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Structure of a geranyl-α-pyrone from *Mimulus aurantiacus* leaf resin

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Abstract

A previously reported but misidentified geranyl-α-pyrone, in addition to six known compounds, was isolated from the leaf resin of *Mimulus aurantiacus*. HMBC NMR analyses of the geranyl-α-pyrone resolved uncertainties in the site of attachment of the two side chains and necessitated a revision of the previously reported structure. This compound is shown to be 3-geranyl-4-hydroxy-6-(2-hydroxypropyl)-2-pyrone.

Keywords: *Mimulus aurantiacus*; Scrophulariaceae; Sticky Monkeyflower; Geranylpyrone

1. Introduction

The chaparral subshrub, *Mimulus* (= *Diplacus*) *aurantiacus* Curtis (Scrophulariaceae) has intrigued chemical ecologists because of the large quantity of leaf surface resins that it produces, comprising nearly 30% of the dry weight of the leaves (Lincoln, 1980). This resin reduces growth and survival of its primary herbivore, the larvae of *Euphydryas chalcedona* (Lepidoptera: Nymphalidae; Lincoln et al., 1982; Lincoln, 1985). The resin also may provide some protection from UV light and help reduce water loss during drought conditions (Lincoln et al., 1986).

Most of this resin is comprised of at least five geranylflavonoids whose structures have been elucidated previously (Lincoln et al., 1986; Wollenweber et al., 1989; Phillips et al., 1996). Additional resin components have been noted but not completely identified (Lincoln et al., 1986; Wollenweber et al., 1989). A tentative structure of one such component, a geranyl-α-pyrone with a molecular weight of 306, was proposed, although there was some uncertainty as to the attachment of substituents to the pyrone ring (Wollenweber et al., 1989). As part of a larger study to investigate the geographic and temporal variation in the production of individual leaf resin components within and among several southern California populations of *M. aurantiacus* (Hare, 2002a,b), we re-examined the resin components produced by these plant populations. Here, we report the revision of the structure of the previously reported geranyl-α-pyrone, which we propose to call aurantiacone (**1**), based on additional NMR spectroscopic experiments.

2. Results and discussion

A compound (**1**) with a UV maximum absorbance at 293 nm and an HPLC retention time shorter than the known geranylflavanoids of *M. aurantiacus* yielded a molecular ion at *m*/*z* 306 and produced the same fragmentation pattern as the compound previously reported by Wollenweber et al. (1989). The structural information from our NMR spectroscopic studies, however, is not in accord with the previous structure (**2**) suggested by (Wollenweber et al., 1989). The assignment of the geranyl side chain to C-6 in **2** seemed to be most consistent with the structures of other known pyrones with long aliphatic side chains, although the alternative structure **1** could not be ruled out. Moreover, the unambiguous assignment of ^{13}C NMR signals due to C-2, C-4, and C-6 could not be made at that time (Wollenweber et al., 1989).

Assignments of the ^{1}H and ^{13}C spectra of **1** in CD_3OD and DMSO-d_6 are given in Table 1. For each ^{1}H chemical shift reported in Table 1 the corresponding

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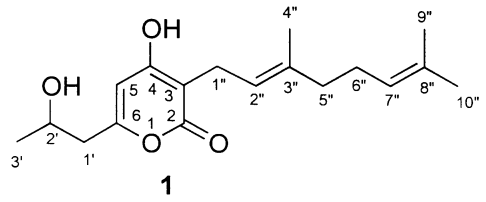

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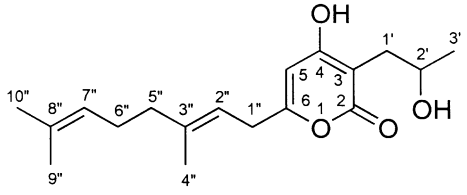

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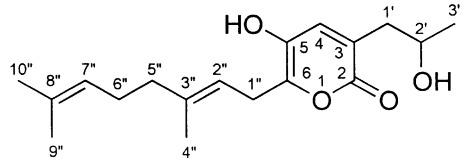

3

^{13}C chemical shift represents the observed gHMQC correlation. Comparison of the spectra with those obtained for the known geranylflavonoids from *M. aurantiacus* showed this compound to also contain a geranyl side chain. In addition, a 2-hydroxypropyl side chain was evident in the gCOSY spectrum. With the assignment of the two side-chains there remains only five unaccounted carbons. The gHMBC cross peaks observed for H-1″, OH-4, H-5 and H-1′ indicate that these five remaining carbons are sequentially connected. This, along with the observed unsaturation, indicates a ring structure but not a five membered ring. Taking into consideration the MS data, the observed unsaturation and the number of quaternary carbons, the remaining 32 unaccounted mass units must be due to two oxygens. One must be a carbonyl and the other must be placed in the ring connecting the terminal carbons (C-2 and C-6) of the five carbon segment. The resulting six membered ring with three of the quaternary carbons (C-2, C-4, and C-6) having chemical shifts greater than 160 ppm indicate that this ring is a substituted pyrone (Waterman and Crichton, 1980; Fehr et al., 1999). These data, along with the mass spectral data, indicate that **1** is indeed a geranyl-α-pyrone and most likely the same compound previously reported. The observed pattern of three deshielded carbons with chemical shift greater than 160 ppm and two shielded carbons at ca. 103 ppm can only fit an α-pyrone with symmetrical oxygenation at C-2, C-4 and C-6. No other pattern would give the observed ^{13}C chemical shift distribution. Our assignments for C-2, C-4 and C-6 not only follow the observed pattern of HMBC correlations, but also is analogous to the assignment of a simpler substituted 2-pyrone, 4,5,6-trimethyl-2H-pyran-2-one (Fehr et al., 1999). The hydroxyl group must be at C-4 as indicated by gHMBC cross peaks with C-3 and C-5. The lone ring ^{1}H is attached to C-5 as the gHMQC shows a direct correlation. The previous observations eliminate **3** as a possible structure as well as the complimentary structure to **3** in

Table 1
NMR spectroscopic data for **1** in CD_3OD and DMSO-d_6 (25 °C)

Position	δ_C		δ_H		HMBC (cross peaks)
	CD_3OD	DMSO-d_6	CD_3OD	DMSO-d_6	
2	168.92	164.76			
3	103.65	100.73			
4	168.36	164.48			
5	103.53	100.88	6.04 (*s*)	6.01 (*s*)	3,6,1′
6	162.31	160.98			
1′	44.19	42.77	2.54 (*m*)	2.43 (*m*)	2′,3′,5,6
2′	66.37	64.01	4.10 (*m*)	3.90 (*m*)	6
3′	23.53	23.39	1.22 (*d* 6.30)	1.10 (d 6.10)	1′,2′
1″	22.96	21.69	3.07 (*d* 7.25)	2.94 (d 7.30)	2,3,2″,3″
2″	122.78	121.70	5.16 (*t* 7.25)	5.10 (t 7.30)	4″,5″
3″	136.57	134.50			
4″	16.42	15.90	1.72	1.66	2″,3″, 5″
5″	41.00	39.20	1.96 (*m*)	1.90 (*m*)	2″,3″,4″,6″,7″
6″	27.84	26.11	2.05 (*m*)	1.99 (*m*)	3″,5″,7″,8″
7″	125.55	124.05	5.07 (*t* 7.25)	5.04 (*t* 7.30)	9″,10″
8″	132.24	130.67			
9″	17.86	17.50	1.57 (*s*)	1.53 (*s*)	7″,8″,10″
10″	26.01	25.25	1.64 (*s*)	1.61 (*s*)	7″,8″,9″
OH4	Not observed in CD_3OD			11.09 (*s*)	3,4,5
OH′	Not observed in CD_3OD				

which the side chains are attached as in **2**. To this point we are in agreement with the structure proposed by Wollenweber et al. (1989). However, our data do not support the side chain attachment as shown in **2**. Our data support a reversal of those assignments. The 2-hydroxypropyl group must be attached to C-6 as there is a gHMBC correlation between it and a deshielded (O-linked) carbon. The geranyl side chain must be attached to C-3, as there is a gHMBC correlation between H-1″ and this shielded ring carbon. This is also supported by the observed NOE in DMSO-d_6 between OH-4 and H-1″. Neither we nor Wollenweber et al. (1989) observed a chemical shift for OH-2′. Neither did we observe any NOE between H-5 and H-2′. We speculate that this could be caused by hydrogen bond formation between OH-2′ and the ring oxygen, thereby causing H-2′ to rotate away from H-5. The proposed structure (**1**), with a series of oxygen atoms attached to four alternate carbons (C-2, C-4, C-6 and C-2′) also is more reasonable from a standpoint of acetogenin biosynthesis than the other structures. We conclude that the structure of the geranylpyrone from *M. aurantiacus* is 3-geranyl-4-hydroxy-6-(2-hydroxypropyl)-2-pyrone (**1**), and we propose the name, aurantiacone, for this structure. The patterns of geographic, and genetic variation in the concentrations of aurantiacone and other leaf resin components from six southern California populations of *M. aurantiacus* are presented elsewhere (Hare, 2002a,b).

3. Experimental

3.1. Plant material

Individual leaves from six *M. aurantiacus* populations in southern California were collected in the spring of 1998. The location of all plant populations are described elsewhere (Hare, 2002a), and voucher specimens are deposited in the University of California Riverside herbarium.

3.2. Extraction and isolation

A sample of leaves from the different plant populations were pooled (total sample of 100 g, fr. wt) and surface-extracted in MeOH three times for 5 min each. The MeOH extracts were pooled and concentrated under rotary evaporation. The extract was partitioned 3× against hexane to remove non-polar components and the hexane layers were discarded. An equal volume of H_2O was then added to the MeOH extract, and this mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was concentrated to dryness under rotary evaporation, and the initial 100 g of leaves yielded 7.46 g of crude resin.

An aliquot of crude resin (200 mg) was dissolved in 1 ml 95% aqueous EtOH and subjected to flash-chromatography on a 25 mm×18 cm column of C_{18} silica gel, eluting with 100-ml portions of 50, 60, 70, and 80% aqueous EtOH, respectively, taking 10 ml fractions. Fractions were combined into five groups based on RP–TLC of the fractions (70% aq. EtOH). The first group did not contain any resin components and was discarded. Groups 2 through 5 were further purified by preparative RP-HPLC (Alltech "Econosil" C_{18}, 10 μm particle size, 250 mm×10 m i.d.). An eluent of 45% CH_3CN and 55% H_2O to which 0.075% TFAA was added, at a flow rate of 5 ml/min, was used to separate the compounds in Group 2; 60% CH_3CN was used to separate the components in Groups 3 and 4; and 62% CH_3CN was used to separate the components in Group 5.

Group 2 yielded eriodictyol 7,3′ dimethyl ether (9.8 mg) and **1** (22.8 mg), while Groups 3–5 yielded the geranylflavonoids previously known to be components of *Mimulus* leaf surface resin. The quantities and order of elution of these compounds was as follows: diplacol (28.2 mg), 3′-*O* methyldiplacol (6.9 mg), diplacone (53.6 mg), mimulone (7.8 mg), and 3′-*O* methyldiplacone (10.8 mg). The identities of the geranylflavonoids were determined by comparing their mass, ^1H, and ^{13}C NMR spectra with published data (Lincoln et al., 1986; Wollenweber et al., 1989; Phillips et al., 1996). The identity of eriodictyol 7,3′ dimethyl ether also was determined by comparing its mass,^1H, and ^{13}C NMR spectra with published data (Arene et al., 1978; Wollenweber, 1981; Rauter et al., 1989; Vascooncelos et al., 1998). This is, to our knowledge, the first report of eriodictyol 7,3′ dimethyl ether from *Mimulus*. We also confirmed the reversal of chemical shifts for H-6 and H-8 for eriodictyol 7, 3′ dimethyl ether, depending upon whether the solvent for NMR analyses was CD_3OD or $CDCl_3$ (Vasconcelos et al., 1998).

3.3. NMR and MS spectroscopy

1D and 2D NMR spectra were obtained in CD_3OD, $CDCl_3$ and DMSO-d_6 on a Varian Inova 500 MHz spectrometer equipped with triple axis gradients. The chemical shift values δ for both ^1H and ^{13}C are reported as parts per million (ppm) relative to tetramethylsilane (TMS), and coupling constants ($J_{H,H}$) are in hertz (in parentheses). ^1H assignments were made using 2D gCOSY and gNOESY experiments whereas ^{13}C assignments were made using 2D gHMQC and gHMBC experiments. HREIMS data were obtained using a VG7070E mass spectrometer in positive ion mode with an ionization voltage of 50 eV.

3.4. 3-Geranyl-4-hydroxy-6-(2-hydroxypropyl)-2-pyrone (**1**)

MP and UV spectra have been previously reported (Wollenweber et al., 1989); EIMS: *m*/*z* 306 $[M]^+$ (43),

291 (3), 288 (4), 237 (34), 193 (20), 184 (42), 183 (43), 175 (25), 166 (24), 139 (94), 123 (68), 109 (33), 81 (22), 69 (100), 67(35), 45 (34), 43 (27); HREIMS $m/z=$ 306.1827 (calc. for $C_{18}H_{26}O_4$, 306.1831); ^1H and ^{13}C NMR data, see Table 1.

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