J|A|C|S
ARTICLES

Published on Web 10/09/2008

Spiroacetal Biosynthesis in Insects from Diptera to Hymenoptera: The Giant Ichneumon Wasp *Megarhyssa nortoni nortoni* Cresson

Brett D. Schwartz,[†] Christopher J. Moore,[‡] Fredrik Rahm,[†] Patricia Y. Hayes,[†] William Kitching,[†] and James J. De Voss*,[†]

Department of Chemistry, The University of Queensland, Brisbane, Australia, and Department of Primary Industries and Fisheries, Yeerongpilly, 4105 Queensland, Australia

Received May 15, 2008; E-mail: j.devoss@uq.edu.au

Abstract: The volatile components of the mandibular gland secretion generated by the Giant Ichneumon parasitoid wasp *Megarhyssa nortoni nortoni* Cresson are mainly spiroacetals and methyl ketones, and all have an odd number of carbon atoms. A biosynthetic scheme rationalizing the formation of these diverse components is presented. This scheme is based on the results of incorporation studies using ^{2}H-labeled precursors and [^{18}O]dioxygen. The key steps are postulated to be decarboxylation of β-ketoacid equivalents, β-oxidation (chain shortening), and monooxygenase-mediated hydroxylation leading to a putative ketodiol that cyclizes to spiroacetals. The generality of the role of monooxygenases in spiroacetal formation in insects is considered, and overall, a cohesive, internally consistent theory of spiroacetal generation by insects is presented, against which future hypotheses will have to be compared.

Introduction

The spiroacetal structural motif is widespread in a variety of natural products isolated from prokaryotes and eukaryotes and from marine and terrestrial organisms.[1] In particular, simple spiroacetals represent a novel class of insect-derived semiochemicals that have been employed for both intraspecific (pheromonal) and interspecific communication. These deceptively simple molecules provide organisms with a catalogue of opportunities for conveying information by subtle structural and stereochemical variations. For example, insect-derived compounds having [4.4], [4.5], [4.6], [5.5], and [5.6]dioxaspiroalkane skeletons with a variety of substitution patterns, leading to over 30 structurally distinct parents, have been reported. In addition, the configuration of the spirocenter can be inverted via a simple ring-opening/ring-closing sequence at this acetal center. The most stable configuration of the spirocenter reflects the free energy balance between the anomeric effect and the configuration of any substituted ring carbons.

For the widely distributed dioxaspiroalkane 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**1**) (Chart 1), the SRS isomer (*E*,*E*)-**1** is ~4.8 kcal/mol more stable than the SSS diastereomer (*Z*,*Z*)-**1** that differs only in the configuration at the spirocenter.[2,3] Despite this, both of these diasteromers of **1** have been observed in nature, suggesting both a kinetically controlled cyclization in its biosynthesis and the possibility that the less-stable isomer may convey time- and or environment-dependent information. Insect spiroacetals have thus attracted much synthetic[4] attention in their own right and have served as model target systems for method development for more complex systems. However, with respect to their biosynthesis in insects (see below), little is known. We present here for the first time an experimentally founded biosynthetic route from primary metabolites to a suite of related spiroacetals against which any future proposals will have to be compared.[1]

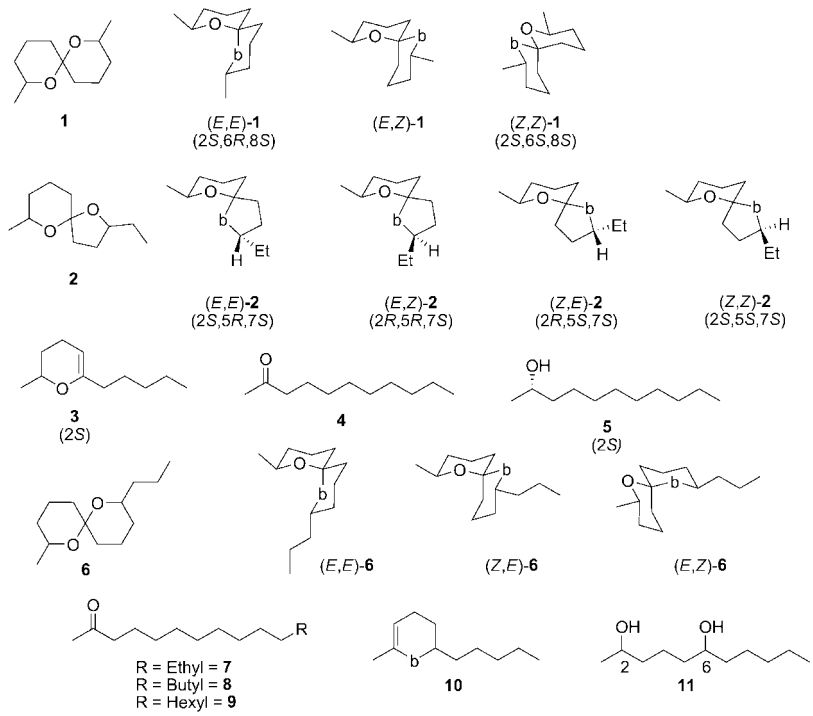
Information on the biosynthesis of insect spiroacetals was provided by our recent studies involving several Dipteran (fruit fly) species.[5] Incorporation studies employing ^{2}H-labeled precursors have shown that oxygenated spiroacetals derive from oxidation of the parent compounds and that tetrahydropyranols are suitable precursors for the production of a variety of spiroacetals via oxygenation. The proposed intervention of monooxygenases in these transformations was confirmed by an investigation of the patterns of ^{18}O incorporation into spiroacetals when both ^{18}O-labeled dioxygen and water were employed.[6] Subtleties in the formation and processing of the dioxygenated intermediate were revealed by the contrasting results obtained with *Bactrocera cucumis* (cucumber fly) as opposed to both *Bactrocera oleae* and *Bactrocera cacuminata.* In the latter two species, careful consideration of ^{2}H exchange observed in precursors suggested that a diol rather than a tetrahydropyranol was the natural spiroacetal precursor.[7] Additionally, the contrasting oxygen incorporation profiles for **1**

[†] The University of Queensland.
[‡] Department of Primary Industries and Fisheries.

(1) Francke, W.; Kitching, W. *Curr. Org. Chem.* **2001**, *5*, 233.
(2) Deslongchamps, P.; Rowan, D. D.; Pothier, N.; Sauve, G.; Saunders, J. K. *Can. J. Chem.* **1981**, *59*, 1105.
(3) Kitching, W.; Lewis, J. A.; Fletcher, M. T.; Drew, R. A. I.; Moore, C. J.; Francke, W. *J. Chem. Soc., Chem. Commun.* **1986**, 853.
(4) Schwartz, B. D.; Hayes, P. Y.; Kitching, W.; De Voss, J. J. *J. Org. Chem.* **2005**, *70*, 3054.
(5) Fletcher, M. T.; Kitching, W. *Chem. Rev.* **1995**, *95*, 789.
(6) (a) Fletcher, M. T.; Wood, B. J.; Brereton, I. M.; Stok, J. E.; De Voss, J. J.; Kitching, W. *J. Am. Chem. Soc.* **2002**, *124*, 7666. (b) Fletcher, M. T.; Mazomenos, B. E.; Georgakopoulos, J. H.; Konstantopoulou, M. A.; Wood, B. J.; De Voss, J. J.; Kitching, W. *Chem. Commun.* **2002**, 1302.

10.1021/ja8036433 CCC: $40.75

Chart 1



from *B. cucumis* (with one oxygen atom from water and the other from dioxygen)[6a] and for 1,7-dioxaspiro[5.5]undecane from *B. oleae* and *B. cacuminata* (both oxygen atoms from dioxygen) revealed contrasting origins for the dioxygenated intermediates. However, the origin of the dioxygenated precursor was unknown at the commencement of the present work.

A broader issue was whether the biosynthetic paradigm we had developed for the latter stages of spiroacetal biosynthesis in *Bactrocera* species (sp.) was generalizable across different insect genera and orders. This is of particular interest because many of the known spiroacetals have been isolated from Hymenopteran (bee and wasp) as well as from Dipteran (fly) species.[8] The North American Giant Ichneumon wasp, *Megarhyssa nortoni nortoni* (Cresson), appeared to offer the opportunity for simultaneous examination of these questions.[9] *M. nortoni* is a parasitoid wasp having a remarkable appearance (up to 15 cm in length) and lifecycle as well as some commercial importance. It detects the larvae of its host species, the wasp *Sirex noctilo*, by the volatiles emitted by the *S. noctilo* symbiotic fungus (*Amylosterum areolatum*). *M. nortoni* then employs its ovipositor (up to 10 cm in length) to drill through the wood to the *S. noctilo* larva, which is stung and paralyzed. An egg is deposited, which hatches and consumes the host larva. As *S. noctilo* infests and causes significant damage to commercial pine species, *M. nortoni* has been introduced into a number of countries as a biological control agent for it. Early work in 1985 by Davies and Madden[9a] suggested that *M. nortoni* employs a mandibular secretion to aid in aggregation, precopulatory species recognition, and defensive behavior. Chemical analysis[9a] of this secretion revealed a number of compounds, including the spiroacetal systems **1** and 2-ethyl-7-methyl-1,6-dioxaspiro-[4.5]decane (**2**) as well as the biosynthetically suggestive dihydropyran **3**, 2-undecanone (**4**), and 2-undecanol (**5**). Here we report further analyses of this secretion and then a pathway for the biosynthesis of **1** and **2** in *M. nortoni* that is based on the combined results of intermediate-isolation and precursor-incorporation studies. This proposed biogenesis pathway not only reinforces the results obtained with *Bactrocera* sp. but also provides a cohesive platform for future guidance and comparisons.

Results

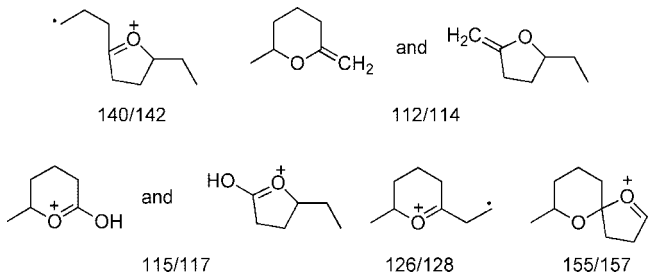
Isolation. Gas chromatography−mass spectroscopy (GC−MS) examination of pentane extracts of the excised heads of female wasps as well as solid-phase microextraction (SPME)-coupled GC−MS analyses of the released volatiles revealed a more complex range of spiroacetals than had been previously described.[9a] The major spiroacetals were the (*E*,*E*) and (*E*,*Z*) isomers of **1** and the (*E*,*E*) isomer of **2**, as reported previously.[9a] In addition, we also detected low levels of the (*E*,*Z*) and (*Z*,*Z*) isomers of **2** as well as three stereoisomers [(*E*,*E*), (*E*,*Z*), and (*Z*,*E*)] of 2-*n*-propyl-8-methyl-1,7-dioxaspiro[5.5]undecane (**6**). Furthermore, use of an enantioselective β-cyclodextrin phase in the GC−MS system confirmed through comparison with authentic standards[4] that (*E*,*E*)-**1** was the (2*S*,6*R*,8*S*) enantiomer (>98% ee) and additionally that all of the other chiral components were highly enantiomerically enriched, particularly

(7) Schwartz, B. D.; McErlean, C. S. P.; Fletcher, M. T.; Mazomenos, B. E.; Konstantopoulou, M. A.; Kitching, W.; De Voss, J. J. *Org. Lett.* **2005**, *7*, 1173.

(8) A survey of the occurrence of spiroacetals in insects across the orders Hymenoptera, Hemiptera, Diptera, and Coleoptera is presented in ref 1. In addition, see: (a) Tu, Y. Q.; Hübener, A.; Zhang, H.; Moore, C. J.; Fletcher, M. T.; Hayes, P.; Dettner, K.; Francke, W.; McErlean, C. S.; Kitching, W. *Synthesis* **2000**, *13*, 1956. (b) Zhang, H.; Fletcher, M. T.; Dettner, K.; Francke, W.; Kitching, W. *Tetrahedron Lett.* **1999**, *40*, 7851.

(9) (a) Davies, N. W.; Madden, J. L. *J. Chem. Ecol.* **1985**, *11*, 1115. (b) Also see: Nuttall, M. J. In *New Zealand: Insect Parasites of Sirex*; New Zealand State Forest Service Bulletin, 1980, vol. 47. (c) For a color photo of this spectacular wasp, see: Fletcher, M. T.; Wood, B. J.; Schwartz, B. D.; Rahm, F.; Lambert, L. K.; Brereton, I. M.; Moore, C. J.; De Voss, J. J.; Kitching, W. *Arkivoc* **2004**, *10*, 109 (issue dedicated to Professor R. Rickards).

Chart 2



(*E*,*E*)-**2**, with the most likely configuration shown in Chart 1, which corresponds to that of (*E*,*E*)-**1**.

Davies and Madden[9a] also reported that **3**, **4**, and **5** were present in the mandibular secretion of *M. nortoni*. We were able to confirm these findings and substantially extend them, as **4** is accompanied by trace amounts of a suite of other higher odd-numbered methyl ketones: 2-tridecanone (**7**), 2-pentadecanone (**8**), and 2-heptadecanone (**9**). Additionally, re-examination of the GC−MS data following the precursor-incorporation studies (see below) revealed that trace amounts of 6-methyl-2-pentyl-3,4-dihydro-2*H*-pyran (**10**) (an isomer of **3**) and 2,6-undecanediol (**11**) were also present.

[^{18}O]Dioxygen Incorporation. An apparatus described and illustrated elsewhere[9c] was used to conduct studies of ^{18}O incorporation from [^{18}O]dioxygen via SPME-coupled GC−MS analysis. The sensitivity of the technique is such that a single wasp provided sufficient volatiles for analysis in any given experiment. Mass-spectral analysis confirmed that *one oxygen atom* from dioxygen was incorporated into each of the identified spiroacetals. Careful analysis of the mass-spectral fragmentation pattern allowed the location of labeled oxygen in the nonsymmetrical spiroacetals **2** and **6** to be determined. For example, in the spiro[4.5]decane isomer **2,** fragment ions containing both oxygen atoms or selectively reporting on an oxygen atom in only one of the two rings were identified (Chart 2 and Figure 1).

The daughter ion at *m*/*z* 140 in the unlabeled compound (Figure 2) contains only the oxygen in the five-membered ring, while the ion at *m*/*z* 126 incorporates the tetrahydropyranyl oxygen.[10] The mass spectrum of (*E*,*E*)-**2** isolated after exposure of *M. nortoni* to $^{18}O_2$ (Figure 1) clearly indicated a mixture of [$^{16}O_2$]-**2** (M = 184) and [^{16}O,^{18}O]-**2** (M = 186), the former arising from endogenous **2** present in the wasp at the start of the experiment, and the ∼80% level of $^{18}O_2$ enrichment achievable.[11] Examination of the fragment ions at *m*/*z* 126 and 128 and at *m*/*z* 140 and 142 clearly indicated that the ^{18}O label resided entirely in the five-membered ring.

Similar analyses indicated that for **6**, the single ^{18}O label was located in the *n*-propyl-bearing ring[4,6a] (such oxygen atoms are shown as ● in the structures in Figure 1). The trace levels of dihydropyran **10** and diol **11** observed in these experiments were also found by careful mass-spectral analysis to contain a single ^{18}O atom derived from dioxygen. For **11**, the quality of the available mass spectrum prevented unambiguous identification of the label position, but the data were consistent with ^{18}O located at C6. [A significant ion from $C_6H_{13}{}^{18}O$ at *m*/*z* 103 resulting from C5−C6 (α) cleavage was observed.] *No* incorporation of ^{18}O from [^{18}O]dioxygen into dihydropyran **3**, the suite of methyl ketones (**4**, **7**, **8**, and **9**), or undecanol **5** occurred.

Synthesis of ^{2}H-labeled Precursors and 10. The three key potential precursors **4**, **5**, and **11** were synthesized in deuterium-labeled form. These syntheses were based upon alkyne anion chemistry that at once allowed construction of the desired compounds and incorporation of the ^{2}H labels via Pd/C-catalyzed reduction in the presence of $^{2}H_2$ (Scheme 1). Straightforward modification of **14** to hydroxyketone **19** followed by cyclization/dehydration by preparative gas chromatography afforded the previously unreported dihydropyran **10** (Scheme 2).

Separate administration of the ^{2}H-labeled monooxygenated precursors [$^{2}H_4$]-**4** and [$^{2}H_4$]-**5** (as a racemate) to female wasps demonstrated their efficient incorporation into the C_{11} spiroacetals **1** and **2**, as determined on the basis of the changes in the observed mass-spectral fragmentation patterns. Additionally, both of the dihydropyrans **3** and **10** were found to be ^{2}H-labeled, and interconversion between the alcohol [$^{2}H_4$]-**5** and the ketone [$^{2}H_4$]-**4** was observed when either was administered to the wasps. The alcohol [$^{2}H_4$]-**5** appeared to be more efficiently incorporated than the corresponding ketone [$^{2}H_4$]-**4**, but caution must be exercised in interpreting these results. Environmental and physical factors, as well as location in the biosynthetic sequence, can influence the level of incorporation. The ^{2}H-labeled precursor [$^{2}H_4$]-**11** was also very efficiently incorporated into the C_{11} spiroacetal systems **1** and **2** as well as into both of the dihydropyrans **3** and **10** by a male wasp.

Experimental Section

General Procedures. ^{1}H NMR spectra were recorded at 400 or 500 MHz using the signal of residual $CHCl_3$ in $CDCl_3$ (δ 7.24) or benzene in C_6D_6 (δ 7.15) as an internal standard. ^{13}C NMR spectra were recorded at 100 or 125 MHz with the central peak of either the $CDCl_3$ triplet (δ 77.00) or the multiplet of benzene (δ 128.0) as an internal standard. *J* values are reported in Hz. The GC−MS data were recorded at 70 eV on a gas chromatograph−mass spectrometer fitted with a 30 m × 0.25 mm BP5 column. The standard program was set as follows: 2 min at 100 °C, a temperature increase to 250 °C at a heating rate of 16 °C/min, and holding for 10 min at 250 °C. Preparative gas chromatography was performed to the following specifications: Column, 10% OV3 Chrom P; flow rate, 100 kg/cm^3; column temperature, 150 °C (isothermal); detector temperature, 200 °C; injector temperature, 250 °C. Flash chromatography was carried out using Merck Kieselgel 60 (230−400 mesh) or Scharlau silica gel (200−400 mesh). Moisture- or air-sensitive experiments were conducted in oven- or flame-dried glassware under an atmosphere of nitrogen. Anhydrous tetrahydrofuran (THF) and diethyl ether were distilled off sodium wire; dichloromethane was distilled off CaH_2 under a nitrogen atmosphere. Compounds that contained deuterium were also synthesized in their unlabeled form, and their subsequent spectral data has been reported. Specimens of *M. nortoni nortoni* were supplied by Dr. Charlma Phillips (Forestry SA, Mount Gambier SA) and then caged and fed on sugar water. Fabrication of the glassware was performed by the University of Queensland Glassblowing Services.

Administration of Labeled Precursors and Analysis. Potential precursors were administered on sugar to *M. nortoni* in 250 mL conical flasks fitted with septa. The compound was dispersed onto 30−50 mg of sugar (0.25% w/w) and placed in the conical flask. SPME samples were taken at 10 h and after 2 days or wasp death, and then the insect heads were crushed into pentane and analyzed by GC−MS. [^{18}O]Dioxygen (>99 atom % ^{18}O) was obtained from Isotech (Miamisburg, Ohio). *M. nortoni* were monitored by SPME

(10) For further discussion of the underlying arguments, see ref 6a and its Supporting Information.
(11) The sheer size of *M. nortoni* prevented utilization of the protocol for $^{18}O_2$ incorporation studies described in ref 9c. A partial evacuation/refill strategy was adopted that minimized harm to the wasp and generated an atmosphere that was an ∼4:1 $^{18}O_2/^{16}O_2$ mixture.
(12) Marshall, J. A.; Wang, X. J. *J. Org. Chem.* **1991**, *56*, 960.

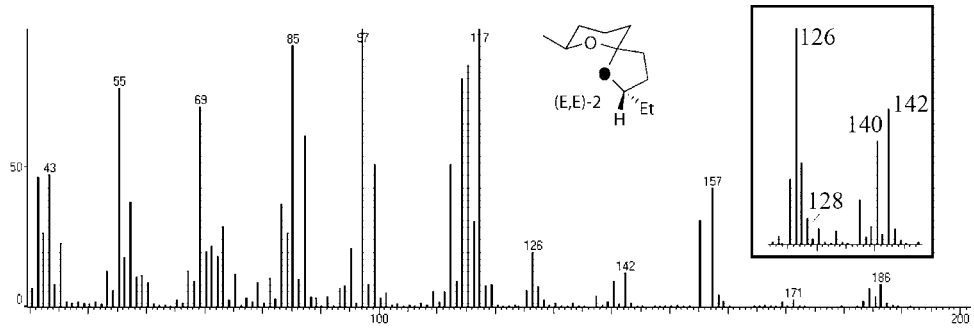


Figure 1. Mass spectrum of (*E*,*E*)-**2** from *M. nortoni* exposed to [^{18}O]dioxygen (• = ^{18}O).

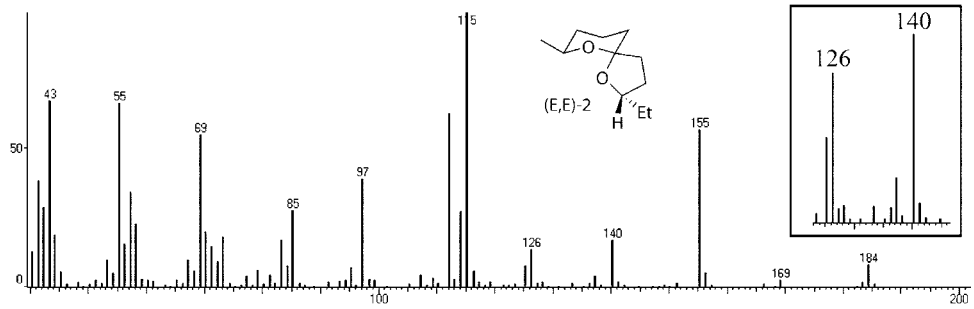
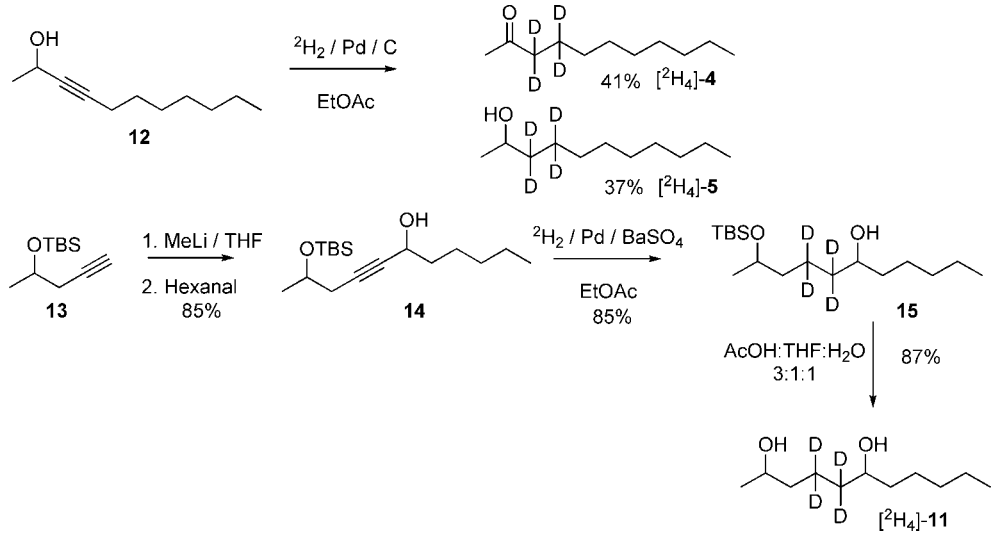
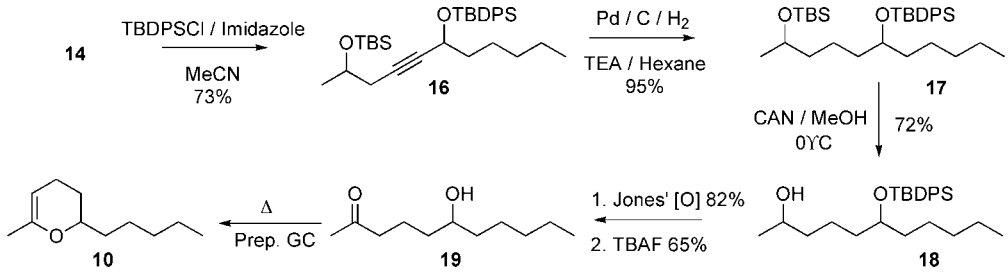


Figure 2. Mass spectrum of (*E*,*E*)-**2** from *M. nortoni* in normal air.

Scheme 1



Scheme 2



during exposure to $^{18}O_2$ in a purpose-built chamber.[9c] After 18 h, the insect heads were crushed into pentane and analyzed by GC−MS. SPME analyses were conducted with a Carboxen PDMS fiber (Supelco). Nonenantioselective GC−MS analyses were conducted on a J&W Scientific DB-5MS 30 m × 0.25 mm × 0.25 μm column with the following method: 2 min at 40 °C, a temperature increase to 260 °C at 10 °C/min, then holding at 260 °C for 6 min (total run time of 30 min). Enantioselective GC experiments were conducted on a J&W Scientific CDX-B (Cyclodex-B) 30 m × 0.25 mm, 0.25 μm film column with the following temperature program: 2 min at 40 °C, increased to 200 °C at 5 °C/min and then without delay to 240 °C at 20 °C/min, where it was held for 20 min (total

run time of 56 min). All of the insect GC−MS analyses were conducted on a VG TRIO-2000 instrument (VG Biotech, Altrincham, U.K.).

[3,3,4,4-$^2H_4$]-2-Undecanone ([$^2H_4$]-4) and [3,3,4,4-$^2H_4$]-2-Undecanol ([$^2H_4$]-5). Palladium catalyst (10% on carbon) was added to a solution of undec-3-yn-2-ol[12] (**12**) (200 mg, 1.20 mmol) in EtOAc (10 mL) and left to stir under a deuterium atmosphere. After 3 h, the reaction was diluted with ether and filtered through Celite. The solution was concentrated in vacuo and purified by flash chromatography on silica gel (1:5 EtOAc/hexane), furnishing [$^2H_4$]-**4** (86 mg, 41%) and [$^2H_4$]-**5** (78 mg, 37%) as colorless oils. The ^{13}C NMR spectra clearly showed that there were no residual unlabeled methylenes at either C3 or C4 (see the Supporting Information), although a significant amount of monodeuteration occurred at C3 and C4.

Data for [$^2H_4$]-**4**: ^1H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, J = 6.7 Hz, 3H), 1.24 (m, 12H), 1.51−1.56 (m, 1H, from scattered reduction), 2.11 (s, 3H), 2.40−2.41 (m, 1H, from scattered reduction). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.6, 22.9−23.8 (m), 28.9−29.0 (m), 29.2, 29.3−29.4 (m), 29.4, 29.8, 31.8, 43.1−43.4 (m), 209.5 (m). GC−MS EI *m/z* (%): 174 [$M^{+\bullet}$] (6), 159 (2), 143 (1), 131 (1), 114 (4), 99 (3), 86 (9), 74 (27), 60 (91), 43 (100).

Data for [$^2H_4$]-**5**: ^1H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, J = 7.1 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H), 1.18−1.29 (m, 12 H), 1.34−1.43 (m, 1.96H, OH and 1H from scattered reduction), 3.76 (quintet, J = 6.1 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 22.7, 23.3−23.4 (m), 24.4−25.7 (m, $COCD_2CD_2$), 28.2−29.6 (m, 4C), 31.9, 38.0−39.3 (m, $COHCD_2$), [67.96 (s, $COHCD_2$), 68.0 (s, *C*OHCDH), 68.1 (s, *C*OHCH$_2$)]. GC−MS EI *m/z* (%): 176 [$M^{+\bullet}$] (2), 158 (1), 145 (0.4), 133 (1), 114 (3), 101 (2), 88 (6), 75 (14), 62 (79), 43 (100).

2-(*tert*-Butyldimethylsilyloxy)undec-4-yn-6-ol (14). *tert*-Butyldimethyl(pent-4-yn-2-yloxy)silane[13] (**13**) (5.80 g, 29.2 mmol) was cooled to −20 °C (EtOH/H_2O/CO_2) in THF (25 mL) under N_2, and then methyllithium (20.9 mL, 29.2 mmol, 1.4 M in Et_2O) was added dropwise. The solution was allowed to stir at −20 °C for 30 min, and then hexanal (2.92 g, 29.2 mmol) in THF (25 mL) was added over 5 min. After 2 h, the mixture was poured onto ice-cold aqueous NH_4Cl, and the resulting mixture was extracted into ether (3 × 50 mL). The organic layers were combined, washed with brine (30 mL), and dried ($MgSO_4$). The ethereal solution was concentrated in vacuo, and the residue was purified by flash chromatography (eluting with 1:10 EtOAc/hexane) to yield alkynol **14** (7.40 g, 85%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$): δ 0.038 (s, 3H), 0.046 (s, 3H), 0.86 (br s, 12H), 1.20 (d, J = 5.9 Hz, 3H), 1.23−1.33 (m, 4H), 1.37−1.45 (m, 2H), 1.57−1.69 (m, 2H), 1.85 (br s, OH), 2.27 (dddd, J = 16.4, 7.1, 2.0, and 1.0 Hz, 1H), 2.35 (ddd, J = 16.4, 5.5, and 1.9 Hz, 1H), 3.89 (m, 1H), 4.31 (ddt, J = 12.0, 6.5, and 1.9 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), mixture of diastereomers: δ −4.8, −4.7, 14.0, 18.1, 22.6, 23.3, 24.9, 25.8 (3C), 29.6, 31.5, 38.1, 62.7, 67.64 and 67.65, 82.64 and 82.65, 82.82 and 82.84. GC/MS EI *m/z* (%): 298 [$M^{+\bullet}$] (0.03), 283 (0.1), 241 (4), 159 (49), 119 (100), 103 (11), 75 (82), 73 (64), 43 (21). Anal. Calcd for $C_{17}H_{34}O_2Si$: C, 68.39; H, 11.48. Found: C, 68.27; H, 11.77.

[4,4,5,5-$^2H_4$]-2-(*tert*-Butyldimethylsilyloxy)undecan-6-ol ([$^2H_4$]-15). Palladium (10 mg, 5% on $BaSO_4$) was added to a solution of alkyne **14** (1.50 g, 5.03 mmol) in 2-propanol (20 mL) and triethylamine (1 mL) and left to stir under a deuterium atmosphere. After 15 min, the mixture changed color from brown to black and was then left to stir for 1 h, after which GC−MS analysis revealed that reduction was complete. The suspension was filtered through Celite, concentrated in vacuo, and purified by flash chromatography on silica gel (1:5 EtOAc/hexane), furnishing [$^2H_4$]-**15** as a colorless oil (1.31 g, 85%).

(13) Dimitriadis, C.; Gill, M.; Harte, M. F. *Tetrahedron: Asymmetry* **1997**, *8*, 2153.
(14) DattaGupta, A.; Sing, R.; Singh, V. K. *Synlett* **1996**, 69.

Data for [$^2H_4$]-**15**: ^1H NMR (500 MHz, $CDCl_3$): δ 0.02 (br s, 6H), 0.86 (s, 9H), 0.87 (t, J = 7.0 Hz, 3H), 1.09 (d, J = 6.1 Hz, 3H), 1.21−1.45 (m, 10H), 3.56 (br t, 1H), 3.77 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$), mixture of diastereomers: δ −4.7 and −4.69, −4.4, 14.1, 18.2, 21.0−22.0 (m), 22.7, 23.75 and 23.79, 25.3, 25.9 (3C), 31.9, 36.6−37.2 (m), 37.4, 39.3−39.8 (m), 68.4−68.6 (m), 71.7−71.9 (m). GC−MS EI *m/z* (%): 305 [$M^{+\bullet}$ − 1] (0.04), 247 (1), 229 (1), 215 (0.2), 203 (0.3), 172 (0.4), 159 (7), 147 (1), 133 (2), 119 (14), 98 (15), 84 (16), 75 (100), 55 (51), 43 (53), 41 (43).

Data for unlabeled **15** (mixture of diastereomers): ^1H NMR (400 MHz, $CDCl_3$) δ 0.026 (s, 6H), 0.86 (9H), 0.87 (t, J = 6.9 Hz, 3H), 1.10 (d, J = 6.1 Hz, 3H), 1.24−1.50 (m, 14H), 3.54−3.60 (m, 1H), 3.73−3.80 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.4, 14.0, 18.2, 21.7 and 21.9, 22.6, 23.75 and 23.80, 25.29 and 25.31, 25.9 (3C), 31.90 and 31.91, 37.4, 37.45 and 37.55, 39.6 and 39.7, 68.5 and 68.6, 71.9 and 72.0. Anal. Calcd for $C_{17}H_{38}O_2Si$: C, 67.48; H, 12.66. Found: C, 67.50; H, 12.52.

[4,4,5,5-$^2H_4$]-2,6-Undecanediol ([$^2H_4$]-11). Alcohol [$^2H_4$]-**15** (550 mg, 1.80 mmol) was dissolved in 6:2:2 (v/v) AcOH/THF/H_2O (10 mL) and heated at 50 °C for 12 h. The solution was concentrated in vacuo, and the residue was purified by flash chromatography (1:1 EtOAc/hexane) to give [$^2H_4$]-**11** (302 mg, 87%) as a colorless paste.

Data for [$^2H_4$]-**11**: ^1H NMR (400 MHz, $CDCl_3$): δ 0.83 (t, J = 6.9 Hz, 3H), 1.12 (d, J = 6.2 Hz, 3H), 1.18−1.45 (m, 10H), 2.68 (br s, 2H), 3.51−3.52 (br m, 1H), 3.70−3.77 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), mixture of diastereomers: δ 13.9, 20.5−21.6 (m), 22.5, 23.3 and 23.4, 25.3, 31.8, 36.0−37.1 (m), 37.3 and 37.4 (br), 38.6−40.0 (m), 67.4 and 67.7 (br), 71.3−71.6 (m). GC/MS EI *m/z* (%): 192 [$M^{+\bullet}$] (0.03), 175 (0.1), 174 (0.1), 158 (0.2), 145 (1), 129 (1), 119 (1), 101 (19), 83 (45), 72 (13), 55 (53), 45 (74), 43 (100), 41 (47).

Data for unlabeled **11**: ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 7.2 Hz, 3H), 1.14 (d, J = 6.4 Hz, 3H), 1.20−1.32 (m, 5H), 1.33−1.48 (m, 9H), 2.24 (br s, 2H), 3.50−3.58 (m, 1H), 3.71−3.80 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$), mixture of diastereomers: δ 14.0, 21.6 and 21.8, 22.6, 23.4 and 23.5, 25.4, 31.9, 37.0 and 37.2, 37.4 and 37.6, 39.0 and 39.1, 67.7 and 67.9, 71.6 and 71.7. GC/MS EI *m/z* (%): 171 [$M^{+\bullet}$ − 17] (0.1), 170 (0.2), 155 (0.4), 145 (1), 127 (1), 117 (2), 99 (21), 83 (17), 81 (46), 70 (18), 57 (37), 55 (82), 45 (46), 43 (100), 41 (62).

2-(*tert*-Butyldimethylsilyloxy)-6-(*tert*-butyldiphenylsilyloxy)undec-4-yne (16). To a solution of **14** (1.53 g, 5.13 mmol) in CH_3CN (30 mL) was added TBDPSCl (1.64 g, 5.98 mmol) followed by portionwise addition of imidazole (610 mg, 9.0 mmol). The reaction mixture was allowed to stir under a nitrogen atmosphere overnight and then concentrated in vacuo. The residue was then suspended in hexane (50 mL) and stirred vigorously for 5 min. The mixture was filtered and concentrated in vacuo, and the resulting oil was purified by flash chromatography (1:30 EtOAc/hexane) to give **16** (2.05 g, 73%). ^1H NMR (400 MHz, $CDCl_3$) δ 0.030−0.042 (m, 6H), 0.85 (t, J = 7.2 Hz, 3H), 0.86−0.88 (br s, 9H), 1.07 (br s, 9H), 1.11 (dd, J = 7.0 and 6.1 Hz, 3H), 1.17−1.28 (m, 4H), 1.35−1.42 (m, 2H), 1.58−1.64 (m, 2H), 2.09 (dddd, J = 16.4, 8.2, 2.0, and 0.6 Hz, 1H), 2.21−2.28 (m, 1H), 3.72−3.80 (m, 1H), 4.33−4.37 (m, 1H), 7.31−7.44 (m, 6H), 7.66−7.71 (m, 2H), 7.73−7.78 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$), mixture of diastereomers: δ −4.77 and −4.74, −4.69 and −4.68, 14.0, 18.1, 19.3, 22.5, 23.15 and 23.2, 24.62 and 24.63, 25.8 (3C), 27.0 (3C), 29.6, 31.4, 38.6, 64.1, 67.70 and 67.74, 82.29 and 82.3, 83.10 and 83.12, 127.25 and 127.26, 127.5, 127.6, 129.4, 129.5, 129.6, 134.0, 134.1, 134.2 and 134.10, 135.5, 135.8, 136.0. Anal. Calcd for $C_{33}H_{52}O_2Si_2$: C, 73.82; H, 9.76. Found: C, 73.82; H, 9.78.

2-(*tert*-Butyldimethylsilyloxy)-6-(*tert*-butyldiphenylsilyloxy)-undecane (17). Palladium catalyst (10% on carbon) was added to a solution of alkyne **16** (1.19 g, 2.22 mmol) in EtOAc (10 mL) and TEA (1 mL) and left to stir under a hydrogen atmosphere. After 1 h, GC−MS analysis revealed that reduction was complete, and the suspension was filtered through a plug of Celite and

concentrated in vacuo. The residue was purified by flash chromatography (1:30 EtOAc/hexane), affording **17** (1.15 g, 95%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$), mixture of diastereomers: δ −0.012 and −0.008 (s, 3H), −0.001 (s, 3H), 0.79 and 0.80 (t, J = 7.1 Hz, 3H), 0.84 and 0.85 (s, 9H), 1.03 (br m, 12H), 1.05−1.42 (m, 14H), 3.60−3.72 (m, 2H), 7.31−7.41 (m, 6H), 7.62−7.68 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ −4.7, −4.4, 14.0, 18.2, 19.4, 21.1 and 21.2, 22.6, 23.71 and 23.74, 24.5 and 24.6, 25.9 (3C), 27.1 (3C), 31.9, 36.18 and 36.2, 36.3 and 36.5, 39.8 and 39.9, 68.6 and 68.7, 73.2 and 73.3, 127.34 (4C), 129.3 (2C), 134.8 (2C), 135.9 (4C). Anal. Calcd for $C_{33}H_{56}O_2Si_2$: C, 73.27; H, 10.43. Found: C, 73.52; H, 10.57.

6-(*tert*-Butyldiphenylsilyloxy)undecan-2-ol (18). The procedure was analogous to that of Singh et al.[14] To an ice-cold solution of protected diol **17** (1.00 g, 1.85 mmol) in MeOH (20 mL) was added ammonium cerium(IV) nitrate (1.01 g, 1.85 mmol) in one portion. After the solution was stirred for six hours at 0 °C, brine (35 mL) was added to the reaction mixture, which was then extracted with hexane (3 × 30 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated in vacuo to give a crude oil, which was purified on silica (1:20 to 1:10 EtOAc/hexane) to afford **18** (565 mg, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.81 (t, J = 7.3 Hz, 3H), 1.04 (s, 9H), 1.08 (d, J = 6.2 Hz, 3H), 1.11−1.27 (m, 10H), 1.37−1.44 (m, 4H), 3.59−3.67 (m, 1H), 3.70 (m, 1H), 7.31−7.42 (m, 6H), 7.64−7.68 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$), mixture of diastereomers: δ 14.0, 19.4, 21.0 and 21.1, 22.6, 23.27 and 23.34, 24.6, 27.1 (3C), 31.9, 36.2 and 36.3, 36.3 and 36.4, 39.32 and 39.34, 67.95 and 68.0, 73.1 and 73.2, 127.4 (4C), 129.4 (2C), 134.68 and 134.70, 134.8, 135.9 (4C). Anal. Calcd for $C_{27}H_{42}O_2Si$: C, 76.00; H, 9.92. Found C, 76.06; H, 10.01.

6-(*tert*-Butyldiphenylsilyloxy)undecan-2-one. To an ice-cold solution of **18** (550 mg, 1.3 mmol) in acetone (20 mL) was added Jones' reagent dropwise until the solution remained yellow. 2-Propanol (~1 mL) was then added, and the reaction mixture was left to stir for 30 min. The mixture was then decanted and concentrated in vacuo to afford a residue that was purified by flash chromatography on silica gel (1:20 EtOAc/hexane), furnishing 6-(*tert*-butyldiphenylsilyloxy)undecan-2-one (450 mg, 82%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.81 (t, J = 7.3 Hz, 3H), 1.04 (s, 9H), 1.06−1.12 (m, 2H), 1.14−1.24 (m, 4H), 1.34−1.45 (m, 4H), 1.51 (quintet, J = 7.7 Hz, 2H), 2.03 (s, 3H), 2.21 (t, J = 7.5 Hz, 2H), 3.70 (quintet, J = 5.6 Hz, 1H), 7.33−7.42 (m, 6H), 7.65−7.67 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 19.2, 19.4, 22.5, 24.5, 27.1 (3C), 29.7, 31.8, 35.6, 36.2, 43.7, 72.9, 127.38 (2C), 127.41 (2C), 129.4 (2C), 134.60, 134.61, 135.9 (4C), 209.0. Anal. Calcd for $C_{27}H_{40}O_2Si$: C, 76.36; H, 9.49. Found: C, 76.40; H, 9.53.

6-Hydroxyundecan-2-one (19). To a solution of 6-(*tert*-butyldiphenylsilyloxy)undecan-2-one (310 mg, 0.73 mmol) in THF (1 mL) was added TBAF (1.45 mL, 1.45 mmol, 1 M in THF), and the solution was left to stir for 48 h. The reaction mixture was diluted with $CuSO_4$ (10 mL, 5%), transferred to a separatory funnel, and extracted into ether (3 × 15 mL). The combined ethereal layers were washed again with $CuSO_4$ (15 mL, 5%) and then with brine (15 mL), dried (Na_2SO_4), and concentrated in vacuo. The product was purified on silica (1:5 EtOAc/hexane) to give a mixture of **19** and the corresponding tetrahydropyranol 2-methyl-6-pentyltetrahydro-2*H*-pyran-2-ol (88 mg, 65%) as a colorless oil. The interconverting forms (cyclic tetrahydropyranol and open-chain hydroxyketone) resulted in complex NMR spectra. ^{1}H NMR (500 MHz, C_6D_6): δ 1.02 (t, open-chain, J = 7.2 Hz, 3H), 1.02 (t, cyclic, J = 7.0 Hz, 3H) 1.15−1.82 (m), 1.41 (s, cyclic, 3H), 1.76 (s, open-chain, 3H), 2.10 (t, J = 7.1 Hz, 2H), 3.46 (quintet, open-chain, J = 6.6 Hz, 1H), 3.99 (dddd, cyclic, J = 12.0, 7.3, 5.0, and 2.2 Hz, 1H). ^{13}C NMR (125 MHz, C_6D_6): δ 14.0 (2C), 19.4, 19.7, 20.7, 22.76, 22.79, 25.5, 29.0, 30.7, 31.0, 32.0, 32.1, 34.7, 36.6, 36.9, 37.7, 42.9, 69.7, 70.9, 94.9, 206.7. GC/MS EI m/z (%): 168 [$M^{+\bullet}$ − 18] (13), 153 (0.4), 139 (4), 125 (1), 115 (5), 110 (11), 97 (24), 71 (97), 58 (45), 55 (44), 43 (100), 41 (44). HRMS m/z Calcd for $C_{11}H_{22}O_2$: 186.1620. Found: 186.1619.

6-Methyl-2-pentyl-3,4-dihydro-2*H*-pyran (10). Preparative gas chromatography of a portion of hydroxyketone **19** provided dihydropyran **10** by thermal cyclization/dehydration. ^{1}H NMR (500 MHz, C_6D_6): δ 0.97 (t, J = 7.2 Hz, 3H), 1.25−1.64 (m, 9H), 1.69−1.78 (m, 1H), 1.87−1.88 (m, 3H), 1.91−1.99 (m, 1H), 2.02−2.11 (m, 1H), 3.79 (dddd, J = 9.9, 7.2, 4.8, and 2.3 Hz, 1H), 4.56 (ttd, J = 4.4, 2.1, and 1.1 Hz, 1H). ^{13}C NMR (125 MHz, C_6D_6): δ 14.2, 20.4, 21.0, 22.9, 25.4, 27.7, 32.2, 35.7, 75.4, 95.0, 151.2. GC/MS EI m/z (%): 168 [$M^{+\bullet}$] (15), 153 (1), 139 (4), 125 (3), 110 (11), 97 (13), 95 (7), 71 (100), 55 (30), 43 (71), 41 (35). HRMS m/z Calcd for $C_{11}H_{20}O$: 168.1514. Found: 168.1516.

Discussion

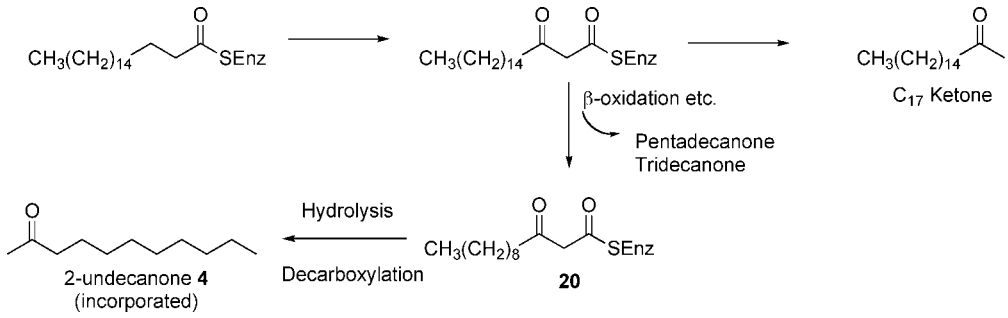
Spiroacetals constitute a structural class that is widely observed in insects and are postulated to function as semiochemicals in the organisms in which they occur.[1] However, little is known to date about their biogenesis. Early work demonstrated the incorporation of some simple precursors such as acetate, citrate, and malonate into spiroacetals, presumably via the TCA cycle.[15] Subsequently, a variety of origins were considered, including polyketide pathways,[16] propionate polymerization, and the involvement of oxodicarboxylic acids.[15] Definition of the pathway has been provided only for the penultimate steps in selected Dipteran (*Bactrocera* sp.) fruit flies: as we have previously demonstrated,[6] these insects utilize a monooxgenase-catalyzed functionalization of an unactivated C−H bond to produce a ketodiol equivalent that spontaneously cyclizes to the spiroacetal. The results now presented permit the description of a pathway *from primary metabolites to spiroacetals* in the Hymenopteran wasp *M. nortoni* (Schemes 3 and 4).

The first committed metabolite in the biosynthesis of the C_{11} spiroacetals **1** and **2** appears to be **4**, which occurs naturally in *M. nortoni* and is incorporated into **1** and **2** when exogenously administered in ^{2}H-labeled form (Table 1). Methyl ketone **4** presumably arises from decarboxylation of a biological equivalent of the C_{12} β-ketoacid **20** (Scheme 3), the thioester of which would participate in primary metabolic processing of fatty acids by β-oxidation. Such a biosynthetic origin is supported by the co-occurrence in *M. nortoni* of a suite of methyl ketones containing an odd number of carbons, with the ones observed putatively arising from decarboxylation of C_{12}, C_{14}, C_{16}, and C_{18} fatty acid precursors. Additionally, β-oxidation incorporates oxygen from water rather than molecular oxygen, in accord with the observed absence of oxygen incorporation from $^{18}O_2$ into the methyl ketones and the single dioxygen-derived oxygen in the spiroacetals of *M. nortoni*.

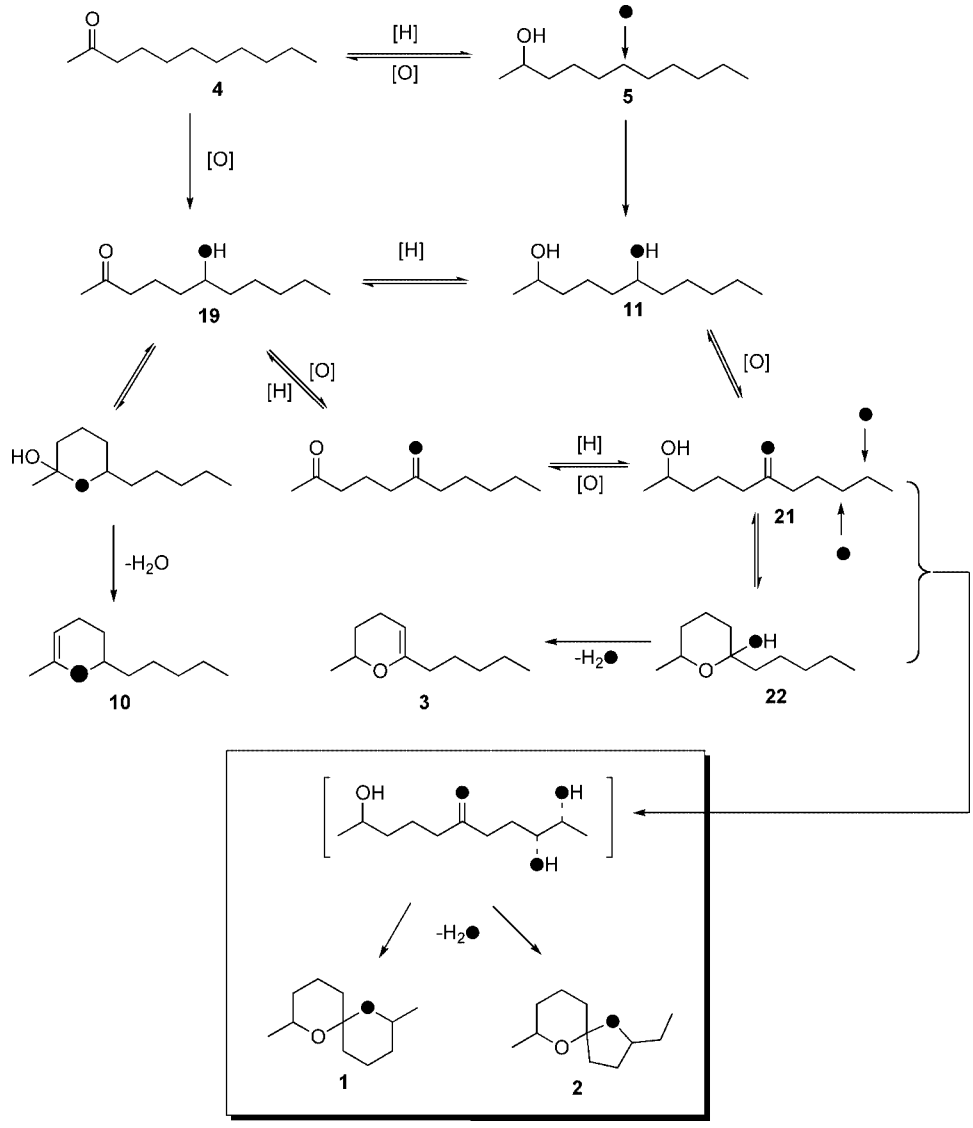
In progressing from **4** to an advanced precursor such as **21**, reduction of the C2 carbonyl as well as hydroxylation and subsequent oxidation at C6 must occur (Scheme 4). Thus, a reasonable pathway proceeds through the (*S*) isomer of **5** to **11** and then to the ketoalcohol **21**. Such a progression is supported by the observation that both **5** and **11** occur naturally in *M. nortoni* and that dihydropyran **3**, which results from dehydration of **14**, occurs in the extracts and emitted volatiles. Additionally, when ^{2}H-labeled **5** and **11** were administered via diet to *M. nortoni*, both were incorporated

(15) Pomonis, J. G.; Mazomenos, B. E. *Int. J. Invertebr. Reprod. Dev.* **1986**, *10*, 169.
(16) Vanderwel, D.; Oehlschlager, A. C. *Pheromone Biochemistry*; Academic Press: New York, 1987.
(17) Stok, J. E.; Lang, C.-S.; Schwartz, B. D.; Fletcher, M. T.; Kitching, W.; De Voss, J. J. *Org. Lett.* **2001**, *3*, 397.

Scheme 3



Scheme 4



into the spiroacetals **1** and **2** as well as into the dihydropyran **3**. Also, as expected for more advanced precursors, administration of [$^2H_4$]-**5** and [$^2H_4$]-**11** resulted in higher levels of ^{2}H-labeled spiroacetals than administration of [$^2H_4$]-**4** (Table 1). Finally, in accord with this biosynthetic progression, the diol **11** found in wasps after exposure to an atmosphere of $^{18}O_2$ contained a *single labeled oxygen atom*, and the mass-spectral characteristics of this isotopomer were consistent with the label being located at C6.

Although **5** could be synthesized in highly enantiomerically enriched form and the enantiomers separated by enantioselective GC, the very low natural levels did not permit a confident direct assignment of its absolute configuration. However, given the apparent ubiquity of the (*S*) configuration at the methyl-substituted centers of spiroacetals in both *M. nortoni* (see above) and elsewhere,[1,5] it is very likely that **14** occurs as the (2*S*) isomer.

Table 1. Comparison of Percentage Deuterium Incorporation into (*E*,*E*)-**1** and (*E*,*E*)-**2** after Administration of Substrates to *M. nortoni*[a]

	% deuteration	
substrate	(*E*,*E*)-**1**	(*E*,*E*)-**2**
[$^2H_4$]-**4**	4	5
[$^2H_4$]-**5**	16	16
[$^2H_4$]-**11**	91	72

[a] Incorporation calculated by comparing the integration of the GC−MS single-ion trace for the unlabeled spiroacetal with the sum of the single-ion traces for the labeled spiroacetals (1−3 deuterons).

Despite the intrinsically appealing simplicity of the above path from **4** to **1** and **2**, it should be noted that dihydropyran **10** was also observed in *M. nortoni* and that it incorporated ^2H from labeled **4**, **5**, and **11** as well as an oxygen atom from [^{18}O]dioxygen. **10** could arise from dehydrative cyclization of ketoalcohol **19** (Scheme 4). These results suggest the operation of a metabolic grid in which the various compounds are interconnected via oxidation−reduction reactions. The existence of such transformations is supported by the present observation of interconversion between labeled **4** and **5** fed to the wasp and the transformation of **11** into both of the dihydropyrans **3** and **10**. Determination of the metabolic flux through each of the individual arms of the grid must await isolation of the relevant enzymes and characterization of their substrate specificities.

Completion of spiroacetal biosynthesis requires monooxygenase-catalyzed hydroxylation of hydroxyketone **21** (Scheme 4) followed by spontaneous cyclization of the resultant ketodiol. Such steps are in accord with the incorporation of a single ^{18}O label from $^{18}O_2$ into spiroacetal systems **1**, **2**, and **6** as well as the extensive data that we have accumulated on the analogous steps in *Bactrocera* sp. fruit flies.[17,18] The biosynthetic origins of the C_{13} spiroacetal system **6** can be similarly explained by an analogous sequence of steps commencing with the observed 2-tridecanone. This proposed biogenesis, initiated by the decarboxylation of a fatty acid, nicely explains the predominance in insects of spiroacetals with an odd number of carbon atoms.[1,5]

The final step proposed for spiroacetal biosynthesis, monooxygenase-mediated hydroxylation of a tetrahydropyranol, is notable in several respects. First, it links results from two different insect orders, Hymenoptera (bees, wasps) and Diptera (true flies), supporting the thesis of a commonality of biosynthetic origin for insect-derived spiroacetals. Second, it appears to be a remarkable example of natural efficiency in which considerable molecular diversity is generated in a single step. Five different components [(*E*,*E*)-**1**, (*E*,*Z*)-**1**, (*E*,*E*)-**2**, (*E*,*Z*)-**2**, and (*Z*,*Z*)-**2**] of the molecular signature of *M. nortoni* are generated from a single precursor **21**, most likely in a hydroxylation reaction catalyzed by a single monooxygenase. The regio- and stereoselectivity, or lack of it, in this hydroxylation step allows access to a range of spiroacetals, whereas the specific ratio of the oxidation products determines the exact composition of the emitted volatiles. For example, in *M. nortoni*, the $\omega - 1$ hydroxylation that leads to **1** is not stereoselective and thus leads to an ~1:1 mixture of the diastereomers (*E*,*E*)-**1** and (*E*,*Z*)-**1**, while the $\omega - 2$ oxidation is quite specific and leads mainly to (*E*,*E*)-**2**. However, in *B. cucumis*, even though the same precursor **21** is employed and processed similarly to the spiroacetal systems **1** and **2**, the emitted volatiles are quite distinct from those of *M. nortoni*, having one predominant regio- and stereochemistry of oxidation that leads mainly to (*E*,*E*)-**1** and (*Z*,*Z*)-**1**, which account for 89% of the spiroacetal volatiles observed.[19] It is tempting to speculate further that members of the C_{13} spiroacetal system **6** observed in *M. nortoni* are products of the same set of biosynthetic enzymes that result from a lack of substrate specificity of the biosynthetic machinery but further modify the molecular signature of the insect. Proof of these hypotheses awaits isolation and characterization of the enzymes involved in these pathways.

Only recently, the role of a decarboxylase was proposed in the biosynthesis of (*S*)-4-methyl-3-heptanone from three proprionate units[20] and in honeybee biosynthesis of various C_{10} acids from stearic acids, with the sequence involving hydroxylation followed by chain shortening and finally alcohol oxidation.[21] Equally of interest is the variety of processing steps (probably from fatty acids) toward this putative intermediate. The present work describes the first cohesive and internally consistent hypothesis for the generation of spiroacetals in insects, against which subsequent information and interpretations will have to be compared.

Acknowledgment. The authors are grateful to the Australian Research Council (ARC) for support of this work, to Stiftelsen Bengt Lundqvist Minne for financial support to F.R., and to Dr. Charlma Phillips (Forestry SA, Mount Gambier SA) for specimens of *M. nortoni nortoni*.

Supporting Information Available: Annotated GC−MS analyses of *M. nortoni* extracts after exposure to [^{18}O]dioxygen, including mass spectra of key labeled and unlabeled metabolites, and spectral characterization of [$^2H_4$]-**4**, -**5**, -**11**, and **15** and unlabeled **10**, **11**, **14**, **15**, and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

JA8036433

(18) A pathway in which hydroxylation of 2,6-undecanediol to the triol precedes C6 oxidation is also possible and is analogous to one recently proposed for *B. oleae* and *B. cacuminata*. However, the significant amount of dihydropyran **3** found in *M. nortoni* (formed via dehydrative cyclization of **21**) suggests that **21** is a bona fide intermediate.

(19) Kitching, W.; Lewis, J. A.; Perkins, M. V.; Drew, R.; Moore, C. J.; Schurig, V.; Konig, W. A.; Francke, W. *J. Org. Chem.* **1989**, *54*, 3893.

(20) (a) Jarvis, A. P.; Liebig, J.; Hölldobler, B.; Olaham, N. J. *Chem. Commun.* **2004**, 1196. Also see: (b) Islam, W.; Bacala, R.; Moore, A.; Vanderwel, D. *Insect Biochem. Mol. Biol.* **1999**, *29*, 201.

(21) Plettner, E.; Slessor, K. N.; Winston, M. L.; Oliver, J. E. *Science* **1996**, *271*, 1851.