

Diastereomers of the Insect Repellent

3-Acetyl-2-(2,6-dimethyl-5-heptenyl)oxazolidine: Evidence from Glass Capillary Gas Chromatography and Mass Spectrometry

Samples of 3-acetyl-2-(2,6-dimethyl-5-heptenyl)oxazolidine (1), a complex new type of insect repellent first described by S. C. Johnson and Son, were separated into a diastereomeric pair (5:6 peak area ratio) by glass capillary gas chromatography.

In 1972, a series of novel oxazolidines and 1,3-tetrahydrooxazines were patented as potential insect repellents (Lajiness, 1972). An additional claim on the repellent properties of these heterocycles has also been made (Lajiness, 1974). The synthetic compound identified as 3-acetyl-2-(2,6-dimethyl-5-heptenyl) oxazolidine (1; Figure 1) has shown promise as a biting fly repellent (Gilbert and Smith, 1972) and has been registered for use in some countries. In Canada, the common name of citronyl was assigned for 1 when it was registered in 1977 as a personal insect repellent. This compound also occurs as an active component in fiber-impregnated repellent jackets.

Four stereoisomers of 1 are possible because of the two asymmetric centers in the molecule, but an analytical technique for detecting these isomers was not available. In this work, 1 has been examined by high-resolution gas chromatography in an attempt to separate the diastereomers of this insect repellent.

EXPERIMENTAL SECTION

Chemicals. An analytical standard of 1 and a sample of 96.4% purity (labeled as R-69 repellent) were supplied by S. C. Johnson and Son, Inc. (Racine, WI). A jacket retreatment solution (containing 46.5% of 1) was provided by Johnson Diversified of Canada (Burlington, Ontario), a Johnson-Wax Associate.

Synthesis of 3-Acetyl-2-(2,6-dimethyl-5-heptenyl)-oxazolidine (1). *Method A.* Citronellal (Eastman; 38.55 g, 0.25 mol) was added dropwise during 30 min to a mechanically stirred suspension of ethanolamine (Eastman; 30.5 g, 0.5 mol), potassium hydroxide (17 g, 0.3 mol), and benzene (150 mL). After 3.5 h, acetic anhydride (51 g, 0.5 mol) was added dropwise to the yellow mixture while maintaining the bath at 20 °C. After the mixture was stirred overnight, benzene and water were added, and the organic phase was washed with water, aqueous sodium bicarbonate, and again with water. The benzene was removed on a rotary evaporator, and the residue was distilled under reduced pressure to give 44.87 g (75% yield) of 1. A pure sample, as a colorless oil, was obtained by column chromatography on silica gel (silicAR cc-7) eluting with a hexane-ether mixture (1:1 v/v): bp 117-118 °C (0.1 mmHg); IR (neat) 1660 cm⁻¹; 60-MHz NMR (CDCl₃) δ 5.56-4.90 (2 H, m), 4.22-3.36 (4 H, m), 2.08 (3 H, s), 1.70 (3 H, s), 1.62 (3 H, s), 2.27-1.10 (7 H, m), 1.00 (3 H, m); high-resolution MS, mass found (ion composition) [calcd mass] 239.189 (C₁₄H₂₅NO₂) [239.188], 154.087 (C₉H₁₂NO₂) [154.087], 136.125 (C₁₀H₁₆) [136.125], 114.056 (C₆H₈NO₂) [114.055], 86.060 (C₄H₈NO) [86.061], 72.045 (C₃H₆NO) [72.045].

Method B. A magnetically stirred solution of citronellal (3.1 g, 0.02 mol.) and ethanolamine (2.69 g, 0.044 mol.) in benzene (30 mL) was heated under reflux for 4 h with continuous removal of water in a Dean-Stark trap (Vogel, 1978). After the mixture was cooled to room temp., acetic anhydride (4.5 g, 0.044 mol.) was added and the mixture was stirred overnight. The organic phase was washed with water, aqueous sodium bicarbonate, water, and finally with

saturated sodium chloride. The benzene extract was dried (MgSO₄) and evaporated. The crude residue was purified (as described in method A) to give 2.77 g of 1 (57.6% yield).

Thin-Layer Chromatography. Precoated 5 × 20 cm silica gel 60 TLC plates (0.25 mm layer thickness) were employed (EM Laboratories, Inc., Elmsford, NY). After development, 1 was detected by spraying the plate with a 5% solution of phosphomolybdic acid in ethanol, followed by heating the plate at 120 °C for 10 min. In the solvents examined, 1 gave a single spot with the following TLC R_f values: 0.06 (hexane-ether, 1:1); 0.17 (toluene-ethyl acetate, 1:1); 0.20 [petroleum ether (bp 30-60 °C)-methanol, 10:1]; 0.25 (ether-ethyl acetate, 10:1); 0.25 (hexane-ether-methanol, 10:10:1); 0.40 (hexane-acetone, 1:1); 0.50 (ether-methanol, 12:1); 0.60 (ether-methanol, 4:1).

Gas Chromatography. A Hewlett-Packard 5838A instrument equipped with a Model 18835B inlet system was used. The glass column (60 m × 0.29 mm i.d.) was dynamically coated with Carbowax 20M-terephthalic acid (TPA) according to the method of Grob and Grob (1976). The velocity of the carrier gas (helium) was 28 cm/s (measured at 35 °C), and nitrogen (30 mL/min) served as the makeup gas for the flame ionization detector. The temperatures were as follows: injector, 225 °C; detector, 250 °C; column oven initially at 175 °C for 0.1 min and then programmed to 200 °C at 0.5 °C/min. One to two micrograms of sample in hexane was injected split and the slope sensitivity was set at 0.03.

Mass Spectrometry. The high-resolution mass spectrum was determined by Dr. A. Hogg (Department of Chemistry, University of Alberta, Edmonton) using an AEI MS50 instrument. The sample was introduced by a direct insertion probe. For low-resolution electron-impact mass spectrometry (EI-MS), a Hewlett-Packard Model 5985B GC-MS-computer system was used with a glass capillary column (Carbowax 20M-TPA; 55 m × 0.31 mm i.d.) and helium as the carrier gas. The conditions were an ionizing energy of 70 eV, an injection port temperature of 225 °C, a column temperature of 180 °C, an interface temperature of 210 °C, and a source temperature of 200 °C. The positive chemical ionization mass spectrum (CI-MS) was recorded on the same GC-MS system with methane as the reagent gas.

RESULTS AND DISCUSSION

Lajiness (1972, 1974) prepared 1 by the reaction of citronellal with ethanolamine and acetic anhydride. In this work, 1 was synthesized by that sequence, employing potassium hydroxide in benzene at room temperature (method A), and by a procedure from Just et al. (1976) with a benzene reflux in the absence of a strong base (method B). In both methods, the first-formed products were not isolated but were reacted in solution with acetic anhydride. From previous work on related condensation reactions [for example, Walker and Moore (1961)], the intermediates to 1 probably exist as a mixture of imino alcohol and epimeric oxazolidine. After acetylation, the crude product was

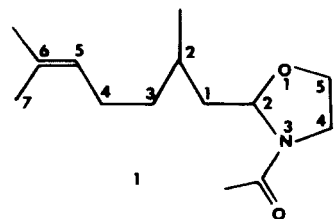


Figure 1. Structure and numbering system of 3-acetyl-2-(2,6-dimethyl-5-heptenyl)oxazolidine.

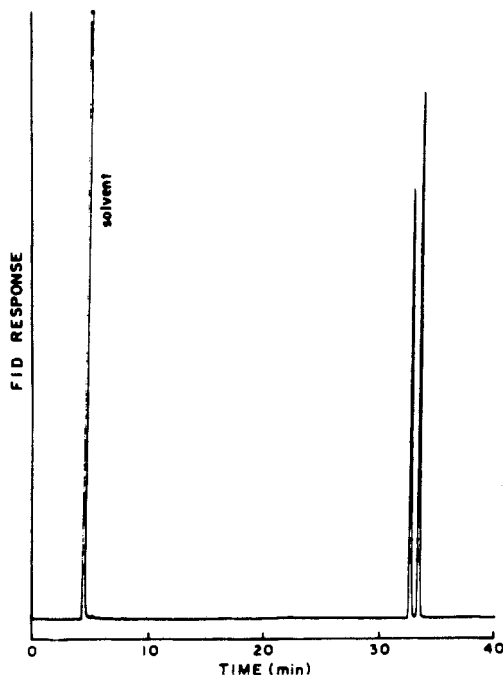


Figure 2. Glass capillary gas chromatogram demonstrating the separation of 1 into a diastereomeric pair. The column (60 m \times 0.29 mm) was coated with Carbowax 20M-TPA. For chromatographic conditions, see Experimental Section.

isolated as a light yellow oil. The purified material from each method was identical by IR and NMR to the analytical standard of 1, which was received as a colorless liquid with a faint terpene odor.

It was initially expected that a pair of diastereomers of 1 would be observed by conventional techniques, such as by packed column GC and by NMR spectroscopy. However, samples of 1 gave a single peak on a variety of packed columns, and the diastereotopic protons coincidentally overlapped with other NMR signals, which lead to inconclusive results. Thin-layer and column chromatography with silica gel also failed to separate the diastereomers. This difficult separation was then attempted by glass capillary GC.

Three common liquid phases (OV-101, OV-17, and Carbowax 20M-TPA) wall coated on short (10–20-m) glass capillaries were initially examined. The OV-101 phase gave only one peak, but each of the other liquid phases showed partial resolution of two components in all samples of 1. The last eluting component always represented the largest peak. By use of a 60-m capillary column coated with Carbowax 20M-TPA, the two components in 1 were completely separated (Figure 2).

As evidence that the two GC components in 1 were diastereomers, their mass spectra were recorded. Under conditions for combined GC-MS, the total ion mass chromatogram (Figure 3) was very similar to the GC tracing of Figure 2. When recorded at the apex of the two peaks, the EI-MS were nearly superimposable (Figure 4).

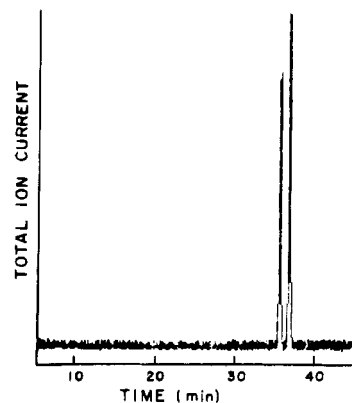


Figure 3. Total ion mass chromatogram demonstrating the separation of 1 into a diastereomeric pair. Conditions are given under Experimental Section.

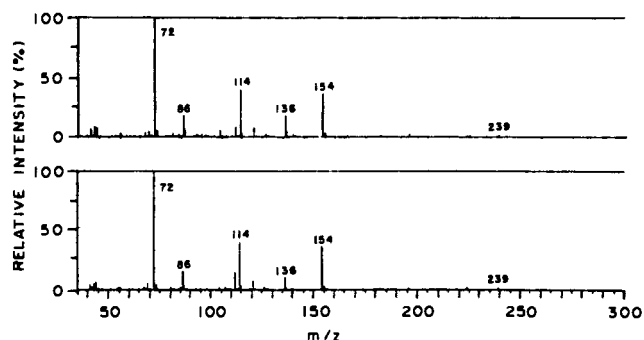


Figure 4. Graphs depicting the low-resolution EI-MS (70 eV) of a major diastereomer of 1 (above) and a minor diastereomer of 1 (below).

Table I. Comparison of Diastereomeric Ratios in Samples of 1 by Glass Capillary Gas Chromatography with Carbowax 20M-TPA^a

source of 1	integrated peak areas, % ^b	
	first eluting diastereomer ^c	second eluting diastereomer ^d
analytical standard	46.1	53.9
technical-grade material	44.8	55.2
jacket-retreatment solution ^e	44.7	55.3
method A ^{f,g}	44.8	55.2
method B ^{f,g}	46.1	53.9
method B ^{f,h}	44.9	55.1
method B ^{f,i}	45.8	54.2

^a For the GC conditions, see Experimental Section.

^b By electronic integration of areas under the two peaks and calculating the percent of each diastereomer in the mixture. Values given are the mean of three determinations for each sample. The SD was ≤ 0.2 . ^c Elution temperature (retention time) of 191.3 °C (32.7 min). ^d Elution temperature (retention time) of 191.7 °C (33.4 min). ^e After partitioning the commercial sample between water and hexane and evaporating the organic extract. ^f The crude sample before purification. ^g Citronellal with $[\alpha]_D^{+8}$ (Eastman Kodak Co., Rochester, NY) was employed for these experiments. ^h For this experiment, citronellal with $[\alpha]_D^{+14}$ was obtained from Tridom/Fluka, Hauppauge, NY. ⁱ For this experiment, citronellal with $[\alpha]_D^{-11}$ was purchased from Aldrich Chemical Co., Milwaukee, WI.

In addition, the CI-MS were identical and the most intense ion, in each spectrum, occurred at m/z 240 (MH^+).

It was of interest to compare the relative amounts of the diastereomers by examining samples of 1 from different sources. By capillary GC analysis of 1 from seven samples

of varying purity, the same diastereomer was always found in slight ($\approx 5\%$) excess (Table I). In fact, the mean ratio, 45.3% to 54.7%, was essentially constant (± 0.7 SD) for these samples.

This preliminary work shows the utility of glass capillary GC for detecting the diastereomers of 1. Further research is necessary to isolate and characterize these isomers and to compare their ability to repel biting insects.

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