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COUPLING A HIGH-PERFORMANCE LIQUID CHROMATOGRAPH WITH A LIQUID SCINTILLATION DETECTOR: EXAMPLE IN THE FIELD OF PYRETHROIDS

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SUMMARY

The resolving power of high-performance liquid chromatography (HPLC) and the high specificity and sensitivity of in-line scintillation detection (SD) were used to study the transformation of tralomethrin, a new synthetic pyrethroid. It was believed that the insecticidal activity of tralomethrin resulted from its transformation to the active deltamethrin. Radiolabelled tralomethrin was first incubated with two different types of excitable membranes. The samples were then analysed by HPLC–SD and shown to contain intact tralomethrin. These data and electrophysiological experiments were used to rule out deltamethrin as the cause of insecticidal activity in tralomethrin.

INTRODUCTION

Radiolabelled molecules are useful tools for investigating the disposition of pharmaceuticals in animal organisms. However, although the radioactivity permits the detection of minute amounts of a compound, it is not suitable for specifying whether the compound is transformed or not. Indeed, the fate of the compound depends on its possible biotransformation. After such a transformation, the analysis of the reaction mixture is effected by means of a chromatographic procedure that allows the separation and, possibly, the identification of the transformed products. Thin-layer chromatography, followed by either autoradiography or liquid scintillation counting, may be used in such instances[1–3]. A few examples[4,5] highlight the advantages of coupling high-performance liquid chromatography (HPLC) with a radioactivity detector able to monitoring continuously the eluate from the chromatographic column,

Tralomethrin {(*S*)-α-cyano-3-phenoxybenzyl-(1*R*,3*S*)-2,2-dimethyl-3-[(*R*,*S*)-1,2,2,2-tetrabromoethyl]cyclopropane carboxylate} (RU 25474), a new pyrethroid insecticide, consists of two isomers, RU 24784 and RU 24785. Three of its four asymmetric carbon atoms have a well defined configuration, 1*R*,3*S*,α*S* (the same as in deltamethrin[6]), whereas the fourth may exist in either the *R* (RU 24784) or the *S* (RU 24785) configuration (Fig. 1).

The insecticidal activity of tralomethrin was suspected to result from its pos-

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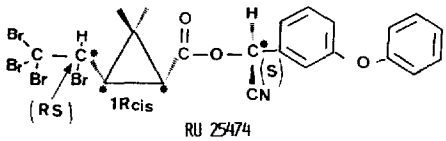

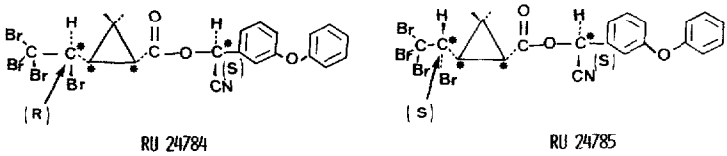

Fig. 1. Structures of the two epimeric components of tralomethrin.

sible transformation to deltamethrin[1,2,7]. In this study, radiolabelled tralomethrin, bound to the abdominal nerve cords of the American cockroach (*Periplaneta americana*), was analysed by means of HPLC coupled with liquid scintillation detection (SD). It was first observed that tralomethrin intoxication behaves differently from that by deltamethrin[8]. Here, the lack of biotransformation of tralomethrin is confirmed. Indeed, the neurotoxic activity of compounds, *e.g.*, pyrethroids, and in this instance tralomethrin, has been studied *in vitro* with two different excitable membranes: insect axonal membrane and mammalian neuroblastoma cells in culture[8]. For a precise knowledge concerning the fate of tralomethrin, we have used the radiolabelled compound. Analysis of these preparations by HPLC–SD allowed us to characterize and identify this compound unequivocally.

EXPERIMENTAL

Materials

Tralomethrin (RU 25474) was obtained by chemical synthesis[9]. The ^{14}C compound was labelled at the two *gem*-methyl groups of the cyclopropane ring (59.3 mCi/mmole).

[^{14}C]Deltamethrin was also labelled at the two *gem*-methyl group of the cyclopropane ring (60 mCi/mmole).

The internal standard was commercially available [^{14}C]β-naphthol (CMM 218) (Commissariat à l'Énergie Atomique, Saclay, France).

Apparatus and procedures

The HPLC apparatus consisted of an M380 Chromatem pump (Touzart et Matignon, Berkeley, CA, U.S.A.), a Rheodyne 7125 injector with a loop of 20 μl and a LiChrosorb Si 60, 5-μm column (25 cm × 0.25 in. I.D.) (Merck, Darmstadt, F.R.G.). The Flo-one/DR (Radiomatic Instrument & Chemical Co., FL, U.S.A.) liquid scintillation detector was connected to the column outlet; keyboard operation permitted the selection of pump flow-rate and scale multiplier. The flow-rate of the pump was adjustable between 0.1 and 10 ml/min in order to provide the optimal flow. A microprocessor processed the data from scintillation counting and provided the results by printing the number of counts and drawing a curve on a chart recorder.

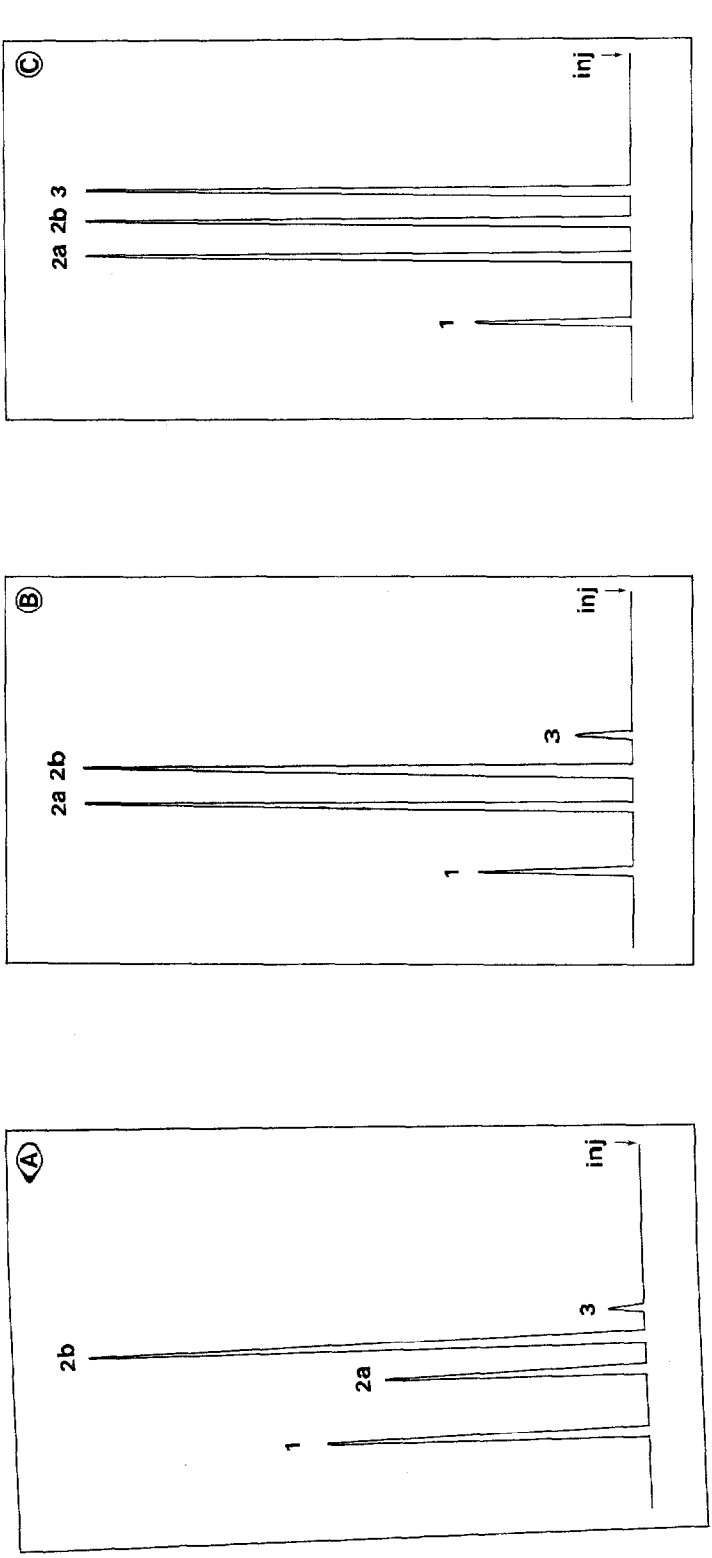

Fig. 2. Separation of tralomethrin (two epimers) by HPLC coupled with a liquid scintillation detector. Peaks: 1 = internal standard, [^{14}C]β-naphthol; 2a and 2b = pure [^{14}C]tralomethrin epimers; 3 = [^{14}C]deltamethrin (pre-existing impurity). (A) Chromatographic profile of the medium before incubation with nerve cord cells. 1, 6 μCi; 2, 9.9 μCi; 3, 0.4 μCi. (B) Chromatographic profile of the extract obtained 1 after incubation with the medium analysed in A. 1, 0.2 μCi; 2, 2.1 μCi; 3, 0.1 μCi. (C) Chromatographic profile of the nerve cord extract (B), showing an increase in the [^{14}C]deltamethrin peak after addition of this compound.

Analytical conditions

The eluent (spectrographic grade materials from Fluka, Buchs, Switzerland) consisted of 1700 ml of hexane, 140 ml of pentane, 25 ml of acetonitrile, 45 ml of dioxane, 15 ml of 2-propanol and Lumaflow I fluid scintillator (Kontron, Zurich, Switzerland) at a flow-rate of 1 ml/min. The flow-rate of the Chromatem pump was 0.6 ml/min.

Under these conditions, the sensitivity of this method was 20 pCi/mmole, corresponding to 15 pmole of tralomethrin applied to the column.

RESULTS

Analysis of [^{14}C]tralomethrin (RU 25474), extracted from the incubation medium prior to its application to the cockroach nerve cells, showed the two diastereoisomeric constituents (RU 24784 and RU 24785, 2a and 2b) (Fig. 2A). It also revealed a low content (about 4%) of an impurity, 3, with a retention time identical with that of deltamethrin. This impurity is probably the result of the preparation method of tralomethrin.

After incubation with cockroach nerve cords for 1 h and extraction, the chromatographic profile of RU 25474 (Fig. 2B) was not altered, and the impurity content (about 4.5%) was not significantly modified. As the addition of [^{14}C]deltamethrin results in an increase in the impurity peak (Fig. 2C), it is assumed to be due to that compound.

CONCLUSIONS

From these results, it can be deduced that no significant biotransformation of tralomethrin molecules occurs when the latter are applied to cockroach nerve cords for 1 h. As it has been demonstrated that tralomethrin acts in less than 1 h and as the electrophysiological response to tralomethrin intoxication differs noticeably from that of deltamethrin, it can be concluded that the low amount of deltamethrin present in tralomethrin as an impurity cannot be held responsible for the insecticidal activity of tralomethrin.

This study exemplifies the ability of HPLC, coupled with liquid scintillation detection, to differentiate between diastereoisomers with regard to their retention times and to quantify them accurately on the basis of their radioactivity.

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