From the Institute of Pharmacy at the Friedrich-Schiller University Jena
(Director: Prof. Dr. H. Bräuniger)

About the Hydrolytic Decomposition of IMET 3393

By W. Fürst, E. Biedermann, W. Ross, S. Hentschel, and M. Hähnel*)

With 5 illustrations

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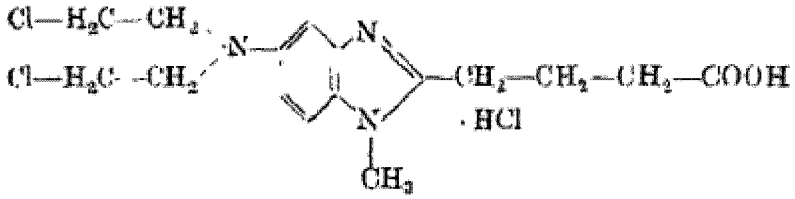
The cytostatically effective compound IMET 3393,γ-[1-methyl-5-bis-(β-chloroethyl)-amino-benzimidazolyl-(2)]-butanoic acid hydrochloride was presented by W. Ozegowski and D. Krebs[1]) at the Institute for Microbiology and Experimental Therapy in Jena from the perspective of combining purine and amino acid antagonism as well as the alkylating effect of nitrogen mustard compounds in one molecule. There are already studies available by H. Baufeld, P. Hesse, P. Köhler, and G. Anger[2]) about the clinical application. Recently, this substance has also been attributed with an immunosuppressive effect.



IMET 3393

At first, the study of the hydrolytic decomposition products which appear under different conditions was of interest both for the pharmaceutical processing of IMET 3393 and the clarification of the metabolism as well as the further behavior under physiological conditions. It was already possible to paper chromatographically detect a quick hydrolytic splitting of the Cl atoms as well as the appearance of several hydrolysis products while preparing a monograph proposal for DAB 7. While, for example, only one hydrolysis product can be detected with cyclophosphamide during alkaline hydrolysis, with IMET 3393, it was always possible to detect several compounds in the reaction products obtained under different conditions. For the paper chromatographic detection, the strong fluorescence of IMET 3393 and its decomposition products under the quartz lamp, which can be attributed to the benzimidazole ring, was particularly favorable. With other detection agents, particularly potassium iodobismuthate solution, it was only possible to always detect the same fluorescent substances. Since the fluorescence is intertwined with the unchanged benzimidazole ring, it can be inferred that there is no breakdown of this ring system during hydrolysis, and only the nitrogen mustard structure is involved in this process.

The speed of the Cl splitting both in purely aqueous solution and buffer solution of different pH values is multiple times that of cyclophosphamide. The forming of the hydrolysis products depends greatly on the pH value and further components in the solution. In the purely aqueous solution of the substance, which has a pH value of approximately 3, it is possible, by

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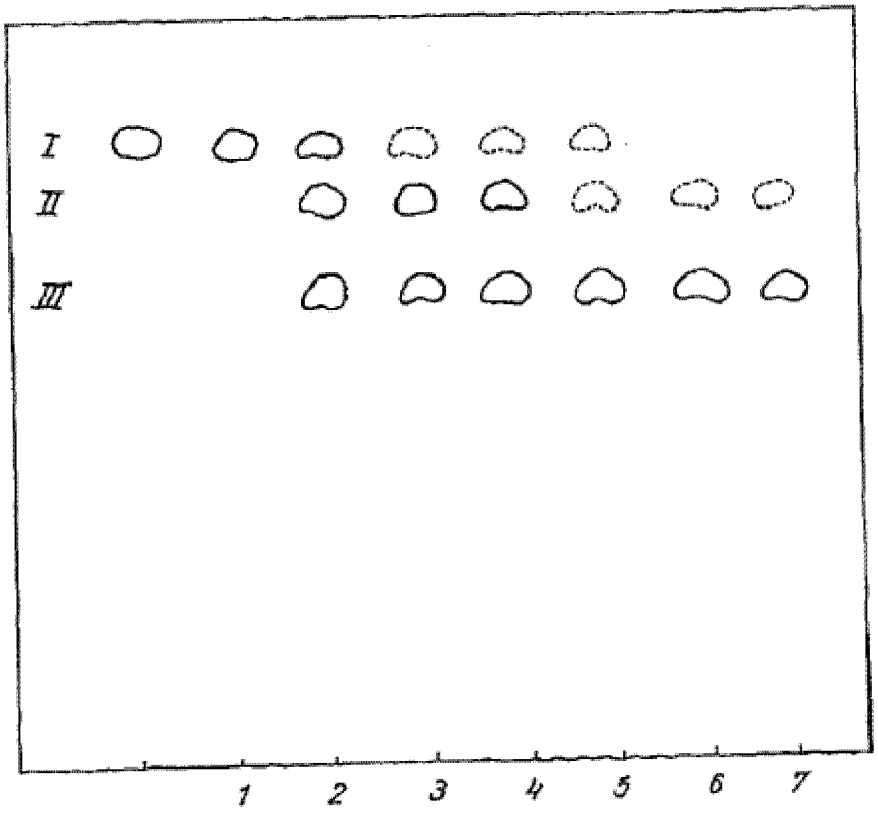
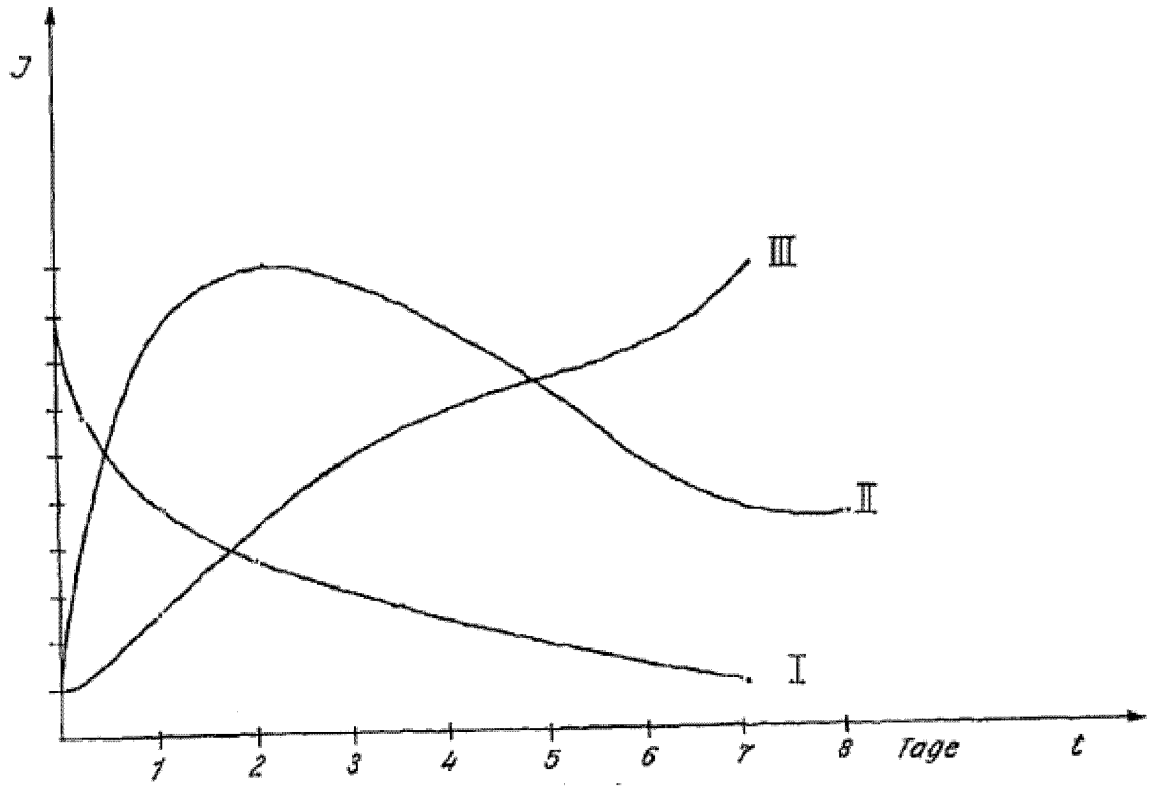
*) Students W. Ross, S. Hentschel, and M. Hähnel participated in these studies within the course of the student competition.

[1]) W. Ozegowski and D. Krebs, J. prakt. Chem. [4] **20**, 178 (1963).
[2]) H. Baufeld, P. Hesse, P. Köhler, and G. Anger, Dtsch. Gesd. Wes. **22**, 1979 (1967).



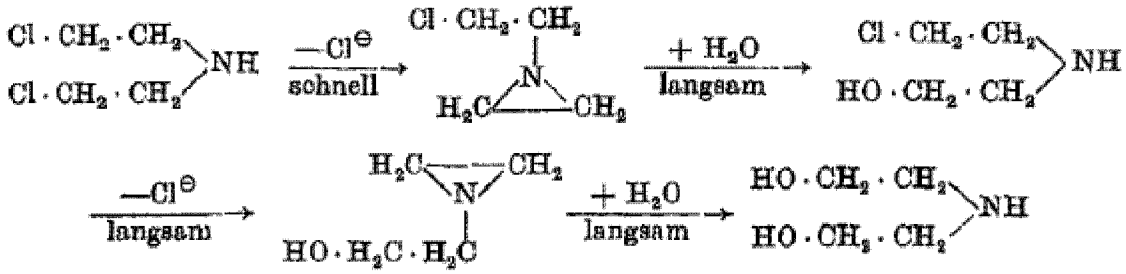
Ill. 1
Paper chromatogram of the reaction products of IMET 3393 in aqueous solution after a reaction time of 1-7 days



Ill. 2

immediately executing the chromatography to already detect in IMET 3393 (I) a further fluorescent substance (II) with a slightly lower R_F value in addition to the substance with the highest R_F value. After leaving the aqueous solution for several hours at room temperature, a further substance (III) with an even lower R_F value emerges. After letting the aqueous solution rest further, I and subsequently II disappear from the solution, so that it eventually only contains III.

Comparison with authentic material has shown that in III, both Cl atoms of IMET 3393 are replaced by OH groups, thus resulting in γ-[1-methyl-bis-(β-chloroethyl)-amino-benzimidazolyl-(2)]-butanoic acid hydrochloride. As a structure for II, the formulas established by H. Arnold and H. Klose[3]) as well as H. M. Rauen, A. Reisch, and H Schriever[4]) for the hydrolytic decomposition of cyclophosphamide and/or bis-(β-chloroethyl)-amine, an appropriate monohydroxy compound of IMET 3393 came particularly into consideration. According to H. M. Rauen and associates, the hydrolytic decomposition of bis-(β-chloroethyl)-amine takes places in accordance with the following formula



schnell = fast
langsam = slow

whereby the aziridines must be viewed as the effective alkylating intermediate stages.

With quantitative analysis of the hydrolysis products of I, it was possible to show that II initially grows at the expense of I, that it subsequently reaches a maximum and that eventually everything passes into III. For the quantitative determinations, the fluorescent spots obtained during chromatography were cut out, eluted and fluorometrically analyzed. Since an isolation of II in substance appeared not to be possible from a preparative point of view due to the overall insufficient quantities even with the use of several paper chromatograms, another path was chosen for demonstrating the structure of II as a monohydroxy compound of IMET 3393. II is sufficiently stable, so that an elution from the chromatograms is possible. II was isolated from several paper chromatograms, which were produced from a preparative perspective, and treated with a mixture of pyridine and acetic anhydride. This resulted in the compound IIa which moved on the chromatogram with the solvent front. IIIa, obtained by acetylation of III, also showed a similar paper chromatographic behavior. After leaving IIa in an aqueous solution for several hours, a further substance IIb was paper chromatographically detected, while IIIa under these conditions remained unchanged. An alkaline hydrolysis of IIa, IIb, and IIIa each resulted in III. This result supports the conclusion that the free OH group is initially acetylated by II, forming

IIa. IIa is subsequently again subject to a hydrolytic splitting into IIb in the aqueous solution and eventually changes into III during alkaline hydrolysis.

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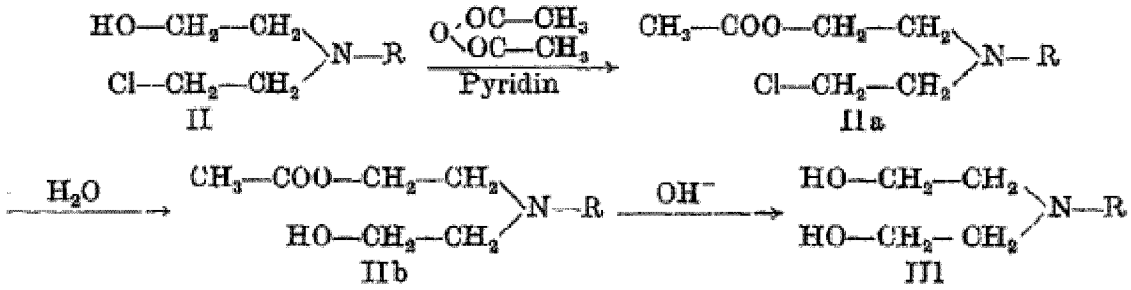
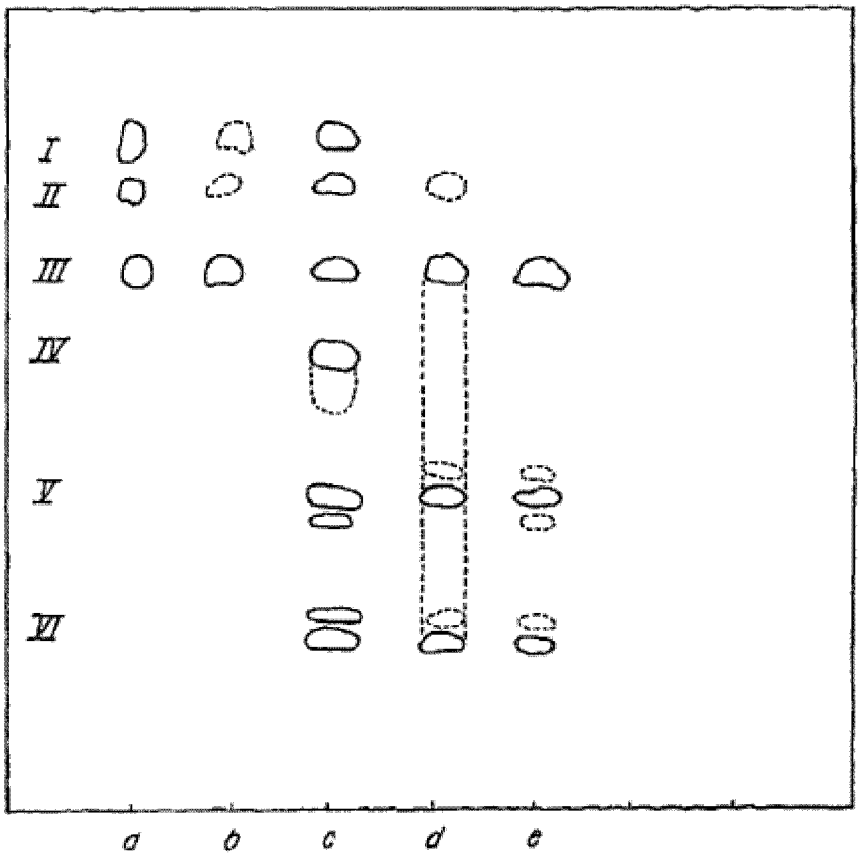
[3]) H. Arnold and H. Klose, Arzneimittel-Forsch. 11, 159 (1961).

[4]) H. M. Rauen, A, Reisch, and H. Schriever, Arzneimittel-Forsch. 14, 176 (1964).



If the hydrolysis is executed in buffer solutions with pH values between 5 and 10, a number of further compounds with significantly lower R_F values are detectable in the reaction product in addition to the aforementioned compounds II and III. Particularly the compounds IV, V, and VI, when observed with a UV lamp, show strong fluorescence. In addition, a number of further compounds can also be detected, but at significantly smaller quantities. In 0.1 n lye, only the two compounds II and III, which are also present in acidic solutions, can be found. Of course, the hydrolysis speed is significantly increased in alkaline solution when compared to the purely



Ill. 3

Paper chromatogram of the reaction products of IMET 3393

a) aqueous solution b) 0.1 n potash lye c) phosphate buffer pH 7.2 after 1 day d) same solution after 2 days
e) same solution after 11 days

aqueous solution. Attempts to isolate particularly substance IV, which is present at the relatively greatest quantity, from several paper chromatograms in crystallized form have not yet lead to success. The compound is sufficiently stable and proved to be chromatographically pure; however, so far, it has not yet led to defined compounds with the precipitating agents applied. It is very difficult to extract VI through organic solvents from aqueous phases. A certain distribution effect can be achieved with higher alcohols. The first assumption that it is a substance with quaternary nitrogen was not confirmed by a paper electrophoretic examination. During electrophoresis, the compounds I, III, and VI showed the same behavior with regard to the isoelectric point. At pH value 4, all three substances did not migrate in the electric field, while at a lower pH value, they flowed as cation, and at a higher pH value, they flowed as anion. Contrary to paper chromatographic separation, whereby VI has a significantly lower R_F value than I and III, the migration speed of VI in the electric field is higher. It can be assumed that the aziridines present are instrumental in forming the substances IV-VI which are also present in IMET—similar to formula 1. For them, such conditions are only present within a specific pH range, which allow for a reaction with each other or with I itself and subsequently lead to the compounds IV, V, and VI.

Tests with the addition of sodium thiosulfate resulted in a further significant clue about the mechanisms of the hydrolysis. According to C. Golumbic, J. S. Fruton, and M. Bergmann[5]) as well as B. Brock and H. Hohorst[6]), the intermediately appearing aziridines react with thiosulfate ions. This is a direct substitution on the *β*-C-atom by the nucleophilic ion. From this perspective, the titrimetric determination of the alkylating activity of the cyclophosphamide was executed by N. Brock and H. Hohorst. When the activity was compared in accordance with this method, IMET 3393 proved to be many times more effective than cyclophosphamide and bis-(*β*-chloroethyl)-amine.

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