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Synthesis of the aggregation pheromone of the Colorado potato beetle from its degradation product

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ABSTRACT

Incubation of the Colorado potato beetle aggregation pheromone, (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one, with antennal or leg extracts from this beetle gave 6-methyl-5-hepten-2-one as the major product. This ketone was used as a substrate in a stereoselective synthesis of the pheromone. It was attached to the butanediacetal of glycolic acid with good stereoselectivity and the desired isomer was further enriched by purification of the product of this reaction on silica gel.

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The aggregation pheromone of the Colorado potato beetle was identified by Dickens and co-workers in 2002 as (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8**.[1] It is synthesized by males of this species and only the *S* enantiomer is active in both sexes, whereas the *R* enantiomer is inactive and its content in mixtures bigger than 13% prevents behavioral response of this beetle to the pheromone.[2]

So far six stereoselective methods of the synthesis of this pheromone have been described. To confirm the structure of the isolated pheromone Oliver and co-workers synthesized both the *R* and *S* enantiomers and the racemic mixture.[3] The racemic compound was obtained from geraniol, and the active *S* enantiomer was prepared from (*S*)-linalool isolated from coriander, which prevented preparation of the pheromone in larger quantities. In 2005 Tashiro and Mori described the synthesis of (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8** from nerol.[4] A key step in this method is the enantioselective esterification of (±)-2,3-epoxynerol with lipase PS from *Pseudomonas cepacia*. Although this reaction gave the (2*S*,3*R*)-epoxyneryl acetate (a key intermediate in this synthesis) with high enantiomeric excess (98.8%), the yield was only 16%. An optically active acetonide of glyceraldehyde was applied as a starting material in the method developed in 2009 by Babu and Chauhan, who obtained (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8** with 98.6% enantiomeric excess.[5] Faraldos and co-workers[6] detected the pheromone as a side product during purification of the 2,3-epoxide obtained from 2-fluoronerol or 2-fluorogeraniol—an intermediate in the synthesis of (*R*)-2-fluorolinalool. However, the enantiomeric excess of (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8** obtained in this method was only 68–91%. Recently, a very simple, protecting group-free procedure for the synthesis of the pheromone from geraniol was developed by Wu and co-workers, who applied the Sharpless epoxidation and selective oxidation of the secondary alcohol, which substantially reduced the number of steps.[7] Although the yield obtained in this preparation was excellent (80%), the enantiomeric excess was again quite low (86%). Finally, Li and co-workers applied the Sharpless epoxidation of geraniol and subsequent recrystallization of the 4-bromobenzoate derivative, which provided the pheromone with 99% ee.[8]

Several lines of evidence demonstrate that enzymatic inactivation of pheromones is important for the function of the insect olfactory system.[9–13] We therefore intended to study the degradation pathway of the aggregation pheromone of the Colorado potato beetle. We also decided to develop a new procedure for its synthesis applying butanediacetals as building blocks.

While working on the synthesis of the enantiomerically pure (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8** we prepared the

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racemic compound according to the published procedure[3] and attempted to obtain potential products of its enzymatic degradation. The most likely target for enzymes in this compound is the primary alcohol, which could be oxidized to an aldehyde or carboxylic acid by an alcohol dehydrogenase and aldehyde dehydrogenase/oxidase. An analogous inactivation pathway has been determined for bombykol, the sex pheromone of the silkmoth, *Bombyx mori*.[14] No degradation involving the other functional groups—the ketone and the tertiary alcohol has been described so far. We have therefore subjected 1,3-dihydroxy-3,7-dimethyl-6-octen-2-one to Swern or Dess–Martin oxidation. However, regardless of the oxidation method used the only product that could be isolated from the reaction mixture was 6-methyl-5-hepten-2-one **1**. This ketone was previously also detected during the GC analysis of the aggregation pheromone of the Colorado potato beetle,[1,3] which implied thermal instability of the pheromone. It was also formed when *tert*-butyldiphenylsilyl-protected precursor of the pheromone was oxidized with pyridinium chlorochromate.[3] Decomposition of the pheromone was also reported during removal of the TBDPS group from the primary alcohol in the final step of its synthesis by Tashiro and Mori.[4] They attributed this decomposition to the basicity of TBAF and compared this reaction to the decomposition of fructose under basic conditions.

It should be noted that when 1,3-dihydroxy-3,7-dimethyl-6-octen-2-one was first described as one of the oxidation products of geraniol by *Pseudomonas incognita*, 6-methyl-5-hepten-2-one **1** was also detected as one of these products.[15] A degradation pathway of geraniol by this bacterium was then proposed, which actually involved several steps analogous to those employed in the synthesis of 1,3-dihydroxy-3,7-dimethyl-6-octen-2-one from this substrate,[3] that is, regioselective epoxidation of the proximal double bond, epoxide hydration, and oxidation of the secondary alcohol.[15] 6-Methyl-5-hepten-2-one **1** has also been described as the degradation product of monoterpene alcohols—geraniol and nerol, and aldehydes—geranial and neral, in several fungal species: *Botrytis cinerea*,[16] *Penicillium italicum*,[17] and *Penicillium digitatum*.[18,19] In this degradation pathway oxidation of the alcohols (geraniol, nerol) to aldehydes was followed by their decomposition, which was attributed to a specific enzyme named citral lyase.[19,20] Biotransformation of farnesol by several fungal species (*Fusarium culmorum*, *B. cinerea*, *Rhodotorula rubra*, *Rhodotorula marina*) produced an analogous ketone—6,10-dimethyl-5,9-undecadien-2-one (geranylacetone).[21] It seems therefore that such ketones are common degradation products of terpenes in microorganisms.

Performing a retrosynthetic analysis we came up with an idea of using a reverse reaction to synthesize the pheromone from 6-methyl-5-hepten-2-one **1** by attaching it to the butanediacetal derivative of glycolic acid **2**. Stereoselective ketone aldol reaction with butanediacetals of glycolic acid was first studied by Ley and co-workers.[22] In this reaction the desired configuration at the generated chiral center can usually be obtained when the two chains attached to the carbonyl group differ substantially in size and 6-methyl-5-hepten-2-one **1** fulfills this requirement. The butanediacetal of glycolic acid, (5*R*,6*R*)-5,6-dimethoxy-5,6-dimethyl-1,4-dioxanyl-2-one **2**, was prepared according to published procedures.[23] 6-Methyl-5-hepten-2-one **1** was then attached to its enolate generated with lithium bis(trimethylsilyl)amide. The reaction proceeded with good yield (88%) and gave a mixture of two diastereoisomers with the *S* and *R* configuration at the newly generated chiral center ((3*S*,5*R*,6*R*)-5,6-dimethoxy-5,6-dimethyl-3-[(1*S*)-1,5-dimethyl-1-hydroxyhex-4-enyl]-1,4-dioxan-2-one **3*S*** and (3*S*,5*R*,6*R*)-5,6-dimethoxy-5,6-dimethyl-3-[(1*R*)-1,5-dimethyl-1-hydroxyhex-4-enyl]-1,4-dioxan-2-one **3*R***) in 1:0.21 ratio (determined by ^{1}H NMR, Fig. S1). These diastereoisomers were easily separated by column chromatography on silica gel giving the *S* isomer **3*S*** with 98% de. Removal of the protecting group was performed by transesterification with hydrogen chloride generated from trimethylsilyl chloride in methanol, which gave the corresponding methyl ester **4** with 63% yield. Deprotection of the diacetal by acid hydrolysis with trifluoroacetic acid in water was unsuccessful. Such problems were previously also reported for other butanediacetal derivatives of glycolic acid but their cause has not yet been explained.[22] The ester **4** was then reduced with lithium aluminum hydride to the triol **5** in 57% yield. Next steps followed the procedures described previously by Oliver and coworkers[3] (Scheme 1) giving (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8** with an overall yield of 26%. Enantiomeric excess of the TBDPS-protected pheromone **7** determined by chiral HPLC was 98% (Fig. S2). The chemical purity of the final product determined by GC was >99% and its specific optical rotation was +1.6 ± 2.1 (*c* 0.79, $CHCl_3$).

The pheromone (0.25–2.5 mM) was then incubated with protein extracts from the antennae (50–200 equiv) or legs (10–20 equiv) of the Colorado potato beetle for 1–96 h at 22 or 30 °C in 0.5–1.5 ml of 20 mM sodium phosphate buffer, pH 7.5 with or without NAD^+ at equal concentration. Aliquots were extracted with an equal volume of diethyl ether, the solvent volume was reduced ca. 20 times and the samples were initially analyzed by TLC. These experiments indicated that 6-methyl-5-hepten-2-one **1** was the major degradation product in these reactions, although they usually required 96 h incubations of the 1 mM pheromone with 200 antenna-equivalents in 1.5 ml of buffer to proceed to near completion. Such reaction conditions, however, are usually required, when unlabeled pheromones are used at relatively high concentrations with antennal protein extracts.[24] We then adjusted the conditions to minimize the background amounts of 6-methyl-5-hepten-2-one **1** in GC analysis and were able to demonstrate the degradation of (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8** also by this technique. The pheromone was stable during the incubation in a buffer with/without NAD^+, but was degraded to 6-methyl-5-hepten-2-one **1** in the presence of antennal protein extracts. The degradation was always faster when NAD^+ was added (Fig. 1). Similar degradation was also observed with leg extracts. No degradation was detected when the protein extracts were thermally inactivated by boiling for 5 min. Very little conversion to 6-methyl-5-hepten-2-one **1** was seen in reactions with male antennal extracts from the silkmoth (25 antenna-equivalents) or the nun moth (*Lymantria monacha*, 20 antenna-equivalents) with or without NAD^+ (Table S1).

Enzymes, whose participation in pheromone inactivation has been established, are quite diverse, but they generally attack particular functional groups: esters, aldehydes, alcohols, epoxides, sometimes alkyl groups attached to heteroatoms.[9] However, none of the reactions identified so far involves the breakdown of the carbon–carbon backbone, as we have demonstrated for the aggregation pheromone of the Colorado potato beetle. Moderate enhancement of the reaction rate by addition of NAD^+ to the crude protein extracts suggests the participation of a dehydrogenase. We suspected that the reaction may involve oxidation of the primary alcohol to an aldehyde. It has been demonstrated previously that citral decomposes to 6-methyl-5-hepten-2-one **1** and acetaldehyde via 3-hydroxycitronellal in the presence of high concentration of amino acids,[25] which suggested that β-hydroxyaldehydes are unstable and decompose by retro-aldol reactions to carbonyl compounds. However, it was difficult to envisage a plausible mechanism explaining such a decomposition in the case of the Colorado potato beetle pheromone, which contains a ketone at C2. Nevertheless we have incubated (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8** with recombinant equine alcohol dehydrogenase to see whether enzymatic oxidation of the primary alcohol may lead to its decomposition. 6-Methyl-5-hepten-2-one **1**

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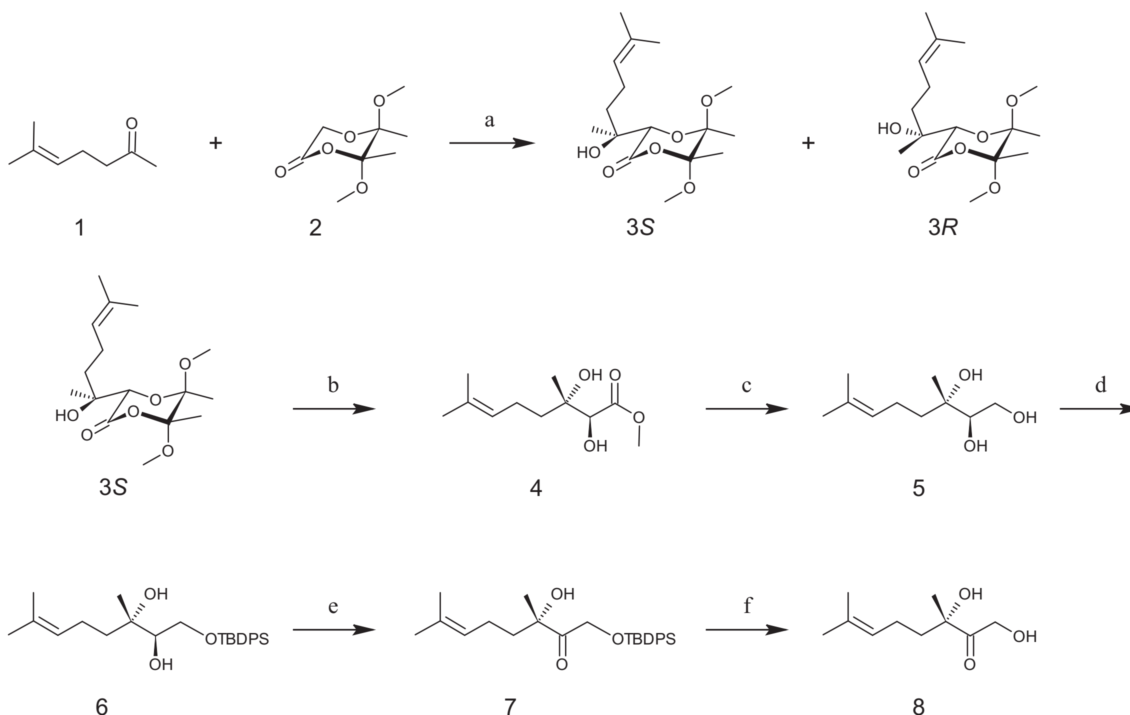


Scheme 1. Synthesis of (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8** from 6-methyl-5-hepten-2-one **1** and the butanediacetal derivative of glycolic acid **2**. Reagents and conditions: (a) LHMDS, THF, −78 °C; (b) HCl, MeOH; (c) $LiAlH_4$, Et_2O; (d) TBDPSCl, Et_3N, DMAP, CH_2Cl_2; (e) SO_3:Py, DMSO, Et_3N, CH_2Cl_2, 0 °C–room temp; (f) TBAF, THF, 0 °C.

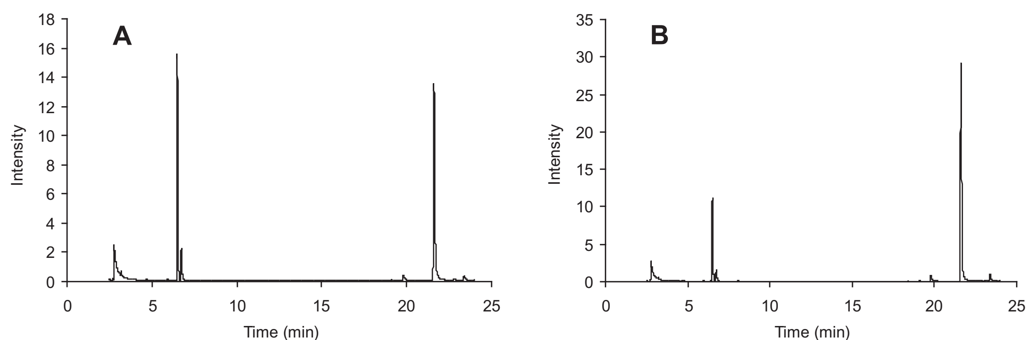
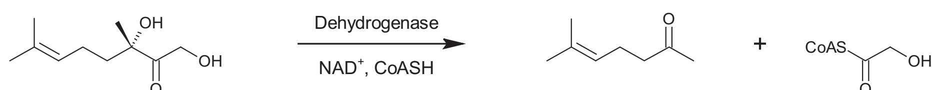


Figure 1. Gas chromatograms of diethyl ether extracts obtained from mixtures of 1 mM (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8** with a protein extract from 200 Colorado potato beetle antennae and 1 mM NAD^+ (A) or without this cofactor (B) incubated for 96 h. The retention times are 21.6 min for the pheromone **8** and 6.5 min for 6-methyl-5-hepten-2-one **1**.



Scheme 2. Proposed mechanism of decomposition of (*S*)-1,3-dihydroxy-3,7-dimethyl-6-octen-2-one **8** to 6-methyl-5-hepten-2-one **1** by antennal protein extracts from the Colorado potato beetle.

was detected as the product of this reaction, but it proceeded very slowly (Table S1), suggesting that a different mechanism should be taken into consideration. In the decomposition of citral involvement of a specific lyase has been proposed.[19,20] However, comparison of the pheromone structure to the wide-spread microbial metabolite acetoin suggested a simpler mechanism. Acetoin, which contains neighboring ketone and secondary alcohol groups, is degraded to acetaldehyde and acetyl-CoA by acetoin dehydrogenase belonging to the β-ketoacid dehydrogenase family, which utilizes thiamine pyrophosphate as a cofactor.[26] Although the aggregation pheromone of the Colorado potato beetle contains a tertiary alcohol, its degradation by a similar enzyme requiring NAD^+ as the electron acceptor would lead to 6-methyl-5-hepten-2-one **1** and glycolyl-CoA (Scheme 2).

Because of the long incubation times, which we had to apply in degradation reactions with the unlabeled pheromone, participation of microbial enzymes cannot be entirely excluded. However, much slower degradation by the antennal extracts from male silkmoths and nun moths suggests that if this is the case the microorganisms responsible for this reaction are not a contamination coming from the laboratory environment but are associated with the Colorado potato beetle. To resolve these ambiguities, the reactions will most likely have to be conducted with the radiolabeled pheromone, preparation of which is now in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.06.083. These data include MOL files and InChiKeys of the most important compounds described in this article.

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