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ACTIVITY OF ENANTIOMERS OF SULCATOL ON APTERAE OF *Rhopalosiphum padi*

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Abstract—GC-MS analysis of volatiles released from wheat infested with a high density of aphids showed the presence of 6-methyl-5-hepten-2-ol (sulcatol). The proportion of enantiomers present in the volatiles was determined by esterifying the mixture with (1*S*)-(−)-camphanic chloride and quantifying the esters. The mixture consisted of 75% (*R*)-(−) and 25% (*S*)-(+). The mixture of enantiomers as well as the racemate showed significant repellency towards apterous *Rhopalosiphum padi* in an olfactometer (15.7% and 14.4%, respectively, with 10 ng of stimulus). Single enantiomers or a mixture containing 25% (*R*)-(−)- and 75% (*S*)-(+)-enantiomers were inactive. The results are discussed in relation to the achievement of specificity by aphids in different pheromone-mediated behaviors.

Key Words—Sulcatol, aphid, spacing pheromone, enantiomeric synergism.

INTRODUCTION

Many intraspecific chemical signals have been involved in processes related to population dynamics of insects such as reproduction, spacing, aggregation, and defense (Silverstein, 1984; Löfstedt, 1991; Pickett et al., 1992). Among the mechanisms developed by insects to attain species specificity through these chemical signals are: differences in chemical structure, constitutional isomerism, stereoisomerism, different ratios in multiple component pheromone complexes, and chiral isomerism (Silverstein, 1984; Mori, 1989; Seybold, 1993). In most cases of chiral isomerism, the naturally occurring enantiomer is much more active than the antipode (Dickens and Mori, 1989; Tengö et al., 1990). Never-

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theless, the antipode may inhibit the response to the naturally occurring enantiomer (Pierce et al., 1987; Camacho et al., 1993). Furthermore, enantiomeric synergism has been shown when both enantiomers are naturally occurring (Borden et al., 1976; Millar et al., 1985; Oehlschlager et al., 1987, 1988).

Enantiomers of sulcatol (6-methyl-5-hepten-2-ol), an aggregation pheromone of the ambrosia beetle *Gnathotrichus sulcatus* (Byrne et al., 1974), have been shown to exhibit enantiomeric synergism. Borden et al. (1976) showed that *G. sulcatus* responded to a broad range of mixtures of enantiomers of sulcatol including the racemate, while *Gnathotrichus retesus* (Le Conte) produced only the *S* enantiomer and did not respond to the racemate (Borden et al., 1980). Recently, Quiroz et al. (1997) found that the naturally occurring mixture of 6-methyl-5-hepten-2-one, (−)- and (+)-sulcatol, and 2-tridecanone released from wheat seedlings heavily infested with aphids (9 aphids/cm^2) was repellent to apterous *Rhopalosiphum padi* (Homoptera: Aphididae) in olfactometric assays and produced population deaggregation of aphids feeding on wheat. Furthermore, the ketones in the natural mixture were found individually to produce repellency in the olfactometer (Quiroz et al., 1997). In this paper, we report the use of (1*S*)-(−)-camphanic chloride to resolve a racemic mixture of sulcatol. We also report the effect of single enantiomers and mixtures on apterae of *R. padi.*

METHODS AND MATERIALS

Aphids. Colonies of *R. padi* were started with individuals collected in grass fields near the Facultad de Ciencias, Universidad de Chile, and kept on oat (*Avena sativa* L. cv. Nahuén) in a growth room at 18–22°C and a light regime of 18L:6D.

Olfactometry. We used a common bioassay for aphids (Campbell et al., 1993; Pettersson et al., 1994, 1995) in which an aphid placed in a Perspex olfactometer can be exposed to volatiles coming from containers attached to each of its four side arms (two opposed containers contain the treatment, the other two the control). The observation area is divided into four arm zones and one central indifferent zone. The time the aphid spends in each arm is registered during 15 min. Double solvent and blank control showed no bias in the olfactometer. Pseudoreplication (Hurlbert, 1984) was avoided by changing the treatment and control stimuli and the test aphid and by disassembling and cleaning the olfactometer after each repetition. Each experiment was replicated 10 times, and results were analyzed using Wilcoxon's one-tailed rank-sum test for two groups).

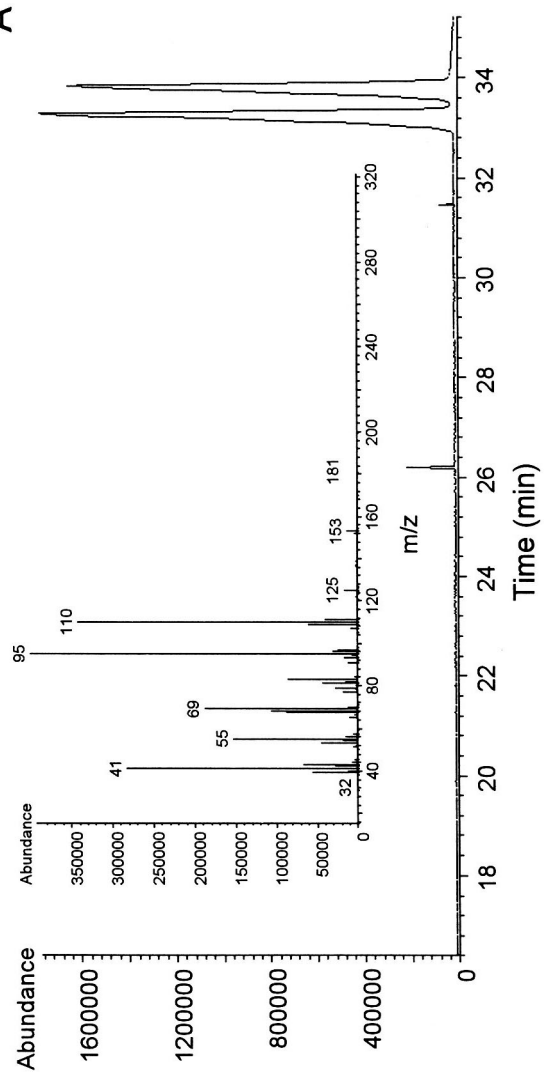
Capture and Analysis of Volatiles. Twenty wheat seedlings in decimal

growth stage 20 (Zadoks et al., 1974) infested with apterae of *R. padi* (ca. 1500) at high density (ca. 9 aphids/cm^2) were placed in bell jars permeated by air dried and purified by passage through activated 5 Å molecular sieves and charcoal. The air was drawn at 1 l/min for 48 hr, and the volatiles captured on Porapak Q (Blight, 1990). The volatiles were desorbed from the Porapak with 2.5 ml of freshly distilled diethyl ether, and the extract was concentrated to 50 μl under a stream of nitrogen. Aliquots (1 μl) of the concentrated extract were used in GC-MS analysis on a capillary α-DEX 120 Supelco GLC column (25 m × 0.2 mm ID) directly coupled to a mass detector and an integrated data system (GC model HP-5890, MD model HP-5972). Ionization was by electron impact at 70 eV and 280°C. The GC oven was maintained at 40°C for 6 min, then programmed to increase at 15°C/min to 150°C, then 10°C/min to 200°C, and finally 0.1°C/min to 220°C.

Preparation of Diasteroisomeric Esters of (±)-Sulcatol. To 3 ml of dry pyridine containing 2 g (9.2 mmol) of (±)-sulcatol (Aldrich Chem. Co.) was added 0.7 ml (4.6 mmol) of (1*S*)-(−)-camphanic chloride (Aldrich Chem. Co.). The mixture was stirred at room temperature. After 1 hr, it was cooled in ice-water, and water (50 μl) was added. TLC (ether–light petroleum 1 : 1) showed two products (R_f = 0.65 and 0.80). The mixture was extracted with diethyl ether (8 ml) and CH_2Cl_2 (4 ml), and the organic phase washed successively with saturated aqueous KCl, 1 N HCl, saturated aqueous KCl, and saturated aqueous $NaHCO_3$ (16 ml each). The organic phase was dried with $MgSO_4$. Evaporation of the solvents gave a yellow syrup (1.3 g) that was purified by preparative liquid adsorption chromatography with accelerating gradients (Baeckström, 1996) using Silicagel 60 and mixtures of diethyl ether and light petroleum. The residue obtained was 1.23 g (87%).

The mixture was separated on a Waters Nova-Pak HPLC column (30 cm × 7.8 mm ID) using hexane–2-propanol (500 : 1) at 3 ml/min. The separation was controlled at 203 nm and yielded 90 mg of (−)-1,5-dimethyl-4-hexenyl-(*S*)-(−)-camphanate and 120 mg of (+)-1,5-dimethyl-4-hexenyl-(*S*)-(−)-camphanate from 250 mg of the ester mixture. The esters were identified by: (1) different retention times in the gas chromatograph and identical mass spectra (Figure 1); (2) generation of the respective enantiomeric alcohols by hydrolysis; and (3) pattern of mass fragmentation.

Preparation of (S)-(+)- and (R)-(−)-Sulcatols. To 10 ml methanol containing 120 mg NaOH was added 120 mg of (+)-1,5-dimethyl-4-hexenyl-(*S*)-(−)-camphanate. The mixture was heated under reflux for 1 hr. TLC (diethyl ether–light petroleum 1 : 1) showed complete conversion of the starting material to a single product. Subsequently, water (10 ml) was added and the solution extracted three times with chloroform. The organic phases were dried with $MgSO_4$. Evaporation of the solvent gave 65 mg of (*S*)-(+)-sulcatol (92% yield)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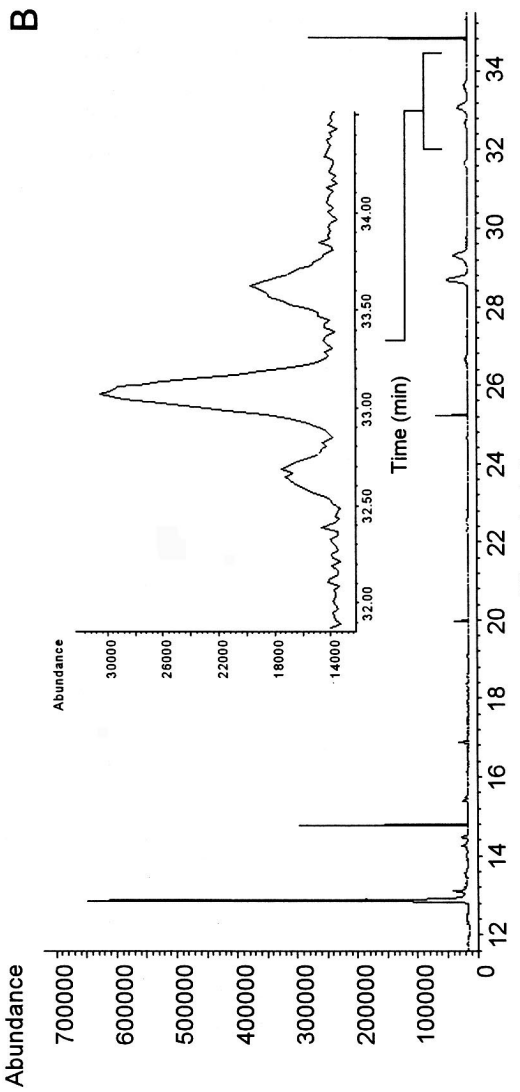

FIG. 1. Total ion current of: (A) (−)- and (+)-1,5-dimethyl-4-hexenyl-(*S*)-(−)-camphanates, including a mass spectrum of the esters, and (B) extract of volatiles released from wheat seedlings with aphids in high density (9 aphids/cm^2) esterified with (1*S*)-(−)-camphanic chloride, including an enlarged area that shows the ratio between diasteroisomeric esters.

with $[\alpha]_J^{20} = +15.3$ (578 nm, c = 0.013, MeOH). Similar treatment of 90 mg of (−)-1,5-dimethyl-4-hexenyl-(*S*)-(−)-camphanate gave 50 mg of (*R*)-(−)-sulcatol (94% yield) with $[\alpha]_J^{20} = -14.1$ (578 nm, c = 0.01, MeOH).

Determination of Enantiomeric Composition of Sulcatol Present in Entrained Mixture of Volatiles. To 500 μl of hexane containing 250 ng of volatile compounds released from wheat infested with a high density of aphids was added 500 μl of hexane containing 100 ng of (1*S*)-(−)-camphanic chloride. The mixture was shaken at room temperature for 3 hr, after which GC-MS did not show the presence of sulcatol. The esters were quantified by GC from calibration curves made with the pure esters. As a control, the reaction of each enantiomer (0.78 mmol) with (1*S*)-(−)-camphanic chloride (0.78 mmol) and 4-dimethylaminopyridine (1 mmol) gave only the respective esters, thus ensuring that the microesterification of the extract would not give collateral products due to acid release.

RESULTS

The enantiomeric mixture of sulcatol was determined as 75% (*R*)-(−)- and 25% (*S*)-(+)-sulcatol (Figure 1). In olfactometric bioassays single enantiomers or a 25% (*R*)-(−)/75% (*S*)-(+)-sulcatol mixture did not show activity towards apterous *R. padi*, while the naturally occurring ratio of (*R*)-(−)- and (*S*)-(+)-enantiomers (75:25) generated a repellency comparable to that obtained when the stimulus was the racemic mixture (Table 1).

TABLE 1. OLFACTOMETRIC RESPONSES OF ONE APTEROUS *R. padi* TO DIFFERENT MIXTURES OF SULCATOL (6-METHYL-5-HEPTEN-1OL) ENANTIOMERS

Stimulus applied	Average time spent in each arm (min)[a]	P[b]
10 ng of (*R*)-(−)-sulcatol	3.45 ± 0.86	0.99
Hexane	3.47 ± 0.59	
10 ng of (*R*)-(−)- and (*S*)-sulcatol (75:25)	2.95 ± 0.45	0.04
Hexane	4.05 ± 0.57	
10 ng of synthetic racemic mixture of sulcatol	2.85 ± 0.35	0.03
Hexane	3.81 ± 0.45	
10 ng of (*R*)-(−)- and (*S*)-(+)-sulcatol (25:75)	2.95 ± 0.78	0.78
Hexane	3.15 ± 0.95	
10 ng of (*S*)-(+)-sulcatol	3.37 ± 0.59	0.80
Hexane	3.27 ± 0.94	

[a] Average ± standard error.
[b] Wilcoxon one tailed rank-sum test for two groups.

DISCUSSION

The synthesis of each enantiomer of sulcatol and the resolution of the racemic mixture were reported earlier. The (*S*)-(+) enantiomer was prepared from L-fucose (Schuler and Slessor, 1977), (*S*)-(−)-lactate (Johnston and Slessor, 1979), (*S*)-(+)-ethyl-3-hydroxybutyrate (Mori, 1981), and by reduction of the respective ketone with biological systems such as bakers's yeast, the anaerobic bacterium, *Clostridium tyrobutyricum*, and the thermophilic anaerobic bacterium, *Thermoanaerobium brockii* (Belan et al., 1987). The (*R*)-(−) enantiomer was prepared chemically from 2-deoxy-D-ribose (Schuler and Slessor, 1977) and biologically by enzymatic reduction of the respective ketone (Belan et al., 1987). Resolution of racemic sulcatol was performed by fractional crystallization of the brucine salt of the phthalic hemiester (Plummer et al., 1976) and by enzymatic resolution (Belan et al., 1987).

In this work, an alternative strategy was employed for obtaining single enantiomers of the alcohol. The racemic mixture was treated with a pure chiral reagent (1*S*)-(−)-camphanic chloride. Subsequently, the esters were separated by preparative HPLC, and finally the enantiomers were generated by basic hydrolysis. Optical rotation values obtained for (*S*)-(+)-sulcatol($[\alpha]_J^{20} = +15.3$) and for (*R*)-(−)-sulcatol ($[\alpha]_J^{20} = -14.1$) compared well with those previously reported by Belan et al. (1987). This classical approach yielded enantiomers with a high optical purity (>99% ee) in a short time (6 hr). Hence, the method proved suitable for the enantiomeric resolution of this secondary alcohol.

The methodology originally designed for the determination of the enantiomeric ratio of sulcatol in our extract of volatiles was based on: (1) synthesis of the enantiomers, (2) enantiomeric analytical separation using a chiral capillary column (α-DEX 120, Supelco), and (3) gas chromatographic analysis of the volatiles using the column mentioned above. However, despite variation of GC parameters such as gas flow, temperature program, and pressure, the column did not separate adequately the racemic mixture of sulcatol. Therefore, the naturally occurring ratio of sulcatol enantiomers was determined in an indirect way. The extract of volatiles, obtained from the system composed of wheat seedling and aphids in high density, was esterified with (1*S*)-(−)-camphanic chloride. Peaks in the GC trace corresponding to the camphanates (same retention time and mass spectra) were quantified. The naturally occurring ratio of enantiomers of sulcatol was determined to be 75(−):25(+).

The olfactometric avoidance response of apterous *R. padi* to racemic sulcatol and to the naturally occurring 75:25 mixture of (*R*)-(−) and (*S*)-(+)-sulcatol [a dose–response curve for this mixture was reported previously by Quiroz et al. (1997)], together with the lack of response to single enantiomers, suggests a synergism between the sulcatol enantiomers. Similar results were described by Borden et al. (1976), who found that *G. sulcatus* only responded to the aggregation pheromone sulcatol when both enantiomers were present and

that the aggregation activity shown by the racemic mixture was greater than the naturally occurring mixture consisting of (35 : 65) of (*R*)-(−)- and (*S*)-(+)-enantiomers.

Although the origin (biological source) of the substance in our system is still unknown, the fact that sulcatol has been reported as a population aggregation pheromone of the beetle *G. sulcatus* (Byrne et al., 1974) and the avoidance behavior shown by apterous *R. padi* towards the naturally occurring mixture of sulcatol suggest that this aphid might use this specific ratio of sulcatol enantiomers as a spacing pheromone.

Earlier reports have shown the low variability of chemical structures used by aphids as pheromones. For instance, the alarm pheromone for several species of aphids has been shown to be (*E*)-β-farnesene (Bowers et al., 1972; Edwards et al., 1973), and the sex pheromone in many species has been shown to comprise the compounds (+)-(4a*S*,7*S*,7a*R*)-nepetalactone and (−)-(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (Dawson et al., 1990; Pickett et al., 1992), species specificity being related to the production of these components in different ratios (Guldemond et al., 1993; Thieme and Dixon, 1996). In that sense, synergism between enantiomers of sulcatol could be a means of achieving species specificity in the spacing behavior of aphids. Studies of the spacing behavior of other aphid species and the compounds responsible for it are underway.

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