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RELATIVE CONFIGURATION OF A δ-LACTONE ISOLATED FROM MANDIBULAR GLAND SECRETION OF *Calomyrmex* sp. MALES

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Abstract—The relative configuration of a δ-lactone isolated from the mandibular gland extracts of *Calomyrmex* sp. males has been determined to be (3*SR*,5*RS*,6*SR*)-3,5,6-trimethyltetrahydropyran-2H-one after the synthesis of the four possible racemates and comparison of their mass spectra and gas chromatographic properties with those reported in the literature for the natural product.

Key Words—*Calomyrmex* sp. males, mandibular gland secretion, 3,5,6-trimethyltetrahydropyran-2-ones, synthesis.

INTRODUCTION

In studies with Australian desert ants (*Calomyrmex* sp.), Brough reported that a complex mixture of substances was secreted by the mandibular glands of disturbed workers, and the mixture was shown to have an important role in alarm communication and defense (Brough, 1976, 1977, 1978). Later, the secretion from mature workers was found to have antimicrobial activity when tested against selected soil microorganisms (Brough, 1983).

The mandibular gland of *Calomyrmex* sp. workers is extremely well developed, and the color of its secretion varies with age from white in the youngest workers to deep orange in the mature ones. However, a colorless secretion occurs in *Calomyrmex* sp. males, suggesting different roles for the mandibular gland secretion in workers and males (Brough, 1977). Considering that the

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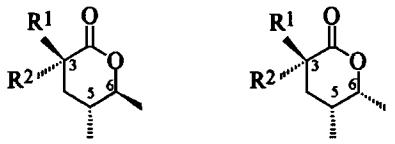


1a R^1=H, R^2=Me **1c** R^1=H, R^2=Me
1b R^1=Me, R^2=H **1d** R^1=Me, R^2=H

FIG. 1. Structures of the four possible racemates of 3,5,6-trimethyltetrahydropyran-2H-one.

mandibular gland secretions of other hymenopteran males function as sex attractants, a similar role in *Calomyrmex* sp. males is possible (Lloyd et al., 1975; Brand et al., 1973).

Brown and Moore (1979) studied the chemical constituents of the mandibular gland secretion of workers, gynes, and males by gas chromatography. While the mixture secreted by workers was shown to be composed of 6-methylhept-5-en-2-one and its corresponding alcohol, terpenoids, and pyrazines, the analysis of the pentane extract of male heads indicated two major components in approximately equal amounts: nerol and a δ-lactone that was presumed to function as a sex pheromone. It was 3,5,6-trimethyltetrahydropyran-2H-one (Figure 1) based upon mass spectrum analysis and preparation of a mixture of its four possible racemates (**1a–d**) from the condensation of 1-chloro-2-methylbutan-3-one and sodium diethyl methylmalonate. However its relative configuration was not established.

METHODS AND MATERIALS

^{1}H NMR spectra were recorded in $CDCl_3$ solution at 300 MHz and ^{13}C NMR spectra in $CDCl_3$ solution at 75.2 MHz (unless otherwise noted) with a Varian Gemini 2000 or a Bruker AC-300P instrument. Chemical shifts are expressed in parts per million relative to tetramethylsilane followed by multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; qt, quintet, m, multiplet), coupling constant, and number of protons. Infrared spectra were recorded on a Perkin-Elmer 399B or 1600 series spectrophotometer. Mass spectra were obtained via electron impact (30 eV) on a Varian MAT 311A spectrometer. Elemental analyses were performed at Instituto de Quimica, Unicamp. High-resolution mass spectra were kindly provided by Professor Armin de Meijere, Georg-August Universität, Göttingen, Germany.

GC analyses were carried out in capillary columns (30 m × 0.53 mm) with 1% phenylmethylsilicone (HP-1) or cross-linked polyethylene glycol (Carbowax

20 M) as stationary phases. GC-MS analyses were performed on a Hewlett-Packard 5890 series II gas chromatograph coupled to a MSD 5970 mass detector equipped with a capillary column (Carbowax 20 M, 25 m × 0.20 mm × 0.33 μm).

Column chromatography was performed with silica gel (70–230 mesh), except when stated otherwise, and reactions were monitored by TLC (plates from Macherey-Nagel, Germany).

Chemicals. Ether and tetrahydrofuran were treated with sodium–benzophenone and distilled immediately prior to use. Dichloromethane, triethylamine, diisopropylamine, and benzene were treated with calcium hydride and distilled immediately prior to use. Acetaldehyde, oxalyl chloride, acetic, and propionic anhydride were distilled prior to use. The remaining reagents employed were purchased from commercial suppliers and used without further purification. The reactions involving anhydrous solvents were carried out under argon atmosphere.

*(2SR,3SR)-2′,6′-Di-*tert*-butyl-4′-methylphenyl-3-hydroxy-2-methyl-butanoate* (**3**). To a LDA solution prepared from diisopropylamine (1.1 ml, 7.9 mmol) and 1.6 M *n*-BuLi in hexane (4.5 ml, 7.2 mmol) in THF (3.0 ml), we added dropwise at −78°C a solution of ester **2** (2.0 g, 7.2 mmol) in THF (2.0 ml). After 1 hr at −78°C, a solution of acetaldehyde (1.03 g, 23.5 mmol) in THF (2.0 ml) was added and the reaction stirred for 30 min and then quenched by the addition of satd. NH_4Cl (7.2 ml). The reaction mixture was diluted with ether (10 ml), and the organic phase was washed with 1% aq. HCl (3 × 5.0 ml), satd. $NaHCO_3$ (3 × 5.0 ml), and satd. NaCl (3 × 5.0 ml). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude mixture was purified by recrystallization from hexane to afford **3** (1.38 g, 4.32 mmol), as a colorless solid in 60% yield.

Mp: 109.9–110.7°C (found: C, 75.51%; H, 10.39%; $C_{20}H_{32}O_3$ requires: C, 74.96%, H, 10.06%). IR (KBr): 3360 and 1785 cm^{-1}. ^1H NMR: δ 1.30 (d, J = 6.3, 3H, CH_3-2), 1.32 [s, 9H, $-C(CH_3)_3$], 1.33 [s, 9H, $-C(CH_3)_3$], 1.47 (d, J = 7.5, 3H, CH_3-1), 2.32 (s, 3H, CH_3-4′), 2.72 (q, J = 7.5, 1H, CH-3), 3.70 (d, J = 3.30, 1H, OH), 4.02–4.08 (m, 1H, CH-2), 7.13 (s, 2H, CH-3′ e 4′). ^{13}C NMR: δ 13.1, 20.4, 21.5, 31.4, 31.5, 35.2, 35.3, 47.7, 68.9, 127.0, 127.2, 134.8, 141.8, 142.0, 145.8, 176.3. MS (*m/z*): 220 (100%), 205 (100%), 103 (23%), 73 (25%), 57 (63%), 45 (97%).

*(2RS,3SR)-1-*O-p-*Toluenesulfonyl-2-methyl-1,3-butanediol* (**4**). To a suspension of $LiAlH_4$ (0.20 g, 5.3 mmol) in THF (15 ml) at 0°C was added a solution of **3** (0.86 g, 2.7 mmol) in THF (8 ml). The reaction was stirred overnight at room temperature and then treated with water (0.20 ml), 10% aq. NaOH (0.20 ml), and water (0.60 ml), successively. The organic layer was separated, the solids were washed with ether (2 × 10 ml), and the combined organic phase was evaporated under reduced pressure. The crude product was purified by column chromatography (1 : 1 hexane–ethyl acetate) to afford (*2RS,3SR*)-2-methyl-1,3-butanediol (0.20 g, 1.9 mmol) in 70% yield.

To a solution of the above mentioned diol (0.20 g, 1.9 mmol), Et_3N (0.29 ml, 2.1 mmol) and 4-*N*,*N*-dimethylaminopyridine (0.010 g) in CH_2Cl_2 (2.0 ml) at 0°C was added *p*-toluenesulfonyl chloride (0.38 g, 2.0 mmol). The reaction mixture was kept overnight at −15°C and then diluted with CH_2Cl_2 (10 ml) and washed with 1% HCl (2 × 3 ml), satd. $NaHCO_3$ (2 × 5 ml), and satd. NaCl (2 × 5 ml). The organic layer was dried over $MgSO_4$, evaporated under reduced pressure, and the crude product was purified by column chromatography (15% ethyl acetate in hexane) to afford **4** (0.44 g, 1.7 mmol), in 90% yield, as a colorless oil (found: C, 55.79%; H, 6.97%; $C_{12}H_{18}O_4S$ requires: C, 55.79%; H, 7.02%).

IR (film): 3422 (br), 1355 and 1175 cm^{-1}. ^1H NMR: δ 0.91 (d, J = 6.7, 3H, CH_3-2), 1.15 (d, J = 6.3, 3H, CH_3-4), 1.75–1.82 (m, 1H, CH-2), 2.02 (s, br, 1H, OH), 2.45 (s, 3H, CH_3-$ArSO_2$), 3.65–3.69 (m, 1H, CH-3), 4.05 (dd, J = 9.6 and 4.6, 1H, CH_2-1), 4.11 (dd, J = 9.8 and 5.5, 1H, CH_2-1), 7.35 (d, J = 8.1, 2H, Ar), 7.79 (d, J = 8.1, 2H, Ar). ^{13}C NMR: δ 13.2, 20.9, 21.6, 40.5, 68.6, 72.8, 128.1, 130.1, 133.3, 145.0. MS (*m*/*z*): 217 (8%), 173 (100%), 172 (50%), 155 (27%), 91 (88%).

*(2*RS,*3*SR*)-1-*O-p-*Toluenesulfonyl-3-*O*-propionyl-2-methyl-1,3-butanediol* (**5**). To a solution of **4** (0.18 g, 0.70 mmol) in CH_2Cl_2 (2.0 ml) was added Et_3N (0.15 ml, 1.1 mmol), 4-*N*,*N*-dimethylaminopyridine (0.010 g), and propionic anhydride (0.10 ml, 0.78 mmol). The mixture was stirred 30 min at room temperature and then diluted with CH_2Cl_2 (10 ml). The organic phase was washed with 1% HCl (2 × 5.0 ml), satd. $NaHCO_3$ (2 × 5.0 ml), and satd. NaCl (2 × 5 ml). The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by column chromatography (5% ethyl acetate in hexane) to afford **5** (0.19 g, 0.60 mmol), in 85% yield, as a colorless oil (found: C, 57.43%; H, 6.68%; $C_{15}H_{22}O_5S$ requires C, 57.30%; H, 7.05%).

IR (film): 1730, 1355 and 1170 cm^{-1}. ^1H NMR: δ 0.94 (d, J = 6.7, 3H, CH_3-2), 1.07 [t, J = 7.6, 3H, $-OC(O)CH_2C\mathbf{H_3}$], 1.15 (d, J = 6.4, 3H, CH_3-4), 1.95–2.10 (m, 1H, CH-2), 2.21 [q, J = 7.6, 2H, $-OC(O)C\mathbf{H_2}CH_3$], 2.45 (s, 3H, CH_3ArSO_2), 3.91 (dd, J = 9.5 and 6.2, 1H, CH_2-1), 4.01 (d, J = 9.5 and 4.8, 1H, CH_2-1), 4.79 (qt, J = 6.6, 1H, CH-3), 7.36 (d, J = 8.2, 2H, Ar), 7.78 (d, J = 8.3, 2H, Ar). ^13C NMR: δ 13.0, 16.9, 21.6, 27.7, 37.7, 70.8, 71.3, 127.9, 129.8, 132.8, 144.8, 173.6.

*(3*SR,*5*RS,*6*SR)- *and (3*RS,*5*RS,*6*SR)-*3,5,6-Trimethyltetrahydropyran-2H-one* ***1a/1b***. To a solution of *tert*-BuOK (0.15 mg, 1.3 mmol) in THF (10 ml) at −20°C was added dropwise a solution of **5** (0.10 g, 0.32 mmol) in THF (6.0 ml). After stirring for 20 min, the reaction was quenched with conc. HCl (0.1 ml), dried with $MgSO_4$, and the solvent was removed under reduced pressure. Purification by column chromatography (Florisil, 2% ethyl acetate in hexane) afforded a 3 : 2 mixture of lactones **1a**/**1b** (0.023 g, 0.16 mmol), in 50% yield.

IR (film): 1732 cm^{-1}. MS (*m*/*z*): 142 (3%), 127 (3%), 98 (18%), 70 (16%),

57 (13%), 56 (100%), 55 (13%). HR-MS calcd. for $C_8H_{14}O_2$: 142.0993; found: 142.0994.

1a. ^{1}H NMR: δ 0.99 (d, *J* = 6.5, 3H, CH_3-5), 1.29 (d, *J* = 7.0, 3H, CH_3-3), 1.36 (d, *J* = 6.3, 3H, CH_3-6), 1.36 (m, 1H, CH-5), 1.6–1.7 (m, 1H, CH_2-4), 1.91 (ddd, *J* = 3.1, 6.2 and 13.3, 1H, CH_2-4), 2.48–2.52 (m, 1H, CH-3), 3.89–4.20 (m, 1H, CH-6). ^{13}C NMR: δ 17.3, 17.3, 20.2, 36.0, 36.4, 37.8, 83.7, 174.6.

1b. ^{1}H NMR: δ 1.01 (d, *J* = 5.8, 3H, CH_3-5), 1.22 (d, *J* = 6.9, 3H, CH_3-3), 1.35 (d, *J* = 6.2, 3H, CH_3-6), 1.66–1.76 (m, 3H, CH_2-4 and CH-5), 2.53–2.57 (m, 1H, CH-3), 3.89–4.20 (m, 1H, CH-6). ^{13}C NMR: δ 16.5, 17.9, 19.5, 32.7, 33.5, 35.4, 80.1, 176.5.

*(2RS,3SR)-1-*O*-p-Toluenesulfonyl-3-*O*-benzyl-2-methyl-1,3-butanediol* (**7**). To a suspension of NaH (0.029 g, 1.2 mmol) in DMF (2.0 ml) at 0°C was added benzyl bromide (0.09 ml, 0.8 mmol), followed by a solution of **4** (0.10 g, 0.40 mmol) in DMF (1.5 ml). The reaction was quenched by addition of satd. NH_4Cl (4.0 ml) after 45 min at room temperature. The aqueous phase was extracted with CH_2Cl_2 (3 × 10 ml), and the organic phase washed with water (3 × 10 ml), dried over $MgSO_4$, and the solvent evaporated under reduced pressure. Purification of the crude product by column chromatography (5% ethyl acetate in hexane) afforded **7** (0.084 g, 0.24 mmol) in 60% yield as a colorless oil (found: C, 65.07; H, 7.06%; $C_{19}H_{24}O_4S$ requires C, 65.49%; H, 6.94%).

IR (film): 1360 and 1177 cm^{-1}. ^{1}H NMR: δ 0.90 (d, *J* = 7.0, 3H, CH_3-2), 1.08 (d, *J* = 6.3, 3H, CH_3-4), 1.82–1.87 (m, 1H, CH-2), 2.36 (s, 3H, CH_3ArSO_2), 3.35 (qt, *J* = 6.4, 1H, CH-3), 3.93 (dd, *J* = 4.7 and 9.5, 1H, CH_2-1), 3.94 (dd, *J* = 5.6 and 9.5, 1H, CH_2-1), 4.22 (d, *J* = 11.7, 1H, OCH_2Ar), 4.44 (d, *J* = 11.7, 1H, OCH_2Ar), 7.12–7.24 (m, 7H, Ar), 7.66 (d, *J* = 8.3, 2H, Ar). ^{13}CNMR: δ 12.8, 16.1, 21.2, 38.5, 70.2, 71.4, 74.8, 127.0, 127.2, 127.7, 127.9, 129.4, 133.9, 138.5, 143.6. MS (*m/z*): 176 (7%), 155 (7%), 108 (7%), 107 (30%), 91 (100%), 70 (26%).

E/Z-(4RS,5SR)-Ethyl-5-O-Benzyl-2,4-dimethyl-5-hydroxy-2-hexenoate (**8**). A 5 M KOH solution (9.0 ml) was added to a solution of **7** (0.49 g, 1.4 mmol) in DMSO (1.5 ml). The reaction mixture was kept at 85°C for 12 hr, and then it was extracted with ether (3 × 30 ml). The organic phase was washed with 10% HCl (2 × 20 ml) and satd. NaCl (2 × 20 ml). The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (10% ethyl acetate in hexane) to afford (*2RS,3SR*)-3-*O*-benzyl-2-methyl-1,3-butanediol (0.16 g, 0.84 mmol) in 60% yield.

Swern oxidation (Mancuso and Swern, 1981) afforded the corresponding aldehyde (0.14 g, 0.73 mmol), in 87% yield after purification by column chromatography (2% ethyl acetate in hexane), which was dissolved in benzene (3 ml) and treated at 0°C with a benzene solution (3 ml) of the sodium salt of triethyl 2-phosphonopropionate (0.29 g, 1.1 mmol).

The reaction was stirred 1 hr, after which it was diluted with ether (5 ml) and washed with water (2 × 3 ml), 1% HCl (2 × 3.0 ml), satd. $NaHCO_3$ (2 × 3 ml), and satd. NaCl (2 × 3 ml). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (2% ethyl acetate in hexane) to afford **8** (0.14 g, 0.51 mmol) as a 2 : 1 mixture of the *E* and *Z* isomers, in 70% yield, as a colorless oil (found: C, 74.19; H, 8.70; $C_{17}H_{24}O_3$ requires: C, 73.88; H, 8.75).

(E)-***8***. IR (film): 1711 cm^{-1}. ^1H NMR (CCl_4): δ 1.02 (d, J = 6.9, 3H, CH_3-4), 1.12 (d, J = 6.2, 3H, CH_3-6), 1.30[t, J = 7.1, 3H, $C(O)OCH_2C\mathbf{H_3}$], 1.79 (d, J = 1.4, 3H, CH_3-2), 2.59–2.68 (m, 1H, CH-4), 3.37 (qt, J = 6.2, 1H, CH-5), 4.14 [q, J = 7.1, 2H, $C(O)OC\mathbf{H_2}CH_3$], 4.39 (d, J = 12.1, 1H, OCH_2Ph), 4.55 (d, J = 12.1, 1H, OCH_2Ph), 6.54 (dq, J = 1.4 and 10.0, 1H, CH-3), 7.10–7.30 (m, 5H, Ar). ^{13}C NMR (CCl_4): δ 12.4, 14.3, 15.3, 16.4, 38.5, 59.5, 70.2, 76.9, 126.0, 127.1, 127.6, 127.8, 139.0, 143.1, 166.3. MS (*m/z*): 232 (5%), 141 (14%), 135 (10%), 113 (33%), 91 (100%).

(Z)-***8***. ^1H NMR (CCl_4): δ 1.01 (d, J = 6.5, 3H, CH_3-4), 1.07 (d, J = 6.2, 3H, CH_3-6), 1.29 [t, J = 7.1, 3H, $C(O)OCH_2C\mathbf{H_3}$], 1.88 (d, J = 1.4, 3H, CH_3-2), 3.30–3.50 (m, 2H, CH-4 and CH-5), 4.14 [q, J = 7.1, 2H, $C(O)OC\mathbf{H_2}CH_3$], 4.40 (d, J = 12.1, 1H, OCH_2Ph), 4.54 (d, J = 12.0, 1H, OCH_2Ph), 5.80 (dq, J = 1.4 and 9.6, 1H, CH-3), 7.10–7.30 (m, 5H, Ar). ^{13}C NMR (CCl_4): δ 14.2, 16.1, 16.7, 20.7, 37.7, 59.2, 70.0, 77.4, 126.8, 127.1, 127.6, 127.7, 138.6, 144.6, 166.2. MS (*m/z*): 232 (5%), 141 (14%), 135 (10%), 113 (33%), 91 (100%).

*(3*SR,*5*RS,*6*SR*)- and (3*RS,*5*RS,*6*SR*)-3,5,6-Trimethyltetrahydropyran-2H-one* (***1a/1b***). A suspension of a catalytic amount of 10% Pd/C in a solution of ester **8** (0.41 g, 1.5 mmol) in ethanol (8.0 ml) was stirred 48 hr under hydrogen (3 atm) at room temperature. The mixture was filtered on Celite, the solvent removed under reduced pressure, the crude product diluted with benzene (2.0 ml), and a catalytic amount of *p*-toluenesulfonic acid (10 mg) was added. After stirring 12 hr at room temperature, the solvent was removed under reduced pressure and the crude product was purified by column chromatography (Florisil, 2% ethyl acetate in hexane) to afford a 2 : 1 mixture of lactones **1a/1b** (0.13 g, 0.90 mmol) in 60% yield.

*(4*RS,*5*SR*)-5-Hydroxy-2,4-dimethyl-2-*O*-trimethylsilyl-3-hexanone* (***10***). To a solution of diisopropylamine (0.88 ml, 6.3 mmol) in THF (10.5 ml) at −78°C was added a 2.5 M solution of *n*-BuLi (2.3 ml, 5.7 mmol). After stirring 15 min, a solution of ketone **9** (1.0 g, 5.3 mmol) in THF (2.0 ml) was added dropwise and the reaction was stirred for 1 hr at −78°C; then a solution of acetaldehyde (0.70 g, 16 mmol) in THF (2.0 ml) was added. After 45 min at −78°C, the reaction was quenched by addition of satd. NH_4Cl (2.5 ml) and the mixture was warmed to room temperature. The reaction mixture was diluted with ether (10 ml) and the organic phase was washed with 1% HCl (2 × 5 ml), satd. $NaHCO_3$ (5 ml), and satd. NaCl (5 ml). The organic phase was dried over $MgSO_4$ and the

solvent was removed under reduced pressure. The crude product was purified by Kugelrohr destillation (2 mm Hg, 65–70°C) to afford **10** (1.11 g, 4.77 mmol) as colorless oil in 90% yield.

IR (film): 3432 and 1709 cm^{-1}. ^1H NMR (CCl_4): δ 0.19 [s, 9H, $OSi(CH_3)_3$], 1.08 (d, J = 6.4, 3H, CH_3-6), 1.09 (d, J = 7.0, 3H, CH_3-4), 1.33 (s, 3H, CH_3-1), 1.36 (s, 3H, CH_3-2), 2.69–2.85 (s, br, 1H, OH), 3.18 (dq, J = 3.7 and 7.0, 1H, CH-4), 3.89 (dq, J = 3.7 and 6.4, 1H, CH-5). ^{13}C NMR (CCl_4): δ 2.2, 11.0, 20.0, 27.0, 27.3, 44.4, 67.3, 80.1, 218.0. MS (m/z): 132 (12%), 131 (100%), 75 (26%), 73 (75%), 45 (12%).

*(4*RS,*5*SR*)-5-O-Acetyl-2,4-dimethyl-2,5-dihydroxy-3-hexanone* (***11***). To a solution of **10** (1.73 g, 7.45 mmol) in CH_2Cl_2 (15 ml) were added Et_3N (1.24 ml, 8.93 mmol), acetic anhydride (0.84 ml, 8.9 mmol), and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine. The reaction mixture was stirred for 30 min at room temperature, diluted with CH_2Cl_2 (10 ml), and the organic phase was washed with water (2 × 5 ml), 5% HCl (2 × 5 ml), satd. $NaHCO_3$ (2 × 5 ml), and satd. NaCl (2 × 5 ml). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by Kugelrohr distillation (2 mm Hg, 85–90°C) and the *O*-acetyl derivative (1.73 g, 6.33 mmol, 85% yield) was dissolved in THF (13 ml) and a solution of acetic acid (3.7 ml) in water (12.5 ml). After 14 hr at room temperature, the reaction mixture was extracted with ether (2 × 25 ml) and washed with satd. $NaHCO_3$ (3 × 20 ml) and satd. NaCl (2 × 15 ml). The organic phase was dried over $MgSO_4$, the organic solvent removed under reduced pressure, and the crude product was purified by column chromatography (20% ethyl acetate in hexane) to afford **11** (1.02 g, 5.06 mmol) as a colorless oil in 80% yield.

IR (film): 3485, 1734 and 1711 cm^{-1}. ^1H NMR: δ 1.15 (d, J = 6.9, 3H, CH_3-4), 1.22 (d, J = 6.5, 3H, CH_3-6), 1.38 (s, 3H, CH_3-1), 1.40 (s, 3H, CH_3-2), 2.05 [s, 3H, $OC(O)CH_3$], 3.27 (qt, J = 6.9, 1H, CH-4), 3.61–3.69 (s, br, 1H, OH), 5.17 (qt, J = 6.5, 1H, CH-5). ^{13}C NMR: δ 14.6, 17.8, 21.2, 26.3, 26.5, 43.9, 72.0, 77.2, 170.7, 216.1. MS (m/z): 119 (16%), 83 (11%), 59 (100%), 56 (49%), 43 (55%).

*(E/Z)-(4*SR,*5*SR*)-Ethyl-5-O-acetyl-2,4-dimethyl-5-hydroxy-2-hexenoate* (***12***). To a solution of **11** (0.98 g, 4.85 mmol) in methanol (10 ml) at 0°C was added $NaBH_4$ (0.37 g, 9.7 mmol) portionwise. The reaction was stirred 1 hr at 0°C and acidified with 5% HCl (pH 4–5). The reaction mixture was saturated with solid NaCl and extracted with ether (3 × 15 ml). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was dissolved in ethanol (30 ml) and aqueous $NaIO_4$ solution (0.20 M, 60 ml) was added dropwise at 0°C, followed by aqueous NaOH (0.25 M, 2.0 ml). The reaction was kept 1 hr at 0°C, poured in water (20 ml) and extracted with CH_2Cl_2 (3 × 10 ml). The organic phase was washed with water (3 × 10 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure to furnish the (2*RS*,3*SR*)-3-*O*-acetyl-

2-methyl-2-hydroxybutyraldehyde (0.49 g, 3.4 mmol) in 70% combined yield (2 steps), which was used without further purification.

The aldehyde was dissolved in benzene (3.3 ml) and treated at 0°C with a benzene solution (4.4 ml) of the sodium salt of triethyl 2-phosphonopropionate (1.34 g, 5.16 mmol). The reaction was stirred 1 hr at 0°C, diluted with ether (20 ml), and washed with water (2 × 10 ml), 1% HCl (2 × 5 ml), satd. $NaHCO_3$ (2 × 5 ml), and satd. NaCl (2 × 5 ml). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (2% ethyl acetate in hexane) to afford **12** (0.39 g, 1.7 mmol) in 50% yield as a 1.2 : 1 mixture of the *Z* and *E* isomers, and **13** (0.23 g, 1.4 mmol) in 40% mmol) in 40% yield (found: C, 63.34; H, 9.02; $C_{12}H_{20}O_4$ requires, C, 63.14; H, 8.83).

IR (film): 1735 and 1711 cm^{-1}. MS (*m/z*): 184 (7%), 142 (100%), 141 (19%), 114 (30%), 43 (30%).

(Z)-***12***. ^1H NMR (CCl_4): δ 0.97 (d, *J* = 6.8, 3H, CH_3-4), 1.114 (d, *J* = 6.3, 3H, CH_3-6), 1.30 (t, *J* = 7.1, 3H, OCH_2CH_3), 1.89 (d, *J* = 1.5, 3H, CH_3-2), 1.97 [s, 3H, $OC(O)CH_3$], 3.21–3.41 (m, 1H, CH-4), 4.16 (q, *J* = 7.1, 2H, OCH_2CH_3), 4.68 (qt, *J* = 6.3, 1H, CH-5), 5.63 (dq, *J* = 1.5 and 10.2, 1H, CH-3). ^{13}-C NMR (CCl_4): δ 12.6, 14.2, 15.9, 17.5, 20.9, 38.0, 59.8, 73.3, 128.0, 142.6, 167.1, 169.5.

(E)-***12***. ^1H NMR (CCl_4): δ 1.02 (d, *J* = 6.8, 3H, CH_3-4), 1.16 (d, *J* = 6.3, 3H, CH_3-6), 1.29 (t, *J* = 7.1, 3H, OCH_2CH_3), 1.82 (d, *J* = 1.5, 3H, CH_3-2), 1.98 [s, 3H, $OC(O)CH_3$], 2.26–2.28 (m, 1H, CH-4), 4.14 (q, *J* = 7.1, 2H, OCH_2CH_3), 4.73 (qt, *J* = 6.3, 1H, CH-5), 6.47 (dq, *J* = 1.5 and 10.3, 1H, CH-3). ^{13}C NMR (CCl_4): δ 12.6, 14.2, 16.1, 17.3, 20.9, 37.7, 60.2, 73.3, 128.7, 141.8, 167.2, 169.7.

13. ^1H NMR (CCl_4): δ 1.28 (t, *J* = 7.1, 3H, OCH_2CH_3), 1.74 (d, *J* = 6.8, 3H, CH_3-6), 1.83 (s, 3H, CH_3-4), 1.94 (s, 3H, CH_3-2), 4.14 (q, *J* = 7.1, 2H, OCH_2CH_3), 5.64 (q, *J* = 6.8, 1H, CH-5), 6.99 (s, 1H, CH-3). ^{13}C NMR (CCl_4): δ 13.8, 13.9, 14.3, 15.9, 59.7, 125.3, 129.4, 133.1, 142.1, 167.5.

*(3*SR,*5*RS,*6*RS*)- and (3*RS,*5*RS,*6*RS*)-3,5,6-Trimethyltetrahydropyran-2H-one* (***1c*** and ***1d***). A suspension of a catalytic amount of 10% Pd/C in a solution of **12** (0.18 g, 0.79 mmol) in ethanol (40 ml) was stirred 17 hr under a hydrogen atmosphere (3 atm). The mixture was filtered on Celite and the solvent was removed under vacuum. The residue was dissolved in methanolic KOH (1.0 M, 4.0 ml), and the reaction mixture was refluxed for 2 hr. The solvent was removed under reduced pressure, the residue was treated with 5% HCl until acidic (pH 3–4), and the aqueous phase was saturated with solid NaCl and extracted with ether (3 × 10 ml). The solvent was removed under reduced pressure and the residue was dissolved in benzene (2.0 ml) and treated overnight with a catalytic amount of *p*-toluenesulfonic acid. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (Florisil, 2% ethyl acetate in hexane) to afford a 4 : 1 mixture of **1d**/**1c** (0.067 g, 0.47 mmol) in 60% yield.

IR (film): 1734 cm^{-1}. MS (*m/z*): 142 (2%), 127 (3%), 98 (11%), 70 (16%), 57 (11%), 56 (100%), 55 (20%). HR-MS calcd. for $C_8H_{14}O_2$: 142.0993; found: 142.0994.

1c. ^1H NMR (CCl_4): δ 0.94 (d, *J* = 7.0, 3H, CH_3-5), 1.14 (d, *J* = 6.7, 3H, CH_3-3), 1.27 (d, *J* = 6.6, 3H, CH_3-6), 1.62–1.72 (m, 1H, CH-5), 2.02–2.24 (m, 2H, CH_2-4), 2.43–2.49 (m, 1H, CH-3), 4.43 (dq, *J* = 6.6 and 3.3, 1H, CH-6). ^{13}C NMR (CCl_4): δ 15.4, 16.3, 16.7, 31.1, 32.9, 34.6, 75.3, 171.2.

1d. ^1H NMR (CCl_4): δ 1.01 (d, *J* = 7.0, 3H, CH_3-5), 1.23 (d, *J* = 7.1, 3H, CH_3-3), 1.27 (d, *J* = 6.6, 3H, CH_3-6), 1.62–1.72 (m, 1H, CH-5), 1.84–1.98 (m, 2H, CH_2-4), 2.43–2.49 (m, 1H, CH-3), 4.44 (dq, *J* = 6.7 and 3.3, 1H, CH-6). ^{13}C NMR (CCl_4): δ 11.8, 17.9, 18.0, 30.7, 30.8, 35.8, 78.3, 171.2.

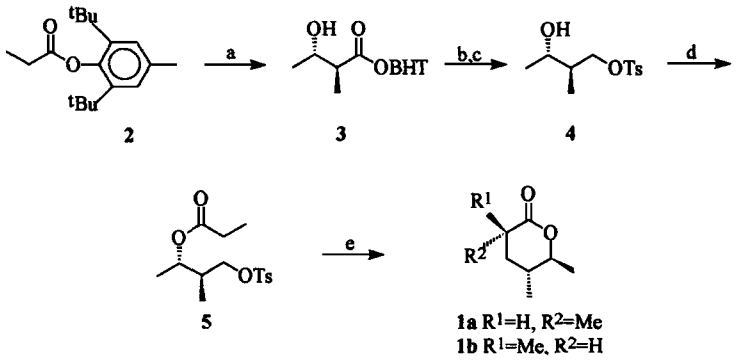
RESULTS AND DISCUSSION

Based on our previous experience on the total synthesis of ant pheromones (Pilli and Murta, 1993), we decided to approach the determination of the relative configuration of the trisubstituted tetrahydropyran-2H-one isolated from male *Calomyrmex* sp. ants by chemical synthesis of authentic samples of δ-lactones **1a/1b** and **1c/1d**. We expected that GC analyses on apolar (100% dimethylpolysiloxane) and polar (polyethylene glycol) stationary phases would allow us to determine the relative configuration of the natural lactone, since it has been previously reported (Brown and Moore, 1979) that the first eluting isomer in the synthetic mixture of the four possible racemates matched the retention time, melting point, and mass spectrum of the natural product.

Among the methodologies available for the construction of the tetrahydropyran-2H-one system, the utilization of an intramolecular alkylation (Nakai et al., 1985; Mori et al., 1991; White et al., 1992) seemed to be particularly attractive as the relative configuration of the stereogenic centers at C-5 and C-6 (Scheme 1) could be unambiguously established by stereoselective aldol condensation (Heathcock, 1993), and the stereogenic center at C-3 could be established during the intramolecular alkylation step. We previously used this plan during the total synthesis of (±)-invictolide (Pilli and Murta, 1993), a constituent of the queen recognition pheromone of the fire ant *Solenopsis invicta* and (−)-serricornine, the sex pheromone of the cigarette beetle *Lasioderma serricorne* (Pilli and de Andrade, 1994).

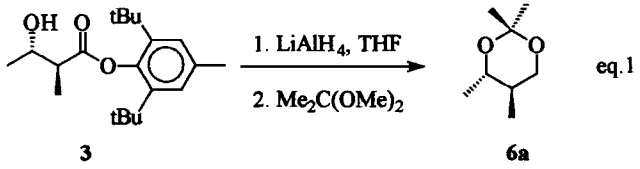
The *trans* relationship at C-5/C-6 of **1a** and **1b** was secured through the stereoselective aldol condensation of the *E*-lithium enolate derived from 2,6-di-*tert*-butyl-4-methylphenyl propionate **2** (Heathcock *et al.*, 1981) and acetaldehyde (Scheme 1).

The *anti*-hydroxy ester **3** was obtained in 60% yield as a colorless solid (mp 109.9–110.7°C) after recrystallization from hexane. The *anti* configuration of hydroxy ester **3** was unambiguously established after its conversion to acetonide



SCHEME 1. (a) i. LDA, THF, −78°C; ii. MeCHO (60%); (b) $LiAlH_4$, THF, rt. (70%); (c) TsCl, Et_3N, CH_2Cl_2, DMAP(cat.), −15°C (90%); (d) $(EtCO)_2O$, Et_3N, CH_2Cl_2, rt. (85%); (e) *t*-BuOK, THF, −20° to 0°C (50%).

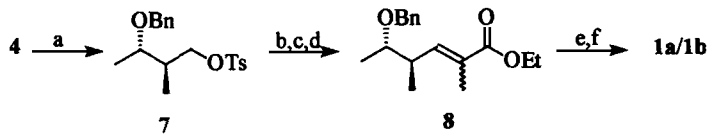
6a (equation 1) and analysis of its ^1H NMR spectrum (Lipshutz et al., 1988; Rychnovsky et al., 1993).



H-5 appeared as a multiplet at δ1.40–1.52 ppm and H-4 as a double quartet at δ3.46 ppm (J = 6.1 and 10.0 Hz), which, upon irradiation of the methyl group at C-4, collapsed to a doublet (J = 9.2 Hz), as expected for the *trans* relationship between H-4 and H-5.

Attempts to carry out the intramolecular alkylation of **5** with lithium or potassium diisopropylamide either in THF or ether did not yield lactones **1a/1b**. However, the reaction proceeded smoothly when a solution of **5** in THF was added dropwise to a THF solution of 4.0 equivalents of freshly sublimed potassium *tert*-butoxide to afford a 3 : 2 mixture of lactones **1a/1b** in 50% yield.

The configuration of the stereogenic center created during the intramolecular alkylation was established through analysis of the ^1H and ^{13}C NMR spectra of the mixture: H-3 in the major isomer appeared at higher field (multiplet, δ2.46–2.58 ppm) than in the minor isomer (multiplet, δ2.58–2.72 ppm), which was the same trend observed for (±)-invictolide and its C-3 epimer (Pilli and Murta, 1993). In the ^{13}C NMR spectra, C-2 in the major isomer and C-6 in the



SCHEME 2. (a) BnBr, NaH, DMF, rt. (60%); (b) KOH, H_2O, DMSO, 80–90°C (60%); (c) $(COCl)_2$, DMSO, CH_2Cl_2, −78°C; then Et_3N, −78°C to rt. (87%); (d) $(EtO)_2P(O)CH(Me)CO_2Et$, NaH, C_6H_6, 0°C (70%); (e) H_2, Pd/C, EtOH; (f) *p*-TsOH, C_6H_6, rt. (60%, two steps).

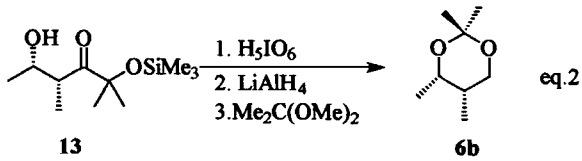
minor one appeared more shielded (δ174.6 and 80.90 ppm, respectively) than the corresponding carbons of their C-3 epimers (δ176.5 and 83.7 ppm, respectively), the same behavior previously observed for (±)-invictolide and its C-3 epimer (Pilli and Murta, 1993).

Despite the conciseness of the route described above for lactones **1a/1b**, the key step in such an approach (intramolecular alkylation) failed to provide reproducible yields. In many instances, lactones **1a/1b** were isolated in disappointingly low yields (ca. 10%). Although the route described in Scheme 1 did provide us lactones **1a/1b** for further comparisons, we decided to pursue an alternative procedure to prepare lactones **1a/1b** and **1c/1d**.

The alternative route (Scheme 2) was based on the chain elongation of protected diol **7** via its conversion to aldehyde and Wittig-Horner homologation via the sodium salt of triethyl 2-phosphonopropionate. A 2 : 1 mixture of unsaturated esters **8** favoring the *E* isomer was obtained, converted to the corresponding saturated hydroxy ester and lactonized to afford a 2 : 1 mixture of lactones **1a/1b**.

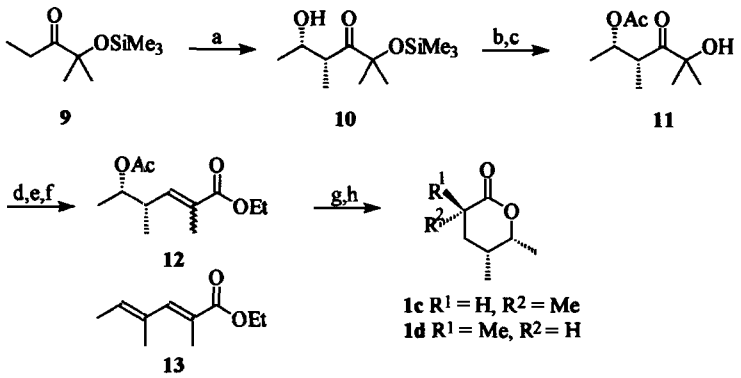
Similarly, a mixture of δ-lactones **1c/1d** (Scheme 3) was prepared starting with the stereoselective aldol condensation of acetaldehyde and the lithium enolate of the *syn*-selective silyloxyketone **9** (Heathcock et al., 1980).

The *syn*-hydroxy ketone **10** was obtained in 90% yield as a colorless oil. The *syn* stereochemistry was established after its conversion to acetonide **6b** (equation 2) and analysis of its ^{1}H-NMR spectrum (Lipshutz et al., 1988; Rychnovsky et al., 1993).



eq.2

The carbinolic proton (H-4) appeared as a double quartet (J = 6.4 and 2.6 Hz)



SCHEME 3. (a) i. LDA, THF, −78°C; ii. MeCHO (90%); (b) Ac_2O, Et_3N, DMAP(cat), CH_2Cl_2 (85%); (c) aq. HOAc, THF (80%); (d) $NaBH_4$, MeOH, 0°C (90%); (e) $NaIO_4$, H_2O, EtOH, 0°C (78%); (f) $(EtO)_2P(O)CH(Me)CO_2Et$, NaH, C_6H_6, 0°C (**12**, 50%; **13**, 40%); (g) H_2, Pd/C, EtOH (95%); (h) i. KOH, MeOH, reflux; ii. *p*-TsOH, C_6H_6, rt. (60%).

at δ 4.04 ppm and a collapsed to a doublet (J = 2.2 Hz) upon irradiation of the methyl group at C-4.

Hydroxy ketone **10** was converted to unsaturated esters **12** (68% yield), which yielded a 4 : 1 mixture of δ-lactones **1d/1c** (Scheme 3).

Analogously to the pattern described for (−)-serricornine (Redlich et al., 1988), in the ^{1}H NMR spectrum of the mixture, the methyl group at C-3 appeared downfield in the major isomer (δ 1.23 ppm) when compared with the corresponding protons in the minor one (δ 1.14 ppm).

GC-MS analysis (Carbowax 20 M and HP-1 columns, 25 m × 0.20 mm ID, film thickness 0.33 μm) of the synthetic lactones **1a/1b** and **1c/1d** revealed the same fragmentation pattern for lactones **1a**, **1b** and **1c**, **1d** as described by Brown and Moore (1979) for the natural lactone including the molecular ion at m/z 142, the characteristic loss of CO_2 (m/z 98), and the base peak at m/z 56, which corresponded to the loss of methylketene. Moreover, lactone **1a** was the first eluting component of the synthetic mixture of lactones (retention time: 11.87, 12.44, 13.18, and 13.46 min. for lactones **1a**, **1b**, **1d**, and **1c**, respectively) as observed by Brown and Moore (1979) when the natural lactone was compared with a synthetic mixture of the four stereoisomers (Figure 2).

Based on the results described above, the structure of the δ-lactone present in the mandibular gland secretion of *Calomyrmex* sp. males and described by Brown and Moore (1979) as 2,4-dimethylhexan-5-olide was assigned as (3*SR*,5*RS*,6*SR*)-3,5,6-trimethyltetrahydropyran-2H-one (**1a**).

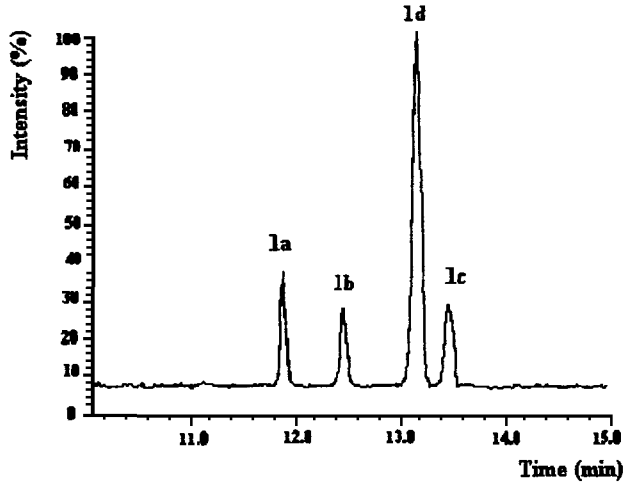


FIG. 2. GC analysis of the synthetic mixture of lactones **1a**, **1b**, **1c**, and **1d**.

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