Asian Journal of Chemistry; Vol. 33, No. 5 (2021), 1176-1182

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23158

Kinetic and Mechanistic Studies of Acidic Hydrolysis of Goniothalamin

R.P.T. KIM[1], M.N. KHAN[1], S.Y. LIEW[2] and K. AWANG[1,3,*]

[1]Department of Chemistry, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[2]Chemistry Division, Centre for Foundation Studies in Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[3]Centre for Natural Products and Drug Discovery (CENAR), Universiti Malaya, 50603 Kuala Lumpur, Malaysia

*Corresponding author: Fax: +60 3 79674193; Tel : +60 3 79674064; E-mail: khalijah@um.edu.my

Received: 8 February 2021; *Accepted*: 2 April 2021; *Published online*: 16 April 2021; AJC-20342

The acidic hydrolysis of goniothalamin was studied on the spectrophotometric kinetic study at different concentration of hydrochloric acid and temperature to determine the stability of the compound. Stability tests were performed using UV-VIS detection. This is a two-step reaction that involves formation of intermediate product. Rate constant of reactant forming intermediate product obeyed pseudo-first-order kinetic, while the second step to form final product is independent on the concentration of HCl. The structure of final products was identified by NMR and MS. The acidic hydrolysis pathway was proposed to involve the opening of lactone ring, followed by dehydration and formation of a double bond.

Keywords: Goniothalamin, Degradation, Kinetic study, Acidic, Hydrolysis.

INTRODUCTION

Goniothalamin is styryl lactone commonly found in the genus *Goniothalamus*. It possess cytotoxic properties against a wide range of cancer cell lines [1-5], it also displayed potent antibacterial [6], antifungal [7] and mosquito larvicidal activities [8]. These studies show that it is a highly potent drug. Therefore, it is vital to ensure that the biological and potentially clinical studies are conducted on the intact lead compound but not a degraded byproduct. To the best of our knowledge, mechanism of degradation studies mostly tested on existence drugs. According to literature, the first natural product underwent kinetic and mechanism study was an alkaloid named securinine. The compound was isolated from the genus *Securinega* and reported to be effective in the treatment of paralysis and physical disorders. The study has shown plausible mechanisms for alkaline hydrolysis and acid cyclization reactions [9].

Another study was done on lithospermic acid B (LAB) degradation in aqueous solution. This is the most abundant and common compound found in one of the Chinese traditional medicine, "Danshen". Different buffer concentration, pH and temperature were tested to identify the stability of the compound. The degradation products were separated and detected by HPLC, followed by characterization using LC-MS [10].

Zahari *et al*. [11] reported on the acid-base equilibria of several aporphine alkaloids isolated from genus *Alseodaphne* and *Dehaasia*. From this study, the pK_a value of those alkaloids were determined. It has been shown that those alkaloids are also stable at physiological pH which is about pH 7-9. Complete degradation of the drug in solution as a function of temperature and pH should be determined before a specific drug can be evaluated for its potential use. Chemical kinetic studies constitute the study of chemical transformation occurs in time to a certain mechanism with regularities characteristics [12]. It has valuable potential to break down complex mechanisms of chemical reactions into sequences of simple reactions.

In kinetics, reactants disappear and the incease amount of products in reactions are always being measured with respect to the reaction time. The method used to monitor kinetics depends on the species involved and the rapidity of which their concentrations change during the course of reactions. The UV spectrophotometry, the measurement of intensity of absorption in a particular spectral region, is widely applicable. It is especially useful when one substance in the reaction mixture has a strong characteristic absorption in a conveniently accessible region of the electromagnet spectrum.

Our interest is to investigate the chemical stability of goniothalamin at various concentration of HCl and temperatures. Thus, in the present work, the stability of goniothalamin in aqueous acidic conditions and various temperatures are reported. The mechanism reaction is also proposed based on the spectrometric data.

EXPERIMENTAL

All the solvents used in extraction and isolation with column chromatography were of analytical reagent grade. Those used for bulk extraction were distilled prior to use. The solvents used were hexane, dichloromethane and methanol. Silica gel 60, 230-400 mesh ASTM (Merck 9385) was used for column chromatography. A slurry of silica gel 60 (approximately 30:1 silica gel to sample ratio) in hexane solvent system was poured into a glass column of appropriate size with gentle tapping to remove trapped air bubbles. Deuterated solvent ($CDCl_3$) was used to dissolve the compound for the purpose to acquire 1D- and 2D-NMR spectra. Acetonitrile, hydrochloric acid and sodium chloride used in kinetic studies were commercially available reagent grade chemicals. Stock solutions (4.00×10^{-3} M) of goniothalamin was prepared by dissolving 20 mg of goniothalamin in 25 mL of acetonitrile.

The UV-visible spectrophotometer used was a Shimadzu UV-1800 with solvent of spectroscopic grade MeOH. The infrared spectra were obtained through Perkin-Elmer FT-IR Spectrometer RX1 with spectroscopic grade chloroform as the solvent. All ^{1}H, ^{13}C, HSQC, COSY and HMBC NMR spectra were recorded in $CDCl_3$ as solvent at room temperature on a 400 MHz JEOL ECA 400 spectrometer. All chemical shifts (δ) were reported in ppm relative to TMS. The mass spectra were obtained on an Agilent Technologies 6530 Accurate-Mass-Q-TOF LC/MS.

Plant material: The stem bark of *G. tapisoides* were collected from Sarawak, Malaysia and the botanical identification was carried out by Prof. Fasihuddin bin Ahmad, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Sarawak, Malaysia. A voucher specimen (HUMS 000108) is deposited at the Herbarium of Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia.

Extraction and isolation of goniothalamin: Dried plant materials (1.5 kg) were extracted first with *n*-hexane for three days period and then filtered. The filtrate was dried using rotary evaporator and 25 g of *n*-hexane extract was obtained. The plant materials were then re-extracted with CH_2Cl_2 and filtered. An amount of 43 g of CH_2Cl_2 extract was obtained after dried with rotary evaporator. The extraction procedures were repeated by soaking the plant materials with MeOH and 24 g of MeOH extract was obtained. The CH_2Cl_2 extract was subjected to column chromatography over silica gel with *n*-hexane, CH_2Cl_2 and MeOH as the mobile phase with gradient elution system. The solvent system used was hexane:CH_2Cl_2 (100:0 v/v), hexane: CH_2Cl_2 (80:20 v/v), hexane:CH_2Cl_2 (50:50 v/v), hexane: CH_2Cl_2 (20:80 v/v), CH_2Cl_2:MeOH (100:0 v/v), CH_2Cl_2:MeOH (98:2 v/v), CH_2Cl_2:MeOH (95:5 v/v), CH_2Cl_2: MeOH (90:10 v/v), CH_2Cl_2:MeOH (80:20 v/v), CH_2Cl_2:MeOH (50:50 v/v) and lastly CH_2Cl_2:MeOH (0:100 v/v). This fractionation process has afforded 11 fractions. Goniothalamin (1.3 g) was purified from fraction 3 (F3) by performing column chromatography over silica gel with solvent system of hexane: CH_2Cl_2 (50:50 v/v).

Kinetic measurements: The rate of hydrolysis of goniothalamin, in an acidic medium was studied by monitoring the changes of UV spectrum at 340 nm as the reaction was ongoing. Initially, 9.90 mL of reaction mixture contained 0.21 mL of 4.775 M HCl, 1.8 mL of 5.0 M NaCl and 7.89 mL H_2O was prepared. NaCl was added was to establish 1.0 M ionic strength in the reaction mixture. The 9.90 mL of mixed solution was then placed into a thermostated oil bath and allowed to equilibrate for 10 min at desired temperature. The reaction was then initiated by adding 0.10 mL of stock solution (4 mM) of goniothalamin (prepared in acetonitrile) with a micro syringe to the temperature equilibrated reaction mixture. The final total volume of the reaction mixture in each kinetic run was 10 mL. The reaction mixture was then mixed well and quickly transferred to a quartz cuvette to take the UV reading. An aliquot of around 2.5 mL was poured from the reaction mixture periodically and quickly to a 3 mL quartz cuvette that was then placed into the cell compartment of the spectrophotometer. The time lag of aliquot transferred and the recording of the absorbance was always ≤ 30 s. The absorbance values (A_{obs}) recorded at certain wavelength against time (t) represent the progress of the reaction in terms of appearance of the product. The temperature of spectrophotometer cell compartment was maintained at 50 ºC to reduce the temperature differences as small as possible. The spectrophotometer was standardized with distilled water both as reference and blank samples. The total volume of reaction mixture was kept constant at 10 mL.

To investigate the effect of hydrochloric acid concentration on the reaction rate, the procedure mentioned above was repeated from 0.1 M to 1.0 M of HCl at 80 ºC. Meanwhile, to study the effect of temperature on the reaction rate, the prepared solutions was inserted into a thermostatic oil bath at 40, 50, 60, 70 and 80 ºC at 1.0 M of HCl. Arrhenius plots were used to determine the activation energy (E_a) of the reaction. In order to determine the wavelength and optimal concentration of goniothalamin for the spectrophotometric kinetic measurement during acidic hydrolysis, a few trial run of spectral study for typical kinetic runs was carried out. The observed data was inserted into BASICA software in order to acquire the calculated data.

Degradation of goniothalamin: Goniothalamin (20 mg) was dissolved in 2 mL of acetonitrile. This reactant with 0.05 M of goniothalamin was then transferred into a glass stoppered conical flask containing 400 mL of 2.0 M HCl to initiate the reaction. The flask was left in thermostated oil bath at 50 ºC. Complete degradation was monitored *via* UV spectrometer by pipette ~2.5 mL from the reaction mixture periodically and transferred back into the conical flask once taken the reading. After complete degradation, the solution was allowed to cool slowly to room temperature. It was then mixed with 400 mL of ethyl acetate, as liquid-liquid (water-ethyl acetate) extraction method was applied in order to extract the product from acidic solution. The obtained degradation products were identified by NMR and mass spectrometry.

RESULTS AND DISCUSSION

Effect of HCl concentration: Kinetic runs of acidic hydrolysis of goniothalamin were studied at 80 ºC for 0.1 M HCl. The progress of acidic hydrolysis of goniothalamin was quickly scanned at different time intervals until completion of the reaction. The UV spectra at different reaction time for acidic hydrolysis of goniothalamin are shown in Fig. 1. It appears that the absorbance at wavelength 340 nm increases until it reaches a highest value and then decreases until it reaches as a lowest constant value.

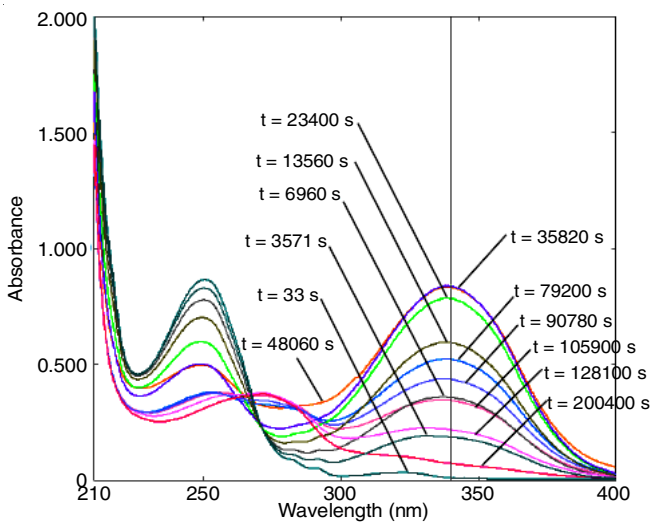

Fig. 1. UV spectra of acidic reaction mixture of containing 4.0×10^{-5} M of goniothalamin at 80 °C in aqueous solvent for 0.1 M HCl

The observed data (A_{obs} *versus* reaction time) recorded for 0.1 M acidic hydrolysis, together with the calculated absorbance and percentage of relative error acquired from BASICA software is tabulated in Table-1. Together with other three curves of absorbance against time at concentration 0.3-0.5 M HCl (Fig. 2), another five plots with concentration of HCl in between 0.6 to 1.0 M are shown in Fig. 3.

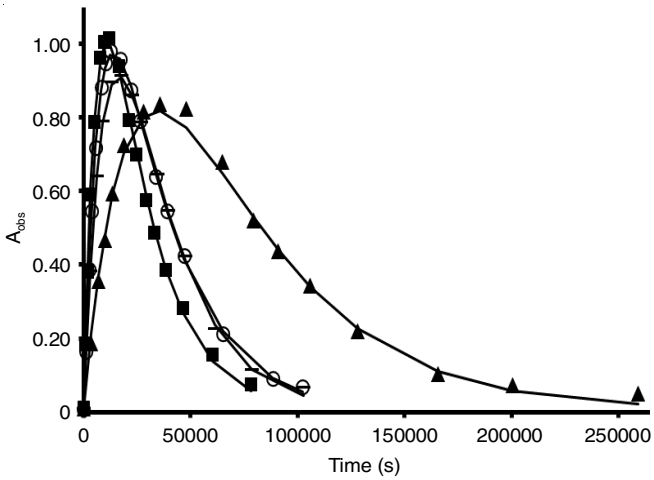

Fig. 2. Plots showing the absorbance at 340 nm *versus* time of 0.1 M (▲), 0.3 M (—), 0.4 M (○) and 0.5 M (■) HCl for acidic hydrolysis of goniothalamin. The solid lines are drawn through the calculated data points

TABLE-1
OBSERVED DATA, TIME *versus* ABSORBANCE AT 340 nm, FOR ACIDIC HYDROLYSIS OF GONIOTHALAMIN AT 0.1 M HCl[a]

Time (s)	A_{obs}	A_{calcd}[b]	RE (%)[c]
33	0.009	0.009	4.87
3571	0.186	0.213	-14.64
6960	0.356	0.371	-4.30
9840	0.467	0.481	-2.89
13560	0.593	0.592	0.10
18840	0.726	0.704	2.98
27900	0.815	0.801	1.73
35820	0.837	0.818	2.27
48060	0.824	0.773	-6.75
64860	0.679	0.648	4.54
79200	0.521	0.530	-1.71
90780	0.436	0.441	-1.09
105900	0.344	0.340	1.21
128100	0.218	0.226	-3.73
165660	0.102	0.110	-7.74
200400	0.072	0.056	21.54
259200	0.049	0.021	57.88

[a][1] = 4×10^{-5} M, [HCl] = 0.1 M, 1.0 M ionic strength, λ = 340 nm, 80 °C; [b]Calculated from eqn. (5.12) with $k_{1\ obs} = 3.70 \times 10^{-5}$ s^{-1}, δ_{app} = 43500 M^{-1} cm^{-1} and $A_0 = 0.0064$; [c]Percentage of relative error, difference between observed and calculated absorbance.

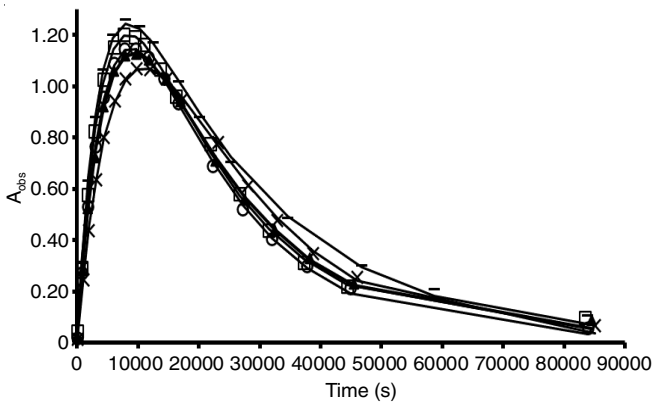

Fig. 3. Plots showing the absorbance at 340 nm versus time of 0.6 M (×), 0.7 M (▲), 0.8 M (○), 0.9M (□) and 1.0 M (—) HCl for acidic hydrolysis of goniothalamin. The solid lines are drawn through the calculated data points

The observed data (Table-1) and Fig. 3 were found to fit to eqn. 1 as shown below:

$$A_{obs} = \frac{A_3 A_1}{A_2 - A_1}\left(e^{-A_1 t} - e^{-A_2 t}\right) + A_4 \qquad (1)$$

where $A_1 = k_1$ (rate of reaction when absorbance increases), $A_2 = k_2$ (rate of reaction when absorbance decreases), $A_3 = \delta_{ap}$ $[A_0]$ and $A_4 = A_0$ ($t = 0$), all of these are empirical constant. $[A_0]$ represents initial concentration of goniothalamin.

During acidic hydrolysis, goniothalamin, as reactant, undergoes reaction catalyzed by hydronium ion to form intermediate product (Int). Subsequently, intermediate product was then gone through second reaction to form final product. It is interesting that the rate constants for these two reactions are different. The first rate constant k_1 found to obey pseudo-first-order reaction. In contrast, second rate constant k_2 does not demonstrate consistency to determine the order of the reaction.

TABLE-2
VALUES OF k_{obs}, A_0 AND $\delta_{ap}[A_0]$ FOR ACIDIC HYDROLYSIS OF GONIOTHALAMIN AT DIFFERENT [HCl][a]

[HCl] (M)	$10^4 k_{1obs}$ (s^{-1})	$10^5 k_{2obs}$ (s^{-1})	$\delta_{ap}[A_0]$ (cm^{-1})	A_0	$10^{-4}\ \delta_{ap}$ ($M^{-1}\ cm^{-1}$)
0.1	0.37 ± 0.09[b]	2.19 ± 0.51[b]	1.738 ± 0.895[b]	0.0064 ± 0.0167[b]	4.35
0.3	0.71 ± 0.10	5.18 ± 0.73	2.111 ± 1.076	0.0117 ± 0.0069	5.28
0.4	1.25 ± 0.47	3.52 ± 0.14	1.618 ± 0.055	-0.0074 ± 0.0074	4.05
0.5	1.48 ± 0.09	3.33 ± 0.20	1.584 ± 0.067	0.0053 ± 0.0129	3.96
0.6	1.31 ± 0.08	6.01 ± 0.38	2.043 ± 0.194	0.0152 ± 0.0087	5.11
0.7	1.74 ± 0.10	6.07 ± 0.34	1.920 ± 0.119	0.0391 ± 0.0107	4.80
0.8	1.81 ± 0.08	6.40 ± 0.29	1.994 ± 0.102	0.0192 ± 0.0090	4.99
0.9	2.00 ± 0.11	6.18 ± 0.33	1.957 ± 0.102	0.0455 ± 0.0119	4.89
1.0	2.51 ± 0.11	4.34 ± 0.21	1.773 ± 0.047	0.0161 ± 0.0145	4.43

[a]$[A_0] = 4 \times 10^{-5}$ M, 1.0 M ionic strength, λ = 340 nm, 80 °C, the aqueous solvent for each kinetic run contained 2% v/v CH_3CN and 98% v/v H_2O. [b]Error limits are standard deviations.

The data of A_1, A_2, A_3 and A_4 obtained within [HCl] range 0.1 M to 1.0 M HCl are presented in Table-2. The non-linear least square technique has been used to calculate the values of A_1, A_2, A_3 and A_4. And such calculated values of A_1, A_2, A_3 and A_4 at 0.1 M HCl are 0.37 ± 0.09 s^{-1}, 2.19 ± 0.51 s^{-1}, 1.738 ± 0.895 and 0.0064 ± 0.0167, respectively. Table-2 showed that reaction rate k_{1obs} (reactant to intermediate product) was increased gradually by increasing the HCl concentration. While reaction rate k_{2obs} (intermediate to final product) values fluctuated between 2.19×10^{-5} s^{-1} to 6.40×10^{-5} s^{-1} and there is no consistent relationship with concentration of HCl.

The plot of k_{1obs} *versus* [HCl] reveals a linear straight line, but k_{2obs} values are fluctuated (Table-3). Therefore, it is concluded that k_{1obs} is pseudo-first-order reaction, while k_{2obs} values are independent of [HCl] (Fig. 4). Linear least-squares techsnique gave slope and intercept of linear plot of k_{1obs} *versus* [HCl] of goniothalamin as $(2.2 \pm 0.2) \times 10^{-4}$ M^{-1} s^{-1} and $(1.93 \pm 1.27) \times 10^{-5}$ s^{-1}, respectively.

TABLE-3
RATE OF ACIDIC HYDROLYSIS OF GONIOTHALAMIN AT 0.1-1.0 M HCl[a]

[HCl] (M)	$10^4 k_{1obs}$ (s^{-1})	$10^4 k_{calc}$ (s^{-1})	$10^5 k_{2obs}$ (s^{-1})	RE (%)
0.1	0.37 ± 0.09[b]	0.409	2.19 ± 0.51[b]	-10.41
0.3	0.71 ± 0.10	0.840	5.18 ± 0.73	-18.31
0.4	1.25 ± 0.47	1.056	3.52 ± 0.14	15.54
0.5	1.48 ± 0.09	1.272	3.33 ± 0.20	14.08
0.6	1.31 ± 0.08	1.487	6.01 ± 0.38	-13.53
0.7	1.74 ± 0.10	1.703	6.07 ± 0.34	2.12
0.8	1.81 ± 0.08	1.919	6.40 ± 0.29	-6.01
0.9	2.00 ± 0.11	2.135	6.18 ± 0.33	-6.73
1.0	2.51 ± 0.11	2.350	4.34 ± 0.21	5.99

[a][1] = 4×10^{-5} M, [HCl] = 0.1-1.0 M, 1.0 M ionic strength, λ = 340 nm, 80 °C.

Activation energy: In order to establish the activation energy, kinetic study of goniothalamin was performed as a function of temperature where five thermostatically controlled oil baths used was set at 40, 50, 60, 70 and 80 ºC. The influence of temperature on the reaction rate constant was given by Arrhenius equation as follows:

$$\ln k_{obs} = \ln A - \frac{E_a}{RT} \quad (2)$$

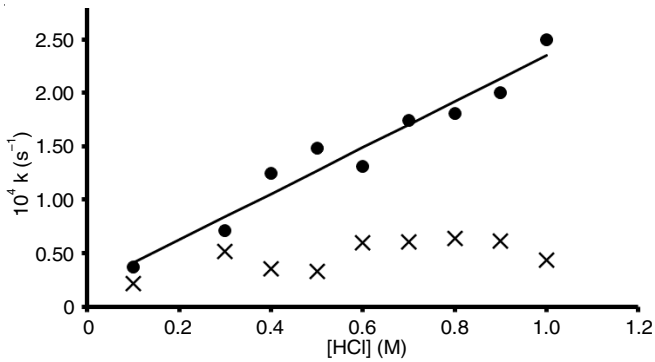

Fig. 4. Plots showing the dependence of k_{1obs} (•) and independence k_{2obs} (×) *versus* [HCl] for the reaction of goniothalamin at 0.1-1.0 M HCl. The solid line is drawn through the calculated data points

where k_{obs} is the reaction rate constant, A is the frequency factor, E_a is the activation energy, R is the universal gas constant and T is the absolute temperature. Table-4 illustrates the linear relationship between ln k_1 and 1/T, where, gradient (m) and and intercept (*c*) were found to be –11014 and 22.7, respectively. Activation energy of the reaction can be calculated by substitute the values into the equation:

$$E_a = -mR = -(-11014 \times 8.314) = 91570 \text{ J mol}^{-1} = 91.6 \text{ kJ mol}^{-1}$$

TABLE-4
EFFECT OF TEMPERATURE ON THE OBSERVED PSEUDO-FIRST-ORDER RATE CONSTANTS (k_1) FOR ACIDIC HYDROLYSIS OF GONIOTHALAMIN IN 1.0 M HCl

T (°C)	10^3 1/T (K^{-1})	$10^5\ k_{1obs}$ (s^{-1})	ln k_{1obs}	ln k_{1calc}[a]
40	3.183	0.48 ± 0.04[b]	-12.24	-12.32
50	3.085	1.13 ± 0.02	-11.39	-11.24
60	2.993	3.99 ± 0.16	-10.13	-10.23
70	2.906	8.43 ± 0.30	-9.38	-9.27
80	2.824	25.10 ± 1.10	-8.29	-8.37

[a]Calculated from eqn. 2; [b]Error limits are standard deviation.

Therefore, the activation energy of acidic hydrolysis of goniothalamin to form intermediate product is 91.6 kJ mol^{-1}. But the activation energy of the sequential process is unable to be identified from the collected data as the second rate constant (k_2) are fluctuated.

NMR analysis and mass spectrometry of degradation products: The structure of degradation product formed from

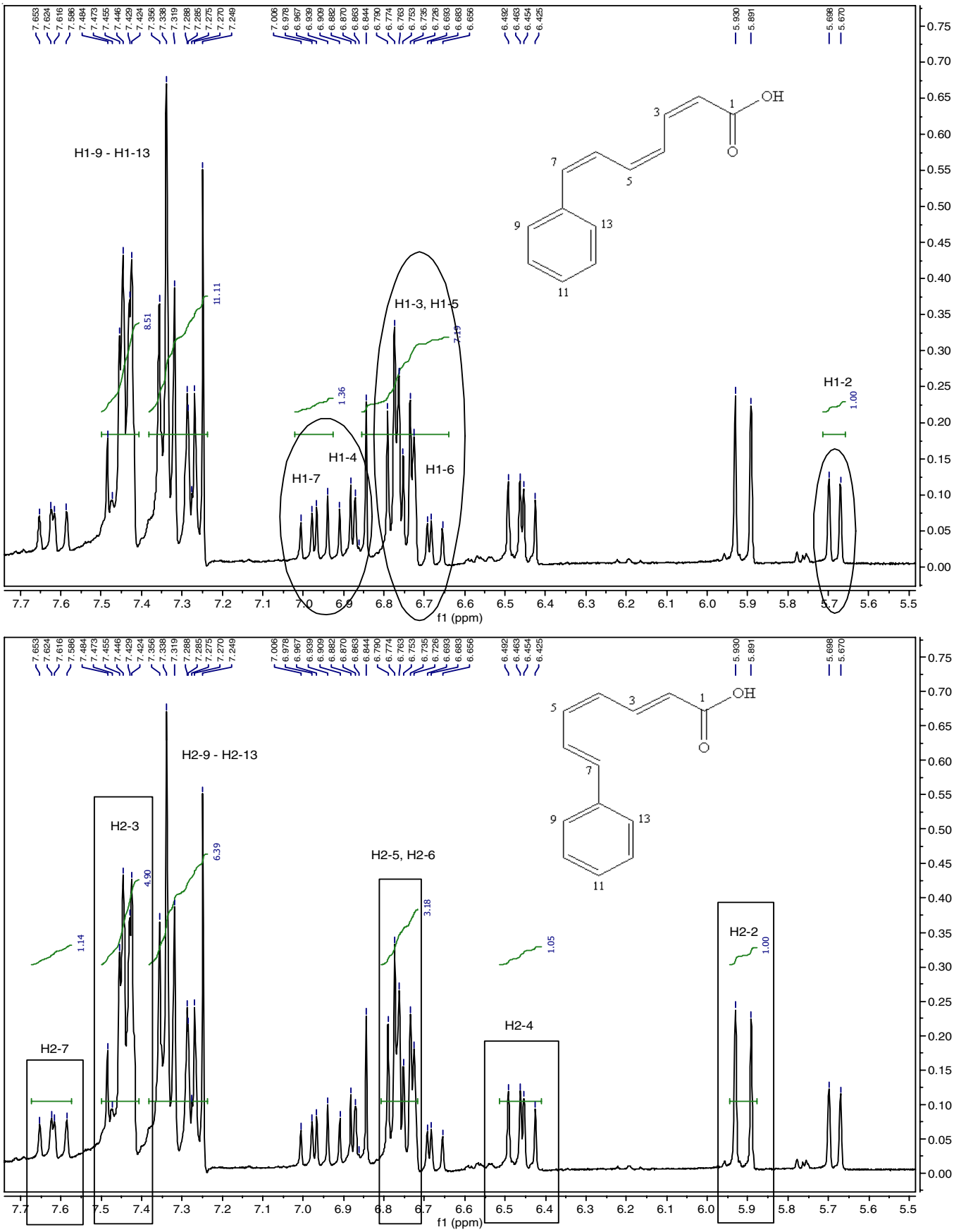

Fig. 5. ^{1}H NMR spectra of minor product (**2**) and major product (**3**). Circles are drawn on the peaks of minor product **2** while the rectangles are drawn on the peaks of major product **3**

acidic hydrolysis was elucidated by NMR and mass spectrometry. Acidic hydrolysis of goniothalamin [13,14] in an aqueous reaction mixture was allowed to progress for the reaction period of more than 10 half-lives. The degradation product was then extracted from aqueous mixture with ethyl acetate by using solvent extraction method. NMR spectra (Fig. 5) illustrated the existence of two products in the extract of acidic hydrolysis. The ratio of amount for these two products in the mixture is significant, therefore integration value in ^{1}H NMR spectra is used to differentiate and analyzed their structures.

(+)-Goniothalamin: White crystal; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.16-7.26 (*m*, 5H, Ph), 6.75 (*ddd*, 1H, *J* = 9.9, 4.6, 3.2 Hz, H-4), 6.60 (*d*, 1H, *J* = 16.2 Hz, H-8), 6.18 (*dd*, 1H, *J* = 16.2, 6.4 Hz, H-7), 5.94 (*dd*, 1H, *J* = 9.9, 1.4 Hz, H-3), 4.89-4.93 (*m*, 1H, H-6), 2.32-2.37 (*m*, 2H, H_2-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 164.1 (C-2), 145.5 (C-4), 135.9 (C-9), 133.0 (H-8), 128.8-126.8 (all Ph C), 125.9 (C-7), 121.2 (C-3), 78.1 (C-6), 29.8 (C-5). LC-MS $[M+H]^+$ *m/z* 201.1044 (calcd. for $C_{13}H_{12}O_2$: 201.0916).

Minor product of HCl hydrolysis: Yellow amorphous; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.16-7.35 (*m*, 5H, Ph), 6.94 (*dd*, 1H, *J* = 15.4, 11.0 Hz, H-7), 6.87 (*dd*, 1H, *J* = 15.6, 11.0 Hz, H-4), 6.75 (*m*, 1H, H-3), 6.73 (*m*, 1H, H-5), 6.68 (*dd*, 1H, *J* = 15.4, 11.0 Hz, H-6), 5.68 (*d*, 1H, *J* = 11.0 Hz, H-2). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 172.3 (C-1), 146.9 (C-3), 142.8 (C-6), 137.4 (C-5), 136.7 (C-8), 128.9, 128.6, 127.0 (all Ph C), 128.6 (C-7), 127.9 (C-4), 116.1 (C-2). LC-MS $[M+H]^+$ *m/z* 201.1004 (calc. for $C_{13}H_{12}O_2$: 201.0916).

Major product of HCl hydrolysis: Yellow amorphous; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.16-7.35 (*m*, 5H, Ph), 7.62 (*dd*, 1H, *J* = 14.6, 11.9 Hz, H-7), 7.45 (*m*, 1H, H-3), 6.77 (*dd*, 1H, *J* = 11.2, 6.9 Hz, H-5), 6.75 (*m*, 1H, H-6), 6.46 (*dd*, 1H, *J* = 15.1, 11.2 Hz, H-4), 5.91 (*d*, 1H, *J* = 15.6 Hz, H-2). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 171.8 (C-1), 146.9 (C-3), 142.1 (C-6), 137.6 (C-5), 136.6 (C-8), 130.0 (C-4), 129.1 (C-7), 128.9, 128.7, 127.0 (all Ph), 119.8 (C-2). LC-MS $[M+H]^+$ *m/z* 201.1004 (calcd. for $C_{13}H_{12}O_2$: 201.0916).

These two products are diastereomers with different *cis-trans* configuration at double bond C2 and C3. The olefinic protons split into doublet at δ 5.68 have coupling constant of 11.0 Hz. This indicated *cis*-configuration of C2 and C3 in minor product. The major product shows *trans*-configuration with coupling constant of 15.6 Hz.

Proposed degradation mechanism: Based on the spectral analysis of the product of acidic hydrolysis of goniothalamin, the mechanism of reaction was proposed as illustrated in Fig. 6. It is suggested that the cleavage of lactone ring occur due to the catalyzation of hydroxonium ions. An intermediate product is formed and it react with hydrogen ions to undergo dehydration, give rise to a double bond at C-4 and C-5. Thus, a mixture of two diastereomers product were formed as final product. The UV spectra of acidic hydrolysis (Fig. 1) at 350 nm kept increasing as reaction going-on. The UV spectrum is compatible with the structure of products formed in acidic hydrolysis. Because the products with three conjugated double bonds contribute to UV absorbance at higher wavelength.

Conclusion

Acidic hydrolysis of goniothalamin is a multi-step reaction (consecutive reaction) as there is an intermediate stage before final product was formed. The UV spectra demonstrated increases of wavelength absorption at 340 nm, followed by decreases in wavelength absorption. The first rate constant, k_{1obs}, is the rate of acidic hydrolysis forming intermediate product. This k_{1obs} is was calculated from the increase of absorbance value, and it obeyed pseudo-first-order reaction with rate constant of 0.37

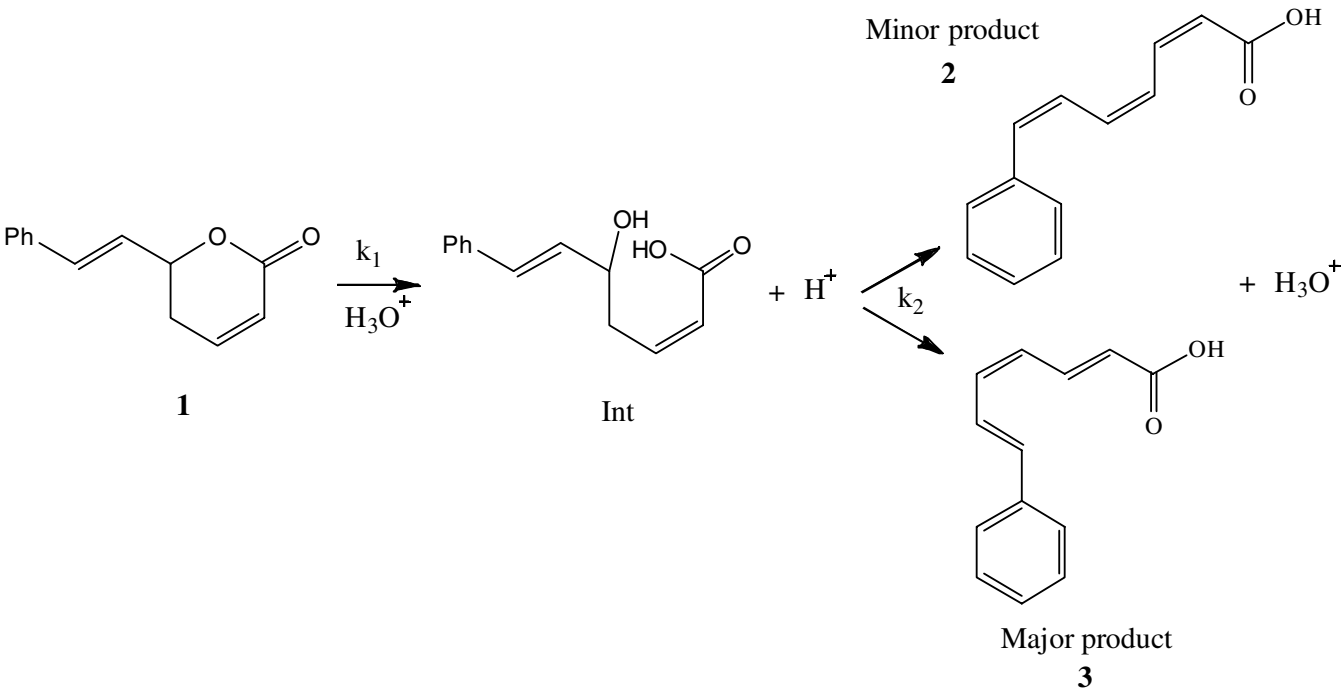

Fig. 6. Hydronium ion-catalyzed opening of the lactone ring and dehydration reaction

$\times 10^{-4}$ s^{-1} at 0.1 M of HCl (80 ºC). While the second rate constant, k_{2obs}, was evaluated from the decrease of absorbance value, when the intermediate product is converting to final product. The k_{2obs} values do not increase or decrease constantly as concentration of HCl increases. Therefore, it is concluded that k_{2obs} (second rate constant) are independent on the concentration of hydrochloric acid. The NMR spectra data analysis showed that final product acquired from acidic hydrolysis of goniothalamin appeared to be diastereomers with *cis-trans* configuration.

ACKNOWLEDGEMENTS

The authors thank Fasihuddin bin Badruddin Ahmad (Universiti Malaysia Sarawak) and Kamarudin Mat Salleh (Universiti Kebangsaan Malaysia) for plant collection and botanical analysis, respectively. This work was supported by Fundamental Research Grant Scheme (FRGS/1/2016/STG01/UM/01/3) and Universiti Malaya research grant (PG138/2013A).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. M.A. Seyed, I. Jantan and S.N.A. Bukhari, *BioMed Res. Int.*, **2014**, 1 (2014); https://doi.org/10.1155/2014/536508
2. C.C. Chiu, P.L. Liu, K.J. Huang, H.M. Wang, K.F. Chang, C.K. Chou, F.R. Chang, I.W. Chong, K. Fang, J.S. Chen, H.W. Chang and Y.C. Wu, *J. Agric. Food Chem.*, **59**, 4288 (2011); https://doi.org/10.1021/jf200566a
3. J.Y. Wach, S. Guttinger, U. Kutay and K. Gademann, *Bioorg. Med. Chem. Lett.*, **20**, 2843 (2010); https://doi.org/10.1016/j.bmcl.2010.03.049
4. N.V. Bihud, N.E. Rasol, S. Imran, K. Awang, F.B. Ahmad, C.-W. Mai, C.-O. Leong, G.A. Cordell and N.H. Ismail, *J. Nat. Prod.*, **82**, 2430 (2019); https://doi.org/10.1021/acs.jnatprod.8b01067
5. N.E. Rasol, F.B. Ahmad, C.-W. Mai, N.V. Bihud, F. Abdullah, K. Awang and N.H. Ismail, *Nat. Prod. Commun.*, **13**, 1575 (2018); https://doi.org/10.1177/1934578X1801301203
6. M.A. Mosaddik and M.E. Haque, *Phytother. Res.*, **17**, 1155 (2003); https://doi.org/10.1002/ptr.1303
7. C.V.B. Martins, M.A. de Resende, D.L. da Silva, T.F.F. Magalhães, L.V. Modolo, R.A. Pilli and A. de Fátima, *J. Appl. Microbiol.*, **107**, 1279 (2009); https://doi.org/10.1111/j.1365-2672.2009.04307.x
8. K.E. Kabir, A.R. Khan and M.A. Mosaddik, *J. Appl. Entomol.*, **127**, 112 (2003); https://doi.org/10.1046/j.1439-0418.2003.00716.x
9. N.H. Lajis, M. Niyaz Khan and H.M. Noor, *J. Pharm. Sci.*, **84**, 126 (1995); https://doi.org/10.1002/jps.2600840128
10. Y.-X. Guo, Z.-L. Xiu, D.-J. Zhang, H. Wang, L.-X. Wang and H.-B. Xiao, *J. Pharm. Biomed.*, **43**, 1249 (2007); https://doi.org/10.1016/j.jpba.2006.10.025
11. A. Zahari, A. Ablat, N. Omer, M.A. Nafiah, Y. Sivasothy, J. Mohamad, M.N. Khan and K. Awang, *Sci. Rep.*, **6**, 21517 (2016); https://doi.org/10.1038/srep21517
12. E. Denisov, O. Sarkisov and G. Likhtenshtein, Chemical Kinetics: Fundamentals and Recent Developments, Elsevier Science (2003).
13. R.P.T. Kim, V. Bihud, K.B. Mohamad, K.H. Leong, J.B. Mohamad, F.B. Ahmad, H. Hazni, N. Kasim, S.N. Halim and K. Awang, *Molecules*, **18**, 128 (2012); https://doi.org/10.3390/molecules18010128
14. A. de Fatima, L.K. Kohn, M.A. Antonio, J.E. de Carvalho and R.A. Pilli, *Bioorg. Med. Chem.*, **13**, 2927 (2005); https://doi.org/10.1016/j.bmc.2005.02.007