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High performance thin layer chromatographic analysis of hydrolyzed tinidazole solutions II. Hydrolysis kinetics of tinidazole[1]

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Abstract

In a citrate–borate–phosphate buffer, 5 mM tinidazole solutions exhibited maximum stability around pH 4.0–5.0. The hydrolysis of tinidazole was mostly a first-order reaction. At pH 10.0 and 60–80°C, tinidazole had an activation energy of 122 kJ mol^{-1} for hydrolysis. It was postulated that tinidazole decomposes by different mechanisms under basic and neutral/acidic conditions.

Keywords: Degradation mechanism; Hydrolysis kinetics; Thin layer chromatography; Tinidazole

1. Introduction

Tinidazole (**2**), an N^1-substituted 2-methyl-5-nitroimidazole, is an antiprotozoal and antimicrobial drug, which is known to be susceptible to both photolysis and hydrolysis. The photolysis of 5-nitroimidazoles has been extensively studied and the mechanism nicely elucidated in the literature [1–3]. The hydrolysis of 5-nitroimidazoles is less well documented. The hydrolysis of metronidazole, another N^1-substituted 2-methyl-5-nitroimidazole, has been studied more closely [4–6] but only one report [7] has been published covering the hydrolysis kinetics of **2** at basic pH. The reactions were shown to be first order [4,6,7].

The hydrolysis products of tinidazole (**2**) have previously been identified as the 4-nitroisomer (**1**) and 2-methyl-5-nitroimidazole (**3**) [8], the latter being one of the starting materials for the synthesis of **2** [9]. **1** and **3** are also the only specifically named structurally related impurities limited by the requirements of the monograph of **2** in the European Pharmacopœia [10]. **1** was formed almost quantitatively in the presence of a catalytic amount of a base [11], but higher concentrations of base resulted in **3** instead. **3** was further reported to yield **1** as a result of N-realkylation on the other nitrogen [7]. Metronidazole, however,

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was reported to yield ammonia and acetic acid almost quantitatively in 0.5 M sodium hydroxide [5]; a compound containing a nitrogen with an available hydrogen was also present in the reaction mixture [4,5]. In the presence of a catalytic amount of a base, metronidazole was not isomerized [11].

This is the second part of a study of hydrolyzed tinidazole (2) solutions; part I is the preceding paper [12]. The present paper reports the results of a series of kinetic studies on tinidazole (2) hydrolysis over a wide pH range (1–12). The results are compared with those obtained by high performance liquid chromatography (HPLC) in basic conditions [7] and with those for metronidazole [4,5]. The formation of 1 and 3 upon degradation of 2 in acidic conditions is shown to be non-quantitative, and the mechanism is discussed.

2. Experimental

5 mM solutions of 2 were prepared in a citrate–phosphate–borate buffer (pH 1.0–12.0 ± 0.05) [13] by dissolving the solid in 20.0 ml of the buffer solution at the appropriate temperature (60–80°C) in a 25 ml volumetric flask submerged in a water bath, whose temperature was kept within ± 0.5°C of the claimed value. 1 ml aliquots were drawn at intervals, added to either 5 ml of 0.1 M HCl (pH 8.0–12.0) or water (pH 1.0–7.0) in an ice-water bath and diluted to 10.0 ml with water. Each sample applied in duplicate, the analyses were carried out using the stability-indicating high performance thin layer chromatography (HPTLC) method and materials described in Part I [12].

3. Results and discussion

The citrate–phosphate–borate buffer was chosen as it provides a large pH range without the need to change the buffer species. To determine the order of the reaction, the least-squares method was applied to both zero- and first-order equations. The line with the better value of r was taken to represent the order of the reaction. The hydrolysis of 2 followed apparent first-order kinetics in all the studied cases except for pH 9.0 and pH 3.0–6.0 at 80°C, where a decision could not be made (Table 1). At pH 12 and 70–80°C, the half-life of 2 was considerably less than 5 min, and the order of the reaction was not determined. All the lines had a value of $r > 0.987$ except for pH 3.0–6.0 at 80°C, where for practical reasons the period studied was considerably shorter than the half-life of 2.

From the collected data it can be clearly seen that at 80°C tinidazole (2) is hydrolytically most stable when the pH is approximately 4.0–5.0 (Fig. 1); metronidazole also exhibits maximum stability around pH 5.0 [4,14]. Although Baveja and Rao [4] used 3 mM metronidazole solutions with 0.05 M phosphate, metronidazole clearly seems to be more stable in basic conditions than 2. Since it has been shown that there is no general acid/base catalysis of metronidazole caused by either acetate, phosphate or borate [6], the difference might be caused by either the citrate of the buffer in our experiment, a salt effect or the intrinsic stability of metronidazole.

The degradation rate constant at pH 11.0 and 80°C for 2 is 150 times greater than that of metronidazole (Fig. 1); the difference is unlikely to be caused by any salt effect alone. However, it

Table 1
First-order decomposition rate constants (h^{-1}) for 5 mM tinidazole solutions in citrate–phosphate–borate buffer. See text for the data in italics

pH	Temperature (°C)				
	80	75	70	65	60
1.0	0.0112				
2.0	0.00551				
3.0	*0.0011*				
4.0	*0.00070*				
5.0	*0.00077*				
6.0	*0.0029*				
7.0	0.0219				
8.0	0.113				
9.0	*0.369*		0.119		
10.0	2.00	1.11	0.619	0.302	0.168
11.0	6.12		2.17		0.559
12.0					4.81

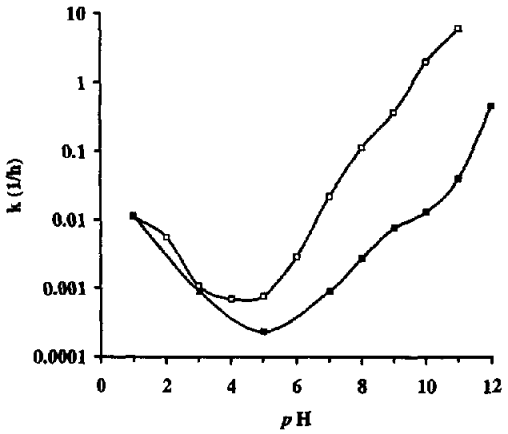

Fig. 1. pH profiles of tinidazole (2) (□) and metronidazole (■). The data are based on Table 1 and Ref. [4].

can be a contributing factor, and it should be emphasized that constant ionic strength is desirable in kinetic studies to preclude salt effects. However, the role of citrate could also be debated as its concentration is constant at every pH but the difference between the rate constants of the two compounds diminishes as the solutions become more acidic. It should be noted that citrate has been reported [15] to stabilize metronidazole as an excimer ion in photolytic studies. Whether this reaction is possible with 2 is not known. In any case, it can be ignored since the metronidazole excimer ion was observed only after exposure to daylight for at least 18 months, but the present tests were performed with protection from direct sun-light and fluorescent light in less than a week.

A plausible explanation would be that 2 decomposes by a different mechanism in basic and acidic conditions while metronidazole is degraded by a single mechanism, which could be identical to that of 2 in acidic conditions. This postulation is further substantiated by the different product profiles of acid- and base-catalyzed hydrolysis of 2. At 80°C and pH 10.0, the amounts of 2 and 3 in the test solution after one half-life correspond to approximately 96% of the initial amount of 2; the rest is accounted for by the small amount of 1 formed. These results are characteristic of the basic (pH 8.0–12.0) conditions studied. In neutral and acidic conditions, however, the product formation is strikingly different; only traces of 1 and 3 are present in the solution at any one time even if 2 is degraded to a substantial extent.

As Rao et al. [11] stated, the alkyl ethyl sulphone side-chain present in tinidazole (2) is essential for the isomerization. The current results would indicate that the N-dealkylation is energetically favored in basic solutions while this pathway is blocked in acidic/neutral conditions leaving the decomposition of the imidazole ring responsible for the degradation of 2. Earlier reports [4,5,11] suggest that metronidazole, which has a hydroxyethyl side-chain attached to the imidazole ring, is not susceptible to isomerization and will undergo ring decomposition at any pH. The difference observed between the degradation rate constants can therefore be mainly explained by the stability of the aromatic 6π-electron structure of the imidazole ring.

The temperature dependence of hydrolysis was studied at pH 10.0 (Table 1). The least-squares method applied to the Arrhenius equation gives for the line a slope of -14.7 ± 0.280, an intercept of 42.3 ± 0.816 and a correlation coefficient of -0.9995 (Fig. 2). The activation energy can be calculated to be 122 kJ mol^{-1} (29.2 kcal mol^{-1}), which is in good accordance with the previous value (130.9 kJ mol^{-1}) reported [7]. If the activation energy remains unchanged in the temperature range 20–80°C, the half-life of 2 at room temperature will be 72 days even in this moderately basic solution. If the pH profile in Fig. 1 is not dramatically changed in shape as the temperature de-

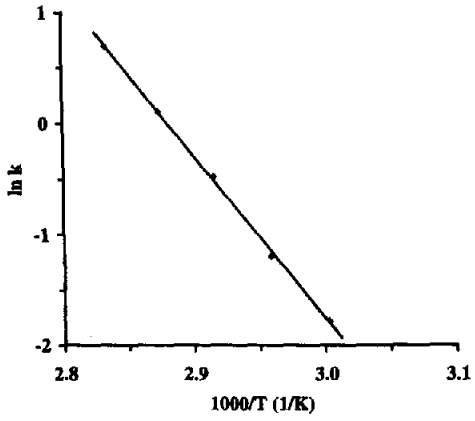

Fig. 2. Arrhenius plot for tinidazole at pH 10.0 and 60–80 °C.

creases, **2** can be regarded as being very stable in solution at room temperature around pH 4.0–5.0.

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