Kinetic Study on the Anelation of Heterocycles. 3. Pyrazino[2,3-*d*]pyrimidine Derivatives Synthesized by the Hinsberg Reaction

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A kinetic study on the obtainment of pyrazinopyrimidine derivatives (pteridines) was performed. The regioselective synthesis of compounds 6-methylpyrazino[2,3-*d*]pyrimidin-7(8*H*)-one (**5a**) and 7-methylpyrazino[2,3-*d*]pyrimidin-6(5*H*)-one (**5b**), in good yields, was expected by the Hinsberg reaction because the synthesis of both isomers analytically pure was never reported to date. This Hinsberg reaction gave good results to obtain compound **5a**, regioselectively and in good yields, either in *p*H 5 aqueous buffer solution or in methylene chloride, among several organic solvents. However, structural and solvation factors prevented the synthesis of the isomer **5b**, which was regioselectively formed in very acid solutions (Ho = -0.89), but in very poor yield.

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Introduction.

Four years ago we studied the regioselective synthesis of benzene-substituted quinoxalines **I** [1] as precursors of derivatives which had shown interesting antineoplastic activity [2]. Recently, several authors have reported that the antineoplastic activity increases if one or two carbon atoms of the quinoxaline benzene ring are replaced by nitrogen atoms [3-6].

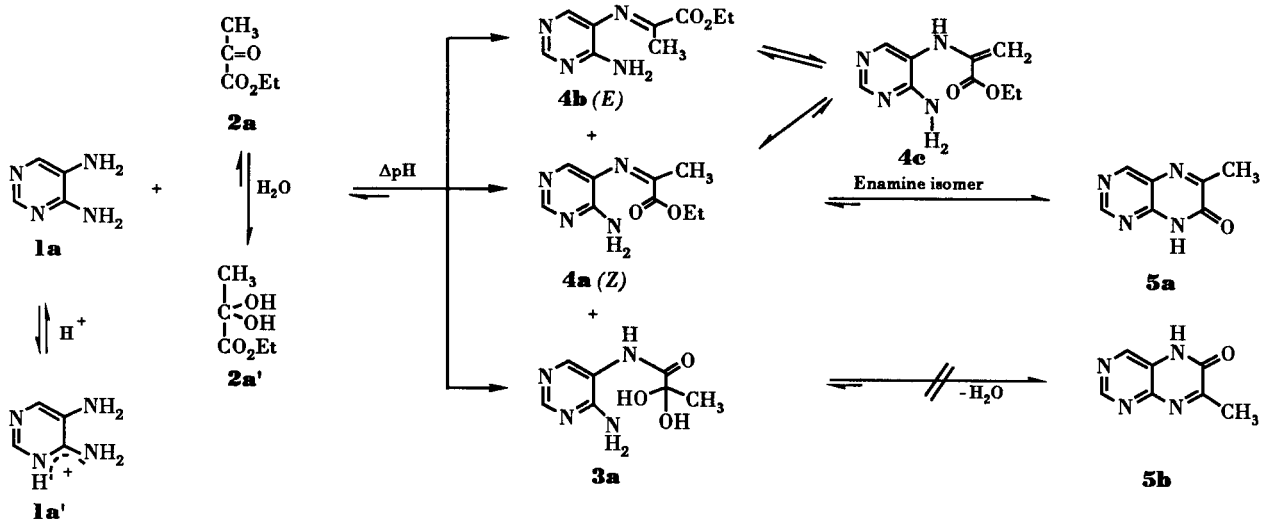
Therefore, we have just reported the study of the regioselective synthesis of several pyridopyrazines **II** by the Hinsberg reaction using 2,3- or 3,4-diaminopyridine and excess glyoxylic, pyruvic or benzoylformic acids as reactants [7-8], trying to obtain precursors in the synthesis of N-1 substituted compounds of **II**, from which higher anticancer activity could be expected [9].

Here we report the study of the synthesis of pyrazinopyrimidines **III** (pteridines) by the Hinsberg reaction in order to observe the feasibility of the reaction when the pyrimidine π-deficient substrate is used and, how the introduction of another nitrogen atom (N-7) will affect the antineoplastic activity of N-1 substituted derivatives which we plan to synthesize in the future.

The achievement of regioselective reactions requires the study of their kinetics and mechanisms when different experimental conditions are employed. Thus these aspects of this problem are presented here.

1) Reaction of 4,5-Diaminopyrimidine **1a-a'** with Ethyl Pyruvate **2a** in Aqueous Buffer Solutions.

Scheme 1



Reactions of **1a-a'** with **2a** were first followed by uv spectrophotometry in buffers of different *p*H values from -0.89 to 11.5. However, in every aqueous solution the peaks of **1a** and the supposedly synthesized products **5a-b** were superimposed and the kinetics could not be followed. The second and fourth derivatives were computationally applied trying to solve this problem but this new method to follow kinetics [10] also failed. The hplc technique gave good results. These are presented in the Table and allowed us to postulate that the reaction proceeds as out-

Table

Reaction Between 4,5-Diaminopyrimidine and Ethyl Pyruvate in Different *p*H Aqueous Buffer Solutions and in Organic Solvents

Aqueous Solutions						Organic Solvents					
*p*H	time	t_r (min)	Compound	% Area	k_{obs} h^{-1} (**5a**)	Solvent	time	t_r (min)	Compound	% Area	k_{obs} h^{-1} (**5a**)
11.50	12 days	2.05	**1a**	100	---			2.15	Open Product	5.29	
								2.42	**1a**	5.25	
		2.30	**1a**	99.977			23 minutes	2.69	Open Product	0.38	
	22 minutes	4.81	**5a**	0.021				3.40	Open Product	1.65	
		8.05	**5b**	0.002				4.76	(Z)	87.43	
		1.90	Open Product	0.05				1.89	Open Product	4.97	
	5.02 days	2.30	**1a**	99.86				2.15	Open Product	10.66	
		4.80	**5a**	0.09			6.94 days	2.43	**1a**	4.17	
7.40					1.12×10^{-9}			4.76	(Z)	40.91	
		2.31	**1a**	99.75	(uncertain)			5.77	**5a**	39.29	
	12 days	4.81	**5a**	0.13		CH_2Cl_2					4.3×10^{-7}
		8.03	**5b**	0.12				1.90	Open Product	7.29	
								2.15	Open Product	10.40	
		2.30	**1a**	99.52			12 days	2.43	**1a**	3.11	
	16 days	4.80	**5a**	0.24				4.76	(Z)	10.08	
		8.02	**5b**	0.24				5.78	**5a**	69.12	
	41 minutes	1.90	Open Product	22.47				1.90	Open Product	3.81	
		3.95	(Z)	77.53				2.15	Open Product	4.36	
							16 days	2.42	**1a**	0.01	
		1.91	Open Product	15.88				4.75	(Z)	0.02	
	3 days	3.96	(Z)	54.09				5.77	**5a**	91.80	
		5.00	**5a**	30.02							
5.14					7.70×10^{-7}			1.94	Open Product	2.09	
		1.91	Open Product	14.30				2.26	Open Product	1.10	
	5.02 days	3.96	(Z)	35.60			23 minutes	2.62	**1a**	24.31	
		5.01	**5a**	50.10				4.15	(Z)	68.12	
								5.35	**5a**	4.38	
		1.90	Open Product	2.30				1.96	Open Product	6.02	
	7 days	3.36	Open Product	0.02				2.25	Open Product	1.49	2.88×10^{-7}
		3.95	(Z)	26.38		THF	6.98 days	2.74	Open Product	3.76	(uncertain)
		5.01	**5a**	71.30				4.16	(Z)	61.63	
								5.36	**5a**	27.10	
		2.14	**1a**	70.05							
	1 hour	3.18	Open Product	0.12				1.95	Open Product	2.47	
		4.41	(Z)	29.83				2.25	Open Product	1.66	
							12 days	2.72	Open Product	9.25	
		2.13	**1a**	16.43				4.15	(Z)	40.57	
	5.02 days	3.17	Open Product	30.35	5.61×10^{-8}			5.35	**5a**	46.05	
1.2		440	(Z)	53.22							
								1.47	Open Product	0.86	
		2.14	**1a**	5.21				1.68	Open Product	3.51	
	12.05 days	3.18	Open Product	2.12				2.18	Open Product	5.62	
		4.41	(Z)	83.66			20 minutes	2.46	**1a**	60.67	
		5.05	**5a**	9.01				4.24	(Z)	29.33	
								15.42	**5b**	0.01	
		2.03	**1a**	94.13				1.69	Open Product	5.12	
	80 minutes	3.45	Open Product	3.10				2.18	Open Product	1.68	

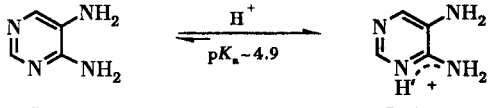
Table (continued)

Aqueous Solutions						Organic Solvents					
*p*H	time	t_r (min)	Compound	% Area	k_{obs} h^{-1} (**5a**)	Solvent	time	t_r (min)	Compound	% Area	k_{obs} h^{-1} (**5a**)
		3.86	Open Product	0.02		DMF	7 days	2.47	**1a**	50.18	1.94×10^{-10}
		5.04	**5a**	2.75				3.04	Open Product	2.53	(uncertian)
								4.24	(Z)	40.47	
		2.03	**1a**	90.44				15.42	**5b**	0.02	
		2.97	Open Product	0.05							
-0.89	5.05 days	3.45	Open Product	0.04	8.05×10^{-8}			1.69	Open Product	7.15	
		4.29	Open Product	0.20	(uncertian)			2.18	Open Product	0.13	
		5.05	**5a**	3.22			12 days	2.47	**1a**	40.22	
		11.55	Degradation Product	6.05				4.24	(Z)	52.46	
								15.41	**5b**	0.04	
		2.03	**1a**	9.97				1.94	Open Product	2.09	
		3.45	Open Product	0.01				2.26	Open Product	5.54	
	11.98 days	5.06	**5a**	0.11			20 minutes	2.62	**1a**	24.33	
		11.55	Degradation Product	10.07				4.12	Open Product	0.34	
								5.35	(Z)	67.70	
		14.96	Degradation Product	79.84							
								1.96	Open Product	5.74	
								2.28	Open Product	30.55	
						MeOH	6.85 days	2.62	**1a**	18.26	8.99×10^{-8}
								5.37	(Z)	34.44	(uncertain)
								15.66	**5b**	11.01	
								1.95	Open Product	16.66	
								2.25	Open Product	29.05	
							12 days	2.61	**1a**	8.01	
								5.37	(Z)	26.78	
								15.59	**5b**	18.50	

lined in Scheme 1.

It can be seen in the Table that the reaction does not take place when 0.1*N* sodium hydroxide is used as the solvent. This fact points out that anelation requires acid catalysis to proceed. When the *p*H value is decreased to 7.14 compound **5a** slowly begins to appear having a retention time of 4.8 minutes. But the nucleophilicity of the 5-NH_2 group of **1a** does not differ much from that of the 4-NH_2 group at this *p*H value, so, the other isomer **5b** also appears, even more slowly (t_r = 8.02-8.03 minutes). Therefore, at *p*H 7.14 the classic "Hinsberg mixture" is obtained in low yields. Observed rate constants for both isomers were approximately 1.1×10^{-9} h^{-1} (Table).

In turn, when the reaction was performed at the *p*H value of maximum stability of the intermediate Schiff bases **4a-c** (*p*H 5) [11], 70% of the anelation was achieved in seven days and **5a** was regioselectively obtained. It is interesting to point out that reactants disappeared at this *p*H value in less than 40 minutes during the fast step of the reaction.



1a **1a'**

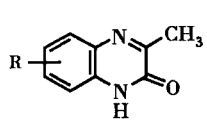
Lowering the *p*H to very acid values, for example 1.2 or -0.89 (Table) nucleophilicity of the 5-NH_2 group in **1a'** decreases due to the equilibrium **1a** ⇌ **1a'** and reactions to obtain **5a** are not favorable. The same occurs when 10% sulfuric acid is used. In this case degradation reactions take place in a short time (Table).

The same equilibrium **1a** ⇌ **1a'** supports the fact that only the -NH_2 group at C-5 can undergo nucleophilic attack upon the *alpha* C=O group of **2a** to give 6-methylpyrazino[2,3-*d*]pyrimidin-7(8*H*)-one (**5a**) as the *sole* product of the reaction. It can be concluded that in aqueous solutions **5a** can be smoothly obtained regioselectively by applying acid catalysis.

2) Reaction of 4,5-Diaminopyrimidine **1a-a'** with Ethyl Pyruvate **2a** in Anhydrous Organic Solvents.

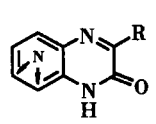
Reaction of **1a** with **2a** leads to the open products **4a-c** during the fast step of the reaction. These products appear during the period of different retention times between 1.90 and 4.80 minutes. Afterwards, the *Z*-isomer anelates to give **5a** during the slow step of the reaction.

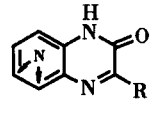
We can see in the Table that anelation, at room temperature, comes up to 40% of **5a** in seven days and 92% of **5a** in 16 days when anhydrous methylene chloride is used as the solvent. On the other hand, 50% of **5a** is achieved in



I

R = H, CH_3, OCH_3, NH_2, NO_2



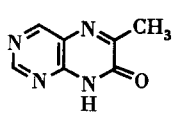


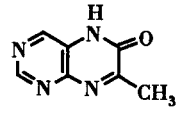
II

R = H, CH_3, C_6H_5

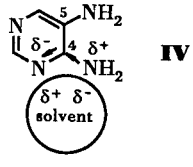
12 days when anhydrous THF is used.

Obviously, the major solvation by these two non-polar solvents (dichloromethane and THF) through a polarized accommodation between N-3 and 4-NH_2 groups (**IV**) facilitates the attack of the 5-NH_2 group upon the α-keto group of **2a** to give **5a** in a shorter time (Scheme). We must also consider that both solvents favour the solubility of the intermediates, so the anelation of the *Z*-isomer is more feasible.





III



IV

When the aprotic polar solvent dimethylformamide (DMF) was used, (Table) we expected a higher rate of reaction to give **5a**. However, anelation to obtain **5a** did not occur and, instead, traces of **5b** were observed. We presume that decomposition reactions can take place in this solvent or a higher degree of solvation can occur at the Schiff bases level which inhibits the anelation of the *Z*-isomer to give **5a**. Though **5b** is obtained in DMF, yields are very poor. Nevertheless, the reaction in this solvent is regioselective.

The same happened when methanol was used as solvent. The good solubility of **1a** and **2a** in this solvent favours the reaction but the possibility of hydrogen bonds during the formation of the open intermediates may retard anelation and only 19% of **5b** appears after twelve days (Table).

Other methods reported in the literature did not give good results when **5a** or **5b** were intended to be synthesized [12-13], and always the Hinsberg mixture was obtained mixed with other open intermediate products.

We could obtain **5a** in good yields (>70%) *via* the Hinsberg reaction but this is not a good method to synthesize the isomeric 7-methylpyrazino[2,3-*d*]pyrimidin-6(5*H*)-one (**5b**).

Thus, we can conclude that the better regioselective synthesis of **5a** is the Hinsberg modified anelation in aqueous solution at *p*H 5.14 or in anhydrous dichloromethane.

Therefore, the investigation of a good regioselective method to obtain **5b** still remains open.

EXPERIMENTAL

The kinetic measurements were performed with a Hewlett-Packard 1050 hplc spectrophotometer. The nmr spectra were obtained on a Varian FT 80A spectrometer with tetramethylsilane as the internal reference. The ir spectra were recorded on a Beckman IR-20A spectrophotometer using potassium bromide pellets. Analytical samples on the starting materials were used to perform the kinetic studies.

6-Methylpyrazino[2,3-*d*]pyrimidin-7(8*H*)-one (**5a**).

Compound **5a** was synthesized by the Hinsberg reaction from 0.5 g (4.54 mmoles) of **1a** and 5 ml of **2a** (45.6 mmoles) in aqueous buffer solution of *p*H 5.14 (10 ml) at room temperature with stirring. The resulting solid crystallized from ethanol (white needles) affording **5a** (70% yield), mp 198-199°. Spectral properties of compound **5a** are described in the literature [12-13]. Reference compound **5a** was synthesized according to [12].

7-Methylpyrazino[2,3-*d*]pyrimidin-6(5*H*)-one (**5b**).

Reference compound **5b** was synthesized according to the method of Albert and Reich [12]. Yellow powder was obtained and crystallized from methanol (20% yield), mp 270-271°. The mp had never been reported until now. Spectral properties of **5b** are described in [12].

Kinetic Measurements.

Reactions were performed at 25° using buffers of *p*H -0.89, 1.2, 5.14, 7.40 and 0.1*N* sodium hydroxide. The *p*H of each solution above 0.40 was measured at 25° in a Metrohm E632 *p*H meter using a standardized glass electrode. Values 1.2 and -0.89 were taken from Hine [14]. Reactions performed with initial concentrations 2×10^{-2} to 2×10^{-4} *M* of **1a-a'** showed a first-order dependence on the pyrimidine derivative at every hydrogen concentration at which anelation occurred. All rate constants were obtained from 1.40×10^{-4} *M* initial concentration of **1a-a'** and 9.80×10^{-2} *M* of **2a-a'**.

The appearance of **5a-b** and also the *Z*-isomer was followed by tlc on Silica gel $F_{254-366}$ plates and kinetics were followed by hplc with a Hewlett-Packard 1050 apparatus, using a C_8 column (2.1 x 200 mm), λ = 290 nm, methanol:2-propanol:water (50:10:40) as the mobile phase and Q = 0.250 ml/minute.

Rate constants (Table) were obtained from the area under the peaks, and linear profiles accounted for pseudo-first order kinetics.

General Kinetic Procedure.

Solutions (1.40×10^{-4} *M*) of **1a-a'** and (9.80×10^{-2} *M*) of **2a-a'** in buffers or organic solvents were prepared and thermostated at 25° ±0.1°. Both solutions were mixed and an aliquote was injected in the hplc apparatus. The appearance of **5a** was followed at 290 nm until 70-80% of its final concentration was achieved. When this was not possible, observed rate constants were reported as "uncertain".

Acknowledgement.

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[9] We are now trying to obtain N-1 substituted derivatives of **II** replacing the N_1 hydrogen by 5-piperazinopentyl, 5-aminopentyl, 3-*n*-propylaminopropyl, and 4-*n*-propylpiperazinopropyl groups which will be submitted for screening as antineoplastic agents to the National Cancer Institute, USA.

[10] This method was developed by us to follow and compare kinetics of *o*-phenylendiamine with ethyl pyruvate. Results were good and the observed rate consants were within a variation of $\pm 0.05\%$. The new method was more precise than the classic uv spectrophotometry. (Unpublished results).

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