

AN OXYGEN-18 STUDY OF THE HYDROLYSIS OF N-PHENYLMALEISOIMIDE
 UNDER ACIDIC, BASIC AND NEUTRAL CONDITIONS

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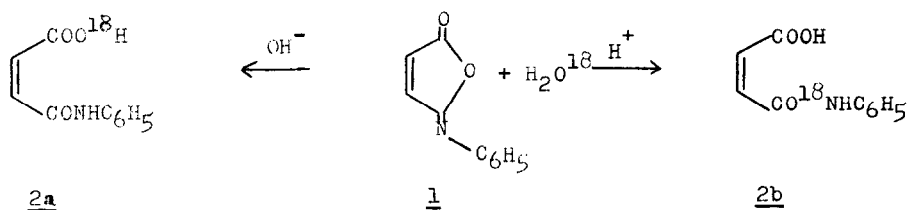
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It has recently been suggested that cyclic isoimides may play a role in amide-catalyzed hydrolyses of carboxylic acid derivatives (1). Since suitably arranged amide and carboxylic acid derivatives often occur in proteins, isoimides may also occur in these systems and thus they may participate in some way in the mechanisms of enzyme reactions. For these reasons, we undertook the present oxygen-18 study of the hydrolysis of N-phenylmaleisoimide 1 in order to determine the site of attack of the water molecule on 1 to give N-phenylmaleamic acid 2 as a function of the acidity of the solution.

A previous study by Paul and Kende (2) of the basic hydrolysis in labeled water of N-n-butylmaleisoimide, indicated that attack on the isoimide occurs at the carbonyl group and Ernst and Schmir (1) have suggested that specific acid-catalyzed hydrolysis of N-phenylphthalisoimide proceeds by the addition of water to the protonated imino function. Thus it could be predicted that the location of an introduced oxygen-18 label from the hydrolysis of 1 in labeled water would be a function of the pH of the solution as shown in Scheme I. We wish to report experiments which demonstrate the validity of this hypothesis.

Scheme I



N-Phenylmaleisoimide (3) was hydrolyzed according to the Paul and Kende procedure (2) and a 70% recrystallized (95% ethanol) yield of N-phenylmaleamic acid, 2a, m. p. 201-202° was isolated. Oxygen-18 analysis (4) demonstrated the presence of 8.3^a atom percent excess oxygen-18 in the molecule. Similarly 2b containing 7.9 atom percent excess oxygen-18 was isolated in 59% recrystallized yield when 1.04 g H₂O¹⁸, 0.11 g of concentrated sulfuric acid and 0.78 g of 1 were mixed in 4.0 ml dry tetrahydrofuran and stirred for ten minutes.

The positions of the labels in these compounds were established by the following experiments. When N-phenylmaleamic acid 2a was subjected to dehydration with N,N'-dicyclohexylcarbodiimide (DCC), the resulting N-phenylmaleisoimide 3a was found to contain 4.2 atom percent excess oxygen-18 and the N,N'-dicyclohexyl urea was found to contain 4.3 atom percent excess oxygen-18. Similarly 2b yielded 3b containing 7.4 atom percent excess oxygen-18 and 4b containing 0.5 atom percent excess oxygen-18. These data suggest that the major pathway shown in Scheme II and previously suggested by others (2,5) for similar reactions is correct. It may be concluded that hydrolysis in strong acid leads to ca 87% addition of water to the imino function and ca 13% addition to the carbonyl function. Exchange reactions cannot account for the

Table I

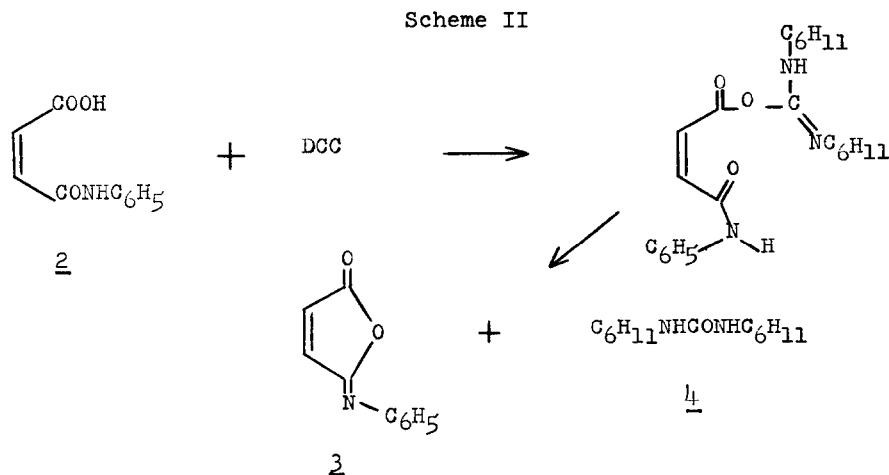
Summary of Oxygen-18 Analyses*

Conditions	Maleamic Acid	Isoimide	Urea
Basic	2a 8.3 ± 0.2	3a 4.2 ± 0.1	4a 4.3 ± 0.0
Acidic	2b 7.9 ± 0.1	3b 7.4 ± 0.1	4b 0.5 ± 0.1
Neutral	2c 8.2 ± 0.1	3c 6.5 ± 0.0	4c 1.6 ± 0.1
Neutral	2d 3.2 ± 0.1		4d 0.6 ± 0.0

*Atom percent excess oxygen-18 ± average deviation for two or more analyses. Mass spectra of labeled CO₂ were run on a Perkin Elmer Hitachi RMU-7 or a Consolidated-Nier Isotope Ratio Model 21-201 spectrometer.

(a) The labeled water was 10 atom percent oxygen-18 from Bio-Rad Laboratories.

presence of the label in the carboxyl group since exposure of 2b to the acid hydrolysis reaction conditions using unlabeled water led to no loss of label.



These data support the previously proposed mechanism (1) involving nucleophilic attack at the carbonyl by hydroxide in the reaction run in potassium hydroxide, water and tetrahydrofuran. In the reaction run in 10% sulfuric acid in tetrahydrofuran, the distribution of the label provides experimental proof that addition of water to the protonated isoimide occurs primarily at the immonium center, although a small amount of addition to the carbonyl function is observed.

Two experiments were performed to determine the position of the incorporated label when the hydrolysis occurs in the absence of added acid or base. When a mixture of 245 mg 1 in 1.97 g tetrahydrofuran containing 520 mg H_2O^{18} was allowed to stir for 25 hours, a 28% recrystallized yield of N-phenylmaleamic acid 2c containing 8.2 atom percent excess oxygen-18 was isolated. Oxygen-18 analyses of the dehydration products 3c and 4c shown in Table I, indicated that 39% of the label was located in the carbonyl function and 61% was located in the amide function. Furthermore, when a 5% slurry of 1 was hydrolyzed in a stirred 3:1 mixture of unlabeled water to labeled water containing 2% acetonitrile, 2d containing 3.2 atom percent excess oxygen-18 was isolated. The label appeared to be distributed between the carboxyl and

amide functions in a manner similar to the previous experiment, since the dehydration procedure produced urea 4d containing 0.6 atom percent excess oxygen-18 (the isoimide was lost). When the reaction in water-acetonitrile was carried out on a larger scale, the pH dropped rapidly to 4.0 and then slowly dropped to 2.8 presumably as a result of the formation of 2 and its hydrolysis products, maleic acid and aniline. (6).

These data indicate that most of the "neutral" hydrolysis with oxygen-18 occurred with acid catalysis. Since the fractional distribution of the reaction between the acid-catalyzed and neutral pathways cannot be estimated with accuracy, the location of oxygen-18 in the products cannot be interpreted so as to identify the site of the neutral water reaction. Two possible mechanisms were previously suggested (1) for the neutral hydrolysis of N-phenylphthalisoimide: (a) either nucleophilic attack of water upon the carbonyl of neutral isoimide or (b) reaction of protonated isoimide with hydroxide ion. The latter pathway can probably be ruled out for N-phenylphthalisoimide because the second order rate constant for such a reaction step calculated from the neutral rate constant reported in reference 1 would probably exceed the diffusion rate, i.e. k would be $1.53 \times 10^{10} \text{M}^{-1} \text{sec}^{-1}$ for $\text{pK}_a = 0$ for the protonated isoimide (7).

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- (7) We wish to thank the referee for this suggestion.