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Mechanistic investigation on 2-aza-spiro[4,5]decan-3-one formation from 1-(aminomethyl)cyclohexylacetic acid (gabapentin)

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ABSTRACT

The intramolecular cyclization of the amino acid gabapentin has been studied in the pH range 2.24–11.15 at 80 °C in buffered solutions and constant ionic strength, and monitoring the progress of the process by fluorimetric method and proton NMR spectroscopy. From the profile of log k_0 versus pH two different acid–base equilibria are involved. The maximum rate is observed above pH 9.80 and the minimum rate has been measured between pH 5.15 and 6.21. The pK_{a1} and pK_{a2} have been determined by potentiometric titration to be 3.72 and 9.37, respectively. The buffer effect and the solvent kinetic isotopic effect suggest that the reaction is subject to general acid and general base catalysis. The process is sensitive to the gabapentin concentration (pH 10.45) and the pseudo-first order rate constant decrease with increasing the reagent concentration above 5.50×10^{-2} M.

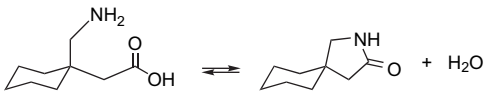
1. Introduction

The study of the stability and reactivity of active pharmaceutical ingredients (APIs) is a basic requirement for the design and validation of the manufacturing processes, as well as for the formulation and storage. At the same time, more information on possible metabolism pathways in humans is achieved. Usually, these data are presented in patents, which cover the field and very seldom published in scientific journals.

Our attention has been focused on gabapentin (1-(aminomethyl)cyclohexylacetic acid), a Pfizer active ingredient, that since October 2004 has been launched as a generic drug.[1] Gabapentin is an anticonvulsant medication indicated in the treatment of epilepsy and neuropathic pain. It is also used in the treatment of bipolar disorder and may be effective in reducing pain and spasticity in multiple sclerosis.

Gabapentin is a GABA analogue that does not bind to GABA receptors or alter GABA metabolism in the brain. The gabapentin binding site appears to be located on neurons in brain areas rich in glutaminergic synapses that do not bind other antiepileptic drugs. Gabapentin is a weak inhibitor of GABA aminotransferase, stimulates glutamate dehydrogenase, and is a potent, competitive inhibitor of brain branched-chain amino acid aminotransferase, an enzyme involved in the glutamate synthetic pathway.[2] Its therapeutic action on neuropathic pain is thought to involve voltage gated calcium ion channels.

Gabapentin is an achiral γ-amino acid, which under appropriate conditions may give intramolecular cyclization to form the five-membered cyclic lactam 2-aza-spiro[4,5]decan-3-one, Scheme 1. The study of the conditions that favor this process is particularly important considering that the lactam **2**, in spite of its demonstrated neuroprotective effect,[3] displays a certain toxicity and must therefore be avoided as far as possible in the formulated drug. For example, gabapentin has a toxicity (DL_{50}, mouse) of more than 8000 mg/Kg, while for the corresponding lactam **2**, values of 300 mg/Kg and 125 mg/Kg (acute toxicity in water), respectively, have been reported.[4,5] Consequently, the presence of lactam **2** as impurity in the active ingredient and its potential formation during storage of pharmaceutical compositions must be reduced to a minimum for reasons of safety.[6,7]



1 2

Scheme 1. Gabapentin lactamization.

The formation of the amide bond is a spontaneously occurring process, which is often observed in the case of reactive amino acids. For example, 3-(2-aminophenyl)propionic acid cyclizes at room temperature and this unusual high reactivity has been attributed to the close proximity of the amine and the carboxyl groups (high effective molarity).[8] In the case of gabapentin the reaction appears to be less favored and, indeed, the cyclization process has been

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investigated at 80 °C to speed up the reaction.[9] To our knowledge, this is the only published study of this cyclization process and due to the pharmacological relevance of gabapentin a deeper mechanistic investigation is clearly desired.

We report here our studies on the cyclization process and in particular we have investigated the effects of pH and of the concentration of the reagent, the solvent kinetic isotopic effect, and the buffer catalysis.

2. Result and discussion

Figure 1 shows the pH-rate profile for the cyclization of 1-(aminomethyl)cyclohexylacetic acid to 2-aza-spiro[4,5]decan-3-one. The pseudo-first order rate constants have been extrapolated to zero buffer concentration and are reported in Table S1. These experiments have been carried out at 80 °C, with a reagent concentration of 2.9×10^{-3} M, buffer concentration of 0.025–0.10 M, and constant ionic strength (μ=0.5 M, NaCl). The reaction progress has been monitored determining the gabapentin concentration with a fluorimetric method, see Section 4, specific for primary amines.[10,11] Several reactions have been also monitored by proton NMR (5% D_2O in H_2O) following both the decrease of gabapentin and the formation of the lactam, see Section 4. Very reproducible data were obtained with both techniques. NMR experiments also show that the reaction is clean and the formation of the lactam as the only product is observed. The profile of the plot of Figure 1 suggests the presence of two acid–base equilibria. The equilibria between the different forms of gabapentin as function of the pH are reported in Scheme 2. Starting from the right side of Figure 1, a plateau of maximum rate is obtained when all the amine is present as free base (nucleophile). Then increasing the amount of protonated amine the reaction rate decreases up to pH 5.70[12] when all the amine is protonated. From the sigmoidal profile of k_0 versus pH (range 5.70–11.15) the pK_{a2} (at 80 °C) was calculated to be equal to 9.31 in good agreement with the value of 9.37 obtained from potentiometric titrations as described in Section 4. At pHs lower than 5.70 the reaction rate increases since the acid function is going to be protonated up to pH 2.24. The values of rate constant in this pH interval (5.70–2.24) may be used to calculate the pK_{a1} relative to the acid function of gabapentin if we assume that at pH 2.24 all the acid is in the protonated form. The profile of k_0 versus pH in this region is not complete but considering a plateau for pH<2.24 a tentative fitting of the data gives a pK_{a1} value of 3.5 close to the value determined by potentiometric titration, that is, 3.72.

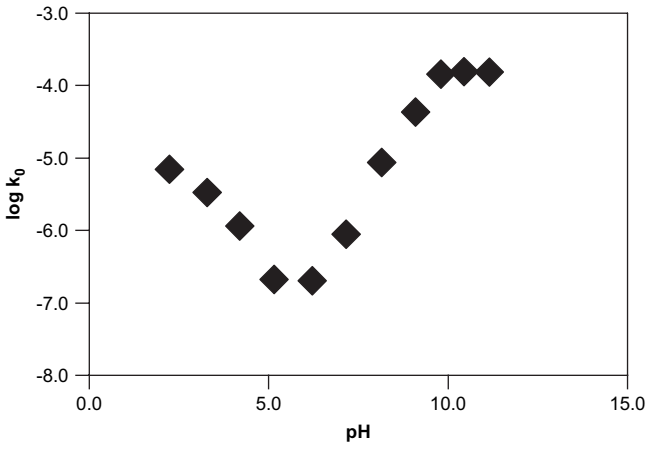
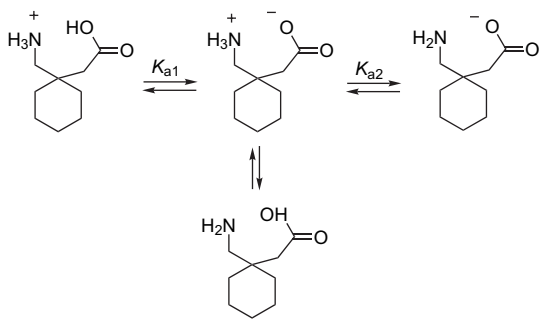


Figure 1. Log of the pseudo-first order rate constant at zero buffer concentration (k_0, s^{-1}) against pH for the cyclization of gabapentin **1** to lactam **2**. Conditions: [**1**]=2.9×10^{-3} M, μ=0.5 M (NaCl), 80 °C.



Scheme 2. Equilibria between different forms of gabapentin as function of pH.

2.1. Buffer effect

The study of the cyclization reaction at different buffer concentrations shows a dependence of the rate constant for pH 2.24–9.10 (buffers: sulfate, acetate, phosphate, borate) with the exception of pH 8.15. At this value of pH we could not observe buffer catalysis both with borate and phosphate buffers. Plots of the pseudo-first order rate constant versus buffer molar concentration at different pH are reported in Supplementary data. These results suggest that the reaction is subject to general acid catalysis and to general base catalysis. The lack of buffer catalysis observed at pH 8.15 is in contrast with the solvent kinetic isotope effect that will be discussed below.

2.2. Solvent kinetic isotopic effect

Reactions carried out in D_2O, under the same conditions described for the reactions in light water, allow the determination of the solvent kinetic isotopic effect (SKIE). The experimental data are reported in Table 1 where they are compared to the k_{obs} measured in H_2O. At all pH values the reaction in water is faster than the reaction in heavy water, except for pH 5.15, and this supports general acid and general base catalysis. The rationalization of the data reported in Table 1 is rather difficult since the k_H/k_D ratio may be the sum of primary, secondary, and solvent effects. The occurrence of X–H bonds breaking and forming may contribute to a different extent to the SKIE depending on how advanced or late in the rate-determining step (rds) the rupture or the formation of these bonds occurs. This and also the coexistence of cationic, neutral, and anionic species at different concentrations as function of pH cause the observed variability of the solvent kinetic isotope effect versus pH. The very high value of k_H/k_D at pH 8.1 seems to have a substantial primary contribution likely due to the protonation of the intermediate.

Table 1
Observed rate constants for the cyclization of gabapentin in water or D_2O at different pH and pD values[a]

pH/pD[b]	$k_D\times10^6$ (s^{-1})	$k_H\times10^6$ (s^{-1})	SKIE
2.24	1.25	8.08	6.5
3.29	2.30	4.74	2.1
4.19	1.40	1.65	1.2
5.15	0.352	0.346	1.0
6.21	0.136	0.356	2.6
7.16	0.244	1.69	6.9
8.15	0.949	8.67	9.1
9.10	14.2	71.0	5.0
9.80	39.7	143	3.6
10.45	66.1	155	2.3
11.15	71.3	154	2.2

[a] Conditions: [**1**]=2.9×10^{-3} M, [buffer]=0.10 M, μ=0.5 M (NaCl), 80 °C.
[b] Measured at 80 °C.

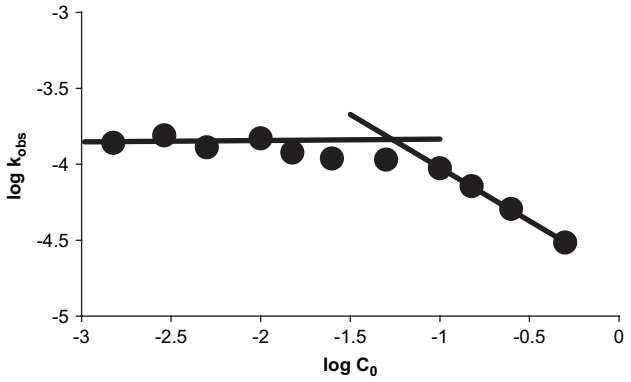


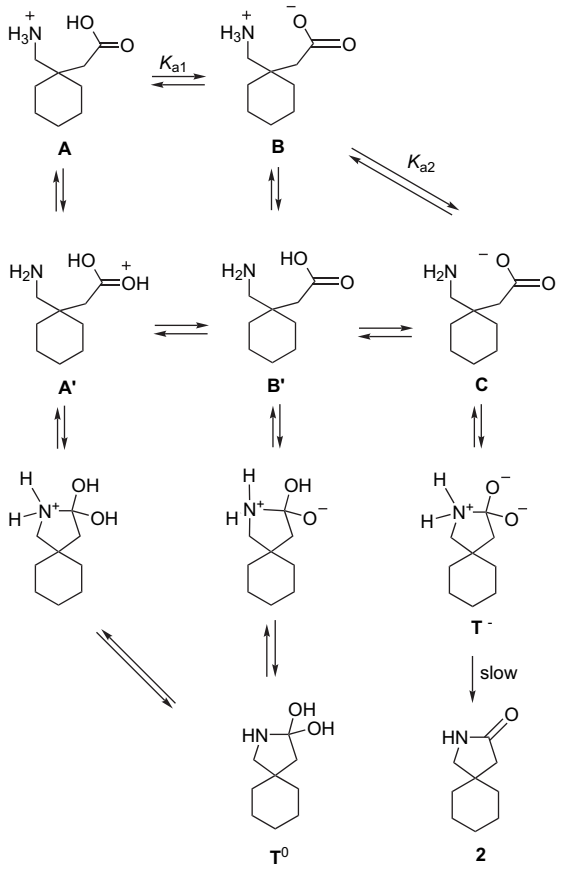
Figure 2. Effect of the increasing concentration of gabapentin (C_0, M) on the cyclization rate constant (k_{obs}, s^{-1}). Conditions: pH 10.45, [buffer]=0.05–0.10 M, μ=0.50 M (NaCl), 80 °C.

2.3. Influence of the gabapentin concentration on the reaction rate

Experiments carried out at different gabapentin initial concentrations (from 1.5×10^{-3} to 5.0×10^{-1} M, pH 10.45) show a decrease of the cyclization rate constants, as shown in Figure 2. The rational we may offer for this behavior is that above a 'critical' concentration aggregation of the substrate may occur giving rise to clusters of gabapentin.[13] Due to the deprotonated carboxylic function the cluster is highly anionic and this may affect the local pH in a way similar to that observed with anionic micelles.[14] The apparent decrease of the local pH would therefore results in a lower reactivity. Although this explanation is speculative at the present, the observation of a dependence of the cyclization rate on the concentration of gabapentin is important from an applicative point of view. Indeed, for the reason highlighted in Section 1, it would be highly desirable to find conditions in which this reaction is suppressed or, at least, minimized.

2.4. Proposed mechanism

The intramolecular gabapentin cyclization has been studied in the pH range 2.24–11.15. The pH-rate profile for the reaction (Fig. 1) shows a minimum at about pH 6.0 and significant reactivity of both protonated (pH=2.2) and anionic form (pH>10). The data here reported for the lactam formation in acidic conditions (below pH=6) suggest that a mechanism similar to those reported for 3-(2-aminophenyl)propionic acid[8] and the corresponding methyl ester[15] might also be operative in the present case (Scheme 3). Taking into consideration that only the neutral amino group can act as nucleophile the zwitterionic **B** and the cationic form **A** of gabapentine should be unreactive. Therefore, the observed reactivity in this pH region has been interpreted as the attack of the free amino group on the protonated **A′** or free carboxyl group **B′** (depending on pH) leading to the neutral tetrahedral intermediate **T°**. The general acid-catalyzed breakdown of such an intermediate has been postulated to be the rate-determining step of the reaction. By analogy, the slow step of the lactamization of gabapentin would involve breakdown of the tetrahedral intermediate, and general acid catalysis could occur via the addition of a buffer proton to the leaving hydroxyl group. This hypothesis is consistent with the SKIE observed, although conclusions on the basis of this effect are not easily drawn for the reasons already exposed above. The lower reactivity of gabapentin with respect to 3-(2-aminophenyl)propionic acid ($t_{1/2}\cong1$ day at 80 °C compared with $t_{1/2}\cong3.5$ h at 39 °C and pH 2) is probably to be ascribed to two main factors: a lower effective molarity of the amino group due to less rigid and pre-organized structure of the substrate (vide infra) and due to the formation of a five-membered ring, more strained than a six-membered ring.



Scheme 3. Proposed mechanism for gabapentin lactamization.

On moving from acidic to basic conditions the analogy with the reported cyclization of 3-(2-aminophenyl)propionic acid[7] is not valid anymore. Indeed, while Kirby reports that the anionic form of the substrate is completely unreactive, we observe the maximum reactivity at pH's above 9 where the anionic form of gabapentin **C** is dominant. This difference is probably related to the nucleophilicity of the amino group investigated. In the case of 3-(2-aminophenyl)propionic acid the aromatic amine is a poor nucleophile able to attack at reasonable rate only the neutral CO_2H group or its conjugate acid. On the contrary, the aliphatic amine of gabapentin is a much more powerful nucleophile and shows a relevant reactivity also toward the conjugate base of the CO_2H group. This observation is in accord with the data reported by Morawetz and Otaki[16] who measured the rates of amide formation in aqueous solution from simple aliphatic amines and carboxylic acids and concluded that the only reaction they observed involved the free amine and the carboxylate ion. Following this argument, the intramolecular attack will lead to the anionic intermediate T^-, which eventually breakdowns to the final product. Again on the basis of the general base catalysis and the SKIE observed we could speculate that this last step is the rate-determining one.

Taking as a reference value for the bimolecular amide formation the reaction between propionic acid and methylamine in basic condition investigated by Morawetz and Otaki[16] we can estimate an effective molarity (EM)[17] of 2600 M for the intramolecular cyclization of gabapentin in similar condition. This value can be compared with the available EMs for related cyclization reactions between amino groups and carboxylic esters; the reaction of more rigid and pre-organized substrates such as methyl

3-(2-aminophenyl)propionate[13] and methyl 2-aminomethylbenzoate[18] are characterized by EM values about two order of magnitude higher, while the intramolecular aminolysis of substituted phenyl γ-dimethylaminobutyrates,[19] a less constrained substrate, shows EM values comparable to that obtained in the cyclization of gabapentin. Therefore, we can assume that the high effective molarity of the amino group is important in determining the observed reactivity of gabapentin although this effect is limited by the relatively high conformational flexibility respect to more constrained and pre-organized system.

3. Conclusions

The intramolecular amide bond formation of gabapentin in aqueous solution is very slow at room temperature, $t_{1/2}\cong 1$ day at 80 °C and pH 2 whereas the cyclization of 3-(2-aminophenyl)propionic acid at 39 °C occurs with an half life of about 3.5 h at pH 2.24.[8] Responsible for this is probably the less rigid structure of the substrate, which results in a lower effective molarity of the amino group and the formation of a five-membered ring, more strained than a six-membered ring. The inertness of gabapentin to cyclization reaches a maximum at pH values between 5 and 7. The process presents the maximum rate at basic pH values above pH 9.80 and this reactivity, not observed in the case of 3-(2-aminophenyl)propionic acid, is related to the higher basicity (pK_a=10.57 at 25 °C) and nucleophilicity of the aliphatic NH_2 group. The most surprisingly outcome of our studies is the decrease of reactivity on increasing the amino acid concentration (at pH 10.45). We can speculate that increasing the gabapentin concentration up to the solid state the cyclization may be very much depressed or even absent and we are actively investigating on this important finding.

4. Experimental section

4.1. General

Fluorescence measurements were carried out on a Perkin–Elmer Luminescence Spectrometer LS 50B using fluorimeter cuvettes in polymethacrylate ($\lambda_{excitation}$=390 nm, $\lambda_{emission}$=480 nm, scan speed=100 nm/min, scan range=430–530 nm, excitation and emission slit=3.5 nm). NMR experiments were performed with a Jeol 400 MHz NMR instrument operating at 399.78 MHz for ^{1}H and at 100.53 MHz for ^{13}C and equipped with a variable temperature unit. All kinetic experiments were performed at 80 °C. The pH values of the reaction mixtures were determined at 80 °C with a pH meter Basic 20 equipped with a Crison glass electrode. The glass electrode was standardized with buffer solution thermostatted at 80 °C following the indications of the producer. The apparent dissociation constants of gabapentin were determined at 25, 35, and 45 °C by potentiometric titration using Ω Metrohm pH meter 716 DMS Titrino equipped with a Ω Metrohm glass electrode 6.0204.100. Authentic samples of gabapentin and 2-aza-spiro[4,5]decan-3-one were synthesized by the Fine Chemicals Business Unit of Zambon Group S.p.A. Both compounds have been prepared with the high purity required for pharmaceutical drugs and have been fully characterized (^{1}H NMR, ^{13}C NMR, mass-spectrometry, and elemental analysis). Commercial reagents were purchased from standard chemical suppliers. All experiments were conducted with bidistilled water or deuterated water (99.9% CIL).

4.2. Kinetic measurements

4.2.1. Fluorimetric method

Kinetic experiments were carried out by addition of 100 μL of a stock solution of gabapentin (50 mg/mL) to 10 mL of the buffered solution that was kept at 80 °C in an oil bath. In a typical kinetic run the initial concentration of gabapentin is 0.0029 M. All reactions were studied at a fixed ionic strength (μ=0.5 M with NaCl). At appropriate time intervals samples of 50 μL were withdrawn and quenched in an ice-water bath. Each sample of 20 μL was added to 2 mL of borate buffer (0.1 M, pH 9.0) in fluorimeter cuvettes and these solutions were treated with 100 μL of acetone solution of fluorescamine (10 mM). The decrease in the concentration of gabapentin was followed by measuring the fluorescence emission at 480 nm. The concentration of gabapentin was calculated using a calibration curve obtained by measuring the emission intensity of solution at known concentration of gabapentin. The linearity range of the assay was determined (up to a final concentration of 30 μM) and the proper dilution in order to operate in the linearity range was used.

Sulfate buffer, made by mixing the proper amount of $NaHSO_4$ and Na_2SO_4, was used for reactions at pH 2.24 and 3.29, acetate buffer was used for reactions at pH 4.19 and 5.15, phosphate buffer was used for reactions at pH 6.21, 7.16, and 8.15, and borate buffer was used for reactions from pH 8.15 to 11.15. In each kinetic run the pH of the reaction mixture was checked before and after the reaction and no change in pH was observed. The rates of the reactions from pH 2.24 to 9.10 were determined at three different concentrations of buffers (0.025, 0.060, and 0.10 M) at a fixed pH, while the rate constants of the reactions from pH 9.80 to 11.15 were determined at two different concentrations of borate buffer (0.05 and 0.10 M). When a buffer effect was observed (pH<9.80) the first order buffer-independent rate constant k_0 was obtained from the intercept of plots of k_{obs} versus the total buffer concentration; when no buffer effect was observed (pH>9.80) the first-order kinetic constants reported are average values of different kinetic runs.

The experiments in D_2O were performed from pD 2.24 to 11.15 at buffer concentration 0.1 M. The pD value were measured in the usual way using a standard pH meter and applying the following correction: $pD=pH_{measured}+0.4$.[20]

At least four independent experiments were carried out for each reaction and the reported kinetic constants are average values.

4.2.2. NMR method

Experiments were performed at pH 10.45, with concentrations of gabapentin >0.10 M. The experiments were carried out keeping at 80 °C in aqueous solution (5% D_2O, pH 10.45, [borate]=0.10 M, μ=0.5 M with NaCl) with the concentration of gabapentin reported in Table 1 in NMR tubes equipped with airtight screw caps. The reactions have been monitored at time intervals of 30 min. The decrease of the concentration of gabapentin was followed by measuring the ^{1}H NMR integrated area of the methylenic resonances at 3.01 and 3.58 ppm. The increase of the concentration of lactam was followed by measuring the ^{1}H NMR integrated area of the correspondent peaks at 2.86 and 3.83 ppm. The concentration of gabapentin C_g was calculated by equation: $C_g=(a/(a+b))C_0$ where a and b are the areas of gabapentin and lactam peaks, respectively, C_0 is the initial concentration of gabapentin. At least four independent experiments were carried out for each reaction and the reported kinetic constants are average values.

4.3. Determination of pK_{a1} and pK_{a2}

The pK_{a1} and pK_{a2} of gabapentin were determined at 25, 35, and 45 °C by potentiometric titration. An aqueous gabapentin solution (50.6 mL, 1.18 mM) containing 1 equiv of HCl and having an ionic strength of 0.10 M (NaCl) were titrated with 1.5 mL of 1.016×10^{-1} M NaOH standard solution. The pK_{a1} and pK_{a2} values at 80 °C were obtained by linear extrapolation of pK_a values versus temperature.

Acknowledgements

We are indebt to Prof. Paolo Tecilla for the very helpful discussions and we would like to thank Dr. Fabrizio Mancin for potentiometric titrations.

Supplementary data

Table of pseudo-first order rate constants k_0 at different pH values, effects of buffers concentrations at different pH, and plot of log k_0 against pD are provided. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.05.010.

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