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Identification of a Grain Beetle Macrolide Pheromone and Its Synthesis by Ring-Closing Metathesis Using a Terminal Alkyne

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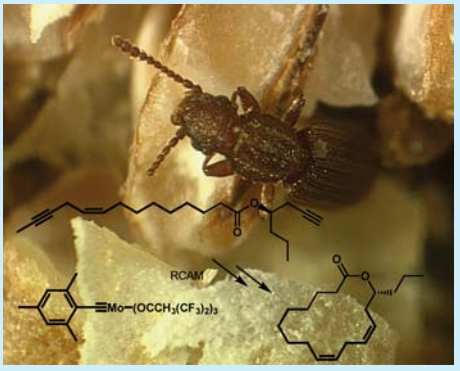
Supporting Information

ABSTRACT: A major C_{18}-macrolide was found during analysis of the frass of the storage beetle *Oryzaephilus surinamensis* to be (9*Z*,12*Z*,15*R*)-octadeca-9,12-dien-15-olide (**10**, cucujolide XI). The synthesis used ring-closing alkyne metathesis as a key step. The highly active 2,4,6-trimethylbenzylidyne molybdenum complex [MesCMo{OC$(CF_3)_2$Me}$_3$] (**12**) allowed the use of a terminal alkyne and afforded the product in excellent yield. Bioassays proved the activity of the *R*-enantiomer **10** in the aggregation of the beetle. Cucujolide XI is the first macrolide pheromone oxidized at the ω-4 position.



Macrolides are an important class of compounds used by several animals for communication via pheromones.[1] Beetles of the genera *Cryptolestes* (Laemophloeidae) and *Oryzaephilus* (Silvanidae), which are important grain storage pests, use a variety of such macrolides as aggregation and sex pheromones, often in species specific mixtures. Groundbreaking work about 30 years ago by the group of Oehlschlager led to the identification of cucujolides I to IX, unsaturated macrolides from C_{10} to C_{14} containing one or two double bonds.[2] We became interested in the pheromones of the sawtoothed grain beetle *Oryzaephilus surinamensis*, previously investigated by Oehlschlager,[3] because these compounds might be used by parasitoids of the beetle for host finding. These parasitoids are potentially useful in storage protection. We recently identified cucujolide X, (5*Z*,8*Z*,12*R*)-tetradeca-5,8-dien-12-olide, as a female attracting pheromone in *O. surinamensis*.[4] This is the first macrolide carrying an ethyl side chain. All others are either unbranched or carry a methyl group, only.

During the analysis of volatiles released from the frass of *O. surinamensis* we obtained a fraction that was highly attractive to the parasitoids. Besides small amounts of cucujolides V and X, large amounts of fatty acids such as linolenic acid were present. After removal of the acids with basic alumina, a new major peak was found by GC/MS analysis, previously obscured by the broad tailing of linolenic acid (Figure 1). This compound **A** showed a mass spectrum closely related to that of linolenic acid (see Supporting Information, Figure S2). Without the alumina treatment it would have escaped our attention. It was accompanied by a second compound **B** with an identical molecular mass of 278. Compound **B** was readily identified by comparison with a synthetic sample to be coriolide known from *Heliconius* butterflies.[5,6]

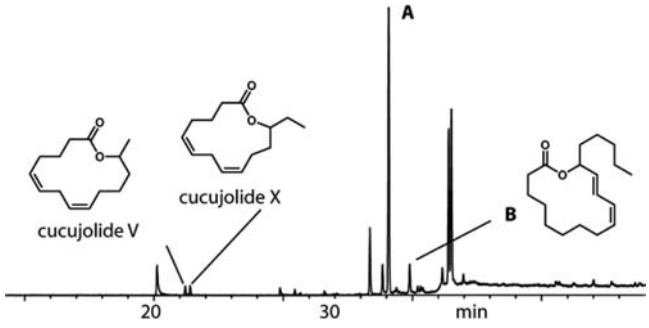


Figure 1. Total ion chromatogram of active fraction containing unknown compounds **A** and **B**, as well as known cucujolides V and X.

To obtain more structural information, the extract containing compound **A** in the μg range was subjected to microhydrogenation with Pd/C catalysis. The resulting compound **C** exhibited the mass spectrum shown in Figure 2. The molecular ion shift from m/z 278 to 282 revealed the presence of two double bonds in **A**. The large ion at m/z 264 (M-18)

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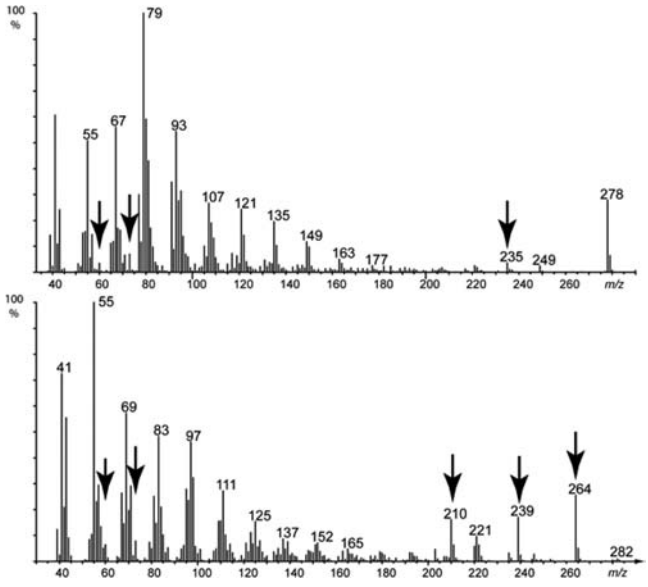


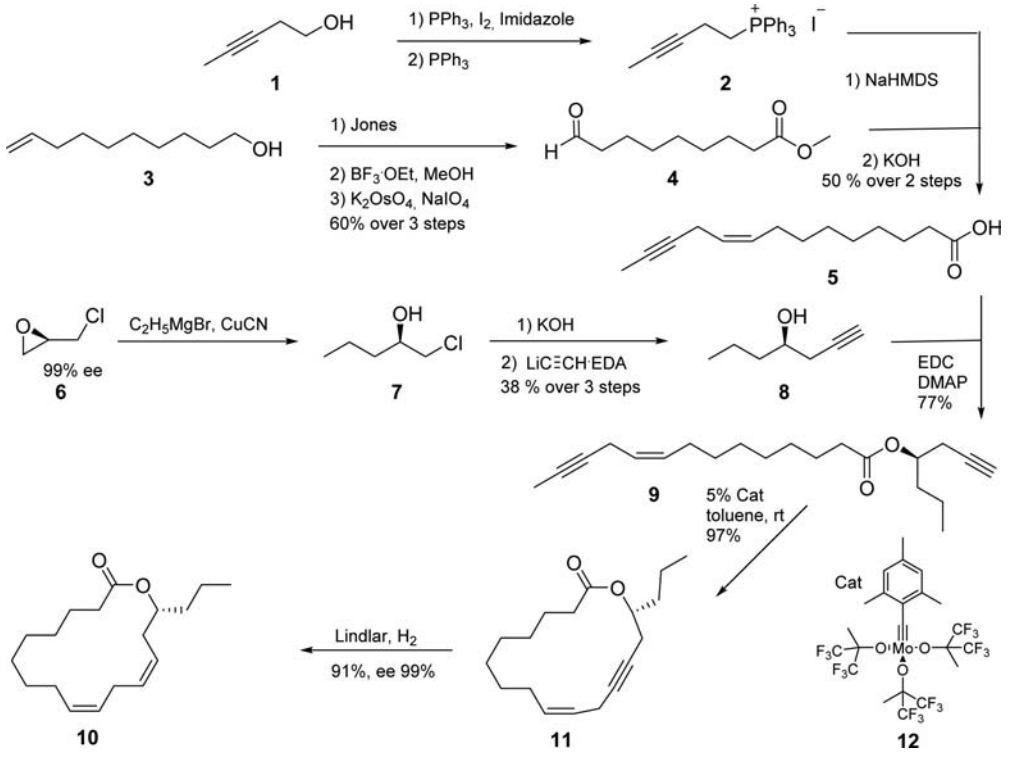
Figure 2. EI-mass spectra of compound **A** (top) and its hydrogenation product **C** (bottom). Diagnostic ions are marked.

together with the ions at m/z 60 and 73 indicated a macrocyclic lactone, because many published mass spectra of macrolides show such fragments. Two diagnostic ions can be found, m/z 239 (M-43) and m/z 210 (M-72), suggesting a 16-membered macrocyclic ring and a propyl group next to the single bound oxygen. We observed this mass spectrometric behavior in various branched macrolides (unpublished results). Assuming a potential biosynthetic relation to linolenic acid, as is the case for cucujolide V and X, we assumed that compound **A** would be (9*Z*,12*Z*)-octadeca-9,12-dien-15-olide (**10**).

To prove our assumption, we planned a synthesis of **10** via ring-closing alkyne metathesis (RCAM) (Scheme 1). With the availability of highly active alkyne metathesis catalysts,[7] RCAM has recently become an important synthetic tool for the preparation of cyclic natural products.[6,6] For recent examples reporting the RCAM of internal alkynes, see ref 8. In previous work, we developed an enantioselective synthesis to cucujolide **X** using a enyne precursor obtained by Wittig methodology.[4] Although the RCAM reaction was catalyzed in a satisfactory manner by an imidazolin-2-iminato tungsten benzylidyne complex,[9] the more recently developed, highly active 2,4,6-trimethylbenzylidyne molybdenum complex [MesCMo{OC-$(CF_3)_2$Me}$_3$] (**12**) might serve as an improved RCAM catalyst.[10] Moreover, this catalyst proved capable of promoting the metathesis of terminal alkynes, which allows avoiding the sometimes tedious installment of an internal triple bond at the ω-1 position of a chain. For determination of the absolute configuration of the natural product and for bioassays, both enantiomers of the target lactone **10** were prepared.

For the synthesis of the *R*-enantiomer, the required enantiomerically pure alkynol **8** was prepared, starting from *R*-epichlorhydrin (**6**) by alkylation, epoxide formation, and its regioselective alkynylation, as reported previously.[11,12] The enyne acid **5** was introduced by the approach reported by us earlier.[4] 9-Undecen-1-ol (**3**) was converted into methyl 9-oxononanoate (**4**) by Jones oxidation, methylation, and K_2OsO_4/$NaIO_4$ cleavage. A Z-selective Wittig reaction with phosphonium iodide **2** furnished (*Z*)-tetradec-9-en-12-ynoic (**5**) acid after saponification. Attempts to obtain a simpler analog, (*Z*)-tridec-9-en-12-ynoic acid carrying a terminal triple bond, failed because the basic conditions of saponification and Wittig reaction favored formation of the conjugated allenic (*Z*)-tridec-9,11,12-trienoic acid instead. The precursor **9** for the RCAM was obtained by 1-ethyl-3-(3-(dimethylamino)propyl)-

Scheme 1. Synthesis of Cucujolide XI (10)



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carbodiimide (EDC) esterification of **8** and **5**. The key RCAM step with the catalyst **12** furnished macrolactone **11** in almost quantitative yield. Final hydrogenation under Lindlar catalysis led to the target compound *R*-**10**. In an identical sequence, *S*-**10** was obtained starting from *S*-epichlorhydrin. Comparison of GC and MS data revealed the natural compound to be indeed (9*Z*,12*Z*)-octadeca-9,12-dien-15-olide (**10**), carrying a propyl side chain. We propose the name cucujolide XI for this compound, in congruence with previous pheromone components of cucujid beetles.[13] The absolute configuration of the natural product is *R*, as determined by gas chromatography on a chiral Hydrodex-6-TBDMS phase (see Supporting Information, Figure S3).

Next the biological activity of **10** was tested. A bioassay was performed to test the attractiveness for both males and females of *O. surinamensis* in a four-chamber olfactometer, as described previously.[4] While males did not respond to 200 ng of **10** at all, females showed a significantly increased walking duration in the test field with the *R*-enantiomer as compared to the control field. The *S*-enantiomer proved to be inactive (Figure 3). The

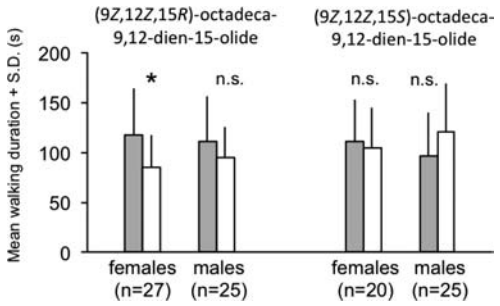

Figure 3. Response of female and male *O. surinamensis* to the *R*-enantiomer (**A**) and the *S*-enantiomer (**B**) of (9*Z*,12*Z*)-octadeca-9,12-dien-15-olide in a four-chamber olfactometer. Gray: test compounds in hexane. White: hexane control. *: $p < 0.05$; n.s. $p > 0.05$ (Wilcoxon-matched-pairs test).

response is similar to that to cucujolide X that is also only attractive to females.[4] Both sexes are attracted to the C_{12} cucujolides IV ((3Z,6Z,11R)-dodeca-3,6-dien-11-olide) and IX ((3Z,6Z)-dodeca-3,6-dien-12-olide), while the C_{14} cucujolide V acts as a synergist.[3,14] The C_{14} cucujolide X and the C_{18} cucujolide XI are active per se, and only on females. Although first experiments showed the activity of cucujolide X to be stronger than that of cucujolide XI, the concentration of the latter in frass is about 20 times higher. How this compound compares to the already known blends[3] and whether it in combination might increase attractivity to the beetle and/or the parasitoid awaits further studies.

Cucujolide XI is likely formed from linoleic acid by oxidation at the ω-3 position, or from linolenic acid by formal water addition to the C15 double bond. Oxidation of acids at the ω or ω-1 position, followed by ring closure, is a common biosynthetic pathway to form fatty acid derived macrolides.[1] Nevertheless, macrolides formed from ω-3 oxidized precursors have, to the best of our knowledge, not been reported before from nature.

RCAM with terminal alkynes has only recently begun to be used in the synthesis of natural products. The molybdenum catalysts developed by Tamm[10,15] and Fürstner[16] are both capable of promoting the metathesis of terminal alkynes. Recently, Fürstner used his catalyst in a total synthesis of mandelalide A, effectively achieving RCAM with a precursor carrying one terminal and one preterminal triple bond,[17] similar to the alkyne motif used in our synthesis. These catalysts proved to be very useful, because the sometimes tedious synthesis of methylated alkynes can thus be avoided.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02461.

Experimental procedures, bioassay method, and enantiomer separation (PDF)

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Notes

The authors declare no competing financial interest.

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