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ANALYTICAL AND PREPARATIVE SEPARATION OF BARK BEETLE PHEROMONES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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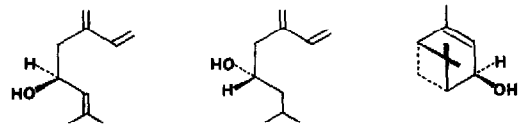
SUMMARY

The analytical scale optical resolution of synthetic racemic ipsdienol, an aggregation pheromone of bark beetles, *Ips* species, with an optically active column is described. Both forms of (±)-ipsdienol benzoate are completely separated by an opticaly active poly(triphenylmethyl methacrylate) column. The use of a chiral reagent to form diastereomers for a preparative scale separation is also described. Both racemic ipsdienol and ipsenol are converted through the hydroxy groups into their diastereomers by treatment with optically pure (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid. The diastereomeric derivatives are then resolved by normal-phase preparative high-performance liquid chromatography in a recycle mode.

INTRODUCTION

In recent years much research has been aimed at establishing alternative means to traditional methods of pest control. One of the most promising alternatives is the use of naturally occurring organic compounds such as insect pheromones. A problem in the large scale synthesis of pheromones is the control of the enantiomeric purity. The enantiomeric purity is often crucial to the biological activity. In some cases, as little as 1% of the wrong enantiomer inhibits the response to the pheromone[1].

Ipsdienol [(*S*)-(+)-2-methyl-6-methylene-2,7-octadien-4-ol], ipsenol [(*S*)-(−)-2-methyl-6-methylene-7-octen-4-ol] and (*S*)-(+)-*cis*-verbenol are components of the pheromones produced by bark beetles in the genus *Ips* species (Fig. 1)[2]. Racemic ipsdienol and ipsenol did not show high activity in field tests (*I. confusus*)[3], in contrast



(*S*)-(+)-Ipsdienol (*S*)-(-)-Ipsenol (*S*)-(+)-*cis*-Verbenol

Fig. 1. Aggregation pheromone of the bark beetles, *Ips* species.

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to the high activity of the natural products in laboratory assays[4]. The initial identification of these three components as the pheromone of *I. paraconfusus* by Silverstein *et al.*[5] involved only the naturally occurring enantiomers. Sufficient quantities of all six enantiomers in high purity have not been available for field and laboratory testing.

One method to acquire the optically pure bark beetle pheromones is the resolution of synthetic racemic compounds by high-performance liquid chromatography (HPLC). An advantage of this method is that it does not require complex synthetic techniques and both enantiomers are obtained simultaneously.

Two efficient methods currently available for separating pairs of enantiomers by HPLC are their direct separation through the use of a chiral HPLC stationary phase, and their conversion into a diastereomer, through the introduction of an additional asymmetric center, and subsequent separation of the diastereomers by normal HPLC methods. After separation, the added asymmetric center is removed to give the optically active material. We, herein, report the analytical and preparative scale separation of synthetic racemic pheromones of the bark beetles by these two HPLC methods.

EXPERIMENTAL

Apparatus

HPLC was carried out with a Rabbit HP solvent-delivery system (Rainin Instrument, Woburn, MA, U.S.A.) equipped with a DuPont variable-wavelength ultraviolet (UV) spectrophotometer operating at 254 nm. The apparatus used in preparative work was a Model LC-09 high-performance preparative liquid chromatograph (Japan Analytical Industry, Tokyo, Japan). A Jasco Chiralpak OT(+) column (50 cm × 4.6 mm I.D.) (Nihonbunko, Tokyo, Japan) and a Partisil PXS 5/25 (5 μm, 25 cm × 4.6 mm I.D.) (Whatman, Clifton, NJ, U.S.A.) were employed for the analytical scale separation. An Alltech custom HPLC column (50 cm × 10 mm I.D.) packed with Nucleosil silica 50 (5 μm) (Alltech, Deerfield, IL, U.S.A.) was employed for the preparative separation.

Materials

The synthetic racemic ipsdienol and ipsenol were obtained from Chemical Samples (Willoughby, OH, U.S.A.). (*R*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) was purchased from Aldrich (Milwaukee, WI, U.S.A.). All other reagents employed were of reagent grade. The solvents used were of reagent or HPLC grade.

Optical resolution with a chiral column

Ipsdienol benzoate was prepared by the reaction of ipsdienol (1 g) with benzoyl chloride (1 g) in the presence of pyridine (0.5 g) in dichloromethane (10 ml). After 1 h at room temperature, saturated sodium bicarbonate solution was added to the reaction mixture. The organic layer was washed with saturated sodium chloride solution (3 × 10 ml). The organic layer was then dried over sodium sulfate, filtered and evaporated, *in vacuo*. The racemic ipsdienol benzoate was isolated by silica-gel column chromatography. The (±)-ipsdienol benzoate was injected into the optically active column, Chiralpak OT(+), for optical purification.

Analytical and preparative separation of diastereomers

Ipsdienol (72 mg) and ipsenol (72 mg) were converted into their diastereomeric esters by the reaction with MTPA (100 mg) in the presence of dicyclohexylcarbodiimide (DCC) (130 mg) and 4-pyrrolidinopyridine (6 mg) respectively. The reactions were performed in dried dichloromethane (2 ml) at room temperature for 4 h. The reaction mixtures were filtered and evaporated *in vacuo*. The racemic residues were isolated by silica-gel column chromatography. Each of the esters was subjected to analytical or preparative recycle HPLC using a normal-phase silica column.

RESULTS AND DISCUSSION

Our initial effort was the direct resolution of racemic ipsdienol with the optically active column, Chiralpak OT(+). This column gets its chirality from the spiral secondary structure of poly(triphenylmethyl methacrylate) (PTrMA)[6]. Various racemic compounds, particularly those having aromatic groups, have been resolved by HPLC on PTrMA columns[7]. However, a direct attempt to resolve (±)-ipsdienol with this column failed. When ipsdienol was converted into its benzoate and applied to this column, baseline separation of racemic ipsdienol benzoate into its enantiomers was achieved (Fig. 2). The optimum mobile phase was 100% methanol at a flow-rate of 1 ml/min. Unfortunately, the maximum capacity of this column (50 cm × 4.6 mm I.D.) was found to be 60 μg of ipsdienol benzoate. Hence, while a good resolution of ipsdienol enantiomers can be obtained with such a column, it is not practical for preparative work. In addition, we found column deterioration was a problem.

For the preparative scale separation, the formation and subsequent normal-phase HPLC isolation of diastereomeric ipsdienol and ipsenol was attempted. Commercially available (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA), which is used as a chiral reagent to determine absolute stereochemistry by NMR spectroscopy[8], was used to form the additional asymmetric center. Both enan-

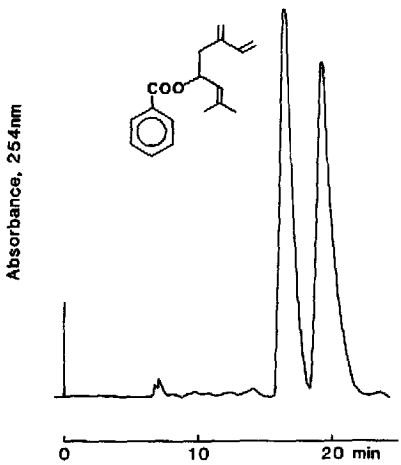

Fig. 2. Separation of ipsdienol benzoate with a Chiralpak OT(+) column. Mobile phase: methanol, flow-rate 1 ml/min.

tiomeric ipsdienol and ipsenol were readily converted into their diastereomeric esters through the hydroxy group by treatment with MTPA in the presence of dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine as a catalyst[9] (Fig. 3). In a previous NMR study, MTPA was shown to react via its acid chloride with chiral alcohols to give a mixture of diastereomeric esters[8]. In the present study, DCC and 4-pyrrolidinopyridine were employed for the direct condensation of MTPA and hydroxy groups. We found that this is not only more convenient than the method involving the acid chloride but also involves milder reaction conditions.

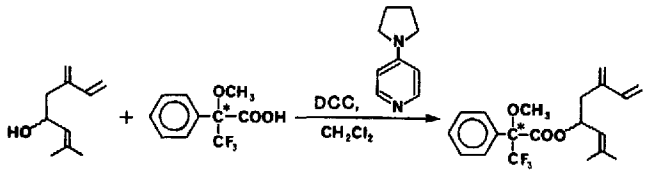

Fig. 3. Reaction of ipsdienol with MTPA.

Several solvent systems were used in attempts to separate the diastereomeric esters of the bark beetle pheromones by normal-phase silica HPLC. The diastereomers could not be separated with an *n*-hexane-based solvent system. However, when an *n*-pentane-based solvent system was used, some separation was achieved. The diastereomers were completely separated by HPLC on an analytical silica column using *n*-pentane–acetone (100:0.1) as mobile phase. A typical chromatogram is shown in Fig. 4. On the basis of the above results, a preparative scale separation was performed using a larger silica column and the diastereomeric derivatives of ipsdienol

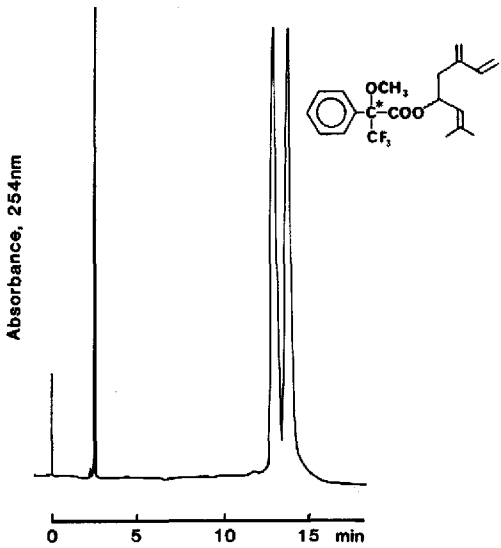

Fig. 4. Analytical separation of ipsdienol MTPA ester. Column: Whatman PXS 5/25 (5 cm × 4.6 mm). Mobile phase: *n*-pentane–acetone (100:0.1), flow-rate 1.7 ml/min.

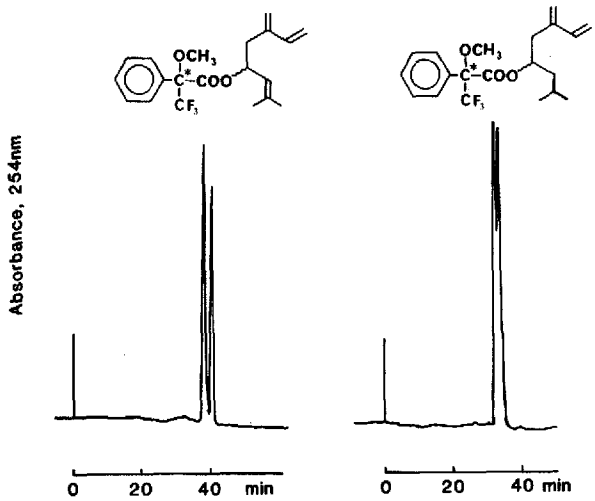

Fig. 5. Preparative HPLC of ipsdienol and ipsenol MTPA esters. Column: Nucleosil silica 50 (50 cm × 10 mm). Mobile phase: *n*-pentane–acetone (100:0.2), flow-rate 3 ml/min.

were again completely separated (Fig. 5). However, even this column's capacity was still insufficient for our purposes. Adequate separation of ipsdienol diastereomers was limited to an injection of 3 mg. In the case of ipsenol the maximum capacity was even less (Fig. 5). Recycling was attempted to improve the system's capacity for these diastereomers.

A much larger quantity of ipsenol MTPA ester (20 mg) was injected into a preparative recycle HPLC system using the same column. The optimum mobile phase was *n*-pentane–acetone (100:0.2) at a flow-rate of 10 ml/min. The recycle chromatogram of diastereomeric ipsenol derivatives is shown in Fig. 6, with the recycling carried out at intervals of 28 min. Complete baseline separation of the diastereomeric ester was obtained in cycle 7.

In the present study, we applied only 20 mg of diastereomeric esters to this HPLC method. It may be possible to perform the separation on a much larger sample volume if the number of recycles is increased. However, we found a 20-mg capacity to be adequate for the quantities required for field assays[10].

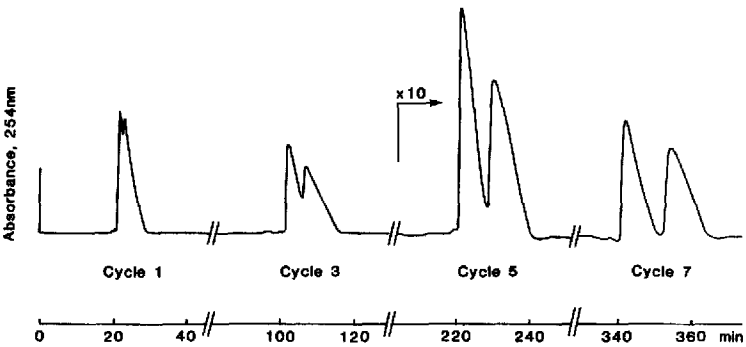

Fig. 6. Recycle preparative HPLC of ipsenol MTPA ester. Column: Nucleosil silica 50 (50 cm × 10 mm). Mobile phase: *n*-pentane–acetone (100:0.2), flow-rate 10 ml/min.

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