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Carbon Hydroxylation of Alkyltetrahydropyranols: A Paradigm for Spiroacetal Biosynthesis in *Bactrocera* sp.

Jeanette E. Stok, Cheng-Shan Lang, Brett D. Schwartz, Mary T. Fletcher, William Kitching, and James J. De Voss*

Department of Chemistry, University of Queensland, Brisbane, 4072 Australia

devoss@chemistry.uq.edu.au

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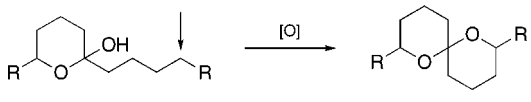
ABSTRACT



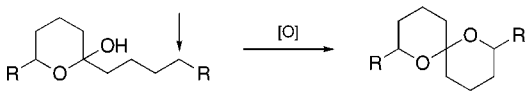
2 (R=H)
4 (R=CH_3)

1 (R=H)
3 (R=CH_3)

In a number of *Bactrocera* species the penultimate step in the biosynthesis of spiroacetals is shown to be the hydroxylation of an alkyltetrahydropyranol followed by cyclization. The monooxygenases that catalyze this side chain hydroxylation show a strong preference for oxidation four carbons from the hemiketal center, to produce the spiroacetal. The hydroxy spiroacetals observed in *Bactrocera* appear to derive from direct oxidation of the parent spiroacetals and not from alternate precursors.

Spiroacetals such as **1** are widely distributed across the insect kingdom and are often found as volatile components of various secretions.[1] In some cases they exhibit pheromonal activity, and their structure, stereochemistry, and biological activity have been actively investigated.[2] Until recently, essentially nothing was known about the biosynthesis of these compounds. However, the possibility of environmentally friendly insect control strategies based on disruption of pheromone formation has aroused interest in their biosynthesis. We have previously demonstrated[3] that the penultimate step in the biosynthesis of 1,7-dioxaspiro[5.5]undecane (**1**) in the female olive fly, *Bactrocera oleae*, is the ω-hydroxylation of 6-*n*-butyltetrahydropyran-2-ol (**2**) (Scheme 1).

Scheme 1



2 (R=H)
4 (R=CH_3)

1 (R=H)
3 (R=CH_3)

This species is widely distributed throughout southern Europe and northern Africa. *B. cacuminata* and *B. cucumis*, two species of fruit fly prevalent in the coastal regions of eastern Australia, also biosynthesize spiroacetals. The major rectal glandular component of male *B. cacuminata* is 1,7-dioxaspiro-[5.5]undecane (**1**), identical to that released by female *B. oleae*.[4] *B. cucumis*, however, produces mainly 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**3**) as an unequal mixture of isomers that have been previously synthesized and characterized.[5] This raised the question whether oxidation of alkyltetrahydropyranols was a general step in the formation of spiroacetals in fruit flies or whether different species utilized different biosynthetic pathways.

To test the generality of this oxidative pathway (Scheme 1) we first examined *B. cacuminata*. The deuterium labeled precursor, [$^2H_3$]-2-*n*-butyltetrahydropyran-2-ol ([$^2H_3$]-**2**),[3] was

(1) Francke, W.; Kitching, W. *Curr. Org. Chem.* **2000**, *4*, 411−431.
(2) Fletcher, M. T.; Kitching, W. *Chem. Rev.* **1995**, *95*, 789−828.
(3) Hungerford, N. L.; Mazomenos, B. E.; Konstantopoulou, M. A.; Krokos, F. D.; Haniotakis, G. E.; Hubener, A.; Fletcher, M. T.; Moore, C. J.; De Voss, J. J.; Kitching, W. *Chem. Commun.* **1998**, 863−864.
(4) Kitching, W.; Lewis, J. A.; Fletcher, M. T.; Drew, R. A. I.; Moore, C. J.; Franke, W. *Chem. Commun.* **1986**, 853−854.

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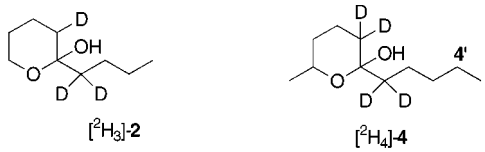
administered to male flies through their diet, and the rectal glandular contents were extracted and examined by GC−MS. The parent spiroacetal (**1**) was significantly [^{2}H]-enriched with an estimated incorporation level of 30%. This confirmed that the final step in the biosynthesis of **1** in both male *B. cacuminata* and female *B. oleae* is the oxidation of **2**.



To explore whether *B. cucumis* produces the bis-homologue, 2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**3**), via the same pathway, [^{2}H$_4$]-labeled 2-methyl-6-pentyl-tetrahydropyran-2-ol[6] ([^{2}H$_4$]-**4**) was required. Simple Grignard addition of *n*-pentylmagnesium bromide to *δ*-hexalactone gave racemic, unlabeled **4**, and deuterium incorporation was accomplished via acid-catalyzed exchange of the α-protons, presumably via a ring-opened form.

[^{2}H$_4$]-**4** was administered to male *B. cucumis* in their diet, and the rectal gland contents were subsequently extracted and analyzed by GC−MS. Spiroacetal **3** was significantly deuterium enriched, thus confirming that the final step in its formation is the hydroxylation of **4**. Therefore, carbon hydroxylation followed by cyclization appears to be a general paradigm for spiroacetal biosynthesis in, at least, *Bactrocera* sp.

One enantiomer of each of the *EE* (2*S*,6*R*,8*S*) and *ZZ* (2*S*,6*S*,8*S*) forms and the *EZ* (2*S*,6*S*,8*R*), *ZE* (2*S*,6*R*,8*R*) diastereomers are seen naturally in *B. cucumis* (Figure 1).[5]

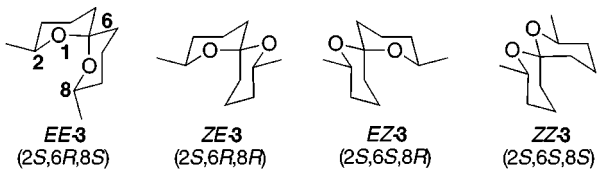
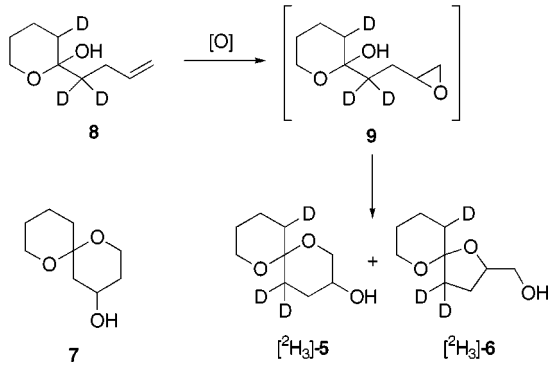


Figure 1.

The *EE* and the *ZZ*-diastereomers differ only in the stereochemistry at the anomeric carbon, both having the *S* configuration at the C2 and C8 positions. They should thus arise from 2*S*-**4** by hydroxylation at the 4′ side chain position to yield an *S* alcohol followed by cyclization. The *EZ* and *ZE* isomers again differ only in the stereochemistry of the anomeric center but have a 2*S*,8*R* configuration. Therefore they may derive either from 2*R*-**4** by C4′ *S*-hydroxylation or from 2*S*-**4** by C4′ *R*-hydroxylation, followed in both cases by cyclization. As the [^{2}H$_4$]-**4** synthesized was racemic, the amount of deuterium incorporation, after administration to male *B. cucumis*, should reveal the importance of the stereochemistry at C-2. If the *S* configuration at C-2 of **4** is essential for recognition by the oxidative enzyme that transforms it into the spiroacetal, selective incorporation of only the *S* isomer would produce the same proportional level of deuterium in all isomers of **3**. However, if both 2*R* and 2*S* **4** can be oxidized to yield a spiroacetal the amount of deuterium incorporation into *EZ* and *ZE*-**3** would be increased relative to the amount in the *EE* and *ZZ* forms, as 2*R*-**4** can only act as a precursor for the *EZ* and *ZE* isomers. We observed approximately 50% deuterium enrichment of the *EZ* and *ZE* isomers, compared with 10−15% incorporation into the *EE* and *ZZ* isomers. Thus, 2*R*-**4** can be hydroxylated in *B. cucumis* and serve as a precursor of *EZ* and *ZE* **3**. However, further experiments are necessary to demonstrate whether this route is the natural pathway to these compounds.

The hydroxy spiroacetals **5** and **7** also occur naturally in both *B. cacuminata* and *B. oleae* (**6** is also found *in B. oleae*). Plausible biosynthetic routes to these compounds include direct hydroxylation of **1** or epoxidation of an alkene such as **8** followed by cyclization (for **5** and **6**; Scheme 2). We

Scheme 2



have previously demonstrated that the most likely biosynthetic route to these hydroxy spiroacetals was hydroxylation of **1**.[3] To determine whether *B. cacuminata* also produced the hydroxy spiroacetals via hydroxylation of **1** or epoxidation of an appropriate alkene, [^{2}H$_3$]-**8**[7] and [^{2}H$_4$]-**1** were administered to *B. cacuminata*.

For such flies fed [^{2}H$_3$]-**8** the level of hydroxy spiroacetals **5** and **6** was observed to increase dramatically and both were deuterium enriched. As expected, no deuterium was detected in the peak corresponding to **7**. Enantioselective GC−MS analysis of the labeled diastereomers of **5** and **6** demonstrated that they were significantly different from those found naturally. Indeed, **6** is not usually seen in *B. cacuminata* but was observed in these extracts and was derived entirely from deuterated precursor. In contrast, when the hydroxy spiroac-

(5) 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*, 3893−3902.

(6) Fletcher, M. T.; Wells, J. A.; Jacobs, M. F.; Krohn, S.; Kitching, W.; Drew, R. A. I.; Moore, C.; Francke, W. *J. Chem. Soc., Perkin. Trans.* **1992**, 2827−2831.

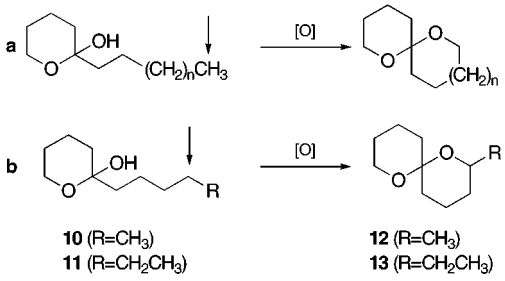
(7) Compounds **1**, **3**, **5**−**8**, **10**, **12**, and **18** were obtained from the Kitching research group collection.

etals were analyzed from *B. cacuminata* fed [$^2H_4$]-**1**, there was no significant change in the stereoisomers from those usually seen.[8] However, both **5** and **7** were observed to be deuterium enriched, indicating that in both *B. oleae* and *B. cacuminata* the hydroxy spiroacetals are formed by oxidation of **1**.

The results from feeding [$^2H_3$]-**8** are consistent with our hypothesis that a monooxygenase is responsible for the hydroxylation of the alkyltetrahydropyranol **2** to produce the spiroacetal **1**. The alkene **8** is an analogue of **2** and would be oxidized by the monooxygenase to yield an epoxide **9**, which could cyclize to the observed hydroxy spiroacetals (Scheme 2). However, the differences in enantiomeric excesses and isomer distribution between those derived from **8** and the ones seen in vivo[8] agree with the natural hydroxy spiroacetals arising from direct oxidation of **1**.

Having established the intermediacy of alkyltetrahydropyranols in spiroacetal biosynthesis, we wished to investigate the specificity of the postulated monooxygenase involved in their hydroxylation. Two possibilities arise as to the specificity of the oxidation to produce **1** in *B. cacuminata*. The first is that the enzyme involved could hydroxylate the terminal carbon of the alkyl substituent (Scheme 3a), acting

Scheme 3



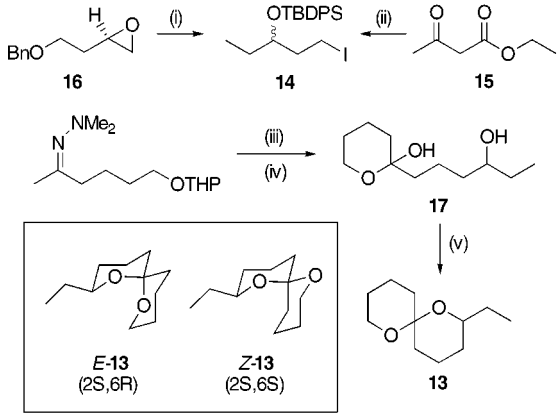
independently of the length of the carbon chain present. The second possibility is that the enzyme is specifically designed to produce a 1,7-dioxaspiro[5.5]undecane and hence hydroxylates four carbons from the hemiketal center, regardless of chain length (Scheme 3b).

To test these hypotheses, tetrahydropyranol precursors **10**[7] and **11** were administered to male *B. cacuminata*. GC−MS analysis of the excised gland extracts revealed that a small amount of the spiroacetals **12** and **13**, respectively, were present. As **10** and **11** were non-natural substrates, the amount of conversion to the corresponding spiroacetals was low, approximately 4% of the parent spiroacetal **1**, but easily detectable by GC−MS. The production of **12** and **13** suggests that the monooxygenase acts four carbons from the hemiketal center rather than being an ω-hydroxylase that only oxidizes the terminal methyl group of an alkyl chain.

Interestingly, GC−MS analysis of the gland extracts after administration of **11** revealed **two** compounds with the mass spectral characteristics expected of the 2-ethylspiroacetal **13**. Treatment of the extract with acid equilibrated the two compounds so that only one remained. We therefore hypothesized that both the *E* and *Z* isomers of **13** were being produced. Similar results were obtained for the spiroacetal **12** produced from **10**. As these examples would constitute the first time that the less stable *Z* isomer of a simple monoalkylspiroacetal had been isolated from a natural source, we investigated this further by synthesis of both isomers of **13**. In addition, as both **12** and **13** are generated with a new stereogenic center we wished to investigate the stereoselectivity of this process and this required racemic and enantiomerically enriched samples of **13**.

(*E*)-2-Ethyl-1,7-dioxaspiro[5.5]undecane **13** has been found previously as a component of the cephalic secretions of a cleptoparasitic bee, but only mass spectral data were reported.[9] The key compound in our synthesis of **13** (Scheme 4) was the TBDPS protected 1-iodopentan-3-ol **14**. This (**14**)

Scheme 4[a]



[a] (i) (a) MeMgBr/CuI, (b)TBDPS-Cl, (c) H_2/Pd, (d) Ph_3P/I_2; (ii) (a) $NaBH_4$, (b) $LiAlH_4$, (c) TosCl, (d) TBDPS-Cl, (e) NaI; (iii) (a) LDA, (b) **14**, −78 °C; (iv) (a) H^+/methanol/H_2O, (b) F^-; (v) benzene/CH_3COOH.

was simply derived from ethylacetoacetate **15** for the racemic series and from (*R*)-(2-benzyloxyethyl)oxirane **16**,[10] readily available from (*S*)-aspartic acid, for the enantioselective synthesis. The iodide **14** was used to alkylate a known hydrazone[11] (Scheme 4), and acid-catalyzed removal of protecting groups yielded the lactol **17**. Cyclization of **17** in benzene/acetic acid proceeded slowly to give a mixture of the *E* and *Z* spiroacetals **13,** which were characterized by ^1H and ^{13}C NMR and GC−MS. Treatment of the mixture with TFA resulted in equilibration to yield only the more stable *E* isomer. The synthetic samples of *E* and *Z* spiroacetals had GC retention times and MS fragmentation patterns identical with those of the compounds generated by *B. cacuminata*, to which **11** had been administered.

(8) Fletcher, M. T.; Jacobs, M. F.; Kitching, W.; Krohn, S.; Drew, R. A. I.; Haniotakis, G. E.; Francke, W. *Chem. Comm.* **1992**, 1457−1459.

(9) Tengo, J.; Bergstrom, G.; Borg-Karlson, A. K.; Groth, I.; Francke, W. *Z. Naturforsch. C.* **1982**, *37*, 376−380.

(10) Frick, J. A.; Klassen, J. B.; Bathe, A.; Abramson, J. M.; Rappoport, H. *Synthesis* **1992**, 621.

(11) Mitra, R. B.; Reddy, G. B. *Synthesis* **1989**, 694−698.

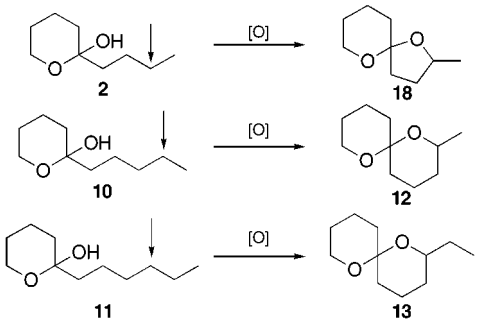
In addition, the enantiomers of both the *E* and *Z* isomers of **13** were well resolved by enantioselective GC (*β*-cyclodextrin column) and 2*S*-**13**, synthesized from *S*-aspartic acid had an ee of 96%. Similarly, we demonstrated that the predominant isomers of **13** produced by the fly had the 2*S* configuration (approximately 84% ee).

2*S*-**13** eluted before the 2*R* isomer during enantioselective GC, and this appears to be a general phenomenon for alkyl-substituted spiroacetals.[12] Racemic **12**[7] was similarly resolved, and the stereochemistry of the major insect product once again was 2*S* (the first eluting isomer). However the ee (56%) was significantly lower than that for **13** isolated from *B. cacuminata*.

To our knowledge, these are the first disclosures of the identification of a *Z* isomer of a monoalkylspiroacetal from a natural source, and its presence is informative about the environment in which it is formed. In acidic regions, such as sections of the digestive tract, the *Z* isomer would be anticipated to isomerize to the more stable *E* form. Thus the environment in which the spiroacetal is formed by cyclization of a hydroxyhemiketal is concluded to be only mildly acidic.

The stereoselectivity of the monooxygenase involved in the oxidation of **4** to produce **3** in *B. cucumis* was also investigated. The results of GC−MS analysis of gland extracts from *B. cucumis* fed the hemiketals **2**, **10**, and **11**, are shown in Scheme 5.

Scheme 5



The oxidation of **10** unsurprisingly resulted in the production of **12**, as this reaction is essentially the same hydroxylation performed in vivo to form the spiroacetal **3**. With **11**, the spiroacetal **13** was again produced. As observed with *B. cacuminata*, both **12** and **13** were isolated as a mixture of *E* and *Z* isomers, with predominantly the 2*S* configuration (**12***E*:**12***Z*, 56:44; 2*S***12**:2*R***12**, >95:5; **13***E*:**13***Z*, 70:30; 2*S***13**:2*R***13**, 75:25). These results again suggest a monooxygenase is present that acts four carbons from a hemiketal center, as was seen in *B. cacuminata*. However, feeding **2** to *B. cucumis* resulted not in the formation of **1** but in the production of spiroacetal **18**, where oxidation has occurred three carbons from the hemiketal. This result perhaps reflects the known preference for monooxygenases to oxidize methylene rather than methyl moieties because of the differences in CH bond strengths.[13] The stereochemistry of **18** was again determined by comparison of synthetic standards[7] with the isolated material, using enantioselective GC. This revealed that both enantiomers of the *E* and *Z* diasteromers were formed, although the 2*S* isomers greatly predominated (**18***E*:**18***Z*, 1:1; 2*S***18**:2*R***18**, >95:5).

The predominant formation of the 2*S* isomers of the methyl-substituted spiroacetals **12** and **18** is not surprising as the naturally occurring **3** has the same configuration. However, the formation of a small amount of the 2*R* isomers of both **12** and **18** suggest that the monooxygenase responsible for this *ω*-1 hydroxylation is not stereospecific and may explain the natural occurrence of the *EZ* and *ZE* isomers of **3**. Alternatively, as discussed above, 4′*S* hydroxylation of 2*R*-**4** may also give rise to *EZ* and *ZE* **3**. Further experiments with labeled enantiopure **4** would be necessary to completely resolve the biosynthetic route(s) to *EZ* and *ZE* **3**.

Overall, this work has revealed that the biosynthetic route we originally demonstrated for **1** in *B. oleae* presents a general paradigm for the formation of spiroacetals in, at least, *Bactrocera* sp. Thus, an alkyltetrahydropyranol is hydroxylated, and subsequent cyclization yields the spiroacetal. Hydroxy spiroacetals appear to derive from oxidation of the parent spiroacetal. The specificity of the monooxygenases involved in spiroacetal formation seems to conform with the hypothesis that oxidation occurs four carbons from the hemiketal center of the precursor in the species investigated, although the nature of the group oxidized (methylene versus methyl) may also be important. Future work will focus on further characterizing the oxidative enzymes implicated in spiroacetal biosynthesis.

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Supporting Information Available: Experimental procedures including administration and analysis of fly glands, synthetic schemes, and characterization of compounds [$^2H_4$]-**4**, **11** and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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