

Structure Confirmation of the Four *trans* Isomers of Ceralure, a Medfly Attractant, by NMR

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A series of one- and two-dimensional NMR experiments were used to confirm structures of the four racemic *trans* isomers of ceralure, ethyl 4-(or 5)-iodo-*trans*-2-methylcyclohexane-1-carboxylate, a synthetic attractant for the male Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Proton and carbon assignments were made on the basis NMR data obtained from 2D chemical shift correlation maps, attached proton tests, and 2D-¹H homonuclear correlation spectroscopy. Assignments of configuration and conformation, based on NMR, for the four *trans* isomers of ceralure (A, B₁, B₂, and C) were consistent with assignments previously derived from a comparison of the gas chromatographic elution behavior of the *trans* isomers of ceralure and the *trans* isomer of trimedlure, 1,1-dimethylethyl 4-(or 5)-chloro-*trans*-2-methylcyclohexane-1-carboxylate.

Keywords: Ceralure; attractant; NMR; proton; carbon-13

INTRODUCTION

Ceralure (CRL), ethyl 4-(or 5)-iodo-*trans*-2-methylcyclohexane-1-carboxylate, is a synthetic attractant mixture whose components are structurally related to the components of trimedlure (TML), the lure currently used to detect and monitor population outbreaks of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann). High attractancy and persistency (i.e., low volatility) (McGovern and Cunningham, 1988a; Warthen and McGovern, 1990; DeMilo et al., 1994) make CRL a prime candidate for inclusion in a male annihilation technique. Similar to TML, the synthesis of CRL produces a mixture of eight diastereomers (four *trans* and four *cis*). To synthesize highly attractive mixtures of CRL, procedures have been optimized to produce racemic mixtures predominating in the four *trans* isomers, CRL-A, CRL-B₁, CRL-B₂, and CRL-C (Figure 1). While the most attractive isomer in TML is TML-C (McGovern et al., 1966), specifically the 1*S*,2*S*,4*R*-TML-C enantiomer (Sonnet et al., 1984; Doolittle et al., 1991), the most attractive isomer in CRL is CRL-B₁ (Warthen and McGovern, 1990; Warthen et al., 1994). Assignments of configuration and conformation for the four *trans* TML isomers have been made from a series of chemical and spectral (IR, NMR) analyses (McGovern and Beroza, 1966; Sonnet et al., 1984; Warthen and McGovern, 1986; Warthen et al., 1987, 1988).

A recently developed semipreparative high-performance liquid chromatographic (HPLC) procedure has made gram quantities of the four racemic *trans* isomers of CRL (Warthen and McGovern, 1990) available for new studies. The present study was undertaken to confirm assignments of configuration and conformation for the four racemic *trans* isomers of CRL. Previous to this study, assignments were made primarily on the basis of similarities in the syntheses of CRL and TML

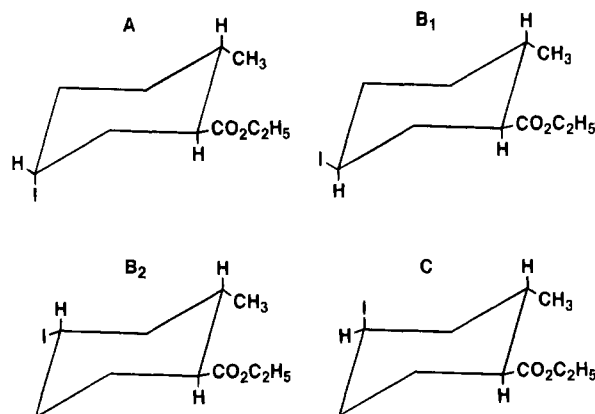


Figure 1. Structures of the four *trans* isomers of ceralure.

as well as similarities in the GC behavior of the four *trans* isomers of CRL and those of TML (TML-A, -B₁, -B₂, -C).

MATERIALS AND METHODS

Chemicals. CRL-B₁ (ethyl *cis*-5-iodo-*trans*-2-methylcyclohexane-1-carboxylate) and CRL-B₂ (ethyl *trans*-4-iodo-*trans*-2-methylcyclohexane-1-carboxylate) were isolated by semipreparative HPLC (Warthen and McGovern, 1990) from a typically prepared synthetic mixture (McGovern and Cunningham, 1988a). CRL-A (ethyl *trans*-5-iodo-*trans*-2-methylcyclohexane-1-carboxylate) and CRL-C (ethyl *cis*-4-iodo-*trans*-2-methylcyclohexane-1-carboxylate) could not be resolved by HPLC; consequently, these isomers had to be obtained by an alternate procedure using the *tert*-butyl ester analogs of *trans* CRL. The A and C *tert*-butyl isomers were isolated by HPLC (Warthen and McGovern, 1990). Then these esters were hydrolyzed to their respective acids, and finally these acids were converted (via their acid chlorides) to the desired ethyl esters. Conditions used for the hydrolysis (McGovern and Cunningham, 1988b), acid chloride formation, and re-esterification (Green and Beroza, 1959) did not induce epimerization.

NMR Experiments. Spectra were obtained with a GE QE-300 spectrometer with a 5 mm ¹H/¹³C probe. Samples were

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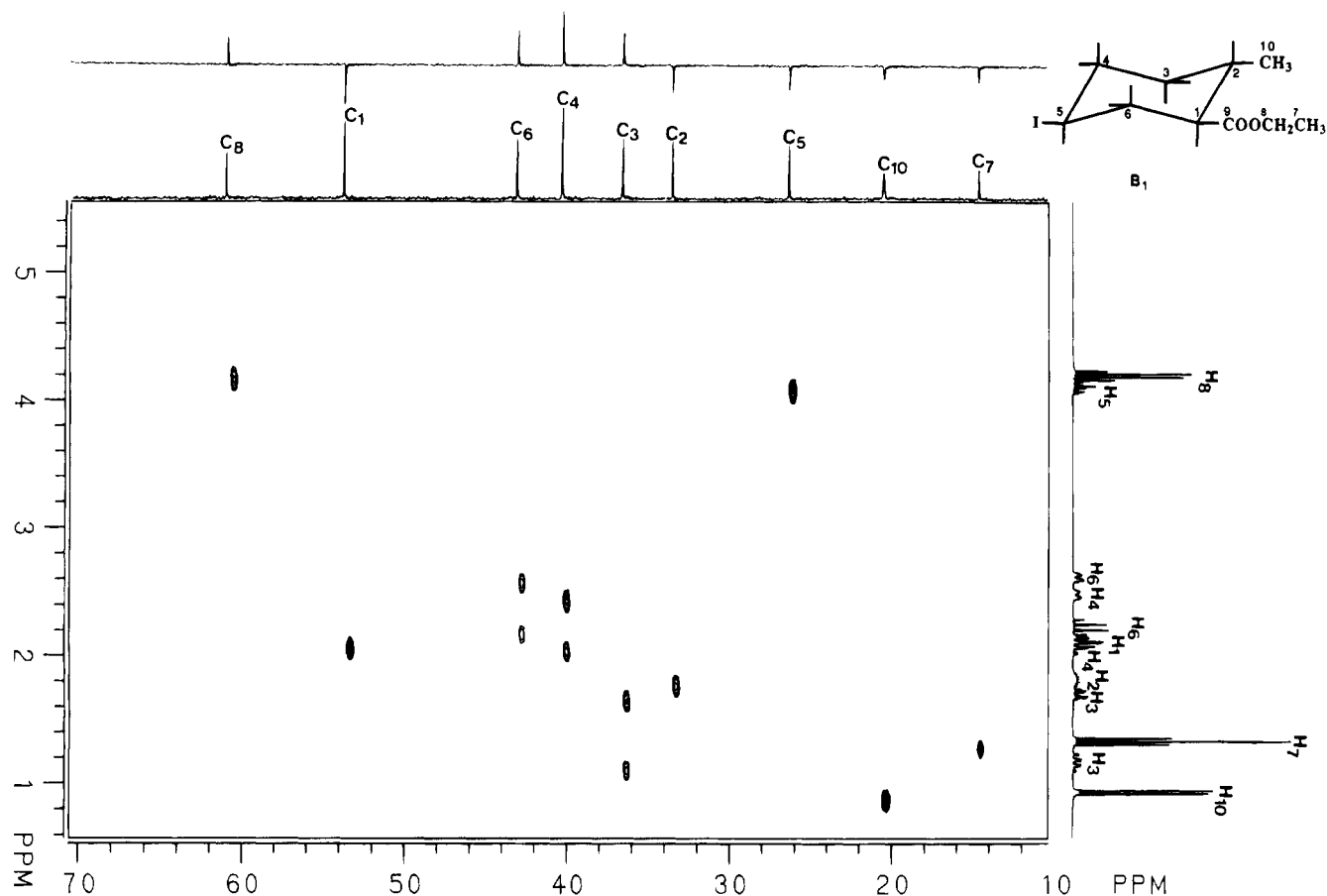
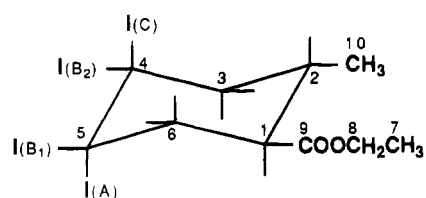


Figure 2. HETCOR (CSCM) of ceralure isomer B₁; APT, attached proton test.

Table 1. ¹³C Chemical Shifts (Parts per Million)



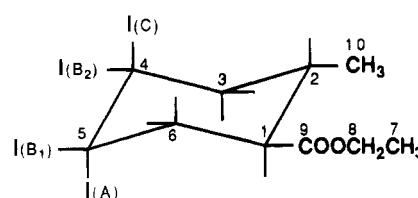
| carbon type | CRL-A | CRL-B ₁ | CRL-B ₂ | CRL-C |
|-------------|--------|--------------------|--------------------|--------|
| 1 | 47.42 | 53.25 | 49.73 | 50.71 |
| 2 | 34.14 | 33.11 | 37.05 | 30.35 |
| 3 | 30.23 | 36.16 | 47.61 | 43.63 |
| 4 | 35.67 | 39.87 | 26.64 | 33.10 |
| 5 | 33.14 | 25.90 | 39.08 | 35.43 |
| 6 | 38.89 | 42.66 | 31.70 | 26.00 |
| 7 | 14.29 | 14.24 | 14.26 | 14.31 |
| 8 | 60.35 | 60.43 | 60.32 | 60.26 |
| 9 | 175.32 | 173.45 | 175.36 | 174.97 |
| 10 | 20.08 | 20.09 | 19.83 | 19.47 |

dissolved in CDCl₃ (ca. 40 mg/0.6 mL) for all experiments, and chemical shifts were reported from tetramethylsilane (TMS) as an internal reference. An attached proton test (APT) (Patt and Shoolery, 1982) chemical shift correlation map (CSCM) (Bax and Morris, 1982), and COSY (2D-¹H homonuclear correlation spectroscopy) (Jeener et al., 1979; Bax et al., 1981) were performed on all isomers. ¹H spectra were also obtained for CRL-A in acetone-*d*₆ at 22.5 and 60 °C.

RESULTS AND DISCUSSION

Since CRL-B₁ is the most attractive isomer in CRL, NMR discussion will be limited primarily to proton and

Table 2. ¹H Chemical Shifts (Parts per Million)^a



| proton type | CRL-A | CRL-B ₁ | CRL-B ₂ | CRL-C |
|-------------|-------|--------------------|--------------------|-------|
| 1 | 2.60 | 2.10 | 2.05 | 2.05 |
| 2 | 1.80 | 1.75 | 1.80 | 2.35 |
| 3a | 1.65 | 1.10 | 1.75 | 1.35 |
| 3e | 1.65 | 1.65 | 2.45 | 2.15 |
| 4a | 1.60 | 2.05 | 4.20 | |
| 4e | 2.15 | 2.40 | | 4.85 |
| 5a | | 4.10 | 2.00 | 1.60 |
| 5e | 4.80 | | 2.50 | 2.20 |
| 6a | 1.70 | 2.20 | 1.60 | 1.80 |
| 6e | 2.25 | 2.55 | 1.85 | 2.05 |
| 7 | 1.25 | 1.30 | 1.25 | 1.25 |
| 8 | 4.20 | 4.15 | 4.15 | 4.20 |
| 10 | 1.00 | 0.85 | 0.85 | 0.95 |

^a Values correspond to the center of the ¹H resonances in the 2D CSCM.

carbon assignments for this biologically important isomer. Similar NMR experiments and analyses were used to make ¹H and ¹³C assignments for the other *trans* CRL isomers. ¹³C and ¹H chemical shift data for all *trans* CRL isomers are reported in Tables 1 and 2, respectively.

The 1D-¹³C spectrum of CRL-B₁ in Figure 2 shows nine peaks (carboxylate carbon C-9 at 173.45 ppm not

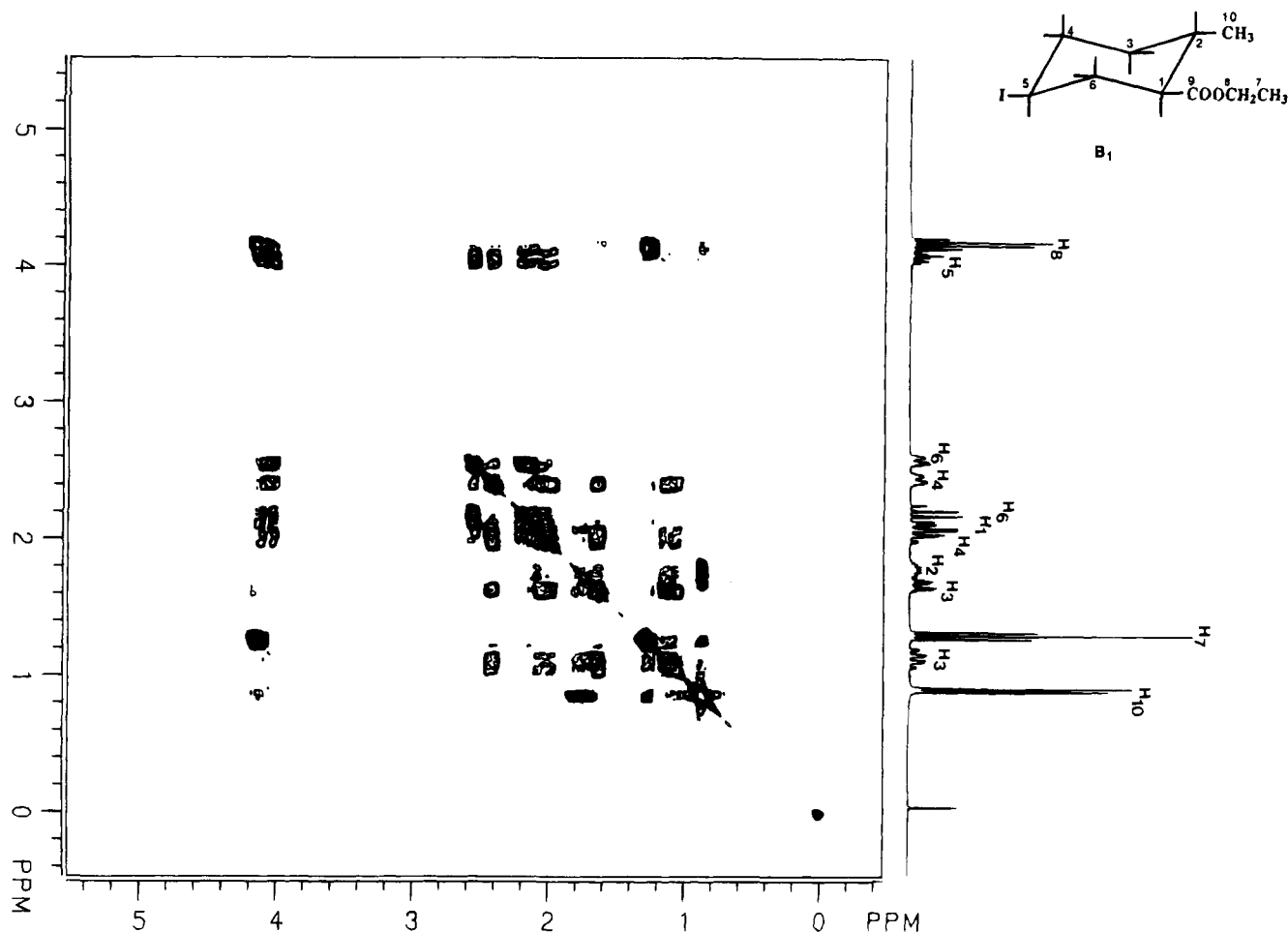


Figure 3. COSY of ceralure isomer B₁.

shown). The APT spectrum shows four positively deflected peaks, indicating 4 CH_2 -type carbons (36.16, 39.87, 42.66, 60.43 ppm), and five negatively deflected peaks, indicating CH - or CH_3 - (14.24, 20.09, 25.90, 33.11, 53.25 ppm) type carbons. From APT and 2D-CSCM data (Figure 2), negatively deflected carbons C-7 (14.24 ppm) and C-10 (20.09 ppm) were assigned CH_3 types due to their strongly shielded chemical shifts and cross peaks that correlated with the large proton doublet (0.85 ppm) and proton triplet (1.30 ppm), indicative of α -CH and α - CH_2 groups, respectively. The remaining negatively deflected carbons were assigned CH types since these carbons correlated with protons that integrated for an area of ca. 1H, at 1.75, 2.10, and 4.10 ppm. The CH-type signal at 25.90 ppm was assigned C-5 since it correlated with a strongly deshielded proton H-5 characteristic of one attached to an iodo-substituted methine group (Silverstein et al., 1981). Since one of the four CH_2 signals, observed at 60.43 ppm, showed a cross peak in the proton portion of the 2D-CSCM spectrum with the quartet (indicative of an α - CH_3) at 4.15 ppm, this carbon was assigned C-8.

Assignments for the remaining carbons (C-1-4, C-6) were made from the CSCM experiment following completion of proton assignments that were derived from cross-peak correlations in the 2D- ^1H homonuclear (COSY) experiment (Figure 3).

^{13}C chemical shift data also supported assignment of an equatorial-equatorial relationship between the methyl at C-2 and the carboxylate group at C-1 for CRL-B₁. Specifically, the observed chemical shift for C-2 (33.11

ppm) was well within the range of equatorial substitution (30-40 ppm) rather than axial (25-30 ppm) (Schneider and Hoppen, 1978). Similarly, a large downfield shift (53.25 ppm) for C-1 indicated an equatorial attachment of the carboxylate group rather than axial (Jancke et al., 1975). 1D- ^{13}C chemical shift data provided support to the assignment of an equatorial conformation of the iodo substituent at C-5 in CLR-B₁; i.e., more shielded chemical shifts for equatorial carbons C-4 (compare 26.64 ppm for CLR-B₂ vs 33.10 ppm for CLR-C) and C-5 (compare 25.90 ppm for CLR-B₁ vs 33.14 ppm for CLR-A) are consistent with shift effects observed in related iodo-substituted cyclohexanes (Schneider and Hoppen, 1978; Abraham and Loftus, 1981). Further support of the equatorial conformation of the iodo substituent at C-5 in CRL-B₁ was evident from proton chemical shift data; i.e., a more shielded shift (4.10 ppm) of H-5 in CRL-B₁ compared to that observed at C-5 in CRL-A (4.80 ppm) suggests an axial conformation (Silverstein et al., 1981) and, therefore, an equatorial conformation of the iodo substituent. Similar reasoning was used to determine conformational assignments for the other isomers.

A rather unusual feature was observed in the 1D- ^1H spectrum of CRL-A. While methylene protons at C-8 were observed at ca. 4.2 ppm as quartets (typical of an AX_3 coupling pattern) for isomers CRL-B₁, CRL-B₂, and CRL-C, the same methylene protons in CRL-A were observed as a multiplet of ≥ 14 peaks (Figure 4A). This unexpected multiplet may be rationalized by the fact that methylene protons at C-8 are in different magnetic environments. Two possible causes for this are (a) inter-

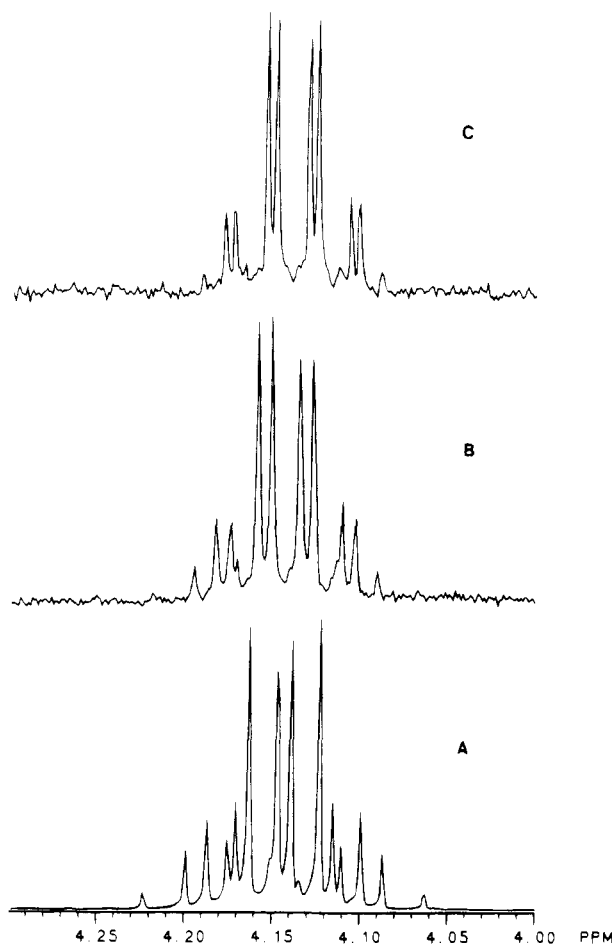


Figure 4. C-8 methylene protons for ceralure *trans* isomers A: (A) spectrum in CDCl_3 (22.5 °C); (B) spectrum in acetone- d_6 (22.5 °C); (C) spectrum in acetone- d_6 (60 °C).

or intramolecular hydrogen bonding which might reduce the mobility of the ethoxy moiety and (b) distortion of the ring from the chair form due to the bulk of the iodine atom in the axial position. Case a is supported by the fact that the multiplet partially coalesces when the spectrum is taken in a more polar solvent (acetone- d_6) (Figure 4B). Case b is supported by the fact that the multiplet coalesces even more at an elevated temperature (60 °C) (Figure 4C). Clearly, further NMR experiments and perhaps molecular modeling studies are needed to determine the cause of this complex multiplet.

In summary, chemical shifts and respective assignments for proton and carbon atoms for the four *trans* isomers of CRL were reported. Assignments of configuration and conformation for these isomers are consistent with stereochemistry previously assigned to TML-A, -B₁, -B₂, and -C (McGovern and Beroza, 1966) and CRL (Warthen and McGovern, 1990). Assignments of configuration for the three ring substituents (1-carboethoxy, 2-methyl, 4-(or 5) iodo) are as follows: CRL-A, 1,2,5-*eea*; CRL-B₁, 1,2,5-*eee*; CRL-B₂, 1,2,4-*eee*; and CRL-C, 1,2,4-*eea*. These NMR data should be useful to scientists engaged in the synthesis and elucidation of the structure of new analogs of CRL as well as those scientists engaged in structure-activity studies for this class of attractants.

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