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Spiroacetal Biosynthesis: (±)-1,7-Dioxaspiro[5.5]undecane in *Bactrocera cacuminata* and *Bactrocera oleae* (Olive Fruit Fly)

Brett D. Schwartz,[†] Christopher S. P. McErlean,[†] Mary T. Fletcher,[†] Basilis E. Mazomenos,[‡] Maria A. Konstantopoulou,[‡] William Kitching,[†] and James J. De Voss*,[†]

Department of Chemistry, The University of Queensland, Brisbane, Australia 4072, and Institute of Biology, NCSR "D", 15310 Ag. Paraskevi Attikis, Greece

j.devoss@uq.edu.au

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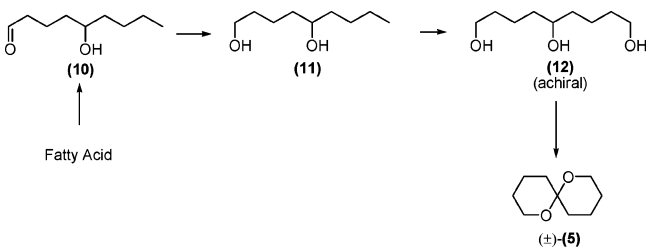
ABSTRACT



A biosynthetic scheme rationalizing the formation of (±)-1,7-dioxaspiro[5.5]undecane (5) in the fruit fly species *Bactrocera cacuminata* and *Bactrocera oleae* (olive fruit fly) is presented. Incorporation studies with deuterium-labeled keto aldehyde (10), 1,5-nonanediol (11), and 1,5,9-nonanetriol (12), and our previous finding that both oxygen atoms of 5 originate from dioxygen, are strongly evidentiary. The racemic condition of the natural spiroacetal 5 is accounted for, and inter alia, it is demonstrated that dihydropyran (18) is not an important intermediate en route to 5.

Spiroacetals are a fascinating class of insect-derived semiochemicals,[1−4] and recently we proposed a general paradigm for spiroacetal biosynthesis in *Bactrocera* sp. that involved monooxygenase-mediated hydroxylation of an intermediate alkyltetrahydropyranol in the penultimate step.[5−7] Specific incorporation of [^{2}H]-labeled alkyltetrahydropyranols was observed, and the patterns of [^{18}O]-oxygen incorporation from both dioxygen and water into spiroacetals from *B. oleae*, *B. cacuminata*, and *B. cucumis* were in harmony with this proposal.[8,9] With respect to *B. cacuminata* and *B. oleae*, both oxygen atoms of the predominant volatile component, (±)-1,7-dioxaspiro[5.5]undecane,[9,10] **5** originated

[†] The University of Queensland.
[‡] Institute of Biology.

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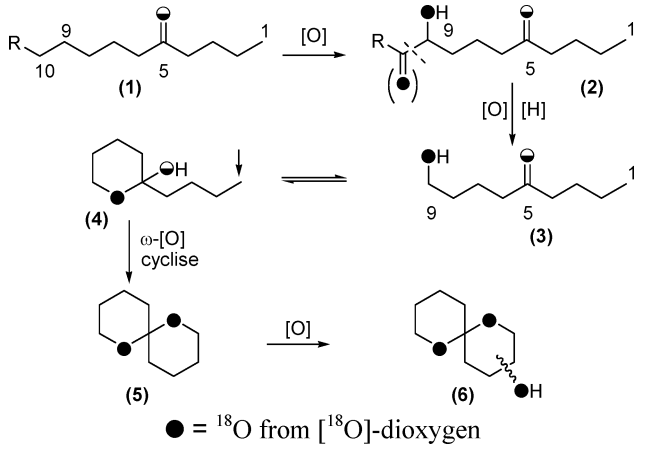
from [^{18}O]-dioxygen. Likely precursors to this oxygenated nine-carbon unit were considered to be those incorporating the oxidatively susceptible O−C−C−O moiety, as present in 1,2-diols, and α-hydroxy or α-ketoacids. A generalized pathway accommodating these[7−9] observations was developed and is summarized below (Scheme 1), although we

Scheme 1



emphasized that the order of oxidative events and the level of oxidation of the oxygenated carbons was not clear.[11]

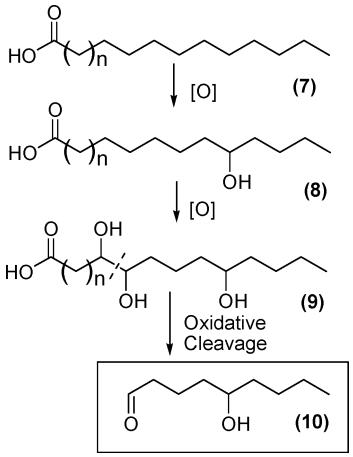
Longer chain fatty acids (C_{14}−C_{18}) frequently occur in the glandular secretions of fruit fly species, and certain observations led to the view that fatty acids were probably the source of the nine-carbon unit of **5**, as mono-oxygenated nine-carbon units, including nonanoic acid, 1-nonanol, nonanal, 5-nonanone, and 5-nonanol, were not incorporated into **5** when ^{2}H-labeled versions were administered to *B. cacuminata*.[12]

Consequently, we hypothesized that hydroxylation of a longer chain fatty acid would furnish an oxidatively susceptible unit (e.g., 1,2-diol) that, after cleavage, would yield a 1,5-dioxygenated nine-carbon unit. This sequence is shown below (Scheme 2) and is seen to be accommodated, other than for oxidation levels, within Scheme 1.

Scheme 2 identifies the hydroxyaldehyde (**10**) as the initially available nine-carbon unit for transformation to **5**. Consistent with this, [$^2H_4$]-**10** (see Scheme 3) was very efficiently incorporated into **5** in *B. cacuminata*. As reduction must be achieved if aldehyde **10** is a bona fide intermediate on the route to **5**, [$^2H_4$]-nonane-1,5-diol, [$^2H_4$]-**11**, was then administered and also shown to incorporate very efficiently into the spiroacetal **5** in both *B. cacuminata* and *B. oleae*. This experiment was informative in other ways, as labeled dihydropyran (**18**) was also produced. (Under natural conditions, the dihydropyran **18** and tetrahydropyranol (**16**) co-occur in the glandular extract of *B. cacuminata* with the spiroacetal **5** and some other minor components.)[2]

Although the spiroacetal **5 retained all four deuterium atoms** from the tetradeuterated [$^2H_4$]-nonane-1,5-diol,

Scheme 2



[$^2H_4$]-**11**, this was not the case for the dihydropyran **18**, which retained **one or two deuterium atoms**, but **not three**. (One ^{2}H must be lost in the dehydration step to form the dihydropyran [$^2H_3$]-**18** from the putative dihydropyranol [$^2H_4$]-**16**.) This is shown in Scheme 3b. The gas chromatographic trace and the mass spectra make this clear, as shown in Figure 1. These results demonstrate that no significant opportunity can be available for H−D exchange on the major pathway to the spiroacetal from nonane-1,5-diol. In contrast, the dihydropyran has lost more than one ^{2}H-atom, and this requires that it cannot be part of the major pathway to the spiroacetal.

It was unsurprising that the dihydropyran **18** was not an intermediate on the pathway to **5**, as we have previously observed that, although it can be processed to the spiroacetal, it is less readily incorporated than the corresponding tetrahydropyranol **16**.[5] However, as deuterium loss in the dihydropyran can only occur as part of the dehydration−hydration (D−H exchange) that interconverts the dihydropyran and the tetrahydropyranol or by prior exchange of deuterium in the tetrahydropyranol, it follows also that the tetrahydropyranol is not part of the major pathway to the spiroacetals (see Scheme 3b).

As we now believe that 1,5-nonanediol **11** is a bona fide intermediate, two oxidative events are then required prior to formation of the spiroacetal **5**, namely, oxidation at C5 and hydroxylation at C9. The tetrahydropyranol **16**, or its open-chain form, the hydroxyketone (**15**), both differ in oxidation level at C-5 compared with **11**, and the labeling results above suggest that these are not intermediates.

However, hydroxylation of the diol **11** at C-9 would provide 1,5,9-nonanetriol (**12**). Thus, we synthesized [$^2H_4$]-**12** and found that it is very efficiently incorporated into **5** in both *B. cacuminata* and *B. oleae*. This triol, on secondary alcohol oxidation, would provide ketodiol (**13**), which would spontaneously cyclize to spiroacetal **5**.

Interestingly, wherever this spiroacetal **5** has occurred naturally (in *B. olea*, *B. cacuminata*, and *B. umbrosa*), it is strictly **racemic**,[2] whereas it is known that the enantiomers (shown in Scheme 3a) are optically stable (at pH ∼7)[2] and

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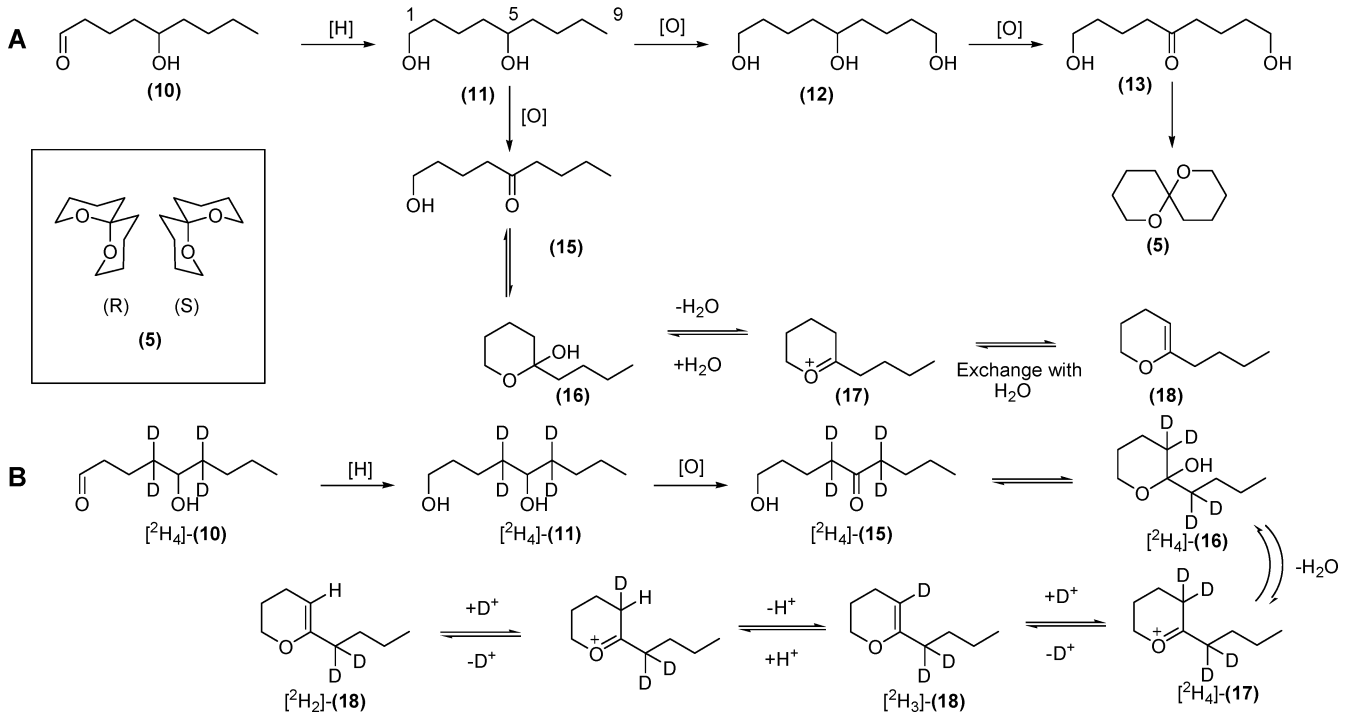
Scheme 3



easily separable by enantioselective gas chromatography.[13] This finding is consistent with the intermediacy of the achiral 1,5,9-nonanetriol **12**, and ketodiol **13**. Nonenzymatic dehydrative spirocyclization of **13** must afford (±)-**5**. Labeled 1,5-nonanediol and 1,5,9-nonanetriol are also very efficiently processed by female *B. oleae* to give a product and incorporation profile very similar to that of *B. cacuminata*. It is likely that the pathway in Scheme 3 is employed also by the olive fly in generating (±)-**5**.

In contrast to the racemic nature of the spiroacetal **5**, we now predict that a chiral intermediate **11** is an intermediate in the biosynthesis. To investigate any enantioselectivity inherent in the pathway, [$^2H_4$]-labeled **enantiomers** of 1,5-nonane diol **11** were synthesized as shown in Scheme 4.[14,15] Enantioselective gas chromatography of the diols and their trifluoroacetates using cyclodextrin-based phases established the ee values to be at least 95%.[16] Administration of these enantiomers to *B. cacuminata* was conducted in the normal

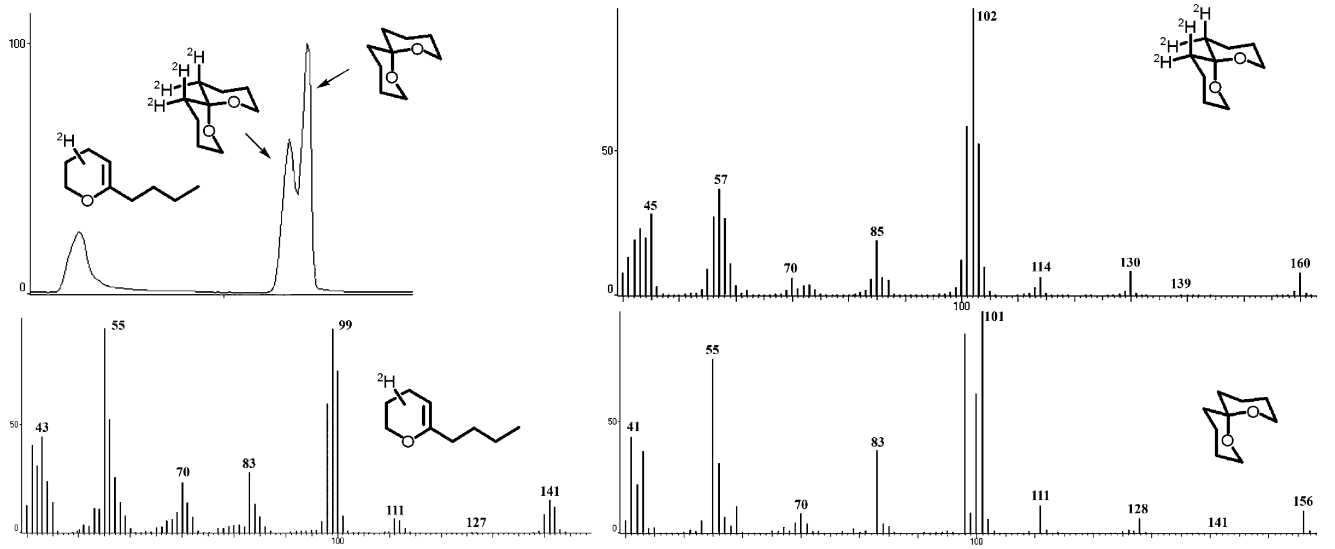
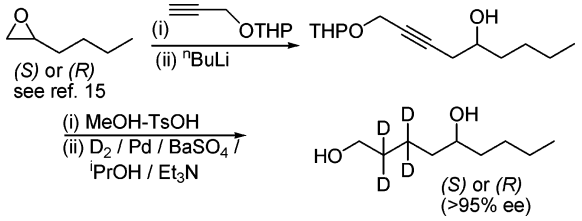


Figure 1. Gas chromatographic trace showing the formation of [^{2}H]-labeled dihydropyran **18** and labeled spiroacetals **5** after administering [$^2H_4$]-nonane-1,5-diol **10**. [$^2H_4$]-**5** elutes prior to unlabeled **5**. EIMS shows that the dihydropyran **18** (M = 140) consists of predominantly the [$^2H_1$] and [$^2H_2$] isotopomers (M = 141, 142). In contrast, **5** (M = 156) is overwhelmingly [$^2H_4$]-**5**, (M = 160).

Scheme 4



way, but GCMS showed essentially no discrimination between the two enantiomers, with both being equally and efficiently incorporated into **5**. It was possible that this apparent lack of selectivity was the result of strong enzymic selection of the minor enantiomer present in the unnatural isomer **11**. Thus, we undertook feeding experiments in which the [$^2H_4$]-(*S*)-**11** was mixed with an equimolar amount of unlabeled [$^2H_4$]-(*R*)-**11** and vice versa. In these experiments, enzymic selection would lead to unlabeled **5** when the [$^2H_4$]-**11** that was not a precursor was administered to the flies. However, once again, no discrimination was observed between the enantiomers of **11**. This may not be indicative of the natural pathway but may simply reflect the remoteness of the site of hydroxylation (the methyl group) from the existing C5 stereogenic center to give achiral **12**.

Incorporation studies were also conducted in an attempt to provide support for the proposal that a fatty acid was the immediate precursor of the hydroxyaldehyde **10**. Dietary administration of ^{2}H-labeled octadecanoic acid (^{2}H at C_9, C_{10}, C_{12}, C_{13}) and hexadecanoic acid (^{2}H at C_{12}, C_{13}) and their methyl esters led to no discernible ^{2}H-incorporation into **5** in *B. cacuminata*. The coadministration of the β-oxidation inhibitor 3-tetradecylthiopropionic acid[17] did not change this, although such compounds have previously been reported to facilitate incorporation of rapidly metabolized polyketide precursors.[18] Labeled hydroxy fatty acids such as 12-hydroxyhexadecanoic acid (^{2}H at C_{11}, C_{13}) (and its methyl ester) and 7,8-dihydroxyhexadecanoic acid (^{2}H at C_{10},C_{11}) (alone and with the inhibitor, 2-fluorostearic acid)[19] were administered. These two hydroxy-substituted C_{16} acids on further hydroxylation and cleavage could provide appropriately oxygenated C_9 units. However, once again no incorporation was detected. There may be several reasons for this. Extensive dilution with endogenous fatty acids would occur, and with the fatty acid at the "front end" of a lengthy sequence, there could be competition for it from other processing opportunities. More specific, advanced fatty acid precursors are currently being investigated in the hope that they will be directed to spiroacetal formation rather than general metabolism.

Thus, the general paradigm advanced previously (Scheme 1) for spiroacetal biosynthesis appears to be valid, although the oxidative events for the formation of **5** differ from those originally proposed.[5] Mono-oxygenation of the methyl group of the C_9 precursor preceded C5 oxo formation, and nonane-1,5-diol rather than the corresponding tetrahydropyranol is an intermediate in the biosynthesis of **5**. Investigation of spiroacetal biosynthesis in other species is continuing, as are inhibition studies of the oxidative events central to these processes.

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Supporting Information Available: Precursor administration methods; synthesis and characterization of [4,4,6,6-$^2H_4$]-**10**, [4,4,6,6-$^2H_4$]-**11**, (*R*)-**11**, (*S*)-**11**, (*S*)-[2,2,3,3-$^2H_4$]-**11**, (*R*)-[2,2,3,3-$^2H_4$]-**11**, and [4,4,6,6-$^2H_4$]-**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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