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DERMATOTOXIC PHENOLICS FROM GLANDULAR TRICHOMES OF *PHACELIA CAMPANULARIA* AND *P. PEDICELLATA*

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Key Word Index—*Phacelia campanularia; P. pedicellata;* Hydrophyllaceae; trichomes; contact dermatitis; quinones.

Abstract—The principal contact allergens of the glandular trichomes of *Phacelia campanularia* and *P. pedicellata* were identified, and their potential to elicit allergic contact dermatitis was tested with guinea pigs. The active compounds from *P. campanularia* are novel derivatives of farnesylhydroquinone. The sensitizer of *P. pedicellata*, also a new compound, is 2,4-dihydroxy-6-geranylphenyl acetate.

INTRODUCTION

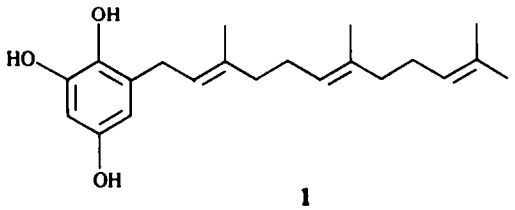
Contact with the glandular trichomes of several species of *Phacelia* causes many persons to suffer a severe allergic dermatitis [1]. *Phacelia campanularia* and *P. pedicellata* are two annual species of the California Mojave Desert which have this property. We determined to identify the compounds responsible for the dermatitis.

RESULTS AND DISCUSSION

The glandular exudate from the trichomes of each species was collected by immersing intact fresh plants briefly (5–10 sec) in acetone. This extract was compared by TLC to an extract of individual trichomes in order to confirm that the compounds isolated were constituents of the trichome glands.

Four phenolic compounds which had dermatitic activity were isolated from the extract of *P. campanularia*. The principal constituents were compounds **1**, **2** and **3**. Compounds **1** and **2** were unstable making an accurate assessment of their quantities in the original extract uncertain. Positions of phenolic hydroxyl groups are apparent from the coupling constants of the two aromatic protons of each compound. In **3** chelation of the hydroxyl at C-1 with the carbonyl group at C-1′ is evident in the low frequency of the C=O stretch in the IR spectrum (1640 cm^{-1}), and in the ^1H NMR spectrum from the single hydroxyl signal at δ 13.15. Configuration of farnesyl double bonds at C-2 and C-6 are apparent from the similarity of the vinyl methyl ^1H NMR shifts with those of homologous compounds for which we determined configuration with ^{13}C NMR spectra [2]. Compound **4** is a minor constituent which we reported previously as a major constituent in *P. minor* and *P. parryi* [3]. The ^{13}C NMR spectrum of **4** is reported here to confirm the configuration of the farnesoyl group shown. In structure **4** the placement of a vinyl methyl group at C-6 versus C-7, or *Z* configuration of the C-6 double bond gives substantially different calculated ^{13}C NMR spectra than that observed.

The glandular trichomes of *P. pedicellata* contained two principal phenolic compounds **5** and **6**. The ^1H NMR spectrum of **5** shows a geranyldihydroxyphenyl acetate. The ^1H NMR spectrum of the triacetate shows two non-equivalent *meta*-coupled aryl protons (J = 2.5 Hz), which establishes the benzene oxygenation pattern shown. Placement of the acetate group is apparent from the



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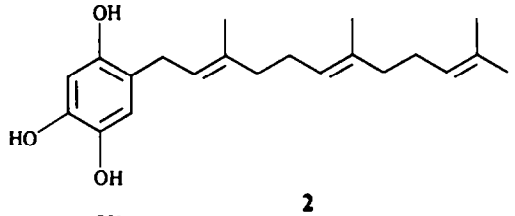

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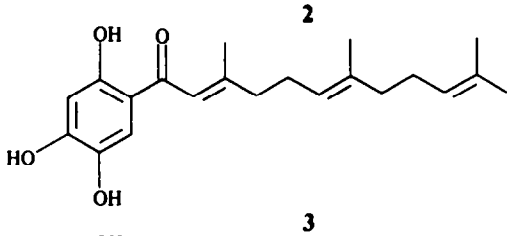

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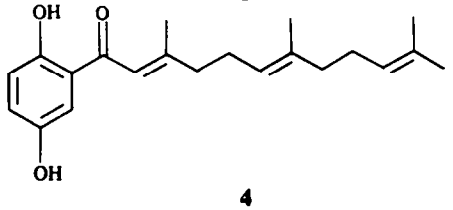

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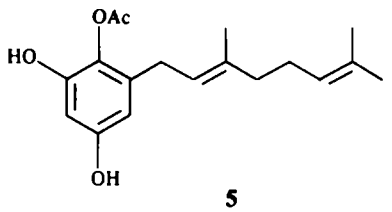

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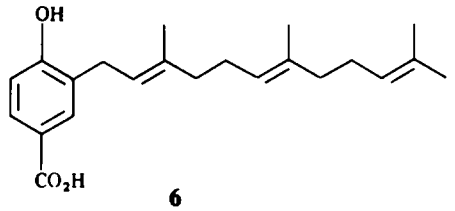

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similarity of the observed ^{13}C NMR spectrum of the aromatic carbons with the estimated spectrum for structure **5** made by applying known benzene substituent effects [4] to the observed ^{13}C NMR spectrum of geranylhydroquinone. This structure was substantiated by methylation of the two free hydroxyls, followed by hydrolysis of the acetate. The ^1H NMR spectra of this derivative showed the signals of geranyl protons on C-1 and C-2 to be shifted downfield in C_5D_5N relative to the spectrum in $CDCl_3$ by 0.35 and 0.32 ppm respectively, indicating that the free hydroxyl obtained by deacetylation is *ortho* to the geranyl group [5]. Compound **6** is a non-allergenic constituent which we isolated previously from the trichomes of *Turricula parryi* (Hydrophyllaceae) [2].

The allergenic potential of compounds **1**–**4** were tested on the shaved skin of guinea pigs sensitized with the crude extract of *P. campanularia*. The skin irritant properties were assayed on the same animals prior to sensitization. One of five animals was not sensitized and gave no reactions to the highest amounts applied (1.5 μmoles on an 8 mm diameter patch). As shown in Table 1, compounds **3** and **4** are potent contact allergens which produced erythematous reactions with as little as 0.002 μmole applications on some animals. Compounds **1** and **2** were applied in a mixture because of major loss of these compounds during their separation. Application of compounds **1** and **2** together produced allergic reactions with 0.018 μmole application, but it is not certain to what degree each is responsible for the dermatitis. Some of the lesser potential of **1** and **2** to elicit allergic reactions can be attributed to the instability of these compounds which visibly polymerized on the surface of the skin within seconds after application. This undoubtedly reduces the amounts of these allergens which reach the cells in the epidermis which initiate the allergic reaction. These compounds are equivalent in allergenic potential to pentadecylcatechol, one of the allergenic components of poison ivy urushiol, which gives allergenic reactions on urushiol-sensitive guinea pigs with 0.005 μmole applications and irritant reactions with 0.1 μmole when tested using the procedures described here.

Table 1. Amounts of compounds (μmoles applied to 8 mm diameter of skin) producing a visible erythema with at least half of animals tested

Compound	Sensitized animals	Unsensitized animals
1, 2	0.055	1.5
3	0.0058	NR*
4	0.019	1.5
5	0.061	0.55
6	1.5	1.5

* NR denotes no reaction at the highest dose applied (1.5 μmoles).

Compound **5** was the principal dermatotoxin from *P. pedicellata*, giving irritant reactions on unsensitized animals with 0.55 μmoles and allergic reactions on sensitized animals with 0.061 μmoles. Compound **6** was irritating with 1.5 μmoles applied, but did not give allergic reactions. These results are consistent with current models of allergic dermatitis in which a phenolic contact allergen is oxidized to a quinone which can then bond covalently to a protein nucleophile to form an antigenic complex [6]. The current understanding of the mechanism of allergic contact dermatitis is described in ref. [7].

EXPERIMENTAL

Phacelia campanularia Gray subsp. *vasiformis* Gillett (1.5 kg fr. wt) and *P. pedicellata* Gray (2.2 kg fr. wt) were collected in the Lucerne Valley area of San Bernardino Co., CA. Voucher specimens 21750 and 21751 are deposited at the Museum of Systematic Biology, University of California, Irvine. Species identification was confirmed by Mr. Fred Roberts of the museum staff. Aerial parts of intact fresh plants were immersed × 3 for 5–10 sec, each time in 4 l. clean Me_2CO containing 0.1% HOAc. The concd extracts were partitioned between C_6H_{14}, CH_2Cl_2 and 70% aq. MeOH. The CH_2Cl_2 extracts were separated on Sephadex LH-20 (Me_2CO) and on silica gel (CH_2Cl_2, 0.4% HOAc, gradient Me_2CO).

6-Farnesyl-1,2,4-trihydroxybenzene (**1**). A light yellow oil. UV λ_{max}^{MeOH} nm: 290. ^1H NMR (90 MHz, $CDCl_3$): δ6.29 (1H, *d*, *J* = 2.1 Hz, H-5), 6.16 (1H, *d*, *J* = 2.1 Hz, H-3), 5.43 (3H, *br*, OH), 5.27 (1H, *t*, *J* = 7 Hz, H-2'), 5.09 (2H, *mt*, H-6', 10'), 3.29 (2H, *d*, *J* = 7 Hz, H-1'), *ca* 2.08 (8H, H-4', 5', 8', 9'), 1.74 (3H, *s*, Me-3'), 1.69 (3H, *s*, H-12'), 1.62 (6H, *s*, Me-7', Me-11'). EIMS (probe) 70 eV, *m/z* (rel. int.): 330 $[M]^+$ (43), 177 $[M-C_{11}H_{19}-2H]^+$ (54), 139 $[M-C_{14}H_{23}]^+$ (65), 69 (100).

5-Farnesyl-1,2,4-trihydroxybenzene (**2**). ^1H NMR (90 MHz, $CDCl_3$): δ6.58 (1H, *s*, H-6), 6.38 (1H, *s*, H-3), 3.21 (2H, *d*, *J* = 7 Hz, H-1'), signals of other protons were the same as for **1**.

5-Farnesoyl-1,2,4-trihydroxybenzene (**3**). Yellow needles (300 mg) crystallized from C_6H_{14}–CH_2Cl_2, mp 77–78°. UV λ_{max}^{MeOH} nm: 257, 292, 278. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3200 (OH), 1640 (C=O), 1600, 1515 (C=C), 1272 (C–O). ^1H NMR (90 MHz, $CDCl_3$): δ13.15 (1H, *s*, OH-4), 7.16 (1H, *s*, H-6), 6.49 (1H, *s*, H-2'), 6.41 (1H, *s*, H-3), *ca* 5.90 (2H, *br*, OH), 5.07 (2H, *m*, H-6', 10'), *ca* 2.22 (4H, H-4', 5'), 2.11 (3H, *s*, Me-3'), *ca* 2.02 (4H, H-8', 9'), 1.68 (3H, *s*, H-12'), 1.63 (3H, *s*, Me-7'), 1.62 (3H, *s*, M-11'). MS (probe) 70 eV, *m/z* (rel. int.): 344 $[M]^+$ (16), 193 $[M-C_{11}H_{19}]^+$ (100), 153 $[M-C_{14}H_{23}]^+$ (46).

2-Farnesoyl-hydroquinone (**4**). A yellow oil (30 mg) was obtained. UV, IR, mass and ^1H NMR spectra are as in ref. [3]. ^{13}C NMR (22.6 MHz, $CDCl_3$): δ196.2 (*s*, C-1'), 161.5 (*s*, C-1), 157.2 (*s*, C-3'), 147.5 (*s*, C-4), 136.5 (*s*, C-7'), 131.6 (*s*, C-11'), 124.3, 124.2 (*d*, C-6', C-10'), 122.8 (*d*, C-2'), 120.6 (*s*, C-2), 119.6, 119.2 (*d*,

C-5, C-6) 115.1 (*d*, C-3), 41.6 (*t*, C-4'), 39.7 (*t*, C-8'), 26.7 (*t*, C-9'), 26.2 (*t*, C-5'), 25.7 (*q*, C-12'), 20.1 (*q*, Me-3'), 17.1 (*q*, Me-11'), 16.1 (*q*, Me-7').

2,4-Dihydroxy-6-geranylphenyl acetate (**5**). A brown oil (1.2 g) was isolated from *P. pedicellata*. UV λ_{max}^{MeOH} nm: 217, 285. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3360 (OH), 1749, 1714 (C=O), 1205 (C–O). ^{1}H NMR (90 MHz, $CDCl_3$): δ6.18 (1H, *d*, J = 2.5 Hz, H-5), 6.13 (1H, *d*, J = 2.5 Hz, H-3), 5.40 (2H, *br*, OH), 5.13 (1H, *t*, J = 7 Hz, H-2'), 5.05 (1H, *m*, H-6'), 3.11 (2H, *d*, J = 7 Hz, H-1'), 2.28 (3H, *s*, OAc), *ca* 2.04 (4H, *m*, H-4' and H-5') 1.68 (3H, *s*, Me-3'), 1.67 (3H, *s*, H-8'), 1.60 (3H, *s*, Me-7'). ^{13}C NMR (67.94 MHz, $CDCl_3$): δ171.3 (*s*, OAc), 153.9 (*s*, C-4), 148.0 (*s*, C-2), 136.9 (*s*, C-3'), 135.6 (*s*, C-6), 131.5 (*s*, C-7'), 130.7 (*s*, C-1), 124.2 (*d*, C-6'), 121.3 (*d*, C-2'), 108.4 (*d*, C-5), 102.4 (*d*, C-3), 39.7 (*t*, C-4'), 28.9 (*t*, C-1'), 26.7 (*t*, C-5'), 25.7 (*q*, C-8'), 20.5 (*q*, OAc), 17.7 (*q*, Me-7'), 16.1 (*q*, Me-3'). EIMS (probe) 70 eV, *m/z* (rel. int.): 304 $[M]^+$ (12), 262 $[M-CH_2CO]^+$ (48), 177 $[M-C_6H_{11}-CH_2CO-2H]^+$ (28), 138 $[M-C_9H_{15}-MeCO]^+$ (64), 69 (100). Methylation of **5** with CH_2N_2 followed by hydrolysis of the acetate (0.5 M KOH, 80% EtOH) gave a dimethyl ether with a free hydroxyl *ortho* to the farnesyl group: ^{1}H NMR (90 MHz, $CDCl_3$): δ6.35 (2H, *s*, H-3 and H-5), 5.35 (1H, *t*, J = 7 Hz, H-2'), 5.3 (1H, *br*, OH), 5.12 (1H, *m*, H-6'), 3.84 (3H, *s*, OMe), 3.77 (3H, *s*, OMe), 3.38 (1H, *d*, J = 7 Hz, H-2'). ^{1}H NMR (90 MHz, C_5D_5N): δ6.67 (2H, *s*, H-3 and H-5), 5.67 (1H, *t*, J = 7 Hz, H-2'), 5.18 (1H, *m*, H-6'), 3.75 (3H, *s*, OMe), 3.73 (2H, *d*, J = 7 Hz, H-1'), 3.63 (3H, *s*, OMe).

3-Farnesyl-4-hydroxybenzoic acid (**6**). A brown oil (0.67 g) was obtained from the *P. pedicellata* extract. Spectral data are given in ref. [2].

Tests for dermatotoxicity. The assay used was essentially the Freunds Complete Adjuvant Test described by Klecak [8]. Five Hartley strain female guinea pigs 4–6 months old were tested for irritant reactions to test compounds prior to sensitization. Three-fold serial dilutions of each compound tested were prepared beginning with a 10% soln in Me_2CO. The flanks of the animals were shaved and a 5 μl drop of each test soln was applied to the skin in an 8 mm diameter circle left uncovered. Animals were inspected daily for reactions for 3 days. Animals were then sensitized with a 5% soln of the crude trichome extract made in Freunds complete adjuvant. Three 0.1 ml intradermal injections were given 2 days apart in the interscapular region of each animal. Two weeks following the last injection the animals were challenged with the test solns in the same manner as in the irritancy test.

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