DOI: 10.1002/anie.200905680

Structure Elucidation

Octamethylbicyclo[3.2.1]octadienes from the Rhizobacterium *Serratia odorifera***

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Dedicated to Professor H. M. R. Hoffmann on the occasion of his 75th birthday

Rhizobacteria, which are associated with plant roots, have recently attracted attention because they can emit volatile organic compounds capable of affecting growth and development of plants, fungi, and other bacteria through largely unknown mechanisms.[1] Rhizobacterial volatiles released by *Serratia odorifera* 4Rx13 have been reported to inhibit growth of *Arabidopsis thaliana*,[2] as well as mycelial growth of fungal plant pathogens such as *Rhizoctonia solani*.[2,3]

Volatiles released by *S. odorifera* are highly dominated by a single compound (**1**). The EI mass spectrum of **1** (70 eV; Figure 1) revealed a signal for the molecular ion at *m*/*z* 218 [M^+] and an unusual cluster of highly abundant fragment ions at *m*/*z* 136 (100), 135 (89), and 134 (70). Coupled GC/HRMS led to the assignment of $C_{16}H_{26}$ as the molecular formula, thus revealing four units of unsaturation. About 1 mg of **1** was collected by adsorption on SuperQ. The sample was obtained from headspace collections of volatiles released by a ten-liter culture of *S. odorifera*.[4] The ^1H NMR spectrum in [D$_6$]benzene exhibited signals for one exocyclic methylene group at $\delta = 4.63$ (s, 2H) and one doublet methyl group at $\delta = 0.73$ (d, $J = 6.9$ Hz, 3H) that is connected to a methine group at $\delta = 2.04$ (q, $J = 6.9$ Hz, 1H). Furthermore, isochoric signals corresponding to two doublet methyl groups at $\delta = 1.12$ (d, $J = 7.3$ Hz, 6H), each connected to its own methine group at $\delta = 2.08$ (q, $J = 7.2$ Hz, 2H), were identified, along with two singlet methyl groups at $\delta = 0.83$ (s, 6H), and two olefinic methyl groups at $\delta = 1.41$ (s, 6H). The integral ratio of 1:2:3:6 for these ^1H NMR signals and the large number of isochoric methyl signals indicated a poly(methylated) compound showing C_s symmetry. Inspection of the ^{13}C PENDANT and HMQC spectra confirmed the presence of one exocyclic methylene group at $\delta = 111.1$ (t) and 156.0 ppm (s), two different types of methine groups at $\delta = 39.9$ and 44.1 ppm, as well as four types of methyl groups at $\delta = 9.6$, 11.0, 17.7, and 19.5 ppm. The remaining four quaternary carbon atoms, which are required for the molecular composition of $C_{16}H_{26}$, were observed at $\delta = 51.5$ and 134.2 ppm in agreement with isochoric pairs of quaternary and olefinic carbons, respectively. Inspection of the COSY-90 spectrum confirmed the identification of five distinguishable partial structures, which could be connected according to the long-range H,C-correlations from the HMBC spectrum, thus, completing the structure of the novel 1,2,4,5,6,7,8-heptamethyl-3-methylene-bicyclo[3.2.1]oct-6-ene (**1**), which we named sodorifen. The stereochemistry of sodorifen was deduced from the NOESY spectrum. NOE interactions between the equivalent doublet methyl groups (at C2 and C4) and the methine proton at C8 indicated an 8-*anti*-2,4-bisaxial configuration.

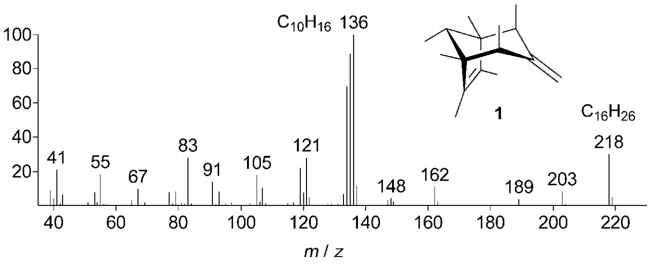

Figure 1. Mass spectrum of sodorifen (**1**) from *Serratia odorifera* was determined by EI (70 eV).

The structure of sodorifen (**1**) was unambiguously proven by synthesis (Scheme 1).[4] Access to the bicyclo[3.2.1]octadiene skeleton through 6π,7C-cycloaddition has been described by Hoffmann.[5] Treatment of commercially available pentamethylcyclopentadiene (**2**) with 2,4-dibromopentan-3-one (**3**) using the sodium iodide/copper route described by Ashcroft and Hoffmann[6] afforded 1:2–1:4 mixtures of 8-*anti*-configured 2,4-bisaxial- (*exo*-) and 2,4-bisequatorial- (*endo*-) 1,2,4,5,6,7,8-heptamethylbicyclo[3.2.1]-oct-6-en-3-ones **4** and **5**, respectively. Trace amounts of the corresponding *syn*-configured epimers at C8 were also detected upon coupled GC/EIMS analysis (< 0.5 %). Care had to be taken to prevent epimerization of **4** under acidic or alkaline conditions or upon distillation, which resulted in the formation of **5** via the unstable mixed axial/equatorial configured (chiral) isomer. The separation of the isomers **4** and **5** was achieved by crystallization at −20 °C and subsequent column chromatography on silica gel. Wittig reaction of triphenylmethylenephosphorane and **4** led to epimerization,

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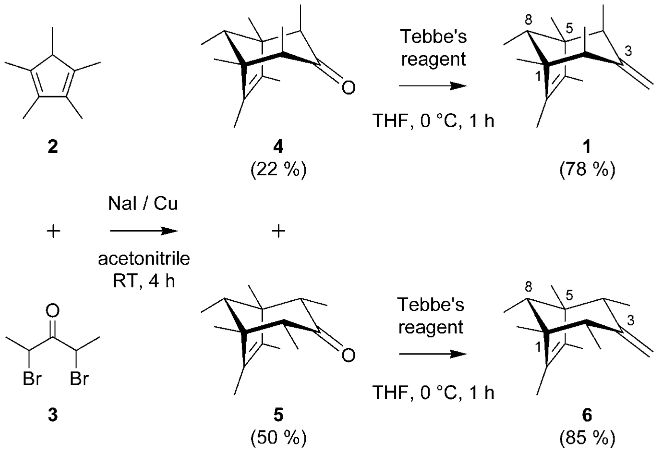
[**] This work was financially supported by the Deutsche Forschungsgemeinschaft (Fr-507/16-1 and PI-153/26-1). We thank Dr. V. Sinnwell and Dr. S. Franke (Hamburg) for measurements of NMR and HRMS spectra.

See the Supporting Information for full experimental details. Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200905680.



Scheme 1. Synthesis of 1,2,4,5,6,7,8-heptamethyl-3-methylenebicyclo-[3.2.1]oct-6-enes **1** and **6**.

whereas **5** did not react at all. In contrast, treatment with Tebbe's reagent[7] afforded the corresponding hydrocarbons **1** and **6** in up to 80% total yield. The isomeric hydrocarbons **1** and **6** were isolated by a sequence of column chromatography on silica gel and preparative gas chromatography. Only 8-*anti*-2,4-bisaxial-1,2,4,5,6,7,8-heptamethyl-3-methylenebicyclo-[3.2.1]oct-6-ene (**1**, sodorifen) exhibited mass spectra and GC retention indices as well as ^{1}H and ^{13}C NMR spectra that were identical to the natural product. The stereoisomer **6**, as well as the corresponding 8-*syn* epimers were not detected in the headspace of *S. odorifera*.

S. odorifera releases a range of new hydrocarbons, among which the major component, sodorifen (**1**), dominates by 85%. It is accompanied by a multitude of smaller amounts of isomers, some of which were also observed by GC/MS upon treatment of **1** with acidic Amberlyst 15 resin, thus suggesting related carbon skeletons. To the best of our knowledge, the structures of these volatiles are unique among natural products, and show one extra skeletal carbon atom attached to each of those of the core system. Preliminary ^{13}C-labeling experiments with [2-^{13}C]acetate, which targeted a polyketide or mevalonate biogenesis, resulted in significant [^{13}C]-enrichment of sodorifen; however, the distribution of the label(s) did not provide conclusive results. Investigations concerning the biological significance of the *S. odorifera* volatiles in general, and specifically that of sodorifen, are presently underway.

Received: October 9, 2009
Published online: February 12, 2010

Keywords: hydrocarbons · natural products · NMR spectroscopy · rhizobacteria · structure elucidation

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