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SEMITERPENOID ESTERS FROM THE VENOM OF THE EUROPEAN HORNET, Vespa crabro (HYMENOPTERA: VESPIDAE)

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Abstract: A series of semiterpenoid esters unique in insects have been identified in the venom of the European hornet.

The non-volatile components of bee and wasp venoms have been analyzed extensively.[1] Only in *Paravespula vulgaris*,[2] a European yellowjacket, have volatiles been identified in venom gland extracts. Here we report that the venom gland of another vespid wasp, *Vespa crabro*,[3] contains trace amounts of a complex mixture of volatile semiterpenoid esters and alcohols.

Vespa crabro is the largest eusocial wasp in the United States. When disturbed, this normally docile wasp becomes aggressive, spraying intruders with a sweet-smelling fluid released from the abdomen, and then usually stings. Crushed venom glands have a similar sweet odor.

Gas chromatographic-mass spectroscopic analysis of methylene chloride extracts of excised venom glands showed the presence of more than a dozen components[4] (Figure 1). Isoamyl acetate (1) (Table 1) eluted first on a polar column followed by a related unsaturated acetate, 3-methyl-3-buten-1-yl acetate (2). The next two compounds were the corresponding alcohols, isoamyl alcohol (3) and 3-methyl-3-buten-1-ol (4). Then eluted a series of saturated and unsaturated esters containing ten carbons. 3-Methyl-1-butyl 3-methylbutanoate (isoamyl isovalerate) (5), the major component of the mixture was followed by the unsaturated esters, 3-methyl-3-buten-1-yl 3-methylbutanoate (6), 3-methyl-2-buten-1-yl 3-methylbutanoate (7), 3-methyl-1-butyl 3-methyl-2-butenoate (8), 3-methyl-3-buten-1-yl 3-methyl-2-butenoate (9) and 3-methyl-2-buten-1-yl 3-methyl-2-butenoate (10). Finally three esters derived from citronellol eluted: citronellyl acetate (11) and two coeluting esters citronellyl 3-methylbutanoate (12a) and 3-methyl-1-butyl citronellate (12b). Only three of these esters exhibited molecular ions, but the mass spectra were definitive (Table 1) providing enough information to place double bonds at specific positions in the molecule. Synthetic samples matched the natural materials' retention times and mass spectra while isomers with double bonds in other positions gave different mass spectra.

Ions corresponding to the acylium ion (RCO+), hydrated acylium (RCO_2H_2+), allylic cleavage (or lack of), and multiple McLafferty rearrangements were extremely useful for structural elucidation of the esters. Combined extracts of 100 glands were necessary for definitive spectra.

We have demonstrated previously that the European hornet secretes a mixture of citronellyl citronellate and citronellyl geranate from its seventh sternal gland.[5] These two C_{20} esters from the seventh gland are directly related to the citronellyl esters and similar to the C_{10} semiterpenoid esters found in the venom. The semiterpenoid esters are related to esters of C_5 alkenols and fatty acids which we have identified in the Dufour's gland secretions of halictid bees.[6,7]

A semiterpenoid alcohol (2-methyl-3-buten-2-ol) plays an important role in bark beetle aggregation responses.[8] The role(s) of volatile esters in the alarm response of these aggressive vespid wasps is actively being pursued in our laboratories.

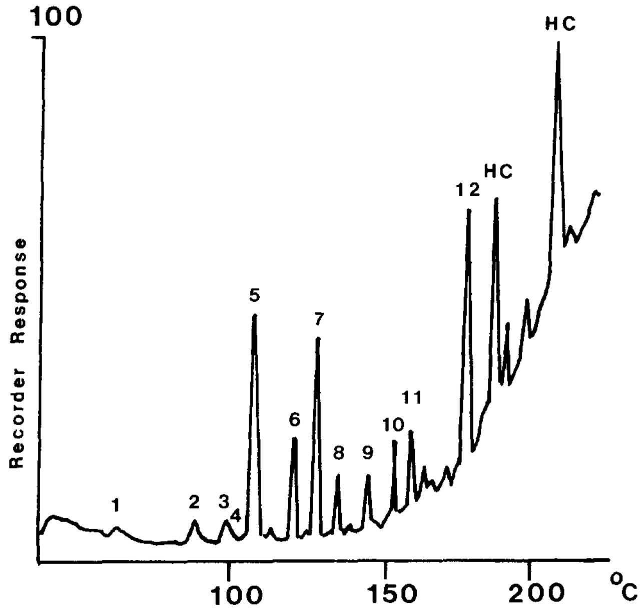

Figure 1. Gas chromatogram of a methylene chloride extract of <u>Vespa crabro</u> venom glands. Numbered peaks are identified in the discussion. HC = hydrocarbon (C_{19}ene and C_{21}ene).

TABLE 1. Mass Spectra of *Vespa crabro* Venom Components

Peak	Compound (M.W.)	Structure	Mass Spectrum
1.	3-Methyl-1-butyl acetate (130)	$(CH_3)_2CHCH_2CH_2OCOCH_3$	41(26),43(100),55(24),61(6),70(20),73(4),87(3)
2.	3-Methyl-3-buten-1-yl acetate (128)	$CH_2=C(CH_3)CH_2CH_2OCOCH_3$	41(12),43(100),53(9),67(29),68(56),73(3)
3.	3-Methyl-1-butanol (88)	$(CH_3)_2CHCH_2CH_2OH$	41(95),42(77),43(65),55(100),70(54)
4.	3-Methyl-3-buten-1-ol (86)	$CH_2=C(CH_3)CH_2CH_2OH$	41(100),53(2),55(24),56(59),67(42),68(53),71(8), 86(13)M+
5.	3-Methyl-1-butyl 3-methylbutanoate (172)	$(CH_3)_2CHCH_2CO_2CH_2CH_2CH(CH_3)_2$	41(74),43(83),55(47),57(60),60(7),70(100),85(71), 103(14),115(3),129(2),130(1)
6.	3-Methyl-3-buten-1-yl 3-methylbutanoate (170)	$(CH_3)_2CHCH_2CO_2CH_2CH_2C(CH_3)=CH_2$	41(78),53(11),57(66),67(33),68(100),85(37), 103(2),110(1),128(0.5)
7.	3-Methyl-2-buten-1-yl 3-methylbutanoate (170)	$(CH_3)_2CHCH_2CO_2CH_2CH=C(CH_3)_2$	41(100),43(26),53(29),57(59),60(37),67(52), 68(58),69(60),85(43),103(1),128(0.5),170(0.8)M+
8.	3-Methyl-1-butyl 3-methyl-2-butenoate (170)	$(CH_3)_2C=CHCO_2CH_2CH_2CH(CH_3)_2$	41(32),43(54),53(11),55(56),70(22),71(18), 83(100),100(38),101(18),126(0.4),170(2)M+
9.	3-Methyl-3-buten-1-yl 3-methyl-2-butenoate (168)	$(CH_3)_2C=CHCO_2CH_2CH_2C(CH_3)=CH_2$	41(37),53(22),55(34),67(20),68(66),83(100), 101(5),113(0.7)
10.	3-Methyl-2-buten-1-yl 3-methyl-2-butenoate (168)	$(CH_3)_2C=CHCO_2CH_2CH=C(CH_3)_2$	41(64),53(42),55(47),67(48),68(50),69(53), 83(100),100(17),101(5),109(1),123(3),153(1), 168(1)M+
11.	Citronellyl acetate (198)	$(CH_3)_2C=CHCH_2CH_2CH(CH_3)CH_2CH_2OCOCH_3$	41(87),43(100),55(43),57(38),67(43),69(59), 81(42),95(29),109(15),123(24),138(13)
12a.	Citronellyl 3-methyl-butanoate (238)	$(CH_3)_2C=CHCH_2CH_2CH(CH_3)CH_2CH_2OCOCH_2CH(CH_3)_2$	41(100),43(23),55(41),57(48),67(37),69(59), 81(62),85(23),59(49),109(15),123(31),138(15)
12b.	3-Methyl-1-butyl citronellate (238)	$(CH_3)_2C=CHCH_2CH_2CH(CH_3)CH_2CO_2CH_2CH_2CH(CH_3)_2$	41(100),55(52),69(74),81(19),95(31),110(22), 123(7),138(2),152(9),153(1)

3-Methyl-1-butyl acetate (isoamyl acetate)[9] and 3-methyl-1-butanol[10] have been identified previously in the sting apparatus of honeybees. Citronellyl acetate is the major component of bumblebee labial gland secretions.[11] Isoamyl isovalerate is a component of the peel of ripe bananas,[12] plums,[13] and fermented cider[14] as well as other oils and spices. However, this ester and the other C_{10} esters identified here are new insect natural products.[15]

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3. Specimens were collected by net in the Washington, D.C. area, placed individually in glass shell vials, and kept on ice. Venom glands were excised under water and extracted with methylene chloride.
4. A Finnigan 3200E combined gas chromatograph-mass spectrometer (EI) with a 1.8 meter x 1 mm 10% SP-1000 column was used. Analysis of four separate extracts spanning three years indicated that all components were consistently present. The esters were synthesized from the corresponding acid chlorides and alcohols. Proton nmr spectra indicated that no isomerization had taken place during synthesis.
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