EurJOC
European Journal of Organic Chemistry

A Journal of ChemPubSoc Europe

Accepted Article

Title: Viaticene A, an Unusual Tetraterpene Cuticular Lipid Isolated from the Springtail Hypogastrura viatica

Authors: Jan Bello, Patrick Stamm, Hans Petter Leinaas, and Stefan Schulz

To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201900224

Link to VoR: http://dx.doi.org/10.1002/ejoc.201900224

Supported by ACES Asian Chemical Editorial Society

WILEY-VCH

10.1002/ejoc.201900224

WILEY-VCH

COMMUNICATION

Viaticene A, an Unusual Tetraterpene Cuticular Lipid Isolated from the Springtail *Hypogastrura viatica*

Jan E. Bello,[a] Patrick Stamm,[a] Hans Petter Leinaas,[b] and Stefan Schulz*[a]

Abstract: The cuticles of springtails are extremely wear- and friction-resistant, super-hydrophobic, non-fouling, and self-cleaning. As such, the chemistry of the lipids covering these cuticles is of great interest as model for biomimetic super-hydrophobic surfaces, although only few of these lipids have been structurally elucidated. *Hypogastrura viatica*, a surface-dwelling springtail, produces highly branched tetraterpene hydrocarbons with an unprecedented [6+2]-terpene connectivity as components of the epicuticular lipid layer. The structure of the major lipid component, viaticene A, was elucidated through isolation, spectroscopic analysis, chemical derivatization, synthesis, as well as stereochemical analysis of the core unit obtained from ozonolysis of the isolated lipid. Viaticenes A and B represent a new class of irregular tetraterpenoid natural products.

Introduction

Springtails (Collembola) are small, wingless, soil dwelling hexapods that consist of approximately 8,700 species found throughout the world.[1] The cuticles of springtails are robust, non-fouling, self-cleaning, and super-hydrophobic surfaces that protect these arthropods from harsh soil conditions.[2] Recent work has shown that the cuticular surfaces of springtails are not only water-resistant but are also able to withstand wetting from several low surface tension liquids.[3] Due to their unique properties, such cuticles are of great interest due to possible applications in biomimetic engineered surfaces. The springtail surfaces contain small nanoscopic comb-like structures covered by a thick lipid layer.[2,3] Although the morphology of Collembola cuticles has been well studied, very little is known about the chemical composition of their epicuticular lipid layers. Cuticular compounds from only two Collembola species, *Podura aquatica* producing the irregular tetraterpene poduran[4] and *Tetrodontophora bielanensis* producing lycopene derivatives,[5–7] have been structurally elucidated. Aside from protection and prevention of dehydration, the cuticular lipids of springtails may also serve a secondary purpose in the chemical communication of these soil dwelling hexapods, releasing information on the species and sex of an individual. Previous studies have shown that Collembola produce pheromones mediating aggregation and reproductive behavior, as well as development,[8] but it is not yet known if springtails utilize cuticular compounds as semiochemicals. For insects, which are closely related to Collembola, cuticular lipids are well known to act as contact sex pheromones inducing sexual behavior in many species at close range.[9–11]

Hypogastrura viatica is a medium sized species (2 mm) commonly found on the soil surface in arctic and temperate coastal areas, feeding on terrestrial cyanobacteria, algae, and decaying seaweed.[12] *H. viatica* may form groups of many thousands animals, a behavior possibly mediated by a yet unknown chemical cue.[13] In this study, the cuticular lipids from a cyanobacteria feeding population of *H. viatica* found in the high arctic Svalbard Islands were analyzed.

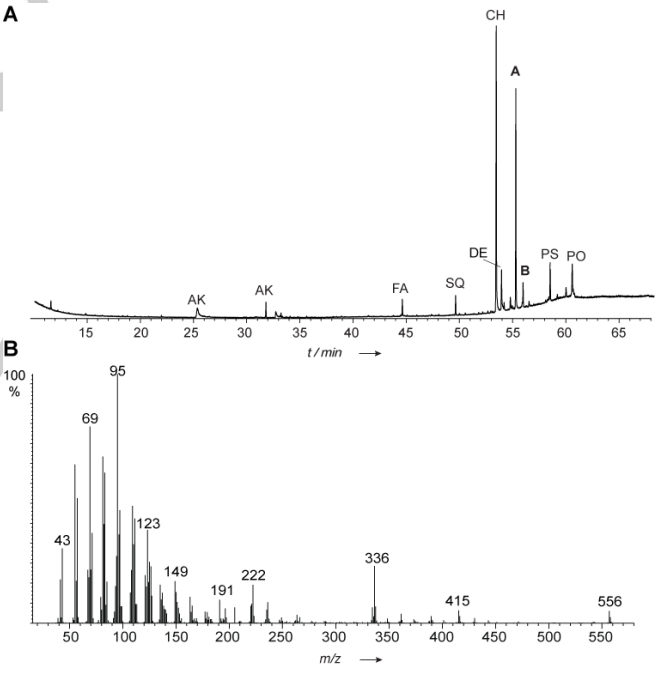


Figure 1. A) Total ion chromatogram of a pentane extract of *Hypogastrura viatica*. Alkaloids (AK), fatty acid amide (FA), squalene (SQ), cholesterol (CH), desmosterol (DE), lipids **A** and **B**, and phythyl palmitate and oleate (PS, PO) were present in the extract. B) EI mass spectrum of unknown compound **A**, identified as viaticene A.

Results and discussion

Short term extraction of the springtails with pentane furnished extracts that were analyzed by GC/MS. Major components were cholesterol and an unknown compound **A** (Figure 1). They were accompanied by minor amounts of some unknown alkaloids and a fatty acid amide, squalene, desmosterol and fatty acid phythyl

[a] Dr. J. E. Bello, P. Stamm, Prof. Dr. S. Schulz
Institute of Organic Chemistry,
Technische Universität Braunschweig
Hagenring 30, 38106 Braunschweig (Germany)
E-mail: Stefan.Schulz@tu-bs.de
Homepage: http://www.oc.tu-bs.de/schulz/index_en.html
[b] Prof. Dr. H. P. Leinaas
Department of Biosciences
University of Oslo
Postboks 1066, Blindern, 0316 Oslo (Norway)

Supporting information for this article is given via a link at the end of the document.

esters. GC/HR-MS of the cuticular compound **A** revealed the molecular formula to be $C_{40}H_{76}$ (*m/z* obs. 556.5947, calc. 556.5944), with three double-bond equivalents (Figure 1). Although the mass spectrum of **A** gave little information about the structure of the compound, the gas chromatographic retention index (*I*) of 3325 suggested a highly branched backbone, hinting that compound **A** was possibly a tetraterpene lipid.

Column chromatography with a gradient solvent system was utilized to isolate 2.3 mg of compound **A** (>90 % purity) from 20 g of *H. viatica* (see SI Figure S2). The IR spectrum of **A**, obtained by GC/IR, showed significant absorptions between 2955 cm^{-1} and 2850 cm^{-1}, which are typical of a hydrocarbon. Absorptions at 3075 cm^{-1}, 1643 cm^{-1}, 995 cm^{-1}, and 909 cm^{-1} indicated the presence of a terminal vinyl group in the structure (see SI Figure S14). Several microscale derivatizations were performed to obtain more structural information for compound **A**. Hydrogenation resulted in a single compound with a molecular mass of 562, indicating the loss of three double-bonds. Microscale ozonolysis of **A** produced three major compounds **1**-**3** (Figure 2), which were structurally elucidated based on mass spectral fragmentation patterns, GC/HR-MS, GC/IR, and calculation of retention indices (see SI section 7). Ozonolysis product **1** was proposed to be 4,8-dimethylnonanal and product **2** to be 2,6-dimethylnonanedial. These suggestions were verified by synthesis of both compounds as shown in the SI (section 7). Product **3** was identified as 3-(3,7-dimethyloctyl)heptane-2,6-dione, also verified by synthesis (see below, Scheme 1). For convenience, compound **3** obtained by ozonolysis from isolated **A** will referenced as natural **3** in this article.

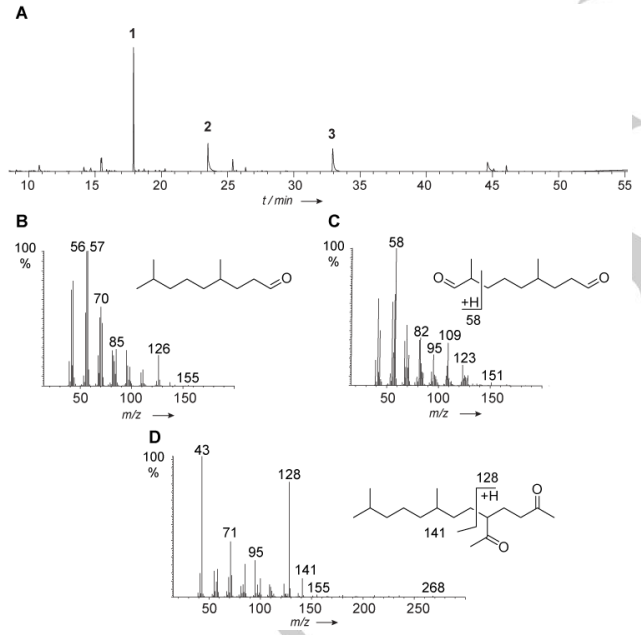
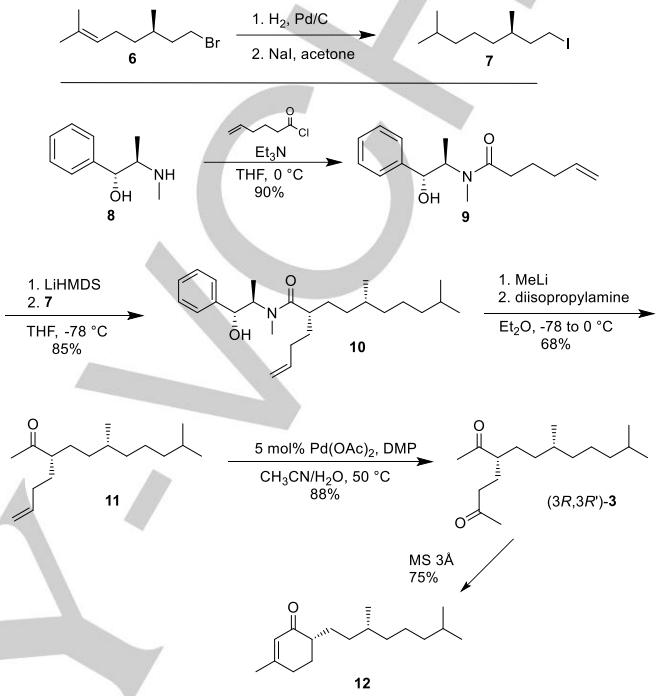


Figure 2. A) Total ion chromatogram of the ozonolysis products of compound **A**. The three major products are numbered. EI mass spectra of ozonolysis products **1**, 4,8-dimethylnonanal (B), **2**, 2,6-dimethylnonanedial (C), and **3**, 3-(3,7-dimethyloctyl)heptane-2,6-dione (D).

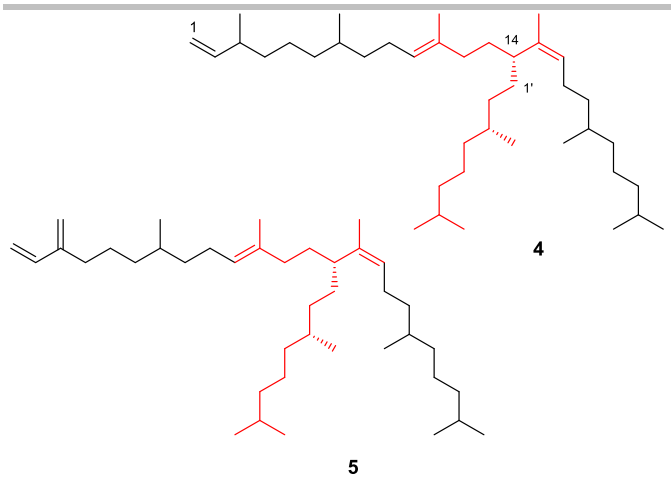
The ^{13}C and ^{1}H NMR spectra of **A** revealed a total number of 40 carbon atoms in agreement with the molecular formula. However, the number of hydrogen atoms could not be clearly determined due to overlapping CH_2 signals in the ^{1}H NMR spectra, which made integration difficult (see SI Figure S3). The ^{13}C NMR resonances were assigned to three sp^2 methine carbons, two sp^2 quaternary carbons and one sp^2 methylene carbon confirming the presence of three double bonds in the structure. The remaining ^{13}C NMR resonances were assigned to sp^3 methylene carbons, 7 sp^3 methine and 10 CH_3 carbons.



Scheme 1: Synthesis of (3*R*,3′*R*)-(3,7-dimethyloctyl)heptane-2,6-dione ((3*R*,3′*R*)-**3**) respective cyclohexenone 12.

Two-dimensional NMR experiments ($^{1}H,^{1}H$-COSY, $^{1}H,^{1}H$-TOCSY, $^{1}H,^{13}C$-HMBC, and $^{1}H,^{13}C$-HSQC) were performed to gain information about the connectivity of the compound. H_2C=CH-CH(CH_3)-CH_2, -H_2C-HC=C(CH_3)-CH_2, and -H_2C-HC=C(CH_3)-CH-(CH_2)$_2$ spin systems were deduced, signifying that one terminal vinyl group, and two trisubstituted double-bonds were present in compound **A**. The last spin system also revealed the presence of a connection point unusual in terpenes in the molecule (see Figures S4-S9 in the SI).

Combination of the results of the microscale derivatization and NMR experiments allowed us to propose a tetraterpene hydrocarbon structure **4** for compound **A** containing five chiral centers, three double-bonds, and unusual connectivity (Scheme 2). The mass spectrum of compound **A** is in full agreement with the proposed structure. The fragmentation can be explained by ions at *m/z* 415, 336, and 222 corresponding to preferred cleavage at the allylic positions and at the main branching point of the molecule (see SI Figure S13). Unlike most tetraterpenoid compounds that are biosynthesized via the reaction of two geranylgeranyl pyrophosphate subunits resulting in a [4+4]-terpene connectivity (see SI section 10 for the introduction of a new terpene terminology),[14] compound **A** has a [6+2] connectivity, involving the head to tail linking of six isoprene units, coupled to a branch consisting of two isoprene units forming a 'Y'-shaped structure.



Scheme 2. Structure of viaticene A (**4**) and viaticene B (**5**). The tetraterpenes show an unusual [6+2]-terpene connectivity. The origin of the diketone **3** core obtained by ozonolysis is highlighted in red.

To confirm the structure of compound **A** and gain insight into the stereochemistry of the natural compound, we synthesized the four stereoisomers of the diketone core (3-(3,7-dimethyloctyl)heptane-2,6-dione) of compound **A** that was obtained as the ozonolysis product **3** of the lipid (Scheme 1). Comparison of the synthetic stereoisomers and the degradation product **3** via chiral-GC would allow for the determination of the absolute configuration of the core structure of compound **A**.

(*R*)-Citronellyl bromide (**6**) served as starting material. Hydrogenation of **6**[15] followed by a Finkelstein reaction afforded (*R*)-1-iodo-3,7-dimethyloctane (**7**).[16] In the next step 5-hexenoic acid was converted into 5-hexenoyl chloride and immediately treated with (1*R*,2*R*)-pseudoephedrine (**8**) to form the tertiary carboxamide **9**.[17,18] Pseudoephedrine amides are easily transformed into highly enantiomerically enriched ketones, which dictated our use of pseudoephedrine as the chiral auxiliary in this synthesis.[17,18] Treatment of **9** with LiHMDS and **7** afforded the alkylated amide **10** in 95:5 diastereomeric ratio. Reaction of **11** with methyl lithium by acidic work-up produced the respective ketone (3*S*,6*R*)-3-(but-3-en-1-yl)-6,10-dimethylundecan-2-one (**11**) with no detectable epimerization of the α-stereogenic center. Ketone **11** was then treated with $Pd(OAc)_2$ and Dess-Martin periodinane (DMP) in a Wacker-type oxidation to afford (3*R,3'R*)-3-(3,7-dimethyloctyl)heptane-2,6-dione (3*R,3'R*)-**3** in 38% overall yield.[19] Synthesis of the three other enantiomers of **3** was performed by respective combinations of enantiomers of **6** and/or **8** in the corresponding steps of the described synthesis to afford the diketones (3*R*,3'S)-**3**, (3*S,3'S*)-**3**, and (3*S*,3'*R*)-**3.** The synthetic diketone diastereomer (3*R,3'R*)-**3**/(3*S,3'S*)-**3** had identical mass spectra and gas chromatographic retention indices to the ozonolysis product **3**, giving further confirmation that the proposed structure of **A** was correct. We propose the name viaticene A for natural compound **A**.

Chiral gas chromatography was then performed to clarify the absolute configuration of the core structure of the natural product. Separation of the diastereomers of **3** was possible on a chiral β-dex-225 column (Figure 3). Coinjection of the synthetic and natural diketone core molecules proved the naturally occurring 3-(3,7-dimethyloctyl)heptane-2,6-dione to be identical to the (3*R*,3'*R*)/(3*S*,3'*S*)-diastereomer of **3**. However, attempts to separate all four stereoisomers on various chiral stationary phases failed. Therefore, we returned again to microscale derivatization, this time of the synthetic and viaticene derived diketones **3** to achieve separation of the resulting derivatives.

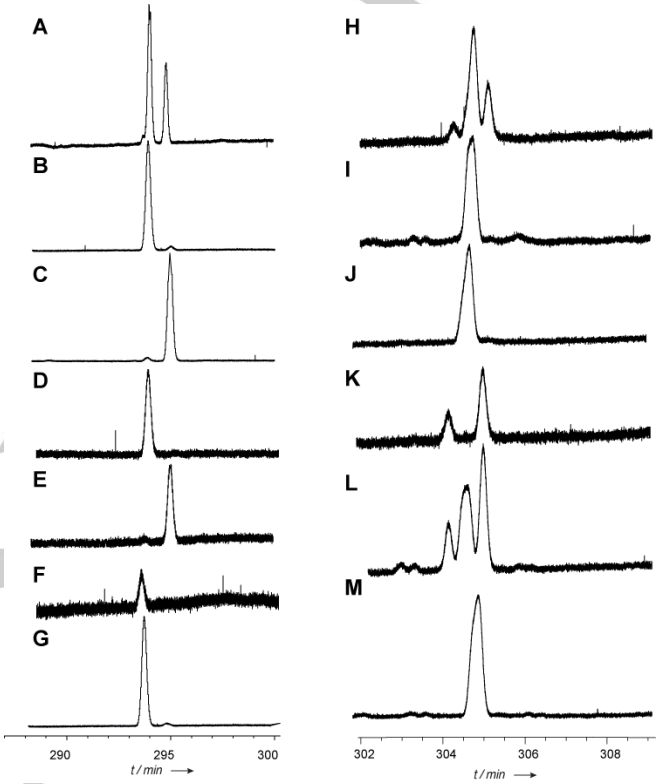


Figure 3. Determination of the absolute configuration of the naturally occurring diketone core (3*R*,3'*R*)-**3** from viaticene A by chiral GC on a βdex-225 column. A) Mixture of all enantiomers of 3-(3,7-dimethyloctyl)heptane-2,6-dione (**3**), B) (3*R,3'R*)-**3**, C) (3*R,3'S*)-**3**, D) (3*S,3'S*)-**3**, E) (3*S*,3'*R*)-**3**, F) **3** derived from naturally occurring **4**, G) coinjection of natural **3** and (3*R*,3'*R*)-**3**, indicating that the (*RS*,*RS*)-**3**-diastereomer occurs naturally, H) mixture of synthetic cyclohexanone derivatives (3*R*,3'*R*)-**12** and (3*S*,3'*S*)-**12**, I) derivative **12** obtained from natural **3**, J) (3*R*,3'*R*)-**12**, K) (3*S*,3'*S*)-**12**, L) coinjection of (3*S*,3'*S*)-**12** and the viaticene derived **12**, M) coinjection of (3*R*,3'*R*)-**12** and the viaticene derived **12**.

Formation of a pyridine ring system derived from the 1,5-diketones was attempted by microscale treatment of a 1:1 mixture of the synthetic diketones with hydroxylamine and 3Å molecular sieves in dry Et_2O.[20] However, the resulting product was not the desired trisubstituted pyridine, but 6-(3,7-dimethyloctyl)-3-methylcyclohex-2-enone (**12**) formed by a regioselective intramolecular base-catalyzed aldol condensation of **3** with 3Å mol sieves serving as the base (see SI section 7). Molecular sieves have been reported to function as a base in aldol and Mannich type reactions.[21] Independent synthesis confirmed the structure of **12** (see SI section 7). Fortunately, the cyclohexenones **12** formed from (3*R*,3'*R*)- and (3*S*,3'*S*)-**3** were separable on the β-dex225 column (Figure 3). The derivatization reaction proceeds without or with only low epimerization (0-20 %).

Co-injection of (3*R*,3'*R*)-**12** and **12** obtained from natural 3 proved that both are derived from (3*R*,3'*R*)-3-(-3,7-dimethyloctyl)heptane-2,6-dione (Figure 3).

The stereochemistry of the stereogenic centers at C7' and C19 could not be assigned because the instability of dial **2** and lack of any successful separation of a 4-methylalkanal such as **1** or their derivatives by chiral GC as of yet.[22] The stereochemistry of the trisubstituted double-bonds of **A** was determined by ^{1}H,^{1}H-NOESY experiments (see SI section 5). Viaticene A can therefore be assigned as (3'*R*,10*E*,14*R*,15*Z*)-14-(3,7-dimethyloctyl)-3,7,11,15,19,23-hexamethyltetracosa-1,10,15-triene.

Compound **B** showed a molecular mass two units lower than **4**, *m/z* 554, indicating an additional double-bond. The mass spectra of **A** and **B** were similar. The ion *m/z* 413 located the additional double-bond outside the geranyl side-chain, while *m/z* 336 placed it between C-1 and C-12. Minor signals in the NMR spectra of **A** that originated from a small impurity of **B** in the sample showed the presence of a terminal 1,3-butadiene-system (see SI Figure S5). Therefore, we propose (3'*R*,10*E*,14*R*,15*Z*)-14-(3,7-dimethyloctyl)-7,11,15,19,23-pentamethyl-3-methylenetetracosa-1,10,15-triene (**5**) as structure for viaticene B.

To determine if diet affects the production of viaticene A in the cuticular lipids of *H. viatica*, a population of *H. viatica* found on the coast of Southern Norway, whose diet consisted of decaying algae, was analyzed. Viaticene A occurred in both populations, but viaticene B was only present in the Svalbard population. Chiral-GC analysis of the 3-methylcyclohex-2-enone derivative of core product **3** from the ozonolysis of the southern Norway viaticene resulted in a product that was a mixture of (3*R*, 3'*R*)-**3** and (3*S*,3'*R*)-**3** stereoisomers, revealing that this population uses as mixture of (14*R*) and (14*S*)-viaticene epimers (see SI section 8). The variation in the stereochemistry of the Svalbard viaticene A and the southern Norway viaticene compound may represent non-adaptive consequences of long geographic segregation, but may also have implications for reproductive isolation of the different subpopulations of *H. viatica*.

Lab cultures of both populations fed solely with cyanobacteria showed formation of viaticene A, while the cyanobacteria themselves did not produce **A** or other related lipids, indicating the endogenous production of these lipids in *H. viatica*. The biosynthesis of viaticene A is still unknown. Unlike most tetraterpenes that are formed by the reaction of two geranylgeranyl pyrophosphate subunits in a [4+4]-terpene biosynthetic pattern (e.g. lycopene and carotenoids),[14] viaticene A has a [6+2]-terpene biosynthetic pattern. The unusual connectivity of viaticene A suggests a biosynthesis involving the reaction of a farnesylfarnesyl pyrophosphate and geranyl pyrophosphate, a biosynthetic sequence for tetraterpenes not yet reported in nature (see SI scheme S6). Tetraterpenes of unusual biosynthetic origin have been identified before from springtails. Poduran, a tetraterpene isolated from *Podura aquatica* (see SI Scheme S7), features tricyclic head group connected to a tail composed of five isoprene units, arranged in a consecutive [8]-terpene pattern.[4]

Conclusion

The viaticenes are the first isolated irregular tetraterpenes, representing [6^{14}+2^{1}]-terpenes. Other irregular terpenes are for example [3^{6}+2^{1}]- and [3^{6}+3^{1}]-terpenes, so called highly branched isoprenoids from diatoms[23] or [3^{3}+3^{1}]-terpenes from brown algae[24]. The synthesis of the four stereoisomers of **3** and chiral-GC analysis revealed that the stereochemistry of the core segment of the molecule has (3'*R*,14*R*)-configuration. Analysis of the different populations of *H. viatica* showed that the viaticene compositions from different populations can vary in composition and is not dependent on the food source. Currently, the total synthesis of viaticene A is in progress to determine the physical properties and ecological significance of this unique lipid.

Acknowledgements

We thank the Alexander von Humboldt Foundation for financial support to JB. We would also like to thank Christian Schlawis, Markus Menke, and Serdar Dilek for their help with GC/IR and chiral GC experiments.

Keywords: super-hydrophobic lipid • Collembola • cuticular lipids • pheromones • tetraterpenes

References

[1] Bellinger, P.F., Christiansen, K.A. & Janssens, F, "Checklist of the Collembola of the World 1996-2018", can be found under http://www.collembola.org, **2018**.

[2] a) H. Gundersen, H. P. Leinaas, C. Thaulow, *PLoS One* **2014**, *9*, e86783; b) H. Gundersen, H. P. Leinaas, C. Thaulow, *Beilstein J. Nanotechnol.* **2017**, *8*, 1714–1722.

[3] R. Hensel, C. Neinhuis, C. Werner, *Chem. Soc. Rev.* **2016**, *45*, 323–341.

[4] S. Schulz, C. Messer, K. Dettner, *Tetrahedron Lett.* **1997**, *53*, 2077–2080.

[5] J. Nickerl, M. Tsurkan, R. Hensel, C. Neinhuis, C. Werner, *J. R. Soc. Interface* **2014**, *11*, 20140619.

[6] G. Brasse, *PhD thesis*, TU Braunschweig, **2005**.

[7] K. Stránský, A. Trka, M. Budesínský, M. Streibl, *Collect. Czech. Chem. Commun.* **1986**, *51*, 948–955.

[8] a) H. A. Verhoef, C. J. Nagelkerke, E.N.G. Joosse, *J. Insect Physiol.* **1977**, *23*, 1009–1013; b) H. A. Verhoef, *J. Insect Physiol.* **1984**, *30*, 665–670.

[9] R. W. Howard, G. J. Blomquist, *Annu. Rev. Entomol.* **2005**, *50*, 371–393.

[10] G. J. Blomquist, A. G. Bagnères (Eds.) *Insect Hydrocarbons*. *Biology, biochemistry, and chemical ecology*, Cambridge University Press, Cambridge, UK, New York, **2010**.

[11] G. J. Blomquist in *Insect Hydrocarbons*. *Biology, biochemistry, and chemical ecology* (Eds.: G. J. Blomquist, A. G. Bagnères), Cambridge University Press, Cambridge, UK, New York, **2010**, pp. 19–34.

[12] a) A. Fjellberg, *The Collembola of Fennoscandia and Denmark*, Brill, Leiden, **1998**; b) K. Hertzberg, N. G. Yoccoz, R. A. Ims, H. P. Leinaas, *Oecologia* **2000**, *124*, 381–390.

[13] J. Mertens, R. Bourgoignie, *Behav. Ecol. Sociobiol.* **1977**, *2*, 41–48.

[14] N. Misawa in *Comprehensive Natural Products Chemistry II* (Eds.: L. N. Mander, H.-W. Liu), Elsevier, Oxford, **2010**, pp. 733–753.

[15] E. Hedenström, F. Andersson, *J. Chem. Ecol.* **2002**, *28*, 1237–1254.

[16] T. W. Baughman, J. C. Sworen, K. B. Wagener, *Tetrahedron* **2004**, *60*, 10943–10948.
[17] A. G. Myers, B. H. Yang, H. Chen, J. L. Gleason, *J. Am. Chem. Soc.* **1994**, *116*, 9361–9362.
[18] A. G. Myers, B. H. Yang, H. Chen, L. McKinstry, D. J. Kopecky, J. L. Gleason, *J. Am. Chem. Soc.* **1997**, *119*, 6496–6511.
[19] D. A. Chaudhari, R. A. Fernandes, *J. Org. Chem.* **2016**, *81*, 2113–2121.
[20] V. G. Kharchenko, L. I. Markova, O. V. Fedotova, N. V. Pchelintseva, *Chem. Heterocycl. Compd.* **2003**, *39*, 1121–1142.
[21] a) F. Zhong, C. Jiang, W. Yao, L.-W. Xu, Y. Lu, *Tetrahedron Lett.* **2013**, *54*, 4333–4336; b) W.-B. Chen, Y.-H. Liao, X.-L. Du, X.-M. Zhang, W.-C. Yuan, *Green Chem.* **2009**, *11*, 1465–1476.
[22] K. Mori, K. Akasaka, *Tetrahedron: Asymmetry* **2016**, *27*, 182–187.
[23] a) S. T. Belt, T. A. Brown, L. Smik, A. Tatarek, J. Wiktor, G. Stowasser, P. Assmy, C. S. Allen, K. Husum, *Org. Geochem.* **2017**, *110*, 65–72; b) E. Douka, A.-I. Koukkou, C. Drainas, C. Grosdemange-Billiard, M. Rohmer, *FEMS Microbiology Letters* **2001**, *199*, 247–251.
[24] T. D. Niehaus, S. Okada, T. P. Devarenne, D. S. Watt, V. Sviripa, J. Chappell, *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 12260–12665.

10.1002/ejoc.201900224

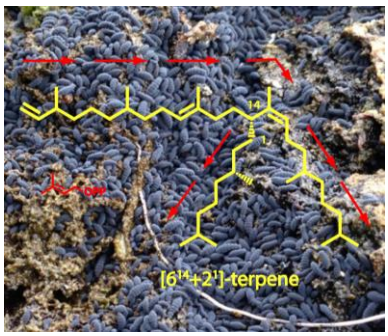
WILEY-VCH

COMMUNICATION

Entry for the Table of Contents

Key topic: Irregular terpenes

COMMUNICATION



Jan E. Bello,[a] Patrick Stamm,[a] Hans Petter Leinaas,[b] and Stefan Schulz*[a]

Page No. – Page No.

Viaticene A, an Unusual Tetraterpene Cuticular Lipid Isolated from the Springtail *Hypogastrura viatica*

A unique highly branched tetraterpene constitutes the epicuticular layer of this Collembola. The structure was elucidated by combination of NMR techniques and chemical synthesis of degradation products. A Y-shaped structure is formed by combination of two terpene units made up six and two prenyl units, respectively. Viaticene is the first member of a new terpene compound class.