Structures of Homofarnesene and Bishomofarnesene Isomers from *Myrmica* Ants

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The homofarnesene and bishomofarnesene isomers from Dufour glands of *Myrmica* ants have been identified by degradation as 7-ethyl-3,11-dimethyldodeca-1,3,6,10-tetraene (2), and 7-ethyl-3,11-dimethyltrideca-1,3,6,10-tetraene (3).

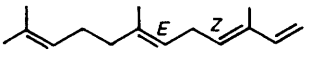
In an investigation of the contents of the Dufour gland of the ant *Myrmica rubra* L. Morgan and Wadhams [1] found small quantities (*i.e.* nanograms per ant) of farnesene and two homologues, identified by their mass spectra and named homofarnesene and bishomofarnesene. From comparison of its mass spectrum and g.l.c. retention times with those of a mixture of isomers prepared from nerolidol by dehydration, and with published data,[2] the farnesene isomer from ants was identified as (*Z*,*E*)-α-farnesene, *i.e.* (*Z*,*E*)-7,3,11-trimethyldodeca-1,3,6,10-tetraene (1), and homofarnesene and bishomofarnesene were given the structures (2) and (3), respectively, on the basis of their mass spectra.[1] Later Morgan and Parry found that in the Dufour gland of *Myrmica scabrinodis* Nyl. the principal constituents [3] were the same (*Z*,*E*)-α-farnesene, homofarnesene, and bishomofarnesene as found earlier, together with a further homologue, trishomofarnesene (4). The amounts available (*ca.* 0.1 μg per ant) precluded normal spectroscopic examination.

Homofarnesene has also been identified in other *Myrmica* species [4] and in a tropical species, *Acromyrmex octospinosus*.[5]

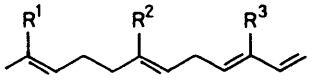
At about the same time, and subsequently, a series of sesquiterpenoid compounds of similar structure, the juvenile hormones [JH 0—III, structures (5)—(8), respectively], have been isolated from a number of insect species; for example JH 0 (5) was isolated recently from the eggs of *Manduca sexta*.[6] A bishomofarnesene compound faranal (9) has been shown to be a trail pheromone in the ant *Monomorium pharaonis*,[7] and recently a different homofarnesene isomer has been reported to occur in the ant *Solenopsis invicta*.[8] Because the position of the extra methylene units in *Myrmica* homofarnesenes is different from that in these other terpenoids, it was considered important to provide unequivocal proof of structure to support the earlier assignment from mass spectra.

Micro-ozonolysis of ant farnesene (1) gave the expected 4-oxopentanal (10), identified by g.l.c. retention time and its mass spectrum, proving the presence of a methyl group at C-7. The same oxo-aldehyde was obtained from ozonolysis of a mixture of synthetic (*Z*,*E*)- and (*Z*,*Z*)-α-farnesene or from (*Z*)- or (*E*)-nerolidol, which have a similar skeleton. The mass spectrum of 4-oxopentanal has the base peak at *m*/*z* 43 ($MeCO^+$). Micro-ozonolysis of homo- and bishomo-farnesene gave a product with a higher retention time than 4-oxopentanal, as expected for one extra carbon atom. The compound was identified as 4-oxohexanal (11); *m*/*z* 57 ($EtCO^+$, base peak). Hence an ethyl group is present at C-7 in both homo- and bishomo-farnesene.

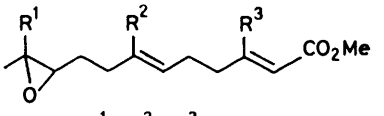
Propanone (12) was found among the ozonolysis products of farnesene (1), homofarnesene (2), and nerolidol arising from the similar structure at C-11. Bishomofarnesene (3) however gave butanone (13), indicating the presence of an ethyl group at C-11.



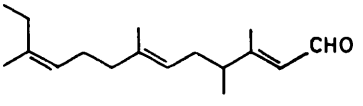
(1)



(2) $R^1 = R^3 = Me$, $R^2 = Et$
(3) $R^1 = R^2 = Et$, $R^3 = Me$
(4) $R^1 = R^2 = R^3 = Et$



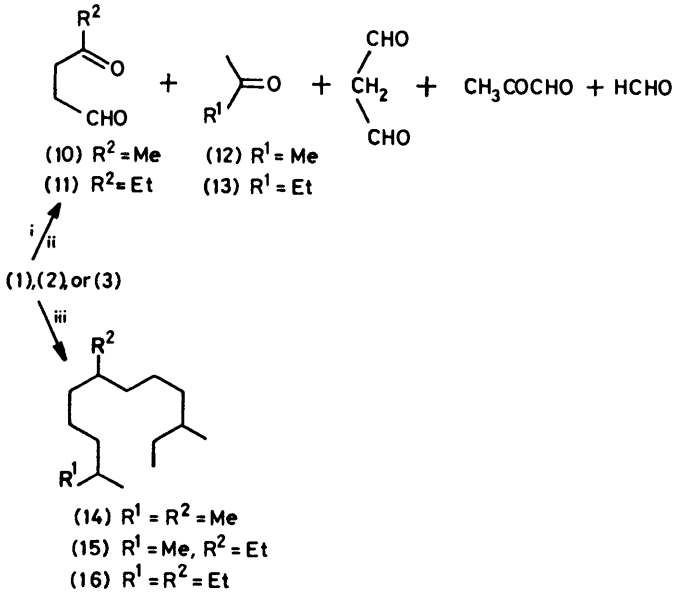
(5) $R^1 = R^2 = R^3 = Me$
(6) $R^1 = Et$, $R^2 = R^3 = Me$
(7) $R^1 = R^2 = Et$, $R^3 = Me$
(8) $R^1 = R^2 = R^3 = Et$



(9)

Propanedial was not seen in the gas chromatograms because of its poor stability and its very low response factor towards the flame-ionization detector, as observed elsewhere.[9] 2-Oxopropanal and methanal also give poor responses.

Micro-hydrogenation of the farnesenes provided further evidence for the location of ethyl groups in homo- and bishomo-farnesene. The $M^+ - 29$ peak is not very significant in the mass spectra of either farnesene (1), which has no ethyl group, or farnesane (14), which has only a terminal ethyl group. The $M^+ - 29$ peak is seen in the spectra of homofarnesene (2) and bishomofarnesene



SCHEME *Reagents*: i, O_3–CCl_4; ii, Ph_3P; iii, H_2–Pd, 140 °C

(3), and more significantly in those of homofarnesane (15) and bishomofarnesane (16), which indicates a similar type of non-terminal ethyl branching in (2) and (3). The peak at *m/z* 141 is stronger than that at *m/z* 127 in the case of bishomofarnesane (16) because the ions produced on α-cleavage at C-7 are of equal mass (*m/z* 141). However, the *m/z* 141 peak is weaker than *m/z* 127 in the spectrum of homofarnesane (15), and absent in that of farnesane (14). Homofarnesane (15) and bishomofarnesane (16) have not been reported previously.

On the basis of the above evidence, homofarnesene (2) was identified as 7-ethyl-3,11-dimethyldodeca-1,3,6,10-tetraene and bishomofarnesene (3) as 7-ethyl-3,11-dimethyltrideca-1,3,6,10-tetraene. By analogy with (1), the 3,4- and 5,6-double bonds in each of the higher homologues are expected to be *Z* and *E*, respectively. No information is available yet on the additional isomers possible at C-10 in (3).

EXPERIMENTAL

G.l.c.–mass spectrometry was carried out with a Pye 104 gas chromatograph linked through a glass jet separator to an A.E.I. MS12 mass spectrometer at 70 eV with helium as carrier gas at a flow rate of 20 ml min^{-1}. G.l.c. refers to analysis with a Pye 104 chromatograph with a flame ionization detector using one of the following columns: (A) 2.75 m × 4 mm packed column of 10% PEGA on Chromosorb W, 100—120 mesh; (B) 2.75 m × 4 mm packed column of 10% PEG 20M on Chromosorb W, 100—120 mesh; (C) 1.5 m × 4 mm packed column of Chromosorb 102, 100—120 mesh. The nitrogen carrier gas flow rate was maintained at 50 ml min^{-1}.

Rearing of Insects.—A colony of *Myrmica scabrinodis* was collected from Chesterton, Staffordshire, and maintained in the laboratory in artificial nests partly filled with moistened plaster and fed with 10% sugar solution and meal worm (*Tenebrio molitor*) larvae.

Isolation of the Volatile Chemicals.—The ants were killed by momentary immersion in liquid nitrogen. The gasters were separated and sealed in a small section of soft glass capillary tubing and introduced into column (A), at 140 °C, using a solid sampling technique.[10] The effluent was split, using an all-glass splitter (100 : 1), with outlet temperature maintained at 170 °C, and collected into U-shaped metal tubing of 1 mm i.d., cooled in a mixture of liquid nitrogen and ethyl acetate.

Separation of Farnesenes.—The total volatile materials from 5 gasters were analysed on column (B), at 160 °C and the three major constituents were characterised as (i) (*Z,E*)-α-farnesene (1), t_R 10.8 min; *m/z* 204 (4%, M^+), 161(5), 135(22), 119(66), 107(61), 105(30), 93(100), 91(53), 81(24), 79(47), 77(34), 69(68), 55(41), 43(37), and 41(95), identical with synthetic (*Z,E*)-α-farnesene; (ii) 7-*ethyl*-3,11-*dimethyldodeca*-1,3,6,10-*tetraene* (2), t_R 13.2 min; *m/z* 218 (11%, M^+), 189(7), 149(20), 133(22), 121(20), 119(20), 107(50), 105(41), 93(78), 79(41), 69(63), 55(59), 44(41), 43(37), and 41(100); and (iii) 7-*ethyl*-3,11-*dimethyltrideca*-1,3,6,10-*tetraene* (3), t_R 18.3 min; *m/z* 232 (8%, M^+), 203(11), 175(8), 149(21), 135(16), 133(25), 121(22), 119(21), 109(14), 107(54), 105(43), 95(30), 93(81), 91(37), 83(32), 81(30), 79(41), 77(22), 69(31), 67(24), 57(24), 55(100), 53(18), 44(32), 43(33), 41(75), 40(46), and 39(22).

Ozonolysis of the Farnesenes.—Farnesene (1) (4 μg), homofarnesene (2) (10 μg) and bishomofarnesene (3) (10 μg), were collected separately from 20 gasters and washed with CCl_4 (×3; 30 μl; preparative-g.l.c.-purified) into a glass micro test tube, cooled in ice. Ozone from a micro-ozonizer [9] was passed through rapidly for 3 min and the product was shaken with triphenylphosphine (0.5 mg). The products (11 μl) were analysed at 140 °C, on column (A) (attenuation ×200). Farnesene (1) gave 4-oxopentanal (10), identical with that obtained from the ozonolysis of a mixture of synthetic (*Z,E*)- and (*Z,Z*)-α-farnesene, or (*E*)- or (*Z*)-nerolidol: t_R 6.5 min; *m/z* 100 (3%, M^+), 99(22), 72(27), 57(9), 55(16), 43(100), and 32(89).

Separate ozonolysis of homofarnesene (2) and bishomofarnesene (3) produced 4-oxohexanal (11), t_R 8.5 min; *m/z* 114 (2%, M^+), 99(15), 86(35), 85(56), 57(100), 43(10), and 32(67).

On analysis of ozonolysis products on column (C), at 160 °C, farnesene (1) and homofarnesene (2) yielded propanone (12), identical with that obtained from ozonolysis of synthetic (*Z,E*)- and (*Z,Z*)-α-farnesene or (*E*)- or (*Z*)-nerolidol: t_R 4.3 min. Bishomofarnesene (3) under the same conditions gave butanone (13), t_R 9.3 min. Propanone and butanone were identified by co-chromatography with authentic materials and by mass spectrometry.

Hydrogenation of the Farnesenes.—1% Palladium catalyst [11] was prepared by evaporating an aqueous solution (150 ml) of palladium chloride (25 mg) and sodium hydroxide (11.2 mg) in contact with 100—120 mesh Chromosorb W (1.5 g) on a rotating evaporator, and dried overnight at 150 °C. It was packed between two glass wool plugs in a glass tube and hydrogen (40 ml min^{-1}) was passed for 60 min at 200 °C, for activation. The activated catalyst was made into a pre-column packing (6 cm) between two glass wool plugs on column (A). Total volatile material from 20 whole gasters was introduced, at 140 °C as described before, but using hydrogen as the carrier gas (40 ml min^{-1}). The total volatile product was collected as described before. The system was constantly checked for gas leaks and the hydrogen from the end of the U-tube was vented. The collected farnesanes were taken up in hexane (25 μl) and analysed on column (B)

at 160 °C. Complete hydrogenation was observed under the above conditions. The three major constituents, in order of increasing t_R value were (i) 2,6,10-*trimethyldodecane* (14), t_R 3.0 min; *m/z* 212 (6%, M^+), 127(13), 113(14), 97(12), 85(37), 71(92), 57(100), 43(57), and 41(24), identical with that obtained from synthetic farnesene; (ii) 6-*ethyl*-2,10-*dimethyldodecane* (15), t_R 3.85 min; *m/z* 226 (1%, M^+), 197(12), 155(3), 141(10), 127(14), 113(12), 111(16), 99(19), 85(64), 83(10), 71(88), 70(19), 69(20), 57(100), 56(17), 55(23), 43(58), and 41(30); and (iii) 7-*ethyl*-3,11-*dimethyltridecane* (16), t_R 5.7 min; *m/z* 240 (0.5%, M^+), 211(13), 141(17), 127(11), 113(13), 111(16), 99(23), 85(64), 71(77), 70(25), 69(14), 57(100), 55(21), 43(51), and 41(27).

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