C-Geranyl Compounds from *Mimulus clevelandii*

William R. Phillips, Nina J. Baj, A. A. Leslie Gunatilaka, and David G. I. Kingston*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212

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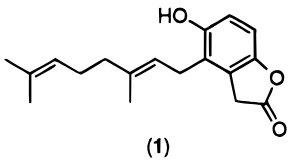
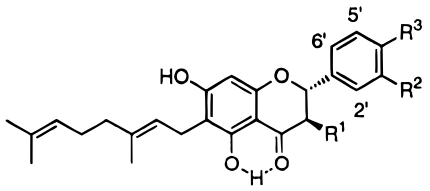
Fractionation of the MeCOEt extract of *Mimulus clevelandii* yielded the novel 4-geranyl-5-hydroxy-2(3*H*)-benzofuranone (**1**) and the five known 6-geranylflavanones diplacone (**2a**), 3′-*O*-methyldiplacone (**2b**), diplacol (**2c**), mimulone (**2d**), and 3′-*O*-methyldiplacol (**2e**). 2D-NMR methods required revision of assignments for diplacone and diplacol and resolved the uncertainty in the site of methylation for the methyl ethers.

As part of our efforts to isolate novel bioactive agents from natural sources, we have screened extracts from local plants using a mechanism-based bioassay[1] which utilizes genetically engineered yeast strains. Out of those investigated, a MeCOEt extract of *Mimulus clevelandii* Brandegee (Scrophulariaceae) showed weak antifungal activity in the RS188N (RAD$^+$) yeast assay. In this paper, we report the isolation and structure elucidation of six compounds from this extract, including a new geranylbenzofuranone (**1**) and the weakly active geranylflavanone, diplacol (**2c**).



(**1**)



(**2a**) R^1 = H, R^2 = R^3 = OH

b) R^1 = H, R^2 = OMe, R^3 = OH

c) R^1 = R^2 = R^3 = OH

d) R^1 = R^2 = H, R^3 = OH

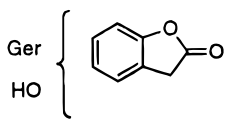
e) R^1 = R^3 = OH, R^2 = OMe

The MeCOEt extract of *M. clevelandii* was subjected to a standard liquid−liquid fractionation, and the weakly active chloroform-soluble fraction was further separated by gel permeation chromatography. The bioactivity was traced to the fraction eluted with CH_2Cl_2−Me_2CO (3:2), and further fractionation by Si gel column chromatography yielded the novel benzofuranone (**1**) and the five geranylflavanones (**2a**−**e**).

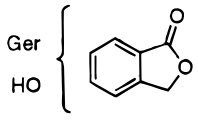
Compound **1** had the composition $C_{18}H_{22}O_3$ (HRE-IMS). From the ^{1}H-NMR spectrum (Table 1), the spin systems C=CHCH$_2$ (triplet and doublet at δ_H 5.16 and 3.29 $J_{vicinal}$ = 7 Hz) and C=CHCH$_2$CH$_2$ (triplet and four-proton multiplet at δ_H 5.01 and 2.06, $J_{vicinal}$ = 6 Hz) together with the three vinyl methyl singlets at δ_H 1.76, 1.64, and 1.56 indicated the presence of a geranyl side chain. Other recognizable groups were two ortho-coupled aromatic protons (doublets at δ_H 6.81 and 6.72, J_{ortho} = 8 Hz), a phenolic group (singlet at δ_H 5.07), and an isolated methylene group (singlet at δ_H 3.62). These features suggested an ortho-disubstituted 2(3*H*)-benzofuranone (**3**) as a partial structure. The alternative formulation as a 1(3*H*)-isobenzofuranone (**4**) was ruled out by the chemical shift of the C-3 methylene group (δ_C 32.9, δ_H 3.62) and the saturated nature of the lactone carbonyl (ν_{max} 1771 cm^{-1}). The corresponding signals for the isobenzofuranone[2] would be expected at δ_C 70.4, δ_H 5.20, and ν_{max} 1730, 1693 cm^{-1}. Long-range coupling from the side-chain methylene protons at C-1′ (δ_H 3.29) to C-3a (δ_C 123.0) and C-5 (δ_C 150.8) restricted the location of the side-chain and phenolic group to C-4 and C-5, respectively, resulting in the structure **1**. Mass spectral fragmentation occurred primarily along the geranyl side chain, giving ions at *m*/*z* 286 (M$^+$), 69 ($C_5H_9^+$), 123 ($C_9H_{15}^+$), and 163 ([M − C_9H_{15}]$^+$). This is the first report of a 2(3*H*)-benzofuranone from a *Mimulus* species. The related 5-hydroxy-7-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl)-2(3*H*)-benzofuranone isolated from the fruits of *Iryanthera grandis*

Table 1. NMR Data for **1** in $CDCl_3$

position	δ_H	δ_C	HMBC
2		174.6 s	
3	3.62 (2H, s)	32.9 t	2, 3a, 7a
3a		123.0 s	
4		124.4 s	
5	5.07 (1H, s, OH)	150.8 s	
6	6.72 (1H, d, *J* = 8 Hz)	115.6 d	4, 5, 7a
7	6.81 (1H, d, *J* = 8 Hz)	108.9 d	3a, 5, 7a
7a		148.2 s	
1′	3.29 (2H, d, *J* = 7 Hz)	27.0 t	3a, 4, 5, 2′, 3′
2′	5.16 (1H, t, *J* = 7 Hz)	119.7 d	4, 1′, 4′, 5′
3′		139.2 s	
4′	1.76 (3H, s)	16.3 q	2′, 3′, 5′
5′	2.06 (4H, m, *J* = 6 Hz)	39.6 t	2′, 3′, 6′, 7′
6′		26.3 t	3′, 5′, 7′, 8′
7′	5.01 (1H, t, *J* = 6 Hz)	123.6 d	5′, 6′, 9′, 10′
8′		132.2 s	
9′	1.64 (3H, s)	25.6 q	7′, 8′, 10′
10′	1.56 (3H, s)	17.7 q	7′, 8′, 9′



(**3**)



(**4**)

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(Myristicaceae) by Vieira[1] is believed to be biosynthesized from homogentisic acid.

Compounds **2a**−**e** were identified as the 6-geranylflavanones, diplacone, 3′-*O*-methyldiplacone, diplacol, mimulone, and 3′-*O*-methyldiplacol, respectively. These are the major components of the leaf resin of *M. aurantiacus*[4] but are being reported for the first time from *M. clevelandii*. Most of the physical data for **2a**−**e** agreed well with those reported in the literature for these compounds except for the significant difference in the mp of our diplacone (**2a**) to that reported by Wollenweber *et al.*[4] Since diplacone and related compounds cannot be crystallized and are only obtained as powders, this mp difference presumably reflects a difference in physical form due to different solvents used for precipitation. Specific rotation data for **2b**−**e** have been reported for the first time. While there was generally good agreement with reported ^{1}H-NMR data, we found it necessary to revise the chemical shift assignments for positions C-2′, C-5′, and C-6′ in diplacone (**2a**) and for positions C-2, C-2′, C-5′, and C-6′ in diplacol (**2c**). In the ^{1}H-NMR spectrum of these two compounds, the ring-B signals appeared as two singlets in the ratio 1:2 at δ_H 6.85 and 6.72. Since the protons H-5′ and H-6′, if assigned different chemical shifts as in the literature (δ_H 6.72 and 6.85, respectively), should appear as doublets with ortho coupling, we reassigned the one-proton singlet at δ_H 6.85 to H-2′ leaving H-5′ and H-6′ to account for the two-proton singlet at δ_H 6.72 by accidental equivalence. Corresponding ^{13}C-NMR assignments consistent with the cross-peaks from direct coupling (HETCOR) and long-range coupling (HMBC) were made.

In the cited paper[4] the proton signals for the ring C of diplacol (**2c**) were given as H-2 (δ_H 5.67, d, J = 7 Hz) and H_{ax}-3 (δ_H 4.48, dd, J = 7, 11 Hz). No explicit statement was made on the origin of the 11 Hz coupling, but the implication was that it arose from vicinal coupling between H_{ax}-3 and 3_{eq}-OH. We observed, however, that the chemical shift of the doublet at δ_H 5.67 was variable (δ_H 5.73 in our case), suggesting it to be an OH signal. As confirmation, this signal exchanged rapidly with D_2O, and its disappearance from the spectrum was accompanied by the collapse of H_{ax}-3 at δ_H 4.46 from a doublet of doublets (J = 6 and 11 Hz) to a doublet (J = 11 Hz). Also, the HETCOR spectrum showed C-2 at δ_C 83.0 to be directly attached to the proton at δ_H 4.92 (doublet, J = 11 Hz). With these revisions, the 11 Hz coupling was the $^3J_{ax,ax}$ between H-2 and H_{ax}-3 [cf. 12.5 and 13 Hz observed for diplacone (**2a**) and 3′-*O*-methyldiplacone(**2b**)] while the 6 Hz coupling arose from vicinal coupling between H_{ax}-3 and OH-3_{eq}.

The location of substituents in ring B of the methyl ethers **2b** and **2e**, previously reported by Lincoln and Walla[5] as 3′-hydroxy-4′-methoxy, was revised to 4′-hydroxy-3′-methoxy by Wollenweber *et al.*[4] on the basis that a small downfield shift is observed at a carbon atom when a *p*-phenolic group is methylated. In view of our reassignments of the ring B signals, we have presented in Figure 1 the essential long-range couplings from the HMBC spectrum of the ether **2b** which support the location of the methoxy group at C-3′. The data quoted are for the HMBC spectrum in $CDCl_3$ because the phenolic protons did not provide prominent cross-peaks with DMSO-d_6 as solvent. Since the change in solvent

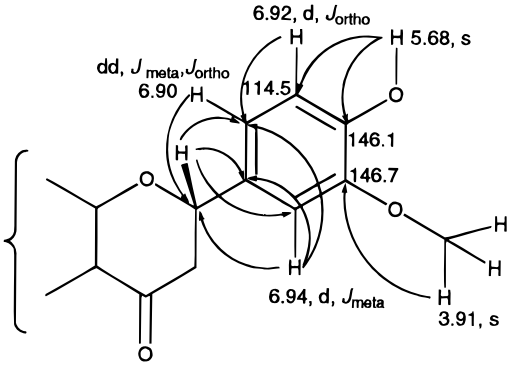

Figure 1. HMBC correlations for ring B of **2b**.

entailed substantial changes in chemical shifts, we obtained the NMR data on **2b** in DMSO-d_6 to establish identity with the reported compound and in $CDCl_3$ to locate the methoxy group with certainty. In particular, the coupling between the phenolic proton (singlet at δ_H 5.68) and C-5′ (at δ_C 114.5) would not have been possible in a 3′-hydroxy-4′-methoxy compound. As confirmation, the sole coupling shown by the methoxy protons (singlet at δ_H 3.91) was with C-3′ (at δ_C 146.7). Similarly, the methoxy group in the ether (**2e**) was shown to be at C-3′.

The weak antifungal activity of the extract was associated with diplacol (**2c**) (IC_{12} 430 μg/mL in RAD^+).

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were taken with a Perkin-Elmer Model 241 polarimeter. IR spectra were determined as mulls in Nujol on a Nicolet Impact 400 FT-IR spectrophotometer. UV spectra were taken in MeOH on a Beckman DU-50 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively, with TMS as internal standard. The DEPT, DQCOSY, HETCOR, and HMBC spectra were determined on the same instrument using standard Varian pulse sequences. In the HMBC determinations, a 9 Hz optimization was employed for the long-range coupling pathways. Column chromatography employed Si gel Merck G60 (230−400 mesh). TLC analyses were performed using precoated Si gel 60 F_{254} plates, and detection was accomplished by UV_{254} irradiation and by spraying with 5% molybdophosphoric acid in H_2SO_4−HOAc followed by heating.

Plant Material. The roots, stem, leaves, and flowers of *Mimulus clevelandii* were collected in California in July 1993. A voucher specimen (WBA no. 2593) was authenticated by Dr. R. W. Spjut of the World Botanical Associates, Laurel, MD.

Extraction and Isolation. The plant material (400 g) was extracted sequentially with hexane, MeCOEt, and MeOH. The MeCOEt extract (6.3 g) was partitioned between hexane and 80% aqueous MeOH. The aqueous MeOH fraction was diluted to 60% with water and extracted with $CHCl_3$. The $CHCl_3$ extract, after evaporation to dryness *in vacuo*, was subjected to Sephadex LH-20 gel permeation chromatography eluting initially with hexane−CH_2Cl_2 (1:4), CH_2Cl_2−Me_2CO (3:2), CH_2Cl_2−Me_2CO (1:4), and finally with MeOH. The fraction eluted with CH_2Cl_2−Me_2CO (3:2) (1.5 g) was column chromatographed on Si gel. The compounds **1**, **2b**, **2a**, and **2c** were obtained as major

Table 2. ^{1}H-NMR Data for **2a**−**e** [δ_H ppm, mult, (*J* Hz)]

H	**2a**[a]	**2b**[b]	**2b**[a]	**2c**[a]	**2d**[a]	**2e**[a]
2	5.32 dd (12.5, 3)	5.24 dd (13, 3)	5.37 dd (13, 2.8)	4.92 d (11)	5.38 dd (13, 3)	4.98 d (11)
3_{ax}	3.14 dd (17, 12.5)	3.06 dd (17, 13)	3.28 dd (17, 13)	4.46 dd (11, 6)	3.22 dd (17, 13)	4.60 dd (11, 6)
3eq	2.66 dd (17, 3)	2.76 dd (17, 3)	2.66 dd (17, 2.8)		2.66 dd (17, 3)	
3eq-OH				5.73 d (6)		5.72 d (6)
5-OH	12.40 s	12.40 s	12.41 s	12.17 s	12.41 s	12.19 s
7-OH	10.71 s	6.24 s	10.72 s	10.76 s	10.72 bs	10.80 bs
8	5.94 s	5.99 s	5.96 s	5.92 s	5.95 s	5.93 s
2′	6.85 s	6.94 d (1.6)	7.06 d (1.9)	6.85 s	7.29 d (8.6)	7.07 d (2)
3′					6.77 d (8.6)	
3′-OMe		3.91 s	3.77 s			3.77 s
3′-OH	9.00 s			8.96 s		
4′-OH	9.05 s	5.68 s	9.12 s	9.01 s	9.57 s	9.10 s
5′	6.72 s	6.92 d (8)	6.77 d (8)	6.72 s	6.77 d (8.6)	6.77 d (8)
6′		6.90 dd (8, 1.6)	6.88 dd (8, 1.9)		7.29 d (8.6)	6.88 d (8,2)
1″	3.10 d (7)	3.35 d (7)	3.11 d (7)	3.12 d (7)	3.11 d (7)	3.11 d (7)
2″	5.11 t (7)	5.24 t (7)	5.12 t (7)	5.12 t (7)	5.11 t (7)	5.11 t (7)
4″	1.68 s	1.79 s	1.69 s	1.69 s	1.69 s	1.69 s
5″	1.89 t (7)	2.07 m (6)	1.89 t (7)	1.89 t (7)	1.89 t (7)	1.89 t (7)
6″	1.98 q (7)		1.99 q (7)	1.99 q (7)	1.99 q (7)	1.98 q (7)
7″	5.03 t (7)	5.03 t (6)	5.03 t (7)	5.03 t (7)	5.03 t (7)	5.03 t (7)
9″	1.59 s	1.65 s	1.59 s	1.59 s	1.59 s	1.59 s
10″	1.52 s	1.57 s	1.52 s	1.52 s	1.52 s	1.52 s

[a] In DMSO-d_6. [b] In $CDCl_3$.

components on gradient elution with $CHCl_3$−EtOAc mixtures in the ratios 96:4, 92:8, 80:20, and 60:40, respectively. Compounds **2d** and **2e** were obtained as minor components from the fraction eluted with $CHCl_3$−EtOAc (92:8). For each of the compounds, an additional chromatographic separation on Si gel was required to obtain pure samples.

4-Geranyl-5-hydroxy-2(3*H*)-benzofuranone (1): colorless powder (0.11 g); mp 131−132 °C; UV (MeOH) λ max (log ϵ) 214 (4.19), 291 (3.52) nm; IR (Nujol) ν max 3304 (OH), 1771 (C═O, saturated lactone) cm^{-1}; ^{1}H-NMR, ^{13}C-NMR, and HMBC data, see Table 1; EIMS (70 eV) *m*/*z* $[M]^+$ 286 (7), 163 (10), 123 (22), 69 (100); HREIMS *m*/*z* $[M]^+$ 286.1575 ($C_{18}H_{22}O_3$ requires 286.1569).

Diplacone (2a): colorless powder (0.10 g); mp 124−125 °C (lit.[4] mp 170−173 °C); $[\alpha]^{25}_D$ −12° (*c* 0.77, MeOH) [lit.[4] $[\alpha]^{25}_D$ −26° ($CHCl_3$)]; UV (MeOH) λ max (log ϵ) 211 (4.20), 287 (3.99) nm; ^{1}H-NMR data, see Table 2; ^{13}C-NMR data, see Table 3; EIMS, in good agreement with published data.[5]

3′-*O*-Methyldiplacone (2b): colorless powder (0.14 g); mp 103−104 °C (lit.[4] mp 102−103 °C); $[\alpha]^{25}_D$ −17° (*c* 0.70, $CHCl_3$); UV (MeOH) λ max (log ϵ) 218 (4.26), 288 (3.88) nm; ^{1}H-NMR data, see Table 2; ^{13}C-NMR data, see Table 3; EIMS, in good agreement with published data.[5]

Diplacol (2c): colorless powder (0.29 g); mp 152−153 °C (lit.[4] mp 150−153 °C); $[\alpha]^{25}_D$ +16° (*c* 1.15, MeOH); UV (MeOH) λ max (log ϵ) 215 (4.39), 290 (4.00) nm; ^{1}H-NMR data, see Table 2; ^{13}C-NMR data, see Table 3; EIMS, in good agreement with published data.[5]

Mimulone (2d): colorless powder (0.01 g); mp 116−118 °C (lit.[4] mp 120−122 °C); $[\alpha]^{25}_D$ +1° (*c* 1.75, MeOH); UV (MeOH) λ max (log ϵ) 207 (4.38), 292 (4.07) nm; ^{1}H-NMR data, see Table 2; ^{13}C-NMR data, see Table 3; EIMS, in good agreement with published data.[5]

3′-*O*-Methyldiplacol (2e): colorless powder (0.02 g); mp 140−141 °C; $[\alpha]^{25}_D$ −4° (*c* 5.4, $CHCl_3$); UV (MeOH) λ max (log ϵ) 211 (4.46), 295 (4.22) nm; ^{1}H-NMR data, see Table 2; ^{13}C-NMR data, see Table 3; EIMS, in good agreement with published data.[5]

Table 3. ^{13}C-NMR Data for **2a**−**e** (δ_C ppm, mult)

C	**2a**[a]	**2b**[b]	**2b**[a]	**2c**[a]	**2d**[a]	**2e**[a]
2	78.3 d	79.1 d	78.6 d	83.0 d	78.3 d	83.1 d
3	42.1 t	45.3 t	42.2 t	71.6 d	42.0 t	71.5 d
4	196.4 s	196.1 s	196.4 s	197.9 s	196.5 s	198.0 s
5	160.5 s	161.2 s	160.6 s	160.4 s	160.6 s	160.4 s
6	107.4 s	106.8 s	107.5 s	107.7 s	107.5 s	107.8 s
7	164.2 s	164.0 s	164.3 s	164.4 s	164.2 s	164.5 s
8	94.3 d	95.7 d	94.3 d	94.3 d	94.3 d	94.4 d
9	160.4 s	161.1 s	160.5 s	160.1 s	160.5 s	160.1 s
10	101.6 s	102.9 s	101.5 s	100.2 s	101.6 s	100.2 s
1′	129.5 s	130.4 s	129.5 s	128.1 s	129.0 s	128.2 s
2′	114.3 d	108.7 d	111.1 d	115.3 d	128.3 d	112.2 d
3′	145.2 s	146.7 s	147.5 s	145.7 s	115.1 d	147.3 s
4′	145.6 s	146.1 s	146.9 s	144.9 s	157.7 s	147.0 s
5′	115.3 d	114.5 d	115.1 d	115.1 d	115.1 d	114.9 d
6′	117.8 d	119.5 d	119.6 d	119.3 d	128.3 d	121.1 d
1″	20.5 t	21.1 t	20.6 t	20.6 t	20.6 t	20.6 t
2″	122.4 d	121.2 d	122.4 d	122.3 d	122.4 d	122.4 d
3″	133.8 s	139.7 s	133.8 s	133.8 s	133.8 s	133.8 s
4″	15.9 q	16.2 q	15.8 q	15.9 q	15.9 q	15.9 q
5″	39.2 t	39.7 t	39.2 t	39.2 t	39.3 t	39.3 t
6″	26.2 t	26.3 t	26.2 t	26.2 t	26.2 t	26.2 t
7″	124.1 d	123.6 d	124.1 d	124.1 d	124.1 d	124.1 d
8″	130.6 s	132.1 s	130.6 s	130.6 s	130.6 s	130.6 s
9″	25.5 q	25.7 q	25.4 q	25.5 q	25.5 q	25.5 q
10″	17.5 q	17.7 q	17.5 q	17.5 q	17.5 q	17.5 q
3′-OMe		56.0 q	55.7 q			55.7 q

[a] In DMSO-d_6. [b] In $CDCl_3$.

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References and Notes

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