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3-Methyl-α-himachalene is confirmed, and the relative stereochemistry defined, by synthesis as the sex pheromone of the sandfly *Lutzomyia longipalpis* from Jacobina, Brazil

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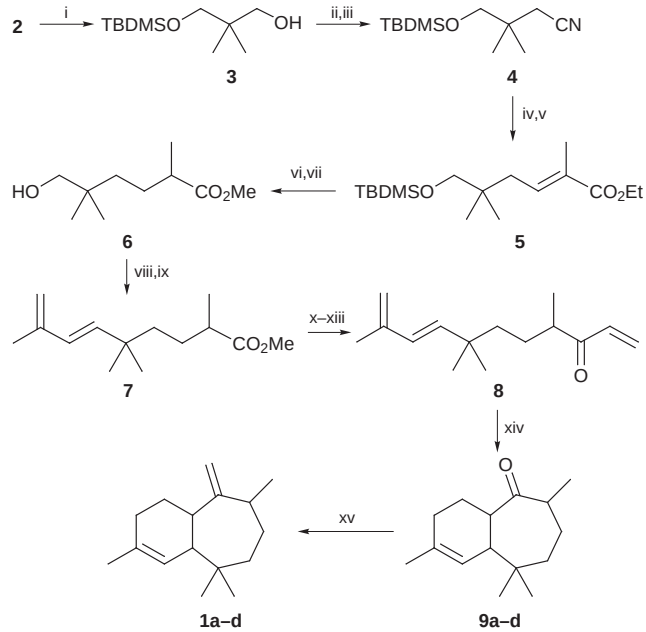
The structure of the sex pheromone produced by the male sandfly *Lutzomyia longipalpis*, from the Jacobina region of Brazil, previously proposed tentatively as the novel homosesquiterpene 3-methyl-α-himachalene is confirmed by synthesis and biological activity; the relative stereochemistry is defined as 1*RS*,3*RS*,7*RS* by comparing the natural product with the four synthetic diastereoisomers.

The sandfly *Lutzomyia longipalpis* (Lutz and Neiva) (Diptera: Psychodidae) is the vector of the protozoan parasite *Leishmania chagasi* (Cunha and Chagas) (Kinetoplastida: Trypanosomatidae), the causative agent of visceral leishmaniasis in the New World. Male *L. longipalpis* release a sex pheromone from glands on the tergites of the abdomen[1] which is highly attractive to females.[2] The sex pheromone gland of *L. longipalpis* from Jacobina (Bahia State) in northeastern Brazil produces several compounds but only the principal volatile component is responsible for the attraction of females.[3] It was proposed, mostly on the basis of mass spectrometry of the natural product and of products of microchemical reactions, that the pheromone comprised the novel homosesquiterpene 3-methyl-α-himachelene.[4] The sex pheromones of other sympatric and allopatric populations of *L. longipalpis* are different;[5] for example, that from the Lapinha region of Brazil (Minas Gerais State) has been tentatively proposed as another novel sesquiterpene, 9-methylgermacrene-B.[6] The purpose of this work was to test the proposed structure for the Jacobina *L. longipalpis* sex pheromone by synthesis of 3-methyl-α-himachalene **1**.

The synthetic route adopted followed the intramolecular Diels–Alder methodology employed by Wenkert and Naemura[7] for α-himachalene and is described in Scheme 1. Diene **7** comprised a mixture of *E*/*Z* isomers (77 : 23–90 : 10) by ^{1}H NMR spectroscopy. The subsequent cyclisation of the keto enediene **8** in xylene under reflux gave a mixture of cyclic products, which could be separated by medium pressure liquid chromatography into enantiomeric pairs of diastereoisomers **9a**–**d**.† Final conversion to the diastereoisomeric 3-methyl-α-himachalenes **1a**–**d**‡ was achieved quantitatively using the Tebbe reagent. Assignment of structures **9a**–**d** was by correlation and two dimensional NOE spectroscopy (Fig. 1). Compounds **9a** and **9c** were designated as *cis*-fused by the ring junction protons showing a NOE to each other. The relative stereochemistry of the methyl group could be determined for **9c** by a NOE to the ring junction proton of C-1, a signal not seen for **9a**. The relative stereochemistries of the C-3 methyl group of *trans*-fused **9b** and **9d** were also elucidated by NOE experiments. A NOE was observed between the C-1 proton of **9b** and one of the C-6 methyl groups, while the *other* C-6 methyl group showed a NOE to the C-3 methyl, which therefore lies on the *opposite* face to the C-1 proton (Fig. 1). The structures of **1a**–**d** were inferred from **9a**–**d** and in comparison with NMR data for natural α-himachalene.[4] Mass spectra were obtained by GC-MS.§

Co-injection of natural product with the diastereoisomers, using high-resolution capillary GC on columns of different polarity (Table 1) gave peak enhancement only with diastereoisomer **1c**. In the case of chiral GC, only one enantiomer of **1c** enhanced the natural product peak. The ^{1}H NMR results reported,[6] although incomplete, showed good agreement with only those for structure **1c**. The mass spectrum for the natural product[6] was almost identical with that for **1c**, with greater differences from those for **1a**, **1b** and **1d**. All had a base peak at *m*/*z* 94, different from that for α-himachalene (*m*/*z* 93), which was suggested as likely when the earlier tentative structural prediction was made.[6] Bioassays¶ involving attraction of female *L. longipalpis* and conducted in a Y-tube olfactometer showed that only diastereoisomer **1c** and the natural pheromone extract were active and both showed high statistical significance. This was in agreement with the chromatographic and spectral data, giving the sex pheromone structure as (1*RS*,3*RS*,7*RS*)-3-methyl-α-himachalene, *i.e.* (1*RS*,3*RS*,7*RS*)-2-methylene-3,6,6,9-tetramethylbicyclo[5.4.0]undec-8-ene (**1c**). The *cis* stereochemistry of the natural product is analogous



Scheme 1 *Reagents and conditions:* i, NaH (1.0 equiv.), TBDMSCl (1.1 equiv.), THF, 94%; ii, TsCl, Py, $CHCl_3$; iii, NaCN, DMSO, heat, 94% (2 steps); iv, DIBALH, CH_2Cl_2, H_3O^+, 67%; v, $CH_3C{=}(PPh_3)CO_2Et$, benzene, reflux, quant.; vi, Mg, MeOH, 88%; vii, aq. HF, Me_3CN, 97%; viii, Swern oxidation; ix, $Ph_3P{=}CHCMe{=}CH_2$, THF, reflux, 56% (2 steps); x, $LiAlH_4$, Et_2O, 92%; xi, PCC, MS 4A, CH_2Cl_2; xii, $CH_2{=}CHMgBr$, THF, 70% (2 steps); xiii, Dess–Martin oxidation, 86%; xiv, xylene, reflux, 62%; xv, Tebbe reagent, quant.

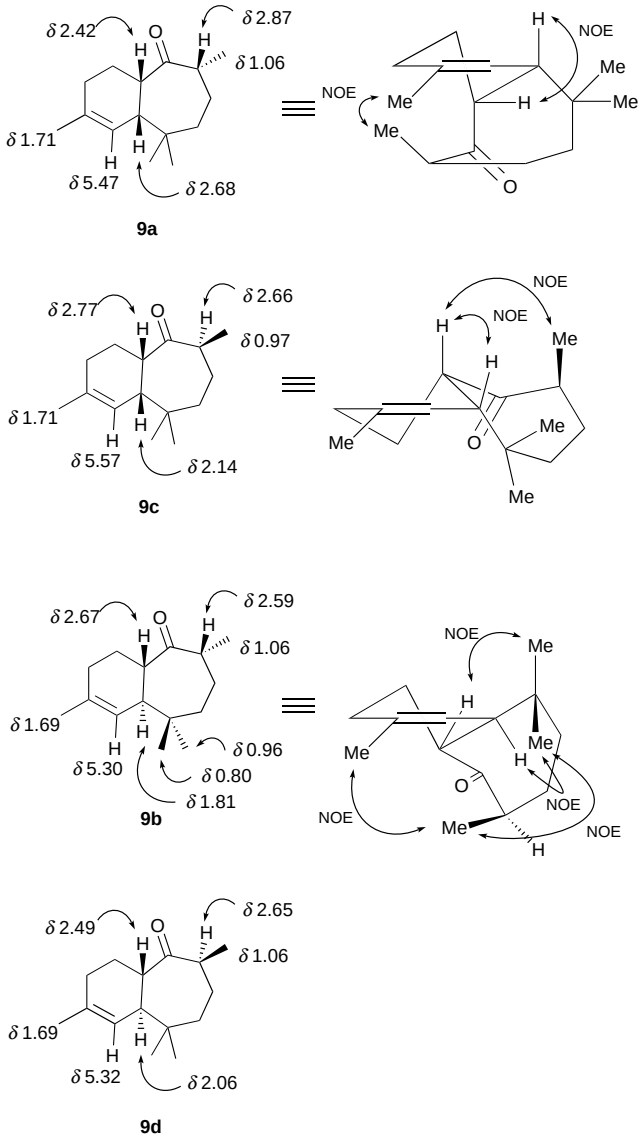


Fig. 1 Assignment of the relative stereochemistry of **9a**–**d**.

Table 1 Retention times of all isomers of 3-methyl-α-himachalene **1a**–**d** by GC and peaks enhanced on coinjection with natural material of *L. longipalpis* from Jacobina

	Retention time/min	
Compound	HP-5 (a siloxane)[a]	Chiral GC (β-cyclodextrin)[b]
1a	16.67	37.01
		No chiral separation
1b	16.03	35.15
		36.35
1c	14.73[c]	32.50
		33.01[c]
1d	14.71	32.58
		33.36

[a] 0.32 mm id × 30 m × 25 μm film thickness, 40 to 150 °C at 5 °C min^{-1}. [b] 0.25 mm id × 30 m × 25 μm film thickness, 40 to 180 °C at 3 °C min^{-1}. [c] Peaks enhanced on coinjection with natural material.

to that of natural α-himachalene, with absolute stereochemistry 1*R*,7*R*.[8] The absolute stereochemistry of the pheromone is now under further investigation, although the bioassay results suggest no interference with biological activity by the sample containing both enantiomers. Attempts are being made to scale-up the synthesis for field and pest control studies.

Notes and references

† *Selected data* for **9a**: R_f 0.64; δ_H 0.70 (s, 3H, 6-CH_3), 1.01 (s, 3H, 6-CH_3), 1.06 (d, *J* 6.5, 3-CH_3), 1.71 (br s, 3H, 9-CH_3), 2.42 (m, 1H, 1-H), 2.68 (br s, 1H, 7-H), 2.87 (m, 1H, 3-H), 5.47 (br s, 1H, 8-H). For **9b**: R_f 0.64; δ_H 0.80 (s, 3H, 6-CH_3), 0.96 (s, 3H, 6-CH_3), 1.06 (d, *J* 6.5, 3-CH_3), 1.69 (br s, 9-CH_3), 1.81 (m, 1H, 7-H), 2.67 (m, 1H, 1-H), 2.59 (m, 1H, 3-H), 5.30 (br s, 1H, 8-H). For **9c**: R_f 0.61; δ_H 0.85 (s, 3H, 6-CH_3), 0.97 (d, *J* 6.5, 3-CH_3), 1.07 (s, 3H, 6-CH_3), 1.71 (br s, 9-CH_3), 2.14 (br s, 1H, 7-H), 2.66 (m, 1H, 3-H), 2.77 (m, 1H, 1-H), 5.57 (br s, 1H, 8-H). For **9d**: R_f 0.61; δ_H 0.77 (s, 3H, 6-CH_3), 1.01 (s, 3H, 6-CH_3), 1.06 (d, *J* 6.5, 3-CH_3), 1.69 (br s, 9-CH_3), 2.06 (m, 1H, 7-H), 2.49 (m, 1H, 1-H), 2.65 (m, 1H, 3-H), 5.32 (br s, 1H, 8-H). R_f values in hexane–EtOAc 10:1.

‡ *Selected data* for **1a**: δ_H 0.81 (s, 3H, 6-CH_3), 0.94 (s, 3H, 6-CH_3), 1.10 (d, *J* 7, 3-CH_3), 1.70 (br s, 3H, 9-CH_3), 4.82 (s, 1H, 2-C$H_a$$CH_b$), 4.86 (s, 1H, 2-$CH_aCH_b$), 5.49 (br s, 1H, 8-H). For **1b**: δ_H 0.71 (s, 3H, 6-CH_3), 0.94 (s, 3H, 6-CH_3), 1.06 (d, *J* = 7, 3-CH_3), 1.69 (br s, 9-CH_3), 4.76 (s, 1H, 2-C$H_a$$CH_b$), 4.86 (s, 1H, 2-$CH_aCH_b$), 5.30 (br s, 1H, 8-H). For **1c**: δ_H 0.96 (s, 3H, 6-CH_3), 1.00 (s, 3H, 6-CH_3), 1.01 (d, *J* 7, 3-CH_3), 1.68 (br s, 9-CH_3), 4.76 (s, 1H, 2-C$H_a$$CH_b$), 4.81 (s, 1H, 2-$CH_aCH_b$), 5.51 (br s, 1H, 8-H). For **1d**: δ_H 0.70 (s, 3H, 6-CH_3), 0.94 (s, 3H, 6-CH_3), 0.97 (d, *J* 7, 3-CH_3), 1.67 (br s, 9-CH_3), 4.75 (s, 1H, 2-C$H_a$$CH_b$), 4.79 (s, 1H, 2-$CH_aCH_b$), 5.31 (br s, 1H, 8-H).

§ GC–MS: 0.32 mm id × 50 m HP-1(a siloxane × 0.52 μm film thickness, 30 to 250 °C at 5 °C min^{-1}; EI at 70 EV, 250 °C (VG-Autospec, Fisons Instruments), elution order **1d**, **1c**, **1b**, **1a**. *Selected data* for **1a**: *m*/*z* 94 (100%), 79 (55), 121 (54), 41 (52), 93 (51), 91 (41), 107 (40), 105 (35), 69 (35), 55 (34), 119 (32), 81 (29), 95 (24), 77 (24), 148 (23), 149 (19), 133 (18), 65 (18), 203 (16), 161 (16), 175 (15), 53 (15), 39 (15), 218 (11, M^+). For **1b**: *m*/*z* 94 (100%), 121 (87), 105 (61), 91 (58), 79 (57), 55 (56), 41 (53), 148 (51), 107 (50), 136 (47), 93 (46), 69 (43), 175 (35), 119 (33), 39 (31), 42 (28), 77 (28), 133 (27), 95 (23), 161 (22), 218 (21), 81 (21), 92 (20), 27 (20), 67 (19), 123 (18), 162 (18), 131 (17), 148 (17), 147 (16), 120 (15), 43 (15). For **1c**: *m*/*z* 94 (100%), 41 (69), 93 (63), 107 (61), 79 (59), 121 (58), 175 (53), 91 (46), 105 (45), 69 (44), 55 (43), 119 (40), 203 (38), 81 (36), 77 (32), 133 (26), 67 (26), 95 (25), 149 (24), 148 (23), 147 (21), 39 (21), 43 (20), 29 (20), 109 (20), 43 (20), 162 (19), 27 (19), 218 (18, M^+), 123 (17), 161 (16), 136 (15), 67 (15). For **1d**: *m*/*z* 94 (100%), 121 (65), 79 (50), 93 (49), 107 (45), 41 (44), 91 (40), 136 (39), 105 (38), 119 (38), 148 (36), 69 (32), 55 (31), 175 (30), 81 (27), 77 (26), 133 (25), 149 (24), 218 (23 M^+), 95 (22), 161 (18), 147 (17), 67 (16), 109 (15), 53 (15).

¶ Virgin female sandflies were removed from larval rearing pots within 10 h after eclosion to ensure that they were unmated. They were provided with a saturated sugar solution on cotton wool and subsequently maintained for 5–6 days in Barraud cages (18 × 18 × 18 cm). Bioassays were conducted in a glass (9 mm internal diameter) Y-tube olfactometer. Zero grade air was passed (2 ml min^{-1}) through two charcoal filters into the test and control arms (10 cm long). The olfactometer was connected to the air supply by Teflon tubing. A filter paper disk (1.5 cm diameter) was inserted into the Teflon tubing at the connection with the olfactometer test and control arms. During bioassays pheromone extracts or synthetic chemicals in hexane were placed on one of the filter paper disks. Hexane in the same quantity as for the test arm was placed on the other filter paper disk. The female sandfly was introduced into the third arm (10 cm long) of the olfactometer and its response observed for 5 min. Female attraction to **1c** and extract prepared from 5–7 day old male *L. longipalpis* was highly significant (χ^2 = 6.2 × 10^{-7} and P = 1.4 × 10^{-26}, respectively). Females were not significantly attracted to **1a**, **1b** or **1d**, nor to a natural α-himachalene sample at the same levels.

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