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Enantioselective Synthesis and Activity of All Diastereoisomers of (*E*)-Phytal, a Pheromone Component of the Moroccan Locust, *Dociostaurus maroccanus*

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ABSTRACT

The Moroccan locust, *Dociostaurus maroccanus* (Thunberg, 1815) (Orthoptera: Acrididae), is a polyphagous pest capable of inflicting large losses in agriculture under favorable environmental and climatic conditions. Currently, control of the pest relies solely on the application of conventional insecticides that have negative effects on the environment and human safety. In the search for a more rational, environmentally-acceptable approach for locust control, we have previously reported that (*Z/E*)-phytal (**1**) is a male-produced candidate sex pheromone of this acridid. This molecule, with two stereogenic centers at C-7 and C-11, has four different diastereomers along with the *Z/E* stereochemistry of the double bond at C-2. In this paper, we present for the first time the enantioselective synthesis of the four diastereomers of (*E*)-phytal and their electrophysiological and behavioral activity on males and females. Our results demonstrate that the (*R,R*)-phytal is the most active diastereomer in both assays, significantly attracting females in a double-choice Y olfactometer, and confirming the previous chromatographic assignment as component of the sex pheromone of the Moroccan locust.

KEYWORDS: *Moroccan locust, Dociostaurus maroccanus, sex pheromone, phytal, , (2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-enal, enantioselective synthesis, electrophysiology, behavioral activity.*

INTRODUCTION

According to data from the Food and Agricultural Organization of the United Nations (FAO) the world food production will need to rise by 70% and to double in the developing countries by 2050. In addition, it is likely that these countries will have to face devastating attacks promoted by locust plagues.[1] Control of outbreaks and plagues of these pests has relied primarily on the application of conventional insecticides to hopper bands and swarms.[2] These treatments have been highly controversial for environmental, human safety and economic reasons and therefore, alternative, environmentally-friendly strategies for locusts control are urgently needed.[2-4] First attempts have been implemented using biopesticides based on the fungus *Metarhizium anisopliae* var. *acridum* as an alternative biological control measure,[5] but chemical control nevertheless remains the most effective defence against locusts so far.[4]

The Moroccan locust, *Dociostaurus maroccanus* (Thunberg, 1815) (Orthoptera: Acrididae), is a polyphagous pest of crops and pastures, particularly in Southern Europe, North Africa and the Middle East, but devastating outbreaks have also been seen in Afghanistan, Iran, and adjacent countries of the former USSR.[6-8] The locust is highly voracious and, as such, considered a "major pest of many crops".[9] As for other acridid species, like *Schistocerca gregaria* (Forskål, 1775) or *Locusta migratoria* (L., 1758),[10] if environmental and climatic conditions are favorable, the Moroccan locust can also shift from solitarious to gregarious phase, increasing dramatically in number with formation of hopper bands and large landscape-devouring swarms.[11] This fact has led to an increasing use of insecticides in infested zones to prevent spreading to non-infested areas. Thus, 651,600 ha were treated in Uzbekistan in 1984, 290,000 ha in Kazakhstan in 1993, 219,700 ha in Turkmenistan in 1996, and ca. 650,000 ha in Spain in the period 2004-2007,[11,12] among others. However, in spite of its economic incidence, no reports have been found in the literature to tackle management of this locust through a more rational, environmentally acceptable approach. In a previous

study,[13] we reported the identification, synthesis and biological activity of (*Z*/*E*)-phytal, **1** (Figure 1) as a male-specific candidate sex pheromone compound of the acridid. Biosynthetically, it has been postulated that phytal proceeds from oxidation of phytol in marine bacteria,[14,15] and later supported by us to occur in the gut of Moroccan locust males.[13] Like phytol, the alcohol moiety of the chlorophyll molecule, the phytal molecule (3,7,11,15-tetramethylhexadec-2-enal, **1**) features four methyl substituents, two of them generating a stereogenic center at C-7 and C-11. Therefore, four different diastereoisomers are possible, in addition to the *Z/E* stereochemistry of the double bond at C-2. Only the natural (*R,R*)-phytal and the racemic compound have been prepared by oxidation of the corresponding phytols[13,16,17] or by reduction of the corresponding nitrile.[18] In this study, we developed an enantioselective synthesis of all diastereoisomers of (*E*)-phytal, **2-5** (Figure 2) from commercially available chiral synthons, and their electrophysiological and behavioral activity on males and females of the Moroccan locust to establish the most active diastereomer as well as the final assignment of the natural pheromone.

MATERIALS AND METHODS

General. Chemicals and solvents were purchased from commercial suppliers or purified by standard techniques. Racemic and (2*E,*7*R*,11*R*)-phytol were commercially available from Sigma-Aldrich (Tres Cantos, Madrid, Spain) and TCI Europe (Zwijndrecht, Belgium), respectively. All reactions employing organometallic compounds were carried out under inert atmosphere. IR spectra were recorded on a Avatar 360 FT-IR spectrometer (Thermo Nicolet, Madison, WI). NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Mercury 400 spectrometer (Varian, Palo Alto, CA). Spectra were recorded in $CDCl_3$ at ambient temperature using the $CHCl_3$ present in the solvent (7.25 ppm for ^{1}H NMR and 77.0 ppm for ^{13}C NMR spectra) as internal standard. Mass spectra (MS) were obtained on a Trace MS (ThermoQuest-Finnigan, Manchester, United Kingdom) coupled to a GC2000 gas

chromatograph (ThermoQuest-Finnigan, Milan, Italy). High resolution mass spectra (HRMS) were run on a Acquity UPLC coupled to an LCT Premier XE mass spectrometer (Waters, Milford, MA) using a 50 mm x 2.1 mm i.d., 1.7 μm, BEH C18 column (Waters, Milford, MA) eluting with CH_3CN/H_2O (80:20, v/v). Elemental analyses were determined on a Flash 2000 Organic Elemental Analyzer (Thermo Scientific, Cambridge, United Kingdom). Optical rotations were measured on a model 341 polarimeter (Perkin-Elmer, Waltham, MA) at 589 nm.

Synthesis of Diastereomers of (*E*)-phytal, 2-5 (Figures 3 and 4)**.**

The general approach is represented by the synthesis of (2*E*,7*S*,11*R*)-phytal, **2**, from the intermediate (2*S*,6*R*)-2,6,10-trimethylundecyl alcohol, **6** (Figure 3), as follows:

*(2S,6R)-2,6,10-Trimethylundecyl methanesulfonate [(2S,6R)-**16**]*. To a solution of chiral alcohol (2*S*,6*R*)-**6** (1.20 g, 5.6 mmol) in dichloromethane (11 mL) was added triethylamine (2.50 mL, 18 mmol) and methanesulfonyl chloride (0.78 mL, 10.1 mmol) at 0 ºC. The reaction was stirred at 0 ºC for 1 h, and at room temperature for 3 h. The mixture was quenched with a saturated NH_4Cl solution (10 mL) and extracted with ether (3 x 40 mL). The combined extracts were washed with brine (20 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was chromatographed (silica, hexane/diethyl ether 85:15) to give mesylate (2*S*,6*R*)-**16** (1.40 g, 86%) as a pale yellow oil. ^{1}H NMR δ 4.07 (dd, *J*= 9.4, 5.7 Hz, 1 H), 3.98 (dd, *J*= 9.4, 6.7 Hz, 1 H), 2.98 (s, 3 H), 1.86 (m, 1 H), 1.50 (m, 1 H), 1.43–1.01 (m, 13 H), 0.97 (d, *J*= 6.7 Hz, 3 H), 0.89–0.80 ppm (m, 9 H). ^{13}C NMR δ 76.7 (CH_2), 39.3 (CH_2), 37.2 (CH_3), 37.1 (CH_2), 37.0 (CH_2), 33.0 (CH), 32.9 (CH_2), 32.6 (CH), 27.9 (CH), 24.7 (CH_2), 24.0 (CH_2), 22.6 (CH_3), 22.5 (CH_3), 19.5 (CH_3), 16.4 ppm (CH_3). IR (film): 2939, 2924, 2871, 1454, 1352, 1322, 1184, 1059, 974, 809 cm^{-1}. MS (EI): *m/z* (%): 291 (1) $[M-1]^+$, 196 (25), 168 (11), 154 (9), 140 (24), 126 (81), 111 (91), 97 (77), 83 (83), 69 (100), 57 (95), 43 (94).

*(3S,7R)-3,7,11-Trimethyldodecanenitrile [(3S,7R)-**17**]***.** To a solution of mesylate (2*S*,6*R*)-**16** (1.40 g, 4.78 mmol) in THF/DMSO (1:1, v/v) (14 mL) was added sodium cyanide (516 mg, 10.50 mmol) and the mixture was refluxed for 3 h. The reaction mixture was cooled to room temperature, quenched with water (10 mL) and stirred for 10 min. The organic material was extracted with ethyl acetate (3 x 35 mL). The combined extracts were washed with brine (20 mL) and water (15 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was chromatographed (silica, hexane/diethyl ether 9:1) to give nitrile (3*S*,7*R*)-**17** (930 mg, 87%) as a colourless oil. $[\alpha]_D^{25}$ = +3.06 (c 1.11, hexane). ^{1}H NMR δ 2.31 (dd, *J*= 16.7, 5.8 Hz, 1 H), 2.22 (dd, *J*= 16.7, 6.8 Hz, 1 H), 1.83 (m, 1 H), 1.50 (m, 1 H), 1.43–1.00 (m, 13 H), 1.05 (d, *J*= 6.7 Hz, 3 H), 0.84 ppm (t, *J* = 6.9 Hz, 9 H). ^{13}C NMR δ 118.9 (C), 39.2 (CH_2), 37.2 (CH_2), 36.8 (CH_2), 36.1 (CH_2), 32.6 (CH), 30.4 (CH), 27.9 (CH), 24.7 (CH_2), 24.5 (CH_2), 24.2 (CH_2), 22.6 (CH_3), 22.5 (CH_3), 19.5 (CH_3), 19.4 ppm (CH_3). IR (film): 2954, 2925, 2868, 2248, 1461, 1383 cm^{-1}. MS (EI): *m/z* (%): 224 (2) $[M+1]^+$, 223 (1) $[M]^{+\bullet}$, 222 (7) $[M-1]^+$, 208 (69), 194 (20), 180 (70), 166 (60), 152 (82), 138 (100), 124 (63), 111 (81), 97 (79), 82 (68), 71 (92), 57 (98), 43 (92). HRMS calc for $C_{15}H_{28}N$: 222.2222 $[M-1]^+$, found 222.2223.

*(3S,7R)-3,7,11-Trimethyldodecanal [(3S,7R)-**18**]*. A 1M solution of diisobutylaluminium hydride (DIBAH) in hexane (5.30 mL, 5.30 mmol) and nitrile (3*S*,7*R*)-**17** (928 mg, 4.09 mmol) was stirred in hexane (68 mL) at room temperature under Ar for 2.5 h. Ethanol (55 mL) and water (35 mL) were added dropwise to the mixture and stirred for 1 h. The mixture was extracted with diethyl ether (3 x 40 mL). The combined extracts were washed with 3M HCl (25 mL), water (25 mL), saturated sodium hydrogen carbonate solution (25 mL) and brine (25 mL). After drying with anhydrous Na_2SO_4, the solvent was evaporated under reduced pressure to furnish aldehyde (3*S*,7*R*)-**18** (916 mg, 97%) as a colourless oil, pure enough for the next step without need of further purification. $[\alpha]_D^{25}$ = -1.22 (c 1.14, hexane).

^{1}H NMR δ 9.74 (t, *J* = 2.3 Hz, 1 H), 2.38 (ddd, *J*= 16.0, 5.7, 2.0 Hz, 1 H), 2.21 (ddd, *J* = 16.0, 7.8, 2.6 Hz, 1 H), 2.03 (m, 1 H), 1.59–0.99 (m, 14 H), 0.94 (d, *J*= 6.6 Hz, 3 H), 0.84 ppm (dd, *J*= 8.7, 6.6 Hz, 9 H). ^{13}C NMR δ 203.1 (CH), 51.1 (CH_2), 39.3 (CH_2), 37.3 (CH_2), 37.2 (CH_2), 37.0 (CH_2), 32.6 (CH), 28.1 (CH), 27.9 (CH), 24.7 (CH_2), 24.3 (CH_2), 22.6(CH_3), 22.5 (CH_3), 19.9 (CH_3), 19.5 ppm (CH_3). IR (film): 2952, 2924, 2868, 1728, 1461, 1377, 1365, 1144, 976 cm^{-1}. MS (EI): *m/z* (%): 226 (1) $[M]^+$, 182 (67), 154 (9), 137 (18), 126 (74), 123 (85), 111 (69), 97 (89), 81 (87), 71 (100), 57 (95), 43 (94). HRMS calc for $C_{15}H_{29}O$: 225.2218 $[M-1]^+$, found 225.2224.

*2,4-Dimethylpent-3-yl 3-methylbut-2-enoate (**19**).* This compound was obtained from dimethylacrylic acid (4.00 g, 40 mmol), 2,4-dimethylpentan-3-ol (14 mL, 100 mmol) in the presence of 4,4'-dimethylaminopyridine (440 mg, 3.6 mmol) in dichloromethane and N,N'-dicyclohexylcarbodiimide (1.0 M in dichloromethane, 48 mL, 48 mmol) to give ester **19** in 51% isolated yield, as previously described.[19]

*((1-(2,4-Dimethylpent-3-yloxy)-3-methylbuta-1,3-dien-1-yl)oxy)trimethylsilane (**20**).* This compound was prepared by reaction of ester **19** (4.08 g, 20.6 mmol) with lithium diisopropylamide (LDA) (22.7 mmol) in hexane followed by the addition of chlorotrimethylsilane (TMSCl) (4.70 mL, 37.08 mmol) to provide enol ether **20** (4.96 g, 89%; 95% pure by ^{1}H NMR), as previously described.[19] The compound was used in the next step without further purification.

*2,4-Dimethylpent-3-yl (2E,5RS,7S,11R)-5-hydroxy-3,7,11,15-tetramethylhexadec-2-enoate [(2E,5RS,7S,11R)-**21**].* A solution of silyl enol ether **20** (3.60 g, 12.63 mmol) in dichloromethane (10 mL) was added at -78 ºC to a mixture of aldehyde (3*S*,7*R*)-**18** (916 mg, 4.04 mmol) in dichloromethane (25 mL) and 1.0 M solution of titanium tetrachloride in dichloromethane (12.5 mL, 12.5 mmol) over 20 min. The mixture was stirred at -78 ºC for 1 h, left to warm to room temperature, and quenched with saturated sodium hydrogen carbonate

solution (80 mL). After extraction with diethyl ether (3 x 40 mL), the extracts were combined, washed with brine (20 mL), dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane/diethyl ether 98:2 to 95:5) to give alcohol (2*E*,5*RS*,7*S*,11*R*)-**21** (246 mg, 14%) as a colourless oil (none of the *Z* isomer was detected). Stereomeric mixture: ^{1}H NMR δ 5.76 (s, 1 H), 4.61 (t, *J*= 6.2 Hz, 1 H), 3.95–3.78 (m, 1 H), 2.25 (m, 2 H), 2.19 (s, 3 H), 1.89 (m, 2 H), 1.75–1.01 (m, 18 H), 0.95–0.81 ppm (m, 24 H). ^{13}C NMR δ 166.5 (C), 155.7 (C), 118.4 (CH), 81.7 (CH), 67.3 (CH), 67.0 (CH), 49.8 (CH_2), 44.8 (CH_2), 39.3 (CH_2), 38.1 (CH_3), 37.3 (CH_2), 37.2 (CH_2), 37.1 (CH_2), 32.7 (CH), 29.4 (CH), 29.2 (CH), 27.9 (CH), 24.7 (CH_2), 24.3 (CH_2), 22.7 (CH_3), 22.6 (CH_3), 20.0 (CH_3), 19.6 (CH_3), 19.5 (CH_3), 19.1 (CH_3), 17.3 ($2CH_3$), 17.2 ppm ($2CH_3$). IR (film): 3384 (b), 2956, 2926, 2867, 1715, 1644, 1462, 1220, 1129 cm^{-1}. MS (EI): *m/z* (%): 423 (1) $[M-1]^+$, 309 (49), 198 (21), 123 (15), 111 (41), 100 (100), 83 (86), 71 (53), 57 (83), 43 (65). HRMS calc for $C_{27}H_{53}O_3$: 425.3995 $[M+1]^+$, found 425.3976.

*2,4-Dimethylpent-3-yl (2E,5RS,7S,11R)-3,7,11,15-tetramethyl-5-(methylsulfonyloxy)hexadec-2-enoate [(2E,5RS,7S,11R)-**22**]*. To a solution of alcohol (2*E*,5*RS*,7*S*,11*R*)-**21** (245 mg, 0.58 mmol) in dichloromethane (2.5 mL) was added anhydrous triethylamine (0.4 mL, 2.9 mmol) and methanesulfonyl chloride (0.14 mL, 1.73 mmol) at 0 ºC. The reaction was stirred at 0 ºC for 1 h and at room temperature for 2 h. The mixture was quenched with a saturated NH_4Cl solution (10 mL) and extracted with diethyl ether (3 x 15 mL). The combined extracts were washed with brine (10 mL) and water (10 mL), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was chromatographed (silica, hexane/ethyl acetate, 98:2 to 96:4) to give mesylate (2*E*,5*RS*,7*S*,11*R*)-**22** (181 mg, 63%) as a pale yellow oil. This compound was used immediately for the next step. Stereomeric mixture: ^{1}H NMR δ 5.76 (s, 1 H), 5.03–4.89 (m, 1 H), 4.61 (t, *J*= 6.2 Hz, 1 H), 2.95 and 2.93 (2s, 3 H), 2.66–2.37 (m, 2 H), 2.20 (s, 3 H), 1.89 (m, 2 H), 1.81–0.99 (m, 14 H), 0.93 (d, *J*= 6.4 Hz, 3 H), 0.90–

0.79 ppm (m, 24 H). ^{13}C NMR δ 166.2 (C), 153.0 (C), 119.1 (CH), 81.9 (CH), 79.6 (CH), 79.2 (CH), 46.8 (CH_2), 46.1 (CH_2), 42.5 (CH_2), 42.2 (CH_2), 39.3 (CH_2), 38.7 (CH_3), 37.5 (CH_2), 37.2 (CH_2), 36.8 (CH_2), 32.7 (CH), 29.3 (CH), 29.0 (CH), 27.9 (CH), 24.7 (CH_2), 24.1 (CH_2), 24.0 (CH_2) 22.6 (CH_3), 22.5 (CH_3), 19.6 (CH_3), 19.5 ($2CH_3$), 19.1 (CH_3), 19.0 (CH_3), 18.9 (CH_3), 17.2 ppm ($4CH_3$). IR (film): 2952, 2925, 2867, 1709, 1629, 1366, 1314, 1171, 1054, 967, 796, 750 cm^{-1}.

*(2E,5RS,7S,11R)-1-Hydroxy-3,7,11,15-tetramethylhexadec-2-en-5-yl methanesulfonate [(2E,5RS,7S,11R)-**23**]*. A 1.2M solution of diisobutylaluminium hydride in toluene (1.5 mL, 1.80 mmol) was added to a cold (-78 ºC) solution of mesylate (2*E*,5*RS*,7*S*,11*R*)-**22** (180 mg, 0.36 mmol) in anhydrous THF (0.7 mL). After 2 h of stirring, the reaction mixture was warmed to -20 ºC and stirred for 2 h more. Then, saturated NH_4Cl solution (1 mL) was added and the reaction mixture allowed to reach room temperature. The mixture was extracted with ethyl acetate (3 x 10 mL) and the combined extracts were washed with brine (2 x 4 mL), dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude was passed through a pad of silica gel eluting with ether to give alcohol (2*E*,5*RS*,7*S*,11*R*)-**23** (138 mg, 96%) as a colourless oil. This compound was used immediately for the next step without purification. Stereomeric mixture: ^{1}H NMR δ 5.51 (t, *J*= 6.5 Hz, 1 H), 4.92 (m, 1 H), 4.13 (m, 2 H), 2.97 and 2.96 (2s, 3 H), 2.50–2.21 (m, 2 H), 1.72 (s, 3 H), 1.64–0.97 (m, 17 H), 0.91 (dd, *J*= 6.3, 3.7 Hz, 3 H), 0.87–0.75 ppm (m, 9 H). ^{13}C NMR δ 134.4 (C), 128.0 (CH), 80.3 (CH), 80.1 (CH), 59.0 (CH_2), 45.9 (CH_2), 45.1 (CH_2), 42.4 (CH_2), 42.2 (CH_2), 39.3 (CH_2), 38.8 (CH_3), 37.6 (CH_2), 37.3 (CH_2), 37.2 (CH_2), 36.8 (CH_2), 32.7 (CH), 30.2 (CH), 29.4 (CH), 29.1 (CH), 27.9 (CH), 24.7 (CH_2), 24.2 (CH_2), 24.0 (CH_2), 22.6 (CH_3), 22.5 (CH_3), 19.7 (CH_3), 19.6 (CH_3), 19.2 (CH_3), 16.4 ppm (CH_3). IR (film): 3380 (b), 2952, 2925, 2867, 1462, 1334, 1171, 902 cm^{-1}.

(2E,7S,11R)-3,7,11,15-Tetramethylhexadec-2-en-1-ol, (2E,7S,11R)-phytol, ***24***. A 1.0M solution of lithium aluminium hydride in ether (0.55 mL, 0.55 mmol) was added dropwise to a solution of compound (2*E*,5*RS*,7*S*,11*R*)-**23** (138 mg, 0.353 mmol) in anhydrous ether (2.0 mL) at room temperature, and the mixture was stirred for 4 h. Then, water (0.3 mL) was added followed by 1M HCl (0.3 mL). The mixture was extracted with ethyl acetate (3 x 10 mL), the combined extracts were washed with brine (2 x 5 mL), dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed (silica gel, hexane/ethyl acetate, 95:5) to give 80 mg of a mixture of (2*E*,7*S*,11*R*)-phytol, **24**, α,β,γ,δ-diunsaturated alcohol (2*E*,4*E*,7*S*,11*R*)-**24a**, and (7*S*,11*R*)-2,3-dihydrophytol in 78:5:17 ratio as a clear oil. **24**: ^{1}H NMR δ 5.39 (tq, *J*= 6.9, 1.3 Hz, 1 H), 4.25 (d, *J*= 7.0 Hz, 2 H), 1.97 (t, J=7.9 Hz, 2 H), 1.64 (s, 3 H), 1.57–0.95 (m, 19 H), 0.90–0.77 ppm (m, 12 H). ^{13}C NMR δ 140.2 (C), 123.0 (CH), 59.4 (CH_2), 39.8 (CH_2), 39.3 (CH_2), 37.3 (CH_2), 37.2 (CH_2), 37.2 (CH_2), 36.7 (CH_2), 32.7 (CH), 32.6 (CH), 27.9 (CH), 25.1 (CH_2), 24.8 (CH_2), 24.4 (CH_2), 22.6 (CH_3), 22.5 (CH_3), 19.6 ($2CH_3$), 16.1 ppm (CH_3). IR (film): 3324 (b), 2952, 2924, 2866, 1669, 1461, 1377, 1000 cm^{-1}. MS (EI): *m/z* (%): 296 (1) $[M]^+$, 196 (4), 179 (3), 151 (4), 137 (11), 123 (76), 111 (39), 95 (68), 81 (77), 71 (100), 57 (75), 43 (74). $[\alpha]_D^{25}$ = -0.91 (c 0.99, $CHCl_3$). **24a** (characteristic data): ^{1}H NMR δ 6.05 (d, J=15.4 Hz, 1H), 5.68 (dt, J=15.0, 7.3 Hz, 1H), 5.55 (bt, J=6.9 Hz, 1H), 4.26 (d, J=7.3 Hz, 2H). ^{13}C NMR δ 134.9 (C), 129.3 (CH), 127.5 (CH), 125.3 (CH), 61.35 (CH_2). (7*S*,11*R*)-2,3-Dihydrophytol (characteristic data): ^{1}H NMR δ 3.72 (m, 2H). ^{13}C NMR δ 61.31 (CH_2).

(2E,7S,11R)-3,7,11,15-Tetramethylhexadec-2-enal, (2E,7S,11R)-phytal, ***2***. Activated MnO_2 (470 mg, 5.40 mmol) was added at room temperature to a solution of (2*E*,7*S*,11*R*)-phytol, **24** (80 mg, 0.27 mmol) in dichloromethane (1.4 mL) and the reaction mixture was refluxed for 24 h. The solid was then filtered through a Celite pad and washed with dichloromethane. The combined organic layers were concentrated *in vacuo*, and the residue was purified by column

chromatography (silica, hexane/diethyl ether, 95:5) to obtain (2*E*,7*S*,11*R*)-phytal, **2** as a clear oil in three batches: first batch, 22 mg, 94% pure by ^{1}H NMR, *E/Z* ratio 96:4, containing 6% of α,β,γ,δ-diunsaturated aldehyde (2*E*,4*E*,7*S*,11*R*)-**2a**; second batch, 12 mg, 86% pure by ^{1}H NMR; *E/Z* ratio 96:4, containing 14% of **2a**; third batch, 20 mg, 76% pure by ^{1}H NMR, containing 12% of **2a**. The calculated yield of pure **2** was 58%. *E* isomer (2*E*,7*S*,11*R*)-**2**: ^{1}H NMR δ 9.99 (d, *J* = 8.1 Hz, 1 H), 5.87 (dq, *J*= 8.1, 1.1 Hz, 1 H), 2.21-2.13 (m, 2 H), 2.15 (d, J=1.2 Hz, 3H), 1.60–0.96 (br, 19 H), 0.88–0.77 ppm (m, 12 H). ^{13}C NMR δ 191.4 (CHO), 164.4 (C), 127.2 (CH), 40.9 (CH_2), 39.3 (CH_2), 37.3 ($2CH_2$), 37.2 (CH_2), 36.5 (CH_2), 32.7 (CH), 32.6 (CH), 27.9 (CH), 24.7 (CH_2), 24.6 (CH_2), 24.3 (CH_2), 22.6 (CH_3), 22.5 (CH_3), 19.6 (CH_3), 19.5 (CH_3), 17.4 ppm (CH_3). IR (film): 2952, 2924, 2867, 1675, 1632, 1461, 1379, 1193, 1116, 864, 735 cm^{-1}. MS (EI): *m/z* (%): 294 (2) $[M]^+$, 279 (3), 276 (3), 263 (7), 194 (8), 191 (10), 179 (10), 163 (25), 150 (26), 149 (64), 140 (61), 139 (56), 121 (66), 111 (76), 97 (84), 84 (100), 71 (75), 69 (77), 57 (79), 55 (79), 43 (78). $[\alpha]_D^{25}$ = +0.55 (c = 0.90, ethanol). HRMS calc for $C_{20}H_{39}O$: 295.3001 $[M+1]^+$, found 295.2988. *Z* isomer (2*Z*,7*S*,11*R*)-**2** (characteristic data): ^{1}H NMR δ 9.95 (d, *J* = 8.3 Hz, 1 H, CHO). ^{13}C NMR δ 190.7 (CHO), 164.8 (C), 128.4 (CH). MS (EI): *m/z* (%): 294 (1) $[M]^+$, 279 (1), 263 (3), 191 (3), 179 (4), 163 (9), 150 (10), 149 (26), 140 (23), 139 (23), 121 (29), 111 (47), 97 (84), 95 (42), 84 (100), 83 (56), 69 (58), 57 (67), 55 (63), 43 (71). α,β,γ,δ-Unsaturated aldehyde (2*E*,4*E*,7*S*,11*R*)-**2a** (characteristic data): ^{1}H NMR δ 10.10 (d, *J* = 8.1 Hz, 1 H, CHO). ^{13}C NMR δ 191.4 (CHO), 154.8 (C), 138.6 (CH). 134.4 (CH), 128.3 (CH). MS (EI) *m/z* (%): 292 (4) (M^+), 277 (1), 208 (2), 179 (2), 137 (6), 110 (16), 109 (40), 96 (52), 95 (100), 81 (37), 71 (38), 57 (51), 55(45), 43 (51).

Synthesis of (2*E*,7*R*,11*S*)-phytal, 3, and (2*E*,7*S*,11*S*)-phytal, 4 (Figure 2).

These compounds were obtained by a route similar to **2** from the corresponding alcohols.

Synthesis of (2*E*,7*R*,11R)-phytal, 5 (Figure 2).

This compound was obtained from commercially available (2*E*,7*R*,11R)-phytol in 85% yield, as previously reported.[13]

Insects. Fourth- and fifth-instar nymphs (N4 and N5) and adults of *D. maroccanus* were collected from the field around Alhama de Aragón (Zaragoza) (840 m altitude, Latitude (41° 20' 58.54'' (ºN)/Longitude (1° 55' 39.73'' (ºW)), Alcudia Valley (Ciudad Real) (685 m altitude, Latitude (38° 34' 49.08'' (ºN)/Longitude (4° 20' 0.60'' (ºW)), and Tierra de Ledesma (Salamanca) (833 m altitude, Latitude (41° 3' 7.00'' (ºN)/Longitude (6° 13' 33.12'' (ºW)) during the period May-July 2013-2018. After collection, insects were placed in 50x50x50 cm metallic cages except the ceiling and the bottom that were of polyvinyl chloride. Next to the lateral walls and outside the cages, 60W incandescent light bulbs were placed to facilitate thermoregulation of the insects. The cages were placed into a climatic room (27±2 ºC, 50±10% RH) under 12:12 L:D photoperiod. Nymphs and adults were fed once a day with fresh alfalfa (*Medicago sativa*) and common reed (*Phragmites australis*). Before last molt, insects were sorted by sex to get virgin adults and after last molt, adults were separated to control the age. When required, insects were sent to the Institute of Advanced Chemistry of Catalonia (CSIC) where they were maintained in similar cages in a climatic room at 29±3 ºC, 50±10% RH and 14:10 L:D photoperiod for electroantennographic (EAG) recordings and behavioral activity.

EAG activity. The electroantennogram apparatus was commercially available from Syntech (Kirchzarten, Germany) and the methodology used was based on standardized protocols.[20] Briefly, one antenna from a non-anesthetized insect was excised, and mounted on an electrode holder (Syntech). A flow of humidified pure air (ca. 750 mL/min) was continuously directed over the antenna to clean the environment and prevent its desiccation. Test stimulations were carried out by giving puffs of air (ca. 200 mL/min) for 200 ms through a Pasteur pipette with the aid of a CS-01 stimulus controller (Syntech). The pipette contained

a piece of Whatman filter paper (2.5 cm diameter) containing either the solvent (*n*-hexane, 10 µL) alone as control or the corresponding amount of the chemical, dissolved in hexane, which was allowed to evaporate before the tests. For each compound, two consecutive stimulations of each testing dose were applied at 60 s intervals over the antennae. Control puffs were applied before and after each pair of stimuli. The output signals were amplified (10×), filtered (DC to 1 kHz) with an IDAC-2 interface (Syntech), further amplified (10×), digitized on a PC and analyzed with the EAG 2000 program (Syntech). The whole EAG preparation was enclosed in a Faraday cage (70×65×60 cm) connected to the ground to prevent extraneous electric signals. The net EAG responses were calculated by subtracting the mean response to control (hexane) before and after each stimulus from the mean response to the corresponding chemical. Two EAG tests were conducted. In the first assay, a dose-response profile with (*R*,*R*)-phytal and the racemic mixture was obtained for males (N=4-10 antennae) and females (N=8-14 antennae) (8-10 weeks-old) using three doses (2, 20 and 200 µg) for each chemical. In the second assay, the electrophysiological activity elicited by 200 µg of the four diastereomers and the racemic mixture was evaluated. This high dose was required according to the only significant response shown by the antennae in the first assay. Each antenna was stimulated with all compounds in a random sequence and a total of 9-11 male antennae and 8-10 female antennae (7-8 weeks-old) were tested. Prior to each assay, adults were individually isolated for 24 h to avoid possible interference of odors from conspecific individuals.

Behavioral activity. Behavioral responses of virgin adults of controlled age (7-15 days-old females and 9-16 days-old males to all diastereoisomers of (*E*)-phytal and ca 5-7 weeks to racemic phytal at 200 µg dose were evaluated in a Y-shaped dual-choice olfactometer. The olfactometer consisted of a main glass tube (21 cm long x 29 mm i.d.) with two 10 cm arms separated by 90º. Each arm was connected to a glass adaptor (10.4 cm long x 21 mm i.d.)

containing a Whatman filter paper (25 mm diameter) with the test sample or as control. Charcoal-filtered air (700-800 mL/min was passed through the arms to carry the stimuli to the test insects. The system was lit by a 60W white bulb centered in the middle of the olfactometer to ensure homogeneous illumination. The experiments were performed from 10:00 a.m. to 6:00 p.m. at 26-29 ºC and 35-50% RH. The locusts were removed from their containers and kept individually in BugDorm round-bottomed polypropylene containers (Entomopraxis, Barcelona, Spain) covered with mesh screen lid for 24 h at 26±2 ºC, 50±10% RH and 14:10 L:D photoperiod. For the experiments, locusts were placed at the base of the main arm, and individuals walking upwind and reaching the middle of one side arm without returning to the intersection point within 5 min were recorded as positive response, and those that failed to choose either arm in the same period were excluded from analysis. A total of 25 insects per diastereomer were tested and assays for each treatment were performed on at least two different days. When half of the insects of an specific experiment was tested, the olfactometer arms were reversed to avoid any possible positional bias. After testing one compound, the Y-tube was cleaned with soap, ethanol, and acetone, allowed to dry and heated in an oven at 200 ºC for 1 h. Then, 10 µL of a 20 µg/µL solution of the diastereomer in nanograde hexane was deployed in the filter paper, the hexane allowed to evaporate and the paper placed in the treatment container. The number of insects directed to either arm was counted and the treatment response was calculated as the number of insects attracted to the compound relative to the total number of insects moving to either arm of the olfactometer (treatment+control).

Statistical analysis. Mean net electroantennographic responses were subjected to one-way ANOVA followed by Fisher's least significant difference (LSD) post-hoc test. If necessary, data were log-transformed to fulfill the assumptions of normality and heteroscedasticity. For behavioral assays and to assess the null hypothesis that the locust adults had no preference for

the treatment and control arms the Chi-square goodness-of-fit test was used. In both types of assays, the significance level was set at $\alpha = 0.05$ and all tests were performed using SPSS Statistics 17.0.

RESULTS AND DISCUSSION

Synthesis of all diastereomers of (*E*)-phytal

It has been reported that phytal, also known as phytenal, is a degradation product of phytol, widely present in plants.[21] Therefore, the easiest way to prepare (7*R*,11*R*)-phytal, **5** is by direct oxidation of the corresponding chiral phytol.[13,16] This diterpenic alcohol had been previously obtained by ring-opening reaction of (*R*)-β-methyl-β-propiolactone with (*R*)-4,8-dimethylnonyl bromide,[22] and by Mukaiyama reaction of (3*R*,7*R*)-3,7,11-trimethyldodecanal with silyl enol ether **20**.[19] For the oxidation to phytal, active MnO_2 in CH_2Cl_2 is the reagent of choice since other oxidizing agents, such as Jones reagent/acetone and pyridinium dichromate/CH_2Cl_2 produce variable amounts (4-10%) of hexahydrofarnesyl acetone, resulting from the double bond oxidative cleavage.[13] Pyridinium chlorochromate/CH_2Cl_2 has also been successfully used as oxidizing agent apparently with no side reaction.[17] Racemic (*E,Z*)-phytal has also been obtained as *E*:*Z* 1.2:1 mixture from the corresponding nitrile precursor by reduction with diisobutylaluminium hydride in CH_2Cl_2.[18] However, no synthetic approaches for the synthesis of the other diastereomers 7*R*,11*S*, 7*S*,11*R*, and 7*S*,11*S* of (*E*)-phytal or the corresponding phytol precursors have been reported.

Synthesis of the diastereomers (2*E*,7*S*,11*R*)-phytal, **2***;* (2*E*,7*R*,11*S*)-phytal, **3***;* and (2*E*,7*S*,11*S*)-phytal, **4** was accomplished through the corresponding building blocks (2*S*,6*R*)-**6**, (2*R*,6*S*)-**6** and (2*S*,6*S*)-**6**[18,23] from (*R*)- or (*S*)-citronellal, **7,** and methyl (*R*)- and (*S*)-3-hydroxy-2-methylpropionate, **11,** as chiral sources (Figures 3 and 4). Key processes of our synthesis involve: i) Li_2CuCl_4-mediated coupling of the differently protected alcohol groups

of chiral 2-methyl-1,3-propanediol, one of them as tetrahydropyranyl (THP) or *tert*-butyldimethylsilyl (TBDMS) ether and the other as a suitable leaving group such as tosylate or mesylate,[24] and ii) Lewis-acid catalyzed Mukaiyama aldol reaction[25] between silyl enol ether **20** with a suitable chiral aldehyde such as (3*S*,7*R*)-**18**, to introduce a 5-carbon prenyl unit.[19] Reduction of (*R*)-citronellal, **7** to (*R*)-citronellol, **8** followed by hydrogenation (H_2, 10% Pt/C, EtOH) provided alcohol (*R*)-**9** in 92% overall yield, which was transformed into the corresponding bromide (*R*)-**10** (N-bromosuccinimide, Ph_3P, CH_2Cl_2, 90%) Tetrahydropyranyl-protected tosylate (*R*)-**14** was prepared by protection of hydroxyester (*R*)-**11** as tetrahydropyranyl ether (*R*)-**12**[26-28] followed by diisobutylaluminium hydride reduction in toluene/THF at -20 ºC, to produce mono-protected diol (*S*)-**13** in 81% overall yield from (*R*)-**11.** It should be noted that the intermediate aldehyde can be isolated in good yield when the reaction is performed in CH_2Cl_2 at -78 ºC.[28] Tosylation of (*S*)-**13** under standard conditions provided the required tosylate (*R*)-**14** in 87% yield. Coupling reaction of (*R*)-**14** with the Grignard reagent derived from (*R*)-**10** under Schlosser's conditions[29] followed by acid hydrolysis afforded the intermediate (2*S*,6*R*)-**6** in 66% overall yield from (*R*)-**14** (Figure 3). The alternative protection of alcohol (*R*)-**11** as *tert*-butyldimethylsilyl ether afforded mixtures of products when the corresponding tosylate or mesylate was reacted with bromide (*R*)-**10** (not shown). Mesylation of (2*S*,6*R*)-**6** followed by cyanation provided nitrile (3*S*,7*R*)-**17** that was reduced with diisobutylaluminium hydride in hexane to aldehyde (3*S*,7*R*)-**18** in 72.6% overall yield from (2*S*,6*R*)-**6** (Figure 4)**.** Introduction of a new prenyl unit into the chain of aldehyde **18** was achieved through Mukaiyama aldol reaction with silyl dienol ether **20.**[19] The protected enol ether[30] was obtained by deprotonation of ester **19** followed by silylation in 45.4% yield from dimethylacrylic acid. Coupling reaction of aldehyde (3*S*,7*R*)-**18** with vinyl ether **20** in $TiCl_4/CH_2Cl_2$ afforded mixture of stereomeric alcohols **21** at C-5, from which isomer (2*E*,5*RS*,7*S*,11*R*)-**21** was obtained after careful column chromatography

purification. None of the *Z* isomer was isolated (Figure 4). The Mukaiyama reaction was used by Fleming et al.[19] to transform aldehyde (3*R*,7*R*)-**18** into (7*R*,11*R*)-phytol as a 3:1 *E*:*Z* mixture after mesylation and lithium aluminium hydride reduction. In our hands, however, direct treatment of mesylate (2*E*,5*RS*,7*S*,11*R*)-**22** with lithium aluminium hydride under different conditions gave an impractical mixture of products. Therefore, after an extensive optimization of the reduction conditions, we found that this process is preferably implemented in two steps: a first chemoselective reduction of the conjugated ester group of **22** with diisobutylaluminium hydride in toluene at -78 ºC to provide hydroxy mesylate (2*E*,5*RS*,7*S*,11*R*)-**23** in almost quantitative yield, followed by lithium aluminium hydride/ether treatment at room temperature. However, the second elimination reaction was not completely chemoselective since a small proportion (5%) of the α,β,γ,δ diunsaturated alcohol (2*E*,4*E*,7*S*,11*R*)-**24a** was also obtained from the competing dehydromesylation reaction. In addition, (7*S*,11*R*)-2,3-dihydrophytol (17%) was also obtained from overreduction. The expected (2*E*,7*S*,11*R*)-phytol, **24** was obtained in 46.6% overall yield from alcohol **21**. Selective oxidation of **24** with active MnO_2 in dichloromethane as previously described,[29] gave, after careful column chromatography purification, a first batch of (2*E*,7*S*,11*R*)-phytal, **2,** 94% pure as 96:4 *E:Z* mixture, containing 6% of the α,β,γ,δ diunsaturated aldehyde [(2*E*,4*E*,7*S*,11*R*)-**2a**. Two more fractions from the column provided the expected phytal **2** less pure (76-86%). The calculated yield of pure phytal was 58% (Figure 4). Since MnO_2 is a well-known selective oxidation reagent for allylic alcohols,[31] the 2,3-dihydrophytol present in the alcohol mixture was not oxidized under the conditions used and was easily removed by column chromatography. It should be noted that the *E* isomer of all synthetic diastereomers partially isomerized to the *Z* isomer in few hours at room temperature to reach a ca. 75:25 *E*:*Z* stereochemical mixture. Therefore, all the diastereomers

were stabilized with 20% w/w of BHT for the electroantennographic and behavioral assays. In previous tests no activity was elicited by the antioxidant.

Electroantennographic activity

In the first experiment aimed to determine the electrophysiological activity elicited by different doses of (2*E*,7*R*,11*R*)-phytal, **5** relative to the racemic material, female antennae were more sensitive than male antennae, particularly at the highest doses tested (20 and 200 μg) (Figure 5). Within each sex, the depolarization responses were always higher to stimulations with (*R,R*)-phytal, except for the 2 μg dose on female antennae, although significant difference ($P \leq 0.05$) was only apparent at the intermediate dose (20 μg). In the second experiment, aimed to assess the electroantennographic responses of each synthetic diastereomer relative to the racemic material, female antennae responded also better to all diastereomers than male antennae, with the depolarizations induced by the *R,R* diastereomer on females being significantly higher than those displayed by any other diastereomer (Figure 6). In males, the same trend was noticed with the highest statistically significant activity being displayed by the *R,R* diastereomer. In this regard, it should be noticed the *R,R* configuration of natural phytol, a very common component of locust food, so it is reasonable to argue that this configuration is maintained also in its degradation products phytal[13] and phytanic acid[15]. In both sexes the following more active diastereomers were *R,S* (42% and 58% relative to *R,R* in males and females, respectively) and *S,R* (53% and 56%, respectively), while the *S,S* and the racemic mixture elicited the weakest response. In no case, the difference was significant.

In all cases, the net electroantennographic depolarizations elicited by the different diastereomers were quite small, probably because of the very low volatility of the pheromone, in spite of the huge amount of the compound tested, and particularly by the small number of chemoreceptor organs in the antenna.[32,33] In this regard, the antennal sensory

system of acridids present a phenotypic variation in the number and type of sensilla arising from differences in food habits, crowding and previous odor environmental experience.[34] In the Moroccan locust, like in other Acrididae,[35] the most abundant sensilla are basiconic followed by coleoconic, trichoid and chaotic.[36] Although knowledge of the molecular mechanisms of olfaction in locusts is highly unknown, it appears established that odorant receptors expressed in neurons located in sensilla coeloconica would be tuned to plant and fruit odors,[37] those expressed in basiconic sensilla would be tuned to odors related to social behaviors,[38] and receptors expressed in neurons of sensilla trichodea are probably related to sexual recognition.[39] As result of the low depolarizations elicited along with the low stability of the signal, only a limited number of antennae were considered for analysis (e.g. in experiment 1 from 19-20 male and female antennae only 4-10 for males and 8-14 for females, respectively, were used). A similar situation had been found with phytol, which gave small, if any, electroantennogram responses although this terpenic alcohol was found to be tuned to a specific receptor cell in *Spodoptera littoralis* eliciting a clear response.[40]

Behavioral activity

In previous experiments, sexually mature 7-15 days-old virgin males and females did not elicit a significant preferential response towards the (*R,R*)-phytal at the dose of 20 µg (data not shown). When sexually mature 7-15 days-old virgin females were tested, the (*R,R*)-phytal at the dose of 200 µg was clearly the most effective diastereomer in a dual-choice Y olfactometer with 15 insects (79% response) being attracted to the chemical-containing arm and 4 individuals (21% response) to the control arm (6 locusts did not respond) (Table 1). The response to the treatment was significant (p=0.012, Chi-square goodness-of-fit test). Similarly, the (*R,S*)- and (*S,S*)-phytal attracted more insects to the arm containing the chemical than the control arm but the difference was not significant (p=0.275 and 0.180, respectively). The (*S,R*)-phytal and the racemic material did not induce any preferential

response on the insects tested although the latter was assayed on field-collected insects of 5-7 weeks-old (Table 1). When all diastereomers were tested on males of similar age, neither one elicited a significant preferential response towards the treatment arm (Table 1). As for females, (*R,R*)- and (*S,S*)-phytal were more attractive than the other two diastereomers but the difference *vs* control was not significant. The racemic material was also ineffective.

Previous experiments conducted with racemic phytal (20 μg) on a bioassay arena[41] with groups of 10-15 adults resulted in a preferred attraction to females over males, but attraction to males was also significant.[13] In this regard, we postulated that the attraction to males could be the result of a male-produced sex pheromone that is eavesdropped on by male congeners to locate possible mating partners.[42] Some points can be considered to explain the apparently contradictory results obtained in both types of assay. First, the different design of the bioassays; second, the age of insects (7-16 days old in the Y olfactometer and 10-30 days old in the arena bioassay); and third, the different state of the insects used, i.e. groups of 10-15 individuals in the arena experiment *vs* single locusts in the Y olfactometer. It may be possible that grouped individuals produce other minor components in addition to phytal to attract other males whereas single insects do not. This speculation will be tackled in future investigations of our group.

In summary, we have synthesized and tested electrophysiologically and behaviorally all diastereomers of (*E*)-phytal, and assessed that the *R*,*R* isomer is the most active, confirming the initial chromatographic identification as component of the sex pheromone of the Moroccan locust.

ASSOCIATED CONTENT

Supporting Information

Synthesis of intermediate (2*S*,6*R*)-**6**, and stereoisomers (2*E*,7*R*,11*S*)-phytal, **3;** (2*E*,7*S*,11*S*)-phytal, **4** and (2*E*,7*R*,11*R*)-phytal **5**. ^{1}H and ^{13}C NMR spectra of all stereoisomers. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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596

597 FIGURE CAPTIONS

598 **Figure 1**. Structure of (±)-(*Z/E*)-phytal, **1**.

599 **Figure 2**. Structure of all diastereoisomers of (*E*)-phytal, **2**-**5**.

600 **Figure 3**. Synthesis of intermediate (2*S*,6*R*)-**6. (**Abbreviations: NBS, N-bromosuccinimide;
601 DHP, 3,4-dihydro-2H-pyran; DIBAH, diisobutylaluminium hydride; TsCl, *p*-toluenesulfonyl
602 chloride; TEA, triethylamine).

603 **Figure 4**. Synthesis of (2*E*,7*S*,11*R*)-phytal, **2**. (Abbreviations: MsCl, methanesulfonyl
604 chloride; TEA, triethylamine; DIBAH, diisobutylaluminium hydride; LDA, lithium
605 diisopropylamine; TMSCl, chlorotrimethylsilane).

606 **Figure 5**. Mean electroantennographic responses (mV ± SEM) from antennae of *D.*
607 *maroccanus* males (N=4-10) and females (N=8-14) to different doses of (*R,R*)- and racemic
608 phytal. Means with different letters within sex are statistically different (one-way ANOVA
609 followed by LSD post-hoc test, α=0.05).

610 **Figure 6**. Mean electroantennographic responses (mV ± SEM) from antennae of *D.*
611 *maroccanus* males (n=9-11) and females (N=8-10) to 200 µg of the four diastereoisomers of
612 (*E*)-phytal and the racemic mixture. Means with different letters within sex are statistically
613 different (one-way ANOVA followed by LSD post-hoc test, α=0.05).

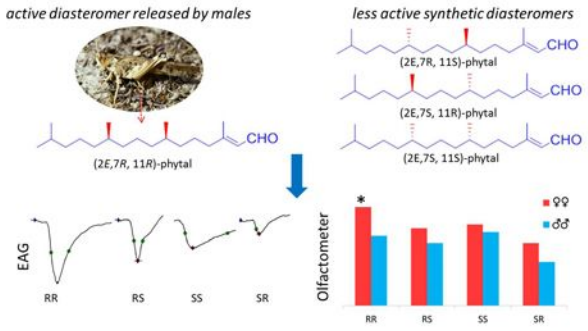
614

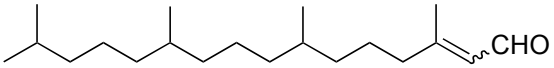
Table 1. Behavioral Response of Virgin *D. maroccanus* Females and Males to all Diastereomers of (*E*)-Phytal and the Racemic Material in a Dual-Choice Y Olfactometer

Compound	N	Sex	Age (d)	Treatment	Control	Treatment response (%)	Significance
(*R,R*)-phytal	25	F	7-15	15	4	78.95	*
(*R,S*)-phytal	25	F	7-15	13	8	61.90	NS
(*S,S*)-phytal	25	F	7-15	13	7	65.00	NS
(*S,R*)-phytal	25	F	7-15	10	10	50.00	NS
(±)-phytal	24	F	--[1]	10	11	47.62	NS
(*R,R*)-phytal	25	M	9-16	13	10	56.52	NS
(*R,S*)-phytal	25	M	9-16	9	9	50.00	NS
(*S,S*)-phytal	25	M	9-16	13	9	59.09	NS
(*S,R*)-phytal	25	M	9-16	8	15	34.78	NS
(±)-phytal	21	M	--[1]	8	10	44.44	NS

*p = 0.012 (Chi-square goodness-of-fit test at $\alpha = 0.05$)
NS = non significant
[1]Unknown age (field collected).

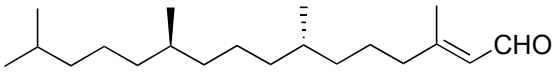
Table of Contents Graphic



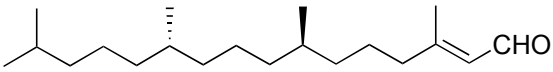


(±)-(*Z*/*E*)-phytal, **1**

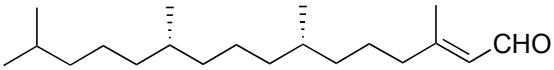
Figure 1



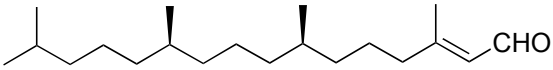
(2*E*,7*S*,11*R*)-phytal, **2**



(2*E*,7*R*,11*S*)-phytal, **3**



(2*E*,7*S*,11*S*)-phytal, **4**



(2*E*,7*R*,11*R*)-phytal, **5**

Figure 2

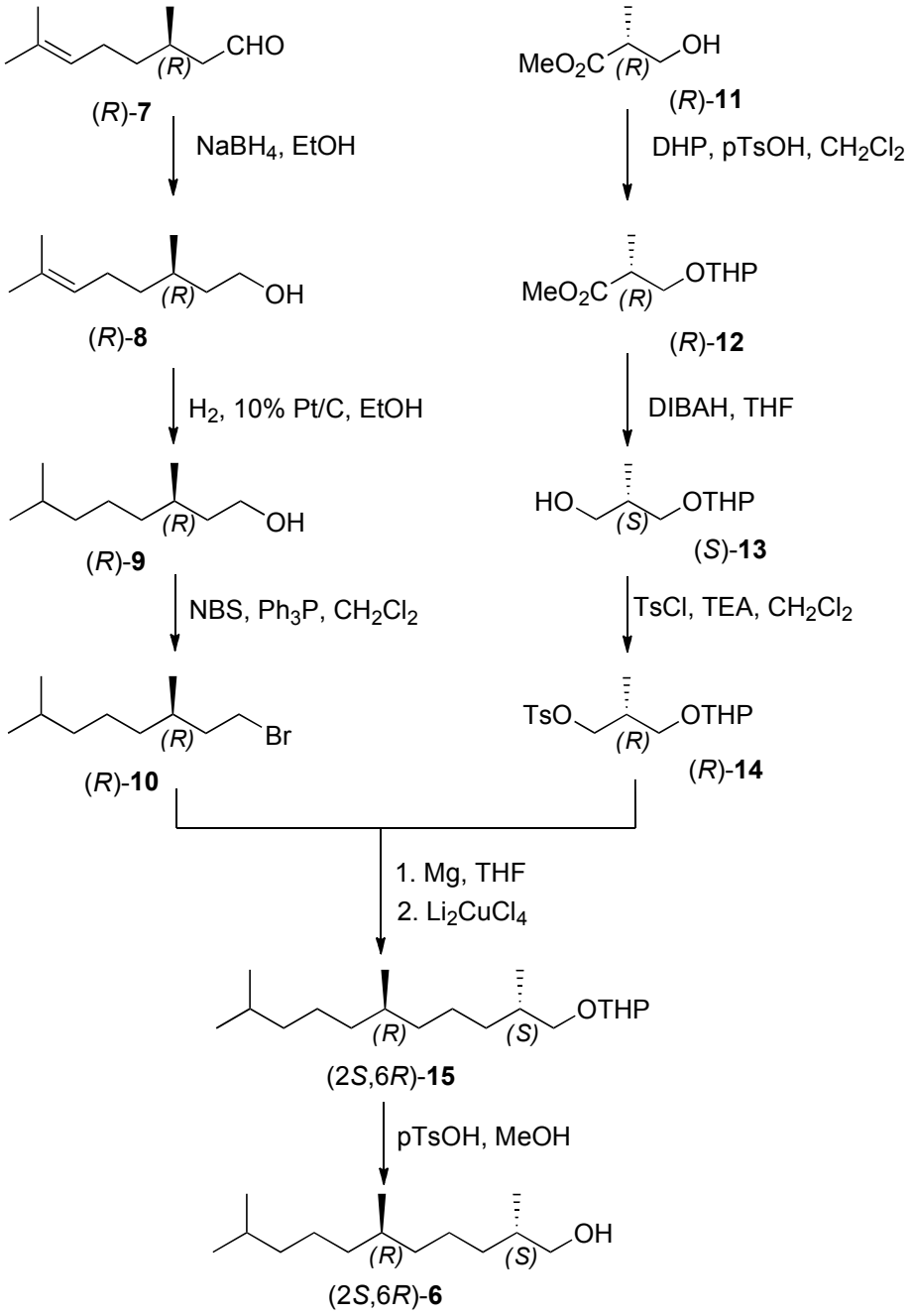

CHO
(R)
(R)-7
NaBH4, EtOH
OH
(R)
(R)-8
H2, 10% Pt/C, EtOH
OH
(R)
(R)-9
NBS, Ph3P, CH2Cl2
Br
(R)
(R)-10
MeO2C
OH
(R)
(R)-11
DHP, pTsOH, CH2Cl2
MeO2C
OTHP
(R)
(R)-12
DIBAH, THF
HO
OTHP
(S)
(S)-13
TsCl, TEA, CH2Cl2
TsO
OTHP
(R)
(R)-14
1. Mg, THF
2. Li2CuCl4
OTHP
(R)
(S)
(2S,6R)-15
pTsOH, MeOH
OH
(R)
(S)
(2S,6R)-6

Figure 3

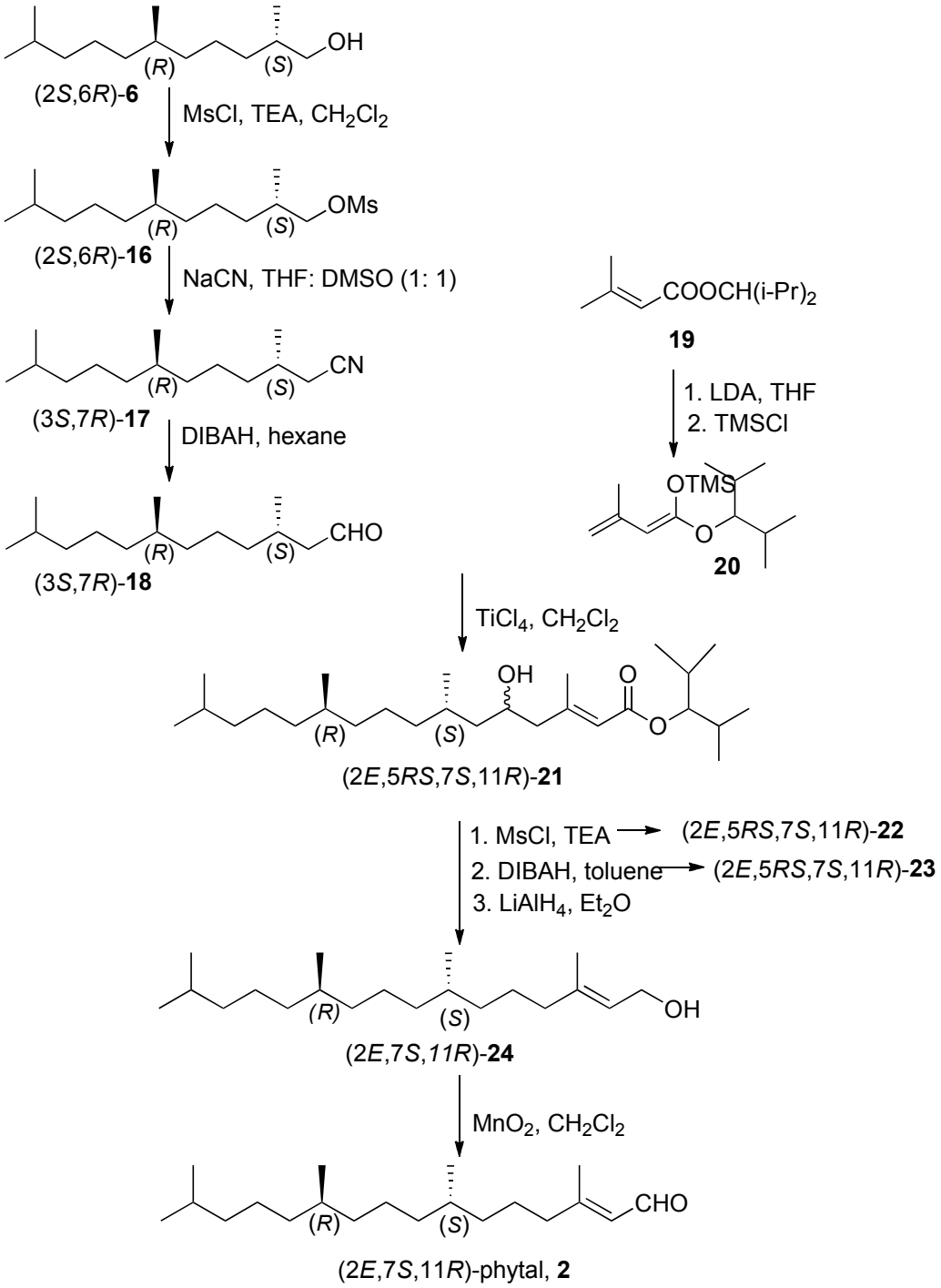

OH
(R)
(S)
(2S,6R)-6
MsCl, TEA, CH2Cl2
OMs
(2S,6R)-16
NaCN, THF: DMSO (1: 1)
CN
(3S,7R)-17
DIBAH, hexane
CHO
(3S,7R)-18
COOCH(i-Pr)2
19
1. LDA, THF
2. TMSCl
OTMS
O
20
TiCl4, CH2Cl2
OH
O
(2E,5RS,7S,11R)-21
1. MsCl, TEA
(2E,5RS,7S,11R)-22
2. DIBAH, toluene
(2E,5RS,7S,11R)-23
3. LiAlH4, Et2O
(2E,7S,11R)-24
MnO2, CH2Cl2
(2E,7S,11R)-phytal, 2

Figure 4

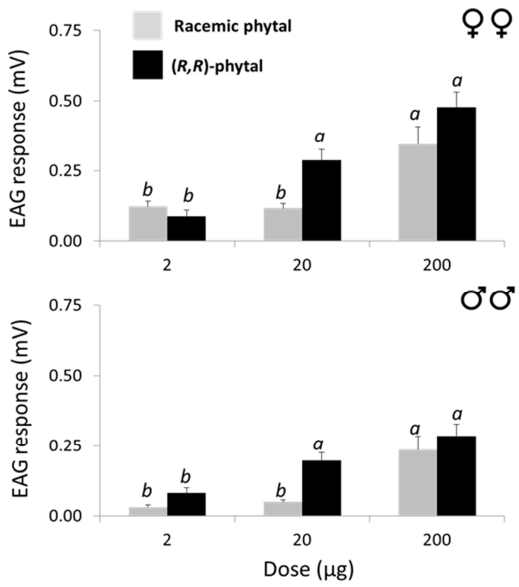

Racemic phytal
(R,R)-phytal
♀♀
♂♂
EAG response (mV)
0.75
0.50
0.25
0.00
2
20
200
Dose (µg)
a
b

Figure 5

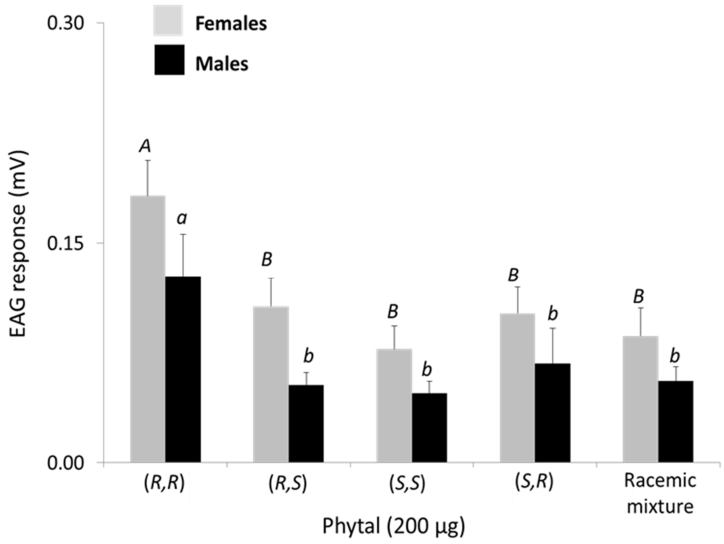

0.30
0.15
0.00
EAG response (mV)
Females
Males
A
a
B
b
B
b
B
b
B
b
(R,R)
(R,S)
(S,S)
(S,R)
Racemic mixture
Phytal (200 µg)

Figure 6