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CHEMICAL CONSTITUENTS OF *GLADIOLUS SEGETUM* KER-GAWL

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من الجزء المذاب فى الكلوروفورم لخلاصة الكحول الميثيلى لكورمات الجلاديولس سيجيتم كير جول تم فصل تسعة مركبات وقد تم التعرف عليها كالآتى: ثلاثة مركبات من اللجنانات: (+) ديميثوكسى بينوريسينول (١) ، (+) بينوريسينول (٢) ، (+) بينوريسينول أحادى ميثيل إيثر (٣). مركب نيولجنان: ( ) ديهدروداى كونيفيريل كحول (٤). ثلاثة مركبات أنثراكينونية: دى أوكسى إرثرولاسين (٥) فيسيون (٦) ، حمض لاسايك دي ميثيل إستر (٧) بالاضافة الى ٦ أ بلميتويل ٣ أ بيتا سيتوستيرول جلوكوبيرانوزيد (٨) وبيتا سيتوستيرول ٣ أ بيتا جلوكوبيرانوزيد (٩).

وقد تم التعرف على المركبات المفصولة بالوسائل الطبيعية والكروماتوجرافية والطرق الطيفية المختلفة والتى تشتمل على الرنين النووى المغناطيسى ومطياف الكتله.

المركبات من ١ ٤ ، ٦ ٩ تفصل لأول مرة من جنس الجلاديولس بينما المركب رقم (٥) فقد تم فصله سابقا من نفس النبات.

*From the chloroform soluble fraction of the methanolic extract of the corms of Gladiolus segetum Ker-Gawl (Iridaceae), nine compounds were isolated and identified as follows: the lignans (+)-demethoxypinoresinol (**1**), (+)-pinoresinol (**2**) and (+)-pinoresinol monomethylether (**3**); the neolignan (–)-dehydrodiconiferyl alcohol (**4**) and the anthraquinones deoxy-erythrolaccin (**5**), physcion (**6**) and laccaic acid D methylester (**7**) together with 6′-O-palmitoyl-3-O-β-sitosterol glucoside (**8**) and β-sitosterol-3-O-glucoside (**9**). The structures of the isolated compounds were determined by physical and spectroscopic methods including NMR and MS spectral analysis. Compounds **1-4** and **6-9** are reported here for the first time from the genus Gladiolus while compound **5** was previously isolated from the same plant.*

INTRODUCTION

Gladiolus segetum Ker-Gawl (F. Iridaceae) is a herbaceous plant cultivated in Egypt as an ornamental plant.[1,2] In the folk medicine, the uses of some *Gladiolus* species were reported as a remedy for dysentery, impotence and for relief of rheumatic pains. Moreover, the smoke from the burning corms is sometimes inhaled for colds.[3] The current literatures revealed that the genus *Gladiolus* showed the presence of flavonols, anthocyanidins, ascorbic acid, saponins, fatty acids, mucilage and anthraquinones.[4-11]

The present study deals with the isolation and structure elucidation of nine compounds from the chloroform soluble fraction of the methanolic extract of the corms of *G. segetum* using different chromatographic techniques and various tools of NMR spectral analysis including H-H COSY and C-H COSY as well as EI MS spectral analysis. The isolated compounds (**1-9**) were identified as the lignans (+)-demethoxypinoresinol (**1**), (+)-pinoresinol (**2**) and (+)-pinoresinol monomethylether (**3**); the neolignan (–)-dehydrodiconiferyl alcohol (**4**) and the anthraquinones deoxyerythrolaccin (**5**), physcion (**6**) and laccaic acid D methylester (**7**) together with 6′-O-palmitoyl-3-O-β-sitosterol glucoside (**8**) and β-sitosterol-3-O-glucoside (**9**).

EXPERIMENTAL

Melting points: uncorrected and measured on Stuart Scientific (SMPI). Optical rotation was measured on Union PM-101 automatic digital polarimeter. Nuclear Magnetic Resonance (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectra were recorded on a

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JEOL JNM α-400 spectrometer using TMS as internal standard. Mass spectra were taken on a JEOL JMS-SX 102 spectrometer by direct inlet method at an ionizing voltage of 70 eV. For column chromatography, Kieselgel 60 (70-230 mesh, Merck) and Sephadex LH-20 (Merck) were used. For thin layer chromatography, silica gel 60 precoated plates, F-254 (Merck) were used with the following solvent systems:

I- $CHCl_3$ - MeOH (9.5 : 0.5)
II- $CHCl_3$ - MeOH (9 : 1)
III- $CHCl_3$ - MeOH (8 : 2)

Plant material

Corms of *Gladiolus segetum* Ker-Gawl were collected from the plants cultivated in the Experimental Station of Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Assiut, Egypt in 1999. The plant was identified by Dr. Salah El-Nagar, Professor of Taxonomy, Dept. of Botany, Faculty of Science, Assiut University.

Extraction and isolation

The air-dried powdered corms of *Gladiolus segetum* (500 g) was extracted three times by maceration with MeOH at room temperature. The methanolic extracts were combined together and concentrated under reduced pressure till dryness. The dried methanolic extract (145 g) was suspended in H_2O and fractionated with *n*-hexane and $CHCl_3$ successively. The $CHCl_3$ fraction (6 g) was chromatographed on silica gel column and eluted with $CHCl_3$ and $CHCl_3$ with increasing gradient of MeOH to give 12 fractions (F-1 to F-12).

Fraction F-3 (27 mg) was chromatographed on Sephadex LH-20 column using MeOH as a solvent system to afford compound **6** (19 mg). Fraction F-4 (127 mg) was chromatographed on silica gel column using $CHCl_3$ - MeOH (9.7 : 0.3) as a solvent system to give compounds **3** (20 mg) and **4** (28 mg). Fraction F-5 (58 mg) was subjected to preparative TLC using silica gel plates and $CHCl_3$ - MeOH (9.5 : 0.5) as a solvent system to afford compound **2** (37 mg). Fraction F-6 (69 mg) was subjected to Sephadex LH-20 column chromatography using MeOH as a solvent system to give compounds **1** (12 mg) and **7** (23 mg). Fraction F-7 (26 mg) was chromatographed on Sephadex LH-20 column using MeOH as a solvent system to afford compound **5** (10 mg). Fraction F-8 (168 mg) was applied on silica gel column chromatography using $CHCl_3$ - MeOH (9 : 1) as a solvent system to give compound **8** (57 mg). Fraction F-9 (563 mg) was crystallized using MeOH to afford compound **9** (394 mg). Fractions F-1 and F-2 were resinous in nature and impure while the quantity of fractions F-10, F-11 and F-12 was very minor.

Compound (1)

Amorphous powder, R_f = 0.42 (System I), $[\alpha]_D^{26}$ +88.2º ($CHCl_3$, 0.04). EI-MS (m/z): 328 (M^+, $C_{19}H_{20}O_5$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.16 (2H, *d*, *J*= 8.5 Hz, H-2′, 6′), 7.14 (1H, *d*, *J*= 8.0 Hz, H-5″), 6.87 (1H, *d*, *J*= 1.8 Hz, H-2″), 6.74 (1H, *dd*, *J*= 8.0, 1.8 Hz, H-6″), 6.71 (2H, *d*, *J*= 8.5 Hz, H-3′, 5′), 4.62 and 4.60 (each 1H, *d*, *J*= 5.1 Hz, H-2, 6), 4.10 and 3.71 (each 2H, *m*, H-4, 8), 3.74 (3H, *s*, -OMe), 3.03 (2H, *m*, H-1, 5). ^{13}C NMR (100 MHz, DMSO-d_6, Table 1).

Compound (2)

Colourless crystals (MeOH), m.p 117-119º, R_f = 0.51 (System I), $[\alpha]_D^{26}$ +76.2º ($CHCl_3$, 0.07) lit.[12] +77.5º. EI-MS (m/z): 358 (M^+, $C_{20}H_{22}O_6$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.71-6.83 (6H, *m*, aromatic H), 4.57 (2H, *d*, *J*= 4.12 Hz, H-2, 6), 4.08 and 3.73 (each 2H, *m*, H-4, 8), 3.78 (6H, *s*, 2x-OMe), 3.04 (2H, *m*, H-1, 5). ^{13}C NMR (100 MHz, DMSO-d_6, Table 1).

Compound (3)

Amorphous powder, R_f = 0.54 (System I), $[\alpha]_D^{26}$ +64.2º ($CHCl_3$, 1.2) lit.[13] +65.6º. EI-MS (m/z): 372 (M^+, $C_{21}H_{24}O_6$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.69-6.85 (6H, *m*, aromatic H), 4.65 (2H, *d*, *J*= 4.8 Hz, H-2, 6), 4.27 and 3.77 (each 2H, *m*, H-4, 8), 3.73 (3H, *s*, -OMe), 3.72 (6H, *s*, 2x-OMe), 3.08 (2H, *m*, H-1, 5). ^{13}C NMR (100 MHz, DMSO-d_6, Table 1).

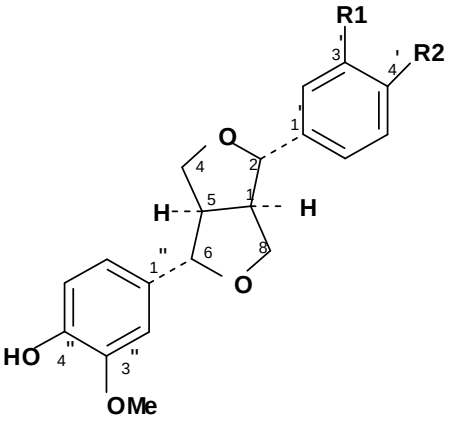
Compound (4)

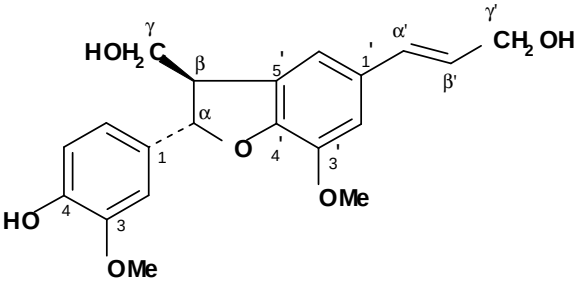
Amorphous powder, R_f = 0.56 (System I), $[\alpha]_D^{26}$ –31.3º ($CHCl_3$, 1.15) lit.[14] –31.6º. EI-MS (m/z): 358 (M^+, $C_{20}H_{22}O_6$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.73-6.89 (5H, *m*, aromatic H), 6.45 (1H, *d*, *J*= 16.1 Hz, H-α′), 6.17 (1H, *m*, H-β′), 5.44 (1H, *d*, *J*= 6.4 Hz, H-α), 4.07 (2H, *br.d*, *J*= 5.1 Hz, H-γ′), 3.84 (2H, *m*, H-γ),

Compound (1): R1= H, R2= OH

Compound (2): R1= OMe, R2= OH

Compound (3): R1, R2= OMe



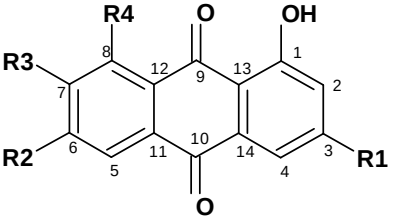


Compound (4)

Compound (5): R1, R2= OH, R3= H, R4= Me

Compound (6): R1= Me, R2= OMe, R3= H, R4= OH

Compound (7): R1, R2= OH, R3= COOMe, R4= Me



3.77 (3H, *s*, -OMe), 3.75 (3H, *s*, -OMe), 3.41 (1H, *m*, H-β). ^{13}C NMR (100 MHz, DMSO-d_6, Table 1).

Compound (5)

Orange crystals from MeOH, m.p 300º (decomp.), R_f = 0.45 (System II). EI-MS (m/z): 270 (M^+, $C_{15}H_{10}O_5$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.24 (1H, *s*, -OH at C-1), 7.42 (1H, *d*, *J*= 2.5 Hz, H-5), 7.04 (1H, *d*, *J*= 2.3 Hz, H-4), 6.99 (1H, *d*, *J*= 2.5 Hz, H-7), 6.55 (1H, *d*, *J*= 2.3 Hz, H-2), 2.70 (3H, *s*, -Me). ^{13}C NMR (100 MHz, DMSO-d_6, Table 2).

Compound (6)

Orange yellow crystals from MeOH, m.p 235-236º, R_f = 0.71 (System I). EI-MS (m/z): 284 (M^+, $C_{16}H_{12}O_5$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.41 and 12.18 (each 1H, *s*, -OH at C-1 and C-8), 7.36 (1H, *d*, *J*= 2.0 Hz, H-4), 7.08 (1H, *d*, *J*= 1.8 Hz, H-5), 6.94 (1H, *d*, *J*= 2.0 Hz, H-2), 6.78 (1H, *d*, *J*= 1.8 Hz, H-7), 3.88 (3H, s, -OMe), 2.66 (3H, *s*, -Me). ^{13}C NMR (100 MHz, DMSO-d_6, Table 2).

Compound (7)

Orange crystals from MeOH, m.p 272-273º, R_f = 0.36 (System I). EI-MS (m/z): 328 (M^+, $C_{17}H_{12}O_7$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.30 (1H, *s*, -OH at C-1), 7.65 (1H, *s*, H-5), 7.13 (1H, *d*, *J*= 2.1 Hz, H-4), 6.74 (1H, *d*, *J*= 2.1 Hz, H-2), 3.60 (3H, *s*, -COOMe), 2.65 (3H, *s*, -Me). ^{13}C NMR (100 MHz, DMSO-d_6, Table 2).

Table 1: ^{13}C NMR spectral data of compounds **1-4** (100 MHz, DMSO-d_6).

C	**1**	**2**	**3**	C	**4**
1	53.6[a] *d*	53.9 *d*	53.6 *d*	1	132.4 *s*
2	85.2[b] *d*	85.6 *d*	84.5 *d*	2	110.3 *d*
4	70.9[c] *t*	71.3 *t*	71.1 *t*	3	147.6 *s*
5	53.5[a] *d*	53.9 *d*	53.6 *d*	4	146.4 *s*
6	85.0[b] *d*	85.6 *d*	84.5 *d*	5	115.3 *d*
8	70.8[c] *t*	71.3 *t*	71.1 *t*	6	118.6 *d*
1′	131.6 *s*	132.6 *s*	132.1 *s*	1′	129.5 *s*
2′	127.6 *d*	110.0 *d*	110.0 *d*	2′	110.4 *d*
3′	115.1 *d*	148.2 *s*	146.2 *s*	3′	143.7 *s*
4′	156.7 *s*	146.5 *s*	144.9 *s*	4′	147.2 *s*
5′	115.1 *d*	115.5 *d*	114.0 *d*	5′	130.6 *s*
6′	127.6 *d*	119.0 *d*	118.2 *d*	6′	115.0 *d*
1″	132.2 *s*	132.6 *s*	133.3 *s*	α	87.2 *d*
2″	110.3 *d*	110.0 *d*	108.4 *d*	β	53.0 *d*
3″	147.5 *s*	148.2 *s*	148.7 *s*	γ	62.9 *t*
4″	145.9 *s*	146.5 *s*	148.1 *s*	α′	129.0 *d*
5″	115.0 *d*	115.5 *d*	110.8 *d*	β′	128.0 *d*
6″	118.6 *d*	119.0 *d*	117.9 *d*	γ′	61.7 *t*
O<u>Me</u>	55.6 *q*	56.1 *q* (2xOMe)	55.5 *q* (2xOMe) 55.6 *q*	O<u>Me</u>	55.5 *q* 55.6 *q*

[a, b, c] values may be interchangeable within each column

Table 2: ^{13}C NMR spectral data of compounds **5-7** (100 MHz, DMSO-d_6).

C	**5**	**6**	**7**
1	164.2 *s*	164.4 *s*	160.0 *s*
2	108.2 *d*	105.8 *d*	107.9 *d*
3	164.5 *s*	145.1 *s*	157.1 *s*
4	112.1 *d*	121.2 *d*	107.0 *d*
5	107.1 *d*	125.1 *d*	112.7 *d*
6	161.7 *s*	162.7 *s*	156.4 *s*
7	124.5 *d*	106.8 *d*	130.0 *s*
8	144.8 *s*	164.7 *s*	140.4 *s*
9	187.9 *s*	187.8 *s*	188.3 *s*
10	182.3 *s*	182.5 *s*	181.7 *s*
11	134.2 *s*	134.1 *s*	132.8 *s*
12	122.3 *s*	112.9 *s*	121.8 *s*
13	109.9 *s*	136.8 *s*	111.3 *s*
14	136.6 *s*	110.9 *s*	136.8 *s*
Me	22.3 *q*	23.6 *q*	20.1 *q*
OMe		56.1 *q*	
<u>C</u>OOMe			167.8 *s*
COO<u>Me</u>			52.6 *q*

Compound (8)

Yellow greasy substance, R_f = 0.65 (System III). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.22 (1H, *m*, H-6), 5.03 (1H, *d*, *J*= 7.8 Hz, H-1′ anomeric proton of the glucopyranosyl moiety), 4.05-4.32 (5H, *m*, sugar protons H-2′ to H-6′), 3.57 (1H, *m*, H-3), 0.68-1.09 (7xMe). ^{13}C NMR (100 MHz, $CDCl_3$, Table 3).

Table 3: ^{13}C NMR spectral data of compound **8** (100 MHz, $CDCl_3$).

β-sitosterol moiety				Glucopyranosyl moiety	
C		C		C	
1	36.9	16	26.9	1′	101.1
2	29.6	17	55.6	2′	73.4
3	78.7	18	11.7	3′	76.4
4	39.1	19	18.0	4′	70.3
5	139.2	20	35.1	5′	76.7
6	119.1	21	18.7	6′	63.6
7	31.6	22	34.0	Palmitoyl moiety	
8	31.5	23	25.3	C	
9	49.1	24	45.1	1″	173.5
10	36.0	25	28.9	2″	34.0
11	20.8	26	18.6	3″-15″	22.3-29.4
12	39.3	27	19.0	16″	13.8
13	40.6	28	22.4		
14	55.8	29	11.9		
15	23.5				

Compound (9)

Amorphous powder, R_f = 0.56 (System III), identified as β-sitosterol-3-O-β-glucopyranoside by TLC co-chromatography with authentic sample.

RESULTS AND DISCUSSION

The air-dried powdered corms of *G. segetum* was extracted with methanol and the extract was fractionated with *n*-hexane and chloroform successively. From the chloroform soluble fraction, nine compounds (1-9) were isolated.

The ^{13}C NMR including DEPT mode measurements (Table 1) and ^{1}H NMR spectral data of compound **1** showed characteristic features of tetrahydrofuran lignans and suggesting that it belongs to the 2,6-diaryl-3,7-dioxabicyclo{3.3.0}octane type lignan.[15,16] The EI-MS spectral analysis of **1** exhibited a molecular ion peak at m/z 328 (M^+) corresponding to a molecular formula $C_{19}H_{20}O_5$ and intense fragments at m/z 93 and 123 for 4-hydroxyphenyl and guaiacyl moieties, respectively. The presence of 4-hydroxyphenyl group was assigned from the ^{13}C NMR spectral data (Table 1) at δ_C 131.6 *s* (C-1′), 127.6 *d* (2C, C-2′ and 6′), 115.1 *d* (2C, C-3′ and 5′) and 156.7 *s* (C-4′) and ^{1}H NMR signals at δ_H 7.16 (2H, *d*, *J*= 8.5 Hz, H-2′, 6′) and 6.71 (2H, *d*, *J*= 8.5 Hz, H-3′, 5′). The signals at δ_C 132.2 *s* (C-1″), 110.3 *d* (C-2″), 147.5 *s* (C-3″), 145.9 *s* (C-4″), 115.0 *d* (C-5″), 118.6 *d* (C-6″) and 55.6 *q* (OMe) with the ^{1}H NMR signals (ABX system) at δ 6.87 (1H, *d*, *J*= 1.8 Hz, H-2″), 7.14 (1H, *d*, *J*= 8.0 Hz, H-5″) and 6.74 (1H, *dd*, *J*= 8.0, 1.8 Hz, H-6″) in addition to the aromatic methoxyl group at 3.74 (3H, *s*) indicated the presence of 4-hydroxy-3-methoxyphenyl group (guaiacyl moiety). The presence of 3,7-dioxabicyclo-{3.3.0}octane was indicated from the NMR data at δ_C 53.6 *d*, 53.5 *d* (C-1 and 5); 85.2 *d*, 85.0 *d* (C-2 and 6) and 70.9 *t*, 70.8 *t* (C-4, 8) with δ_H 3.03 (2H, *m*, H-1, 5), 4.62 and 4.60 (each 1H, *d*, *J*= 5.1 Hz, H-2, 6) and 4.10 and 3.71 (each 2H, *m*, H-4, 8). The above mentioned assignments were confirmed by measurements of C-H COSY and H-H COSY.

Concerning the stereochemistry of compound **1**, it is known that the fusion of the tetrahydrofuranes in this class of lignans to obtain the bicycle is always *Cis.*[16] The ^{1}H NMR signals of the benzylic protons H-2 and H-6 at δ_H 4.62 and 4.60 showed that both possessed a diequatorial stereochemistry since they appeared between δ_H 3.75 and 4.70 whereas in the diaxial series they appear between δ_H 3.25 and 4.0 and the other ^{1}H NMR signals fit well for the stereochemistry.[16]

The above mentioned MS and NMR results of compound **1** are in a good agreement with those reported in the literature for (1*R**, 2*S**,5*R**,6*S**)-2-(4′-hydroxyphenyl)-6-(3″-methoxy-4″-hydroxyphenyl)-3,7-dioxabicyclo {3.3.0}octane which is a demethoxylated derivative of pinoresinol[15], due to the positive value of its optical rotation it can be named as (+)-demethoxypinoresinol. This compound was previously isolated from *Mikania saltensis* (Compositae)[15] but due to the scarcity of the isolated material its ^{13}C NMR spectral analysis was not reported, it is herein given (Table 1).

The ^{13}C NMR (Table 1) and ^{1}H NMR spectral data of compound **2** showed a similarity with those of **1** and indicated that it is a tetrahydrofuran lignan derivative of 2,6-diaryl-3,7-dioxabicyclo{3.3.0}octane type with the presence of two guaiacyl moieties as deduced from the signals at δ_C 132.6 *s* (2C, C-1′ and 1″), 110.0 *d* (2C, C-2′ and 2″), 148.2 *s* (2C, C-3′ and 3″), 146.5 *s* (2C, C-4′ and 4″), 115.5 *d* (2C, C-5′ and 5″), 119.0 *d* (2C, C-6′ and 6″) and 56.1 (2xOMe) with δ_H 6.71-6.83 (6H, *m*, aromatic H) and 3.78 (6H, *s*, 2xOMe). The NMR signals of the two fused tetrahydrofuranes displayed at δ_C 53.9 *d* (2C, C-1 and 5), 85.6 *d* (2C, C-2 and 6) and 71.3 *t* (2C, C-4 and 8) with δ_H 3.04 (2H, *m*, H-1, 5), 4.57 (2H, *d*, *J*= 4.12 Hz, H-2, 6) and 4.08 and 3.73 (each 2H, *m*, H-4, 8). The EI-MS spectral data of compound **2** supported the above assignments, whereas the molecular ion peak displayed at m/z 358 (M^+) corresponding to the molecular formula $C_{20}H_{22}O_6$ and indicated the presence of an additional methoxyl group than compound **1**. For further confirmation, 2D NMR spectral analysis was carried out including H-H COSY and C-H COSY.

From the above mentioned data, compound **2** was identified as (+)-pinoresinol; (1*R**,2*S**,5*R**,6*S**)-2-(3′-methoxy-4′-hydroxy-phenyl)-6-(3″-methoxy-4″-hydroxyphenyl)-3,7-dioxabicyclo{3.3.0}octane which was previously isolated from several plant species.[12,15,17]

The EI-MS spectral analysis of compound **3** exhibited a molecular ion peak at m/z 372 (M^+) coincident with the molecular formula $C_{21}H_{24}O_6$. The ^{13}C NMR (Table 1) and ^{1}H NMR spectral data of compound **3** were similar to those of compound **2** except for the presence of three aromatic methoxyl groups at δ_C 55.9 (2xOMe) and 55.6 (OMe) with δ_H 3.72 (6H, *s*, 2xOMe) and 3.73 (3H, *s*, OMe) instead of two methoxyls in compound **2** which indicated that the two aryl moieties of the lignan derivative **3** are guaiacyl and 4-O-methylguaiacyl moieties.

The above mentioned data together with the measurements of H-H COSY and C-H COSY confirmed the identity of compound **3** as (+)-pinoresinol monomethylether; (1*R**,2*S**, 5*R**,6*S**)-2-(3′,4′-dimethoxy-phenyl)-6-(3″-methoxy-4″-hydroxyphenyl)-3,7-dioxabicyclo {3.3.0}octane which was previously isolated from *Forsythia japonica* and *F. giraldiana*.[13]

The EI-MS spectral analysis of compound **4** showed a molecular ion peak at m/z 358 (M^+) assignable to a molecular formula $C_{20}H_{22}O_6$. The ^{13}C NMR including DEPT mode measurements (Table 1) and ^{1}H NMR spectral data of compound **4** showed a similar pattern to those reported for dihydrobenzofuran type neolignans.[14,18] The presence of α,β-dihydrobenzofuran could be recognized from the NMR spectral data at δ_H 5.44 (1H, *d*, *J*= 6.4 Hz) and 3.41 (1H, *m*) assignable to the methine protons H-α and H-β, respectively with δ_C 87.2 *d* (C-α) and 53.0 *d* (C-β) of the dihydrofuran ring fused to a coniferyl alcohol moiety to form the benzofuran skeleton. The *trans* 3-hydroxyprop-2-enyl group of the coniferyl alcohol at C-1′ was deduced from the signals at δ_H 6.45 (1H, *d*, *J*= 16.1 Hz, H-α′) and 6.17 (1H, *m*, H-β′) for the *trans* olefinic protons, and at 4.07 (2H, *br.d*, *J*= 5.1 Hz) for the hydroxylated methylene H-γ′ with δ_C 129.0 *d* (C-α′), 128.0 *d* (C-β′) and 61.7 (C-γ′). The C-2′ and 6′ of the coniferyl alcohol moiety were displayed at δ_C 110.4 *d* and 115.4 *d*, respectively, and the other aromatic carbons found at δ_C 129.5 *s*, 143.7 *s*, 147.2 *s* and 130.6 *s* were assigned to C-1′, 3′, 4′ and 5′, respectively. The presence of a hydroxymethyl group at C-β of the dihydrofuran ring was deduced from the ^{1}H NMR signal at δ 3.84 (2H, m, H-γ) with δ_C 62.9. Moreover, the ^{13}C NMR spectral data revealed also the presence of a guaiacyl moiety linked to C-α of the dihydrofuran ring from the signals at δ_C 132.4 *s* (C-1), 110.3 *d* (C-2), 147.6 *s* (C-3), 146.4 *s* (C-4), 115.3 *d* (C-5), 118.6 *d* (C-6) as well as the methoxyl group at C-3 displayed at either δ_C 55.5 or 55.6 (interfered with that of the coniferyl alcohol moiety at C-3′). This suggestion was confirmed from the ^{1}H NMR data at δ 6.73-6.89 (*m*) of the aromatic protons H-2, 5 and 6 (interfered with those of H-2′ and 6′).

The measurement of H-H COSY and C-H COSY together with comparing the above mentioned spectral data with those reported in the literature,[14] as well as, the result of the optical rotation analysis of compound **4** (Experimental section) confirmed its identity as the neolignan (–)-dehydrodiconiferyl alcohol which was previously reported from *Cistanche tubulosa*.[14]

The EI-MS, ^{1}H NMR, ^{13}C NMR (Table 2), H-H COSY and C-H COSY as well as comparing the physical properties and spectral data with those reported earlier[8,19,20] indicated that compound **5** is the anthraquinone deoxyerythrolaccin which was previously isolated from the same plant by Abdallah *et al*.,[8] however the numbering system mentioned in this reference is not coincident with the general numbering system of anthraquinones.[19-21] The corrected one according to that reported earlier[19-21] is herein given.

The EI-MS spectral analysis of compound **6** exhibited a molecular ion peak at m/z 284 (M^+) corresponding to the molecular formula $C_{16}H_{12}O_5$. The ^{1}H NMR spectral data showed four *meta*-coupled protons at δ_H 6.94 and 7.36 (each 1H, *d*, *J*= 2.0 Hz) assignable to H-2 and H-4, respectively, while the signals at δ 7.08 and 6.78 (each 1H, *d*, *J*= 1.8 Hz) corresponding to H-5 and H-7, respectively. It showed also two signals of chelated hydroxyl groups at C-1 and C-8 at δ 12.41 and 12.18 (each 1H, *s*) and signals of an aromatic methyl at δ 2.66 (3H, *s*) and aromatic methoxyl at δ 3.88 (3H, *s*).

Comparison the EI-MS, ^{1}H NMR, ^{13}C NMR (Table 2) and physical properties of compound **6** with those reported in the literature indicated that it is the anthraquinone physcion.[21-23]

The EI-MS of compound **7** showed a molecular ion peak at m/z 328 (M^+) corresponding to a molecular formula $C_{17}H_{12}O_7$. The ^{1}H NMR spectral data showed signals at δ13.30 (1H, *s*) assigned to a chelated hydroxyl group at C-1, 7.65 (1H, *s*) assigned to H-5, a *meta*-coupled protons at δ 6.74 and 7.13 (each 1H, *d*, *J*= 2.1 Hz, H-2 and 4), a signal of ester methyl at C-7 at δ 3.60 (3H, *s*, -COOMe) and an aromatic methyl at 2.65 (3H, *s*, Me at C-8). The ^{13}C NMR spectral analysis including DEPT mode measurements (Table 2) confirmed the presence of a methylester group from the signals at δ_C 167.8 *s* and 52.6 *q*, an aromatic methyl at δ 20.1, two carbonyl carbons at δ 188.3 *s* and 181.7 *s* for C-9 and C-10, respectively, and three hydroxylated aromatic carbons at 160.0 s, 157.1 s and 156.4 *s* corresponding to C-1, 3 and 6, respectively. The measurements of H-H COSY and C-H COSY confirmed these results.

Comparing the above mentioned data with those reported earlier[19] indicated that compound **7** is laccaic acid D methylester that was previously isolated from *Aloe saponaria*.[19] Anthraquinones with similar substitution pattern specially those with carboxyl group at *ortho* position to both methyl and methoxyl or hydroxyl functions were previously isolated from the genus *Gladiolus*.[8,10,11]

The ^{1}H NMR and ^{13}C NMR (Table 3) spectral data of compound **8** suggested the presence of β-sitosterol 3-O-β-glucopyranosyl and palmitoyl moieties.[24] The attachment of the palmitoyl moiety at C-6′ of the glucopyranosyl moiety was assigned from the downfield shift of C-6′ to δ_C 63.6. Comparison the NMR data of compound **8** with those of authentic sample indicated that it is 6′-O-palmitoyl-3-O-β-sitosterol glucopyranoside.[24]

Compound **9** can be identified as β-sitosterol-3-O-β-glucopyranoside by co-chromatography with an authentic sample.

Compounds **1-4** and **6-9** are reported here for the first time from the genus *Gladiolus* while compound **5** was previously isolated from the same plant. Finally, the occurrence of lignans, neolignans and anthraquinones in the genus *Gladiolus* is noteworthy from the chemotaxonomical viewpoint.

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