Comparative Kinetic Studies on the Synthesis of Quinoxalinone Derivatives and Pyrido[2,3-*b*]pyrazinone Derivatives by the Hinsberg Reaction

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Kinetic studies on the anelation of quinoxalinone derivatives **3a-c** and pyrido[2,3-*b*]pyrazinone derivatives **5a-c** and **6a-c** synthesized by the Hinsberg reaction is reported. *o*-Phenylenediamine or 2,3-diaminopyridine were treated with bifunctional carbonyl compounds such as glyoxylic, pyruvic and benzoylformic acids under different experimental conditions. When pyridopyrazine derivatives were synthesized both position isomers were achieved applying regioselective reactions. Mixture were avoided by looking for special experimental conditions that led unambiguously to only one of the components of the classic "Hinsberg mixture". Quinoxalinone derivatives **3a-c** were obtained at room temperature in good yields (>90%) using anhydrous methanol or ethanol as solvents. On the other hand, only pyrido[2,3-*b*]pyrazin-3(4*H*)-one (**5a**) was regioselectively attained in aqueous buffer of *p*H 7 while 3-methylpyridopyrazinone derivatives were regioselectively separated using anhydrous methanol for one isomer, **5b**, and anhydrous chloroform for the other isomer, **6b**, at room temperature. Yields were higher than 80%. Reactions with benzoylformic acid did not give good yields and only 2-phenylpyrido[2,3-*b*]pyrazin-3(4*H*)-one (**5c**) could be obtained using anhydrous chloroform (yield <30%) as the solvent. Steric hindrance exerted by the phenyl group of the benzoylformic acid is supposed to be responsible of our difficulties to obtain 2-phenylpyrido[2,3-*b*]pyrazin-3(4*H*)-one (**5c**) in good yields applying this technique. The other isomer, 3-phenyl[2,3-*b*]pyrazin-2(1*H*)-one (**6c**) was always formed together with the former isomer and could not be isolated from the mixture, when other solvents than chloroform were used as the reaction media.

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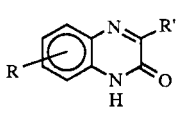
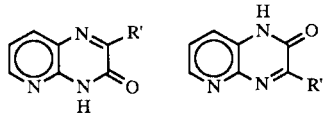
Four years ago our interest turned to the regioselective synthesis of substituted quinoxalinones **I** (R = H, CH_3, NH_2, OCH_3, NO_2; R' = CH_3) as precursors in the synthesis of antineoplastic compounds based on simplified models of the antitumoral agent echinomicine [1]. We are now interested in replacing the methyl group in $C_{2\text{-}3}$ (R') by hydrogen and the phenyl group trying to afford new sites of reaction for the introduction of substituents which could increase the antitumoral activity.

On the other hand, recently we noted [2] that the anticancer activity increases if one or two carbon atoms of the quinoxalinone benzene ring are replaced by nitrogen atoms, **II** [3], so we are interested in comparing both series **I** and **II** when the methyl group is replaced by hydrogen or a phenyl group and R = H in the quinoxalinone series **I**.



I



II

To obtain series **I** and **II** we treated *o*-phenylenediamine **1** (series **I**) and 2,3-diaminopyridine **4** (series **II**) with bifunctional carbonyl compounds such as glyoxylic, pyruvic and benzoylformic acids. We worked at room temperature and used aqueous buffers or organic solvents.

Here we describe the kinetics of the reactions and look for regioselective syntheses in the case of pyridopyrazine derivatives **II** because the presence of the nitrogen atom in the 2,3-diaminopyridine leads to position isomers and low yields when anelation occurs. Consequently, we tried to attain better yields looking for unambiguous conditions when we applied a modified Hinsberg reaction [5] to a π-defficient ring (pyridine).

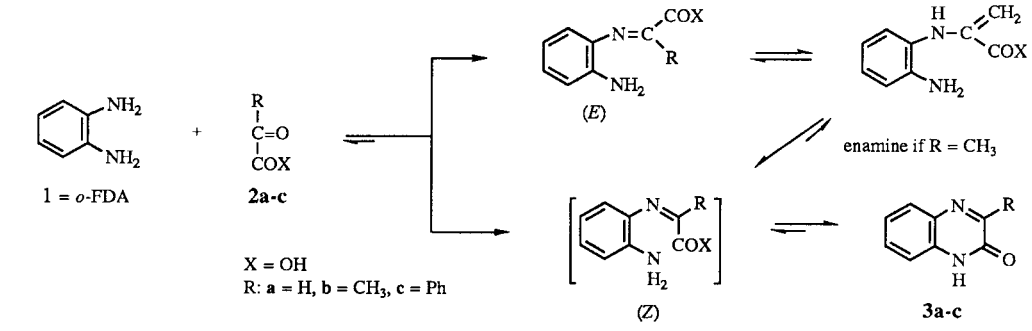
Quinoxalinone Derivatives **3a-c**.

Reaction of *o*-phenylenediamine with pyruvic and benzoylformic acids leads to 3-substituted quinoxalin-2(1*H*)-ones. Position isomers do not exist in these cases and we have concluded early that substituents in the benzene ring of the *o*-phenylenediamine did not favour the condensation, whichever the electronic effect being exerted by them [1].

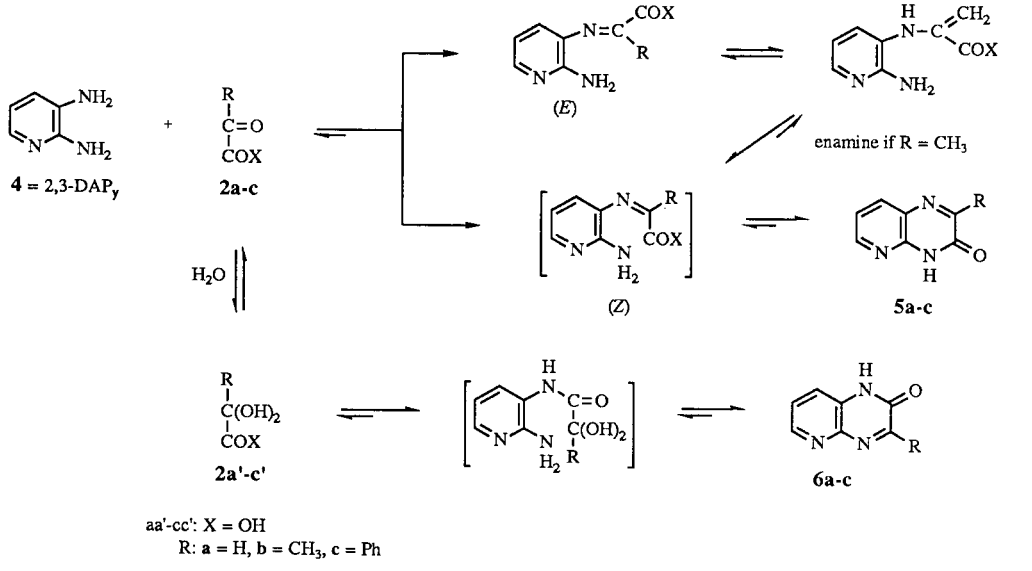
Therefore, we selected *o*-phenylenediamine **1** as reactant for series **I** to study the effect of substituents in C-3 upon the anelation. On the other hand, we selected 2,3-diaminopyridine as the reactant, instead of 3,4-diaminopyridine for obtaining pyrido[2,3-*b*]pyrazinones **II**, because much better yields are obtained with the former [4].

Reactions of *o*-phenylenediamine **1** or 2,3-diaminopyridine **4** with the acid reactants were followed by uv spectrophotometry in 10% sulfuric acid, 0.1*N* sodium hydroxide,

Scheme 1



Scheme 2



and *p*H 5-8 aqueous buffers and also in several organic solvents at room temperature. Reactions occurred as outlined in Schemes 1 and 2.

The appearance of the quinoxalinone derivatives **3a-c** was followed above 320 nm where open intermediates did not absorb. Working with excess of the acid and initial *o*-phenylenediamine concentrations of 10^{-3} to 10^{-5} *M* a first order dependence on the latter was observed.

Reactions in Aqueous Solutions and in Organic Solvents.

Reaction of **1** with glyoxylic acid (**2a**) and benzoylformic acid (**2c**) was followed at the following *p*H values: 0.1, 5, 8 and 11.5. The appearance of **3a** and **3c** (Scheme 1) was followed at 320 nm, being in the case of **3a**, the anelation of the *Z*-isomer the rate determining step of the reaction. However, anelation did not occur when benzoylformic acid (**2c**) was used and only "mixed observed rate constants" (1.1×10^{-2} min^{-1} in alkali and 2.3×10^{-3} min^{-1} in 10% sulfuric acid) could be determined for the formation of open products (Scheme 1). In this case, the first step of the reaction (Schiff bases formation) could be followed because the steric hindrance exerted by the phenyl group of **2c** diminishes the Schiff base rate of formation and determines a kinetic controlled reaction in which only a mixture of open products is obtained.

Working at *p*H 5 the first step is considered to give almost complete transformation of the diamine into the Schiff base isomers. The *Z*-isomer is then rapidly transformed into **3a** (the same occurred with **3b** [1]) during the subsequent slower step of dehydration. Values of observed rate constants for these reactions are listed in Table 1. Those for **1** with the acid reactants are in general, two logarithmic units higher in anhydrous organic solvents than in aqueous solutions when **2a** and **2b** are used, and maintain the same lower level when **2c** is used (Table 1).

Table 1

Rate Constants (min^{-1}) Observed for the Reaction of 1 with 2a, 2b, and 2c (Scheme 1) in Aqueous Media and in Organic Solvents

		Aqueous media				Organic solvents				
Reactants	Products	10% SO_4H_2	*p*H 5	*p*H 8	0.1*N* NaOH	Ethanol	Methanol	Chloroform	Benzene	THF
1 + 2a	3a	7.94×10^{-4}	1.2×10^{-3}	1.2×10^{-3}	1.1×10^{-3}	2.94×10^{-1}	1.3×10^{-1}	3.9×10^{-1}	5.1×10^{-1}	1.3×10^{-1}
1 + 2b	3b	9.80×10^{-5}	1.002	1.003	1.003	1.87×10^{-2}	2.6×10^{-2}	4.5×10^{-2}	6.2×10^{-2}	1.1×10^{-2}
1 + 2c	3c	----	8.5×10^{-4}	7.6×10^{-4}	----	0.91×10^{-3}	4.5×10^{-4}	5.5×10^{-4}	----	----

Table 2

Observed Rate Constants (min^{-1}) for the Reaction of 4 with 2a, 2b, and 2c in Aqueous Media and in Organic Solvents (Scheme 2)

		Aqueous media				Organic solvents			
Reactants	Products	10% SO_4H_2	*p*H 5	*p*H 8	0.1*N* NaOH	Products in methanol		Products in chloroform	
4 + 2a'	5a	----	3.72×10^{-3}	3.08×10^{-3}	5.0×10^{-5}	5a + 6a	2.5×10^{-5} 3.7×10^{-5}	6a + 5a	1.3×10^{-3} 2.0×10^{-4}
4 + 2b'	5b + 6b	6.3×10^{-5} 5.2×10^{-6}	1.25×10^{-3} 6.15×10^{-4}	1.58×10^{-4} 5.60×10^{-5}	1.1×10^{-5} 4.6×10^{-6}	5b	4.68×10^{-6}	6b	3.23×10^{-6} [a]
4 + 2c'	----	----	----	----	----	5c + 6c	4.10×10^{-7} 1.51×10^{-7}	5c	1.3×10^{-4}

[a] Ethylpyruvate was used in this case [4].

Reaction of 2,3-Diaminopyridine (**4**) with Glyoxylic (**2a**), Pyruvic (**2b**) and Benzoylformic (**2c**) Acids in Aqueous Buffer Solutions.

Reaction of **4** with **2a-c** (Scheme 2) was followed by uv spectrophotometry above 330 nm in 10% sulfuric acid *p*H 5 and 0.1*N* sodium hydroxide. Reactions followed Scheme 2 and pyrido[2,3-*b*]pyrazinone derivatives **5** and **6** were obtained only in the case of being the substituent in $C_{2\text{-}3}$ hydrogen, **5a-b**, or methyl group, **6a-b**.

Compounds **5a-b** and **6a-b** were separated and identified by uv through tlc with post-elution. The failure to obtain the 3-phenyl derivative was accounted for by the extreme *p*H values used in basic and acidic media, so we proved that *p*H 5 is in accord with the stability of the intermediate base in aqueous medium [1] (Table 2).

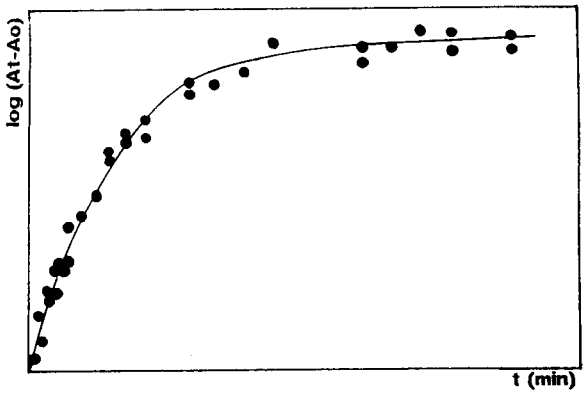


Figure. Characteristic profiles of absorbance *vs* time for the obtainment of pyrido[2,3-*b*]pyrazine derivatives when the "Hinseberg mixture" is obtained.

When **4** was treated with benzoylformic acid (**2c**) (Scheme 2) the corresponding pyridopyrazinones **5c** and **6c** could not be obtained. We deduced that steric effects play an important role in this particular case when the Schiff bases have been formed. Mixtures of compounds **5a-6a** and **5b-6b** were obtained working with **2a** and **2b** respectively (Scheme 2) in aqueous media, as it can be deduced from hyperbolic curves as that shown for example in the Figure. Values of k_1 and k_2 are given in Table 2.

We have postulated that the hydrate form of **2** (Scheme 2) is responsible for the obtainment of **6** [4] because the hydrate form **2a'-c'** is favoured and it is catalysed by H^+ or OH^- [6]. However, using glyoxylic acid (**2a'**) in aqueous solution at *p*H > 5 only one product was regioselectively obtained, pyrido[2,3-*b*]pyrazin-3(4*H*)-one (**5a**) ($k_{obs} \sim 3 \times 10^{-3}$ min^{-1}, Table 2) while if pyruvic acid (**2b**) was used the mixture of both isomers was obtained, 2-methylpyrido[2,3-

b]pyrazin-3(4*H*)-one (**5b**) and 3-methylpyrido[2,3-*b*]pyrazin-2(1*H*)-one (**6b**) (Scheme 2). Compounds **5a**, **5b** and **6b** could be isolated and identified by ^{1}H nmr and tlc with post-elution followed by the uv spectrum development, but yields were very poor.

Reaction of **4** with Glyoxylic (**2a**), Pyruvic (**2b**) and Benzoylformic (**2c**) Acids in Organic Solvents.

We have observed that compound **5b** was regioselectively obtained in anhydrous methanol at room temperature while **6b** was the sole product when anhydrous chloroform was used as solvent [4]. Here we proved several organic solvents using **2a** and **2c** with **4** and paid special attention to reactions in methanol and chloroform. Data from the kinetics are given in Table 2.

Results and Discussion.

As we can see in Table 1 the observed rate constants decrease on increasing the molecular volume of the reactant. Reaction of *o*-phenylenediamine occurs in aqueous media at *p*H 5-8 when benzoylformic acid (**2c**) is used. When organic solvents are used, the reaction of **1** with **2c** gives negative results in benzene and THF.

As we had observed in [1] reactions of **1** with **2b** are not acid or base-catalysed and give an observed rate constant close to unity (Table 1), while in sulfuric acid (H_o range) reaction probably undergoes another mechanism [1].

When reactions with 2,3-diaminopyridine **4** are performed in organic solvents, both amino groups of **4** do not differ much as nucleophiles (in water we determined two pK_a values: 4.89 and -0.58, *i.e.*, radically different), so, when **4** is confronted with a little bulky molecule such as glyoxylic acid, the formation of both isomers **5a** and **6a** is expected (Table 2).

Experiments with greater amounts of the diamine **4** (0.5-1 g) gave good yields with pyruvic acid (>80%) to give **5b** in anhydrous methanol and **6b** in anhydrous chloroform. If ethyl pyruvate is used instead of pyruvic acid, the yield is >90% [4]. If benzoylformic acid was used as the reactant, the reaction in chloroform was slow but led to **5c** as the sole product with a yield lower than 30%.

The Hinsberg mixture was obtained in every case when **2c** was used as the reactant except in anhydrous methanol. This agrees with the fact that only in a polar solvent such as methanol the pK_a value of the reacting amino group may be close to that observed in water (4.89) and can undergo the nucleophilic attack upon the C=O group of pyruvic acid to give **5c** (Table 2).

EXPERIMENTAL

The ultraviolet spectra and kinetic measurements were performed with a Jasco 7850 uv/visible spectrophotometer. The nmr spectra were determined on a Varian FT 80A spectrometer with tetramethylsilane as the internal reference. The ir spectra were recorded on a Beckmen IR 20A spectrophotometer using potassium bromide pellets. The hplc spectra were obtained on a Beckman 110B apparatus. Analytical samples of the starting materials were used to perform the kinetic studies.

Quinoxalin-2(1*H*)-one (**3a**).

Compound **3a** was synthesized from 0.5 g of **1** (4.63 mmoles) and 5 g of **2a** (67.56 mmoles) in anhydrous methanol (15 ml) at room temperature with stirring. The resulting solid crystallized from ethanol (white needles) affording **3a** (92% yield), mp 268-269°. Spectral properties were described by Nishio [8]; uv (methanol): λ max nm 226, 280, 347.

3-Methylquinoxalin-2(1*H*)-one (**3b**).

Compound **3b** was prepared starting from 0.5 g of **1** (4.63 mmoles) and 5 g of **2b** (56.82 mmoles) in anhydrous ethanol (12 ml) at room temperature with stirring. Compound **3b** crystallized as white needles (95% yield), mp (ethanol) 244-245° (lit 245°). Spectral properties were described by Nishio [8]; uv (methanol): λ max nm 216, 255, 325.

3-Phenylquinoxalin-2(1*H*)-one (**3c**).

Compound **3c** was prepared from 0.5 g of **1** (4.63 mmoles) and 6 g (40.0 mmoles) of **2c** in anhydrous methanol (10 ml) at room temperature with stirring. The resulting solid crystallized from ethanol (white powder) affording **3c** (85% yield), mp 250-251°. Spectral properties were described by Nishio [8]; uv (ethanol): λ max nm 290, 300, 320.

2-Methylpyrido[2,3-*b*]pyrazin-3(4*H*)-one (**5b**).

Compound **5b** was prepared from 0.5 g of **4** (4.58 mmoles) and 5 g of **2b** (56.82 mmoles) in anhydrous methanol (10 ml) at room temperature with stirring. The resulting solid crystallized from ethanol (white needles) giving **5b** (84% yield), mp 240° dec, lit [6]; ir: 1700 (C=O), 2700 (N-H), 2900-3040 (C-H) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 8.5-8.6 (dd, 1, py), 8.1-8.25 (dd, 1, py), 7.3-7.5 (q, 1, py), 2.5 (s, 3, CH_3) ppm; uv (methanol): λ max nm 223, 321, 329; hplc: (C_8 mobile phase sodium dihydrogen phosphate-TEA-AcN 5%, λ 330, Q 1.7 ml/min), t_r: 16.22 minutes.

3-Methylpyrido[2,3-*b*]pyrazin-2(1*H*)-one (**6b**).

Compound **6b** was synthesized by the same procedure as above using anhydrous chloroform as the solvent instead of methanol. A pale yellow powder was obtained from ethanol, mp 279° dec, 270° [9]; ir: 1650 (C=O), 2650 (N-H), 2960 (C-H) cm^{-1}; ^{1}H nmr (DMSO-d_6): δ 8.5-8.6 (dd, 1, py), 7.7-7.9 (dd, 1, py-4-aromatic), 7.4,7.6 (q, 1, py), 2.5 (s, 3, CH_3) ppm; uv (methanol): λ max nm 225, 325, 336; hplc: (same experimental conditions as above), t_r: 3.9 minutes.

2-Phenylpyrido[2,3-*b*]pyrazin-3(4*H*)-one (**5c**).

Compound **5c** was synthesized starting from 0.5 g of **4** (4.58 mmoles) and 6 g of **2c** (40.0 mmoles) in anhydrous chloroform at room temperature with stirring. After two days at room temperature a yellow precipitate **5c** appeared that was filtered and crystallized from ethanol (mp >300°); ir: 700 (C-H arom), 1450 (C=C arom), 1550 (C=C arom), 1650 and 1675 (C=O), 2400-3150 (broad signal, N-H and OH) cm^{-1}; uv (ethanol): λ max 216, 280, 365; ^{1}H nmr (DMSO-d_6): δ 12.70 (br s, 1, H), 8.5-8.6 (dd, 1, H py), 8.15-8.30 (dd, 1, H py), 7.25-7.75 (m, 6, H py and Ph) ppm.

Anal. Calcd. for $C_{13}H_9N_3O$: C, 69.94; H, 4.06; N, 18.82. Found: C, 69.98; H, 4.19; N, 18.79.

Kinetic Measurements.

Reactions were performed at room temperature using 10% sulfuric acid, 0.1*N* sodium hydroxide and aqueous buffers *p*H 5-8. 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 below 0.40 were taken as H_o from Hine [7]. Reactions performed with initial concentrations 2 x 10^{-2} to 2 x 10^{-4} *M* of **1** and **4** showed a first-order dependence on the base concentration at every hydrogen concentration at which anelation occurred. All rate constants were obtained from 1.8 x 10^{-4} *M* initial concentrations of **1** and **4** and 9.80 x 10^{-2} *M* of **2a**, **2b** and **2c**. The appearance of **3a-c**, **5a-c** and **6a-c** was followed by uv spectrophotometry at wavelengths above 330 nm at which only quinoxalinone derivatives and pyridopyrazinone derivatives absorb.

Rate constants were obtained from data of log (A_t-A_o) vs time by computational treatments. Linear profiles accounted for pseudo-first order kinetics and hyperbolic profiles (Figure) accounted for biexponential curves according to: $(A_t-A_o) = K_1[1-\exp(-k_1t)] + K_2[1-\exp(-k_2t)]$. This equation was solved and adjusted to our experimental values by a software developed by us to obtain k_1 and k_2 (Table 2). K_1 and K_2 in the equation above are preexponential constants and k_1 and k_2 are exponential factors related to the observed rate constants for the attainment of **3a-c**, **5a-c** and **6a-c** when competitive reactions take place.

General Kinetic Procedure.

Solutions (1.8 x 10^{-4} *M*) of **1** and **4** and (9.8 x 10^{-2} *M*) of the bifunctional acids in the buffers or organic solvents were prepared. Both solutions were mixtured and the appearance of the reaction product was followed by uv spectrophotometry until 80-90% of its final concentration was achieved.

Experimental k_{obs} and adjustment of pK_a values were subject to computational treatments [4].

Acknowledgement.

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[10] As it was observed, the presence of the C=O group causes an increase in melting point in quinoxalines and pyridopyrazines and a marked lowering in the solubility [9]. The same was observed by us in our corresponding derivatives.