Identification and Biosynthesis of an Aggregation Pheromone of the Storage Mite *Chortoglyphus arcuatus*

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Dedicated to Professor Axel Zeeck on the occasion of his 65th birthday

In an effort to identify new pheromones from mites, the headspace of undisturbed colonies of the storage mite Chortoglyphus arcuatus *was analyzed by GC-MS by use of a closed-loop stripping apparatus (CLSA) or solid-phase microextraction (SPME). The major compound emitted from the mites is (4*R*,6*R*,8*R*)-4,6,8-trimethyldecan-2-one (4*R*,6*R*,8*R*-**8**). The structure was elucidated by analysis of the mass spectrum, synthesis of authentic samples, and gas chromatography on a chiral phase. Bioassays show that this compound, for which we propose the trivial name chortolure, is an aggregation pheromone for both sexes of this species. Several related compounds are released in smaller amounts by the mites. The alarm pheromones of these mites, neral and geranial, can only be found in total extracts of the mites, in which **8** occurs only in minute amounts. The method of sampling is therefore crucial for pheromone identification. Feeding experiments with deuterated propionate showed that chortolure is a polyketide, formed by successive addition of four propionate units to an acetate starter.*

Introduction

Mite pheromones have been intensively investigated over the last twenty years, predominately by Kuwahara's group.[1,2] Alarm pheromones, often neral and geranial, have most often been identified, with sex pheromones or aggregation pheromones having been found less commonly. One reason for this discrepancy might be the methods used for extraction of the mites. In earlier studies, whole-mite cultures were sieved to separate the animals from the culture medium, and these were then extracted with a solvent. In more recent work, individual mites were hand-picked and extracted. Alarm pheromones predominate in these extracts, probably because they are stored in order to allow the mite to emit them immediately on a particular incident. Sex or aggregation pheromones do not necessarily have to be stored, because they are given off over longer periods of time. The mite may synthesize them only when needed, a mechanism often found in Lepidoptera, for example.[3,4] Therefore, sex and aggregation pheromones might be present only in low amounts in extracts prepared in the manner described, and so found less often than alarm pheromones.

We chose an alternative method for investigating the pheromones of the storage mite *Chortoglyphus arcuatus* (Tropeau; Acarina, Chortoglyphidae). Comparison of whole-body extracts and headspace analysis of whole-mite cultures should give clues to the functions of identified compounds. The results we obtained by these methods, which led to the identification of a new mite aggregation pheromone, are described in this article.

C. arcuatus is an important storage mite occurring on many farms in rural regions of central Europe. It has recently been deduced to be responsible for allergies in farmers who do not react to allergens from the well-known house dust mites.[5]

Results

Identification

In the first experiment, a fully grown culture of *C. arcuatus* mites was extracted and analyzed by GC-MS. The terpenes geranial and neral and their derivatives were readily identified (see Figure 1 and Table 1). The alarm pheromone activity of neral and geranial in this mite has been reported previously by us. Neral showed the highest repellency towards conspecific mites.[6]

In a second set of experiments, a headspace technique was used for the collection of mite volatiles. Mite cultures were kept in small glass vessels. These vessels were connected to a closed-loop stripping apparatus (CLSA),[7] which allowed continuous circulation of air with low airspeed. A 5 mg active charcoal filter was placed in the flow path of the air to absorb volatiles given off from the mite culture. The volatiles were collected for 6–24 h without any disturbance of the mite cultures

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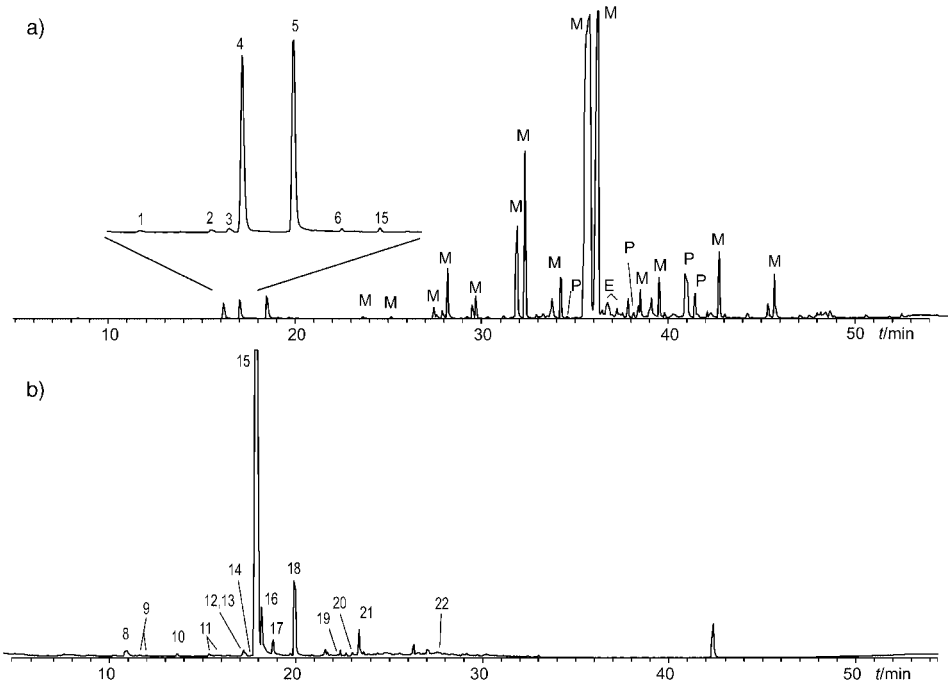


Figure 1. *Gas chromatograms of a) a pentane extract of* C. arcuatus *treated with CH_2N_2 and of b) a headspace CLSA extract. Numbers refer to Table 1. M: Fatty acid methyl esters obtained by the derivatization procedure. E: Naturally occurring fatty acid ethyl esters. P: Naturally occurring fatty acid 2-pentyl esters. The acids are common saturated and unsaturated fatty acids ranging from C_{12} to C_{24}; 25 m DB5, from 60 °C to 280 °C at 6 °C min^{-1}.*

other than the circulating air. To the best of our knowledge, this is the first investigation of an unstressed mite culture.

Table 1. *Compounds identified in headspace and solvent extracts of* C. arcuatus.

No.[a]	Compound	Solvent[b]	Headspace[b]
1	isogeranial (**16**)	+	
2	2,3-epoxyneral (**17**)	+	
3	2,3-epoxygeranial (**18**)	+	
4	neral	+++	
5	geranial	+++	
6	tridecane	+	
7	geranic acid	+++	
8	(4*R**,6*R**)-4,6-dimethyloctan-2-one (**19**)		+
9	2-(1-methylethyl)-5-methylhex-2-enal (**21**)		+
10	4,6-dimethylnonan-2-one (**20**)		+
11	2-(1-methylethyl)oct-2-enal (**22**)		+
12	4,8-dimethyldecan-2-one[c] (**23**)		+
13	6,8-dimethyldecan-2-one[c] (**24**)		+
14	4,6,8-trimethyldecen-2-one[c]		+
15	(4*R*,6*R*,8*R*)-4,6,8-trimethyldecan-2-one (**8**)	+	+++
16	(2*S*,4*R*,6*R*,8*R*)-4,6,8-trimethyldecan-2-ol (**7**)		++
17	4,6,8-trimethyldecen-2-one[c]		+
18	(4*R*,6*R*,8*R*)-4,6,8-trimethylundecan-2-one (**13**)		++
19	4,6,8-trimethyldodecan-2-one[c] (**25**)		+
20	4,6,10-trimethyldodecan-2-one[c] (**26**)		+
21	4,6,8,10-tetramethyldodecan-2-one[c] (**27**)		+
22	4,6,8,10-tetramethyltetradecan-2-one[c] (**28**)		+

[a] No.: number in Figure 1. [b] +++: major component, ++: minor component, +: trace component. [c] Tentative identification based on mass spectrometry, biogenetic considerations, and gas chromatographic retention times.

The filters were then extracted with methyl acetate or CS_2, and the extract was analyzed by GC-MS (Figure 1). The chromatogram was dominated by a single compound (**8**), which had been found only in traces in the total-mite solvent extracts prepared earlier. The compound showed the mass spectrum presented in Figure 2, and was absent in control experiments performed with the culture medium without mites.

The base ion at $m/z = 43$, together with the ions at $m/z = 58$ and 140 ($[M-58]^+$), showed the presence of a methyl ketone. The strong ion peak at $m/z = 85$ was consistent with the presence of a methyl branch at C-4. Despite the low abundance of ions above $m/z = 120$, they did indicate further methyl branches, especially with regard to the ion series $C_nH_{2n-1}O$ and C_nH_{2n-3}, the latter formally arising from the former by loss of water. Thus, the ion pair 127/109 indicated a methyl branch at C-6, while the almost absent ion at $m/z = 169$, together with $m/z = 151$, showed a further branch at C-8. The structure 4,6,8-trimethyldecan-2-one (**8**) was thus proposed from the mass spectrometric data.

Synthesis

We then synthesized a diastereomeric mixture of **8** to verify the structure (Scheme 1). By use of the synthesis of Mori and Kuwahara[8]—who synthesized the homo compound **13** during the synthesis of the mite pheromone lardolure (**15**)—as a model, 2,4,6-trimethylphenol (**1**) was hydrogenated and oxidized to form an enantiomeric mixture of all-*cis*-2,4,6-trimethylcyclohexanone (**2**). Bayer–Villiger oxidation furnished the ε-lactone **3**,[9] which was then opened with sodium methoxide to form the hydroxy ester **4**. During this methanolysis, the C-2 stereogenic center was epimerized, thus a mixture of two diastereomers was obtained. Reduction gave access to two diastereomers of the corresponding diol **5**, which was selectively tosylated at the primary alcohol function to form the tosylate **6**. Under copper catalysis conditions, 1-methylpropylmagnesium bromide was coupled with **6** to form the alcohol **7**. Final oxidation then delivered the target compound **8** as a mixture of all diastereomers, because the stereogenic center at C-2 is destroyed during this process. Investigation of this mixture by GC-MS showed that our structural proposal was correct. Furthermore, the four diastereomers could be separated by GC on an apolar phase. It turned out that the first eluting pair of enantiomers has the same retention time as the naturally oc-

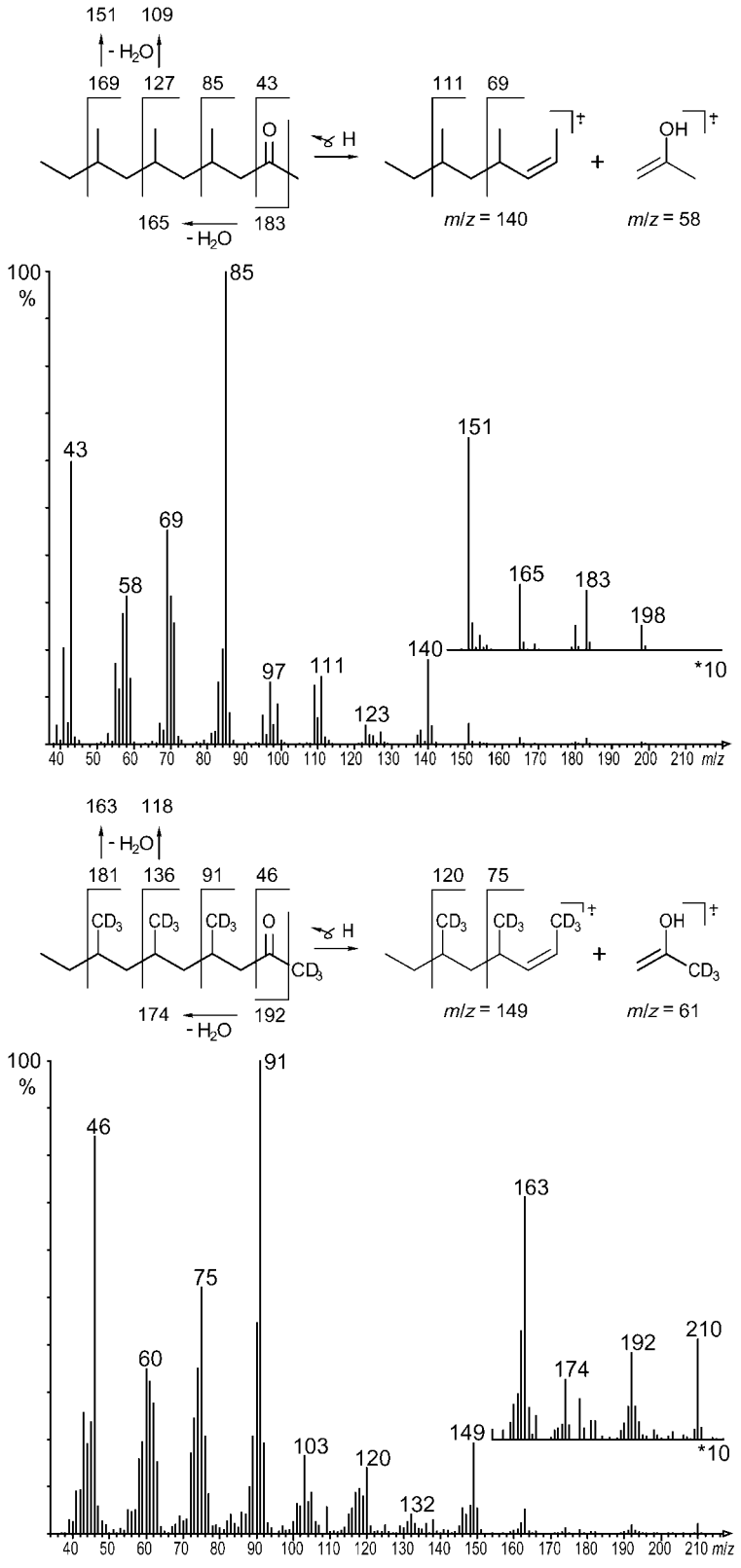


Figure 2. *Mass spectra and fragmentation patterns of 4,6,8-trimethyldecan-2-one (**8**) and [D_{12}]**8**.*

curring compound. We then set out to elucidate the absolute and relative configurations of natural **8** by further synthesis.

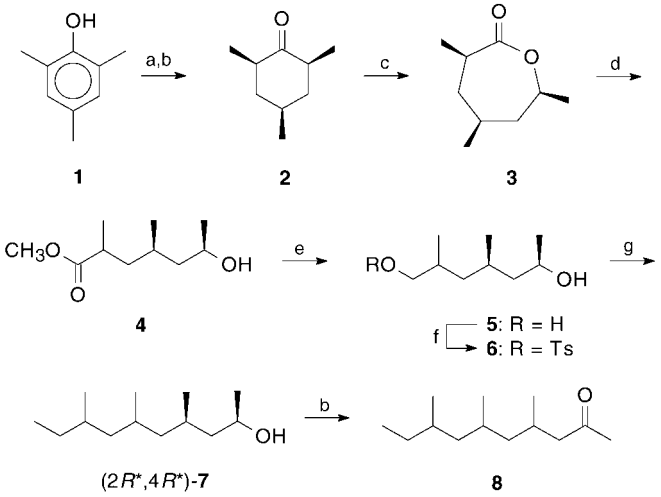
Lactone **3** can be directly transformed into the diol (2*R**,4*S**,6*S**)-**5** by reduction with $LiAlH_4$, thus avoiding epimerization at C-2 of **3**.[8] On application of the described reaction sequence, an enantiomeric mixture of the two 4,6-*syn* diastereomers of **8** was obtained, because the stereogenic center at C-2 of **7** is lost during oxidation. These eluted as the first two peaks in GC, thus confirming the 4,6-*syn* arrangement in the natural **8**. In the mite *Lardoglyphus konoi*, the related aggregation pheromone lardolure **15** has the all-*R* configuration.[15] We therefore chose to synthesize a pure enantiomer of (4*R*,6*R*,8*R*)-**8** in order to compare it with the natural product. This enantiomer had previously been synthesized by Morr et al.,[10] starting from goose fat. We used a technique based on the Yamamoto synthesis of **15**, starting from the (2*R*,4*R*)-pentane-2,4-diol acetal of *cis*-3,5-dimethylcyclohexanone (**9**), to obtain the alcohol **10** in a very high *ee*.[11,12] This alcohol was converted into (4*R*,6*R*,8*R*)-**8** by selective tosylation of the primary alcohol function to yield compound **11**, followed by reduction of the tosylate and oxidation of the resulting alcohol (2*R*,4*R*,6*R*,8*R*)-**7** with Jones' reagent in better than 98% *ee* (Scheme 2).

Chiral gas chromatography was then used to determine the naturally occurring enantiomer of **8** and its purity. The best separation of the racemic mixture of the diastereomers was achieved with a 30 m heptakis(2,6-di-*O*-dimethyl-3-*O*-pentyl)-β-cyclodextrin in 50% OV1701 capillary column at 60 °C. Seven of the eight compounds were separated, and the peak eluting second was (4*R*,6*R*,8*R*)-**8**. The peak area indicated the sixth peak to be the one consisting of two isomers. The natural product, eluting as the second peak, was shown to represent a pure enantiomer; it was identical to our synthetic product, thus confirming our suggestion (Figure 3).

Bioassay

The naturally occurring enantiomer having been identified, our possession of the synthetic product allowed us to perform bioassays to find out the function of **8** in the chemical ecology of the mites.

Bioassays were performed in petri dishes, in which a filter paper with a test solution was placed, with five mites. The numbers of mites present at the filter paper were measured in one-minute intervals with comparison to a test solution (0.5 $\mu L\,mL^{-1}$ hexane) against pure hexane as control. The results (see Table 2) show that both males and females spent significantly more time on the test filter paper than on the control filter paper. We also tested whether both sexes produced **8** by enclosing 20 males or females separately in small vials and analyzing the headspace by SPME (solid-phase microextraction). The results showed that both male and female mites produce the aggregation pheromone **8** (females 12.8 ± 4.7 ng per individual and males 8.5 ± 2.6 ng per individual).



Scheme 1. *Synthesis of a diastereomeric mixture of the ketone* ***8*** *(relative configurations shown). a)* H_2*,* Rh/Al_2O_3*, b)* $Na_2Cr_2O_7/H_2SO_4$*, c)* m*CPBA, d) NaOMe/MeOH, e)* $LiAlH_4$*, f)* p-*TsCl, pyridine, g) (*sec*-butyl)MgBr,* Li_2CuCl_4*.*

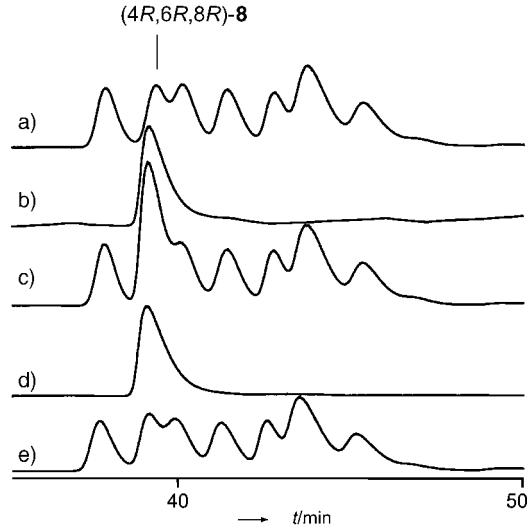
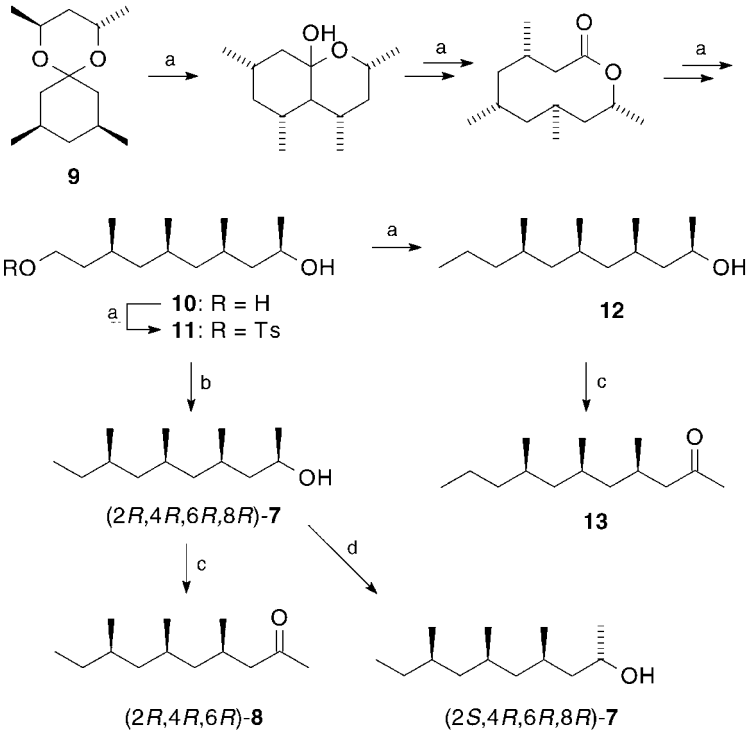


Figure 3. *Separation of* ***8*** *by chiral GC on a heptakis(2,6-di-O-dimethyl-3-O-pentyl)-β-cyclodextrin in 50% OV1701 phase at 60°C. a)* rac-***8****, b) (4*R,6R,8R*)-****8****, c) co-injection a and b, d) natural* ***8****, e) co-injection a and d.*



Scheme 2. *Synthesis of (4*R,6R,8R*)-****8****, (4*R,6R,8R*)-****13****, (2*R,4R,6R,8R*)-****7****, and (2*S,4R,6R,8R*)-****7****. a) Refs. [11, 12]. b)* $LiAlH_4$*, c)* $Na_2Cr_2O_7/H_2SO_4$*, d)* PPh_3*, BzOH, DEAD, then MeONa.*

Biosynthesis

The biosynthesis of methyl branched aliphatic pheromones in insects and other arthropods is not well investigated. All results obtained so far have indicated that methyl branches in the middle of a chain are formed by incorporation of a propionate unit originating from methylmalonate into the chain.[4, 13] We therefore added sodium 3,3,3-trideuteropropionate to the diet and analyzed this culture. After one week, incorporation of up to four propionate units was detected by GC-MS analysis of **8**. Because of the deuterium effect, deuterated compounds elute earlier then their undeuterated counterparts in GC. This resulted in partial separation of the mixture of compounds originating from the incorporation of up to four deuterated propionate units (see Figure 4). The mass spectrum of the compound eluting first ($[M]^+ = 210$, Figure 2) is fully consistent with incorporation of four deuterated propionate units as depicted in Scheme 3. We suggest that **8** is formed by starting with acetate, which is elongated four times with methylmalonate to form acid **14**. Oxidative decarboxylation produces the ketone. The results show that the mites produce the pheromone de novo and that it is biogenetically a polyketide; this has also been proposed, but not experimentally proved, for the related pheromone lardolure **15**.[9]

Comparison of CLSA and SPME sampling

We then checked—by SPME of live, undisturbed cultures—whether the air flow during the CLSA experiment had any effect on the production of volatiles. Again, compound **8** was the dominant constituent.

Table 2. *Reaction of* Chortoglyphus arcuatus *to filter papers treated with (4*R,6R,8R*)-****8*** *(Test) and control filter papers with hexane (Control). Number of individual mites on the filter paper during the test period.*

	Test	Control	p[b]
Females	12.2 ± 8.1[a]	6.0 ± 4.8	0.002
Males	12.4 ± 8.1	7.5 ± 5.9	0.011

[a] Mean ± sd ($n = 25$). [b] Level of significance (Wilcoxon matched-pairs test)

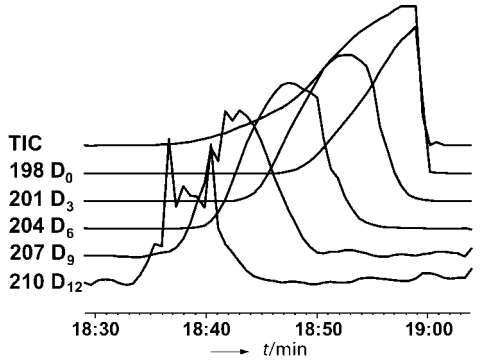
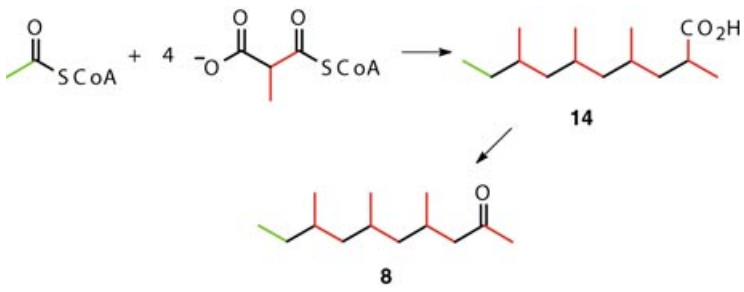


Figure 4. *TIC of headspace CLSA extract of a mite culture supplemented with sodium 3,3,3-trideuteropropionate. Total ion current (TIC) and selected molecular ions characteristic for the incorporation of zero (198), one (201), two (204), three (207), or four (210) propionate units.*



Scheme 3. *Proposed biosynthesis of **8**.*

The large amounts produced by the mites hinder any other component from being adsorbed by the SPME fiber. The SPME experiments therefore showed only a single compound, while the CLSA extracts gave evidence of the presence of several minor components (see Figure 1 and Table 1). This phenomenon should generally be considered when working with SPME.

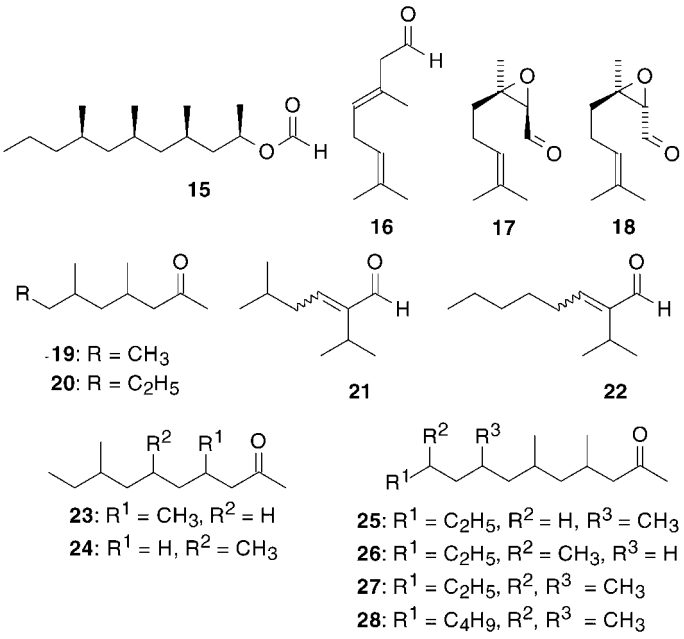
Further components

We then analyzed further components in the headspace extracts. Mite-derived components predominated, while diet constituents could only be found in minor amounts. Most of the compounds had the same biogenetic origin as **8**. Their structures were deduced from their mass spectra, based on the fragmentation pattern presented above, biosynthetic considerations, and retention times (see Supporting Information for individual fragmentation patterns). The second prominent compound proved to be the homologue of **8**, 4,6,8-trimethylundecan-2-one (**13**). Racemic **13** was synthesized by a procedure similar to that shown in Scheme 1, by coupling of **6** with 1-methylbutylmagnesium bromide and subsequent oxidation. The (4*R*,6*R*,8*R*)-enantiomer was synthesized as shown in Scheme 2 by treatment of the tosylate **11** with dimethyllithium cuprate and oxidation. Gas chromatography on a chiral phase allowed good separation into five peaks, but the natural, second eluting peak consisted of one compound only, identical to (4*R*,6*R*,8*R*)-**13** (see Supporting Information). The corresponding alcohol of **8**, 4,6,8-trimethyldecan-2-ol (**7**), eluted slightly after the ketone in the GC and was readily synthesized as a diastereomeric, racemic mixture by reduction of **8**. The (2*R*,4*R*,6*R*,8*R*)-enantiomer was obtained during the course of the synthesis of **8**, while the (2*S*,4*R*,6*R*,8*R*)-enantiomer was synthesized by Mitsunobu inversion of the (2*R*,4*R*,6*R*,8*R*)-enantiomer (Scheme 2). Separation by GC showed that two compounds occur naturally, coeluting with (2*S*,4*R*,6*R*,8*R*)-**7** and its 2*R*-epimer. They were well separated from the other diastereomers and present in a 96:4 ratio. From these results we conclude that the two naturally occurring compounds indeed have the absolute configurations of the synthesized enantiomers. The stereogenic centers containing the methyl branches most probably exhibit the same stereochemistry as the natural ketone, because additional enzymes with other stereospecificity would otherwise have to be active during their biosynthesis.

The short ketone (4*R**,6*R**)-4,6-dimethyloctan-2-one (**19**) was identified by comparison with synthetic compounds, prepared by reaction of tosylate **6** with $(CH_3)_2CuLi$, followed by oxidation. Its biosynthesis can be explained in terms of only three rounds of methylmalonate elongation compared to the biosynthesis of **8**. The two methyl groups possess the *syn* configuration. 4,6-Dimethylnonan-2-one (**20**), the analogue of **13** and also biogenetically formed by only three rounds of methylmalonate elongation, was identified similarly. Several further branched ketones could be tentatively identified (see Supporting Information). The determination of their configurations awaits further synthetic effort, but we know that only one diastereomer of each compound occurs naturally, most probably with the same *all-R* configuration as seen in the ketones **8** and **13**. They are formed by variation of the biosynthetic scheme described in Scheme 3. Incorporation of a fifth propionate unit leads to 4,6,8,10-tetramethyldodecan-2-one (**27**). Addition of a second acetate unit before elongation with methylmalonate furnishes 4,6,8-trimethyldodecan-2-one (**25**) and 4,6,8,10-tetramethyltetradecan-2-one (**28**). The acetate unit can also be shifted between two propionate units, as exemplified by 4,8-dimethyldecan-2-one (**23**), 6,8-dimethyldecan-2-one (**24**), and 4,6,10-trimethyldodecan-2-one (**26**). Finally, double bonds can occur, but their location cannot be unequivocally assigned by analysis of the mass spectra (see Table 2). Unusual branched aldehydes, not previously known from mites, were also present in the CLSA extract and were identified by mass spectral analysis and comparison with synthetic samples. The monoterpene 2-(1-methylethyl)-5-methylhex-2-enal (**21**) is formally formed by aldol condensation of two isopentanal units. The other compound was identified as 2-(1-methylethyl)oct-2-enal (**22**), formally derived from condensation of isopentanal and hexanal. Both aldehydes occur as mixtures of *E* and *Z* isomers and were synthesized by aldol condensation (Scheme 4).

Discussion

Chemical analyses revealed that (4*R*,6*R*,8*R*)-(**8**) is released by both female and male *C. arcuatus* mites as a major component from undisturbed colonies. In bioassays, **8** was found to be attractive to both sexes. Thus, according to Borden's defini-



Scheme 4. *Further compounds identified from* C. arcuatus.

tion,[14] **8** should be seen as an aggregation pheromone of this species. In view of the large amounts produced by the mites, it is reasonable to assume that this pheromone plays an important role in the ecology of *C. arcuatus*. Aggregations produced by the release of aggregation pheromones can have different functions in arthropods.[14] They: i) improve the exploitation of food sources, ii) can serve sexual purposes, bringing males and females together in one place, and can increase the chances of survival iii) in the case of predatory attack or iv) by encouraging migration to areas with better microclimatic conditions. Further studies to examine the ultimate reasons for the formation of aggregations by the release of **8** and to investigate the function of the other identified compounds in *C. arcuatus* are underway. Aggregation pheromones have only rarely been identified in mites. Lardolure (**15**), the formate of the alcohol (2*R*,4*R*,6*R*,8*R*)-**12**, is known from the mite *Lardoglyphus konoi*,[15] while 2-phenylethanol has been reported as an aggregation pheromone of an unknown *Caloglyphus* sp.[2] We also found aggregation activity of 2-phenylethanol for *C. arcuatus* in a preliminary study,[6] but we could not prove its natural occurrence in the present work. The sex pheromone of *Caloglyphus polyphyllae*, the monoterpene β-acaradial (2-(4-methylpent-3-enylidene)butanedial), also shows aggregation activity.[16] The aggregation pheromone **8** identified in this study, for which we propose the trivial name chortolure, is an oxidized nor-derivative of **15**. Interestingly, the closely related alcohol **7**, also formed by *C. arcuatus*, has inverted stereochemistry at C-2 relative to **15**. In a study on the activity of analogues of lardolure,[17] **8** was tested and showed no activity on *L. konoi*, but was as attractive as some other derivatives to *Carpoglyphus lactis*, which does not produce either **8** or **15**. All species belong to the superfamily Acaroidea, but to different families. This polyketide type of pheromone, previously identified only in *L. konoi*, is quite probably more widespread in mites. Biogenetically related 2,4-dimethylheptyl esters of fatty acids were found in the mite *Sancassania shanghaiensis* (Acaridae), but their functions remain unknown.[18] The pheromone **8** is formed by multiple additions of propionate, so successive methyl branchings occur. A similar principle of pheromone biosynthesis has previously been shown in insects: in *Carpophilus* (Nitidulidae) beetles[13] and formicine ants.[19] Whether the mites and insects synthesize the pheromones themselves or do so with the help of symbiotic microorganisms is an open question, because no polyketide genes have so far been found in animals.

Experimental Section

General remarks: ^{1}H and ^{13}C NMR spectra were obtained with Bruker AC 200 and AMX 400 instruments. $CDCl_3$ with tetramethylsilane was used as internal standard for NMR experiments. GC-MS investigations were carried out with a Hewlett–Packard model 5973 mass selective detector connected to a Hewlett–Packard model 6890 gas chromatograph equipped with a 25 m DB-5 capillary column. Alternatively, a Fisons 8060 GC fitted with a 30 m DB-Wax capillary column coupled to a Fisons MD800 quadrupole mass spectrometer was used. Helium was used as the carrier gas, and the mass spectrometers were operated in EI mode at 70 eV. Analytical GC analyses were performed with a CE instruments GC 8000 gas chromatograph fitted with a flame ionization detector and with use of split/splitless injection on a DB-5 capillary column with H_2 as the carrier gas. Reactions were carried out mostly under an inert atmosphere of N_2 in oven-dried glassware. Dry solvents were distilled from CaH_2 (CH_2Cl_2) or K and Na (THF). All other chemicals were commercially available (Fluka, Aldrich) and were used without further treatment if not stated otherwise. All reactions were monitored by thin-layer chromatography on Macherey–Nagel Polygram SIL G/UV_{254} silica plates visualized with heat gun treatment with 10% molybdatophosphoric acid in ethanol. Column chromatography was performed on Merck silica gel 60 (70–200 mesh). All new compounds were determined to be $>95\%$ pure by HPLC, GC, or ^{1}H NMR spectroscopy. Identification of known compounds was accomplished by comparison of mass spectra with those in the databases Wiley7, NIST 2.0, and MassFinder 2.3 (essential oils) and comparison of synthetic samples if not stated otherwise. Chiral GC was carried out on a 30 m heptakis(2,6-di-*O*-dimethyl-3-*O*-pentyl)-β-cyclodextrin in 50% OV1701 capillary column.

Mite culture and analysis: Mites were kept in small open flasks on a diet consisting of dry yeast and wheat baked together at 100 °C, to which an equal amount of dry fish food was added. The mite culture flasks[6] were kept in larger closed vessels at 26 °C at 70% relative humidity (sat. 1:1 KCl/NaCl solution). The flasks were connected to a CLSA apparatus[7] fitted with charcoal or Super-Q filters. Both filters furnished very similar results. The filters were extracted after 6–24 h of sampling with 30 μL CS_2 or methyl acetate and were analyzed by GC-MS. SPME analysis was performed by insertion of a SPME-fiber coated with PDMS/DVB at a film thickness of 100 μm into the vial for 30 min. and subsequent desorption in the injection port of a gas chromatograph for 1 min. To examine the sex-specific release of **8**, 20 male or 20 female mites were placed in a vial (1.5 mL) and analyzed by SPME. The peak areas of **8** were compared with data of a calibration curve produced with 850 ng, 425 ng, and 85 ng ketone. The analysis was repeated three times for each sex. The mite extracts were obtained by sieving mites from the culture medium and putting them directly into pentane. After 30 min. the solvent was decanted and stored until analysis at

−70 °C. Methylation of acids was performed by addition of distilled solutions of CH_2N_2 in pentane to the extracts.

Deuteropropionate feeding: 3,3,3-Trideuteropropionic acid (0.3 mL) was neutralized with NaOH (1 N) and added to the diet materials (1 g). This mixture was lyophilized and added to a fully grown mite culture (3 g). The culture was analyzed after one and four weeks by CLSA and SPME, respectively.

Bioassays: Experiments were performed in a climatic chamber at 25 °C and 70% relative humidity. Five male or five female *C. arcuatus* were placed in a glass dish (diameter 30 mm, 10 mm deep) together with a filter paper disk (diameter 5 mm) treated with (4*R*,6*R*,8*R*)-**8** (5 μL, 425 μg mL^{-1}) (Test) and control filter papers treated with hexane (5 mL, Control). The numbers of individuals at the filter papers were counted each minute for a total of 10 min. The counts from the 10 min. periods were summed up separately for test and control filter papers. The experiment was repeated 25 times for each sex. Each mite was used only once. Summed-up counts on test and control filter papers were compared by the Wilcoxon matched pairs test (see Table 2).

General procedure for the oxidation of alcohols: The appropriate alcohol (4 mmol) was dissolved in acetone (5 mL). This solution was tritrated with Jones' reagent (8 N in 115 mL conc. H_2SO_4 and 500 mL water) until a brownish color persisted for 10 minutes. Excess aq. $NaHSO_3$ (10%) was added, the acetone was removed, and the residues were extracted with diethyl ether. The combined organic phases were washed successively with a small amount of water, sat. aq. $NaHCO_3$, and brine, and finally dried with $MgSO_4$. The crude product was isolated after removal of the solvent and column chromatography on silica (hexane/diethyl ether).

all-*cis*-2,4,6-Trimethylcyclohexanone (2): 2,4,6-Trimethylphenol (**1**, 5.52 g, 40.5 mmol) was dissolved in absolute ethanol (15 mL), and acetic acid (0.5 mL) was added. The mixture was hydrogenated under H_2 atmosphere (35–40 bar) for four days in the presence of Rh (0.3 g, 5% on activated Al_2O_3). The solvent was removed after filtration, and the residue was taken up in water and extracted with diethyl ether. The combined organic phases were washed with sat. aq. $NaHCO_3$ and dried with $MgSO_4$, and the diethyl ether was removed. The crude alcohol was oxidized according to the general procedure. Yield 4.73 g (83%), 93% *de* (by GC). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.4$ (m, 2H; H-2, H-6), 2.05–1.95 (m, 3H; H_{eq}-3, H-4), 1.1 (m, 2H; H_{ax}-3), 1.0 (d, $J = 6.1$ Hz, 6H; CH_3-(C-2, C-6)), 0.9 ppm (d, $J = 6.1$ Hz, 3H; CH_3-C-4); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta =$ 214.8 (C-1), 45.3 (C-3, C-5), 44.2 (C-2, C-6), 32.0 (C-4), 21.2 (CH_3-C-4), 14.5 ppm (CH_3-(C-2, C-6)); MS (70 eV): *m/z* (%): 39 (26), 40 (14), 41 (62), 42 (23), 43 (11), 55 (55), 56 (18), 69 (100), 70 (29), 82 (57), 83 (25), 97 (24), 98 (13), 112 (7), 125 (3), 140 (17).

all-*cis*-3,5,7-Trimethyloxacycloheptan-2-one (3): Compound **3** was prepared from **2** in 91% yield by Mori and Kuwahara's procedure.[9] ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.5$ (m, 1H; H-6), 2.7 (m, 1H; H-2), 1.9 (m, 1H; H-4), 1.8 (m, 1H), 1.6 (m, 1H), 1.35 (d, 3H; H-7), 1.34 (m, 1H), 1.28 (m, 1H), 1.2 (d, $J = 6.9$ Hz, 3H; CH_3-C_2), 0.9 ppm (d, $J =$ 6.6 Hz, 3H; CH_3-C_4); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 177.6$ (C-1), 74.9 (C-6), 44.3 (t), 40.3 (t), 36.8 (d), 34.7 (d), 22.7 (q), 22.6 (q), 18.6 ppm (q).

(4*R,6*R**)-2,4-Dimethylheptane-1,6-diol (5)**: Sodium methoxide (0.5 M, 25 mL) in methanol was added to **3** (1.475 g, 9.4 mmol) in methanol (6 mL), and the mixture was heated under reflux for 4 h. After the mixture had been stirred overnight at room temperature, the solution was neutralized with HCl (6 M). The water/methanol solvent was removed under reduced pressure, and the residue was taken up in dry diethyl ether. This solution was added dropwise to a cooled (0 °C) solution of $LiAlH_4$ (20 mmol) in dry diethyl ether (50 mL). After the addition, the mixture was stirred for 1 h at room temperature, followed by dropwise addition of aq. NaOH (10%) until a white precipitate formed. The mixture was filtered through a Büchner funnel, and the residue was washed several times with diethyl ether. Removal of the solvent furnished an oily residue (1.51 g, 100% yield) sufficiently pure for the next step. The two diastereomers were formed in a proportion of 1:1 (NMR). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.9$ (m, 2H; H-6), 3.5 (m, 4H; H-1), 1.7 (m, 4H), 1.5 (m, 2H), 1.4 (m, 2H), 1.2 (d, $J = 6.1$ Hz, 6H; H-7), 1.1 (m, 2H), 1.0 (m, 2H), 0.93 ppm (d, $J = 6.6$ Hz, 6H), 0.90 (d, $J = 6.6$ Hz, 6H); 2,4-*syn* diastereomer: ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 69.0$ (t), 66.0 (d), 47.3 (t), 41.6 (t), 32.0 (d), 26.8 (d), 24.5 (q), 19.4 (q), 16.7 ppm (q); 2,4-*anti* diastereomer: ^{13}C NMR (101 MHz, $CDCl_3$): $\delta =$ 67.5 (t), 65.4 (d), 46.2 (t), 41.2 (t), 32.5 (d), 26.5 (d), 23.9 (q), 19.7 (q), 17.0 ppm (q).

(2*R,4*R**)-4,6,8-trimethyldecan-2-ol (7)**: Compound **7** was prepared from (4*R**,6*R**)-2,4-dimethyl-1,6-heptanediol via the tosylate **6** by Mori and Kuwahara's procedure[8] in 44% overall yield with use of 1-methylpropylmagnesium bromide. The four diastereomers were formed in a 1:1:1:1 ratio (GC). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 3.94–3.85 (m, 4H; H-2), 1.8–0.7 ppm (m, 116H); MS (70 eV): *m/z* (%): 41 (34), 43 (46), 45 (100), 55 (13), 57 (10), 69 (12), 85 (4), 97 (1), 111 (4), 126 (1), 153 (1), 199 (1).

4,6,8-Trimethyldecan-2-one (8): The alcohol **7** was oxidized by the general oxidation procedure to yield a 1:1:1:1 mixture (GC) of the diastereomers in 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.5$–2.0 (m, 8H; H-3), 2.12 (s, 12H; H-1), 1.6–0.7 ppm (m, 84H); MS see Figure 2.

(2*R*,4*R*,6*R*,8*R*)-Trimethyldecan-2-ol ((2*R*,4*R*,6*R*,8*R*)-7): A solution of **11** (310 mg, 0.84 mmol) in dry diethyl ether (8 mL) was added at 0 °C to a suspension of $LiAlH_4$ (760 mg, 20 mmol) in dry diethyl ether (20 mL). After the mixture had been stirred at room temperature for 2 h, a NaOH solution (10%) was added dropwise until a white precipitate occurred. The mixture was filtered through a Büchner funnel and the residue was washed several times with diethyl ether. The combined organic phases were evaporated, and the residue was purified by column chromatography on silica (hexane/diethyl ether 9:1). $[\alpha]_D^{21} = -20.4$ ($c = 2.03$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.91$ (m, 1H; H-2), 1.80–0.75 (m, 11H), 1.20 (d, $J_{1,2} = 6.1$ Hz, 3H; H-1), 0.89 (d, $J = 6.6$ Hz, 3H; CH_3), 0.86 (t, $J =$ 6.2 Hz, 3H; CH_3), 0.84 (d, $J = 6.1$ Hz, 3H; CH_3), 0.82 ppm (d, $J =$ 6.3 Hz, 3H; CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 66.3$ (C-2), 47.3 (t), 46.4 (t), 44.5 (t), 32.2 (t), 31.0 (C-9), 27.7, 27.0, 24.8, 21.1, 20.4, 20.0, 11.6 ppm (C-10); MS see Supporting Information. *de*: 97% (GC, as TMS derivative).

(2*S*,4*R*,6*R*,8*R*)-Trimethyldecan-2-ol ((2*S*,4*R*,6*R*,8*R*)-7): A mixture of (2*R*,4*R*,6*R*,8*R*)-**7** (20 mg, 0.10 mmol), PPh_3 (29 mg, 0.11 mmol), benzoic acid (17 mg, 0.14 mmol), diethyl diazodicarboxylate (DEAD, 19 μL, 0.12 mmol), and CH_2Cl_2 (1 mL) was stirred for 16 h at 20 °C. The solvent was removed, and the residue was purified by chromatography on silica (hexane/diethyl ether 10:1). Starting material (8 mg) was recovered, while the benzoate (14 mg, 44% yield) was dissolved in methanol (1 mL). A solution of sodium methoxide in methanol (4 M, 1 mL) was added, and the mixture was stirred at room temperature overnight. The solution was then acidified with HCl (1 N) and extracted with diethyl ether. The ethereal extract was washed with sat. $NaHCO_3$ solution and dried with $MgSO_4$, and the solvent was removed. The residue was purified by column chromatography on silica (pentane/diethyl ether 10:1), yielding 5 mg (57%) with an *de* of 97% (GC). The compound had a mass spec-

trum identical to that of (2*R*,4*R*,6*R*,8*R*)-**7**. The TMS derivative of (2*S*,4*R*,6*R*,8*R*)-**7** had a longer retention time (21.43 min) than its epimer (2*R*,4*R*,6*R*,8*R*)-**7** (21.03 min) on an apolar gas chromatographic phase. $[\alpha]_D^{21} = -2.04$ ($c = 1.05$ in $CHCl_3$). The NMR data were identical to those reported by Morr et al.[10]

(4*R*,6*R*,8*R*)-4,6,8-Trimethyldecan-2-one ((4*R*,6*R*,8*R*)-8): The alcohol (2*R*,4*R*,6*R*,8*R*)-**7** was oxidized by the general oxidation procedure to yield the target ketone in 91% yield and with an *ee* > 98%. $[\alpha]_D^{21} = +1.2$ ($c = 5.5$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.41$ (dd, $J =$ 14.8 Hz, 4.3 Hz, 1H; H-3a), 2.14 (dd, $J = 8.7$ Hz, 14.8 Hz, 1H; H-3b), 2.13 (s, 3H; H-1), 1.6–0.8 (m, 9H), 0.89 (d, $J = 6.6$ Hz, 3H; CH_3), 0.86 (t, $J = 6.1$ Hz, 3H; H-10), 0.85 (d, 3H; CH_3), 0.84 ppm (d, $J = 6.1$ Hz, 3H; CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 209.3$ (C-2), 51.0 (C-3), 45.1 (t), 44.6 (t), 31.5 (d), 30.5 (d), 28.9 (C-9), 27.5 (d), 26.8 (C-1), 20.6 (q), 20.6 (q), 19.9 (q), 11.2 ppm (C-10); MS (70 eV): *m/z* (%): 39 (14), 41 (50), 42 (17), 43 (100), 55 (16), 57 (20), 58 (14), 69 (16), 85 (20), 97 (2), 109 (2), 123 (1), 140 (2), 151 (1), 165 (1), 180 (1), 183 (1), 198 (1).

(4*R*,6*R*,8*R*)-Trimethylundecan-2-one ((4*R*,6*R*,8*R*)-13): The compound was prepared by the general procedure from **12**, which was prepared by Yamamoto's procedure.[12] The overall yield over two steps was 91%, with an *ee* > 98%. $[\alpha]_D^{21} = +7.2$ ($c = 5.4$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.42$ (dd, $J = 15.3$ Hz, 4.1 Hz, 1H; H-3a), 2.15 (dd, $J = 8.7$ Hz, 15.3 Hz, 1H; H-3b), 2.13 (s, 3H; H-1), 1.6–0.8 (m, 11H), 0.89 (d, $J = 6.1$ Hz, 3H; CH_3), 0.88 (t, $J = 7.1$ Hz, 3H; H-10), 0.85 (d, $J = 6.6$ Hz, 3H; CH_3), 0.84 ppm (d, $J = 6.6$ Hz, 3H; CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 209.2$ (C-2), 51.1 (C-3), 45.1 (t), 45.1 (t), 58.9 (C-9), 30.5 (d), 29.7 (d), 27.5 (d), 26.7 (C-1), 20.7 (q), 20.6 (q), 20.4 (q), 20.0 (C-10), 11.2 ppm (C-10); MS (70 eV): *m/z* (%): 41 (33), 42 (14), 43 (100), 55 (13), 58 (11), 69 (14), 85 (21), 99 (1), 111 (3), 154 (1), 179 (1), 194 (1), 197 (1), 212 (1).

(4*R,6*R**)-4,6-Dimethyloctan-2-one** (**19**): A solution of methyllithium (5.25 mL, 1.6 M in diethyl ether) was added at 0 °C to a suspension of CuI (0.8 g, 4.2 mmol) in dry diethyl ether (5 mL). After the disappearance of the yellowish color the solution was cooled to −70 °C, and (2*R**,4*R**,6*R**)-**6** (132 mg, 0.42 mmol) in dry diethyl ether (5 mL) was added (this was obtained from **3** as described by Mori and Kuwahara[8]). The solution was stirred for 2 h, and sat. NH_4Cl solution was added. The residue was dissolved with aq. NH_3. The phases were separated, and the aq. phase was extracted with diethyl ether. The combined organic phases were washed with brine and dried with Na_2SO_4, and the solvent was removed. The residue was purified by column chromatography on silica (pentane/diethyl ether 10:1), yielding (2*R**,4*R**,6*R**)-4,6-dimethyl-2-octanol (51 mg, 76% yield). The alcohol was oxidized by the general oxidation procedure to yield the target ketone in 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.41$ (dd, $J_{3a,3b} = 15.5$ Hz, $J_{3a,4} = 4.8$ Hz, 1H; H-3a), 2.17 (dd, $J_{3b,4} = 8.6$ Hz, 1H; H-3b), 2.13 (s, 3H; H-1), 1.45–0.95 (m, 6H), 0.89 (d, $J = 6.3$ Hz, 3H; CH_3), 0.86 (t, $J_{7,8} = 6.8$ Hz, 3H; H-8), 0.85 ppm (d, $J = 6.2$ Hz, 3H; CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 209.2$ (C-2), 51.3 (C-3), 44,4 (C-5), 31.6 (C-6), 30.5 (C-1), 29.1 (C-7), 26.9 (C-4), 20.5 (q), 19.6 (q), 11.2 ppm (C-8).

5-Methyl-2-(1-methylethyl)hex-2-enal (21) and 2-(1-methylethyl)-oct-2-enal (22): A mixture of hexanal (1 mL, 8.34 mmol) and 3-methylbutanal (1 mL, 9.33 mmol) was stirred for 1 h together with KOH in ethanol (7%, 3 mL) at room temperature. The mixture was neutralized with HCl (1 N) and extracted with diethyl ether, and the combined organic extracts were washed with water and sat. brine. After drying with Na_2SO_4, the solvent was removed, and the residue was partly separated by column chromatography with silica (pentane/diethyl ether 10:1). The following products were formed by the aldol condensation: (*E*)-5-methyl-2-(1-methylethyl)hex-2-enal ((*E*)-**21**, 26%), (*Z*)-5-methyl-2-(1-methylethyl)hex-2-enal ((*Z*)-**21**, 4%), (*E*)-2-(1-methylethyl)oct-2-enal ((*E*)-**22**, 62%), (*Z*)-2-(1-methylethyl)oct-2-enal ((*Z*)-**21**, 1%), 2-butyl-5-methylhex-2-enal (2%), 2-butyloct-2-enal (5%).

(*E*)-2-(1-Methylethyl)oct-2-enal ((*E*)-21): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.38$ (s, 1H; H-1), 6.47 (t, $J_{3,4} = 7.5$ Hz, 1H; H-3), 2.25 (q, $J_{4,5} =$ 7.5 Hz, 2H; H-4), 1.82 (sept, $J_{1',2'} = 6.7$ Hz, 1H; H-1′), 1.40–1.26 (m, 6H; H-5, H-6, H-7), 0.97 (d, 6H; H-2′), 0.90 ppm (t, $J_{8,7} = 6.9$ Hz, 3H; H-8); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 195.3$ (C-1), 154.2 (C-3), 144.5 (C-2), 37.8 (t), 30.9 (t), 28.3 (C-1′), 23.8 (t), 22.8 (t), 22.5 (2C, C-2′), 13.9 ppm (C-8); MS see Supporting Information.

(*E*)-5-Methyl-2-(1-methylethyl)hex-2-enal ((*E*)-22): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.34$ (s, 1H; H-1), 6.35 (t, $J_{3,4} = 7.6$ Hz, 1H; H-3), 2.88 (m, 1H; H-5), 2.27 (dd, $J_{4,5} = 7.0$ Hz, 2H; H-4), 1.81 (sept, $J_{1',2'} = 6.7$ Hz, 1H; H-1′), 1.18 (d, $J_{6,5} = 7.1$ Hz, 6H; H-6), 1.0 ppm (d, 6H; H-2′); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 195.6$ (C-1), 154.2 (C-3), 148.5 (C-2), 37.8 (C-4), 28.4 (C-5), 26.0 (C-1′), 22.4 (2C, C-6), 20.3 ppm (2C, C-2′); MS see Supporting Information.

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