THE ENANTIOSELECTIVE TRANSFORMATION OF THE CHIRAL INSECT REPELLENT BAYREPEL® AND ITS MAIN METABOLITE BAYREPEL ACID IN THE AQUATIC ENVIRONMENT

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Abstract
The enantioselective transformation of the chiral insect repellent BAYREPEL® and the formation/further transformation of its main metabolite Bayrepel acid were studied by spiking water samples from the Alster Lake, Hamburg, Germany. Enantioselective gas chromatographic analyses of the water extracts after different periods revealed a preferential transformation of the 2nd and 4th eluting BAYREPEL® enantiomers and an enrichment of the 1st and 3rd eluting enantiomers of the metabolite, be it preferential formation of the first eluting metabolite enantiomers or subsequent faster transformation of the second eluting metabolite enantiomers. A crucial experiment involving spiking the water samples with the metabolite suggests a strong contribution of the latter process.

Introduction

In many parts of the world dangerous diseases, such as malaria, yellow fever, west-nile virus, borreliosis (Lyme Disease) and tick-borne encephalitis are transmitted by mosquitoes, flies, ticks and other insects. In statistical terms, one person dies every 30 seconds from the complications of an insect sting or bite. Malaria alone causes up to 3 million fatalities every year. Attempts all over the world to control or even eradicate insects have been largely unsuccessful and in most cases have probably caused more harm to the environment than to the insects. Methods such as prophylactic medication and vaccination can certainly reduce the risk of contracting insect-born diseases, but they are expensive, specific to individual types of insect and ultimately protect against the disease, but not against the insect bites that cause them. In addition, more and more of the causative agents implicated in diseases are becoming resistant to drugs, and even preventive medication can no longer provide full protection. Repellents like BAYREPEL® (Fig. 1; registered trademark of Bayer AG, Leverkusen, Germany) overcome these disadvantages. They provide effective, convenient and reliable protection against insect bites and stings during outdoor activities. As a result, repellents have developed into a major market segment for consumer-orientated health products in many parts of the world. Once these products have been applied to the skin, they provide hours of protection against mosquitos, flies and ticks. Today *N*,*N*-diethyltoluylamide (DEET) is still the most widely used insect repellent, but BAYREPEL®, a product with an improved mechanism of action and its cosmetic properties, is catching up fast and is thus found in the environment in notable concentrations.[1-7]

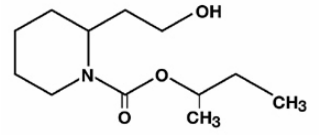

Figure 1: The insect repellent BAYREPEL®
IUPAC name: 1-methylpropyl 2-(2-hydroxyethyl)-1-piperidinecarboxylate

In the present work the fate of BAYREPEL®, after application, in the aquatic environment is investigated by enantioselective chromatography that facilitates discrimination between enzymatic and non-enzymatic transformation processes.

Materials and Methods

Water samples were taken from the Alster Lake located in the centre of Hamburg, Germany, and spiked with BAYREPEL® or its main metabolite Bayrepel-acid, respectively. The transformation of these compounds by the natural mixed microbial community was investigated by extraction of partial aliquots after different periods of time. The analysis of the extracts was performed by enantioselective gas chromatography using a chiral stationary phase. Details of the exact experimental conditions are specified in the Figure captions.

Results and Discussion

In Figure 2 the transformation of the parent compound BAYREPEL® can be followed in three steps (start of the experiment, after 6 days and 1 month), while the enantioselective formation and/or transformation of the

metabolite Bayrepel acid can be inferred from Fig. 3. After about one month (Fig. 2c) the preferential transformation of the 2nd and 4th eluting BAYREPEL® enantiomers becomes obvious. It is worth noting that the

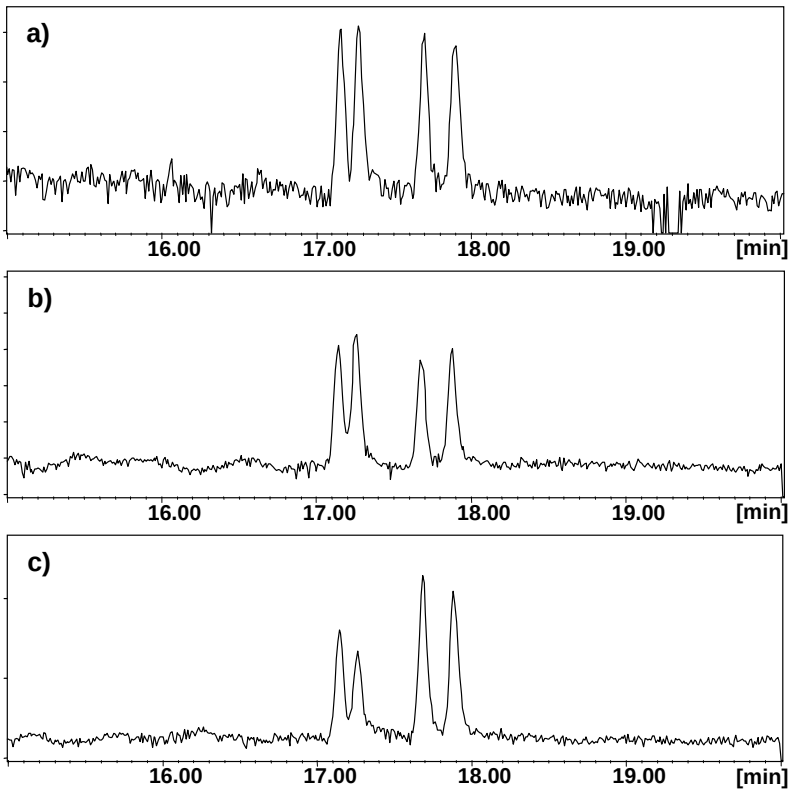

Figure 2: Enantioselective analysis of water sample extracts spiked with Bayrepel®, stored at room-temperature: 2a) sample at t = 0, 2b) sample after 6 days, 2c) sample after 1 month; stationary phase: Hydrodex-ß-6TBDM (25 m*0.25 mm*0.1 µm); temperature program: 70 °C (2 min), 10 °C/min, 180 °C (7 min)

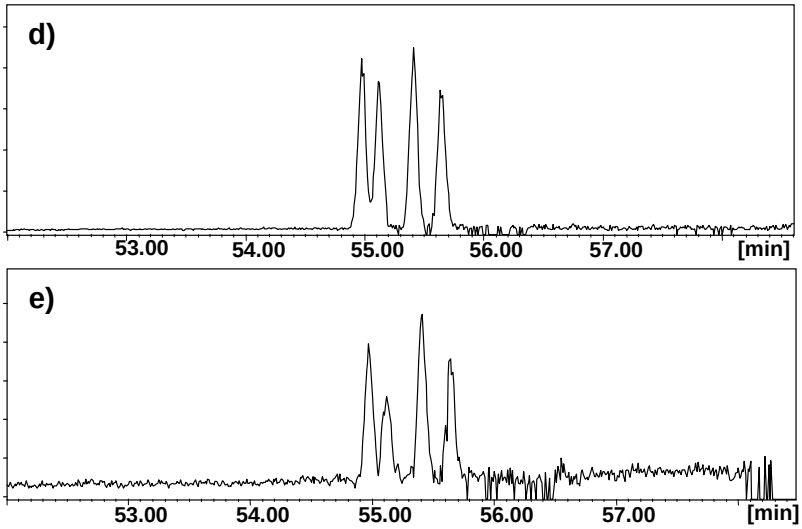

Figure 3: Enantioselective analysis of Bayrepel-acid in extracts of water samples spiked with Bayrepel®, stored at room- temperature: 3d) after 6 days, 3e) after 1 month; stationary phase: Hydrodex-ß-6TBDM (25 m*0.25 mm*0.1 µm); temperature program: 70 °C (15 min), 2.5 °C/min, 180 °C (1 min)

sum of the enantioselective formation and subsequent further metabolisation of Bayrepel acid also shows the same characteristics (Fig. 3e), i.e., the 1st and 3rd eluting enantiomers show the higher concentrations. In this connection it has to be taken into account that two stereogenic centres are present both in BAYREPEL® and in its metabolite Bayrepel acid, thus in both cases resulting in two diasteromeric pairs of enantiomers and, as a consequence, in four peaks in the gas chromatogram. The exact assignment of the four isomers is still under investigation. In order to answer the question regarding the potential contribution of the further transformation of the metabolite Bayrepel acid, a separate experiment was started by spiking the Alster Lake water with the metabolite and analysing the transformation process of this metabolite. As shown in Fig. 4h, the transformation of the metabolite by the microbial community of the Alster Lake reflects a clear structural preference of the 1st eluting enantiomers.

In order to get a clearer answer with regard to the long-term fate of BAYREPEL® in the aquatic environment, the transformation experiments were repeated over a 5 month period, but at lower winter temperatures. The results for water samples spiked with BAYREPEL® are shown in Figure 5, while the respective results for water samples spiked with Bayrepel acid are summarised in Figure 6. It is apparent that the preferential tranformation, as observed in the shorter-term experiments, of the second and fourth eluting BAYREPEL® enantiomers and the preferential formation/transformation characteristics of Bayrepel acid as reflected by the enrichment of the first and third eluting enantiomers is strongly enhanced over the longer term.

References

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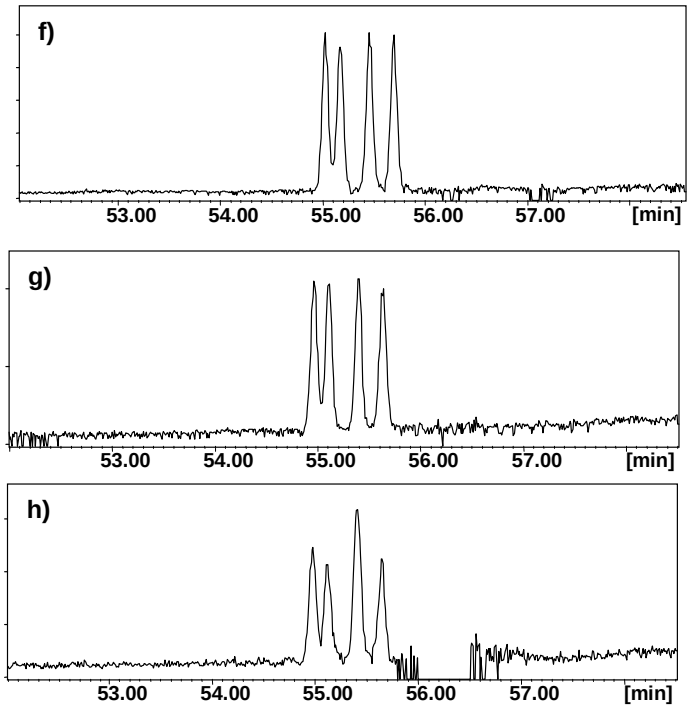

Figure 4: Enantioselective analysis of extracts of water samples spiked with Bayrepel-acid, stored at room temperature: f) sample at the beginning, g) sample after 6 days, h) sample after 1 month; stationary phase: Hydrodex-*ß*-6TBDM (25 m*0.25 mm*0.1 µm); temperature program: 70 °C (15 min), 2.5 °C/min, 180 °C (1 min)

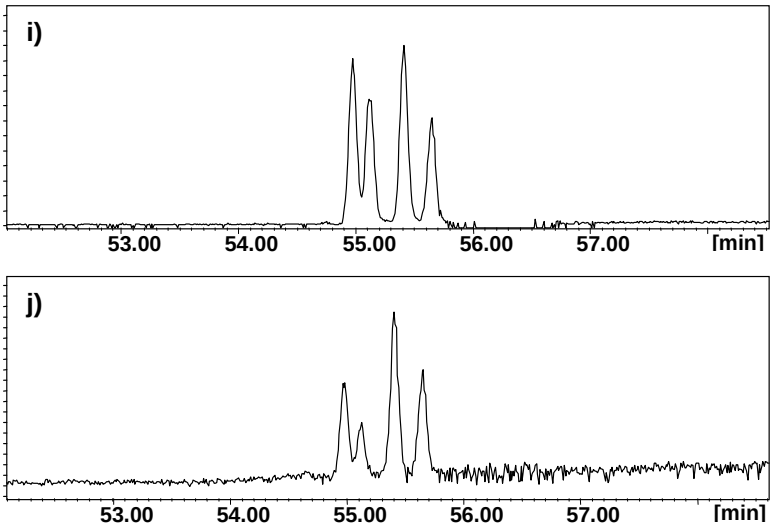

Figure 5: Enantioselective analysis of Bayrepel-acid in extracts of water samples spiked with Bayrepel®, stored at 0-7 °C: i) after 10 weeks, j) after 5 month; stationary phase: Hydrodex-*ß*-6TBDM (25 m*0.25 mm*0.1 µm); temperature program: 70 °C (15 min), 2.5 °C/min, 180 °C (1 min)

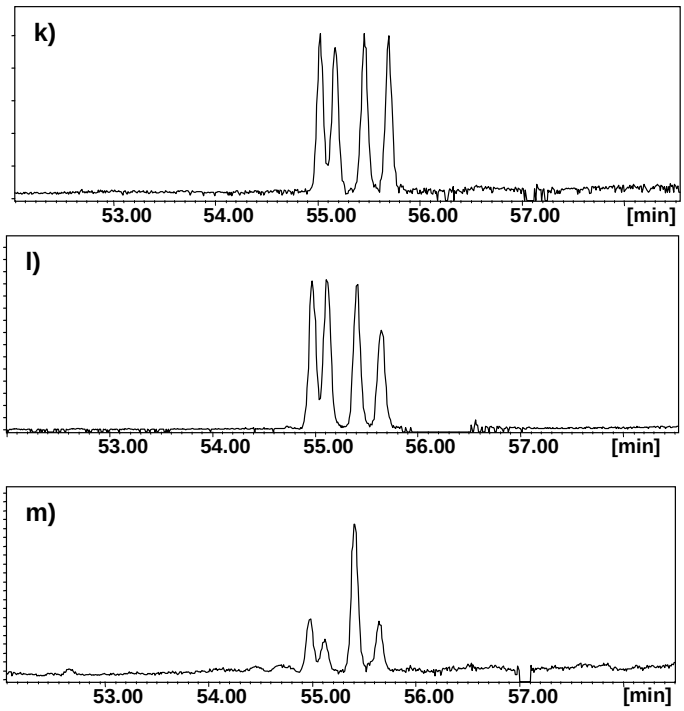

Figure 6: Enantioselective analysis of extracts of water samples spiked with Bayrepel-acid, stored at 0-7 °C: k) sample at the beginning, l) sample after 10 weeks, m) sample after 5 month**;** stationary phase: Hydrodex-*ß*-6TBDM (25 m*0.25 mm*0.1 µm)**;** temperature program: 70 °C (15 min), 2.5 °C/min, 180 °C (1 min)