Determination of the absolute configuration of the sex pheromone of the obscure mealybug by vibrational circular dichroism analysis

Bruno Figadère,†[a] Frank J. Devlin,[b] Jocelyn G. Millar[a] and Philip J. Stephens[b]

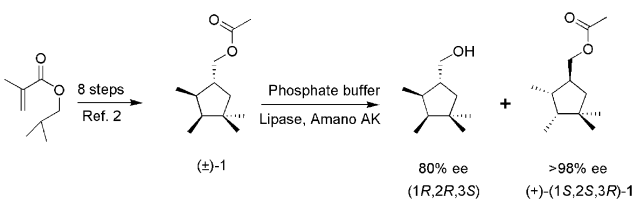
Received (in Cambridge, UK) 12th November 2007, Accepted 4th December 2007
First published as an Advance Article on the web 8th January 2008
DOI: 10.1039/b717440c

Comparison of theoretical and experimental vibrational circular dichroism (VCD) spectra of an enantiopure synthetic sample of the obscure mealybug sex pheromone allowed the determination of the absolute configuration of the insect's pheromone.

We recently reported the identification[1] and diastereoselective synthesis[2] of (1*R**,2*R**,3*S**)-1-acetoxymethyl-2,3,4,4-tetramethylcyclopentane **1**, the female-produced sex pheromone of the obscure mealybug, *Pseudococcus viburni* (Homoptera: Pseudoccidae). This widely distributed insect is a worldwide pest of grape vines, and numerous other crops and ornamental plants. As part of ongoing studies to determine whether the pheromone can be incorporated into insect pest management programs, full identification of the female-produced sex pheromone was required. In previous studies, we unequivocally established the relative configuration of the molecule from NMR spectra, but not the absolute configuration. The few micrograms of the insect-produced compound that were available precluded measurement of its optical rotation. The two enantiomers in the synthetic racemate were resolved to baseline on a chiral stationary phase capillary GC column (Cyclodex B, 30 m × 0.25 mm ID × 0.25 micron film; J&W Scientific, Folsom, California; 40 °C/ 1 min, 3°/min to 200 °C), but in the absence of at least one enantiomer of known configuration, we had no way of determining which enantiomer was which. To break this impasse, we reasoned that if at least one enantiomer could be resolved from the synthetic racemate on milligram scale, it might be possible to determine its absolute configuration by comparison of the experimentally determined vibrational circular dichroism (VCD) spectrum of that enantiomer with the calculated VCD spectra of the two enantiomers. This proved to be the case, and here we describe the preparation of enantiopure (1*S*,2*S*,3*R*)-1-acetoxymethyl-2,3,4,4-tetramethylcyclopentane **1** by kinetic resolution of the synthetic racemate, and the determination of its absolute configuration. This in turn enabled unambiguous assignment of the absolute configuration of the obscure mealybug pheromone.

Thus, (1*R**,2*R**,3*S**)-1-acetoxymethyl-2,3,4,4-tetramethylcyclopentane **1** (50 mg), prepared as previously described,[2] was kinetically resolved by stirring in a slurry of water (6.7 mL), phosphate buffer (1 M, pH = 7, 1.6 mL), and Amano lipase AK (Aldrich, 10.4 mg) at room temperature, following the progress of the enzyme-catalyzed hydrolysis by GC (Scheme 1). After 51 h, GC analysis of an aliquot of the reaction mixture on the chiral phase Cyclodex B column showed a single peak for the acetate **1** and another peak for alcohol **2**, with slightly over 50% conversion. The mixture was then extracted four times with 1 : 1 pentane : Et_2O, the combined organic extracts were washed with saturated $NaHCO_3$ and brine, then dried over anhydrous Na_2SO_4. After concentration at reduced pressure, purification of the crude mixture by flash chromatography (SiO_2, eluting with pentane/Et_2O 5% to 25%) gave acetate **1** ($[\alpha]_D$ = +9.1 (*c* = 0.4, $CDCl_3$)) in 40% yield and ~99% enantiomeric excess, as judged by GC analysis as described above. Alcohol **2** was then converted to the acetate (−)-**1** (Ac_2O, Et_3N in Et_2O) and proved to be 80% ee by GC analysis. The kinetically-resolved (+)-**1** corresponded to the later-eluting of the two enantiomers on the chiral GC column. Co-injection of the insect-produced compound with the racemic standard also resulted in enhancement of the later-eluting peak, confirming that the insect-produced and the kinetically-resolved enantiomer were identical. Injection of the insect-produced compound alone gave only a single peak, proving that the insect produces only a single stereoisomer of the pheromone.

The absolute configuration (AC) of a chiral molecule can be determined using VCD by comparison of its experimental VCD spectrum to the density-functional theory (DFT)-calculated VCD spectra of the two enantiomers.[3–23] In the case of a conformationally flexible molecule, the calculated VCD spectrum is the conformational-population-weighted average of the VCD spectra of the populated conformations.[3–23] The pheromone **1** is clearly conformationally flexible, due to the flexibility of the cyclopentane ring and of the acetoxymethyl substituent.



Scheme 1 Preparation of (+)-**1**.

[a] *Department of Entomology, University of California, Riverside, CA 92521, USA. E-mail: jocelyn.millar@ucr.edu; Fax: +1 951 827 3086; Tel: +1 951 827 5821*
[b] *Department of Chemistry, University of Southern California, Los Angeles, CA 90089, USA. E-mail: pstephen@usc.edu; Fax: +1 213 740 8348; Tel: +1 213 740 4119*
† Permanent address: UMR CNRS 8076, University Paris-Sud, Laboratoire de Pharmacognosie, UFR de Pharmacie, F-92296, Châtenay-Malabry, France, E-mail: bruno.figadere@u-psud.fr, Fax: (+33) 1 4683 5590, Tel: (+33) 1 4683 5592

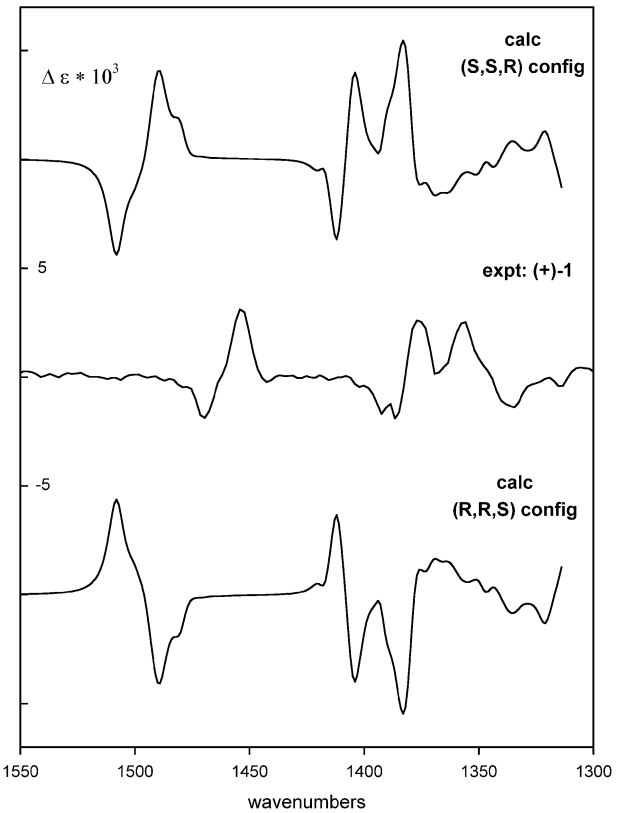


Fig. 1 Comparison of the conformationally averaged B3PW91/TZ2P VCD spectra of (1*S*,2*S*,3*R*)-**1** and (1*R*,2*R*,3*S*)-**1** to the experimental VCD spectrum of (+)-**1**.

The VCD spectrum of (+)-**1** was measured using 0.90 M solutions of (+)-**1** and of (±)-**1** in $CDCl_3$. The resulting spectrum of (+)-**1** in the frequency range 1550–1300 cm^{-1} is shown in Fig. 1. Conformational analysis of **1** was carried out using DFT, at the B3PW91/TZ2P level,[4–18] with the results given in Table 1. There are 15 conformations with free energies within 3 kcal mol^{-1} of the lowest free-energy conformation. The room temperature equilibrium populations resulting from the relative free energies are also given in Table 1. Five conformations have populations >10%, and thus will dominate the VCD spectrum. Calculation of the VCD spectra of all 15 conformations of **1** at the B3PW91/TZ2P level then resulted in the conformationally-averaged VCD spectra of (1*R*,2*R*,3*S*)-**1** and (1*S*,2*S*,3*R*)-**1** shown in Fig. 1. Comparison of the calculated VCD spectra to the experimental VCD spectrum of (+)-**1** unambiguously demonstrates that (+)-**1** has the (1*S*,2*S*,3*R*) configuration. The calculated and experimental VCD spectra at frequencies <1300 cm^{-1} are more complex than in the range 1300–1550 cm^{-1}. As a result, the analysis of the experimental VCD is limited to the range 1300–1550 cm^{-1} (Fig. 1). Because the insect-produced pheromone and kinetically-resolved (+)-**1** were identical, this in turn proves that the insect-produced pheromone must also have the (1*S*,2*S*,3*R*) configuration.

In summary, comparison of experimental and theoretical VCD spectra has again been shown to be a valuable method of determining the absolute configurations of chiral compounds without having to develop enantioselective syntheses of one or both enantiomers, as long as at least one enantiomer can be resolved from the racemate, for example by chromatographic or enzymatic methods. The method should be particularly useful for newly identified natural products, as in the case described here, where the very small amounts of sample may preclude the use of other methods of determining absolute configurations.

Table 1 B3PW91/TZ2P relative energies, relative free energies and populations of the conformations of **1**

Conformer	ΔE^a	ΔG^a	P (%)^b
a	0.00	0.00	23.6
b	0.24	0.19	17.2
c	0.46	0.24	15.6
d	0.39	0.31	14.0
e	0.67	0.50	10.2
f	0.61	0.93	4.9
g	1.34	0.95	4.8
h	0.99	1.09	3.7
i	0.97	1.43	2.1
j	1.07	1.49	1.9
k	1.58	2.02	0.8
l	2.32	2.26	0.5
m	2.64	2.62	0.3
n	2.38	2.70	0.3
o	2.59	3.00	0.2

[a] ΔE and ΔG in kcal mol^{-1}. [b] Populations based on ΔG values, T = 298 K.

JGM and BF thank the Viticulture Consortium for financial support of this work. PJS thanks the National Science Foundation for financial support of this work.

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