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HIGH-PERFORMANCE LIQUID CHROMATOGRAPH COUPLED WITH TWO DETECTORS: A UV SPECTROMETER AND A POLARIMETER

EXAMPLE IN THE FIELD OF PYRETHROIDS: IDENTIFICATION OF ENANTIOMERS

C. MEINARD*, P. BRUNEAU and J. PERRONNET

Division Scientifique, Roussel Uclaf, Procida-CRBA St. Marcel, 13011 Marseille Cédex 11 (France)

SUMMARY

Identification of enantiomers is possible by using two detectors in high-performance liquid chromatography: a UV spectrometer and a polarimeter. In a mixture of enantiomers and diastereoisomers, such as those resulting from a chemical structure with several asymmetric carbons, the UV spectrometer allows the detection of the diastereoisomers, whereas the polarimeter permits the identification of the enantiomers.

Deltamethrin, an optically pure synthetic pyrethroid widely used as an agricultural insecticide, can undergo partial isomerization, especially through the action of light. Every photoisomer in the resulting mixture is a diastereoisomer of deltamethrin with two possible enantiomeric forms. The above method was applied to determine the spatial configurations of the isomers formed, and conclusions were drawn about the mechanism of photoisomerization.

INTRODUCTION

High-performance liquidchromatography (HPLC), coupled with UV spectrometric detection, is widely used for the separation, identification and quantification of diastereoisomers, such as those encountered in the pyrethroid series[1]. However, this method is not suitable for determining the absolute configurations when enantiomeric forms are present, the separation of the latter requiring the use of a chiral chromatographic sorbent[2,3].

An easier way of identifying enantiomers is to use a combination of a UV detector with a polarimetric detector, both coupled with HPLC. The recorder integrator simultaneously indicates the UV absorption and the rotation as a function of retention time. Use of these data permits the analysis of the mixture, provided that a sample of at least one optically pure enantiomer of each diastereoisomer is available[4–6].

Deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate] (RU 22974) is a synthetic pyrethroid widely

used as an agricultural insecticide[7]. It is an optically active compound that contains three asymmetric carbon atoms with the configurations $1R,3R,\alpha S$ (Fig. 1)[8,9]. Through the action of light, deltamethrin undergoes isomerization. Isomerization of all three asymmetric centres could lead to eight isomers (four diastereoisomers, each having two enantiomeric forms). Previous studies[10,11] have indicated the opening and recyclization of the cyclopropane ring (involving the asymmetric centres 1 and 3) and the possible isomerization of the benzylic carbon atom (involving the centre α). Coupling HPLC with both UV spectrometry and polarimetry, and having some optically pure samples at our disposal, thus enabled us to determine the absolute configuration of the photoisomers of deltamethrin. Additional information about the mechanism of photoisomerization was consequently deduced; we can assume that the benzylic carbon atom (α) has not undergone isomerization.
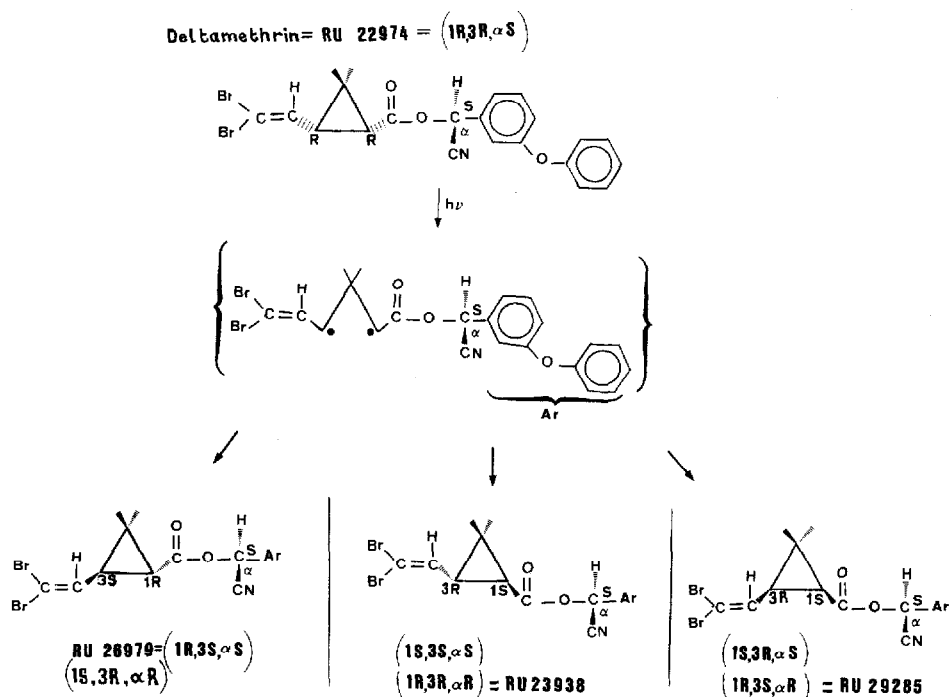

Fig. 1. Photoisomerization of deltamethrin.

EXPERIMENTAL

Materials

Deltamethrin (RU 22974) was obtained by chemical synthesis and is now commercially available*. Three of its diastereoisomers, all having the R configuration at C-1 $1R,3R,\alpha R$ (RU 23938), $1R,3S,\alpha S$ (RU 26979) and $1R,3S,\alpha R$ (RU 29285), were previously prepared by an unequivocal procedure[12]. They are combinations of $1R,3R$ (cis) or $1R,3S$ (trans) acidic moieties with either R or S cyanobenzylic alcohols.

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* Trade name, DECIS (Roussel Uclaf); previous trivial name, decamethrin.

Apparatus and procedures

Deltamethrin, as a thin, crystalline layer (0.1 mg/cm^2), was submitted to irradiation in a SUNTEST apparatus, equipped with a 150 klux xenon vapour lamp (both from Heraeus, Hanau, F.R.G.), which has an emission spectrum approximating that of the sun. After irradiation for 60 h, the resulting photoisomerization mixture was analysed.

The HPLC system consisted of a LiChrosorb Si 60, 5-μm column (250 mm × 4 mm I.D.) (Merck, Darmstadt, F.R.G.). an Altex Model 110A pump (Altex Scientific, Berkeley, CA, U.S.A.) and a Rheodyne 7125 injection valve (Rheodyne Cotati, CA, U.S.A.) with a 20-μl loop.

The detection systems consisted of a Varichrom UV 50 variable-wavelength UV detector (Varian, Palo Alto, CA, U.S.A.) and a Perkin-Elmer 241 LC polarimeter (Perkin-Elmer, Norwalk, CT, U.S.A.) equipped with a 0.65-mm I.D. microflow cell, having a 10-cm optical pathway. The latter was used at 302 nm* and the resulting recording range was ±0.5° full-scale. These two detectors were connected by a stainless-steel capillary tube.

The UV absorption and optical rotation data were fed into an SP 4200 data system (Spectra-Physdics, San Jose, CA, U.S.A.), which traced the corresponding curves and calculated the areas under the peaks.

Analytical conditions and reagents

The analytical flow-rate was 1.3 ml/min. The mobile phase consisted of 1900 ml of hexane, 45 ml of acetonitrile, 100 ml of pentane, 10 ml of dioxane and 1.5 ml of 2-propanol (spectrographic grade, Fluka, Buchs, Switzerland). This eluent was suitable for separating all the apolar components of the photoisomerization mixture. The polar photodegradation products were not investigated. The UV detector was used at 230 nm. Dimethyl isophthalate (Merck), having a UV absorption maximum at 226 nm, was used as an internal standard.

RESULTS AND DISCUSSION

Principle of the determination

The area, *s*, under each peak of the polarimetric curve measures the amplitude of the optical rotation (OR) and is therefore proportional to the amount, *m*, of the diastereoisomer and to its rotary power. When the compound is optically pure (single enantiomer) with a rotatory power α_0, then

$$s = k_1 m \alpha_0 \text{ (OR)}$$

The area, *S*, under each corresponding UV absorption (UV) peak depends only on the amount, *m*, of the compound:

$$S = k_2 m \text{ (UV)}$$

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* The 302 nm wavelength was selected from those provided by a mercury vapour lamp. The maximum of the optical rotatory dispersion for this kind of derivatives is located at 223–230 nm, and these wavelengths would have ensured greater accuracy.

In any case, the ratio s/S (OR/UV) is independent of the amount of the compound and, for a determined pure enantiomer, this ratio is a constant, which depends on the value of its α_0 rotatory power:

$$r = \frac{s}{S} = \frac{k_1 m \alpha_0}{k_2 m} = \frac{k_1}{k_2} \cdot \alpha_0 = k_3 \alpha_0$$

If the diastereoisomer detected by chromatography were a blend of its two enentiomers [in the proportions of $1 - x$ for the enantiomer of rotary power α to x for the antipode $(-\alpha)$], then the UV absorption would be identical, $S = k_2 m$, whereas the optical rotation would have been affected:

$$s' = k_1(1 - x)m\alpha_0 + xm(-\alpha_0)$$

$$= k_1 m(1 - 2x)\alpha_0$$

Therefore, the ratio s'/S would also have been altered:

$$r' = \frac{k_1 m(1 - 2x)\alpha_0}{k_2 m} = k_3(1 - 2x)\alpha_0$$

$$r' = r(1 - 2x); \quad r - r' = 2rx$$

The difference between the values of the ratios r and r' is directly proportional to the content x of the antipodal isomer. If it is equal to zero, then the diastereoisomer is optically pure, and is identical with the standard sample. If the r' ratio has the same absolute value, but is reversed ($r' = -r$), then $x = 1$. The diastereoisomer is optically pure, but is antipodal to the standard sample. If there was no optical rotation, $r' = 0$, then $x = 0.5$, and the diastereoisomer would be the racemic mixture of the enantiomers.

Accuracy of the method

The inaccuracy concerniong the value of the ratio r is a result of experimental errors in determining the values of the areas s and S. Optical rotation determinations are less precise than the UV absorption measurements, and as the value of s is directly proportional to the rotary power α_0 of the compound, the lower the rotatory power, the greater is the inaccuracy. For this reason, amplification of the OR signal has been advised[6]. A statistical study (ten analyses) of a mixture of optically pure samples indicated that the error in the ratio r may be in the range ± 5 to $\pm 10\%$, depending on the compound.

Results

The first practical experiment was the analysis of the artificial mixture of deltamethrin and some of its optically pure diastereoisomers[12] in equal amounts (except RU 26979, for which the amount was tripled because of its low rotatory power). These compounds [RU 22974 (deltamethrin), RU 23938, RU 26979 and RU 29285] were thought to be present in the photoisomerization mixture. On one hand, their retention times could be determined on the basis of the UV absorption curve, and
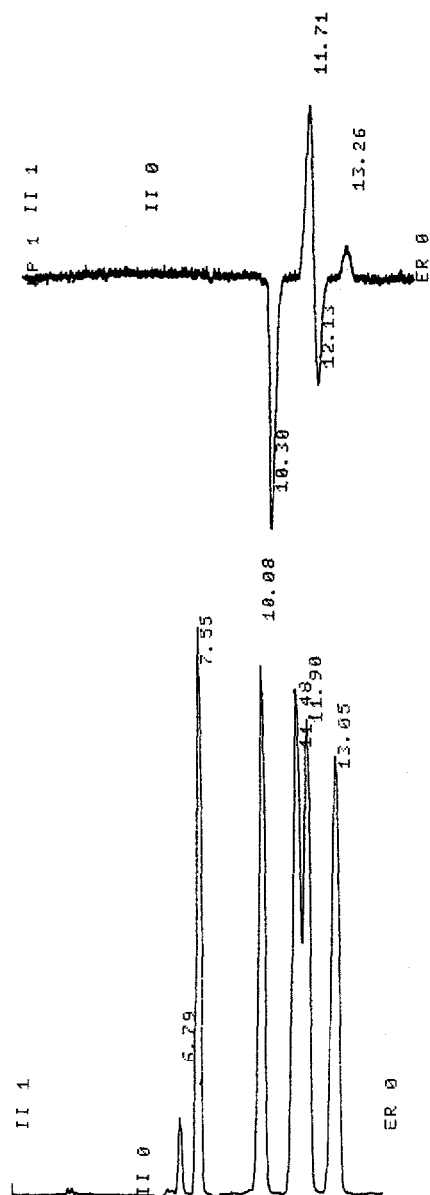
Fig. 2. UV absorption (230 nm) (bottom) and optical rotation (302 nm) (top) of the standard samples as a function of the retention time (min).

Compound	Retention time (min)	
	UV	OR
Unknown impurity	6.79	
Internal standard	7.55	
RU 23938	10.08	10.30
RU 22974	11.48	11.71
RU 29285	11.90	12.13
RU 26979	13.05	13.26

TABLE I

RATIOS r (OR/UV) OF DELTAMETHRIN PRODUCTS

Compound	Configuration	Retention time (min)	Ratio r*	Error (%)
Internal standard		7.6		
RU 22974	$1R,3R,\alpha S$	11.5	+0.32	+ 6.5
RU 23938	$1R,3R,\alpha R$	10.1	−0.42	+ 5
RU 26979	$1R,3S,\alpha S$	13.0	+0.08	+10
RU 29285	$1R,3S,\alpha R$	11.9	−0.16	+ 8.5

* Mean values of ten determinations.

on the other, the polarimetric recorder revealed that two of the samples (RU 22974)and RU 26979) are dextrorotatory, whereas the other two (RU 23938 and RU 29285) are laevorotatory (Fig. 2).

The ratio r (OR/UV) was calculated for each compound and the data are listed in Table I.

The second experiment, carried out under identical conditions, concerned the mixture resulting from the photoisomerization of deltamethrin. The analysis indicated the presence of four main components, A, B, C and D (Fig. 3), and revealed that their retention times correspond to those of the samples investigated in the first experiment.

The UV absorption curve gives an idea of their relative amount. The addition of an internal standard in both the first and the second experiments permits the quantitative determination of the amounts of each detected diastereoisomer. Their total equals 51.3% of the initial amount of deltamethrin. The calculated relative amounts are listed in Table II.

The simultaneously recorded optical rotation indicates that every component of the mixture is dextrorotatory (Fig. 3). The ratios r' (OR/UV) were calculated for the compounds A, B, C and D (Table II). Their absolute values are not significantly different from those corresponding to the standard samples. However, two of them are opposite in sign.

The close parallel between these and the previous data allows us to assume that, within the limits of experimental error, the retention times are equivalent and so are the absolute values of the ratio r (OR/UV).

TABLE II

RATIOS r' (OR/UV) OF DELTAMETHRIN PRODUCTS)

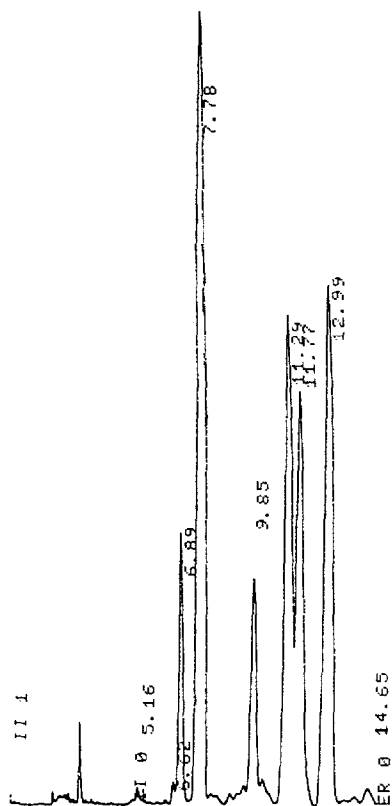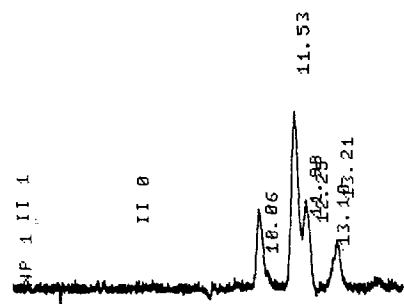| Compound	Retention time (min)	Ratio r'*	Relative amount (%)
Internal standard	7.8		
A	11.3	+0.33	20.7
B	9.9	+0.42	6.2
C	13.0	+0.07	56.7
D	11.8	+0.16	16.5

* Mean values of three determinations.

Fig. 3. UV absorption (230 nm) (bottom) and optical rotation (302 nm) (top) of the photoisomerization products as a function of the retention time (min).

Compound	Retention time (min)	
	UV	OR
Unknown impurity	6.89	
Internal standard	7.78	
B	9.85	10.06
A	11.29	11.53
C	11.77	11.98
D	12.99	13.10

TABLE III

ABSOLUTE COMFIGURATIONS OF DELTAMETHRIN PRODUCTS

Component	Absolute configuration
A	$1R,3R,\alpha S$
B	$1S,3S,\alpha S$
C	$1R,3S,\alpha S$
D	$1S,3R,\alpha S$

CONCLUSIONS

Taking into account the sign of the ratio r', it can be deduced that two components of the photoisomerization mixture are likely to be identical with the standards: A corresponds to RU 22974 (deltamethrin) and C to RU 26979. The two other components are likely to be the antipode of the other two standards: B corresponds to the antipode of RU 23938 and D to the antipode of RU 29285. The absolute configurations of the four components are then likely to be those indicated in Table III.

In each of them the S configuration of the benzylic carbon atom is maintained, while the two asymmetric centres, 1 and 3, may exist in both the R and S configurations. This observation leads to the following conclusions concerning the photochemical pathways of isomerization. Racemization of the asymmetric centres 1 and 3 occurs and probably results from the opening and re-cyclization of the cyclopropane ring, as suggested previously[10,11].

The lack of photoisomers with the R configuration at the benzylic carbon atom negates the question of possible racemization of the alcohol group. This question could not have been answered whilst the detection method was not suitable for characterizing enantiomers. HPLC, coupled with UV spectrometry and polarimetry, is suitable for resolving this kind of problem. Some technical improvements, especially with regard to the light source of the polarimeter, should improve the accuracy of the method.

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