Carbon Hydroxylation of Alkyl-tetrahydropyranols; A Paradigm for Spiroacetal Biosynthesis in *Bactrocera sp.*

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Supplementary Material

General Methods

Synthetic samples were analyzed by split QP5000 GCMS (Shimadzu) containing a J&W DB5 column employing the following temperature program: 100°C for 2minutes; 16°C/minute to 270°C for 25 minutes. NMR spectra were obtained using either a Bruker AC200F or BrukerAMX400 spectrometer using solvents specified.

Administration and analysis of fly glands

The appropriate number of laboratory reared *B. cacuminata* were collected about a week after they had emerged from pupae. The male flies were isolated and starved for 24 hours. The precursor (10µL, ~50-70µmols) which had been dissolved in methanol (20µL), and mixed with water (200µL), and sucrose (200mg) were then fed to the male *Bactrocera* over 24-48 hour period. The flies rectal glands (15-30) were excised and placed into spectrophotometric grade pentane (~0.5mL). This pentane extract was analyzed by splitless QP5050A GCMS (Shimadzu) containing a J&W cyclodexb column (0.25mm×30m) and employing the following temperature program: 40°C for 2 minutes; 20°C/minute to 120°C; 180°C for 40 minutes.

*[$^2H_4$]-2-methyl-6-pentyl-tetrahydropyran-2-ol ([$^2H_4$]-**4**)*

The 4-methyl-2-pentyl-tetrahydropyran-2-ol (**4**)[1] was mixed with THF (5mL) and D_2O (15mL). *d*-TFA (0.27g, 0.0023mmol) was added dropwise and the solution was left stirring overnight in a sealed flask. NaOD (approximately 1M) was added to neutralize the reaction, which was extracted with ethyl acetate, dried ($MgSO_{4)}$ and concentrated. GCMS indicated that between 2-3 deuterons had been incorporated. Purification by flash chromatography (Al_2O_3, 1:1 hexane: ether) gave the pale yellow oil ([$^2H_4$]-**4**). (0.321g, 79%)

*6-n-hexyl-tetrahydropyran-6-ol (**11**)*

Bromohexane (2.61mL, 14.6mmol) in anhydrous THF (10mL) was added dropwise to a mixture of magnesium turnings (0.372g, 15.3mmol) and THF (5mL), under a nitrogen atmosphere. The reaction was kept at 50˚C and left stirring for 2 hours. In a separate flask, δ-valerolactone (1.35mL, 14.6mmol) was dissolved in THF (100mL) and cooled to -40˚C (CO_2/CH_3CN). The Grignard reagent was added dropwise to the lactone over an hour and left to stir overnight. This solution was poured into chilled saturated ammonium chloride solution (50mL), extracted into ether (2x100mL), washed with brine, dried ($MgSO_4$) and concentrated. Purification by neutral alumina flash chromatography, eluting with 50% ether in hexane yielded a colourless oil (710mg, 26%). ^{13}C NMR revealed the presence of a concentration dependent equilibrium between ring open and closed forms of the product (2:1). **GCMS** (70eV) m/z(%) 186(M^+, 0.26), 168(0.34), 143(0.14), 116(7), 113(13), 98(15), 83(19), 57(24), 55(53), 43(100). **^{1}H NMR (400MHz, C_6D_6)** Predominantly Open Form δ 0.87 (t, 3H, *J* = 7.2Hz, C$\underline{H}_3$), 1.1-1.5(m, 14H), 1.96 (t, 4H, *J* = 7.28Hz (C$\underline{H}_2$O)$_2$), 3.25 (q, 2H, *J* = 6.04Hz). **^{13}C NMR (100MHz, C_6D_6)** Ring Closed δ 14.29, 19.4, 23.0, 23.58, 25.98, 30.10, 32.27, 33.53, 43.59, 61.02, 98.85. **^{13}C NMR (100MHz, C_6D_6)** Ring Opened δ 14.23, 20.32, 22.87, 24.03, 29.23, 31.98, 32.52, 42.27, 42.63, 62.10, 210.06.

*E-2-ethyl-1,7-dioxaspiro[5.5]undecane (**13**)*

(3*S*)-(3-Benzyloxy-1-ethyl-propoxy)-*tert*-butyl-diphenyl-silane

To a suspension of copper (I) iodide (107 mg, 0.56 mmol) in dry THF (10 ml) at -40ºC was added methylmagnesium

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bromide (3.75 ml of a 3.0 M solution in THF, 11.25 mmol) dropwise. After 10 minutes, a solution of (2*R*)-2-(2-Benzyloxy-ethyl)-oxirane[2] (1 g, 5.61 mmol) in dry THF (10 ml) was added via syringe slowly. The reaction mixture was stirred at -40°C for 1 h then quenched with saturated NH_4Cl, and diluted with ether. The water layer was extracted with ether (3 x 15 ml) and the combined organic extracts were dried over $MgSO_4$, filtered through a thin pad of silica gel, and concentrated under reduced pressure to give (3*S*)-1-benzyloxy-pentan-3-ol (1.05 g, 96%) as a colourless oil, which was used in the next step without further purification. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 0.94 (t, 3H, $C\underline{H}_3$, *J* = 7.5 Hz), 1.41-1.57 (m, 2H, $C\underline{H}_2CH_3$), 1.71-1.78 (m, 2H, $OCH_2C\underline{H}_2$), 3.62-3.68 (m, 1H, C<u>H</u>OH), 3.70-3.76 (m, 2H, $OC\underline{H}_2CH_2$), 4.53 (s, 2H, $OC\underline{H}_2Ph$), 7.26-7.36 (m, 5H, $C_6\underline{H}_5$). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 9.87, 30.19, 35.91, 69.29, 72.77, 73.32, 127.65, 127.71, 128.44, 137.97. **GC/MS (EI) m/z (%)** 194 (M^+, 0.5), 176 (3.8), 165 (2.8), 147 (7.5), 107 (34.0), 91 (100). **HRMS** Calcd for $C_{12}H_{19}O_2$ [M+H]: 195.1379; Found: 195.1379.

To a solution of (3*S*)-1-benzyloxy-pentan-3-ol (700 mg, 3.61 mmol) and imidazole (490 mg, 7.21 mmol) in dry acetonitrile (15 ml) was added TBDPSCl (1.6 ml, 6.04 mmol) dropwise at room temperature. The mixture was allowed to stir for 1 day and then the suspension was filtered. The filtrate was concentrated under reduced pressure to give the crude product. Purification by flash chromatography on silica gel (hexane-EtOAc 50:1) gave (3*S*)-(3-benzyloxy-1-ethyl-propoxy)-*tert*-butyl-diphenyl-silane (1.46 g, 94%) as a colourless oil. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 0.77 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.4 Hz), 1.04 (s, 9H, $C(C\underline{H}_3)_3$), 1.39-1.51 (m, 2H, $C\underline{H}_2CH_3$), 1.76-1.81 (m, 2H, $C\underline{H}_2CHOSi$), 3.42-3.52 (m, 2H, $OC\underline{H}_2CH_2$), 3.86 (m, 1H, C<u>H</u>OSi), 4.33 (d, 1H, $OC\underline{H}_2Ph$, *J* = 13.6 Hz), 4.36 (d, 1H, $OC\underline{H}_2Ph$, *J* = 13.6 Hz), 7.22-7.42 (m, 11H, $Si(C_6\underline{H}_5)_2$, $CH_2C_6\underline{H}_5$), 7.65-7.69 (m, 4H, $Si(C_6\underline{H}_5)_2$) **^{13}C NMR (100 MHz, $CDCl_3$)** δ 9.06, 19.41, 27.05, 29.58, 35.74, 67.18, 71.90, 72.74, 127.38, 127.44, 127.55, 128.25, 129.40, 129.44, 134.48, 134.67, 135.91, 138.59. **GC/MS (EI) m/z (%)** 403 (M^+-29, 0.2), 297 (1.9), 207 (100), 91 (94). **HRMS (ESI)** Calcd for $C_{28}H_{36}O_2SiNa$ [M+Na]: 455.2379; Found: 455.2382.

*(*1*S*)-tert-Butyl-(1-ethyl-3-iodo-propoxy)-diphenyl-silane ((1*S*)-**14**)

A mixture of (3*S*)-(3-benzyloxy-1-ethyl-propoxy)-*tert*-butyl-diphenyl-silane (1.1 g, 2.55 mmol) and 10% Pd/C (130 mg) in ethanol (25 ml) was shaken under an atmosphere of hydrogen (30psi) at room temperature for 5 hours. The mixture was then filtered through a pad of Celite and the filtrate was concentrated under reduced pressure to give the crude product (3*S*)-3-(tert-butyl-diphenyl-silanyloxy)-pentan-1-ol (838 mg, 96%) as a colourless oil which was used in the next step without further purification. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 0.71 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.5 Hz), 1.06 (s, 9H, $C(C\underline{H}_3)_3$), 1.41-1.60 (m, 2H, $C\underline{H}_2CH_3$), 1.65 (dddd, 1H, $C\underline{H}_2CHOSi$, *J* = 14.1, 6.2, 5.6 and 5 Hz), 1.81 (dddd, 1H, $C\underline{H}_2CHOSi$, *J* = 14.1, 8.3, 5.6 and 4.5 Hz), 3.65 (ddd, 1H, $C\underline{H}_2OH$, *J* = 11, 5.6 and 5.6 Hz), 3.76 (ddd, 1H, $C\underline{H}_2OH$, *J* = 11, 8.3 and 5 Hz), 3.88 (dddd, 1H, C<u>H</u>OSi, *J* = 7.6, 6.2, 4.5 and 4.5 Hz), 7.35-7.46 (m, 6H, $Si(C_6\underline{H}_5)_2$), 7.66-7.73 (m, 4H, $Si(C_6\underline{H}_5)_2$). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 9.36, 19.29, 27.02, 29.12, 37.08, 59.82, 73.38, 127.52, 127.63, 129.65, 129.71, 133.87, 134.21, 135.89, 135.91. **GC/MS (EI) m/z (%)** 297 (M^+-45, 0.2), 285 (13.2), 207 (34), 190 (100), 139 (39.6). **HRMS** Calcd for $C_{21}H_{31}O_2Si$ [M+H]: 343.2088; Found: 343.2088.

To a solution of (3*S*)-3-(tert-butyl-diphenyl-silanyloxy)-pentan-1-ol (775 mg, 2.26 mmol), triphenylphosphine (891 mg, 3.40 mmol) and imidazole (231 mg, 3.40 mmol) in diethyl ether-acetonitrile (3:1) (20 ml) was added iodine (862 mg, 3.40 mmol) in portions at room temperature. The mixture was stirred for 4 hours then filtered through Celite and the filtrate was concentrated under reduced pressure to give the crude product. Purification by flash chromatography on silica gel (hexane) gave (1*S*)-**14** (952 mg, 93%) as a colourless oil. **^{1}H NMR (200 MHz, $CDCl_3$)** δ 0.74 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.5 Hz), 1.05 (s, 9H, $C(C\underline{H}_3)_3$), 1.35-1.52 (m, 2H, $C\underline{H}_2CH_3$), 1.94-2.05 (m, 2H, $C\underline{H}_2CHOSi$), 3.05-3.24 (m, 2H, $C\underline{H}_2I$), 3.63-3.75 (m, 1H, C<u>H</u>OSi), 7.34-7.44 (m, 6H, $Si(C_6\underline{H}_5)_2$), 7.64-7.71 (m, 4H, $Si(C_6\underline{H}_5)_2$). **^{13}C NMR (50 MHz, $CDCl_3$)** δ 2.35, 9.09, 19.41, 27.04, 28.91, 40.14, 74.65, 127.48, 127.61, 129.55, 129.65, 133.93, 134.29, 135.87. **GC/MS (EI) m/z (%)** 395 (M^+-57, 75.5), 367 (9.4), 309 (100), 249 (43.4), 199 (10.4), 181 (14.2). **HRMS** Calcd for $C_{17}H_{20}IOSi$ [M-*t*-Bu]: 395.0328; Found: 395.0334.

2-[(4'*S*)-4'-(*tert*-Butyl-diphenyl-silanyloxy)-hexyl]-tetrahydropyran-2-ol

To a solution of diisopropylamine (390 µl, 2.78 mmol) in THF (8 ml) was added *n*-BuLi (1.5 M, 1.75 ml) slowly at –30°C. The solution was stirred at –30°C for 40 min and then cooled to –78°C. *N*,*N*-Dimethyl-*N*'-[1-methyl-5-(tetrahydropyran-2-yloxy)-pentylidene]-hydrazone[3] (483 mg, 1.99 mmol) in THF (6 ml), was dried over 4A molecular sieves, and then added to the above solution slowly. The resulting solution was allowed to stir at –78°C for 1.5 h. Iodide (1*S*)-**14** (900 mg, 1.99 mmol) in THF (6 ml) was added to the solution slowly at this temperature and the mixture was allowed to warm to room temperature slowly and stir over night. Saturated NaCl solution was added to the mixture and the water layer was extracted with EtOAc (3 x 15 ml). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product. Purification by flash chromatography on silica gel (hexane-EtOAc 10:1) gave (9*S*)-9-(*tert*-butyl-diphenyl-silanyloxy)-1-(tetrahydropyran-2-yloxy)-undeca-5-one (662 mg, 64%) as a colourless oil. **^{1}H NMR (400 MHz, $CDCl_3$)** (two diastereomers) δ 0.78 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.6 Hz), 1.05 (s, 9H, $C(C\underline{H}_3)_3$), 1.30-1.90 (m, 16H), 2.19 (m, 2H, $C(O)C\underline{H}_2$), 2.33 (m, 2H,

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[3] Mitra, R. B.; Reddy, G. B. *Synthesis* **1989**, 694-698.

C(O)C<u>H</u>$_2$), 3.30-3.53 (m, 2H), 3.66 (m, 1H, C<u>H</u>OSi), 3.70-3.88 (m, 2H), 4.56 (dd, 1H, OC<u>H</u>O, J = 4.4 and 2.4 Hz), 7.34-7.69 (m, 10H, Si(C$_6$<u>H</u>$_5$)$_2$). **^{13}C NMR (50 MHz, CDCl$_3$)** (two diastereomers) δ 9.15, 19.15, 19.38, 19.62, 20.57, 25.44, 27.03, 28.80, 29.22, 30.70, 35.01, 42.30, 42.72, 62.33, 67.15, 73.86, 98.84, 127.38, 127.41, 129.40, 134.57, 134.61, 135.88, 210.96. **GC/MS (EI) m/z (%)** 467 (M^+-57, 0.6), 423 (0.4), 365 (5.7), 305 (8.5), 199 (27.4), 139 (11.3), 85 (100). **HRMS** Calcd for $C_{28}H_{39}O_4Si$ [M-*t*-Bu]: 467.2618; Found: 467.2612.

To a solution of (9*S*)-9-(*tert*-butyl-diphenyl-silanyloxy)-1-(tetrahydropyran-2-yloxy)-undeca-5-one (383 mg, 0.73 mmol) in MeOH (4 ml) was added TsOH (25 mg, 0.15 mmol) at room temperature. The mixture was stirred for 1.5 h then saturated $NaHCO_3$ solution was added. MeOH was removed under reduced pressure and the residue was extracted with EtOAc (3 x 5 ml) then the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product. Purification by flash chromatography on silica gel (hexane-EtOAc 10:1 then hexane-EtOAc 1:1) gave a mixture of 2-[(4'*S*)-4'-(*tert*-butyl-diphenyl-silanyloxy)-hexyl]-tetrahydropyran-2-ol and its methyl acetal. This was re-dissolved in THF (4 ml) and water (0.1mL), treated with catalytic amount of TsOH and stirred at room temperature for 30 min. Workup as mentioned above gave the crude product. Purification by flash chromatography on neutral Al_2O_3 (hexane-EtOAc 3:1) gave pure 2-[(4'*S*)-4'-(*tert*-butyl-diphenyl-silanyloxy)-hexyl]-tetrahydropyran-2-ol (290 mg, 90%) as a colourless oil. NMR revealed the presence of a solvent dependent equilibrium between ring-opened and ring-closed isomers of the product (ring-opened isomer is the major form in benzene but the minor one in chloroform). **^{1}H NMR (400 MHz, C$_6$D$_6$)** Ring-opened Isomer δ 0.83 (t, CH$_2$C<u>H</u>$_3$, *J* = 7.4 Hz), 1.20 (s, C(C<u>H</u>$_3$)$_3$), 1.25-1.60 (m, C<u>H</u>$_2$), 1.85 (t, 2H, C(O)C<u>H</u>$_2$, *J* = 7.4 Hz), 1.92 (t, 2H, C(O)C<u>H</u>$_2$, *J* = 7.2 Hz), 3.36 (t, 2H, C<u>H</u>2OH, *J* = 6.3 Hz), 3.75 (qui, 1H, C<u>H</u>OSi, *J* = 5.6 Hz), 7.16-7.82 (m, Si(C$_6$<u>H</u>$_5$)$_2$). Unique peaks of the Ring-closed Isomer δ 3.50-3.60 (m, 2H, OC<u>H</u>$_2$CH$_2$), 3.85-3.94 (m, 1H, C<u>H</u>OSi). **^{13}C NMR (50 MHz, C$_6$D$_6$)** (ring-opened isomer) δ 9.35, 19.50, 19.66, 20.09, 27.32, 29.55, 32.45, 35.51, 42.07, 42.45, 62.09, 74.39, 127.88, 127.90, 128.29, 129.87, 134.97, 136.30, 209.23. **HRMS** Calcd for $C_{27}H_{28}O_2Si$ [M-H_2O]: 422.2641; Found: 422.2649.

(2*S*)-2-ethyl-1,7-dioxaspiro[5.5]undecane (**13**)

To a solution of 2-[(4'*S*)-4'-(*tert*-butyl-diphenyl-silanyloxy)-hexyl]-tetrahydropyran-2-ol (150 mg, 0.34 mmol) in THF (2 ml) was added tetrabutylammonium fluoride (1 M in THF, 3 ml) at room temperature. The mixture was stirred for 1 day then diluted with ethyl acetate (10 ml) and washed with saturated aqueous sodium chloride solution. The water layer was extracted with ethyl acetate (4 x 10 ml) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the crude product. Purification by flash chromatography on silica gel (hexane-EtOAc-Et_3N 1:1:2%) gave 2-((4'*S*)-4'-Hydroxy-hexyl-)-tetrahydropyran-2-ol (4'*S*-**17**) (37 mg, 55%). NMR revealed the presence of an equilibrium between ring-opened and -closed isomers of the product. **^{1}H NMR (200 MHz, C$_6$D$_6$)** (a mixture of 3 possible isomers) δ 0.8-1.0 (m, CH$_2$C<u>H</u>$_3$), 1.0-2.2 (m), 3.15-3.9 (m, OC<u>H</u>$_2$, OC<u>H</u>).

Compound (4'*S*)-**17** (25 mg, 0.12 mmol) was dissolved in d_6-benzene (1ml) then d_4-acetic acid (7 µl, 0.15 mmol) was added to the resulting solution. A mixture of ***E*** and ***Z*** isomers of (2*S*)-**13** were obtained after 3 days (monitored by NMR). Treating the above mixture with *d*-TFA (25 µl, 0.33 mmol) gave only ***E*** isomer after 2 days (with deuterium substituted on C5 and C11). **^{1}H NMR (400 MHz, C$_6$D$_6$/CD$_3$CO$_2$D)** (a mixture of ***E*** and ***Z*** isomers) δ 0.95 (t, 3H, *Z* CH$_2$C<u>H</u>$_3$, *J* = 7.2 Hz), 0.98 (t, 3H, *E* CH$_2$C<u>H</u>$_3$, *J* = 7.4 Hz), 1.0-1.8 (m, 24H), 1.9-2.1 (m, 4H, *E*, *Z* H4$_{ax}$ and H10$_{ax}$), 3.04-3.11 (m, 1H, *Z* H2$_{ax}$), 3.49 (m, 1H, *E* H2$_{ax}$), 3.53-3.70 (m, 3H, *Z* H8$_{eq}$, *E* H8$_{ax}$ and *E* H8$_{eq}$), 4.20 (ddd, 1H, *Z* H8$_{ax}$, *J* = 12.6, 11.2 and 2.8 Hz). **^{1}H NMR (400 MHz, C$_6$D$_6$/CD$_3$CO$_2$D/CF$_3$CO$_2$D)** (***E*** isomer) δ 0.85 (t, 3H, CH$_2$C<u>H</u>$_3$, *J* = 7.4 Hz), 0.9-1.8 (m, 10H), 3.27 (m, 1H, H2$_{ax}$), 3.44-3.6 (m, 2H, H8). **^{13}C NMR (100 MHz, C$_6$D$_6$/CD$_3$CO$_2$D)** (a mixture of ***E*** and ***Z*** isomers) δ 10.49, 10.64, 18.58, 19.04, 19.29, 19.88, 25.82, 26.02, 29.50, 29.68, 30.83, 31.21, 35.85, 36.27, 36.41, 60.18, 61.12, 70.46, 73.85, 95.47, 96.80. **^{13}C NMR (100 MHz, C$_6$D$_6$/CD$_3$CO$_2$D/CF$_3$CO$_2$D)** (***E*** isomer) δ 10.20, 18.11 (t), 18.57 (t), 25.03, 29.01, 30.38, 34.30 (m), 35.40 (m), 60.61, 71.77, 97.56. **GC/MS (EI) m/z (%) *E* isomer:** 184 (M^+, 11), 155 (14), 129 (15), 101 (94), 98 (100), 83 (42), 68 (32), 55 (87), 41 (87); ***Z* isomer:** 184 (M^+, 13), 155 (29), 129 (31), 101 (63), 98 (56), 83 (46), 68 (44), 55 (100), 41 (96).