Chiral-Phase Capillary Gas Chromatography and Mosquito Repellent Activity of Some Oxazolidine Derivatives of (+)- and (−)-Citronellol

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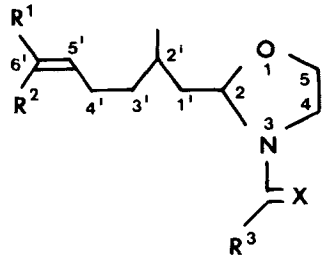
Abstract □ Fourteen stereoisomeric mixtures of six oxazolidine heterocycles and one amino alcohol were obtained from commercial samples of (+)-, (−)-, or (±)-citronellol (**2**) and evaluated in a cloth test system as mosquito repellents. A technical sample of 3-acetyl-2-(2′,6′-dimethyl-5′-heptenyl)oxazolidine (**1**), evaluated initially as a mixture of two pairs of enantiomers, was an effective repellent against two of the three species of mosquitoes. To study repellency–stereochemistry relationships, **1** was isolated as fractions G-1, G-2, I-1, and I-2, corresponding to representative samples of the four possible stereoisomers. The enantiomeric composition of **1**, but not of the other derivatives, could be determined by chiral-phase capillary gas chromatography with Chirasil-Val. Two chiral phases showed that experimental samples of **1**, and hence the commercial samples of **2**, were composed of not more than 90% of one enantiomer. Differences in repellency of isomerically enriched samples were generally small. Fraction G, a mixture of diastereoisomers readily prepared from (+)-**2**, was considerably more effective than standard insect repellents in bioassays with three mosquito species.

(±)-3-Acetyl-2-(2′,6′-dimethyl-5′-heptenyl)oxazolidine (**1**), a chiral terpenoid with insect repellent properties,[1,2] is a registered insect repellent in Canada (Citronyl; S. C. Johnson and Son, Racine, WI). Two pairs of enantiomers of **1** are possible but, to our knowledge, these enantiomers have never been individually tested as insect repellents. Starting with commercial samples of citronellol (**2**), the chiral precursor to **1**, the purpose of this study was to isolate both pairs of enantiomers and to evaluate some of the samples as mosquito repellents using an in vivo cloth test system.[3] Stereoisomeric mixtures of six related compounds (**3**–**8**) were also synthesized, isolated, and tested.

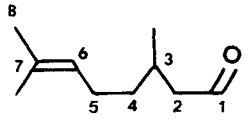
In order to detect the enantiomers of these compounds, we have investigated the technique of chiral-phase capillary gas chromatography (GC), using chiral liquid phases of *N*-*tert*-butyl-*S*-valinamide:polysiloxane (Chirasil-Val)[4] and XE-60-*S*-valine-*S*-α-phenylethylamide (XE-60-*S*-val-*S*-α-pea).[5] These phases are chiral siloxanes of the diamide type.[6]

Capillary GC with achiral liquid phases has previously been utilized to separate **1** into a pair of diastereoisomers,[7] which were designated in subsequent papers[8,9] as **1a** and **1b**. These diastereoisomers, prepared from **2** labeled as the (3*R*)-(+)-enantiomer, were isolated by HPLC with silica gel and characterized.[9] Because the absolute stereochemistry at C-2 of the oxazolidine ring is unknown, **1a** and **1b** cannot be differentiated by the *R*/*S* nomenclature and these diastereoisomers are designated here as (+)-**1a** and (+)-**1b**.[10] The other pair of diastereoisomers, (−)-**1a** and (−)-**1b**,[11] are enantiomers of the former compounds and are accessible from (−)-**2**.

Before this work was initiated, it was uncertain whether commercially available samples of (+)- and (−)-**2** were enantiomerically pure. Optical rotations stated on the labels did not clarify this point because of the inherent variability in polarimetric measurements. Valentine et al.[12] studied the enantiomeric composition of derivatives of citronellic acid by NMR and HPLC. They concluded that **2** from natural sources (Java citronella oil) was ~90% (+)-enantiomer. However, commercial samples of (3*S*)-(−)-**2** were not included for comparison.



1: $R^1 = R^2 = R^3 = CH_3$; X = O
3: $R^1 = R^2 = Cl$; $R^3 = CH_3$; X = O
4: $R^1 = R^2 = CH_3$; $R^3 = CF_3$; X = O
5: $R^1 = R^2 = R^3 = CH_3$; X = CH_2
7: $R^1 = CO_2CH_3$; $R^2 = R^3 = CH_3$; X = O
8: $R^1 = CH_2OH$; $R^2 = R^3 = CH_3$; X = O



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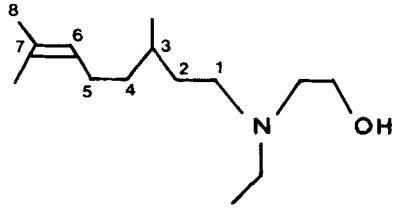

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Experimental Section

Boiling points were recorded during short-path distillations under reduced pressure and are uncorrected. Elemental analyses were performed by Dr. C. Daessle, Montreal, Quebec. IR spectra were taken as liquid films with a Perkin-Elmer 137 instrument. ^{1}H NMR spectra (60 MHz) were obtained with a Varian EM360 spectrometer using tetramethylsilane as internal standard. GC was performed with a Hewlett-Packard 5838A reporting chromatograph equipped with a flame-ionization detector and a Hewlett-Packard 18835B capillary inlet system. Chemical-ionization mass spectra were recorded on a Hewlett-Packard 5985B GC–MS instrument with isobutane as the reagent gas at a source pressure of 0.5 mm Hg. HPLC was performed with a Waters 500A preparative chromatograph equipped with a refractive index detector and Prep PAK silica cartridges (Waters Associates, Milford, MA).

Materials—Three samples of (+)-**2** [sources: A (lot 196085 979, Fluka Chemical Corp., Hauppauge, NY), B (lot A9X, Eastman Kodak Co., Rochester, NY), and C (lot B8C, Eastman)] and two samples of (−)-**2** [sources: D (lot 09297 ME, Aldrich Chemical Co., Milwaukee, WI) and E (lot 3507 DH, Aldrich)] were purchased. A third sample of (−)-**2** (source F) was synthesized[13] from (−)-citronellol (Pfaltz and Bauer, Stamford, CT) using pyridinium chlorochromate and sodium acetate. A technical sample of **1**, prepared from (±)-**2**, was obtained as a gift (S. C. Johnson and Son) and was redistilled (bp 125–129°C at 0.1 mm Hg) before use. Citronellol ethylene acetal (**9**) was prepared[14] using **2** from sources B and E. The isopropyl urethane derivative of (±)-citronellol (lot AB 011177, Aldrich) was prepared with isopropyl isocyanate using the procedure of König et al.[15]

Gas Chromatographic Columns and Conditions—Wall-coated fused-silica capillary GC columns of Chirasil-Val (30 m × 0.25 mm i.d.; Applied Science, State College, PA), XE-60-*S*-val-*S*-α-pea (50 m × 0.22 mm i.d.; Chrompack, Middelburg, The Netherlands), and FFAP (25 m × 0.31 mm i.d.; Quadrex Corp., New Haven, CT) were purchased. A borosilicate glass capillary column of Carbowax 20M-TPA (22 m × 0.33 mm i.d.) was prepared by the method of Grob and Grob.[16] Helium was the carrier gas, at a linear flow velocity of 22–30 cm/s (measured at 35°C). Injector and detector temperatures were 225°C and 250°C, respectively. Samples were dissolved in hexane and injected by the split technique. For calculation of product ratios, peak heights were measured on chromatograms from the chiral columns (results expressed as the mean values from three injections), and peak areas were electronically integrated on chromatograms from FFAP and Carbowax 20M-TPA (single analyses).

Isolation of (±)-3-Acetyl-2-(2′,6′-dimethyl)-5′-heptenyl)oxazolidine (1)—Samples of **1** were prepared by a literature procedure,[9] using **2** (1.0 mol equivalent, sources A, C, D, and F), ethanolamine (2.2 mol equivalents), and acetic anhydride (2.2 mol equivalents). Thus, **1** from source A (30.85 g) gave, after distillation at 116–118°C (0.1 mm Hg), fraction G (39.3 g). GC on fraction G with Chirasil-Val at 140°C (XE-60-*S*-val-*S*-α-pea programmed from 150°C to 170°C at 0.5°C/min) showed 4.8% (5.0%) of (−)-**1a**, 43.1% (45.1%) of (+)-**1a**, and 52.1% (49.9%) of (+)-**1b** plus (−)-**1b**. Compound **1** from source C (3.08 g) gave fraction H as a faint yellow oil (4.27 g). GC (as described for fraction G) showed 4.8% (5.0%) of (−)-**1a**, 43.9% (45.3%) of (+)-**1a**, and 51.3% (49.7%) of (+)-**1b** plus (−)-**1b**. Compound **1** from source D (24.8 g) gave, after distillation at 115–124°C (0.08 mm Hg), fraction I (34.1 g). GC (as described for fraction G) showed 41.9% (40.8%) of (−)-**1a**, 8.7% (10.4%) of (+)-**1a**, and 49.4% (48.8%) of (+)-**1b** plus (−)-**1b**. Source F (3.08 g) gave 4.37 g of **1**, designated as fraction J. GC (as described for fraction G) showed 42.2% (40.7%) of (−)-**1a**, 12.9% (11.9%) of (+)-**1a**, and 44.9% (47.4%) of (+)-**1b** plus (−)-**1b**.

Liquid Chromatography on Fraction G—Using a solvent system of ethyl acetate:methylene chloride (1:1, v/v) and the shave–recycle technique,[9] fraction G (2 g) gave 716 mg of the first HPLC isolate, which was distilled (bp 104–108°C at 0.04 mm Hg) to give fraction G-1 (445 mg). GC (as described for fraction G) showed 8.2% (8.7%) of (−)-**1a**, 83.9% (82.1%) of (+)-**1a**, and 7.9% (9.2%) of (+)-**1b** plus (−)-**1b**. The second component was isolated (3.29 g) by subjecting fraction G to three HPLC runs (2 g each). Distillation (bp 105–106°C at 0.05 mm Hg) gave fraction G-2 (1.72 g). GC (as described for fraction G) showed 0.7% (0.9%) of (−)-**1a** plus (+)-**1a** and 99.3% (99.1%) of (+)-**1b** plus (−)-**1b**.

Liquid Chromatography on Fraction I—Samples of fraction I (6 g total) gave 1.64 g of the first component, after three separations by HPLC. Distillation (bp 110–112°C at 0.05 mm Hg) gave 556 mg of fraction I-1. GC (as described for fraction G) showed 83% (79.9%) of (−)-**1a**, 15.2% (17.0%) of (+)-**1a**, and 1.8% (3.1%) of (+)-**1b** plus (−)-**1b**. The combined fractions of the second HPLC isolate (3.04 g) were distilled (bp 114–117°C at 0.05 mm Hg) to give 1.12 g of fraction I-2. GC (as described for fraction G) showed 1.1% (1.1%) of (−)-**1a** plus (+)-**1a** and 98.9% (98.9%) of (+)-**1b** plus (−)-**1b**.

2-(6′,6′-Dichloro-2′-methyl-5′-hexenyl)-1,3-dioxolane (11)—The starting material, 5-(1,3-dioxolan-2-yl)-4-methyl-1-pentanal (**10**), was prepared from **9** as previously described,[14] using (+)-**2** from source B. To generate (dichloromethylene)triphenylphosphorane[17,18] for use in the Wittig reaction, a mixture of triphenylphosphine (131.1 g, 50 mmol), bromotrichloromethane (13.2 g, 67 mmol), and dry tetrahydrofuran (50 mL) was mechanically stirred in a 250-mL flask for 90 min at 5°C (argon atmosphere). Aldehyde **10** (4.3 g, 25 mmol) was added, and the stirring was continued at 5°C for 3 h then at 20°C for 19 h. Filtration and evaporation gave 4.77 g of a yellow oil. GC and GC–MS analyses with a Carbowax 20M-TPA capillary column showed a mixture of components, with **11** predominating; MS: *m/z* 239 (MH^+, 2.5%), 237 (2), 205 (34), 203 (100).

3-Acetyl-2-(6′,6′-dichloro-2′-methyl-5′-hexenyl) oxazolidine (3)—The aforementioned sample of **11** (4.7 g, 20 mmol) was stirred with 5 M HCl (20 mL) and tetrahydrofuran (100 mL), initially at 5°C for 1 h, then at 20–22°C for 3 d. The mixture was neutralized with solid $NaHCO_3$ and suspended in water. Extraction with methylene chloride gave 4.08 g of a yellow oil. GC–MS analysis showed mostly the intermediate aldehyde **12**. Condensation of this sample (21 mmol) with ethanolamine (2.8 g, 46 mmol) and acetic anhydride (4.7 g, 46 mmol) in benzene[9] gave 3.83 g of a brown oil. Sample cleanup by preparative HPLC with methylene chloride:ethyl acetate (1:1, v/v) gave 2.04 g (35% yield) of the major component. GC and GC–MS analyses with a Carbowax 20M-TPA column showed two diastereoisomers of **3** (46% **3a**; 54% **3b**). Distillation at 130–140°C (0.04 mm Hg) gave 1.28 g of **3**, as a colorless oil. GC analysis with XE-60-*S*-val-*S*-α-pea at 170°C showed components at 73.1, 74.1, and 75.6 min. By analogy to the stereoisomers of **1** detected in fraction G, these components represented (−)-**3a** (4.1%), (+)-**3a** (38.7%), and (+)-**3b** plus (−)-**3b** (57.3%); IR (neat): 1650 cm^{-1} (amide C=O); ^{1}H NMR ($CDCl_3$): δ 5.8 (t, 1, J = 7 Hz, C-5′ H), 5.5–5.2 (m, 1, C-2 H), 4.3–3.3 (m, 4, C-4 H_2 and C-5 H_2), 2.1 (s, 3, $NCOCH_3$), and 2.5–0.6 ppm (overlapping m, 10); MS: *m/z* 280 (MH^+, 100%).

Anal.—Calc. for $C_{12}H_{19}Cl_2NO_2$: C, 51.44; H, 6.83; Cl, 25.31; N, 5.00. Found: C, 51.86; H, 6.62; Cl, 24.98; N, 5.17.

3-Trifluoroacetyl-2-(2′,6′-dimethyl-5′-heptenyl)oxazolidine (4)—This compound was prepared from (+)-**2** (source B, 3.085 g, 20 mmol), ethanolamine (2.69 g, 44 mmol), and trifluoroacetic anhydride (9.24 g, 44 mmol) in methylene

chloride (30 mL). The crude sample (4.6 g) was purified without isomer separation by column chromatography (Silic AR CC-7; Mallinckrodt Chemical Works, St. Louis, MO), eluting first with petroleum ether (bp 30–60°C), then with petroleum ether:10–50% hexane, hexane, and hexane:10–25% ether. The product (815 mg, 14% yield) consisted of a diastereoisomeric mixture of **4a** (45%) and **4b** (55%) by GC (FFAP at 140°C). Distillation gave the same mixture, as a colorless oil, bp 75–85°C (0.1 mm Hg); IR (neat): 1700 cm^{-1} (amide C=O); ^1H NMR ($CDCl_3$): δ 5.6–4.9 (m, 2, C-2 H and C-5′ H), 4.3–3.4 (m, 4, C-4 H_2 and C-5 H_2), 1.7 (s, 3, C-6′ CH_3), 1.6 (s, 3, C-6′ CH_3), and 2.3–0.7 ppm (overlapping m, 10); MS: *m/z* 294 (MH^+, 100%).

Anal.—Calc. for $C_{14}H_{22}F_3NO_2$: C, 57.34; H, 7.56; N, 4.78. Found: C, 57.14; H, 7.90; N, 4.62.

Repetition of this experiment with (−)-**2** (source E) and with benzene as solvent gave another sample of **4**. GC with Chirasil-Val at 130°C gave two main peaks for all samples of **4** at 19.3 and 20.0 min. These corresponded to (+)-**4a** plus (−)-**4a** (48.7%) and (+)-**4b** plus (−)-**4b** (51.3%). Similarly, XE-60-*S*-val-*S*-α-pea at 160°C gave two peaks for **4** at 14.6 min (44.9%) and 15 min (55.1%).

3-Ethyl-2-(2′,6′-dimethyl-5′- heptenyl)oxazolidine (5)—2-(Ethylamino)ethanol (19.61 g, 220 mmol) and (+)-**2** (source C, 15.42 g, 100 mmol) in benzene (500 mL) were heated under reflux for 4 h. Workup in the usual manner followed by distillation gave 15.47 g (69% yield) of **5**, as a faint yellow oil, bp 82°C (0.1 mm Hg); ^1H NMR ($CDCl_3$): δ 5.3–4.9 (m, 1, C-5′ H), 1.7 (s, 3, C-6′ CH_3), 1.6 (s, 3, C-6′ CH_3), and 4.2–0.7 ppm (overlapping m, 20); MS: *m/z* 226 (MH^+, 100%).

Anal.—Calc. for $C_{14}H_{27}NO$: C, 74.61; H, 12.08; N, 6.22. Found: C, 74.71; H, 12.21; N, 6.17.

This experiment was repeated with (−)-**2** (source E). GC analysis gave one main peak for all samples of **5**: FFAP (6.5 min, programmed upward from 130°C at 3°C/min); Carbowax 20 M-TPA (5.2 min, 140°C); Chirasil-Val (8 min, 140°C); XE-60-*S*-val-*S*-α-pea (7.4 min, 165°C).

***N*-(3,7-Dimethyl-6-octenyl)-*N*-ethyl-*N*- (2-hydroxy - ethyl)amine (6)**—A suspension of technical-grade **1** (14.35 g, 60 mmol), $LiAlH_4$ (3.42 g, 90 mmol), and dry ether (240 mL) was heated under reflux for 5 h. Workup in the usual manner followed by column chromatography (Silic AR CC-7) with hexane and hexane:1–80% ether gave 8.17 g (60% yield) of **6** as a faint yellow oil. Distillation gave 6.9 g of **6**: GC retention time (FFAP, 180°C) was 4 min; bp 98–99°C (0.1 mm Hg); ^1H NMR ($CDCl_3$): δ 5.3–4.9 (m, 1, C-6 H), 3.6 (t, 2, J = 5 Hz, CH_2O), 1.7 (s, 3, C-7 CH_3), 1.6 (s, 3, C-7 CH_3), and 3.1–0.7 ppm (overlapping m, 20); MS: *m/z* 228 (MH^+, 100%).

Anal.—Calc. for $C_{14}H_{29}NO$: C, 73.95; H, 12.85; N, 6.16. Found: C, 74.15; H, 12.83; N, 6.29.

GC with Chirasil-Val at 180°C showed one broad peak for **6** at 8.0 min. Repetition of this experiment without excess $LiAlH_4$ (0.25 mol equivalent) gave mostly starting material (31% **1a**; 36% **1b**) plus 11% of **6**, 2% of **2**, and <1% of **5** as determined by GC with FFAP.

Mosquito Bioassays—Repellent activity was determined by the method of Schreck et al.[19] Fifty milligrams of the test compound was placed in a 7.4-mL (2-dram) vial to which 0.75 mL of acetone was added. When the chemical was thoroughly dissolved, a 50-cm^2 (5 × 10-cm) piece of muslin bandage was rolled, placed in the vial to absorb the solution, and the vial sealed until the chemicals were to be tested. The rate of cloth treatment was 1.0 mg/cm^2. After treatment, the cloths were kept in the vials in a refrigerator for at least 24 h.

At the start of a test, the vials were removed from the refrigerator and allowed to warm to room temperature. The cloth was then removed from the vial and stapled over a 4 × 9 cm rectangular opening which had been cut in a 12.7 × 20.3 cm (5 × 8 in.) file card. The cloth was allowed to dry for 15 min before testing. The individual testing the candidate repellents covered his arm with a nylon stocking to keep the treated cloth from contacting the skin and wore a rubber glove over his hand and wrist to protect against bites. The card and attached cloth were then taped over the nylon-covered forearm so that only the treated cloth allowed the mosquitoes access to the skin.

The arm was then exposed in a stock cage containing ~1500 mosquitoes for 1 min. Mosquitoes tested were laboratory reared *Aedes aegypti* (L.), *Anopheles albimanus* Wiedemann, and *Anopheles quadrimaculatus* Say.

More than three bites through the treated cloth in 1 min denoted a failure of the chemical. If the chemical did not fail to repel, it was stored at room temperature and retested in 24 h and daily thereafter until failure.

In addition to duration of repellency, the minimum effective dosage (MED) was determined. The method of testing was the same, except that the rate of treatment was reduced by one-half until more than three bites/min were recorded at the lowest dose. The MEDs were determined only on fresh treatments (air-dried for 15 min) and the 1-d-old treatments. Two repellents, *N,N*-diethyl-*m*-toluamide (deet) and dimethyl phthalate, were used as standards in all tests.

Results and Discussion

Samples of **1** prepared from (±)-**2** were expected to possess the four stereoisomers of this insect repellent and in approximately equal amounts. Based on a 45:55 mixture of diastereoisomers[7-9] and a 1:1 ratio of (+)- and (−)-**2**, the stereoisomeric composition should be 22.5% (+)-**1a**, 22.5% (−)-**1a**, 27.5% (+)-**1b**, and 27.5% (−)-**1b**. Therefore, a typical sample (lot 4542 R25, S. C. Johnson and Son) was examined on the chiral-phase capillary columns. The chromatograms (Fig. 1) showed that it was possible to separate both pairs of enantio-

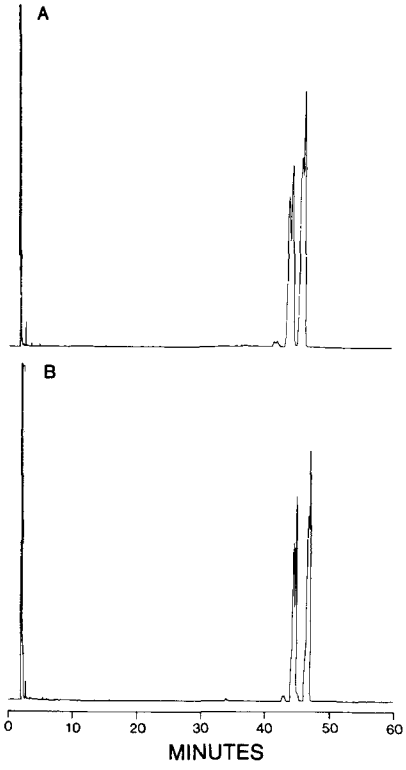


Figure 1—*Chromatograms illustrating the separation of the four stereoisomers of 1 on Chirasil-Val at 140°C (A) and XE-60-S-val-S-α-pea at 160°C (B).*

mers. Subsequent chromatograms obtained on samples of **1** prepared from (+)-**2** and (−)-**2** established the order of elution as (−)-**1a**, (+)-**1a**, (+)-**1b**, and (−)-**1b** on both of these columns. Under a variety of GC conditions, the chromatographic separability factors (α-values)[4,5] for either pair of enantiomers were also similar, ranging from 1.008 to 1.017 for (−)-**1a** and (+)-**1a** and from 1.006 to 1.011 for (+)-**1b** and (−)-**1b**. Peak height measurements from Chirasil-Val indicated a composition of 21.3% (−)-**1a**, 22.7% (+)-**1a**, 25.5% (+)-**1b**, and 30.5% (−)-**1b**. This implied that, if the sample of **2** was exactly a 1:1 mixture of the (+)- and (−)-enantiomers, the error in determining the enantiomeric composition of **2** from the ratio of (−)-**1a** to (+)-**1a** in samples of **1** would not exceed 2%. Utilization of the ratio of (+)-**1b** to (−)-**1b** could lead to errors in excess of 3%.

The column of XE-60-*S*-val-*S*-α-pea gave slightly smaller α-values than Chirasil-Val and showed 20.1% (−)-**1a**, 25.0% (+)-**1a**, 25.0% (+)-**1b**, and 29.8% (−)-**1b** by analysis of the same sample.

Improvements in the resolution of some stereoisomers by coupling an achiral column with Chirasil-Val has been reported.[20] During this investigation, the columns of FFAP and Chirasil-Val were coupled with a capillary column butt connector (Supelco, Inc., Bellefonte, PA). However, this did not improve the α-values for the enantiomers of **1**. In our hands, a longer (50-m) column of Chirasil-Val also failed to increase the α-values when compared to the shorter (30-m) column.

In chromatograms of **1** from (+)-**2** and (−)-**2**, only the first pair of enantiomers, (−)-**1a** and (+)-**1a**, was resolved (Fig. 2). The ratio of these peaks in fractions of **1** prepared from four sources of **2** was determined (Table I). The enantiomeric composition of both samples of (+)-**2** (sources A and C) was 90% (+)-enantiomer. The enantiomeric composition of samples of (−)-**2** was 83% (source D) and 77% (source F) in favor of the (−)-enantiomer. This corresponds to enantiomeric excesses of 80% for (+)-**2** and 66 and 53% for (−)-**2**. From summation of peak heights in chromatograms of **1**, the stereoisomeric composition of fractions G and H was 4.8–5.0% (−)-**1a**, 43.1–45.3% (+)-**1a**, and 49.7–51.3% (+)-**1b** plus (−)-**1b**. The corresponding values for fractions I and J were 40.7–42.2%, 8.7–12.9%, and 44.9–49.4%.

We also tried to separate the (*R*)- and (*S*)-enantiomers of **2**, and of simple volatile derivatives of **2**, by chiral-phase GC using racemic mixtures of **2**, **9**, citronellol, and citronellol isopropyl urethane and the columns of Chirasil-Val and XE-60-*S*-val-*S*-α-pea. Unfortunately, the α-values for these compounds were equal to one. These observations were not entirely surprising because underivatized terpenoids and related chiral compounds are usually not resolved on these columns.[6,21] Isopropyl urethanes of a number of chiral secondary alcohols have been separated on XE-60-*S*-val-*S*-α-pea, giving α-values of 1.011–1.049.[15] In these alcohols, the hydroxyl group was directly attached to the asymmetric center, which contrasts to citronellol, a primary alcohol.

Table I—Ratio of Enantiomers, (+)-1a and (−)-1a, in Fractions Containing 1 by Measurement of Peak Heights from Chiral-Phase GC

Fraction	GC Column	Column Temp.	(+)-1a:(−)-1a
G	Chirasil-Val	140°C	90.0:10.0
	XE-60-*S*-val-*S*-α-pea	160°C	89.9:10.1
H	Chirasil-Val	140°C	90.1:9.9
	XE-60-*S*-val-*S*-α-pea	160°C	90.1:9.9
I	Chirasil-Val	140°C	17.1:82.9
	XE-60-*S*-val-*S*-α-pea	160°C	16.9:83.1
J	Chirasil-Val	140°C	23.4:76.6
	XE-60-*S*-val-*S*-α-pea	160°C	22.6:77.4

The diastereoisomers in fraction G were isolated by achiral-phase HPLC to obtain representative samples of (+)-**1a** (fraction G-1) and (+)-**1b** (fraction G-2) for testing. Similarly, enriched samples of (−)-**1a** (fraction I-1) and (−)-**1b** (fraction I-2) were obtained from fraction I (*Experimental Section*).

Four novel (**3–6**) and two known[8] (**7** and **8**) derivatives of **1** were isolated for testing as mosquito repellents. Compound **3** would be expected to possess enhanced environmental stability since replacement of the isobutenyl group by the dichlorovinyl group is known to improve the photostability of related compounds, such as the synthetic pyrethroid insecticides.[22] This compound was synthesized from the ethylene acetal of **2** enriched in the (+)-enantiomer (Scheme I). The *N*-trifluoroacetyl derivative **4** and *N*-ethyl derivative **5** were prepared from (+)-**2** and (−)-**2** with the idea that volatility, a potentially important variable affecting the duration of topical repellency,[23] might be affected. Since **2** possesses insect repellent properties,[24] **5** can also be considered as a prorepellent because oxazolidines without a stabilizing *N*-acyl group are capable of hydrolysis to the parent carbonyl compound under mild conditions.[25–27] The amino alcohol **6** was isolated from studies on the selective reduction of the amido group in **1**. A variety of reducing agents were investigated.[28–30] These experiments were designed to obtain enantiomerically enriched samples of **5** from enriched samples of **1**. However, complex mixtures of products were isolated.

A summary of the repellency data for the oxazolidines is given in Table II. Tests of the eight compounds were made at different times over a 3-year period. Thus, the data for the two standards, which were repeated each time, were averaged.

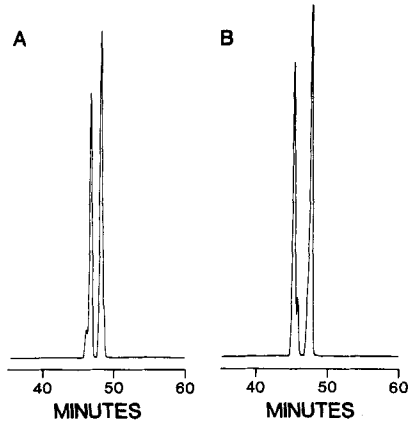
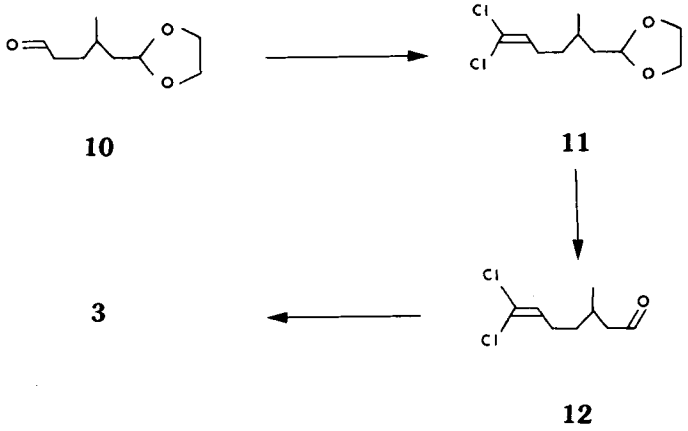


Figure 2—*Chromatograms of samples of 1 prepared from (+)-2 (fraction G) (A) and from (−)-2 (fraction I) (B) on Chirasil-Val (140°C).*



Scheme I

Table II—Effectiveness of Oxazolidines and Related Compounds Against Three Mosquito Species

Compound	Source (Optical Rotation) of **2**	Reference[a]	*Aedes aegypti*			*Anopheles quadrimaculatus*			*Anopheles albimanus*		
			Repellent Activity, d	Minimum Effective Dose, mg/cm²		Repellent Activity, d	Minimum Effective Dose, mg/cm²		Repellent Activity, d	Minimum Effective Dose, mg/cm²	
				15 min	24 h		15 min	24 h		15 min	24 h
1	—[b] (±)		45	0.008	0.063	48	0.016	0.063	0[c]	0.25	>1
	A (+)	fraction G	49	0.008	0.032	55	0.008	0.032	8	0.063	1.0
	A (+)	fraction G-1	56	0.016	0.063	58	0.004	0.063	9	0.125	0.25
	A (+)	fraction G-2	62	0.016	0.063	62	0.008	0.063	0[c]	0.5	>1
	D (−)	fraction I	49	0.008	0.016	55	0.008	0.016	0[d]	0.25	>1
	D (−)	fraction I-1	45	0.016	0.063	31	0.008	0.125	9	0.5	1
	D (−)	fraction I-2	62	0.008	0.032	62	0.008	0.032	0[c]	0.5	>1
2	A (+)		0[c]	0.25	>1	2	0.016	0.25	0[c]	1.0	>1
	E (−)		0[c]	0.25	>1	0[c]	0.125	>1	0	>1	—
3	B (+)		9	0.063	0.25	—[e]	—	—	0[c]	0.5	>1
4	B (+)		0	>1	—	5	0.008	0.5	0	>1	—
	E (−)		0	>1	—	0[c]	0.25	>1	0	>1	—
5	C (+)		3	0.063	0.125	—[e]	—	—	3	0.125	0.25
	E (−)		3	0.125	1	2	0.125	1	0[c]	0.25	>1
6	—[b] (±)		3	0.063	0.125	—[e]	—	—	3	0.063	0.25
7	—[b] (±)	(ref. 8)	0	>1	—	0[c]	0.125	>1	0	>1	—
8	C (+)	(ref. 8)	0	>1	—	0[c]	0.125	>1	0	>1	—
Deet	— —		4	0.018	0.172	4	0.019	0.25	2	0.1	0.5
Dimethylphthalate	— —		2.5	0.043	0.344	5	0.02	0.255	1	0.3	1.0

[a] Unless otherwise noted, the samples are described in the *Experimental Section.* [b] S. C. Johnson and Son, Racine, WI. [c] These compounds demonstrated repellency at 15 min posttreatment but were ineffective at 24 h posttreatment. [d] This compound failed (allowed four bites) at 24 h posttreatment, but provided adequate protection (three bites or less) at 2, 4, and 6 d posttreatment. At 7 d posttreatment, four bites were received and the tests were terminated. [e] Not tested.

Against *Ae. aegypti* and *An. quadrimaculatus*, the technical mixture of **1** was considerably more active than the standards as indicated by the residual effectiveness of 45 and 48 d; the MEDs were also good. Diastereoisomeric mixtures of (+)-**1a** plus (+)-**1b** (fraction G) and (−)-**1a** plus (−)-**1b** (fraction I) were approximately equivalent to **1** against these two species. Fraction G also repelled *An. albimanus* for 8 d. Of the enantiomerically enriched samples, fractions G-2 and I-2 repelled *Ae. aegypti* and *An. quadrimaculatus* for nearly 9 weeks but were ineffective after 15 min against *An. albimanus*. Fractions G-1 and I-1, however, were effective for 1 to 8 weeks against all three mosquito species.

Two of the four possible stereoisomers of the dichloro oxazolidine **3** were tested against *Ae. aegypti* and this mixture of (+)-**3a** and (+)-**3b** had about twice the residual effectiveness of deet but a higher MED. The mixture was only marginally effective in tests with *An. albimanus*.

The four stereoisomers of the trifluoroacetyloxazolidine **4** were evaluated as mixtures containing (+)-**4a** plus (+)-**4b** and (−)-**4a** plus (−)-**4b**. The first diastereoisomeric mixture was about equal to the standards in residual repellency against *An. quadrimaculatus*. A diastereoisomeric mixture of *N*-ethyloxazolidine **5** and a racemic mixture of amino alcohol **6** were about equal to deet against *An. albimanus*. Mixtures of ester **7** and alcohol **8** were practically inactive in all three tests.

Although some of the stereoisomers of **2**–**8** demonstrated repellency on cloth towards one or more of the mosquito species, these compounds were not considered sufficiently promising to warrant additional isolation and testing. This contrasts with **1**, which continues to undergo efficacy trials on human skin.[31,32]

Conclusions

The structural modifications made to **1** decreased its mosquito-repellent activity and strong correlations between repellency and stereochemistry were usually absent. Since only fractions G, G-1, and I-1 were effective in bioassays with *An. albimanus*, it may be advantageous to utilize pure or enriched samples of the stereoisomers of **1** to repel some insects. For economical reasons, fraction G appeared to be the best choice for repelling the three test species. Before practical recommendations on the optimum stereoisomeric composition can be made, additional insect repellent tests under field conditions are needed, using highly purified samples of **1**.

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