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CIS-2-METHYL-5-HYDROXYHEXANOIC ACID LACTONE IN THE MANDIBULAR GLAND SECRETION OF A CARPENTER BEE

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Males of the carpenter bee *Xylocopa hirutissima* establish territories on mountain tops where they hover above projecting trees or shrubs.[1] Females are attracted to these territorial sites by compounds in the mandibular gland secretions of the males which function as sex pheromones.[2] We have analyzed the mandibular gland secretion of *X. hirutissima* and identified *cis*-2-methyl-5-hydroxyhexanoic acid lactone as the major constituent of this pheromonal blend.

Methylene chloride extracts of excised mandibular glands were examined by combined gas chromatography-mass spectroscopy.[3] Minor amounts (Table I) of each of the following compounds were identified by comparison of their mass spectra with those in a computer file of mass spectra[4] as well as comparison with authentic samples: benzaldehyde, *p*-cresol, benzoic acid, pentadecane, hexadecane, vanillin, heptadecane, and octadecane.

Table I

Compound	Elution Temperature	%	mw
Benzaldehyde	80	21.8	106
p-cresol	95	4.2	108
128 Lactone	100	62.5	128
Benzoic Acid	130	0.5	122
Pentadecane	130	0.5	212
Bis homolog of 128	140	1.3	156
Hexadecane	145	1.1	226
Vanillin	150	0.9	152
Heptadecane	150	5.7	240
Octadecane	155	1.5	254

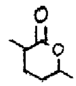
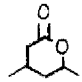
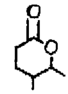
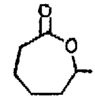
The major volatile component, eluting between p-cresol and benzoic acid on a 1% OV-17 column exhibited a molecular ion m/e 128 with large peaks at m/e 113, 99, 84, 69, 67, 56 (base peak), 55, 43, 42 and 41. Loss of 44 mass units (m/e 84) followed by loss of 28 (m/e 56) was confirmed by the presence of a metastable ion. This and the large peaks at m/e 56 and m/e 42 suggested a lactone ring with a methyl substitutent on the $\delta(\epsilon)$-carbon (loss of CH_3CHO).[5]

Synthesis of the lactone of 2-methyl-5-hydroxyhexanoic acid from 2,5-dimethylcyclopentanone by Baeyer-Villiger oxidation gave a δ-lactone whose mass spectrum and retention time were identical to the bee volatile. The mass spectra and retention times of the isomeric δ-lactones of 6-hydroxyheptanoic acid and 3 (or 4) methyl-5-hydroxyhexanoic acids were significantly different from the volatile compound present in the bee mandibular glands (see Table II).

The cis and trans isomers of the synthetic 2-methyl-5-hydroxyhexanoic acid lactone were separated by preparative gas chromatography and examined by proton and ^{13}C magnetic resonance.[6] The trans lactone exhibited a doublet at δ 1.31(J=7)(CH_3-CH-CO) and a second doublet at δ 1.38 (J=6.3)(CH_3-CH-O) in its pmr spectrum consistent with its diequatorial conformer. Upon cooling to -50° these doublets remained. The cis isomer exhibited a doublet at δ 1.22(J=6.5)(CH_3-CH-CO) and a second doublet at δ 1.35(J=6.10)(CH_3-CH-O) which broadened and resolved into two additional doublets upon cooling to -50°. The cis isomer which eluted second on a 10% SP-1000 column was identical to the bee volatile upon coinjection. ^{13}C spectra of the two isomers showed differences but no assignment could be made due to lack of adequate model compounds.

TABLE II

MASS SPECTRA OF 128 LACTONES

							
m/e	trans	cis	(1)c/t(2)		(1)c/t(2)		
128	3	3	6	5	4	4	2
113	5	4	9	12	2	4	3
85	11	9	10	10	6	7	5
84	32	31	36	35	32	37	54
69	25	20	39	37	8	8	9
67	8	7	4	4	3	3	9
57	13	11	11	12	7	8	5
56	100	100	100	100	100	100	68
55	49	41	28	24	17	18	100
43	46	41	46	46	31	24	23
42	98	84	90	88	38	43	27
41	52	47	51	51	33	33	52

A small amount of what appears to be a bis homolog of the 128 lactone (m/e 156) is also present but has not been identified.

In carpenter ants (Camponotus species) a lactone produced in the mandibular glands of males is believed to function as a sexual excitant for females during the initiation of the mating flight.[7] Macrocyclic lactones have been identified as Dufour's constituents of bees in the genera Halictus and Colletes but in these cases the compounds do not appear to function as sex pheromones.[8] In the Oriental hornet Vespa orientalis δ-hexadecanolactone produced in the mandibular glands of the queen plays the role of a queen substance[9] whereas in a variety of other insects γ and δ lactones are utilized as defensive compounds.[10]

The role of the compounds identified in the mandibular gland secretion of X. hirutissima is now being investigated. It may be significant that we could not detect any compounds other than typical lipids in the mandibular gland secretion of males of Xylocopa virginica, a North American species. However, unlike *X. hirutissima* males, those of *X. virginica* do not establish territorial sites with sex pheromones but rather, copulate with females which they overtake while cruising in areas which they patrol.

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3. A combined gas chromatograph-mass spectrometer (LKB-9000) was used with two columns: A 1% OV-17 temperature programmed from 70° and B, 10% SP-1000 temperature programmed from 40° at 8% min. both on Supelcoport 80-100 mesh (Supelco, Bellefont, Pa.). We thank Dr. H.M. Fales of NHLI, Bethesda, Md. for access to his instrument.
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