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COMPARISON OF THREE DERIVATIVES FOR THE ENANTIOMERIC SEPARATION OF CHIRAL ALCOHOLS AND THE ABSOLUTE CONFIGURATION OF *MYRMICA* ANT 3-OCTANOL

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SUMMARY

A comparison of ease of preparation, retention times and gas chromatographic separation factors of three derivatives for the microscale determination of enantiomeric composition of chiral alcohols is described using 3-octanol as the model compound. Of the three derivatives, N-trifluoroacetyl-(*S*)-(+)-alanyl ester, N-trifluoroacetyl-(*S*)-(−)-prolyl ester and (+)-*trans*-chrysanthemoyl ester, the last was found to be the most useful and was employed to establish the elution order of the (+)-*trans*-chrysanthemate esters of 3-octanol. Thereby 3-octanol, a pheromone component from the mandibular glands of three species of *Myrmica* ants was shown to consist essentially of the *R* enantiomer with a small amount of *S* enantiomer.

INTRODUCTION

In structural investigations of insect pheromones and other biologically active compounds, it is essential to determine the enantiomeric composition of chiral molecules because "odour" receptors in insects can discriminate between enantiomers[1]. Sometimes a "non-biological" enantiomer is inactive or may even express a repellent effect[2]. The amount of insect pheromone components normally isolated precludes an accurate determination of optical rotation by conventional methods.

Plummer *et al.*[3] used chiral shift reagents in Fourier transform nuclear magnetic resonance spectroscopy, to determine the enantiomeric composition of several insect pheromone alcohols, but this technique requires at least 0.5 mg of pure material and highly sophisticated instrumentation.

Gas chromatographic (GC) techniques are preferred for enantiomer composition studies as they are sensitive, need less sophisticated instrumentation and can be applied even to impure biological samples. Direct resolution of enantiomers has been achieved on optically active stationary phases[4] but it has been widely applied only to polar nitrogen-containing compounds, especially amino acids. Ôi *et al.*[5] have pre-

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pared a variety of low-molecular-weight chiral phases which show stereo-selectivity for alcohols, but the retention times of the compounds studied so far are very long. Recently König *et al.*[6] resolved isopropylurethane derivatives of chiral alcohols using a glass capillary column coated with a chiral stationary phase.

An alternative to the direct separation of enantiomers is the GC separation of a diastereomeric derivative formed with an optically pure derivatising agent, on achiral stationary phases, which are less expensive and more widely available. Among the most effective of these derivatives for alcohols are the N-trifluoroacetyl (TFA)-(*S*)-(+)-alanyl esters[7], N-trifluoroacetyl-(*S*)-(−)-prolyl esters[8], (*R*)-(−)-mentyl carbonates[9], (+)-*trans*-chrysanthemoyl esters[10,11], (*S*)-acetoxypropionyl esters[12] and (*R*)-(+)-1-phenylurethanes[13].

3-Octanol and 3-octanone are the major components of the secretion of the mandibular glands of a number of species of *Myrmica* ants[14–16]. 3-Octanol acts alone or synergetically with 3-octanone, to attract worker ants, increase their linear speed and decrease their sinuosity of movement[15,16]. A method was needed to determine the enantiomeric composition of microgram quantities of 3-octanol available from preparative GC of whole heads of ants. As only a few comparative data of the various methods that can be utilized are available, we compared three candidate methods and subsequently used the formation of the (+)-*trans*-chrysanthemate, to determine the enantiomeric composition of 3-octanol in the mandibular glands of *Myrmica* ants.

EXPERIMENTAL

Reagents and materials

N-TFA-(*S*)-(−)-prolyl chloride was kindly donated by Regis (Chicago, IL, U.S.A.). 3-(±)-Octanol was purchased from Koch-Light (Colnbrook, Great Britain) and trifluoroacetic anhydride and (*S*)-alanine from Aldrich (Milwaukee, WI, U.S.A.).

Apparatus and chromatographic conditions

The analytical work was performed with a Carlo Erba Fractovap 2150 gas chromatograph fitted with a flame ionization detector (FID), using glass columns of either (A) 20 m × 0.3 mm I.D., OV-1, wall-coated open tubular (WCOT), at 150°C with helium carrier gas at 2 kg/cm^2 or (B) 60 m × 0.22 mm I.D., DEGS, at 160°C, with helium carrier gas at 1.8 kg/cm^2.

The preparative work was performed with a Pye 104 gas chromatograph with a flame ionization detector, using a packed column (C) of 2.75 m × 4 mm, 10% PEG 20M on Chromosorb W (100–120 mesh), with nitrogen carrier gas flow-rate at 50 ml/min.

GC–mass spectrometry (MS) was performed with a Finnigan 4000 instrument with an on-line INCOS data system. A 20 m × 0.3 mm I.D. fused-silica OV-1 capillary column (D) was linked directly into the mass spectrometer. The GC oven temperature was increased from 60 to 260°C at 6°C/min and the helium carrier gas flow-rate was maintained at 1.5 ml/min. The ionization energy was 40 eV and the source temperature was 250°C.

Rearing of insects

Colonies of *Myrmica rubra*, *M. ruginodis* and *M. scabrinodis* were collected from Chesterton, Staffordshire, Great Britain and maintained in the laboratory, in artificial nests partly filled with moistened plaster and fed with 10% sugar solution and mealworm (*Tenebrio molitor*) larvae.

Isolation of natural 3-octanol

The ants were killed by momentary immersion in liquid nitrogen. The whole heads were separated and 30 heads were sealed in a small section of soda glass capillary tubing (35 mm × 1.8 mm), kept for 5 min in the injection port at 200°C and introduced onto column C at 120°C using a solid injection technique[17]. The effluent was split using an all-glass splitter[18] (95:5, trap:FID) with the outlet heater temperature maintained at 170°C and the desired peak was collected into U-shaped metal tubing of 1 mm I.D., cooled in a mixture of liquid nitrogen and ethyl acetate. The material collected was washed with 100 μl of dichloromethane into a 0.33-ml Reacti-vial containing about 1 mg of anhydrous magnesium sulphate.

(*S*)-3-Octanol was collected from oil of Japanese peppermint (*Menthae japonicae*) in the same way by injecting (5 μl × 5) samples and collecting the small octanol peak.

An insect extract for chrysanthemoylation was obtained by grinding 15–25 worker ant heads with toluene in a small tissue grinder. The extract was dried by elution through powdered sodium sulphate (0.5 g) in a pasteur pipette. This extract was used directly for the preparation of the (+)-*trans*-chrysanthemate ester.

Synthesis of the derivatives

N-TFA-(S)-alanyl 3-octyl ester. N-TFA-(*S*)-alanyl chloride was prepared by a variation of the method by Souter[19]. (*S*)-Alanine (100 mg) in a dry, glass stoppered flask was cooled in an ice-bath and completely dissolved in 1 ml of trifluoroacetic anhydride by occasional shaking. The excess anhydride was evaporated by a stream of dry nitrogen and 1 ml of freshly distilled thionyl chloride was added to the chilled flask. The excess thionyl chloride was evaporated under dry nitrogen and the residue was dissolved in 500 μl of dichloromethane. To 3-octanol in 50 μl of dichloromethane, 50 μl of N-TFA-(*S*)-alanyl chloride solution was added and the reaction mixture was allowed to stand for 3 days at room temperature before examining the products at 150°C on column A.

N-TFA-(S)-prolyl 3-octyl ester. The N-TFA-(*S*)-prolyl ester of racemic 3-octanol was prepared by a variation of the method of Halpern and Westley[20]. 3-Octanol (10 mg) was placed in a 3-ml Reacti-vial (Pierce and Warriner, Chester, Great Britain), and a solution containing N-TFA-(*S*)-prolyl chloride (0.15 mmol in 1.5 ml of chloroform) and 0.1 ml of pyridine were added. The sealed vial was heated (90°C, 10 min), when cool, hydrochloric acid (1 ml, 1 *M*) was added and shaken. The lower organic layer was separated and dried over sodium sulphate (0.1 g).

(+)-trans-Chrysanthemic 3-octyl ester. The (+)-*trans*-chysanthemic acid had an optical purity of 94% as determined by GC of the (−)-menthyl esters[21] and by polarimetry($[\alpha]_D^{25}$ + 13.31°, 1.5% abs. ethanol; lit.[26] (+)-*trans*-chrysanthemic acid, +14.16°, abs. ethanol). The 3-octanol was chrysanthemoylated by a previously described method[10]. Typically, (+)-*trans*-chrysanthemic acid (2 mg) was treated (60°C,

TABLE I

COMPARISON OF GC PROPERTIES OF 3-(±)-OCTANOL DERIVATIZED WITH DIFFERENT CHIRAL RESOLVING AGENTS ON APOLAR (OV-1) AND POLAR (DEGS), WCOT CAPILLARY COLUMNS

Analyses performed isothermally at 150°C (OV-1) and 160°C (DEGS).

Derivative	*OV-1*						*DEGS*					
	Retention time (min)		α	*R*	*Elution order*		*Retention time (min)*		α	*R*	*Elution order*	
	1st isomer	*2nd isomer*			*1st isomer*	*2nd isomer*	*1st isomer*	*2nd isomer*			*1st isomer*	*2nd isomer*
N-TFA(S)-(+)-alanyl ester	2.7	2.8	1.03	1.2	*R*	*S*	3.1	3.2	1.03	1.2	*R*	*S*
N-TFA-(S)-(−)-prolyl ester	8.6	8.9	1.04	1.8	*R*	*S*	11.4	11.7	1.03	1.2	*R*	*S*
(+)-*trans*-Chrysanthemate ester	8.3	8.6	1.03	1.9	*S*	*R*	2.4	2.5	1.04	1.1	*S*	*R*

1 h) with freshly distilled thionyl chloride (200 μl, BDH). Excess thionyl chloride was removed in a stream of dry nitrogen. The alcohol in toluene (sodium dried) was treated (40°C, 2 h) with chrysanthemoyl chloride (three molar proportions) in toluene. The ester was purified by thin-layer chromatography (TLC) (silica gel G, using diethyl ether–hexane, 96:4, R_F same as methyl palmitate).

RESULTS AND DISCUSSION

Preparation of derivatives

Three diastereomeric derivatives of 3-(±)-octanol have been prepared, namely: the N-TFA-(*S*)-(+)-alanyl ester, the N-TFA-(*S*)-(—)-prolyl ester and the (+)-*trans*-chrysanthemoyl ester. Of these the N-TFA-(*S*)-(—)-prolyl ester was the most conveniently prepared as it is readily formed from highly optically pure, commercially available N-TFA-(*S*)-(−)-prolyl chloride. The long time required (3 days at room temperature) for the formation of the N-TFA-(*S*)-(+)-alanyl ester lessens its usefulness and a higher temperature resulted in partial racemization. The other two derivatives form much more readily; the N-TFA-(*S*)-(−)-prolyl ester was produced in sufficient amounts for analysis in 10 min at 90°C and the (+)-*trans*-chrysanthemoyl ester in 2 h at 40°C.

Gas chromatography

Gas chromatography of all three derivatives of 3-(±)-octanol at 150°C on column A or at 160°C on column B resulted in baseline separation of diastereomeric pairs into slightly asymmetrical doublets with separation factors, α, of 1.03 or more (Table I).

Gas chromatography–mass spectrometry

The electron impact (EI) mass spectra of neither the N-TFA-alanyl nor prolyl esters of 3-octanol gave observable molecular ions. However the EI mass spectrum of 3-octyl chrysanthemate exhibited a molecular ion (*m/z* 280, 1%) as noted previously for 2-octyl chrysanthemate[10]. The base peak (*m/z* 123) was attributed to the norchrysanthemyl[10] ion, $C_9H_{15}^+$.

The presence of a molecular ion in the chrysanthemate spectrum is particularly advantageous in the analysis of enantiomers present in complex mixtures where assignment of diastereomers from their retention indices on chromatography is sometimes difficult.

Configuration of 3-octanol in Myrmica ants

(+)-*trans*-Chrysanthemic acid was decided as the most useful derivatizing reagent to study the naturally occuring 3-octanol, since the derivative esters could then be conveniently monitored by mass fragmentography of key ions in the mass spectrum.

GC of the single peak obtained for 3-(*S*)-octyl chrysanthemate [prepared from 3-(*S*)-octanol from oil of *Menthae japonicae*[22,23]] on OV-1 and DEGS columns with the doublet obtained for the racemate, and GC and GC–MS of the ester on OV-1 (Fig. 1), established the elution order as *S* followed by *R* on both phases, consistent with the elution order of the chrysanthemates of 2-octanol on SE-30 stationary

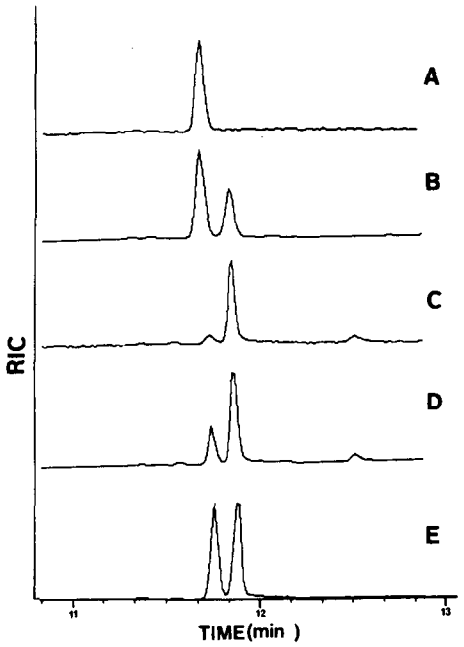

Fig. 1. Reconstructed ion chromatograms (RICs) of the (+)-*trans*-chrysanthemate esters of 3-octanol from: A, oil of *Menthae japonicae*; B, oil of *Menthae japonicae* with commercial racemate; C, *Myrmica* ants; D, *Myrmica* ants with commercial racemate and E, commercial racemic mixture. Analysis performed on a 20 m × 0.3 mm I.D. fused-silica OV-1 capillary column.

phase[10]. The 3-octanol from Japanese peppermint oil was 100% *S* enantiomer only (Fig. 1, trace A), this also shows that no racemization occurs during derivative preparation.

Co-chromatography of the 3-octanol (as its chrysanthemate ester) isolated from the *Myrmica* ants with the racemate revealed that the ant alcohol was essentially the later-eluting *R* enantiomer. Fig. 1 shows reconstructed ion chromatograms of octyl chrysanthemates. The mass spectra of the chrysanthemate esters of 3-octanol from all three sources were identical {*m/z* 280 (M^+, 1%), *m/z* 168 ($C_{10}H_{16}O_2^+$, 5%), *m/z* 151 ($C_{10}H_{15}O^+$, 13%), *m/z* 123 ($C_9H_{15}^+$, 100%), *m/z* 107 (9%), *m/z* 93 (7%), *m/z* 81 (32%), *m/z* 71 (40%), *m/z* 69 (24%), *m/z* 57 (63%), *m/z* 55 (26%)].

To the best of our knowledge this is the first report of 3-(*R*)-octanol from a biological source. Silverstein[2] has reviewed the chiral insect pheromones and in those examples so far reported, nearly always only one enantiomer or a specific blend of enantiomers occurs naturally, rarely the racemic mixture. The mandibular alarm pheromones of *Atta texana* and *A. cephalotes* is (*S*)-4-methyl-3-heptanone only[1]. Pasteels *et al.*[12] found the absolute configuration of a pheromone from the head of another *Myrmicinae* ant *Tetramorium impurum* to be (3*R*,4*S*)-4-methyl-3-hexanol. Though we must avoid a generalization on the two fragments of information available, it will be interesting now to see if the C-3 chiral center of the many mandibular gland pheromonal alcohols of *Myrmicinae* ants listed by Blum and Hermann[24] are all of the *R* configuration. The 4*S* configuration of the *Atta* heptanone and the *Tetramorium* hexanol (above) is also found in the 4-methyl-3-heptanols which are

found in the aggregation pheromones of bark beetles[25]. Further experiments are in progress to determine the specific biological activity of (*R*)-(−)-3-octanol.

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