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9-Methylgermacrene-B is confirmed as the sex pheromone of the sandfly *Lutzomyia longipalpis* from Lapinha, Brazil, and the absolute stereochemistry defined as *S*

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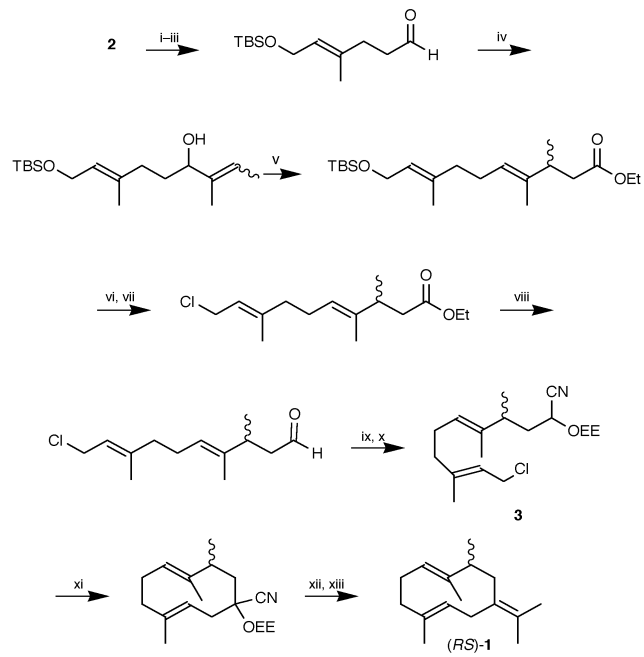
The structure of the sex pheromone produced by the male sandfly *Lutzomyia longipalpis*, from the Lapinha Cave (Minas Gerais State) region of Brazil, previously proposed tentatively as the novel homosesquiterpene 9-methylgermacrene-B, is confirmed and the absolute stereochemistry defined as *S* by comparing physical and biological properties of the synthetic enantiomers with the natural product.

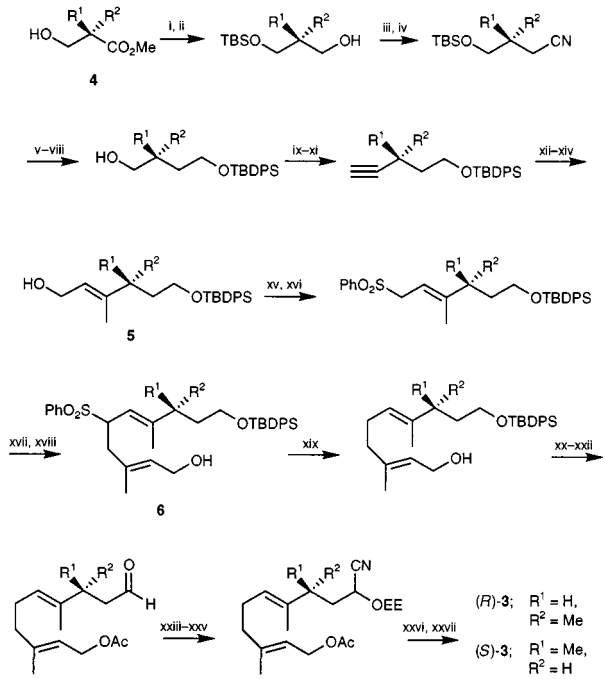
The sandfly *Lutzomyia longipalpis* (Lutz and Neiva) (Diptera: Psychodidae) is the vector of the protozoan parasite *Leishmania chagasi* (Cunha and Chagas) (Kinetoplastida: Trypanosomatidae), the causative agent of visceral leishmaniasis in the New World. Male *L. longipalpis* release a sex pheromone, from glands on the tergites of the abdomen,[1] which is highly attractive to females.[2] The sex pheromone gland of *L. longipalpis* from the Lapinha region (Minas Gerais State) in Brazil produces one principal volatile component that is responsible for the attraction of females.[3] It was proposed, mostly on the basis of mass spectrometry on natural material and on products from microchemical reactions, that the pheromone comprised the novel homosesquiterpene 9-methylgermacrene-B **1**.[4] The sex pheromones of other sympatric and allopatric populations of *L. longipalpis* are different;[5] for example, that from the Jacobina region of Brazil (Bahia State) has been confirmed as another novel sesquiterpene, 3-methyl-α-himachalene, with relative stereochemistry defined as 1*RS*,3*RS*,7*RS*.[6] The purpose of this work was to test the proposed structure for the Lapinha *L. longipalpis* sex pheromone by synthesis of 9-methylgermacrene-B and to define the absolute stereochemistry at C-9 by comparison between the natural product and the synthetic enantiomers (*R*)-**1** and (*S*)-**1**.

The overall synthetic approach adopted has recently been developed for sesquiterpene germacrene-B and involves the establishment of the cyclodecadiene ring system by an intramolecular alkylation before finally elaborating the isopropylidene group.[7] Initially, the racemic 9-methylgermacrene-B (**1**) was prepared as in Scheme 1 from geraniol **2**, in order to test the initial structural hypothesis for this pheromone.[4] The NMR data from racemic **1**† were similar to the limited data obtained originally for the natural product.[4] Co-injection on GC‡ (siloxane column) of racemic **1** with the natural product gave one enhanced peak (18.22 min). The mass spectrum of synthetic compound by GC-MS§ was similar to that published for the natural product.[4] Having confirmed the structure of the pheromone as 9-methylgermacrene-B **1**, the enantiomers were then synthesised by the same cyclisation approach but with methyl 3-hydroxy-2-methylpropionate **4** as chiral precursor, *i.e.* (*S*)-**4** to (*R*)-**1** and (*R*)-**4** to (*S*)-**1** as in Scheme 2. The enantiomers of **4** were obtained commercially and (*R*)-**1** was synthesised first, hence some improvements in yield for (*S*)-**1**. The chiral syntheses required introduction of two stereogenic double bonds at **5** and **6** which were both achieved with an *E*/*Z* ratio of >99% by NMR and the final products possessed an ee of >95%. Both (*R*)-**1** and (*S*)-**1** gave NMR and mass spectra essentially the same as for racemic **1**,†§ as expected, and with $[\alpha]_D^{18}$ + 61.4 and $[\alpha]_D^{20}$ −61.3 ($CHCl_3$), respectively. On chiral GC‡, the *R* and *S* isomers of **1** eluted in that order (38.04, 38.20 min) and, when co-injected with racemic **1**, the natural product gave enhancement of the second peak, defining the absolute stereochemistry of the pheromone as (*S*)-9-methylgermacrene-B (*S*)-**1**. It should be noted, as explained previously,[4] that the pheromone is not stable and readily undergoes acid catalysed cyclisation and a Cope rearrangement to the corresponding elemene structure on heating, hence the requirement for cold on-column injection or low injector port temperatures for GC.‡

Bioassays¶ involving attraction of female *L. longipalpis* and conducted in a Y-tube olfactometer resulted in racemic **1** giving 77% attraction compared with 72% (though not statistically significantly different) for an equivalent amount of the natural product. Attraction to (*S*)-**1** was 67% compared with 56% to the (*R*)-**1** isomer. The response to (*S*)-**1** was not significantly different from that of the natural pheromone, whereas the response to the (*R*)-**1** isomer was significantly lower (χ^2 p =



Scheme 1 Yields are for routes to (±)-**1**, and from compound **3** to **1** also are quoted for routes to (*R*)-**1** then (*S*)-**1** in that order. *Reagents and conditions:* i, TBSCl, imidazole, DMF, 96%; ii, MCPBA, $CHCl_3$, 74%; iii, $HIO_4.2H_2O$, Et_2O, THF, 52%; iv, CHMe=CMeMgBr, THF, 55%; v, $CH_3C(OEt)_3$, $C_2H_5CO_2H$, 71% (*E*/*Z*: 98:2); vi, PPTS, MeOH, 87%; vii; Ph_3P, CCl_4; viii, DIBAL-H, CH_2Cl_2; ix, TMSCN, KCN, 18-crown-6, then $BnNMe_3F$, THF, H_2O; x, CH_2=CHOEt, C_6H_6; xi, NaHMDS, THF, 19% (5 steps), 47%, 42%; xii, PPTS, MeOH then aq. NaOH, Et_2O, 18%, 51%, 50%; xiii, Me_2CBr_2, Sm, $CrCl_3$, SmI_2, THF, 56%, 60%, 64%.



Scheme 2 Yields are quoted for routes to (*R*)-**1** then (*S*)-**1** in that order. *Reagents and conditions:* i, TBSCl, imidazole, DMF, quant., 99%; ii, DIBAL-H, CH_2Cl_2, 85%, 94%; iii, TsCl, Py; iv, NaCN, DMSO, 79%, 84%, 2 steps; v, DIBAL-H, CH_2Cl_2; vi, $NaBH_4$, EtOH, 58%, 65%, 2 steps; vii, TBDPSCl, imidazole, DMF, 89%, 99%; viii, AcOH, THF, H_2O, 84%, 86%; ix, Swern oxidation; x, PPh_3, CBr_4, CH_2Cl_2, 87%, 93%, 2 steps; xi, BuLi, Et_2O, 98%, 98%; xii, Me_3Al, Cp_2ZrCl_2, CH_2Cl_2, H_2O; xiii, BuLi, hexane; xiv, $(CH_2O)_n$, THF, 80%, 80%, 3 steps; xv, PPh_3, CCl_4; xvi, $NaSO_2Ph$, DMF, 76%, 83%, 2 steps; xvii, BuLi, THF, HMPA then (*E*)-$TBSOCH_2C$-$Me{=}CHCH_2Cl$,[8] 78%, 87%; xviii, AcOH, THF, H_2O, 85%, 81%; xix, [$PdCl_2$(dppp)], Super-Hydride, THF, 82%, 81%; xx, Ac_2O, Py, 87%, 95%; xxi, TBAF, THF, 90%, 83%; xxii, Dess–Martin oxidation, 75%, 87%; xxiii, TMSCN, KCN, 18-crown-6; xxiv, $BnMe_3NF$, THF, H_2O; xxv, CH_2=CHOEt, TsOH, C_6H_6, quant., 94%, 3 steps; xxvi, H_2CO_3, MeOH, 87%, 86%; xxvii, LiCl, MsCl, DMF, *s*-collidine, 95%, 90%.

0.009). Thus, while the *S* isomer of **1** is highly active, the *R* isomer is itself also active but does not appear to interfere with the activity of its antipode. This will reduce the need to create highly stereochemically pure (*S*)-**1** for field development of the pheromone in control of these important disease vectors.

The demonstration that the sex pheromone produced by male *L. longipalpis* from the Lapinha region is (*S*)-9-methylgermacrene-B, (*S*)-(*E*,*E*)-7-isopropylidene-4,9,10-trimethylcyclodeca-1(10),4-diene (*S*)-**1**, would suggest that the absolute stereochemistry of that from Jacobina would be (1*S*,3*S*,7*S*)-3-methyl-α-himachalene, since the C-3 methyl is analogous in putative biosynthetic terms to the C-9 methyl of the homogermacrene **1** and the relative stereochemistry has already been established,[6] but this will be reported separately when the hypothesis has been fully tested.

Notes and references

† *Selected NMR data* for (±)-**1**: Spectra were recorded using a JEOL JNM-LA 500 MHz spectrometer. δ_H($CDCl_3$, 500 MHz) 1.04 (3H, d, *J* = 7.0), 1.44 (3H, s), 1.55 (3H, s), 1.68 (3H, s), 1.70 (3H, s), 2.4–1.8 (8H, m), 3.06 (1H, br d, *J* 14.0), 4.39 (1H, br d, *J* 11.0), 4.72 (1H, dd, *J* 12.0, 3.0); δ_C($CDCl_3$, 125 MHz) 11.1, 16.4, 20.4, 20.7, 20.9, 25.3, 34.4, 38.9, 40.7, 46.3, 125.7, 127.0, 128.7, 130.9, 133.7, 140.2.

‡ *GC conditions*: HP-5 (a siloxane) 0.32 mm id × 30 m × 25 μm film thickness, 40 to 150 °C at 5 °C min^{-1}; chiral GC (β-cyclodextrin) 0.25 mm id × 30 m × 25 μm film thickness, 40 to 180 °C at 3 °C min^{-1}.

§ *GC-MS*: 0.32 mm id × 50 m HP-1 (a siloxane × 0.52 μm film thickness, 30 °C on-column injection, then 30 to 200 °C at 5 °C min^{-1}; EI at 70 eV, 250°C (VG-Autospec, Fisons Instruments). *Selected data* for (±)-**1**: *m/z* 121 (100%), 93 (75), 107 (59), 41 (57), 67 (51), 135 (47), 91 (35), 55 (34), 79 (30), 105 (30), 119 (28), 53 (26), 77 (24), 43 (23), 81 (22), 39 (21), 133 (19), 136 (18), 29 (17), 147 (17), 71 (16), 175 (16), 95 (14), 122 (13), 203 (13), 161 (11), 218 (10, M^+).

¶ 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 and 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.

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