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PREDICTION OF BIODEGRADABILITY FROM STRUCTURE: IMIDAZOLES*

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A project for the development of Structure–Activity Relationship for Biodegradation is presented. The aim of the project is to assemble sets of structural rules governing the potential microbial degradability of (classes of) chemicals. These rules will provide tools to take into account the biodegradation aspects of a product—and all precursors in the production process—early in the product development. The modeling concept is to take all experimental biodegradation data available and combine structural trends in the data with mechanistical information from degradation pathways. The rules that are derived should give insight into the possibility of biodegradation for specific classes of chemicals, thereby revealing why a compound is biodegradable or not.

For the class of imidazole derivatives such rules are derived, and a model degradation mechanism is proposed in analogy to the urocanate-hydratase mechanism from histidine metabolism. The model is validated using 12 imidazole-compounds, which are all predicted correctly to be poorly biodegradable. It is demonstrated that both data analysis and information on enzymatic reaction mechanisms are necessary to yield valid Structure–Biodegradation Relationship.

Keywords: Structure–biodegradation relationships; Imidazoles; Histidine; Urocanate-hydratase

INTRODUCTION

Biodegradability highly determines the persistence of chemicals in our environment. The ability of ambient microorganisms to utilize chemicals as nutrient sources secures the transformation of many man-made chemicals that enter the environment. If and how a chemical is biodegraded in the environment will often determine, for a large part, the overall environmental fate of a chemical. The potential of a chemical to be biodegraded also determines whether production process wastes can be treated in an industrial waste-water treatment plant, or have to be treated with other (often more cost-intensive) chemical waste processing methods. Testing the biodegradability of compounds however, is tedious and time-consuming, and negative results in a specific (standardized) test do not necessarily mean that a compound is not biodegradable. This makes it difficult (time- and cost-intensive)

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to take the biodegradability of research candidates for new products into account at a very early stage of product development. Therefore, reliable prediction of the biodegradability in the environment of chemicals, based on their chemical structure, would be very useful in risk assessment and environmental fate analyses of existing chemicals. Even more convenient would these predictions be in the development of new chemicals and production processes, where models can give information about chemicals that have not even been synthesized yet.

A BASF project has therefore been initiated, to gather all available information on biodegradation behavior of chemicals—test results as well as metabolism information—and to look for trends in the data for specific classes of chemicals. External partners are the Laboratory for Microbial Ecology from the University of Konstanz, and the Laboratory for Toxicology and Ecotoxicology from the University of Trier. It is not the aim of the project to develop general, statistical models with a broad applicability, which give a certain probability that a chemical will be biodegradable or not biodegradable in a specific test, since such models have already been the subject of much research in the past [1–5]. Such models do not fulfill the specific need for more fundamental understanding of biodegradability [1–3], which is required in the selection and proposal of research candidates in the development of new products.

The aim of the project is therefore to develop mechanistically based (substructure) models for classes of chemicals which are thought to biodegrade via a common degradation pathway or mechanism. As an example of our approach, one of these newly developed models is presented here, for the class of imidazole compounds.

BIODEGRADATION DATABASE

For this project, a database has been assembled gathering all available data on biodegradation test results, physico-chemical data and metabolic pathway information. This database contains at the moment information on almost 5000 compounds, with chemical structure assigned to over 4000 compounds of these, and the results of more than 15,000 separate biodegradation tests. Metabolic information comes from single literature references as well as from combined available resources such as the Boehringer Metabolism Pathways‡ and the Bioremediation and Biodegradation Database§. The database can be searched on substructures and chemical similarity, making it easy to assemble homologous series of chemicals, which can then be analyzed on biodegradation trends.

For validation purposes the German environmental protection agency (UmweltBundesAmt [6]) has provided the project with a database of 1363 compounds with their chemical structures and biodegradation test results, which were provided for the registration of new chemicals on the German market in the past years. These data are confidential, and are therefore only used to validate our findings within the project. The data (structure and biodegradation test results) can not be shown.

DATA ANALYSIS

A search for imidazole derivatives in our database yielded 23 compounds containing an imidazole ring. Two of these were dyes and one was a polymer. Since these compounds are

‡Boehringer Mannheim Biochemical Pathways on the ExPASy server, Geneva, Switzerland. (http://www.expasy.ch/cgi-bin/search-biochem-index)

§The University of Minnesota Biodegradation and Bioremediation Database (UMBBD). [http://dragon.labmed.umn.edu/umbbd/index.html]

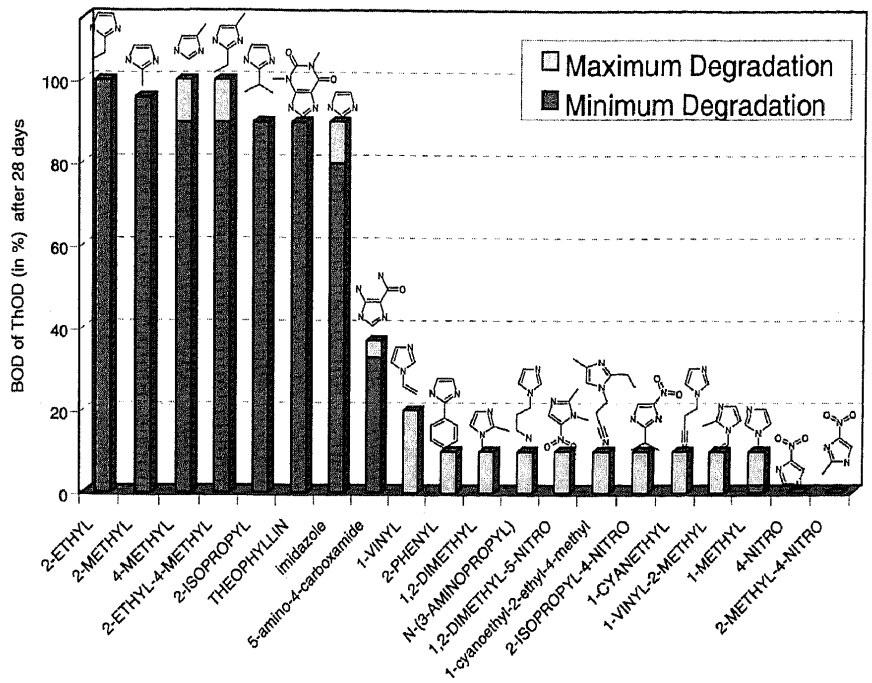

FIGURE 1 Biodegradability expressed in percentage (%) Biological Oxygen Demand (BOD) of theoretical oxygen demand (ThOD) after 28 days, for 20 imidazole-ring containing compounds.

expected to be non-biodegradable by purpose, and as biological degradation is greatly hindered by the mere size of these compounds, they are discarded from the Structure-Biodegradation Relationship (SBR) analysis. This leaves a set of 20 different imidazole derivatives with their biodegradation data. The most relevant quantitative data for these 20 structures are shown in Fig. 1.

The following observations are made:

- Imidazole and its ring-C-substituted derivatives with methyl-, ethyl-, and isopropyl-substituents are ultimately biodegradable
- Phenyl-, cyano- and nitro-substituted imidazoles seem to be poorly biodegradable
- All N-substituted imidazole derivatives are poorly biodegradable

MECHANISTIC INFORMATION

Literature research on biodegradation pathways of imidazole derivatives led us to the histidine metabolism† [7,8]. Catabolic pathways for substituted imidazole compounds may be similar to the breakdown of urocanate, the first metabolite in the histidine biodegradation pathway [7,8]. Below, in Fig. 2, the proposed biodegradation pathways for the breakdown of substituted imidazole-ring compounds is given, in close analogy to the histidine degradation pathway.

The details of the enzymatic mechanism of the urocanase attack is shown in Fig. 3, as proposed by Klepp *et al.* [8]. The product of the hydratase of urocanate is a 5-oxoimidazole derivative, which is subject to (abiotic) hydrolytic cleavage and ring opening. The oxo-substituent of the product greatly reduces the aromatic character of the imidazole ring, enabling cleavage of the ring structure, which is necessary for further microbial degradation.

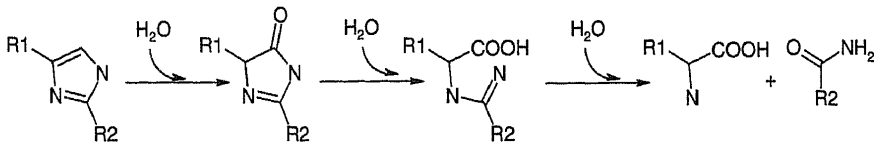

FIGURE 2 Degradation of imidazole-derivatives in analogy to the histidine-degradation pathway.

The hydratase mechanism as shown in Fig. 3 involves electronic rearrangements of the imidazole ring system, induced by an electrophilic attack of urocanase-bound NAD + . The imidazole-ring will be deactivated towards attack of NAD+ by substituents, like nitro-, cyano- or halogen-groups, having an negative electronic effect on the ring, either through inductive or resonance effects. The attack on the imidazole ring will be activated by electron-donating substituents, like alkyl-, amino-, or hydroxyl-groups. The fact that not imidazole, but 2-ethylimidazole is the most readily biodegraded substance in our series (see Fig. 1) also indicates that the imidazole ring can possibly be activated towards electrophilic attack, making the methyl-, ethyl-, and even isopropylimidazoles better biodegradable than their mother compound imidazole.

Combination of the data analysis and the mechanistic information leads to the following rule for predicting the biodegradability of imidazoles in general:

Imidazole derivatives are biodegradable in the aquatic environment, if the substituents are:

(a) attached to the imidazole-ring carbon atoms, and
(b) do not have an electron-withdrawing effect on the imidazole ring through resonance- or inductive-effects.

VALIDATION

The above formulated rule was externally validated with all compounds in the UBA-database [6] containing an imidazole ring. Twelve compounds with an imidazole ring were present.

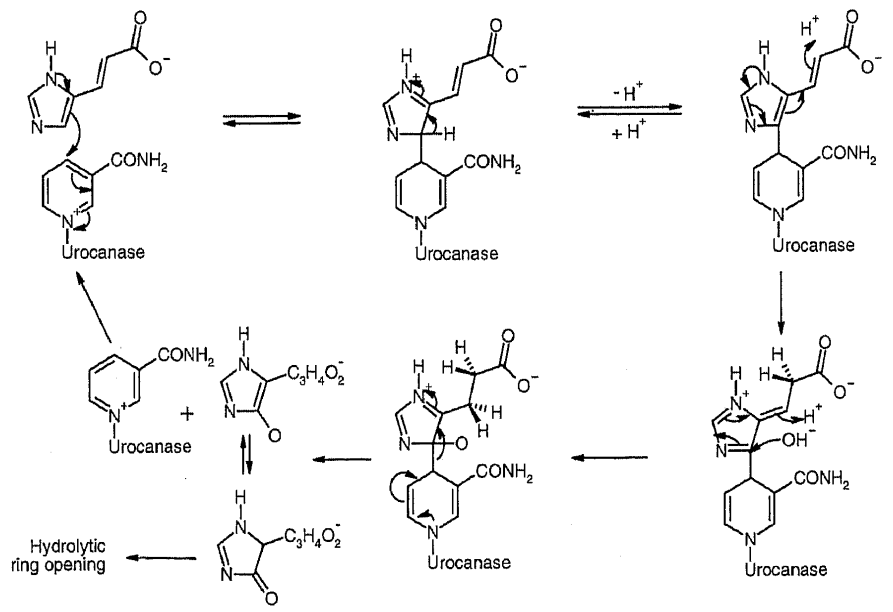

FIGURE 3 Proposed mechanism for the metabolism of urocanate by urocanate-hydratase, EC 4.2.1.49† [8].

All of them were correctly predicted as poorly biodegradable, because of their substitution patterns and/or their calculated atomic HOMO electron density at the carbon 4 or 5 position of the imidazole ring. Details of the validation data cannot be shown because of the confidentiality of the data.

DISCUSSION

Only looking at the proposed degradation mechanism for imidazole derivatives (Figs 2 and 3), it is not obvious that 1-N-substituted imidazoles will be hindered in their degradation via a urocanase-like metabolism. Similarly it is not obvious from the data-analysis that all C-substituents on the imidazole ring that have an electron withdrawing effect through resonance or induction will deactivate the imidazole ring towards NAD+ attack, thus making biodegradation more difficult.

2-Phenyl-imidazole is poorly biodegradable compound, whereas the imidazole-ring substituent is not considered to be electron-withdrawing. This seems to be contradicting the above formulated rules. Preliminary results of quantum-chemical calculations performed on this set of imidazole (results not shown) indicate that the 2-phenyl-substituent to the imidazole ring is not overall electron-withdrawing, but that this substituent has a very distinguished local electron-withdrawing effect on the 4- and 5-imidazole ring position, exactly those positions which would be prone to a electrophilic attack by urocanase bound NAD + .

Apparently, N-substitution of the imidazole ring can block the urocanase mechanism completely. From the preliminary results of the quantum-chemical calculations, it is concluded that the N-substitution does not decrease the biodegradability of the imidazole ring by making the ring less susceptible to electrophilic attack. Possibly imidazole N-substitution hinders the degradation mechanism by disabling the electronic rearrangements in the imidazole ring in the reaction steps after the nucleophilic attack of the urocanase bound NAD+ (see Fig. 3, steps 3 and 4).

For the class of imidazoles it is shown that it is necessary to use both data-analysis (statistical and/or manual methods) and interpret information from catabolic mechanisms, to come up with meaningful structural rules for biodegradation, which have general validity and thus predictive value.

OUTLOOK

The validation performed with 12 imidazole derivatives from the UBA-database is very limited, since no biodegradable compounds were present in this database. At present, the model can therefore be validated only for predictions of non-biodegradability. Some compounds which had to be biodegradable according to our rules will be tested in our laboratory in the future (given their availability), to validate the ability of the model and also to predict biodegradability of imidazole derivatives.

Within the project several classes of chemicals have been analysed, a.o. N-heterocyclic compounds (of which the imidazoles are a part) [9], sulfonated aromatic and sulfonated aliphatic compounds [10], and nitro-aromatic compounds to name a few. As the project continuous, more classes of chemicals will be analyzed and rules for their biodegradation will be established. By identifying the possibility or impossibility of biological attack for specific classes of compounds, it should be feasible to make reliable predictions for complex compounds on time, which might belong to several classes at once.

Acknowledgements

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