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Trail Pheromone of the Ponerine Ant *Leptogenys peuqueti* (Hymenoptera: Formicidae): A Multicomponent Mixture of Related Compounds Pheromones 104 [1]

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Ants are known to utilize trail pheromones to recruit nestmates during food gathering and nest movement [2]. In several species of the subfamily Ponerinae the poison gland has been identified as the source of the trail pheromone [3, 4]. (3*R*, 4*S*)-4-Methyl-3-heptanol, the first reported ponerine trail pheromone, elicits as single substance trail-following activity in a manner virtually indistinguishable from that evoked by a poison gland extract of *Leptogenys diminuta* [5]. Recently we discovered that the same compound is present in the poison gland of another species of this genus. This species, which we call *L. species 5*, is morphologically similar to *L. assamensis* (voucher specimens are available at the Museum of Comparative Zoology, Harvard University, and Natural History Museum, London). In fact, workers of *L. species 5* followed trails made of synthetic 4-methyl-3-heptanol (unpublished data). Therefore it is not surprising that *L. diminuta* and *L. species 5* workers are able to follow artificial trails made of poison gland extracts of either species. However, another species of this genus, *L. peuqueti*, which showed trail-following responses to its own poison gland extracts failed to follow those of *L. diminuta* or *L. species 5*. In this report we describe the identification of the trail pheromone of *L. peuqueti*.

During foraging *L. peuqueti* workers retrieve small prey, usually tiny woodlice, without any assistance from nestmates [6]. However, ants of this species occasionally overwhelm relatively large woodlice by recruiting nestmates for a coordinated attack. While searching for food, worker ants lay orientation trails by touching the substrate with the tip of their gaster and depositing its poison gland secretion.

For the present investigation eight colonies of *L. peuqueti* were collected from sites near Ulu Gombak Field Studies Center in western Malaysia. Each colony consisted of approximately 20–60 worker ants. The ants were kept in plastic boxes and maintained on a diet of live woodlice collected from the ants' natural habitat and laboratory reared isopods (e.g., *Porcellio glaber*). Ten excised poison glands of *L. peuqueti* were sealed in a glass capillary and subjected to gas chromatography by means of a solid-sampling technique [7, 8] using a Hewlett-Packard 5890 instrument fitted with a 25×0.22 mm fused-silica capillary column coated with SE-52. The chromatogram obtained in this way showed several peaks, indicating the presence of many volatile constituents in varying amounts in the poison gland (Fig. 1). Electron-impact mass spectra were obtained by GC-MS (Varian 3400 GC and Finnigan MAT 90 mass spectrometer) for all components. Based on mass spectral and retention time data we propose chemical structures for each compound (Table 1). To confirm the proposed structures of these methyl-branched secondary alcohols and acetates we synthesized all the compounds (Table 1) using synthetic pathways shown in Fig. 2.

The starting material, 2-methylpentanol *1*, was treated with triphenyl phosphite and iodine to form the corresponding alkyl iodide *2* [9]. Reaction of *2* with the lithio salt of 2,4,4-trimethyl-2-oxazoline afforded a 2-alkyloxazoline which was hydrolyzed to the homologated acetic acid *3* [10]. Treatment of 3 with thionyl chloride resulted in 4-methylheptanoyl chloride *4* which was converted to the desired products by either route 1 or 2. In route 1, acyl chloride *4* was cross-coupled with various Grignard reagents in the presence of a catalytic amount of tris(acetylacetonate)iron(III) to give the respective ketones [11], which were reduced with $NaBH_4$ to yield the secondary alkohols *D, E, G, H, I,* and *M*. In the alternative route 2, the acid chloride *4* was first reduced with bis(triphenylphosphine) tetrahydroboratocopper(I) to the aldehyde *5* [12], which was then treated with the Grignard reagents. The alcohols *D, E, G, H, I,* and *M* were subsequently acetylated to give the corresponding acetates *A–C, F, J–L,* and *N*. Alcohols and acetates that we identified from *L. peuqueti*, except *L* which is symmetric, bear at least two chiral centers and may therefore exist as stereoisomeric forms. To study whether the ants respond preferentially to a particular stereoisomer we synthesized both enantiomers of the homochiral alcohol *H* and the corresponding acetate *J* [13] (Fig. 3). The alcohol *H* was selected since it is one of the most abundant constituents (Fig. 1).

Racemic 2-methylpentanoic acid was separated into *6a* and *6b* via the diastereomeric phenylglycinalamides according to the Helmchen procedure [14–17]. The 2-methyl-pentanols *R-7* and *S-7* were obtained by reduction with $LiAl(OCH_3)_2H_2$ and transformed into the iodides *8a* and *8b* by treat-

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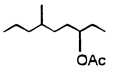
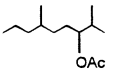
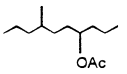
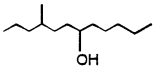
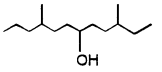
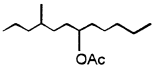
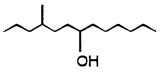
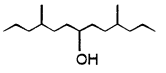
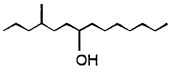
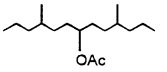
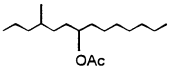
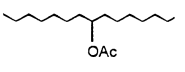
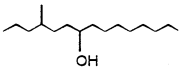
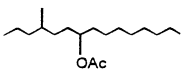
Table 1. Alcohols and acetates identified in the poison gland of *L. peuqueti*

Peak label in Figure 1, name, and mean amount (±SD) of each compound	EI (70 eV) mass spectral data and GC purity	Structure
A: 1-Ethyl-4-methylheptyl acetate; 140±79 ng	*m/z*: 140 (11%), 129 (2), 111 (20), 101 (16), 97 (25), 84 (40), 69 (29), 55 (49), 43 (100); GC: 96%	
B: 1-Isopropyl-4-methylheptyl acetate; 170±29 ng	*m/z*: 154 (11%), 129 (1), 115 (6), 111 (39), 97 (4), 84 (17), 71 (6), 69 (27), 55 (22), 43 (100); GC: 97%	
C: 1-Propyl-4-methylheptyl acetate; 10±8 ng	*m/z*: 154 (8%), 143 (1), 129 (1), 115 (16), 111 (30), 97 (5), 84 (31), 71 (10), 69 (21), 55 (23), 43 (100); GC: 97%	
D: 4-Methy-7-dodecanol; 50±32 ng	*m/z*: 184 (4%), 139 (2), 129 (20), 111 (20), 101 (27); 97 (7), 84 (28), 83 (72); 69 (84), 55 (100), 43 (78); GC: 98%	
E: 3,9-Dimethyl-6-dedecanol; 170±110 ng	*m/z*: 196 (19%), 153 (2),; 129 (37), 115 (34), 111 (39), 97 (91), 84 (50), 83 (35); 69 (100), 55 (93), 43 (75); GC: 97%	
F: 1-Pentyl-4-methylheptyl acetate; 120±45 ng	*m/z*: 182 (27%), 143 (4), 139 (8), 111 (20), 97 (24), 84 (59), 83 (34), 69 (46), 55 (68), 43 (100); GC: 96%	
G: 4-Methyl-7-tridecanol; 20±18 ng	*m/z*: 196 (9%), 153 (5); 141 (3), 129 (40), 115 (35), 111 (37), 97 (81), 84 (34), 83 (20), 69 (97), 55 (100), 43 (65); GC: 98%	
H: 4,10-Dimethyl-7-tridecanol 180±91 ng	*m/z*: 210 (21%), 167 (4); 129 (46), 111 (55), 97 (28), 84 (65), 83 (30), 69 (100), 55 (66), 43 (88); GC: 98%	
I: 4-Methyl-7-tetradecanol; 160±67 ng	*m/z*: 210 (15%), 167 (4), 129 (45), 111 (38), 97 (23), 84 (44), 83 (26), 69 (100), 55 (62), 43 (68); GC: 97%	
J: 1-(3-Methylhexyl)-4-methylheptyl acetate; 5±3 ng	*m/z*: 210 (8%), 199 (1), 171 (1), 167 (2), 140 (1), 111 (46), 97 (15), 84 (48), 69 (27), 55 (38), 43 (100); GC: 96%	
K: 1-(3-Methylhexyl)-octyl acetate; 200±88 ng	*m/z*: 210 (21%), 199 (1), 171 (1), 167 (7), 140 (4), 111 (51), 97 (47), 84 (73), 69 (48), 55 (63), 43 (100); GC: 97%	
L: 1-Heptyloctyl acetate; 20±12 ng	*m/z:* 210 (10%), 171 (4), 111 (31), 97 (7), 83 (9), 69 (15), 55 (22), 43 (100); GC: 98%	
M: 4-Methyl-7-hexadecanol; 30±21 ng	*m/z*: 238 (2%), 195 (1), 157 (17), 139 (1), 129 (28), 111 (36), 97 (31), 84 (37), 83 (62), 69 (100), 55 (74), 43 (80); GC: 96%	
N: 1-(3-Methylhexyl)-decyl acetate; 50±26 ng	*m/z*: 238 (15%), 195 (1), 139 (1), 125 (5), 111 (19), 97 (44), 84 (90), 69 (53), 55 (82), 43 (100); GC: 98%	

ment with $P(OC_6H_5)_3/I_2$ [9]. Treatment of *8a*, and *8b* with triphenylphosphine gave the phosphonium salts *9a*, and *9b*, which were deprotonated with sodium hexamethyldisilazid to the corresponding phosphoniumylids [18]. The following Wittig reaction of *9a*, and *9b* with formaldehyde leads to both enantiomers of 3-methyl-1-hexene (*10a* and *10b*). This reaction proceeds without racemization. To receive enantiomeric coupling product 4,10-dimethyltridecan-7-one *11a* or *11b* the terminal olefins were hydroborated and oxidized according to a method described by Brown et al. [19]. Reduction of the ketones with $NaBH_4$ gave the homochiral alcohols (R,R)-*H* ($[a]_D^{20}$=–1.70°, c=5.20, $CHCl_3$) and (S,S)-*H* ($[a]_D^{20}$=+1.60°, c=4.30, $CHCl_3$). Fi-

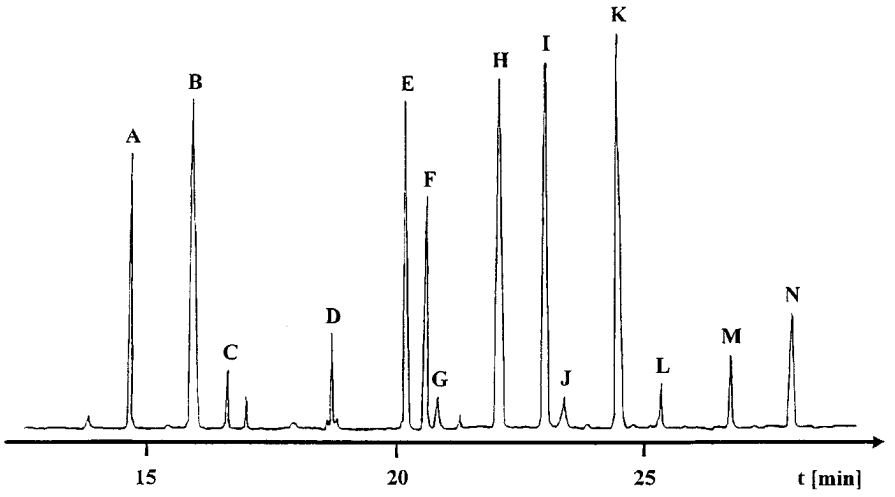


Fig. 1. Gas chromatogram of volatiles from ten poison glands of *L. peuqueti* workers obtained by GC-MS. (SE-52 coated fused-silica capillary column; 60°C for 4 min, 6°C/min to 260°C). The poison gland contents were introduced by a solid-sampling technique. See Table 1 for peak lables

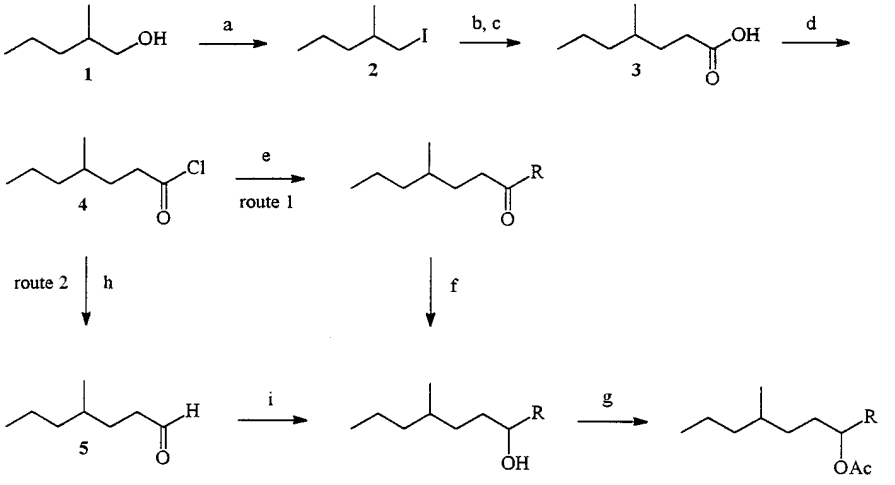


Fig. 2. Synthesis of the alcohols and acetates found in the poison gland of *L. peuquetti*. *a*, I_2/$P(OPh)_3$; *b*, 2,4,4-trimethyl-2-oxazoline, n-BuLi, THF, –78°C; *c*, H^+/H_2O; *d*, $SOCl_2$; *e*, BrMgR, $Fe(acac)_3$; *f*, $NaBH_4$; *g*, Ac_2O, pyridine; *h*, $(PPh_3)_2CuBH_4$, PPh_3; *i*, BrMgR. *R*=-ethyl, -propyl, -isopropyl, -pentyl, -hexyl, -3-methylpentyl, -heptyl, -nonyl

nally, the acetates (*J*) were obtained by treatment with acetic anhydride and pyridine. We must mention that we failed to synthesize and separate the two mesoforms of *H* and *J*; therefore these stereoisomers were not available for trail-following experiments.

Gas chromatographic retention times of SE-52 and SE-30 coated fused-silica capillary columns, as well as mass spectral data of synthetic compounds *A–J* were congruent with those obtained from poison gland compounds. In this way we confirmed the chemical structures of alcohols and acetates found in *L. peuqueti* poison gland (Table 1). The average amount of each compound per individual worker was evaluated by comparison of gas chromatographic peak areas of the natural extract with known amounts of authentic samples. For all compounds significant individual variations were observed (Table 1; n=10).

To study the trail-following activity of each synthetic compound we used a standard trail test consisting of two intercrossing S-shaped lines (each 20 cm). The trails were drawn with a lead pencil on a white piece of cardboard, and hexane solutions of each test chemical were applied with a 10-µl disposable glass capillary tube. One line was streaked with a synthetic test compound while the other was treated with either solvent as control or a gland extract. In each test 20 ants were used. The response of each ant that followed the trail to the end was considered as a positive result, and each test was repeated ten times.

Each synthetic component *A–J* was tested at 0.1 equivalent of the average total amount of that component found in one individual poison gland. Similarly, we tested a synthetic mixture containing equal amounts of each compound at a concentration of 50 pg/cm per component against an artificial trail made from an extract of an individual poison gland. Unfortunately, we were unable to carry out extensive studies with mixtures of different ratios of synthetic compounds because of the difficulty of maintaining nests of *L. peuqueti* for a sufficiently long period of time under our laboratory conditions.

All responses for the single compounds were significantly higher (25–65%) than the values obtained from solvent controls (0–2%). We observed that the trails drawn with a synthetic mixture of all components found in the poison gland exhibited a trail-following activity (89%) closely comparable to that of a poison extract (98%). These results suggest that all chemicals are necessary to simulate the activity of the trail pheromone.

In addition, we examined the trail-following behavior elicited by (4*R*, 10*R*) and (4*S*, 10*S*) enantiomers of 4,10-dimethyl-7-tridecanol (*H*) and their corresponding acetates *J*, and those of the two racemic mixtures. For both alcohol *H* and acetate *J* the workers preferred to follow trails made of (4*R*, 10*R*)-isomer as opposed to that of the (4*S*, 10*S*)-isomer or the respective racemic mixtures. Not only components *H* and *J* but also all the other compounds (except *L*) found in the poison gland bear two or three (*E*) chiral centers. Although we consider that *L. peuqueti* workers might prefer a certain stereoisomer of these other com-

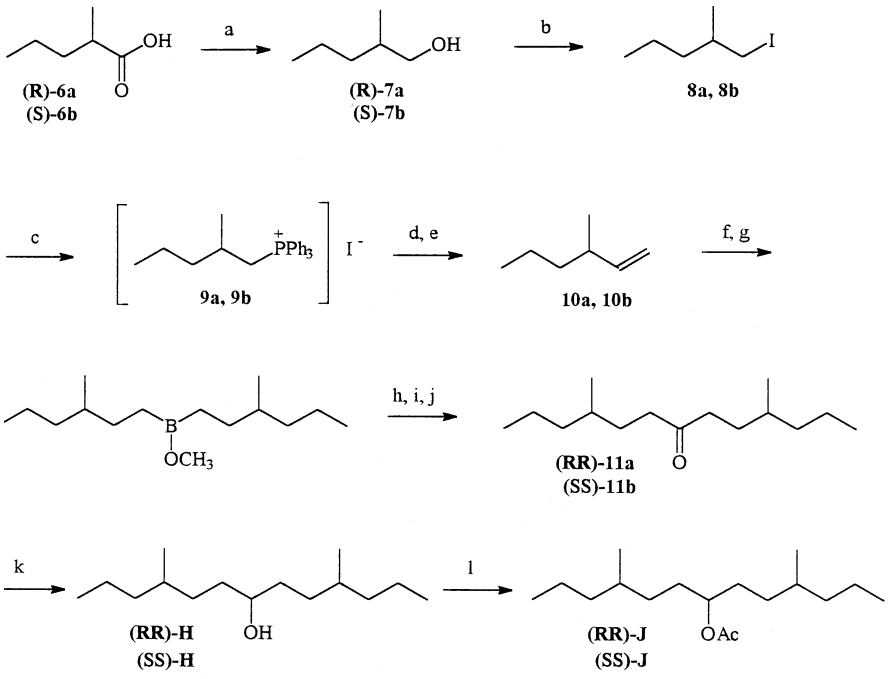


Fig. 3. Synthetic route for stereoisomers of 4,10-dimethyl-7-tridecanol *H* and its corresponding acetate *J*. *a*, $LiAl(OCH_3)_2H_2$; *b*, $I_2/P(OPh)_3$, *c*, PPh_3; *d*, [Na $N(SiCH_3)_2$]; *e*, CH_2O; *f*, BH_2Cl; *g*, $NaOHC_3$; *h*, DCME (dichloromethylether); *i*, $(C_2H_5)_3COLi$; *j*, H_2O_2/OH-; *k*, $NaBH_4$; *l*, Ac_2O, pyridine

pounds, similar to the observations we made with *H* and *J*, we did not attempt to test this hypothesis during the present set of experiments.

Obviously, this is the most complex trail pheromone mixture reported from any ant species. This result is particularly remarkable, since it is entirely different from what we observed for another ponerine, *L. diminuta*, for which a single compound was able to mimic the trail-following behavior of that evoked by an equivalent dose of a poison gland extract [5, 20]. In contrast, in *L. peuqueti* each of the 14 identified compounds was able to release some trail-following behavior, and the mixture of all substances was even more effective than any single compound. With the exception of *L*, all the identified alcohols and acetates bear a common methyl-branched moiety [CH_3-$(CH_2)_2$-$CH(CH_3)$-$(CH_2)_2$–] at the oxygenated carbon atom while the other linear or methyl-branched alkyl group varies in structure. The OH or acetate functional group is common to all components. It appears that the functional group and the conserved sidechain are essential for trail-following activity.

To compose a synthetic mixture which emulates ethological activities indistinguishable from those evoked by a poison gland extract, the relative and absolute amount, and the enantiomeric composition, of each component must be carefully controlled. It will not be surprising if such a blend shows relatively more activity than the mixture that we tested in our present experiments.

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1. Kern, K., Klein, R.W., Janssen, E., Bestmann, H.J., Schäfer, D., Maschwitz, U., Attygalle, A.B.: Pheromones 103, J. Chem. Ecol. (in press)
2. Hölldobler, B., Wilson, E.O.: The ants. p. 265. Berlin: Springer 1990
3. Fletcher, D.J.: J. Entomol. (A) *46*, 27 (1971)
4. Maschwitz, U., Mühlberg, M.: Oecologia *20*, 65 (1975)
5. Attygalle, A.B., Vostrowsky, O., Bestmann, H.J., Steghaus-Kovač, S., Maschwitz, U.: Naturwissenschaften *75*, 315 (1988)
6. Maschwitz, U., Steghaus-Kovač, S.: Naturwissenschaften *78*, 105 (1991)
7. Morgan, E.D., Wadhams, L.G.: J. Chromatogr. Sci. *10*, 528 (1972)
8. Attygalle, A.B., Herrig, M., Vostrowsky, O., Bestmann, H.J.: J. Chem. Ecol. *13*, 1299 (1987)
9. Coe D.C., Landauer S.R., Rydon, H.N.: J. Chem. Soc., 2281 (1953)
10. Meyers, A.I., Temple, D.L., Nolen, R.L., Mihelich, E.D.: J. Org. Chem. *39*, 2778 (1974)
11. Cardellicchio, C., Fiandanese, V., Marchese, G., Ronzini, L.: Tetrahedron Lett. *28*, 2053 (1987)
12. Fleet, G.W.J., Harding, P.J.C.: Tetrahedron Lett. *11*, 975 (1979)
13. Bauriegel, L.: Dr. Thesis, University of Erlangen-Nürnberg (1990)
14. Helmchen, G., Nill, G., Flockerzi, D., Youssef, M.: Angew. Chen. *65*, 91 (1976), Internat. Engl. Ed. *18*, 63 (1979)
15. Flockerzi, D.: Dr. Thesis University of Stuttgart, Germany (1978)
16. Glasbrenner, J.: Dr. Thesis, University of Erlangen-Nürnberg (1987)
17. Bestmann, H.J., Frighetto, R.T.S., Frighetto, N., Vostrowsky, O.: Liebigs Ann. Chem. *877* (1988)
18. Bestmann, H.J., Vostrowsky, O., Stransky, W.: Chem. Ber. *109*, 1694 (1976)
19. Brown, H.C., Ravindran, N., Kulkarni, S.U.: J. Org. Chem. *44*, 2417 (1979)
20. Steghaus-Kovač, S., Maschwitz, U., Attygalle, A.B., Frighetto, R.T.S., Frighetto, N., Vostrowsky, O., Bestmann, H.J.: Experientia *48*, 690 (1992)