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Stereoisomeric Analysis of 6,10,14-Trimethylpentadecan-2-ol and the Corresponding Ketone in Wing Extracts from African *Bicyclus* Butterfly Species

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Abstract Gas chromatography (GC) and mass spectrometry (MS) were used to determine the stereoisomeric compositions of 6,10,14-trimethylpentadecan-2-ol and 6,10,14-trimethylpentadecan-2-one in wing extracts from 17 *Bicyclus* butterfly species from different regions of Africa. All samples were purified using solid phase extraction (SPE). Since some species contained both alcohol and ketone, these were separated and the ketone was reduced to the alcohol before analysis as either (*R*)-*trans*-chrysanthemoyl or (*S*)-2-acetoxypropionyl esters. A novel asymmetric synthesis was developed for a reference mixture of (2*R*/*S*,6*S*,10*R*)-6,10,14-trimethylpentadecan-2-ol with known composition of the eight stereoisomers. The mixture then was used as the (*R*)-*trans*-chrysanthemoyl esters to correlate each of the eight gas chromatographic peaks to a specific stereoisomer of the extracted wing compounds. Seven butterfly species showed (2*R*,6*R*,10*R*)-configuration of the alcohol, four species contained minute amounts of alcohol too small to determine the stereochemistry, nine species showed (6*R*,10*R*)-configuration of the ketone, and one species contained minute amounts of ketone too small to determine the stereochemistry. No other stereoisomers of alcohol or ketone could be detected in the extracts, and the quantities of the compounds in the wing extracts varied from 5 to 900 ng per sample for each species.

Keywords Asymmetric synthesis · GC/MS · 6,10,14-Trimethylpentadecan-2-one · Wing compounds · Stereoisomers

Introduction

Pheromones and other semiochemicals found in nature are often chiral compounds that have one or more stereogenic centers and can exist as two or more stereoisomers. Consequently, the biological functions of the different stereoisomers need to be considered since in some cases small amounts of stereoisomers might act as synergists or antagonists in biological assays. The importance of the optical purity of such volatile organic compounds used for communication within a species is demonstrated by the sex pheromone found in the pine sawfly species *Neodiprion sertifer*. Female *N. sertifer* use the acetate of (2*S*,3*S*,7*S*)-3,7-dimethylpentadecan-2-ol as a sex pheromone. However, even a small amount (<0.2 %) of the (2*S*,3*R*,7*R*)-stereoisomer inhibits the attractiveness (Anderbrant 1999; Anderbrant et al. 2000). This example emphasizes the need for analytical methods that effectively separate stereoisomers of biologically-active compounds (Bång et al. 2011, 2012) in order to interpret and understand the results of bioassays.

Another example of a bioactive secondary alcohol that can exist as eight stereoisomers is 6,10,14-trimethylpentadecan-2-ol that is used as sex pheromone by pest insects such as the rice moth, *Corcyra cephalonica*, the African sugar-cane borer moth, *Eldana saccharina*, and the oil palm bunch moth, *Tirathaba mundella* (Burger et al. 1993; Hall et al. 1987; Mori et al. 1991;

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Sasaerila et al. 2003). The same compound also is produced by many African butterfly species of the genus *Bicyclus* (Nieberding *et al.* unpublished information and results in this paper) and in particular, *Bicyclus anynana* where 6,10,14-trimethylpentadecan-2-ol is a confirmed pheromone component together with (*Z*)-9-tetradecen-1-ol and hexadecanal (Nieberding et al. 2008). Recently the stereochemistry of the wing compound was verified as (2*R*,6*R*,10*R*)-6,10,14-trimethylpentadecan-2-ol (Nieberding et al. 2012). So far there are no reports of (2*R*,6*R*,10*R*)-6,10,14-trimethylpentadecan-2-ol in other *Bicyclus* species but 6,10,14-trimethylpentadecan-2-ol and the corresponding ketone have been found in several other species, and differences in the stereochemistry of these compounds may potentially contribute to species specificity.

Gas chromatography (GC) with mass spectrometry (MS) has been the conventional method for analyzing derivatized secondary alcohols such as 6,10,14-trimethylpentadecan-2-ol in extracts from male *B. anynana* butterflies (Nieberding et al. 2008). The general approach to separate stereoisomers of secondary alcohols is to use pre-column derivatization, resulting in chiral or non-chiral derivatives in combination with chiral or non-chiral columns. However, combinations of different derivatives and columns often have been necessary to separate all the stereoisomers satisfactorily (Bång et al. 2011; Bergström et al. 1995; Wassgren et al. 1992). Preparation of all eight stereoisomers individually is normally necessary to be able to determine the retention time of each stereoisomer, and this involves laborious and time consuming synthetic work. Thus, to be able to screen different species efficiently for the composition of their wing volatile blend and the stereoisomeric compositions of 6,10,14-trimethylpentadecan-2-ol and 6,10,14-trimethylpentadecan-2-one, we developed a novel synthetic route for a reference mixture of the alcohol with a known composition of stereoisomers in order to determine the GC-retention time for each stereoisomer. In this study, we used GC/MS to determine the stereoisomeric composition of 6,10,14-trimethylpentadecan-2-ol and 6,10,14-trimethylpentadecan-2-one in wing extracts from seventeen species of African *Bicyclus* butterflies. We improved the conditions for the GC/MS analysis of (*R*)-*trans*-chrysanthemoyl (Bång et al. 2012) and (*S*)-2-acetoxypropionyl derivatives (Bång et al. 2011) of 6,10,14-trimethylpentadecan-2-ol, including the identification of the elution order of each stereoisomer of the alcohol.

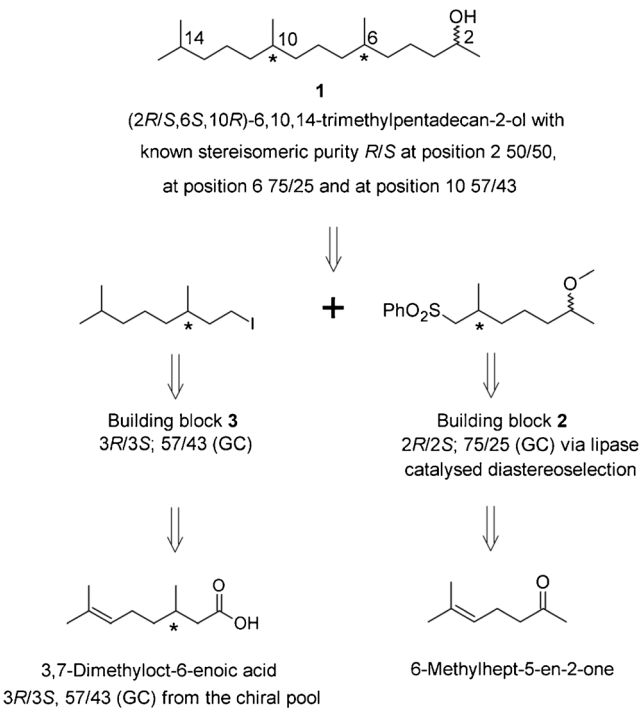
Methods and Materials

Chemicals The solvents used were of spectrophotometric grade or higher and purchased from Sigma-Aldrich, Schnelldorf, Germany. (3*R*)-3,7-Dimethyloct-6-enoic acid, (*S*)-2-acetoxypropionyl chloride (Fluka, puriss.), pentadecan-2-one were purchased from Sigma-Aldrich and the pentadecan-2-ol came from The Sigma-Aldrich Library of Rare Chemicals, Milwaukee WI, USA.

Synthesis The (2*S*,6*R/S*,10*R/S*)- and (2*R*,6*R/S*,10*R/S*)-6,10,14-trimethylpentadecan-2-ol mixtures were synthesized from (2*E*,7*R/S*,11*R/S*)-3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol) following the protocol in Nieberding et al. (2008). By applying the same protocol but starting from (2*E*,7*R*,11*R*)-phytol the pure stereoisomers of (2*S*,6*R*,10*R)*-6,10,14-trimethylpentadecan-2-ol and (2*R*,6*R*,10*R)*-6,10,14-trimethylpentadecan-2-ol were synthesized (Nieberding et al. 2012).

A novel asymmetric synthetic strategy was developed in order to produce a mixture of 6,10,14-trimethylpentadecan-2-ol isomers with known isomeric composition (Scheme 1), as described in the Results section and Supplementary Material.

Insect Extracts We analyzed the volatile chemicals produced in the butterfly wings from a total of seventeen *Bicyclus* species found in different regions of Africa. For each species, the wing samples were collected from three male and two female individuals. The wings were dissected into several pieces according to the androconia location and the pieces extracted separately in 1.5 ml glass vials with 100 μl of redistilled *n*-heptane containing 1 ng μl^{-1} of (*Z*)-8-tridecen-1-yl acetate as internal standard. For samples of whole wings, the hindwing and forewing sections were extracted separately



Scheme 1 Retrosynthetic analysis for a stereoisomeric mixture of (2*R*/*S*,6*S*,10*R*)-6,10,14-trimethylpentadecan-2-ol (**1**) containing all eight isomers with known stereoisomeric composition

in 300 μl of *n*-heptane containing the same internal standard at a concentration of 0.33 ng μl^{-1}.

Analysis of Crude Extracts Initial screening was performed to establish the content of 6,10,14-trimethylpentadecan-2-ol and the corresponding ketone in 32 *Bicyclus* species (Nieberding *et al.* unpublished data) in order to be able to select the seventeen species that contained relatively high amounts of the target compounds for stereochemical analysis. Partial and whole wing extracts from the selected species then were analyzed on an Agilent 6890 N GC (Agilent, Walbronn, Germany) with a polar FactorFOUR VF-23 ms column (30 m×0.25 mm i.d., d_f=0.25 μm Varian, Palo Alto, CA USA) and an HP 5973 MS detector (Agilent) in electron impact (EI) ionization mode. The carrier gas was helium (1 ml min^{-1}); 1 μl of the sample was injected splitless, the injector temperature was 250 °C, and the auxilliary temperature was 280 °C. For identification of the alcohol and ketone in crude extracts, the full scan mode was used, and the column temperature increased from 50 °C at 10 °C min^{-1} up to 230 °C and held at 230 °C for 10 min.

Purification of Insect Extracts Two internal standards (IS), pentadecan-2-one (73–1099 ng) and pentadecan-2-ol (90–1017 ng), were added to the crude hexane extracts from each species in amounts adjusted to match the amount of the analyzed compound in each sample. Each extract then was purified by liquid chromatography (LC) using gradient elution stepwise with 1–11 % ethyl acetate in pentane in steps of 1 % (0.75 ml) on a 500 mg Strata SI-1 Silica Teflon coated solid phase column (Skandinaviska Genetec AB, Västra Frölunda, Sweden). The collected ketone fractions (4–8; 0.75 ml) were pooled and reduced to alcohol with $LiAlH_4$ before derivatization, and were then analyzed as described below. The alcohol fractions (9–11) were pooled and after derivatization they were also analyzed as described below. The internal standards were used both as a control of the purification method and for quantification of ketone and alcohol applying the response factor ratios of 1:1.

Derivatisation of Purified Extracts To determine the stereochemistries of the 6,10,14-trimethylpentadecan-2-ol and 6,10,14-trimethylpentadecan-2-one, the pooled alcohol fraction was derivatized with (*R*)-*trans*-chrysanthemoyl chloride according to Bång et al. (2012) or with (*S*)-2-acetoxypropionyl chloride according to Bång et al. (2011). The pooled ketone fraction with 6,10,14-trimethylpentadecan-2-one was first reduced to alcohol with $LiAlH_4$ before derivatization, as described above. When the target compounds were present in low concentration, the (*S*)-2-acetoxypropionyl chloride method was used, otherwise the extracts were derivatized with (*R*)-*trans*-chrysanthemoyl chloride.

Stereochemical Analysis Improved conditions were developed for separation of (*R*)-*trans*-chrysanthemoyl (Bång et al. 2012) and (*S*)-2-acetoxypropionyl derivatives (Bång et al. 2011) of 6,10,14-trimethylpentadecan-2-ol in GC/MS analyses. Equipment for the GC/MS analyses was as above. The selective ion monitoring mode (SIM) (*m/z* 105, 115, 133, 210, 252) was used for identification of the (*S*)-2-acetoxypropionyl derivatives, and the column temperature was increased from 50 °C at 10 °C min^{-1} up to 110 °C, from 110 °C by 0.02 °C min^{-1} up to 116 °C, and from 116 °C by 10 °C min^{-1} up to 230 °C, and held at 230 °C for 10 min. For identification of (*R*)-*trans*-chrysanthemoyl derivatives, SIM (*m/z* 123, 124, 153, and 168) was used and the column temperature was increased from 50 °C by 10 °C min^{-1} up to 100 °C, from 100 °C by 0.1 °C min^{-1} to 140 °C, and from 140 °C by 10 °C min^{-1} up to 230 °C and held at 230 °C for 10 min.

The enantiomeric compositions of intermediates in the synthesis were determined by enantioselective GC analysis using a β-dex225 column (30 m×0.25 mm, d_f=0.25; Supelco), operated isothermally at 70 °C.

Results and Discussion

Synthesis A novel asymmetric synthesis strategy was developed in order to produce a mixture of 6,10,14-trimethylpentadecan-2-ol stereoisomers with known isomeric composition (Scheme 1), and detailed procedures are described in the Supplementary Material.

Building block **2** was obtained in ten steps from commercially available 6-methyl-5-hepten-2-one. This was reduced to the alcohol with lithium aluminum hydride in ether, and the alcohol was methylated with sodium hydride and methyl iodide. Allylic oxidation was performed with *tert*-BuOOH and SeO_2 (Salimova et al. 2003; Umbreit and Sharpless 1977), and the saturated compound was obtained by hydrogenation with Ra-Ni (Högberg et al. 1992; Shibata et al. 2002). The alcohol was oxidized to the 6-methoxy-2-methylheptanoic acid with Jones reagent (Nagamitsu et al. 1996) using *iso*-propanol in excess to react all the chromium reagent.

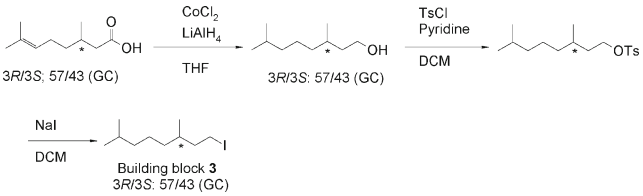
The key stereoselective step in the synthesis of building block **2** was the lipase-catalyzed resolution of this acid. In earlier syntheses, we successfully used lipase-catalyzed resolution of 2-methylcarboxylic acids (See for example Hedenström et al. 2002). Thus, the 6-methoxy-2-methylheptanoic acid was esterified with 2,2-dimethylpropanol under catalysis of *Candida rugosa* lipase (CRL) immobilized on Accurel in isooctane (Sabbani et al. 2006) to obtain the remaining (*R*)-acid in optical purity of 2*R*/2*S* 75:25, as measured by GC analysis on the β-dex225 column, at a conversion of 30 %. The (2*R*)-6-methoxy-2-methylheptanoic

acid obtained was reduced to the alcohol using lithium aluminum hydride in ether, and the product had the same enantiomeric purity as the starting acid. The alcohol then was transformed to (2*R*)-1-iodo-6-methoxy-2-methylheptane *via* a tosylate (Burns et al. 1997) and the iodo-compound was reacted with $PhSO_2Na$ in dry DMF (Kandula and Kumar 2006; Nakamura and Mori 2000) to furnish the sulfone building block **2** (Scheme 2).

Building block **3** was obtained in three steps from commercially-available (*R*)-3,7-dimethyloct-6-enoic acid ((*R*)-(+)-citronellic acid; SigmaAldrich) which was reduced to the alcohol with simultaneous reduction of the double bond (Ashby and Lin 1978). The stereoisomeric purity of the 3,7-dimethyloct-6-enoic acid and the corresponding alcohol was determined by enantioselective GC analysis as 3*R*/3*S* 57:43. (*R*)-3,7-Dimethyloctan-1-ol was transformed to the corresponding iodide *via* a tosylate (Enders and Schüsseler 2002; Kimura et al. 2001) (Scheme 3).

Alkylation of the anion derived from **2** with the iodide **3** resulted in a product with the desired carbon skeleton which yielded the methyl-protected alcohol after sulfone reduction (Nakamura and Mori 2000; Shibata et al. 2002). The methoxy ether was subjected to oxidative cleavage, and the ketone obtained was reduced to the required reference mixture of eight stereoisomers of (2*R*/*S*,6*S*,10*R*)-6,10,14-trimethylpentadecan-2-ol (**1**) (Scheme 4).

GC/MS Analysis of Stereoisomers of 6,10,14-Trimethylpentadecan-2-ol The different reference mixtures were derivatized as (*R*)-*trans*-chrysanthemoyl and (*S*)-2-acetoxypropionyl esters, and the (*R*)-*trans*-chrysanthemoyl



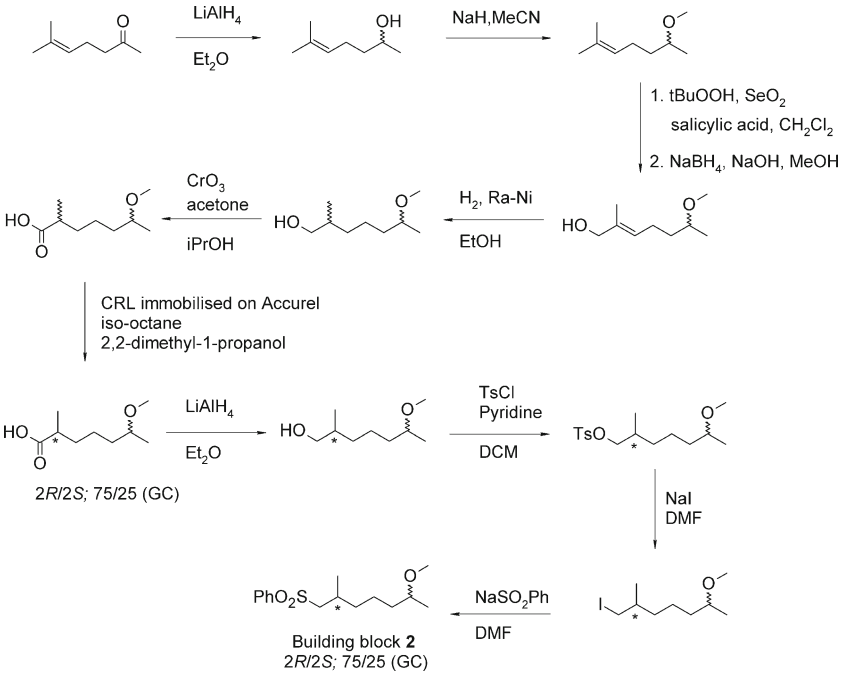
Scheme 3 Synthesis of building block **3** with known enantiomeric purity

esters resulted in better separation on GC/MS analysis (See Figs. 1 and 2). Thus, the first derivatization and analytical method was used to verify the GC elution order of the eight stereoisomers of 6,10,14-trimethylpentadecan-2-ol.

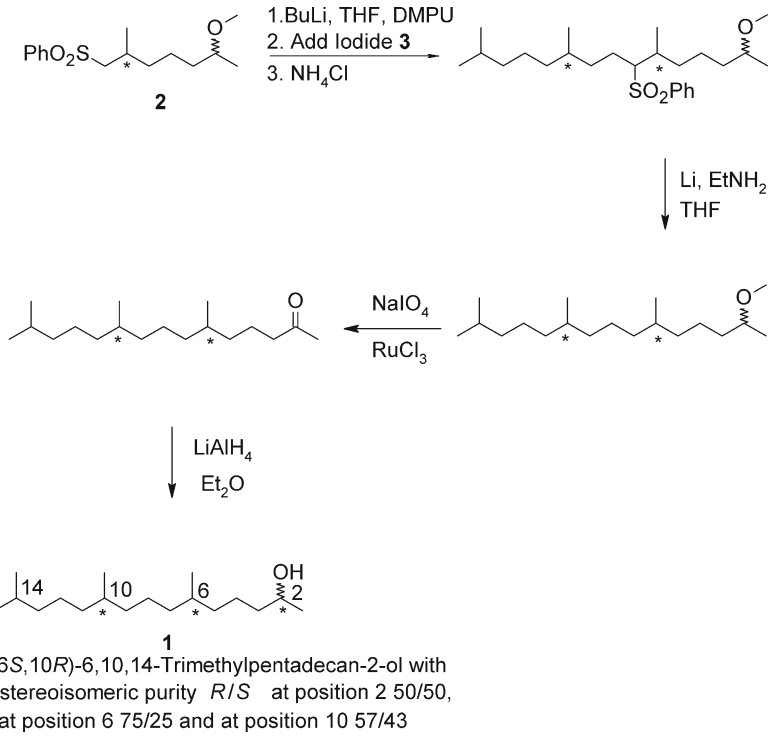
By comparing the stereoisomeric ratio of the synthetic reference alcohol (2*R*/*S*,6*S*,10*R*)-6,10,14-trimethylpentadecan-2-ol (**1**) calculated from the stereoisomeric purity of building blocks (See Table 1) with the relative peak area obtained by GC/MS analysis of the mixture after derivatization with (*R*)-*trans*-chrysanthemoyl (Fig. 1a), we concluded the elution order from the GC-column of the eight stereoisomers to be; (2*S*,6*R*,10*S*) : (2*S*,6*R*,10*R*) : (2*S*,6*S*,10*R*) : (2*S*,6*S*,10*S*) : (2*R*,6*S*,10*R*) : (2*R*,6*S*,10*S*) : (2*R*,6*R*,10*S*) : (2*R*,6*R*,10*R*).

We previously reported (Nieberding et al. 2008) that when applying a lipase catalyzed stereoselective acylation of the eight-component stereoisomeric mixture of (2*R*/*S*,6*R*/*S*,10*R*/*S*)-6,10,14-trimethylpentadecan-2-ol, it was possible to isolate (2*S*,6*R*/*S*,10*R*/*S*)-6,10,14-trimethylpentadecan-2-ol and (2*R*,6*R*/*S*,10*R*/*S*)-6,10,14-trimethylpentadecan-2-ol as separate, four-component stereoisomeric mixtures. After applying the same derivatization and analytical method to these two reference mixtures, we confirmed the four-component mixture of (2*S*,6*R*/*S*,10*R*/*S*)-6,10,14-trimethylpentadecan-

Scheme 2 Synthesis of building block **2** with known stereoisomeric purity



Scheme 4 Synthesis of (2*R*/*S*,6*S*,10*R*)-6,10,14-trimethylpentadecan-2-ol (**1**) with known stereoisomeric composition by coupling the two building blocks **2** and **3**



2-ol eluted before the four-component mixture of (2*R*, 6*R/S*,10*R/S*)-6,10,14-trimethylpentadecan-2-ol (Fig. 1b and c, also reported in Nieberding et al. 2008). Two other synthetic reference mixtures *i.e.*, the 2*R*/*S*,6*R*,10*R*)-6,10,

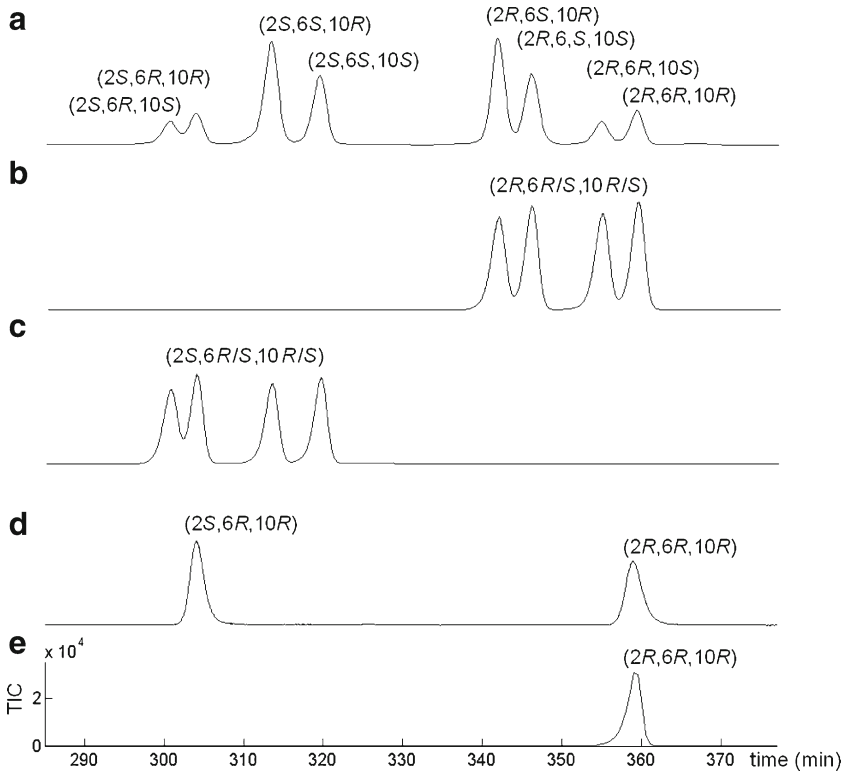


Fig. 1 GC/MS chromatograms of synthetic references **a**) (2*R/S*,6*S*,10*R*)-6,10,14-trimethylpentadecan-2-ol (**1**) with known stereoisomeric composition, **b**) (2*R*,6*R/S*,10*R/S*)-mixture of four stereoisomers, **c**) (2*S*,6*R/S*,10*R/S*)-mixture of four stereoisomers, **d**) (2*R/S*,6*R*,10*R*)-mixture of two stereoisomers, **e**) pure (2*R*,6*R*,10*R*)-stereoisomer. All samples were derivatized with (*R*)-*trans*-chrysanthemoyl chloride and analyzed on a FactorFOUR VF-23 ms column

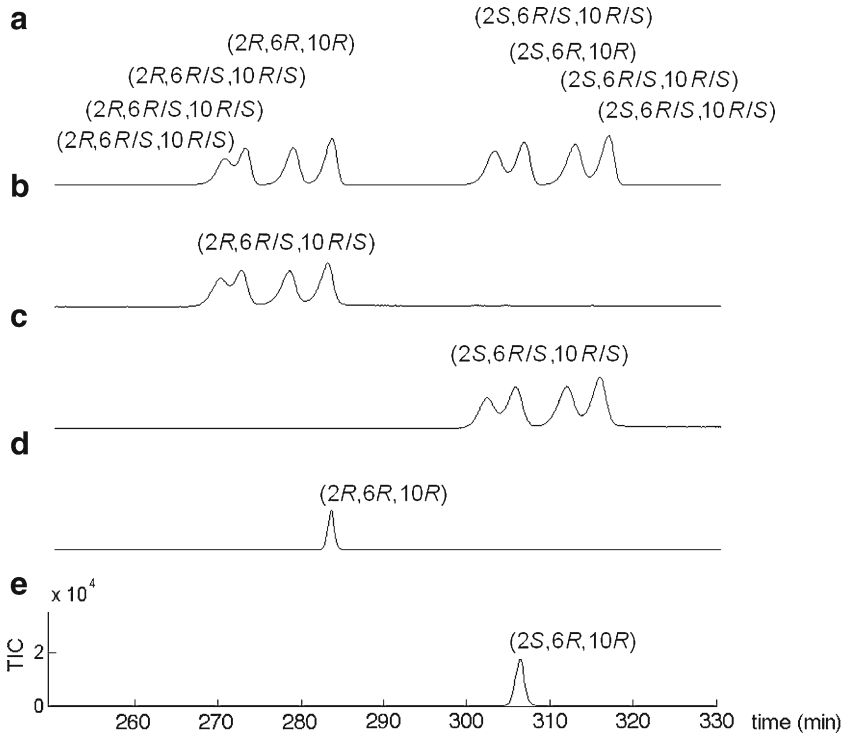


Fig. 2 GC/MS chromatograms of synthetic references **a**) (2*R*/*S*,6*R*/*S*,10*R*/*S*)-6,10,14-trimethylpentadecan-2-ol an eight stereoisomeric mixture, **b**) (2*R*,6*R*/*S*,10*R*/*S*)-mixture of four stereoisomers, **c**) (2*S*,6*R*/*S*,10*R*/*S*)-mixture of four stereoisomers, **d**) pure (2*R*,6*R*,10*R*)-stereoisomer, and **e**) pure (2*S*,6R,10*R*)-stereoisomer. All samples were derivatized with (*S*)-2-acetoxypropionyl chloride and analyzed on a FactorFOUR VF-23 ms column

14-trimethylpentadecan-2-ol and the stereoisomerically pure (2*R*,6*R*,10*R*)-6,10,14-trimethylpentadecan-2-ol, also were derivatized and analyzed by the same method (Fig. 1d and e). The analytical result obtained further strengthened the proposed elution order shown in Table 1.

The (2*S*,6*R*/*S*,10*R*/*S*)-mixture eluted before the (2*R*,6*R*/*S*,10*R*/*S*)-mixture when derivatized as (*R*)-*trans*-chrysanthemoyl esters but eluted after the (2*R*,6*R*/*S*,10*R*/*S*)-mixture when derivatized as (*S*)-2-acetoxy-propionyl esters (See Figs. 1 and 2). The synthetic reference samples (2*R*,6*R*,10*R*)- and (2*S*,6*R*,10*R*)-6,10,14-trimethylpentadecan-2-ol also confirmed this elution order as (*S*)-2-acetoxypropionyl esters when compared with (*R*)-*trans*-chrysanthemoyl esters. One might speculate that the other stereoisomers of (*S*)-2-acetoxypropionyl esters may follow the same pattern of elution as the (*R*)-*trans*-chrysanthemoyl esters, but this was not tested.

Table 1 Identification of the peaks in the GC/MS chromatogram the (*R*)-*trans*-chrysanthemoyl ester of (2*R*/*S*,6*S*,10*R*)-6,10,14-trimethylpentadecan-2-ol (**1**) with known stereoisomeric composition

Stereoisomer	Calculated stereoisomer content[a] (%)	Stereoisomer content by GC/MS analysis (%)
(2*S*,6*R*,10*S*)	0.5×0.25×0.43×100=5	5
(2*S*,6*R*,10*R*)	0.5×0.25×0.57×100=7	7
(2*S*,6*S*,10*R*)	0.5×0.75×0.57×100=21	23
(2*S*,6*S*,10*S*)	0.5×0.75×0.43×100=16	15
(2*R*,6*S*,10*R*)	0.5×0.75×0.57×100=21	24
(2*R*,6*S*,10*S*)	0.5×0.75×0.43×100=16	15
(2*R*,6*R*,10*S*)	0.5×0.25×0.43×100=5	5
(2*R*,6*R*,10*R*)	0.5×0.25×0.57×100=7	7

[a] Using the stereoisomeric purities of the two building blocks **2** for C6 (*S*/*R* 75:25) and **3** for C10 (*R*/*S* 57:43)

Bicyclus Extracts The initial screening for 6,10,14-trimethylpentadecan-2-ol and the corresponding ketone content in thirty-two B*icyclus* species (Nieberding *et al.* unpublished data) resulted in a selection of 17 species (Table 2) that appeared to contain enough of the target compounds for stereochemical analysis. Raw extracts from the selected species were analyzed to quantify the amount of alcohol and ketone in all wing samples (Table 2). The method of derivatization was chosen based on the amount determined in the target compound in each sample. Derivatization with (*R*)-*trans*-chrysanthemoyl chloride proved to give the best GC-separation in the analysis of the reference samples but resulted in less pure samples when used for wing extract analysis. Consequently, this method was used when the wing samples contained relatively high amounts of 6,10,14-trimethylpentadecan-2-ol and 6,10,14-trimethylpentadecan-2-one, and the derivatization method with (*S*)-2-acetoxypropionyl chloride was used when the compounds were present in lower concentrations. The (*R*)-*trans*-chrysanthemoyl derivative of *Bicyclus anynana* wing extract was spiked with the (2*R*,6*R*/*S*,10*R*/*S*)-mix (shown in Nieberding et al. 2008) to prove that the

Table 2 Stereochemistry and amounts of 6,10,14-trimethyl-pentadecan-2-ol and 6,10,14-trimethyl-pentadecan-2-one in wing extracts from 17 African *Bicyclus* butterfly species as determined by GC/MS analyses of derivatized samples (nd not detected; d detected but amount too small to determine stereochemistry or quantity reliably)

Bicyclus species	6,10,14-Trimethyl-pentadecan-2-ol	6,10,14-Trimethyl-pentadecan-2-one[a]	Amount in sample (ng)	Comment
B. anisops	(2*R*,6*R*,10*R*)[b]	(6*R*,10*R*)	300/900	partial wing
B. anynana (2007)	(2*R*,6*R*,10*R*)[b,c]	nd	219	whole wing
B. anynana	d[b]	(6*R*,10*R*)[d]	–/20	partial wing
B. buea	nd	(6*R*,10*R*)[d]	20	partial wing
B. dentata/ dentatus	nd	(6*R*,10*R*)	60	partial wing
B. dorothea	nd	d	5	whole wing
B. ephorus	(2*R*,6*R*,10*R*)[b,c]	nd	70	partial wing
B. golo	nd	(6*R*,10*R*)	80	whole wing
B. graueri	(2*R*,6*R*,10*R*)[c]	nd	20	trace, partial wing
B. heteropsis peitho	nd	d	5	partial wing
B. ignobilis	d[2]	nd	25	partial wing
B. mandanes	nd	(6*R*,10*R*)	130	trace, whole wing
B. mollitia	(2*R*,6*R*,10*R*)[c]	d	–	partial wing
B. safitza	(2*R*,6*R*,10*R*)[b]	nd	100	partial wing
B. sambulos	nd	(6*R*,10*R*)	150	partial wing
B. sandace	nd	(6*R*,10*R*)	95	whole wing
B. sebetus	(2*R*,6*R*,10*R*)[c]	d	30/–	reconcentrated, whole wing
B. vulgaris	nd	(6*R*,10*R*)	700	partial wing

[a] Analyzed as (*S*)-2-acetoxypropionyl ester after reduction to alcohol

[b] Analyzed as (*R*)-*trans*-chrysanthemoyl ester

[c] Analyzed as (*S*)-2-acetoxypropionyl ester

[d] Amount close to the detection limit

extract contained (2*R*,6*R/S*,10*R/S*)-alcohol. Recently we also determined that the stereochemistry of the secondary alcohol in *B. anynana* was (2*R*,6*R*,10*R*)-6,10,14-trimethylpentadecan-2-ol (Nieberding et al. 2012). All 17 wing extracts were analyzed with one or both methods (Table 2), and seven species showed (2*R*,6*R*,10*R*)-configuration of the 6,10,14-trimethylpentadecan-2-ol, four species contained amounts of alcohol too small to determine the stereochemistry, nine species showed (6*R*,10*R*)-configuration of the corresponding ketone, and one species contained amounts of ketone too small to determine the stereochemistry (Table 2). No other stereoisomers of alcohol or ketone could be detected in the extracts.

The butterfly *Pieris brassicae* produces 6,10,14-trimethylpentadecan-2-ol from natural intake of nutrition (Schulz et al. 2011). Our study of the 17 *Bicyclus* species showed that only (2*R*,6*R*,10*R*)-6,10,14-trimethylpentadecan-2-ol and/or (6*R*,10*R*)-6,10,14-trimethylpentadecan-2-one were present in the wing samples. The occurrence in all the species of the same stereoisomers of the two compounds, with rather complex structures that can have eight stereoisomers, might indicate that these compounds are related to substances found in the food sources and that the stereochemistry is already determined by their diet.

To summarize, we were able to correlate each of the eight gas chromatographic peaks to a specific stereoisomer by using different synthetic reference mixtures of 6,10,14-trimethylpentadecan-2-ol derived as (*R*)-*trans*-chrysanthemoyl esters. Thus, GC/MS was used to determine the configuration of 6,10,14-trimethylpentadecan-2-ol and 6,10,14-trimethylpentadecan-2-one found in 17 African *Bicyclus* butterfly species tested, and it was found that the extracts contained only (2*R*,6*R*,10*R*)-6,10,14-trimethylpentadecan-2-ol and/or (6*R*,10*R*)- 6,10,14-trimethylpentadecan-2-one. We also present a novel synthesis of an essential reference mixture of (2*R*/*S*,6*S*,10*R*)-6,10,14-trimethylpentadecan-2-ol with known composition of stereoisomers.

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