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Synthesis of [^{3}H]Cyfluthrin

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Summary

For the elucidation of the distribution and biotransformation of Cyfluthrin in tse-tse flies the tritium-labelled compound was needed. A brominated precursor was dehalogenated with tritium gas using a special noble metal catalyst to give the desired [^{3}H]Cyfluthrin. The labelling position was confirmed by ^{3}H-NMR spectroscopy. After synthesis and purification, the [^{3}H]Cyfluthrin obtained showed a specific activity of 23.7 Ci/ mmol (877 GBq/mmol).

Key words: [^{3}H]Cyfluthrin, tritium labelling, dehalogenation.

Introduction

Cyfluthrin is a synthetic insecticide from the pyrethroid class. The compound has three chiral C-atoms (1, 3 and α) and is consequently a mixture of 8 diastereomers. Two of these, the 1R, 3R, αS- and the 1R, 3S, αS-diastereomer are responsible for the insecticidal activity (see fig. 1) [1].

Cyfluthrin can be separated by column chromatography on silica gel into four racemates (I-IV):

Racemate I	:	1R, 3R, α R	+	1S, 3S, α S
Racemate II	:	1R, 3R, α S	+	1S, 3S, α R
Racemate III	:	1R, 3S, α R	+	1S, 3R, α S
Racemate IV	:	1R, 3S, α S	+	1S, 3R, α R

For a study of the distribution and biotransformation in tse-tse flies tritium labelled Cyfluthrin was needed. Its synthesis, purification and analytical identification are described in the paper.

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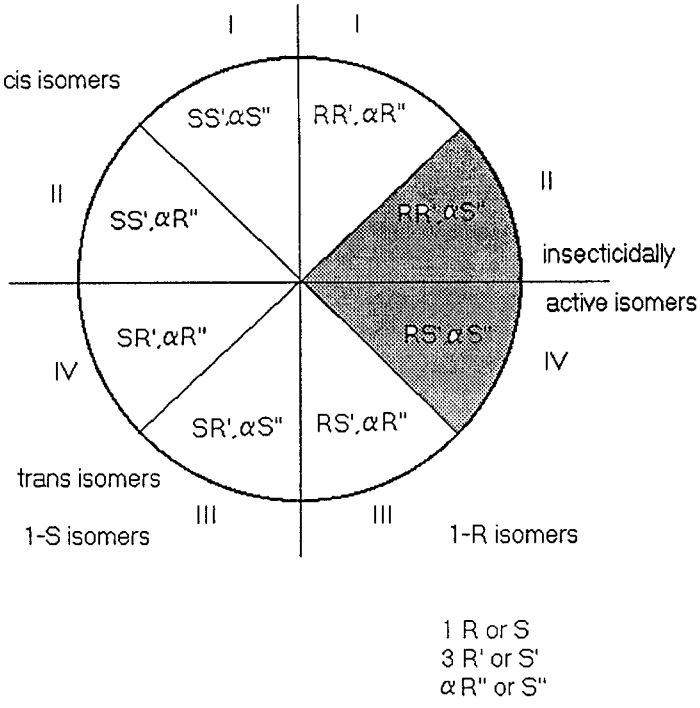
Figure 1: Stereochemical relations of the eight diastereomers of Cyfluthrin.

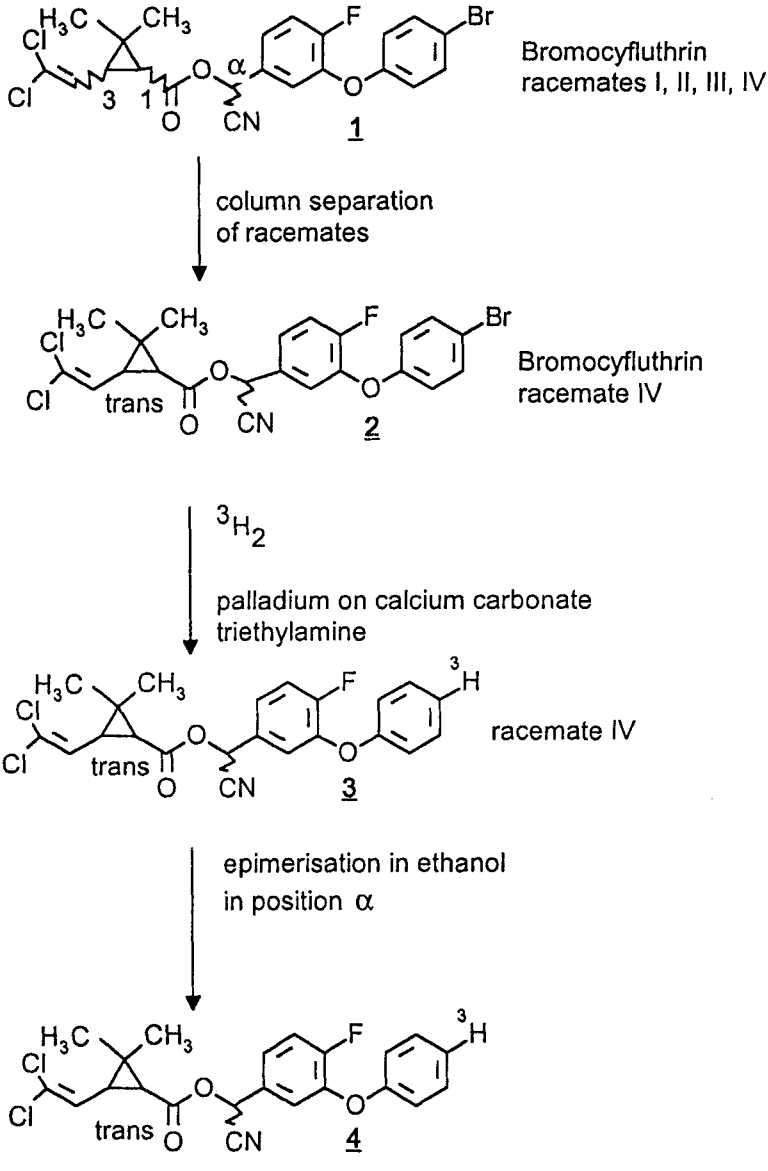


Results and discussion

The route chosen for the radiosynthesis of Cyfluthrin is shown in the reaction scheme. Starting from the brominated precursor **1** the radiolabel should be introduced by catalytic dehalogenation. The precursor **1** consists of the four racemates I - IV. When using this precursor containing the above mentioned racemates in our first dehalogenation reactions, we found a very complex reaction mixture, due to incomplete dehalogenation of the racemic mixture as well as side reactions. This complex mixture did not allow us to determine the optimal hydrogenation conditions. Therefore, we decided to simplify the reaction by using only the racemate IV. This decision was supported by the fact, that the corresponding debrominated racemate IV is the main component of the commercial product Beta-Cyfluthrin (Bulldock®) and contains one of the two biologically active enantiomers. Racemate IV **2** was obtained by a complex chromatographic separation procedure from bromocyfluthrin **1**.

In several experiments, using deuterium, the optimal reaction conditions for the dehalogenation were determined. Undesired side reactions were always observed independent of the chosen reaction conditions. To limit the side reactions, the dehalogenation had to be stopped before complete reduction of the halogenated precursor had taken place. In the presence of triethylamine, palladium on calcium carbonate proved to be the best catalyst for the dehalogenation of bromocyfluthrin. The resulting crude product contained 23 % [^{3}H]Cyfluthrin (racemate IV) **3**. The reaction mixture was separated by HPLC. After purification, the specific activity was found to be 23.7 Ci/mmol (877 GBq/mmol), corresponding to 82 % labelling

Scheme



[^{3}H]Cyfluthrin (mixture of racemates III and IV)

at position 4 in the aromatic ring (see reaction scheme). The degree of incorporation was the same as in the deuteration experiments. The total activity was 60.9 mCi (2253 MBq) in a total amount of 1.12 mg [^{3}H]Cyfluthrin, **3**.

For the application of [^{3}H]Cyfluthrin to tse-tse flies the radioactive substance had to be dissolved in ethanol with a radiochemical concentration of 1.0 mCi/ml (37 MBq/ml). A check of the radiochemical purity by HPLC (see fig. 4) showed two peaks with retention times

identical to those of the Cyfluthrin racemates III and IV. Indeed it is known [1], that in protic solvents like ethanol the α-C-position can epimerize yielding the corresponding diastereomer. Thus, the pure racemate IV forms a mixture of racemates III and IV, as can be seen in the radiochromatogram (fig. 4). If an ethanol solution of the racemate IV is stored over two days the ratio of III and IV is 1:1.

The labelling position was checked by ^{3}H-NMR spectroscopy. The ^{1}H-decoupled ^{3}H-NMR spectrum shows a doublet at 7.17 ppm (see fig. 2). This is in accordance with the fact that the corresponding proton signal of the aromatic position 4 has the same chemical shift. The small splitting-up of the signal is caused by the epimerization to the racemate III, occuring in dimethyl sulphoxide too. The ^{1}H-coupled spectrum shows a triplet caused by coupling with the two adjacent protons (fig. 3). The splitting-up attributed to both of the racemates (II and IV) can also be seen in the spectrum (see fig. 3).

Experimental

Synthesis of 1-[α-Cyano-4-fluoro-3-[4-^{3}H]phenoxybenzyl]-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate, [^{3}H]Cyfluthrin, 4

Before tritium labelling racemate IV 2 was separated from the bromocyfluthrin 1 under the following conditions: column: Lichrosorb® Si 60, 7 μm, 250 x 25 mm (Merck, D-6100 Darmstadt); eluent: n-heptane + dioxan = 1000 + 20, (v + v); flow rate: 15 ml/min, UV detection at 254 nm. 700 mg 1 were separated in 3 equal portions. The eluate containing 2 was fractionated on the basis of the UV-signals. After evaporation of the solvent 160 mg of the raw material 2 were obtained. Due to the unsufficient purity of this material the separation was repeated under the following conditions: column: Lichrospher® Si60, 10 μm, 250 x 10 mm (Merck, D-6100 Darmstadt); eluent: n-heptane + dioxan = 1000 + 20, (v + v); flow rate: 6 ml/min; UV detection at 254 nm. The eluate containing racemate IV was evaporated yielding 132 mg 2. The purity was > 99 % determined by GC-FID and HPLC. The structure was confirmed by ^{1}H-NMR spectroscopy.

10 mg (19.5 μmol) 2 were stirred for 35 minutes in a tritium-labelling apparatus [2] with 13 mg palladium on calcium carbonate (5 % Pd) in a mixture of 1 ml dioxan and 10 μl triethylamine with 7.8 Ci (288.6 GBq) tritium gas at a pressure of 52.4 kPa. After readsorption of non-incorporated tritium the strongly radioactive solvent was adsorbed in a cold trap packed with active carbon. The dry residue was taken up in 0.5 ml dioxan and the catalyst was filtered off using a Millex®-FH_{13} filter (Waters Millipore GmbH, D-6236 Eschborn). The catalyst was washed with 0.5 ml dioxan and the combined filtrates were freeze-dried. To remove the exchangeable tritium, the raw material was dissolved in 1 ml of a 3 + 1 mixture of dioxan and methanol and subsequently freeze-dried. This procedure was repeated five times.

The dry crude product was taken up in a mixture of 0.3 ml dioxan and 0.7 ml hexane and purified by HPLC in 10 equal portions under the following conditions: column: Lichrosorb® Si 60, 7 μm, 250 x 10 mm (Merck, D-6100 Darmstadt); eluent: n-heptane + dioxan = 1000 + 20 (v + v) ; flow rate: 5 ml/min; UV detection at 228 nm; retention time for [^{3}H]Cyfluthrin 3: = 27.0 minutes.

The eluates containing [³H]Cyfluthrin were fractioned on the basis of the UV signal and the fractions were combined (total amount = 48.7 ml). The total activity in the solution was determined to be 60.2 mCi (2227 MBq). The solution was evaporated to dryness under vacuum and redissolved in 11.9 ml ethanol to give a stock solution. The concentration of [³H]Cyfluthrin (racemates III and IV) 4 in this solution was determined by comparing the UV-absorbance of solutions with known Cyfluthrin concentrations. By this method the total quantity was determined to be 1121 μg (2.6 μmol) [³H]Cyfluthrin 4 corresponding to 13.3 % of the theory. The calculated specific activity was 23.7 Ci/mmol (877 GBq/mmol). To minimize radiolysis, the radioactive substance was stored in ethanol with a concentration of 1.0 mCi/ml (37 MBq/ml) in a refrigerator at 4 °C.

Purity test:

The purity test for [³H]Cyfluthrin 4 was performed under the following conditions: LiChrosorb® Si 60, 5 μm, 250 x 4 mm (Merck, D-6100 Darmstadt); solvent: n-heptane + dioxan 1000 + 20 = (v + v); flow rate: 1 ml/min; UV detection at 230 nm (UV 100 detector, Varian GmbH, D-6100 Darmstadt); radioactivity detector Ramona®4, (Raytest, D-7541 Straubenhardt); retention time of racemate III ≙ 24.07 minutes and racemate IV ≙ 26.36 minutes. 10 μl of the stock solution were injected corresponding to 52.3 μCi (1.9 MBq).

Under these conditions the radiochemical purity of racemate III was > 30.8 % and of racemate IV > 68.9 %. The addition of the two peaks (30.8 % + 68.9 %) gives a total purity of > 99.7 % for [³H]Cyfluthrin 4 (see fig. 4). Due to the high specific activity the UV signal was very low. Thus it was impossible to determine the chemical purity.

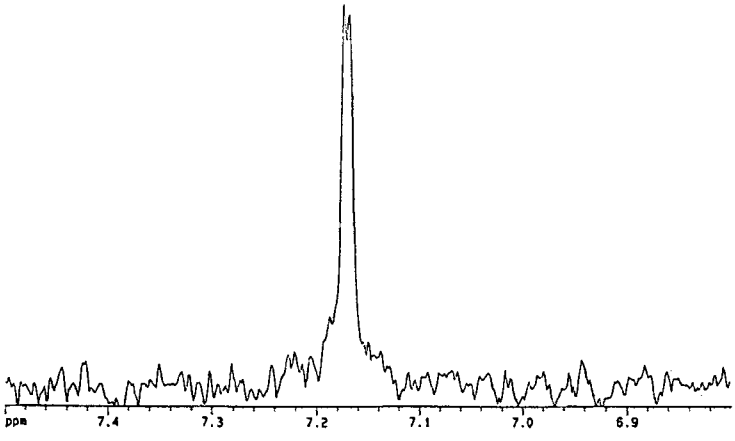


Figure 2: ^{1}H-decoupled ^{3}H-NMR spectrum of [³H]Cyfluthrin 4.

Tritium-NMR spectroscopy:

The tritium-NMR spectrum was recorded using a Bruker AM 300 nuclear magnetic resonance spectrometer with a selective measuring head. The measurement frequency was 320.136 Hz. A ^{1}H-decoupled and a ^{1}H-coupled spectrum were recorded. As already described [3], the standard was an artificial reference signal derived from the ^{1}H resonance of tetramethylsilane. 3 ml (15.7 mCi) of the stock solution of [^{3}H]Cyfluthrin 4 was lyophilized and the dry residue was dissolved in 200 μl [D_6]dimethylsulfoxide and transferred into the measuring cell as already described. To record the ^{1}H-decoupled spectrum, 320 interferograms were accumulated (pulse angle 7 μsec $\triangleq$ 90°, total measuring time 16 minutes, digital resolution = 0.492 Hz/pt). To record the ^{1}H-coupled spectrum, 2612 interferograms were accumulated (total measuring time 132 minutes).

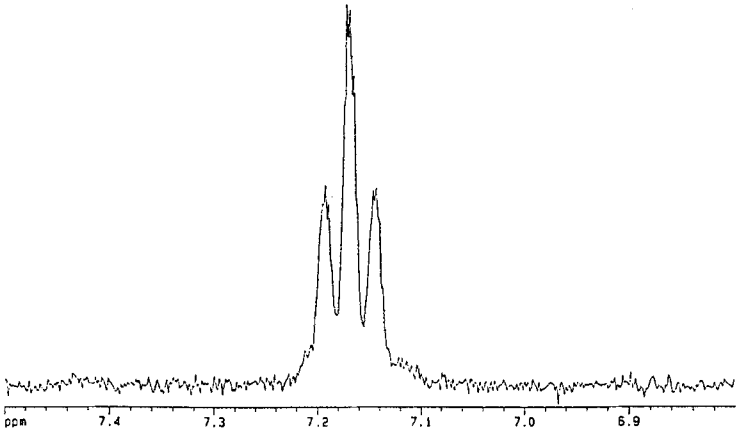


Figure 3: ^{1}H-coupled ^{3}H-NMR spectrum of [^{3}H]Cyfluthrin 4.

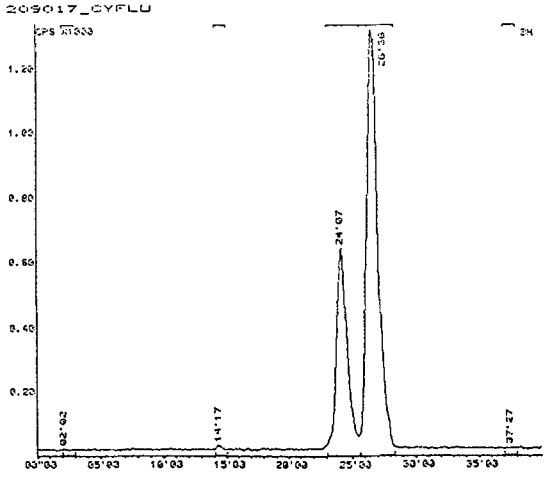


Figure 4: Check of radiochemical purity of [^{3}H]Cyfluthrin 4.

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