Gabriela A. Rodrigo, Sergio G. Renou, Diana G. Bekerman,
M. Inés Abasolo and Beatriz M. Fernández*

Department of Organic Chemistry, Faculty of Pharmacy and Biochemistry,
University of Buenos Aires, (1113) Junin 956, Buenos Aires, Argentina
Received October 1, 1996

Some novel 3-substituted benzoquinoxalinones [R = H, CH_3, C_6H_5, $(CH_2)_2COOH$] were synthesized by the Hinsberg reaction between 2,3-diaminonaphthalene and several α-dicarbonyl compounds. The course of the reactions was followed by the second uv/visible Derivative Spectroscopy Method at different *p*H values (-0.89 to 9.0) and also in organic solvents at 25°. The compound non-substituted at C-3 was the only one that could be obtained in every media in good yields (80%), having pseudo first-order anelation rate constants of relative high values (1×10^{-1} - 1×10^{-2} min^{-1}). On the other hand, only methanol could be used as the organic solvent for the synthesis of all of the other compounds; aqueous media always provided better results. In the 3-methyl derivative, as well as in the 3-phenyl derivative the change of the reaction *p*H medium modified the stoichiometry of the anelation, turning a non-quantitative reaction into a quantitative one. This is explained by a change in the mechanism of the reaction on going to lower hydrogen concentrations, a fact that was supported by complementary quantitative hptlc experiments. In general, pseudo first-order rate constants for the anelation were one or two logarithmic units lower than those of the non-substituted compound (R_{C-3} = H), but yields were above 60% in every case. A reaction scheme is presented which includes a probable mechanism.

J. Heterocyclic Chem., **34**, 505 (1997).

The purpose of this work is to find useful synthetic routes to afford nuclear chromophores which may be precursors in the synthesis of antineoplastic compounds. A number of years ago our interest turned to regioselective synthesis of substituted quinoxalinones [1]. We have noticed that anticancer activity increases if the cyclic chromophore is a tricyclic compound instead of a bicycle, probably due to enhanced intercalation efficiency with DNA. Therefore, we undertook a study of the kinetics of the synthesis of some novel 3-substituted benzoquinoxalinone derivatives searching for specific and unambiguous conditions with good yields.

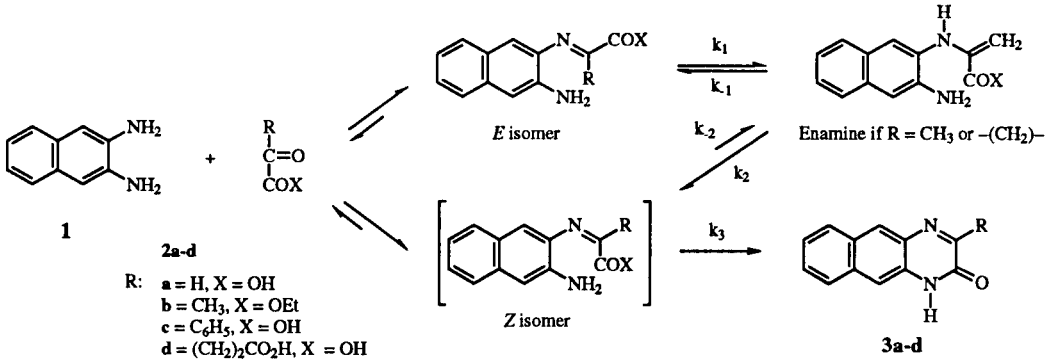
Compounds **3** (Scheme 1) were obtained by the Hinsberg reaction [2] between 2,3-diaminonaphthalene (**1**) and α-bifunctional carbonyl compounds **2a-d**.

The kinetics of the reaction were followed by our second derivative uv spectroscopy method [3] at an adequate wavelength because the spectral bands of reactants and reaction products overlap in the fundamental uv spectra (Figure 1a). From the kinetic point of view, the synthesis of each 3-substituted benzo[*g*]quinoxalin-2(1*H*)-one derivative presents its own characteristics so each reaction will be treated individually.

Reaction of 2,3-Diaminonaphthalene (**1**) with Glyoxylic Acid (**2a**).

The kinetic study on the reaction between **1** and **2a** was performed under different experimental conditions. Anelation occurred at room temperature either in acid or alkaline media up to *p*H 9, and in many organic solvents (DMF, THF, ethylenechloride, methanol, etc.). Kinetics were followed by the second derivative UV spectroscopy at 375 nm. Observed apparent first-order kinetic constants for the anelation were of the order $1x10^{-1}$ min^{-1} in aqueous solutions and $1x10^{-2}$ min^{1} in organic solvents. Compound **3a** was the only derivative which could be

Scheme 1



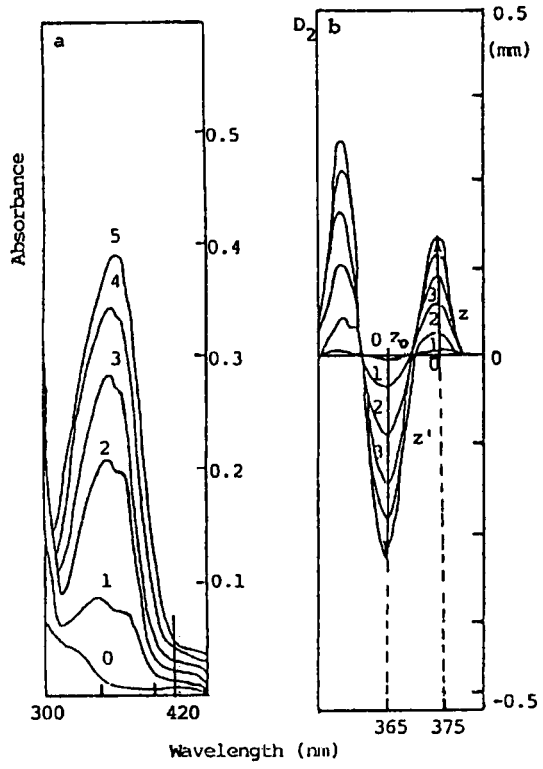


Figure 1. a: Fundamental spectra given in numerical order attainment, at different times (minutes), of reaction **1** + **2b** (ethyl pyruvate) at *p*h 3.0 (Scheme 1). b: Second uv derivative of the spectral data in Figure 1a.

obtained in other organic solvents different from methanol. It has a partial solubility in every reaction [5] media which results in non-quantitative reaction where the final product **3a** must be isolated from the mixture of intermediates of the reaction.

Reaction of 2,3-Diaminonaphthalene (**1**) with Ethyl Pyruvate (**2b**).

A fast reaction is observed when **1** reacts with the ethyl pyruvate (**2b**) in acid medium (*p*H 3) or in neutral medium (*p*H 7) at 25° to give **3b**. Kinetics of the reaction were followed by the second derivative uv spectroscopy at 375 nm complemented by hptlc with post elution [4].

In acid medium (*p*H 3) the hptlc chromatograms showed that this is a consecutive reaction in which the non-catalysed formation of the *E*-isomer and the enamine isomer is the rate determining step of the reaction, while the *Z*-isomer anelates immediately in aqueous media during the fast step of the reaction (Scheme 1).

Anelation at *p*H 3.

Furthermore, hptlc experiments [4] allowed us to see when the steady state of the reaction reached the value of $k_{-2} \neq 0$ (Scheme 1). This fact leads to a non-quantitative reaction in which a mixture of **3b** and intermediate reaction products is finally obtained.

Anelation at *p*H 7.

In this case the reaction intermediates are not salts as in the above case, but free bases. These can account for an enhanced basicity and nucleophilicity of the $-NH_2$ group of the *Z*-isomer at *p*H 7 which causes its instantaneous anelation to give **3b**. The precipitation of **3b** with a constant displacement of the reaction equilibrium towards the final product leads in this case to a *quantitative reaction* ($k_{-2} = 0$). In fact, when the reaction reaches the steady state it is observed by hptlc [4] that only the α-dicarbonyl compound, initially in excess, is present. Values of apparent kinetic constants of anelation are in the order 1 x 10^{-2} min^{-1} in both *p*H media.

Reaction of 2,3-Diaminonaphthalene (**1**) with Benzoylformic Acid (**2c**).

The best yields of the reaction between **1** and **2c** were obtained in acid solution at 25°. In neutral or alkaline media the reaction is slow and gives poor yields. Again two different behaviours are observed according to the *p*H of the reaction medium. Hptlc experiments allow us to verify that this reaction follows the same reaction scheme obviously without the presence of an enamine isomer; it is consecutive, acid catalysed and appears to be *quantitative* at *p*H 0.08 and non-quantitative at *p*H 1.3. The kinetic study was followed by second derivative uv spectroscopy at 375 nm and kinetic constants for the anelation in acid media were in the order 1 x 10^{-3} min^{-1}.

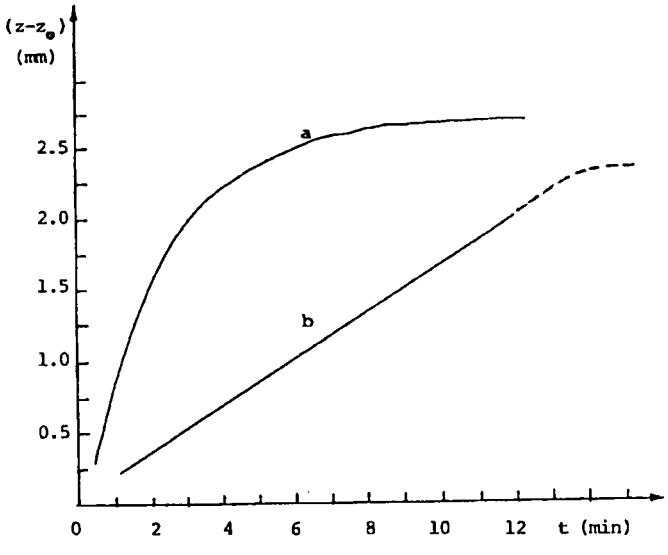


Figure 2. a: Hyberbolic profile for the attainment of compound **3b** at *p*H 3.0 denote that two competitive reactions take place. Values of $(z-z_0)$ were calculated by computational treatments from eq. $(z-z_0) = M[1-\exp(-b_1t)] + N[1-\exp(-b_2t)]$. b: Linear profile for the attainment of the same compound (**3b**) at *p*H 7.0 accounts for a quantitative pseudo first-order reaction according to $(z-z_0) = \ln 2/t_{1/2}$. The value of the apparent rate constant of anelation was 7.3 x 10^{-2} min^{-1} in this case.

Reaction of 2,3-Diaminonaphthalene (1) with α-Ketoglutaric Acid (2d).

Kinetic study of the reaction between 1 and 2d showed a non-catalysed and non-quantitative reaction which occurs at *p*H values between -0.89 and 7.00 at 25°. No reaction was observed in aqueous media at *p*H >7 or organic solvents. Kinetic constants were in the order 1 x 10^{-3} min^{-1} at *p*H >2. Hptlc experiments showed that at every *p*H value $k2 \neq 0$, therefore the final product 3d is obtained in a mixture with the ring-opened intermediate reaction products.

We can conclude that, in the preparation of some 3-substituted benzo[g]quinoxalin-2(1H)-one derivatives the change in *p*H modifies the stoichiometry of the reaction from non-quantitative ($k_2 \neq 0$) to quantitative ($k_2 = 0$) reactions (Scheme 1). On the other hand, the change of water in the reaction medium by an organic solvent (as methanol) do not provide better effectivity of anelation or better yields. Furthermore, high temperatures of reactions above 30° result in severe decomposition and those below 15° cause very slow reactions which exclude the possibility of determining thermodynamic parameters.

EXPERIMENTAL

Melting points were measured on a Buchi apparatus and are uncorrected. The uv absorption spectra and those from the derivative mode were obtained on a Jasco 7850 WMS spectrophotometer. The ir spectra were recorded on a Jasco A200 spectrophotometer using potassium bromide pellets. The ^1H nmr spectra were determined on a Varian FT 80A spectrometer with tetramethylsilane as the internal reference. Chromatographic experiments [4] were carried out on Camag equipment and 5 x 5 x 0.2 mm hptlc F_{254} silica gel chromatoplates were used. The intermediates of the reaction were synthesized by pc using the Chromatotron equipment. Analytical samples of the starting materials were used to perform the kinetic studies.

Benzo[g]quinoxalin-2(1H)-one (3a).

To a methanol solution (17 ml) of 1 (50 mg, 0.34 mmole) was added a methanol solution (2 ml) of 2a (59 mg, 0.64 mmole) at room temperature, and the mixture was stirred for 5 minutes. The resulting yellow precipitate was collected and washed with cold methanol to give 3a in 78% yield, mp (DMF/water) 301-304° dec; ir: 3000, 1690, 1640, 1410, 1170, 880, 730 cm^{-1}; ^1H nmr (DMSO-d_6): δ 7.51 (m, 2H, aromatics), 7.70 (s, 1H, aromatic), 7.97 (dd, 2H, N=C-H), 8.21 (s, 1H, aromatic), 8.42 (s, 1H, aromatic), 12.35 (s, 1H, NH); uv (methanol): λ max 238, 274, 331, 342 nm; hplc, λ 340 nm (methanol); sodium heptanesulfonate 0.1 g in acetic acid:methanol:water (1:40:40) as the mobile phase and Q = 1 ml/minute, t_r (min) 11.8.

Anal. Calcd. for $C_{12}H_8N_2O$: C, 73.46; H, 4.11; N, 14.28. Found: C, 73.49; H, 4.19; N, 14.33.

3-Methylbenzo[g]quinoxalin-2-(1H)-one (3b).

To a methanol solution (17 ml) of 1 (50 mg, 0.34 mmole) was added a methanol solution (2 ml) of ethyl pyruvate 2b (73 mg, 0.63 mmole) at room temperature. The mixture was stirred for 10 minutes. The resulting yellow precipitate was collected and washed with cold methanol to give 3b in 75% yield, mp (methanol/water) 293-295° dec (lit 291°) [5]; ir: 2900, 1680, 1630, 1590, 1420, 1190, 880, 750 cm^{-1}; ^1H nmr (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 7.44 (m, 2H, aromatic), 7.64 (s, 1H, aromatic), 7.94 (dd, 2H, aromatics), 8.30 (s, 1H, aromatic), 12.25 (s, 1H, NH); uv (methanol): λ max 237, 275, 285, 350 nm; hplc, λ 340 nm (methanol); sodium heptanesulfonate 0.1 g in acetic acid:methanol:water (1:40:40) as the mobile phase and Q = 1 ml/minute; t_r (min) 16.33.

3- Phenylbenzo[g]quinoxalin-2(1H)-one (3c).

To a methanol solution (17 ml) of 1 (50 mg, 0.34 mmole) was added a methanol solution (2 ml) of 2c (95 mg, 0.63 mmole) and 0.1 ml of sulfuric acid 2.5%. The mixture was stirred at room temperature. After 45 minutes, 100 ml of water was added and a yellow precipitate appeared. It was collected, washed with water to give 3c in 70% yield, mp (methanol/water) 212-215° dec; ir: 3400, 1610, 1600, 1490, 1390, 990, 870, 740, 690 cm^{-1}; ^1H nmr (DMSO-d_6): δ 7.46 (t, 2H, aromatic), 7.48 (m, 1H, aromatic), 7.53 (m, 2H, aromatics), 7.68 (s, 1H, aromatic), 7.86 (dd, 2H, aromatics), 7.92 (dd, 2H, aromatics), 8.40 (s, 1H, aromatic), 12.30 (s, 1H, NH); uv (methanol): λ max 227, 265, 271, 340 nm; hplc, λ 340 nm (methanol), sodium heptanesulfonate 0.1 g in acetic acid:methanol:water (1:40:40) as the mobile phase and Q = 1 ml/minute; t_r (min) 17.58

Anal. Calcd. for $C_{18}H_{12}N_2O$: C, 79.39; H, 4.44; N, 10.29. Found: C, 79.36; H, 4.34; N, 10.18.

3-[3-Benzo[g]quinoxalin-2(1H)-one]propanoic Acid (3d).

To a methanol solution (17 ml) of 1 (50 mg, 0.34 mmole) was added a methanol solution (2 ml) of 2d (92 mg, 0.63 mmole) at room temperature, and the mixture was stirred for 7 minutes. The resulting yellow precipitate was collected and washed with cold methanol to give 3d in 78% yield, mp (methanol/water) 261-263° dec; ir: 2900, 1710, 1650, 1640, 1560, 1510, 1400, 1300, 1190, 880, 750 cm^{-1}; ^1H nmr (DMSO-d_6): δ 2.73 (t, 2H, CH_2), 3.11 (t, 2H, CH_2), 7.50 (m, 2H, aromatics), 8.03 (dd, 2H, aromatics), 8.32 (s, 1H, aromatic), 12.11 (s, 1H, COOH), 12.31 (s, 1H, NH); uv (methanol): λ max 238, 273, 284, 330, 340 nm; hplc, λ 340 nm (methanol), sodium heptanesulfonate 0.1 g in acetic acid:methanol:water (1:40:40) as the mobile phase and Q = 1 ml/minute; t_r (min) 14.0.

Anal. Calcd. for $C_{15}H_{12}N_2O_3$: C, 67.16; H, 4.51; N, 10.44. Found: C, 67.27; H, 4.59; N, 10.56.

Kinetic Measurements.

Reactions were performed at room temperature using buffers of a definite *p*H value or organic solvents. Reactions performed with initial concentrations 2 x 10^{-2} to 2 x 10^{-4} *M* of 1 showed a first-order dependence of the naphthalene derivative 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 9.80 x 10^{-2} of 2a-d. The appearance of 3a-d was followed by second derivative uv spectroscopy at a satisfactory wavelength. Values obtained by the method "peak to zero" (see below) were plotted *vs.* time (Figure 2). Linear profiles accounted for pseudo-first order kinetics according to the equation $k_{obs} = \ln e/t_{1/2}$, which were obtained in the case of quantitative reactions and hyperbolic profiles accounted for biexponential curves according to

$$(z\text{-}z_0) = M[1\text{-}\exp(\text{-}b_1t)] + N[1\text{-}\exp(\text{-}b_2t)]$$

This equation was adjusted to our experimental values by computational treatments. M and N values are preexponential constants and b_1 and b_2 are exponential factors related to the observed rate constants for the attainment of **3a-d** and the corresponding open-ring intermediates when competitive reactions take place.

General Kinetic Procedure.

Solutions (1.8×10^{-4} *M*) of **1** and (9.8×10^{-2} *M*) of the α-bifunctional carbonyl compound **2a-d** in buffers or organic solvents were prepared. Solutions were mixed, thermostated at 25° and the appearance of the reaction product was followed by the uv derivative spectroscopy mode until 80-90% of its final concentration was achieved.

UV/VIS Derivative Mode. Peak to Zero Method.

A double-beam UV-VIS JASCO 7850 spectrophotometer with capability of the derivative mode was used. The optimized operating conditions recording D_2 spectra, using 1.000 cm quartz cells were: scan speed 100 nm min^{-1}, time constant "fast," spectral band width 2nm.

The D_2 uv spectra were recorded as a function of time. These spectra look like those shown in Figure 1 for compound **3b**, which is presented as an example not having significant differences from the D_2 spectra of the rest of the derivatives **3**. The graphical method "peak to zero" [3] (mm) consisted in measuring the increasing peaks from the basal zero line upwards or downwards ($z\text{-}z_0$ or $z'\text{-}z_0'$) at each corresponding wavelength and plotting each series of values *vs.* time. Observed rate constants were calculated by the corresponding equation as it was explained under "kinetic procedure."

Acknowledgement.

Financial support for this investigation was provided by the Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET). We thank Karina B. Piton, Paula G. Varela and Federico J. Wolman for their careful technical assistance.

REFERENCES AND NOTES

[1] M. I. Abasolo, C. H. Gaozza and B. M. Fernández, *J. Heterocyclic Chem.*, **24**, 1771 (1987).

[2] H. Hinsberg, *Liebigs Ann. Chem.*, **237**, 368 (1887).

[3] M. I. Abasolo, D. G. Bekerman, G. A. Rodrigo and B. M. Fernández, *Anal. Asoc. Quim. Argent.*, in press (1996).

[4] G. A. Rodrigo, D. G. Bekerman, S. G. Renou, M. I. Abasolo and B. M. Fernández, *J. Planar Chromat.*, **8**, 444 (1995).

[5] M. J. Bertrand, L. Maltais, F. Brisse and M. J. Olivier, *Can. J. Chem.*, **63**, 3386 (1985).