H-4 confirming its disposition at C-3. ^{13}C NMR data (Table 2) and DEPT experiments confirmed the presence of two CH_3 four CH and 10 quaternary carbons, of which two carbonyl carbons were observed at δ182.0 and 188.4. The dimethyl ether of **3** was different from that of **2** and, therefore, the methyl group must be at C-6 to support the proposed structure for **3** as 6-methylanthragallol 3-methyl ether.

Compound **4** showed IR bands at 1628 and 1673 cm^{-1} due to two strongly chelated and unchelated carbonyl absorptions, respectively. The EI-mass spectrum displayed $[M]^+$ at *m/z* 300, calculating for $C_{16}H_{12}O_6$. The trisubstitution pattern of ring C was deduced from the aromatic protons singlet at δ7.49 (H-4) and the α-monosubstitution pattern of ring A by the three coupled protons at 7.25 (*dd*, J = 7.6, 1.2 Hz, H-7), 7.63 (*t*, J = 7.6 Hz, H-6) and 7.80 (*dd*, J = 7.6, 1.2 Hz, H-5). Two methoxyl and two *peri*-hydroxyl groups were also detected at δ4.02, 4.05, 12.09 and 12.21, respectively. A NOESY cross-peak correlation was observed between the methoxyl proton signals at δ4.05 and H-4 at δ7.49 and, hence, the two methoxyl groups are located at C-2 and C-3. From the two possible 1,5- and 1,8-dihydroxy-2,3-dimethoxy substitution patterns, the latter was chosen based upon the strong chelation of only one carbonyl absorption in its IR spectrum. Therefore, **4** is as 8-hydroxyanthragallol 2,3-dimethyl ether.

Compound **5** showed IR bands at 3382, 1696 and 1668 cm^{-1} of a free hydroxyl and two unchelated carbonyl groups and the $[M]^+$ at *m/z* 312 calculated for $C_{17}H_{12}O_6$. The ^1H NMR spectrum (Table 1) showed, like **1**, a trisubstituted ring C with two methoxyl and a β-hydroxyl groups and a monosubstituted ring A with

an aldehydic group (δ 10.21). However, the three coupled aromatic protons of ring A exhibited a significant downfield shift of *ca* 0.4–0.7 ppm indicating the disposition of the aldehydic group at C-6 or C-7. ^{13}C NMR data (Table 2) and DEPT experiments displayed signals for two unchelated carbonyl carbons at δ180.0 and 181.0 as well as a strongly downfield shifted one at δ192.6 assigned to that of the aldehyde group. Moreover, reduction of **5** with $NaBH_4$ [12] afforded the corresponding 7-hydroxymethylanthragallol 1,3-dimethyl ether **19**. Consequently, **5** is 7-formylanthragallol 1,3-dimethyl ether.

Compound **6** showed IR bands at 3372, 1627 and 1664 cm^{-1} of a free hydroxyl group and two chelated and unchelated carbonyl absorptions, respectively, and the $[M]^+$ at *m/z* 314 calculated for $C_{17}H_{14}O_6$. The ^{1}H NMR data (Table 1) revealed signals for one methyl and two methoxyl groups at δ2.35, 4.02 and 4.08, respectively, and the aromatic proton signals appeared as a 1-proton singlet at δ7.71 and two 1-proton doublets at δ7.48 and 7.67 (J = 7.6 Hz, each) suggesting that it could be a copareolatin derivative [2]. The substitution pattern was verified by NOE measurements. Irradiation of the methyl protons at δ2.35 produced a 9% enhancement of H-3 at δ7.48. Similarly, irradiation of the methoxyl protons at δ4.08 produced a 16% enhancement of H-8 at δ7.71, while upon irradiation of that at δ4.02 no effect was observed. Moreover, methylation of both **6** and **17** (copareolatin 6-methyl ether) with methyl iodide gave one and the same methylated product. The above findings together with the ^{13}C NMR data (Table 2) confirmed the identity of **6** as copareolatin 5,7-dimethyl ether.

Compound **7** showed IR bands at 3436 and 1625 cm^{-1} of an hydroxyl group and a chelated carbonyl absorption, respectively. The EI-mass spectrum displayed, like **6**, a $[M]^+$ at *m/z* 314, calculating for $C_{17}H_{14}O_6$. The close similarity between **7** and **6** was evidenced from the ^{1}H NMR data (Table 1) by the same substituent groups and splitting pattern of the aromatic protons. NOESY experiments showed cross-peak correlation between the Me-2 at δ2.31 and the aromatic proton at δ7.49 (*d*, H-3) and between the MeO-7 at δ3.98 and the aromatic proton at δ7.41 (*s*, H-8). These spectral data, together with the presence of only one chelated carbonyl absorption at 1625 cm^{-1} support the 1,5-dihydroxy-2-methyl substitution pattern [13] and confirmed the identity of **7** as copareolatin 6,7-dimethyl ether.

Compound **8** showed IR bands at 3431, 1627 and 1672 cm^{-1} of a free hydroxyl and two chelated and unchelated carbonyl groups, respectively and the $[M]^+$ at *m/z* 328, calculated for $C_{18}H_{16}O_6$. The ^{1}H NMR data (Table 1) established, a lucidin-derived structure [2] from the ethoxymethyl proton resonances at δ1.32, 3.71 and 4.96, as well as the hydroxyl protons at δ9.55 and 13.21 of a β-phenolic and a *peri*-hydroxyl group. Ring C was identical to ring C of lucidin ω-ethyl ether **14**, but there is, in addition, a β-methoxyl substituent in ring A resonating at δ3.97. Comparison of the ^{13}C NMR data of **8** with those of lucidin ω-ethyl ether **14** (Table 2) revealed an upfield shift of *ca* 0.5 ppm in the δ value of the C-9 carbonyl carbon of **8** with respect to that of **14** as a result of transfer of electron density by cross-conjugation [3, 6, 14], while those at C-10 were observed at approximately the same δ values. Accordingly, **8** is 6-methoxylucidin ω-ethyl ether.

Compound **9** showed IR bands at 3413, 1665 and 1631 cm^{-1} of a free hydroxyl, unchelated and chelated carbonyl groups and the $[M]^+$ at *m/z* 256 calculated for $C_{14}H_8O_5$. The ^{1}H NMR (Table 1) indicated an α,β-disubstituted ring C from two *meta*-coupled protons at δ6.62 and 7.22 (*d*, 2.3) and a β-substituted ring A from three coupled protons at δ7.56 (*d*, 2.6), 7.17 (*dd*, 8.4, 2.40) and 8.14 (*d*, 8.4). The ^{13}C NMR data (Table 2) revealed a total of 14 carbon signals for five CH and nine quaternary, of which two carbonyl signals at δ181.8 and 185.9 and three C-hydroxyl signals at δ163.2, 164.5 and 165.0

Table 3. ^{1}H and ^{13}C NMR spectral data of compound **10** (400 and 100 MHz, $CDCl_3$)

Position	δ_H	δ_C	^{1}H long-range coupled $^3J_{CH}$	$^2J_{CH}$
1		189.5 *s*		
2	6.87 *d* (1.3)	136.2 *d*	4, 13, 15	
3		150.9 *s*		
4		184.9 *s*		
5	7.75 *d* (7.8)	127.0 *d*	7, 10, 12	
6	7.61–7.70 *m*	131.1 *d*		
7	7.61–7.70 *m*	129.5 *d*		
8	8.42 *d* (8.0)	124.8 *d*	6, 11	
9		163.1 *s*		
10		128.1[a] *s*		
11		136.2 *s*		
12		127.8[a] *s*		
13		109.1 *s*		
14		127.9[a] *s*		
15	2.03 *d* (1.3)	17.1 *q*	2, 4	3
OH-9	14.23 *br, s*	—	12, 13	9
1′		187.8 *s*		
2′		147.6 *s*		
3′		145.2 *s*		
4′		184.0 *s*		
5′	7.96 *d* (8.0)	130.5 *d*	7′, 10′, 13′	
6′	7.61–7.70 *m*	132.1 *d*		
7′	7.61–7.70 *m*	128.9 *d*		
8′	8.57 *d* (7.0)	125.5 *d*	6′, 14′	
9′		162.6 *s*		
10′	8.22 *s*	121.9 *d*	4′, 5′, 12′, 13′	
11′		125.2 *s*		
12′		109.2 *s*		
13′		127.8[a] *s*		
14′		134.1 *s*		
15′	1.73 *s*	14.5 *q*	2′, 4′	3′
OH-9′	13.67 *br, s*		12′, 13′	9′

Signal assignments are based on analysis of 1D (^{1}H, ^{13}C) and 2D (COSY, NOESY, HMQC, HMBC) NMR spectra (δ, in ppm from TMS, J in Hz). $^3J_{CH}$ and $^2J_{CH}$ indicate the protons long-range-coupled with carbons through three and two bonds, respectively observed in the HMBC spectrum.

[a]Signal assignments may be interchanged.

were observed. The choice between the 1,3,6- and 1,3,7-trihydroxylation patterns was deduced from comparison of the ^{13}C NMR data of **9** with xanthopurpurin [14] (Table 2). The upfield shift of the C-9 carbonyl carbon by *ca* 0.6 ppm with respect to that of xanthopurpurin, as a result of cross-conjugation [3, 6, 14], confirmed its identity as 6-hydroxy-xanthopurpurin.

Compound **10** showed IR bands at 1661, 1642 and 1626 cm^{-1} of unchelated and two chelated CO absorptions, respectively. The EI-mass spectrum displayed a $[M]^+$ at *m/z* 474, calculating for $C_{30}H_{18}O_6$ and a significant fragment ion at *m/z* 238, calculating for $C_{15}H_{10}O_3$. The ^1H NMR data (Table 3) revealed signals for two methyl groups at δ2.03 (*d*, 1.3) and 1.73 (*s*), two *peri*-hydroxyl protons at δ13.67 and 14.23, as well as nine aromatic and one quinonoid proton. A total of 30 carbon signals were detected in ^{13}C NMR data (Table 3) and DEPT experiments demonstrated two Me, 10 CH and 18 quaternary carbons of which four are carbonyl at δ189.5, 187.8, 184.9 and 184.0 and two are C-hydroxyl at δ163.1 and 162.6. These spectral findings together with the fragment ion at *m/z* 238 suggested a bianthraquinone skeleton with one methyl and one *peri*-hydroxyl group in each monomer. The NOESY experiments demonstrated a few important cross-peak correlations (Fig. 2) between the methyl protons at δ2.03 and the proton at δ6.87, the methyl protons at δ1.73 and the proton at δ7.75, as well as the protons at δ7.96 and 8.22. The structure **10** was finally established by detailed analysis of HMQC and HMBC spectra. The result of these analyses (Fig. 2, Table 3) revealed the key connectivity between the two monomers by the carbon signals at δ128.1 and 147.6 ascribed to C-10 and C-2′, respectively, through $^3J_{CH}$ correlations with H-5 at δ7.75 and the methyl protons at δ1.73. $^2J_{CH}$ and $^3J_{CH}$ correlations presented in Fig. 2 and Table 3 support the structure of **10** as 10,2′-bi(9-hydroxy-3-methyl-1,4-anthraquinonyl), named bi-sinaiquinone.

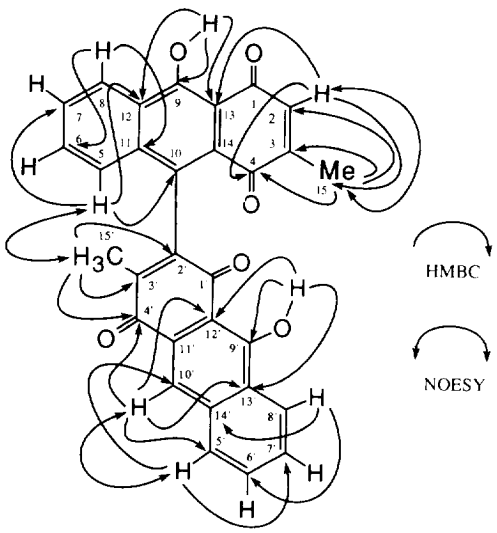

Fig. 2. HMBC and NOESY correlations of compound **10**.

Table 4. IC_{50} values of anthraquinones from *G. sinaicum* in cytotoxic activity against P388 cells

compound	IC_{50} values ($\mu g\,ml^{-1}$)
1	10.1
3	21.0
5	25.0
6	12.5
8	11.2
9	13.0
10	10.9
11	5.8
13	80.0
14	18.0
17	5.5
19	32.0

Anthraquinones isolated from *G. sinaicum* were subjected to the cytotoxic bioassay against P388 leukemic cells. Compounds **11** and **17** showed stronger activity than the other anthraquinones (Table 4).

EXPERIMENTAL

General. Mps: uncorr. ^1H and ^{13}C NMR: $CDCl_3$ or DMSO-d_6 with TMS as int. standard, Bruker AM400. ^{13}C NMR multiplicities were determined by DEPT pulse sequence. 2D NMR (COSY, NOESY, HMQC, HMBC), Bruker AM500. EI-MS: 70 eV. IR: KBr. UV: MeOH. Prep. HPLC: 10 μm ODS column. MPLC: 20 μm ODS column, Kusano Scientific. CC: Kieselgel 60. TLC: silica gel $60F_{254}$ and RP-18 F_{254} (0.25 mm) precoated plates. Spots 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). Cytotoxicity bioassay: Tohso MPR A4*i* microplate reader.

Plant material. Roots of *Galium sinaicum* Boiss. were collected in May 1991 from the mountains of Saint Katherine district, Sinai Peninsula. Identification was verified by Professor Dr N. El-Hadidi (Faculty of Science, Cairo University). A voucher specimen, documenting this collection, is deposited at the Pharmacognosy Department, Faculty of Pharmacy, University of Mansoura.

Extraction and isolation. Air-dried and powdered roots (3 kg) were extracted by percolation with 95% EtOH. The alcoholic extract (96 g; IC_{50}, 14 $\mu g\,ml^{-1}$ to P388 cell line) was diluted with H_2O and successively partitioned with *n*-hexane, CH_2Cl_2 and *n*-BuOH. The n-hexane extract (15 g; IC_{50}, 3.8 $\mu g\,ml^{-1}$ to P388 cell line) was then successively extracted with 1 N Na_2CO_3 and 1 N NaOH to afford extracts A and B (1.5 g, each), respectively. Both extracts were fractionated separately using silica-gel-packed columns eluted with an *n*-hexane–EtOAc gradient; similar frs were pooled together. The frs obtained were subjected either to direct recrystallization or to further purification on RP-18 columns using various chromatographic techniques (HPLC,

MPLC, PTLC) to afford the anthraquinone compounds **1–18**.

7-Methylanthragallol 1,3-dimethyl ether (**1**). Yellow needles (5 mg) from NaOH fr. Mp 197°. UV λ_{max}^{MeOH} nm: 204, 244, 250, 280. IR ν^{KBr} cm^{-1}: 3310, 2923, 1667, 1595, 1561, 1496, 1458, 1347, 1293, 1242, 1168, 1133, 1101, 1047, 905, 874, 790, 732, 692. EI-MS *m/z* (rel. int.): 298 [M]$^+$ (3) (calcd for $C_{17}H_{14}O_5$ 298.0841, found 298.0835), 284 (100), 268 (5), 255 (6), 213 (6.5), 128 (5.3). FABMS *m/z* (rel. int.): 299 [M + 1]$^+$ (100). ^{1}H NMR (400 MHz, $CDCl_3$): Table 1. ^{13}C NMR (100 MHz, $CDCl_3$): Table 2.

7-Methylanthragallol 2-methyl ether (**2**). Yellow crystals (2 mg) from Na_2CO_3 fr. Mp 174–176°. UV λ_{max}^{MeOH} nm: 205, 250, 280, 407. IR ν^{KBr} cm^{-1}: 3392, 2923, 2853, 1673, 1633, 1599, 1580, 1492, 1398, 1363, 1342, 1248, 1213, 1163, 1097, 799, 759, 734, 671. EI-MS *m/z* (rel. int.): 284 [M]$^+$ (100) (calcd for $C_{16}H_{12}O_5$ 284.0685, found 284.0681), 266 (65), 255 (9), 241 (47), 210 (21), 184 (6), 139 (11), 128 (14), 89 (5), 74 (9). ^{1}H NMR (400 MHz, $CDCl_3$): Table 1.

6-Methylanthragallol 3-methyl ether (**3**). Yellow needles (5 mg) from Na_2CO_3 fr. Mp 270–273°. UV λ_{max}^{MeOH} nm: 212, 240 sh, 280, 408. IR ν^{KBr} cm^{-1}: 3353, 2926, 2852, 1652, 1632, 1594, 1570, 1523, 1438, 1371, 1293, 1325, 1311, 1256, 1210, 1188, 1121, 1041, 1005, 892, 815, 782, 752, 731, 672, 623. EI-MS *m/z* (rel. int.): 284 [M]$^+$ (100), (calcd for $C_{16}H_{12}O_5$ 284.0685, found 284.0668), 269 (8), 241 (17), 213 (12), 185 (10), 139 (9), 127 (8), 111 (8), 97 (15), 82 (17), 69 (23), 56 (23). ^{1}H NMR (400 MHz, CD_3OD + $CDCl_3$): Table 1. ^{13}C NMR (100 MHz, CD_3OD + $CDCl_3$): Table 2.

8-Hydroxyanthragallol 2,3-dimethyl ether (**4**). Yellow crystals (2 mg) from NaOH fr. Mp 226–227°. UV λ_{max}^{MeOH} nm: 206, 238 sh, 244 sh, 274, 417. IR ν^{KBr} cm^{-1}: 3436, 2924, 2853, 1673, 1628, 1599, 1505, 1459, 1420, 1371, 1321, 1273, 1241, 1220, 1196, 1163, 1092, 1048, 905, 990, 981, 920, 864, 749, 721. EI-MS *m/z* (rel. int.): 300 [M]$^+$ (38) (calcd for $C_{16}H_{12}O_6$ 300.0634, found 300.0654), 282 (13), 257 (20), 223 (11), 205 (7), 186 (15), 167 (6), 149 (100), 135 (8), 121 (13), 97 (19), 86 (25), 69 (31). ^{1}H NMR (400 MHz, $CDCl_3$): Table 1.

7-Formylanthragallol 1,3-dimethyl ether (**5**). Yellow powder (5 mg) from NaOH fr. UV λ_{max}^{MeOH} nm: 206, 244 sh, 280. IR ν^{KBr} cm^{-1}: 3382, 2960, 2925, 2854, 1696, 1668, 1597, 1560, 1495, 1455, 1381, 1347, 1325, 1289, 1237, 1211, 1161, 1126, 1089, 1043, 991, 882, 819, 800, 790, 735, 726. EI-MS *m/z* (rel. int.): 312 [M]$^+$ (100) (calcd for $C_{17}H_{12}O_6$ 312.0634, found 312.0639), 294 (97), 265 (73), 253 (7), 239 (11), 211 (10), 198 (34), 181 (11), 153 (7), 141 (13), 126 (8), 113 (10), 97 (6), 69 (8), 56 (11). ^{1}H NMR (400 MHz, $CDCl_3$): Table 1. ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

Copareolatin 5,7-dimethyl ether (**6**). Yellow powder (5 mg) from Na_2CO_3 fr. UV λ_{max}^{MeOH} nm: 213, 278, 410, IR ν^{KBr} cm^{-1}: 3372, 2925, 2854, 1664, 1627, 1594, 1561, 1496, 1470, 1457, 1426, 1366, 1323, 1289, 1258, 1199, 1173, 1130, 1100, 1072, 1027, 1001, 904, 872, 847, 811, 792, 738, 717, 688. EI-MS *m/z* (rel. int.): 314 [M]$^+$ (100) (Calcd for $C_{17}H_{14}O_6$ 314.0790, found 314.0808), 296 (58), 281 (26), 267 (43), 255 (6), 241 (10), 213 (9), 200 (27), 172 (7), 144 (10), 128 (12), 115 (23), 89 (7), 77 (15). ^{1}H NMR (400 MHz, $CDCl_3$): Table 1. ^{13}C NMR (100 MHz, $CDCl_3$): Table 2.

Copareolatin 6,7-dimethyl ether (**7**). Yellow needles (2 mg) from NaOH fr. Mp 205–207°. UV λ_{max}^{MeOH} nm: 220, 272, 308 sh, 432. IR ν^{KBr} cm^{-1}: 3436, 2925, 2853, 1625, 1578, 1500, 1445, 1418, 1368, 1303, 1245, 1195, 1180, 1136, 1100, 1076, 1035, 990, 956, 855, 801, 742, 719, 692. EI-MS *m/z* (rel. int.): 314 [M]$^+$ (18) (calcd for $C_{17}H_{14}O_6$ 314.0790, found 314.0812), 299 (11), 270 (18), 256 (28), 236 (15), 221 (7), 199 (13), 185 (15), 167 (18), 149 (76), 135 (12), 123 (17), 111 (28), 97 (49), 85 (36), 71 (60), 70 (100), 56 (98). ^{1}H NMR (400 MHz, $CDCl_3$): Table 1.

6-Methoxylucidin ω-ethyl ether (**8**). Yellow crystals (3 mg) from NaOH fr. Mp 143–145°. UV λ_{max}^{MeOH} nm: 213, 273, 336 sh, 422. IR ν^{KBr} cm^{-1}: 3431, 3235, 2924, 2852, 1672, 1627, 1597, 1482, 1444, 1400, 1376, 1334, 1309, 1293, 1279, 1230, 1185, 1163, 1139, 1090, 1057, 1025, 977, 888, 833, 759, 732. EI-MS *m/z* (rel. int.): 328 [M]$^+$ (13) (calcd for $C_{18}H_{16}O_6$ 328.0947, found 328.0955), 299 (9), 268 (5), 282 (100), 254 (15), 226 (8), 149 (20), 129 (5), 97 (8), 71 (10), 70 (17), 57 (20). ^{1}H NMR (400 MHz, $CDCl_3$): Table 1. ^{13}C NMR (100 MHz, $CDCl_3$): Table 2.

6-Hydroxyxanthopurpurin (**9**). Orange red crystals (2 mg) from NaOH fr. Mp 247–250°. UV λ_{max}^{MeOH} nm: 210, 278, 300 sh, 340 sh, 428. IR ν^{KBr} cm^{-1}: 3413, 2926, 2852, 1665, 1631, 1597, 945, 798, 754, 664. EI-MS *m/z* (rel. int.): 256 [M]$^+$ (30) (calcd for $C_{14}H_8O_5$ 256.0372, found 256.0399), 284 (100), 236 (15), 228 (7), 220 (25), 205 (7), 185 (12), 167 (12), 157 (9), 149 (72), 137 (19), 129 (25), 111 (25), 97 (40), 80 (52), 69 (100). ^{1}H NMR (400 MHz, CD_3OD + $CDCl_3$): Table 1. ^{13}C NMR (100 MHz, DMSO-d_6): Table 2.

Bisinaiquinone (*10,2′-bi(9-hydroxy-3-methyl-1,4-anthraquinonyl)*) (**10**). Red crystals (5 mg) from NaOH fr. Mp 245–248°. $[\alpha]_D \pm 0°$ $CHCl_3$; *c* 0.08). UV λ_{max}^{MeOH} nm: 206, 226, 252 sh, 278 sh, 400 sh, 434 sh. IR ν^{KBr} cm^{-1}: 3436, 2921, 2851, 1737, 1661, 1642, 1626, 1597, 1577, 1499, 1459, 1427, 1399, 1376, 1341, 1303, 1249, 1218, 1103, 986, 954, 903, 857, 834, 806, 786, 759, 750. EI-MS *m/z* (rel. int.): 474 [M]$^+$ (100) (calcd for $C_{30}H_{18}O_6$ 474.1103, found 474.1132), 431 (35), 401 (8), 368 (11), 227 (9), 238 (18), 211 (6), 183 (22), 142 (9), 114 (12), 98 (15), 83 (20), 57 (42). ^{1}H and ^{13}C NMR (40·0, 100 MHz, $CDCl_3$): Table 3.

Cytotoxicity. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was performed in a 96-well plate [15, 16]. 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 leukemia 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° in a humidified atmosphere of 5% CO_2. Various drug concs (10 μl) were added to the cultures at day 1 after 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 of incubation, 100 μl of 10% SDS–0.01 M HCl soln was added to

each well and the formazan crystals in each well were dissolved by stirring with a pipette. *A* measurements were made using a microplate reader (Tohso MPR-A4*i*) with a two wavelength system (550 and 700 nm). In all expts, 3 replicate wells were used to determine each point.

Acknowledgements—The authors are grateful to Dr K. Tagahara (Kobe College of Pharmacy, Japan) for supplying authentic 2,8-dihydroxy-1,3-dimethoxy-7-methyl-9,10-anthraquinone and also to the Ministry of Higher Education in Egypt for financial support.

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