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HYDROCARBON COMPONENTS OF THE TRAIL PHEROMONE OF THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*

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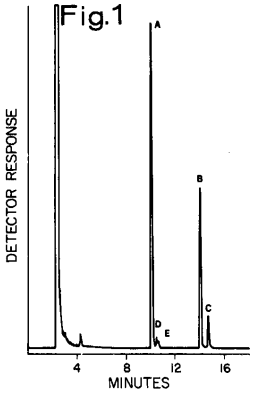
Abstract: Terpenoid trail pheromone components were isolated from whole worker extracts of the red imported fire ant, and identified as *Z,E* and *E,E*-α-farnesene, and the previously unreported homofarnesenes *Z,Z* and *Z,E*-3,4,7,11-tetramethyl-1,3,6,10-dodecatetraene.

The trail pheromone of the red imported fire ant, *Solenopsis invicta* Buren, was shown in 1959 to be produced in the Dufour's gland and dispersed through the sting apparatus.[1] In 1965, partial chemical purification of the trail pheromone was reported[2], and much later the gas chromatographic analyses of the Dufour's gland components of the 4 major fire ant species (*S. invicta*, *S. richteri* Forel, *S. geminata* (F), and *S. xyloni* McCook) were published along with a comparison of species specificity.[3,4] We report here the isolation and identification of 4 hydrocarbon components of the trail pheromone from *S. invicta*.

Chemical isolation of the trail pheromone complex was monitored at every stage by a previously described trail bioassay.[4] Earlier work[3] and results of our own preliminary studies indicated that the trail pheromone components were unsaturated hydrocarbons. Since the extirpation of Dufour's glands in the quantities required was not practical, we devised the following procedure to maximize the yield of unsaturated hydrocarbons from whole-worker extracts. *Solenopsis invicta* workers (939 g) collected from field colonies in the Gainesville area were rinsed several times with hexane to remove cuticular hydrocarbons. The ants were then homogenized in hexane with a Virtis "23"® tissue homogenizer operated at a setting that disrupted the abdomen but generally left the hydrocarbon-rich heads intact. We isolated the hydrocarbons in the hexane extract from lipids by passing the concentrated extract through a gravity silicic acid column (Bio Sil HA, 325 mesh), eluting with hexane. Unsaturated hydrocarbons were separated from the vast majority of saturated and branched hydrocarbons[5,6] by means of low pressure liquid chromatography (Whatman, Silica Gel 80A, 10-20μ), eluting with hexane. Fractions were monitored by gas chromatography and bioassay.[4] Careful preparative gas chromatography of the active fraction (3% OV-17 on 120/140 Gas Chrom Q, 1.8 m x 2 mm ID, 90°, 10:1 splitter, Varian 3700, FID) coupled with bioassays showed components A, B, C, and D+E (Fig. 1, WCOT,

OV-101, 30m capillary column, 120°) to be active. Yields based on GLC integration were 700, 400, 100 and 30µg, respectively.



Component A appeared as a single compound on two packed analytical GLC columns (OV-17, KI 1575 and Superpak 20M KI 1559) and a 30m OV-101 WCOT capillary column. Its electron impact (EI) and chemical ionization (CI) mass spectra (Finnigan Model 1015 SL upgraded to a 3200 GC-MS system) indicated it to be a hydrocarbon with a molecular weight of 204, corresponding to the molecular formula $C_{15}H_{24}$, a hydrocarbon with four sites of unsaturation. Hydrogenation of this compound (10% Pd/C, in hexane at ca. 26°) gave a product with KI 1370 and a molecular formula of $C_{15}H_{32}$ (CI-GC/MS). The shift to lower retention time and its EI mass spectrum was consistent with that of a branched saturated paraffin structure. Therefore, component A was tentatively characterized as a sesquiterpene hydrocarbon with four double bonds. The EI mass spectrum of the pheromone (Table 1) was similar to those reported for α-farnesenes[7], and component A's ultraviolet absorption maximum at 237.5 nm (hexane), indicative of two conjugated double bonds, was identical to that reported for *Z,Z* and *Z,E*-α-farnesene.[7]

Table 1. Mass spectral data for components of the *S. invicta* trail pheromone.

Component A - m/e (%): 204(0.5), 189(0.7), 161(3.0), 135(4.0), 81(18.3), 79(43.1), 77(33.3), 69(40.7), 67(13.2), 55(30.3), 53(16.3), 41(45.7), 39(11.9).

Component B - 218(0.8), 203(0.8), 175(2.6), 149(5.0), 147(1.7), 133(62.4), 121(20.4), 119(17.7), 107(100.0), 105(20.5), 93(28.6), 91(36.9), 79(23.8), 77(19.4), 69(28.6), 67(11.0), 55(18.3), 53(11.7), 41(45.8), 39(14.4).

Component C - 218(1.5), 203(1.3), 175(2.1), 161(1.6), 149(4.3), 147(2.9), 133(28.8), 123(9.9), 121(22.8), 119(13.6), 107(100.0), 95(12.5), 94(31.5), 93(44.7), 91(33.0), 81(9.7), 79(25.3), 77(19.2), 69(44.9), 67(12.8), 55(24.8), 53(12.8), 41(52.1), 39(12.2).

Table 2. Proton Magnetic Resonance Data (δ, 400 MHz, C_6D_6).

Component A‡: 1.529(3H,s), 1.556(3H,s), 1.658(3H,s), 1.778(3H,s), 2.037(2H,t*,J=7.28Hz), 2.124 (2H,t*,J=6.82), 2.876(2H,t*,J=7.39), 5.060(1H,d,J=10.97), 5.204(3H,m), 5.380(1H,t*,J=7.11), 6.870(1H,d of d,J=17.07,10.97).

Component B: 1.516(3H,s), 1.595(3H,s), 1.651(3H,s), 1.694(3H,s), 1.749(3H,s), 2.054(2H,m), 2.115(2H,m), 2.922(2H,d,J=7.36), 5.048(1H,d,J=11.72), 5.203(3H,m), 7.002(1H,d of d,J=18.18,11.72).

Component C: 1.524(3H,s), 1.586(3H,s), 1.652(3H,s), 1.740(3H,s), 1.809(3H,s), 2.050(2H,m), 2.124(2H,m), 2.822(2H,d,J=6.67), 5.037(1H,d,J=11.23), 5.177(3H,m), 6.913(1H,d of d,J=17.89, 11.23).

Z,Z-α-farnesene: 1.524(3H,s), 1.652(6H,s), 1.809(3H,s), 2.124(4H,s), 2.813(2H,t*,J=7.28), 5.037(1H,d,J=10.98), 5.208(3H,m), 5.379(1H,t*,J=7.13), 6.913(1H,d of d,J=17.01, 10.98).

E,Z-α-farnesene: 1.552(3H,s), 1.672(3H,s), 1.680(3H,s), 1.706(3H,s), 2.098(4H,s), 2.853 (2H,t*,J=7.30), 4.954(1H,d,J=11.08), 5.108(1H,d,J=17.68), 5.204(2H,m), 5.511(1H,t*,J=7.07), 6.457(1H,d of d, J=17.45,11.08).

E,E-α-farnesene: 1.536(3H,s), 1.555(3H,s), 1.662(3H,s), 1.689(3H,s), 2.056(2H,m), 2.138 (2H,m), 2.807(2H,t*,J=7.23), 4.946(1H,d,J=10.90), 5.101(1H,d,J=17.81), 5.217(2H,m), 5.505(1H,t*,J=8.38), 6.443(1H,d of d,J=17.61,10.90).

‡Identical to synthetic *Z,E*-α-farnesene. *Broad.

Further evidence supporting this deduction came from the PMR spectra (Bruker WH-400)[8] of component A (Table 2), which included absorptions corresponding to 4 unsplit methyl groups, 2 divinyl methylene protons, and a downfield doublet of doublets that in the α-farnesenes is characteristic of the C_2 proton when the 3-double bond is in the *Z* configuration[7].

We accomplished the synthesis of the 2 β-farnesenes and 4 α-farnesenes by separately dehydrating *Z* nerolidol (Pfaltz & Bauer) and *E* nerolidol (from the preparative GLC separation of a *Z* and *E* nerolidol mixture, Aldrich, 3% OV-17, 1.8 m x 4 mm, TCD) using $POCl_3$/ pyridine[7] or refluxing DMSO for 5 minutes (Fig. 2). In each case the reaction mixture was added to water, extracted with hexane, and the hydrocarbons isolated by Fluorisil chromatography, eluting with hexane. The yield of hydrocarbons (35%) and product ratios (Fig. 2) were essentially the same with either method. Structural assignments were based on GLC retention times, MS data, and UV spectra. *Z,E*-α-farnesene and component A had identical retention times and co-eluted on OV-17 and Superpak 20M columns. Their mass spectra, PMR, UV, and IR spectra (Nicolet 7199 Fourier Transform Interferometer with a 4X beam condenser and a mercury-cadmium telluride-liquid N_2 cooled detector) were identical confirming the structure of component A as *Z,E*-α-farnesene.

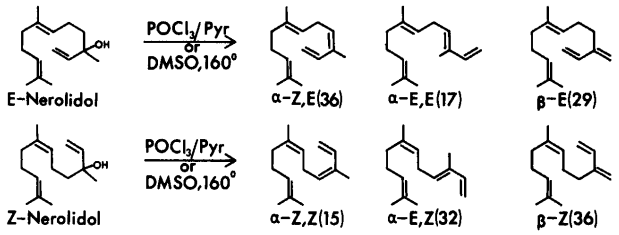


Fig. 2 Synthetic scheme for α- and β-farnesenes (%)

The mass spectra of B and C were similar (Table 1), and both had molecular weights of 218 ($C_{16}H_{26}$, four degrees of unsaturation). On hydrogenation (10% Pd/C in hexane) they yielded the same fully saturated hydrocarbon (a shift from KI 1664 and 1671 to 1438, OV-17) indicative of geometric isomers with 4 double bonds. The PMR spectra of B and C (Table 2) were very similar to the α-farnesenes except for three important features: 1) components B and C had 5 unsplit methyl groups as opposed to 4 in the α-farnesenes, 2) the absorption due to the C_5 methylene changed from a triplet in the α-farnesenes to a doublet in components B and C, indicative of a methyl substitution at C_4 or C_6, 3) the single proton triplet in the α-farnesene olefinic proton region (Table 2) is missing in pheromone components B and C. This absorption was assigned to the C_4 conjugated diene proton based on predicted chemical shifts, literature analogies and the increased λ max of B (243.8 nm) and C (242.7 nm) compared to α-farnesenes (*E,E* and *E,Z*=233, *Z,Z* and *Z,E*=237.5 nm). An increase of 5 nm is expected when an alkyl group is added to a conjugated diene system. Thus the extra methyl group has been established on the 4 carbon. The UV data also suggests that B and C have the double bond configuration of *Z,Z* and *Z,E*-α-farnesene, since the 5 nm λ max increase is only compatible with these isomers. This is substantiated by the chemical shift of the C_2 proton, which in the α-farnesenes is diagnostic of the configuration about the C_3 double bond.[7] When the α-farnesene C_3 double bond is *Z* the PMR absorption of the C_2 proton is shifted downfield relative to its position when the C_3 double bond is in the *E* con-

figuration (6.4 to 6.9 δ). The chemical shifts of the doublet of doublets due to the C_2 proton in components B and C are both consistent with the C_3 double bond in the Z configuration (B= 7.002, C=6.913 δ). On the basis of these data B and C are either *Z,Z* or *Z,E*-3,4,7,11-tetra= methyl-1,3,6,10-dodecatetraene (α-farnesenes with a methyl group in the 4 position) and represent previously unreported natural products. Absolute identification awaits the synthesis of the two homosesquiterpenes.

Component E was identified as *E,E*-α-farnesene. Synthetic *E,E*-α-farnesene coeluted with E on 2 capillary columns (30m WCOT, OV-101 and Silar 10C). The structural assignment is supported by bioassay results which show that the ants are highly sensitive to the *E,E*-isomer (ca 10^{-12} g/cm). Component D has not been identified and does not correspond to a farnesene isomer.

α-Farnesenes have been reported in the Dufour's glands of 3 formicine[9] and 1 myrmecine[10] ant species, but the configuration about the double bonds was not determined and no behavioral function was attributed to them. The Dufour's glands of *Myrmica scrabrinodis*[11] and *M. rubra*[12] contain *Z,E*-α-farnesene and a homofarnesene identified by MS as 7-ethyl-3,11-dimethyl-1,3,6,10-dodecatetraene, but they have no known function. Of the 6 synthesized farnesenes, only the *Z,E* and *E,E* isomers show a positive trail bioassay at a pheromone level. Component A, synthetic or natural, shows significant trail-following activity at 10^{-13}g/cm, which is comparable to that of other reported trail pheromones.[13,14] Additional chemical and biological considerations will appear in future publications.

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