Phytochemistry, Vol. 20, No. 6, pp. 1365–1366, 1981.
Printed in Great Britain.

0031–9422/81/061365–02 $02.00/0
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PRENYLATED HYDROQUINONES: CONTACT ALLERGENS FROM TRICHOMES OF *PHACELIA MINOR* AND *P. PARRYI*

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(*Received* 19 *September* 1980)

Key Word Index—*Phacelia parryi*; *P. minor*; Hydrophyllaceae; trichomes; contact dermatitis; prenylated quinone; 2-(3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl)-1,4-benzendiol; 1-(2,5-dihydroxyphenyl)-3,7,11-trimethyl-2,6,10-dodecatrien-1-one.

Abstract—Geranylgeranylhydroquinone and 1-oxofarnesylhydroquinone were identified as contact allergens in trichomes of *Phacelia minor* and *P. parryi*.

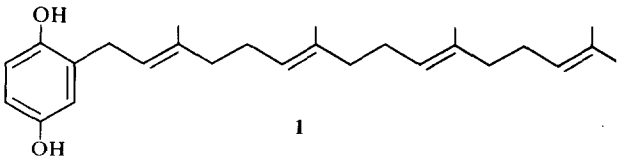
INTRODUCTION

Several species of *Phacelia* (Hydrophyllaceae) cause many persons severe allergic contact dermatitis [1, 2]. Earlier we identified the allergenic constituents of *P. crenulata* [3, 4], the species probably responsible for most reported cases of *Phacelia* dermatitis. The allergens from this species are geranylhydroquinone and geranylquinone which are exuded from the heads of stalked glandular trichomes. In an earlier report [3] we indicated that several other allergenic species, *P. minor*, *P. parryi*, *P. viscida*, and *P. companularia* might also contain geranylhydroquinone. However, we report here that the major trichome constituent and contact allergen of both *P. minor* and *P. parryi* is geranylgeranylhydroquinone (**1**) and that a minor allergenic constituent is 1-oxofarnesylhydroquinone (**2**). Both compounds are new in higher plants; geranylgeranylhydroquinone has been found before in marine sponges [5]. Both species are endemic to the coastal mountains and foothills of southern California, being sympatric in the Los Angeles area, with *P. parryi* extending south into Baja California. The two species are closely related and produce fertile hybrids in nature when populations overlap [6].

RESULTS AND DISCUSSION

The surfaces of whole plants of both *P. minor* and *P. parryi* were washed with Me_2CO to extract primarily the glandular trichome exudate. The TLC of the washings were the same for both species, and were identical to an extract of several trichomes removed from a plant with forceps. The principal constituents were separated by liquid chromatography and were tested for potential to elicit dermatitis on guinea pigs sensitized to the crude extract.

A potent contact allergen was isolated, and was identified as geranylgeranylhydroquinone (**1**). The MS gives a molecular ion 382 with formula $C_{26}H_{38}O_2$, and a dihydroxybenzylium ion ($C_7H_7O_2^+$) at 123 for the base peak. The hydroquinone is apparent from the UV absorption maximum at 294 nm which was not changed by addition of $AlCl_3$, and also from its oxidation to a



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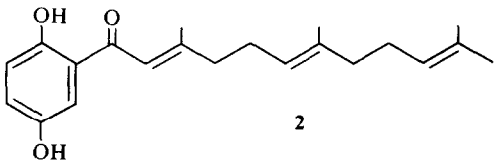

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stable quinone with Ag_2O. The ^1H NMR spectrum is characteristic of a polyprenyl chain of four isoprene units. The 9-proton signal at δ 1.60 and the 3-proton signal at δ 1.68 indicate *trans* configuration at C-6,7 and C-10,11 according to a study of the stereochemistry of trisubstituted double bonds by Bates and Gale [7]. Configuration at C-2,3 of the prenyl chain was determined by correlation with experiments by Inouye *et al.* [8]. Formation of the diacetate caused an upfield shift of the C-3 methyl signal from δ 1.73 to δ 1.68 which is indicative of *trans* configuration at this position.

Present in both species in lesser amount is 1-oxofarnesylhydroquinone (**2**), also a contact allergen. The molecular formula $C_{21}H_{28}O_3$ and structure is obtained from the MS, ^{13}C NMR, and ^1H NMR spectra. The hydroquinone conjugated to a carbonyl is evident from the ^1H NMR shift of the intramolecularly bound hydroxyl proton at δ 12.38 ppm, the ABC splitting of the aryl protons, and the absence of benzylic protons. *Trans* configuration of the C-2,3 double bond of the farnesyl group is indicated by the coupling (J_{AX} = 1.5 Hz) between the C-2 vinyl proton at δ 6.66 and C-3 methyl at δ 2.19 ppm. *Trans* configuration at the C-6,7 double bond is indicated by the methyl signal at δ 1.63 ppm being upfield of that expected for a methyl on a *cis* double bond [7].

Groups of guinea pigs were sensitized to either pure geranylgeranylhydroquinone or to 1-oxofarnesylhydroquinone. Samples in Me_2CO solution were applied to

shaved backs of both sensitized and non-sensitized animals (5 μl in an 8-mm-diameter circle) and left unoccluded. An application of 0.01 μmoles geranylgeranylhydroquinone was required to produce a visible erythema on a half or more of the animals sensitized to either geranylgeranylhydroquinone or to 1-oxofarnesylhydroquinone. An application of 0.02 μmoles 1-oxofarnesylhydroquinone produced reactions in at least half of sensitized animals. Irritant (non-allergic) reactions were produced on the non-sensitized animals by 0.08 μmoles geranylgeranylhydroquinone and by 1.5 μmoles 1-oxofarnesylhydroquinone.

There is evidence that the mechanism by which certain phenolic contact allergens become active antigens is that they are first oxidized within the skin to a quinone [9]. The quinone is susceptible to nucleophilic substitution on the ring by either sulfhydryl or amino groups of cellular protein. The fact that geranylgeranylhydroquinone and 1-oxofarnesylhydroquinone are highly cross-reacting allergens implies that the site and mechanism of their activity *in vivo* are the same, even though 1-oxofarnesylhydroquinone is not readily oxidized to the quinone as is geranylgeranylhydroquinone. It may be that the enol form of 1-oxofarnesylhydroquinone which results from transfer of the phenolic hydroxyl proton to the carbonyl can undergo the same nucleophilic substitution as can the quinone, and thus is able to produce an antigenic site sufficiently similar for cross-reactivity.

EXPERIMENTAL

Phacelia parryi Torr. was collected April, 1980 near Colonet, Baja California, Mexico. *P. minor* (Harv.) Thell. was collected June, 1980 in Silverado Canyon, Orange Co., California. Voucher specimens No. 17913 (*P. parryi*) and No. 17914 (*P. minor*) are at the Museum of Systematic Biology, University of California, Irvine. Whole fresh plants (*P. parryi*—3.6 kg; *P. minor*—2.1 kg) were washed superficially three times for 5–10 sec with Me_2CO. The two principal phenolic constituents were isolated by chromatography over Si gel eluted with C_6H_6–Me_2CO (9:1) and over Sephadex LH-20 eluted with Me_2CO.

Geranylgeranylhydroquinone (**1**). A colorless oil was obtained (2.4 g from *P. parryi*; 1.8 g from *P. minor*). UV λ_{max}^{MeOH} nm: 294. IR ν_{max}^{neat} cm^{-1}: 3400 (OH), 1605, 1500, 1450 (C=C), 1185 (C–O). ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.60 (9 H, *s*), 1.68 (3 H, *s*), 1.73 (3 H, *s*), 2.05 (12 H, *m*), 3.27 (2 H, *d*, J = 7 Hz), *ca.* 4.9 (2 H, broad, –OH), 5.08 (3 H, *m*), 5.24 (1 H, *t*, J = 7 Hz), 6.63 (3 H, *m*). MS (chemical ionization with isobutane, 100 eV) *m/z* (rel. int.): 383 ($M + 1^+$, 17), 205 ($C_{15}H_{25}^+$, 21), 163 ($C_{10}H_{11}O_2^+$, 30), 137 ($C_{10}H_{17}^+$, 50), 123 ($C_7H_7O_2^+$, 100).

1-Oxofarnesylhydroquinone (**2**). A yellow oil was isolated (0.2 g from *P. parryi*; 0.3 g from *P. minor*). UV λ_{max}^{MeOH} nm: 378, 274. ν_{max}^{neat} cm^{-1}: 3390 (OH), 1640 (C=O), 1590, 1490, 1485, 1435 IR(C=C), 1290, 1205 (C–O). ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.60 (3 H, *s*), 1.63 (3 H, *s*), 1.68 (3 H, *s*), 2.06 (4 H, *m*), 2.19 (3 H, *d*, J = 1.5 Hz), 2.24–2.29 (4 H, *m*), *ca.* 5.1 (1 H, broad, –OH), 5.1 (2 H, *m*), 6.66 (1 H, *d*, J = 1.5 Hz), 6.84 (1 H, *d*, J = 9 Hz), 6.98 (1 H, *dd*, J = 9 Hz), 7.21 (1 H, *d*, J = 3 Hz), 12.38 (1 H, *s*, –OH). The ^{13}C NMR spectrum had 21 carbon shifts with C=O at δ 196.2. MS (chemical ionization with isobutane, 100 eV) *m/z* (rel. int.): 329 ($M + 1^+$, 77), 177 ($M^+ - C_{11}H_{19}$, 100), 137 ($M^+ - C_{14}H_{23}$, 89).

Guinea pig assay for allergenicity. Hartley strain female guinea pigs 4–6 months old were sensitized by intradermal injection of the crude plant extract or pure compound mixed in Freund's Complete Adjuvant (FCA), prepared as described in [10]. For each procedure a mixture of 5% suspect allergen in FCA was made. Each of 8 animals received a total of three 0.1 ml injections intradermally in the interscapular area; one injection was given every other day for a total of 15 mg of the suspected allergen. A control group of 7 animals received similar injections of FCA without the allergen. Two weeks after the last injection the animals were depilated and challenged with serial dilutions of the suspected allergens in Me_2CO soln. A 5 μl drop was applied to the back in an 8-mm-diameter circle. The animals were checked at 1, 2 and 3 days for skin reactions.

Acknowledgement—This investigation was supported by Grant No. AI 14646 awarded by the National Institute of Health.

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