Absolute Configuration at a Fourth Activity-Determining Pyrethroid Chiral Center Assigned by NOESY NMR Analysis

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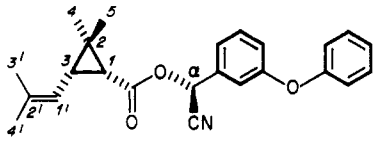
The chirality at $C_{1'}$ of the epoxychrysanthemates, such as that from (1*R*,*cis*,α*S*)-cyphenothrin (1), strongly influences their biological activity. Nuclear Overhauser effect 2D NMR spectral analysis clearly assigns the more neurotoxic diastereomer of (1*R*,*cis*,α*S*)-epoxycyphenothrin (2) as 1′*S* and the major microsomal metabolite as 1′*R*. Microsomal oxidation of 1 preferentially forms the noninsecticidal isomer of 2 and thus represents a detoxification mechanism.

Insecticidal chrysanthemate esters such as cyphenothrin (1) are readily derivatized by addition of oxygen, sulfur, or methylene to the 2-methyl-1-propenyl double bond (Ruzo et al., 1984) or by formation of the aziridine (Holloway et al., 1986), in each case generating a fourth chiral center at $C_{1'}$. These derivatives exhibit reduced insecti-



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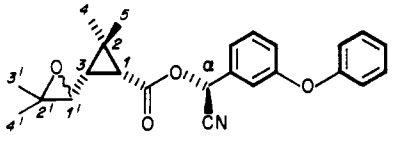

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cidal activity and mammalian toxicity relative to the parent compounds, but some of them are extremely potent in eliciting repetitive discharges in an insect sensory nerve (Ruzo et al., 1984; Holloway et al., 1986; Gammon et al., 1983). The chirality at $C_{1'}$ of the epoxychrysanthemates strongly influences activity; i.e., one of the two diastereomers of epoxycyphenothrin (2) is much more neurotoxic and insecticidal than the other (Ruzo et al., 1984). Photodecomposition of chrysanthemates yields diastereomeric epoxides of differing insecticidal activities (Ueda et al., 1974), and in vitro microsomal epoxidation proeeds with stereoselectivity (Smith and Casida, 1981). Our attempts to assign the configurations of these epoxides by X-ray crystallography following derivatization on acid-catalyzed epoxide ring opening failed due to facile rearrangements of the cyclopropyl group. The present report uses NMR methods to assign the absolute configurations to the diastereomers of 2.

MATERIALS AND METHODS

Spectroscopy. Mass spectrometry (MS) was carried out with a Hewlett-Packard 5985B instrument operated in the chemical ionization (CI) mode with methane (0.8 Torr) as the reagent gas.

Nuclear magnetic resonance spectra were recorded with a Bruker AM 500 NMR spectrometer at 500-MHz ^1H operating frequency in chloroform-*d*. ^{13}C spectra were recorded at 125 MHz. The ^{13}C and ^1H NMR spectral peak assignments of **2a** and **2b** were made based on heteronuclear COSY (Reynolds et al., 1985) and long-range ^1H–^{13}C correlation spectroscopy (COLOC-S) (Krishnamurthy and Casida, 1987).

Nuclear Overhauser effect 2D NMR spectra (NOESY) were obtained with use of the pulse sequence 90°–t_1–90°–τ–90°–Acq (Wider et al., 1984). Identical acquisition, processing, and plotting parameters were used for **2a** and **2b**. A spectral width of 200 Hz was used in F1 and F2 dimensions. For each of the 128 different t_1 values, each consisting of 512 complex points, 32 scans were collected. A 1-s mixing delay (τ) and 5-s relaxation delay were used with a total acquisition time of 9 h. The F2 dimension was transformed after Gaussian multiplication with $\exp(-at - bt^2)$, where $a = 5\pi$ and $b = 5\pi/AQ$ (AQ = acquisition time), while the F1 dimension was modified with the shifted ($\pi/35$) sine bell squared function before Fourier transformation. The final matrix (512 × 512) was symmetrized along the diagonal.

Synthesis and Characterization of the Diastereomers of 2. Diastereomers **2a** and **2b** are readily generated by the reaction of 1 (Roussel Uclaf, Paris, France) with *m*-chloroperoxybenzoic acid in dichloromethane (Ruzo et al., 1984) and isolated by thin-layer chromatography (TLC) (0.5 mm, silica gel 60 F_{254}, carbon tetrachloride–ether (5:1); **2a**, R_f 0.44, 56%; **2b**, R_f 0.32, 38%). These products are also separable by gas chromatography (GC) on a Hewlett-Packard 5840 instrument (interfaced with the MS) equipped with a high-performance methyl silicone capillary column (10 m, 1 mL/min, helium carrier) operated with temperature programming (140–240 °C, 20°/min). Characterization was by CI/MS (Ruzo et al., 1984) and NMR: (**2a**) δ_H 7.4–6.9 (m, 9 H), 6.38 (s, 1 H), 3.11 (d, 1 H), 1.72 (d, 1 H), 1.33 (s, 3 H), 1.32 (s, 6 H), 1.23 (s, 3 H),

Table I. Partial ^{13}C and ^1H NMR Peak[a] Assignments of (1*R*,*cis*,α*S*)-Cyphenothrin Epoxide (2)

	2a			2b		
		δ_H of cross peaks			δ_H of cross peaks	
posn	δ_C	het COSY	COLOC-S	δ_C	het COSY	COLOC-S
5	14.2	1.33	1.23	14.7	1.26	1.20
3′	19.0	1.32	1.32, 3.11	19.4	1.34	1.34, 3.16
4′	24.3	1.32	1.32	24.5	1.34	1.34
3	27.2		1.28	26.9		1.23
4	28.2	1.23	1.31, 1.72	28.3	1.20	1.25, 1.76
1	28.7	1.72	1.28	28.5	1.76	1.23
2	32.3	1.18	1.28	32.8	1.19	1.23
2′	58.4		1.32	58.7		1.34
1′	59.3	3.11	1.32	58.3	3.16	1.34
α	62.2	6.38		62.1	6.38	

[a] All chemical shifts are (ppm) downfield with respect to $(CH_3)_4Si$.

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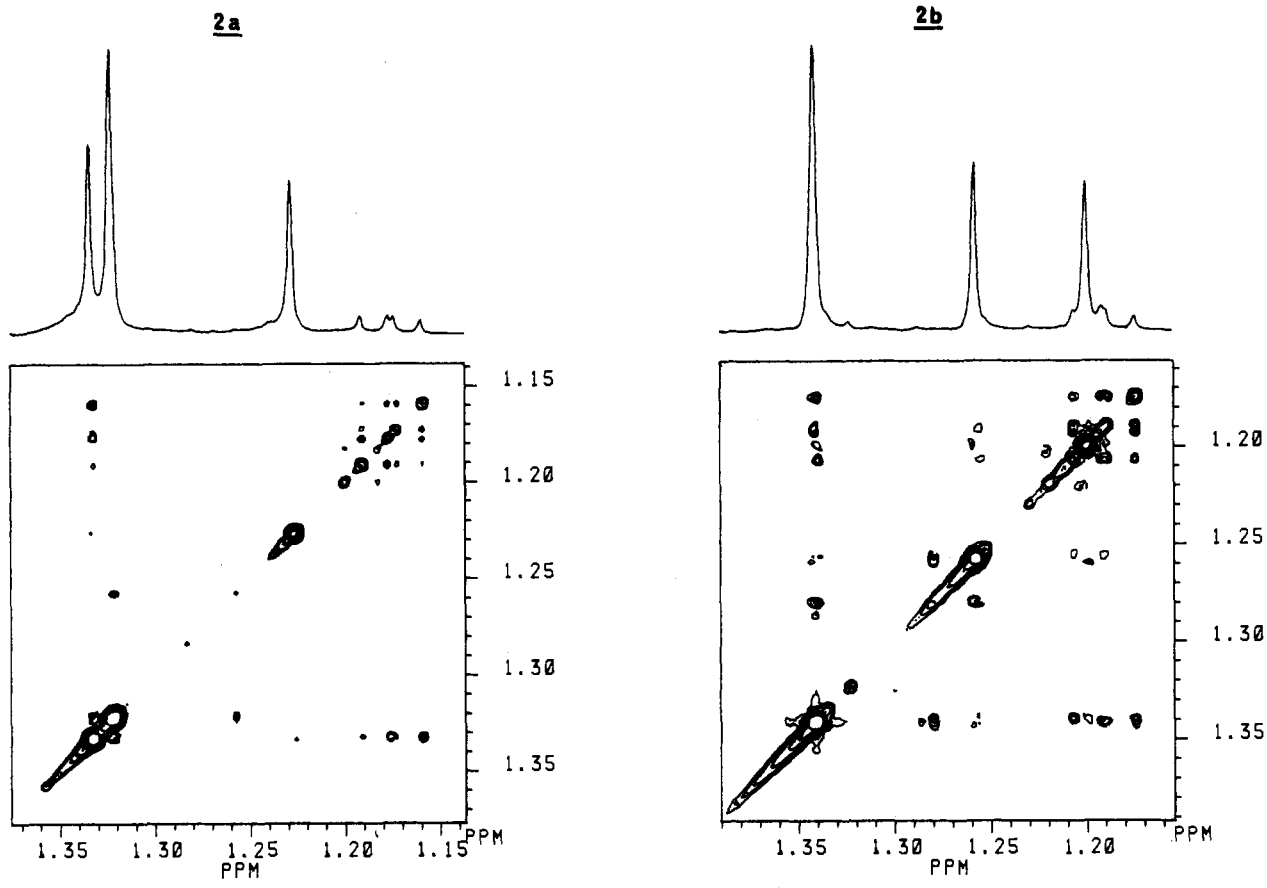

Figure 1. NOESY spectra of **2a** and **2b** recorded in $CDCl_3$ (20 mg/0.5 mL) at ambient temperature.

1.18 (dd, 1 H); (**2b**) δ_H 7.4–6.9 (m, 9 H), 6.38 (s, 1 H), 3.16 (d, 1 H), 1.76 (d, 1 H), 1.34 (s, 6 H), 1.26 (s, 3 H), 1.20 (s, 3 H), 1.19 (dd, 1 H).

RESULTS AND DISCUSSION

The ^1H cross peaks for each of the aliphatic carbon resonances observed in the heteronuclear COSY and COLOC-S spectra and their specific assignments in **2a** and **2b** are listed in Table I. The key observations leading to unambiguous assignment of the methyl proton resonances in **2a** and **2b** are the long-range correlations observed between $C_{3'}$ and $H_{1'}$ and C_4 and H_1 due to trans vicinal couplings.

Space-filling models of the two isomers of **2** reveal that the $C_{3'}$ methyl group trans to the epoxide hydrogen is in close proximity to C_4 in the 1′*R* but not in the 1′*S* diastereomer. The spatial proximity between these methyl groups in the *R* isomer is comparable to that found between an axial methyl group and 3(5)-axial hydrogen(s) in the chair conformation of a cyclohexane ring. Since the nuclear Overhauser effect provides information on the spatial proximity of nuclei, we applied NOE 2D NMR spectroscopy (NOESY) to determine the absolute configurations of the diastereomers of **2**. The observation of a distinct cross peak at 1.34 ppm × 1.20 ppm in the NOESY spectrum (Figure 1) of **2b** and the absence of a similar peak at 1.32 ppm × 1.23 ppm in the spectrum (Figure 1) of **2a** serves to assign **2b** as the *R* diastereomer and **2a** as the *S* isomer.

Epoxidation of **1** by rat liver microsomes was investigated by incubating **1** (10 μg) for 30 min at 37° °C in 1 mL of pH 7.4 phosphate buffer containing rate liver microsomes (1 mg of protein, washed to remove soluble proteins) in the presence and absence of NADPH (1 mg). The presence of **2a** and **2b** was examined by TLC with 4-(4-nitrobenzyl)pyridine reagent (Hammock et al., 1974) and by selected ion monitoring (SIM, *m/e* 392 [MH^+], 374 [$MH^+ - H_2O$]) with GC–CI–MS (R_t: **2a**, 8.4 min; **2b**, 8.9 min). In incubations with NADPH, epoxide **2a** was detected in 2% yield and **2b** in <0.1% yield by both TLC and SIM. Incubations without NADPH did not yield **2a** or **2b**. This process represents a detoxification mechanism since the noninsecticidal isomer (**2a**) is formed preferentially.

Molecular models indicate that the *R* diastereomer is sterically more crowded than the *S* isomer. It is evident that there is a distinct preferene for the formation of the sterically less crowded isomer **2a** on both chemical and biological oxidation.

The absolute configuration was assigned earlier by X-ray crystallography for an analogue of **1** in which the 2-methyl-1-propenyl substituent is replaced by a 1,2-dibromo-2,2-dihaloethyl group, but in this case an activity-determining center may not be involved (Ackermann et al., 1980) since biological thiols rapidly debrominate this proinsecticide to the highly insecticidal 2,2-dihalovinyl derivative (Ruzo et al., 1981). **2** is the first pyrethroid in which the absolute configurations are assigned for four activity-determining chiral centers, providing an additional step in defining the topography of the pyrethroid neuroreceptor.

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Registry No. **1**, 64312-66-9; (1′*S*)-**2**, 108147-01-9; (1′*R*)-**2**, 108147-02-0.

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Residues of Dimethoate and Omethoate in Peaches and Apples following Repeated Applications of Dimethoate

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Repeated applications of dimethoate were carried out on peaches and apple trees in order to establish the maximum number of dimethoate sprays that will give rise to residues complying with international maximum residue limits. Samples were analyzed by gas chromatography for the determination of dimethoate and its oxygen analogue, omethoate, over a period of 3–4 weeks after last spray. Only in the case of apples with seven applications, mean residues of dimethoate exceeded 1 ppm at the end of the preharvest interval (14 days); residue levels of omethoate were always below 0.2 ppm. Considering the eventual need of a preharvest interval not longer than 14 days, restrictions on the number of applications during apples growth cycle should be enforced.

The insecticide dimethoate, *O,O*-dimethyl *S*-[(methylcarbamoyl)methyl] phosphorodithioate, is officially approved in Portugal for use on peach trees with a preharvest interval of 2 weeks and restricted to two applications at 40 g a.i./hl (a.i. = active ingredient), mainly to control the Mediterranean fly, and for most peach varieties no more than two applications will be necessary. Considering the control of other pests on peach trees, namely aphids, we assume that up to three applications of dimethoate during peach growth cycle might be made.

As far as apple trees are concerned, the dimethoate preharvest interval is 2 weeks and its wider use is, by far, against *Carpocapsa pomonella*. Although efforts are being made at national level in order to decrease the number of treatments, the use of dimethoate about every 2 weeks is still current, especially among traditional farmers. This practice can lead to a total of about seven applications during apple growth cycle.

During 1960–1970 considerable work on the disappearance of dimethoate, namely on peaches and apples, was carried out and reviewed by Pietri-Tonelli (1965) and by the Joint Meetings of the WHO/FAO (1968, 1971, 1978). Studies on peaches and oranges were carried out in Portugal by Silva Fernandes (1972, 1973) using the method of analysis of the Joint Dimethoate Residues Panel (1968). In these latter studies residues were determined as the sum of dimethoate and its oxygen analogue metabolite omethoate [*O,O*-dimethyl *S*-[(methylcarbamoyl)methyl] phosphorothioate]. For this reason, no information is given on the levels of this compound, also used as an insecticide and for which different countries have established separate and lower maximum residue limits.

Considering the insufficient information on residues of dimethoate to be expected in the case of some severe agricultural practices still used, together with the interest of determining omethoate residues resulting from the above-mentioned use of dimethoate, residue disappearance studies were carried out in order to assess whether the preharvest interval established in Portugal complies with international maximum residue limits.

MATERIALS AND METHODS

Field Treatments and Sampling. The trial on peach trees was set up at Pegões Experimental Field. One set of nine trees was selected from one row of the orchard for the execution of two applications; a second set of nine trees was selected from a different row for the execution of three applications. In both cases each set of three contiguous trees was considered as a field replicate for sampling. Details of the experiment are described in Table I. Sprays were carried out at high volume to the point of runoff with a wheelbarrow sprayer provided with a two-stroke engine-driven diaphragm pump operated at 20 kg/cm^2.

Average maximum daily air temperatures recorded at Pegões climatological station were 24.4 °C from 23 May up to the end of the month, 26.4 °C in June, and 26.9 °C from then to final sampling. For the same periods total rainfalls were 4.0, 6.6, and 6.8 mm.

Samples of 18 peaches were randomly collected from each field replicate on days 1, 4, 7, 11, 14, and 21 after the last application. Fruits used as controls were picked from trees separated from the treated plots by an unsprayed row. Samples were transported to the laboratory in plastic bags, the stones were removed, and the pulp was chopped in a Hobart food cutter. Three 100-g subsamples of pulp were taken for analysis.

The trial on apple trees was set up at Quinta de São João, Caldas da Rainha. Two sets of nine cordon-grown apple trees (palmette form) were used for the execution

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