Equilibrium and Kinetics of Rotamer Interconversion in Immunosuppressant Prodigiosin Derivatives in Solution

VINCENZO RIZZO,* AMEDEA MORELLI, VITTORIO PINCIROLI, DOMENICO SCIANGULA, AND ROBERTO D'ALESSIO

Contribution from *Pharmacia & Upjohn, viale Pasteur 10, I-20014 Nerviano, Italy.*

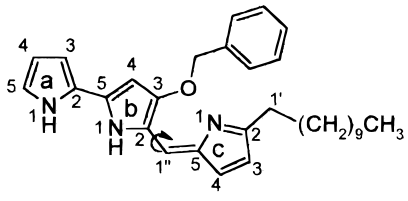
Received May 27, 1998. Final revised manuscript received September 2, 1998.
Accepted for publication September 8, 1998.

Abstract □ The equilibrium and relative rate of rotamer interconversion around the bond joining the 2,2′-bipyrrolyl and pyrromethene moieties in a synthetic analogue of immunosuppressant prodigiosin are investigated as a function of pHapp in a water/acetonitrile mixture (1/1 by volume). Two chromatographically separable isomeric forms are obtained in acid solutions (pHapp < 4), whereas rapid interconversion occurs above neutrality. Furthermore, pH modulates the conformational preference of the molecule according to nitrogen protonation on the three pyrrole rings system ($pK_a = 7.2$). At high pHapp (neutral form), the same conformer that is observed in pure acetonitrile prevails, whereas the other one is preferred by the protonated form. The nuclear magnetic resonance data indicate that the structures of the two conformers mainly differ in the value of the torsion angle around the aforementioned C–C bond. Kinetic and equilibrium data are quantitatively interpreted with a cyclic mechanism including two protonation ($pK_{a1} = 8.23 \pm 0.03$, $pK_{a2} = 5.4 \pm 0.2$) and two conformational rearrangement steps. A molecular interpretation of the observed behavior includes, for the preferred conformer at low pH, formation of a new hydrogen bond between the exocyclic oxygen and the neighboring pyrrole NH upon protonation of the three pyrrole rings system.

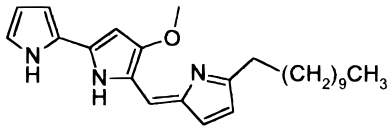
Introduction

Prodigiosins are a class of natural red pigments isolated from *Streptomyces Genus* that are endowed with potent antibacterial and cytotoxic properties.[1] More recently, immunosuppressive properties have been discovered for some members of this class,[2,3] and ascribed to a mechanism of action well distinguished from that exerted by cyclosporin A or other related drugs.[4] In a medicinal chemistry program devoted to the preparation of synthetic analogues of the natural prodigiosins,[5] PNU-156804 (Figure 1) emerged as a lead with very favorable pharmacological properties. These favorable properties prompted a detailed investigation of the solution properties of this analogue and the development of a specific analytical high-performance liquid chromatography (HPLC) method. Anomalous behavior (peak splitting, tailing, etc.) was immediately detected by reversed-phase HPLC under various conditions and attributed to geometrical isomerization in the time scale of chromatography. On the basis of this result, the equilibrium and kinetics of this geometrical transformation in solution were investigated with the aim of both optimizing conditions for chromatography and better understanding the molecular properties of this new class of compounds in water-containing solutions.

PNU-156804 contains the 2,2′-bipyrrolyl−pyrromethene chromophore whose structure is compatible with several



PNU-156804



PNU-156737

Figure 1—Chemical structure of PNU-156804 and PNU-156737. The numeration system used in the presentation af results is shown for PNU-156804 together with the torsion angle that is involved in rotamer interconversion.

geometrical isomers arising from different equilibrium positions around the bonds connecting the three pyrrole rings. The extensive conjugation of this chromophoric system is apparent from the different mesomeric structures that can be written. As a consequence, rotation barriers lower than expected for ordinary double bonds may arise, and interconversion among geometrical isomers may become detectable at ordinary temperatures. In the present investigation we report data on both the equilibrium composition and the rate of interconversion for the two geometrical isomers that are chromatographically resolved at room temperature under acidic conditions. The compound is not soluble enough in pure water, but a study as a function of pHapp in solutions of water/acetonitrile (1/1) proved feasible. The study was carried out for solutions of various pHapp (glass electrode readings) as it was immediately clear that protonation of the molecule greatly affected the conformational equilibrium and the rate of interconversion. Finally, a structural model of the two conformers is proposed on the basis of two-dimensional nuclear magnetic resonance nuclear Overhauser enhancement spectroscopy (2D-NMR NOESY) data.

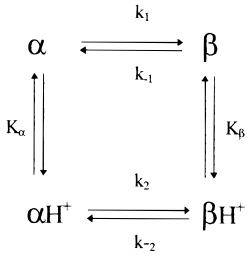
Materials and Methods

Materials—PNU-156804 (either as HCl or as CH_3SO_3H salt) and PNU-156737 were prepared as previously described.[5] The structure of both compounds has been verified with NMR and mass spectroscopy (MS) techniques. HPLC grade acetonitrile and methanol were Carlo Erba products. Methylene chloride RPE, trifluoroacetic acid RPE, and all chemicals for buffer preparation were purchased from Carlo Erba. Water was purified with a Milli-Q apparatus (Waters). Deuterated solvents were purchased from Merck.

Spectrophotometric Titrations and Kinetic Measurements—Data were collected on a Perkin-Elmer Lambda 4 instru-

* To whom future correspondence should be addressed.

10.1021/js980225w CCC: $18.00
Published on Web 11/14/1998



Scheme 1

ment connected to a Perkin-Elmer 7700 computer. The cuvette holder was thermostated at 25 °C with fluid circulation from a water bath (LTD-6, Grant). A fresh solution was prepared for each determination by adding 0.200 mL of a stock solution in acetonitrile (concentration (C) ≈ 0.04 mg/mL) to 2 mL of a titration solvent with pH near the desired value (the final concentration of PNU-156804 was around 0.004 mg/mL). The actual pHapp value (range 2−11) was determined in the cuvette immediately after the spectroscopic measurement (absorbance at 525 nm). The titration solvent was made out of a mixture containing 275 mL of aqueous phosphate 0.02 M, NaCl 0.1 M, and 225 mL of acetonitrile, whose pHapp was adjusted with small additions of 2 N HCl or NaOH to the desired value. The final solvent composition in the cuvette was 1/1 acetonitrile/aqueous phase by volume. Solutions for kinetic measurements were similarly prepared with both titration solvent and stock solution pre-thermostated at 25 °C, and the instrument was set in the time drive mode.

Kinetic Measurements: Stopped Flow—A High Tech SF51 instrument in the light absorption mode was used for fast kinetic measurements. The conformational change was induced by a change of pH and monitored by measuring light absorption at 500 nm at pH values between 3 and 7, and at 430 nm at pH values >7. In detail, the two syringes were filled with either a solution of PNU-156804 in acetonitrile/buffer at about pH 11 (1/1) when jumping from alkaline to neutral/acid region and at about pH 3 when jumping from the acid to the neutral/alkaline region or acetonitrile/buffer at the desired pH (0.02 M phosphate, NaCl 0.1 M). The pHapp of the final solution was determined after mixing equal volumes of the two solutions just mentioned in a test tube, and the final concentration of PNU-156804 in the observation chamber of the stopped flow was similar to that used for the spectrophotometric experiments (C ≅ 0.004 mg/mL).

HPLC Measurements—Chromatograms were collected with a Perkin-Elmer instrument with a LC-410 quaternary pumping system, a 235C diode-array detector, and a Turbochrom data station. Ordinary chromatograms were obtained with a Hipak C8 AB (250 × 4.6 mm, 5 μm) with a mobile phase (flow = 1 mL/min) of methanol/tetrahydrophuran/water (55/25/20 by volume) upon buffering the aqueous phase to the desired pH. Fast resolution was obtained with a C18 cartridge (3 × 3 CR, Perkin-Elmer) with a mixture of water/methanol/methylene chloride/acetonitrile/trifluoroacetic acid (300/400/150/150/1 by volume) as mobile phase at the flow rate of 2 mL/min. This procedure provided sufficient separation of the two isomeric forms in a short analysis time (8 min), which was needed to limit interconversion of the two conformers. Generally, 20 μL of an approximately 0.05 mg/mL solution were injected, and detection was obtained at 500 nm.

NMR Measurements—The ^{1}H NMR data were collected at 28 °C with a Varian Unity 600 instrument operating at 600 MHz. The standard Varian pulse sequences and processing software were used. Mixing times of 2.0 and 0.080 s were used in the 2D experiments NOESY and total correlation spectroscopy (TOCSY), respectively.

Equilibrium and Kinetic Model—Data interpretation was obtained with the following cyclic reaction model (Scheme 1), where α and β indicate the two conformers in neutral form and αH^+ and βH^+ indicate the corresponding protonated forms. No kinetic parameters are indicated for the two protonation steps, which are expected to be much faster than the other two conformational reorientations.

The four equilibrium parameters (two microscopic acidity constants, K_α and K_β, and two equilibrium constants, $K_1 = k_1 / k_{-1}$, $K_2 = k_2 / k_{-2}$) are not independent but are related through the cyclic equilibrium condition as follows:

$$K_\beta = K_\alpha \frac{K_1}{K_2} \tag{1}$$

The macroscopic equilibrium acidity constant, K_a, is consequently given by

$$K_a = K_\alpha \frac{1 + K_1}{1 + K_2} \tag{2}$$

With the assumption that both protonation reactions rapidly equilibrate and that only the two conformational isomerization steps contribute to the observable time dependence of the optical signal, only one reciprocal relaxation time, $k = 1/\tau$, is expected for the kinetic model just described. The derivation of the dependence of k on $[H^+]$ is shortly outlined here according to standard procedures.[6]

The rate equation for conformer α is

$$\frac{d(\alpha + \alpha H^+)}{dt} = -k_1 \alpha - k_2 \alpha H^+ + k_{-1}\beta + k_{-2}\beta H^+ \tag{3}$$

Upon introducing the mass balance condition:

$$C_o = \alpha + \alpha H^+ + \beta + \beta H^+ \tag{4}$$

and the equilibrium condition for the two protonation steps

$$\alpha H^+ = \alpha [H^+]/K_\alpha \qquad \beta H^+ = \beta [H^+]/K_\beta \tag{5}$$

equation 3 transforms into

$$(1 + [H^+]/K_\alpha)\frac{d\alpha}{dt} = -\alpha (k_1 + k_2 [H^+]/K_\alpha) - \alpha(k_{-1} + k_{-2}[H^+]/K_\beta)(1 + [H^+]/K_\alpha)/(1 + [H^+]/K_\beta) + C_o (k_{-1} + k_{-2} [H^+]/K_\beta) \tag{6}$$

which rearranges into a standard first-order rate equation:

$$d\alpha/dt = -k\alpha + \text{const} \tag{7}$$

with const = $C_o (k_{-1} + k_{-2} [H^+]/K_\beta)/(1 + [H^+]/K_\alpha)$ and the first-order rate

$$k = \frac{k_1 + k_2 [H^+]/K_\alpha}{1 + [H^+]/K_\alpha} + \frac{k_{-1} + k_{-2} [H^+]/K_\beta}{1 + [H^+]/K_\beta} \tag{8}$$

At low pH values $k \approx k_2 + k_{-2}$, and the interconversion rate coincides with that of the protonated form; at high pH $k \approx k_1 + k_{-1}$, and the interconversion rate of the neutral form sets the pace.

Data Analysis—Best fit of experimental data to equations was obtained with the nonlinear regression procedure of the MATH menu within program SIGMAPLOT (Jandel). Analysis of first-order kinetics used the equation

$$y = A (1 - e^{-kt}) + \text{offset} \tag{9}$$

Spectrophotometric titration curves and pH dependence of HPLC peak areas and of the interconversion rate were analyzed with the following equation:

$$y = \frac{A + B\ 10^{(pH - pK_a)}}{1 + 10^{(pH - pK_a)}} \tag{10}$$

where A is the limiting value of the observed property at low pH and B is that at high pH.

Analysis of pH dependence of kinetic data was accomplished with 8 (method 1) or with a combination of eqs 8 and 1 to reduce the number of free parameters (method 2) by means of a nonlinear

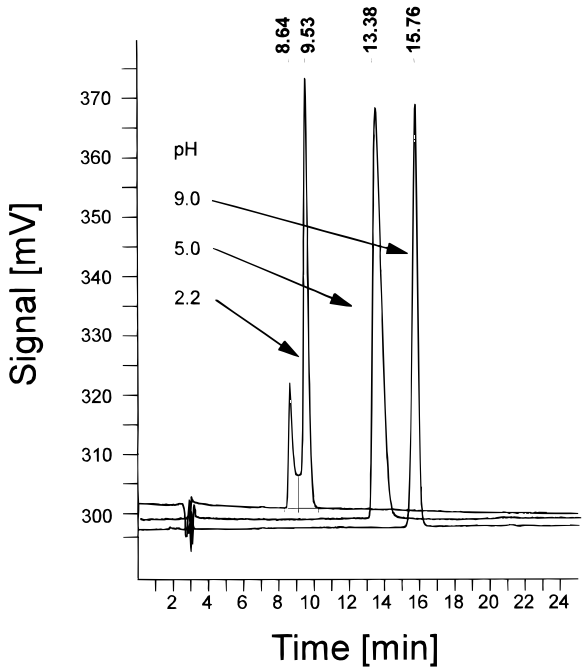


Figure 2—HPLC separation of PNU-156804 rotamers as a function of pH. Three chromatograms obtained with mobile phases buffered at different pH values are superimposed. Two peaks are obtained only with the most acidic mobile phase: the minor peak corresponds to β-rotamer in this case.

weighted least squares procedure (weights = $1/k$) written whithin the program SCIENTIST (Micro-Math).

Results

Spectrophotometric Titration—In the ultraviolet (UV)−visible spectrum of PNU-156804 dissolved in (1/1) water/acetonitrile, the long wavelength absorption band is observed at about 525 nm in acidic solution and, with reduced intensity, at about 460 nm in alkali conditions. This spectroscopic change is the basis for a spectrophotometric titration that leads to $pK_a = 7.20 \pm 0.04$ (average of two determinations: 7.16 and 7.25). This result is attributed to nitrogen protonation in the system of three conjugated pyrrole rings. Data were obtained with solutions equilibrated for at least 30 min at pHapp < 5 and for 10 min above this value, therefore, the pK_a value refers to the equilibrium mixture of two geometric isomers as discussed later.

Equilibrium and Rate of Conformational Change—The HPLC chromatograms of PNU-156804 are greatly affected by the pH of the mobile phase: above neutrality, a sharp peak is obtained that broadens at around pH 5 and splits into two peaks with an acid mobile phase (see Figure 2). Under these latter conditions, the interconversion rate is long in comparison with the chromatographic separation time (the retention times of the two peaks differ by about 1 min) and the relative peak areas approximately correspond to the population of the two conformers in the injected solution, which is a function of the pH and of the composition of this solution. Thus, conformational equilibrium composition of PNU-156804 in solution may be investigated by HPLC.

A pure acetonitrile solution produces a chromatogram similar to that obtained for water containing solutions at high pH (predominance of β-conformer). Preequilibrated solutions at low pH correspond to chromatograms with predominance of α-conformer. The pH dependence of relative peak area (Figure 3) nicely fits a titration curve corresponding to a pK_a of 7.16, which is in very good

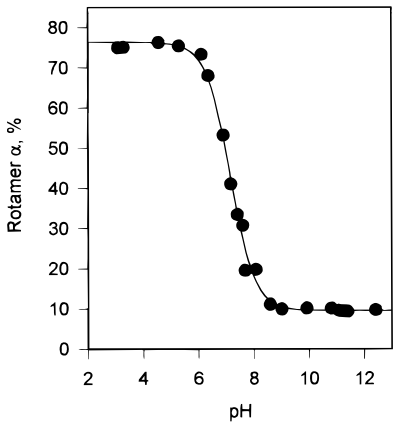


Figure 3—Equilibrium α-rotamer population as determined by HPLC using an acidic mobile phase. The pH dependence is well interpreted with a titration curve corresponding to $pK_a = 7.16$, which is in good agreement with spectroscopic titration data.

agreement with the value obtained from spectrophotometric titration. The limiting value of α-conformer population goes from about 75% at low pH down to 10% at high pH. Actually, high pH data are affected by partial reequilibration during chromatography in the acid mobile phase; thus, the effective limiting population of α-conformer can be much lower than 10%. Perturbation of the equilibrium during chromatographic separation is also likely at low pH, where conformer half-life is about 5 min and thus comparable with the separation time, and may depend on the composition of the mobile phase, which is not the 1/1 water/acetonitrile mixture used in all other experiments. Accordingly, the estimates of equilibrium constants ($K_2 \cong 0.3$ and $K_1 \geq 10$) as obtained with these data should be considered as approximate values.

The UV−visible absorption spectra of the two geometric isomers, as measured with diode array detection in HPLC, are similar but not identical, and the largest difference (about 10% of total absorbance) is observed at around 500 nm. The same spectroscopic change is seen when an acetonitrile solution of PNU-156804 is diluted to acetonitrile/acid buffer (1/1) and the spectrum measured soon after solution preparation is compared with that taken after 30 min. This difference is the basis for kinetic measurements that were made in an ordinary spectrophotometer at pHapp between 2 and 5, and with a stopped-flow apparatus at higher pHapp values by the pH-jump technique. At above pH 7, where the spectrum of the neutral form predominates, the best signal is obtained with detection at 430 nm. For acid solutions, where determination of rates was spectrophotometrically accessible, the two methods gave identical results. Data are analyzed with a first-order kinetic model (eq 4) that leads to determination of the reaction rate k. The dependence of this parameter on pHapp is shown in Figure 4 together with the results of model fitting obtained with eq 8 (method 1, 6 free parameters) and a combination of eq 8 and eq 1 (method 2, 5 free parameters). Values of the two acidity constants (see Table 1) are reasonably well determined and agree in both fitting methods. No error estimate is possible for the rate constants in method 1 because of strong parameter correlation; method 2 provides error estimates for all k values and also suggest a major indetermination for rate constants k_2 and k_{-2}, which are strongly method dependent. Not surprisingly, the estimated values of the equilibrium constants ($K_2 = 0.06$ and $K_1 = 30$, method 2) only qualitatively agree with results of the chromatographic investigation. Most likely as a consequence of the poor evaluation of the kinetic parameters, the estimate $pK_a =$

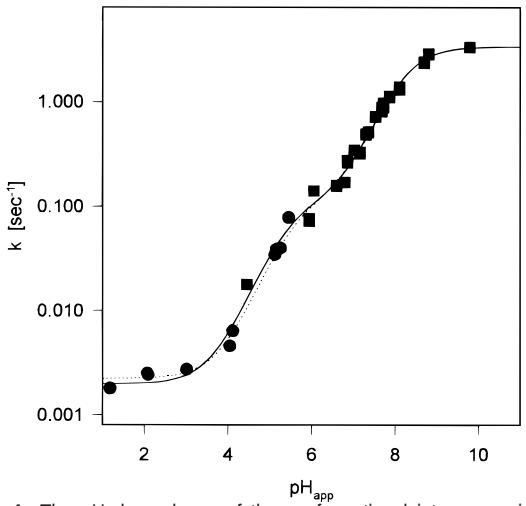


Figure 4—The pH dependence of the conformational interconversion rate constant *k*; the logarithmic scale is used to highlight the two-step behavior. Filled circles are results of spectrophotometric experiments, and squares are stopped-flow data. Fitting by method 1 (all parameters in eq 8 are allowed to vary) produced the solid line, a very similar result (dotted line) is obtained by method 2, where explicit use is made of the cyclic equilibrium condition, thus reducing the number of free parameters. Parameter values are reported in Table 1. The interconversion rate increases about 1000-fold for the unprotonated with respect to the protonated form of PNU-156804.

Table 1—Estimates of Kinetic Parameters from pH Dependence of Interconversion Rate

method[a]	k_1 [s^{-1}]	k_{-1} [s^{-1}]	k_2 [10^{-4} s^{-1}]	k_{-2} [10^{-3} s^{-1}]	pK_α	pK_β
1	3.3	0.1	5	1.5	8.23 ± 0.03	5.4 ± 0.2
2	3.26 ± 0.08	0.11 ± 0.02	1.3 ± 1.1	2.1 ± 1.6	8.23 ± 0.03	(5.5)

[a] Method 1 is nonlinear regression with eq 8 and 6 free parameters; method 2 makes explicit use of the cyclic equilibrium condition to reduce the free parameters to 5. In this latter case, pK_β is derived from the other 5 parameters according to eq 1.

6.7 according to eq 2 is significantly lower than the experimental value (7.2).

NMR Studies—The ^{1}H NMR spectra of PNU-156804, HCl salt, dissolved in $CDCl_3$ or in CD_3CN reveal the presence of only one conformer, which is β according to the notation just presented. Signals of the two conformers are well resolved in solvent mixtures containing H_2O or D_2O at low pH, such as CD_3CN/D_2O (1/1, pH 1.1), where conformer α and β coexist at about 2/1 ratio. This result is at variance with HPLC data (α/β ratio = 3/1). The higher concentration ($\sim$ 0.15 mg/mL) used in NMR work is not the cause of the observed discrepancy because no change of the conformer ratio was detected upon dilution of the NMR sample up to 8-fold. Most likely, the NMR data gave a correct measurement of the unperturbed conformer equilibrium in the 1/1 water/acetonitrile mixture, which is not the case of the HPLC data.

Rapid exchange of NH protons with water protons completely excludes (in D_2O) or greatly reduces (in H_2O) the detection of the corresponding signals and NOESY cross-peaks, which are of great structural significance. Fortunately, the $CH_3SO_3^-$ salt of PNU-156804 in $CDCl_3$ solution is found as a mixture of conformers (α/β = 1/2) in the presence of about 30% molar excess CH_3SO_3 H (see ^{1}H NMR spectrum in Figure 5), where a detailed structural identification of the two conformers is possible. Correspondence of conformers in different solvents is based on chemical shifts: CH_2-1′ protons resonate at 2.0−2.1 ppm in the α-conformer and at 2.6−3.0 ppm in the β-conformer and are highly diagnostic to this purpose. Other protons of the molecule display a definite chemical shift pattern: H-1″, OCH_2, H-4b, and the phenyl group (more deshielded in the α-conformer) or H-3c and NH-1c (more deshielded in the β-conformer). The NOESY data point out that the two conformers mainly differ for the C-2b−C-1″ torsion angle value and are thus rotamers; this angle is close to 0° for the β-rotamer and close to 180° for the α-rotamer (see Figure 6). Conformer α coincides with the reported crystal structure of a prodigiosin analogue[7] containing a sulfur atom in place of nitrogen at ring A (Figure 1). In this case, a hydrogen bond between the protonated nitrogen of the pyrromethene group and the exocyclic oxygen is apparently stabilizing the 180° rotamer. This result agrees

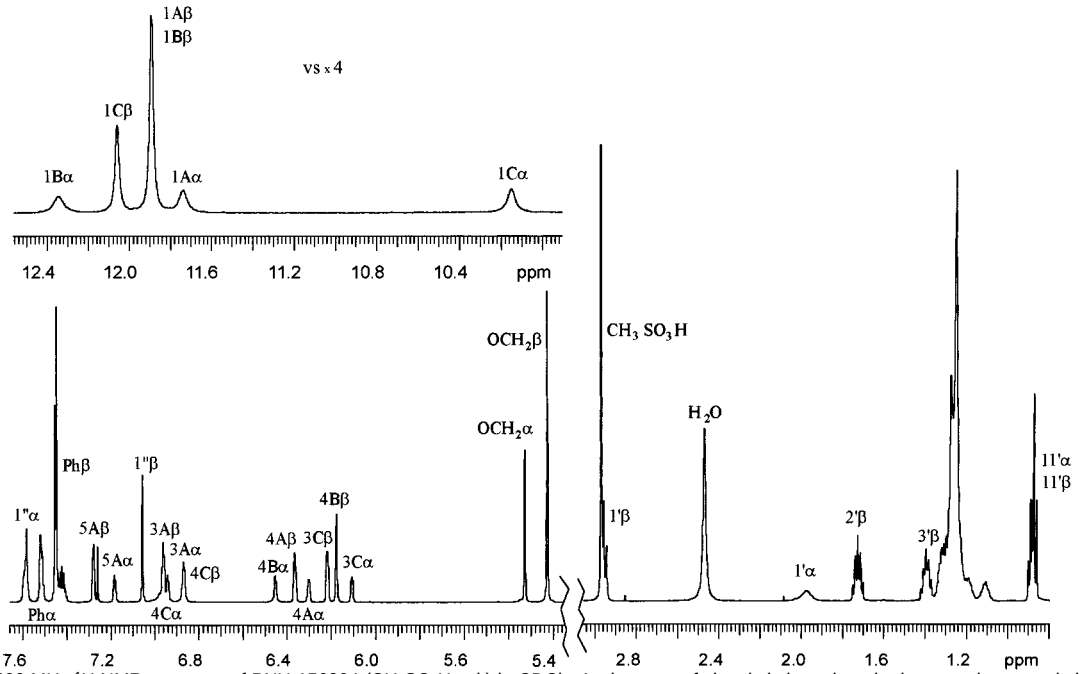


Figure 5—The 600 MHz ^{1}H NMR spectrum of PNU-156804 (CH_3SO_3H salt) in $CDCl_3$. Assignment of signals is based on the homonuclear correlation experiments TOCSY and NOESY. For proton numbering, see at Figure 1. Resonances of each pyrrole ring are recognized in the TOCSY spectrum from the different number of protons in each spin system (4, 2, and 3, respectively) and signal assignment is based on NOE effects with adjacent protons as detected in the NOESY spectrum (e.g., NH-1a/H-5a or CH_2-1′/H-3c).

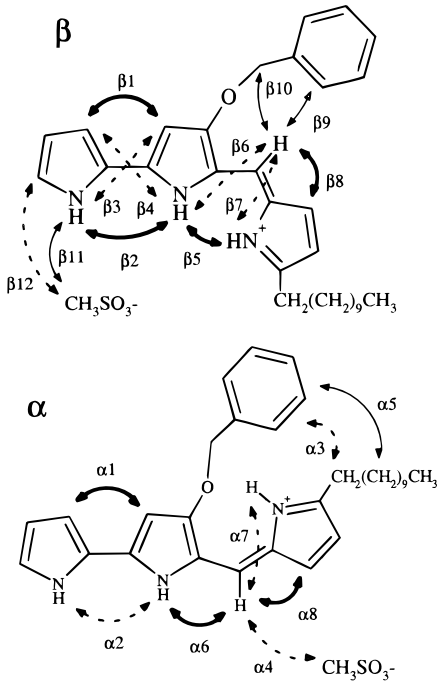


Figure 6—Structure of the α- and *β*-conformers as deduced from NOESY data on PNU-156804 in $CDCl_3$. The significant NOESY cross-peaks are indicated here with double arrows according to the following intensity-related code: heavy lines, strong, thin lines, moderate; and dashed lines, weak. Cross-peak *β*2 is not detected in this spectrum because of NH1a and NH1b signal overlapping. This NOE is detected in the NOESY spectrum of PNU-156804 (HCl salt) dissolved in $CDCl_3$, where only the *β*-conformer is present.

with the predominance of α-conformer at low pH values, as already observed.

Hindered rotation around (C-2a−C-5b) or (C-1″−C-5c) bonds can be excluded as a source of conformational diversity: the same nuclear Overhauser effects (NOEs) are detected for the two conformers in that part of the molecule (cross-peaks corresponding to *β*3 and *β*4 are not detected in the α-conformer because these NOEs are very weak and α is the minor isomer) and suggest a torsion angle close to 0° for both (C-2a−C-5b) and (C-1″−C-5c) bonds. This suggestion is consistent with the X-ray structure of the aforementioned prodigiosin analogue.[7] The most indicative NOEs that distiguish and characterize the two conformers are those labeled α6 (also present in *β* but very weak), α3 and α5 for the α-conformer, and *β*5, *β*9, and *β*10 for the *β*-conformer. The presence of NOEs between the methyl group of the methansulfonate and protons of the molecule (α4, *β*11, and *β*12) is probably due to ion-pairing with formation of hydrogen bonds between the counterion oxygens and NH protons. Chemical shift variation of CH_2-1′ in the two conformers (α, 1.98 ppm; *β*, 2.96 ppm) are simply rationalized in terms of a different C-2b−C-1" torsion angle value: when these protons are oriented toward the phenyl group (conformer α), its shielding cone causes an upfield shift. This interpretation is confimed by the ^{1}H NMR spectrum of PNU-156737 (the natural undecyl-prodigiosin that bears a methoxy group in place of the benzyloxy of PNU-156804, see at Figure 1), which shows no such chemical shift difference between the two conformers (α, 2.77 ppm; *β*, 2.86 ppm) as obtained in $CDCl_3$ solution in the presence of an excess CF_3COOH. Chemical shift changes of NH-1c (α, 10.15 ppm; *β*, 11.89 ppm) and H-1″ (α, 7.59 ppm; *β*, 7.06 ppm) must have a different origin because these are equally well observed for PNU-156737.

Conclusions

The conformational model of Figure 6 satisfactorily explains the results of the present investigation: the two geometrical isomers differ for the polar head structure (the conjugated system is expanded in α and compact in *β*) and for the solvent exposed hydrophobic surface (reduced in the α-conformer by the contact between phenyl and alkyl chain). This result explains the chromatographic separation on a reversed-phase HPLC column. In turn, this result is experimentally feasible only at low pH, where the interconversion rate of the two conformers is comparable or slower than analysis time. In 1/1 water/acetonitrile as solvent, the measured interconversion half-life is about 5 min for the protonated form, but this rate is probably slower in the HPLC mobile phase, which contains less water. Thus, the reasonably good quantitation of the two conformers by HPLC is understandable.

Both the rate of interconversion and the equilibrium distribution of the two conformers are greatly affected by nitrogen protonation. This influence can be attributed to hydrogen bonding between the protonated nitrogen and the exocyclic oxygen, as found in the crystal of a prodigiosin analogue,[7] but a general redistribution of the electron density in the system of three conjugated pyrrole rings after protonation may also play a role. In particular, our data indicate an increase of the rotational energy barrier around C-2b−C-1″ bond in the protonated form and the consequent freezing of the conformational equilibrium. On the basis of a rate-on comparison (k_1 and k_2), if we assume identical preexponential factor, a difference of 25 kJ/mol is estimated, which may be attributed to a strong hydrogen bond.

The different pK_a values of the two conformers, which drive the shift of the equilibrium position from preferred α-form at low pH to almost pure *β*-form at high pH (see eq 1), indicate that the α-conformer is much more easily protonated than the *β*-conformer. This observation is in keeping with the aforementioned extra hydrogen bond in the protonated α-rotamer. However, a clear intepretation of the observed experimental properties must clearly await a detailed theoretical study of the electronic properties of the 2,2′-bipyrrolyl−pyrromethene moiety, which appears well motivated by the important pharmacological effect of this group. In fact, the very limited conformational energy difference in play (few kilocalories, as inferred by the equilibrium constant values) suggests the possibility that equilibrium freezing may occur at the (yet unknown) biological receptor in either the pure α- or the pure *β*-form. In this regard, most intriguing is the analogy with the case of immunophilins, which are enzymes capable of catalyzing cis−trans amide bond isomerization in peptidyl-proline sequences[8] and are the primary target of the well-known immunosuppressant drugs cyclosporin A (cyclophilin) and FK506 (FK binding protein).

References and Notes

1. Williams, R. P.; Hearn, W. R. Prodigiosin. *Antibiotics* **1967**, *2*, 410−432.
2. Nakamura, A.; Nagai, K.; Ando, K.; Tamura G. Selective Suppression by Prodigiosin of the Mitogenic Response of Murine Splenocytes. *J. Antibiot.* **1985**, *39*, 1155−1159.
3. Tsuji, R. F.; Yamamoto, M.; Nakamura, A.; Kataoka, T.; Magae, J.; Nagai, K.; Jamasaky, M. Selective Immunosuppression of Prodigiosin 25-C and FK506 in the Murine Immune System. *Antibiotics* **1990**, *13*, 1293−1301.
4. Liu, J. FK506 and Cyclosporin, Molecular Probes for Studying Intracellular Signal Transduction. *Immunol. Today* **1993**, *14*, 290−295.
5. D'Alessio, R.; Rossi A. Short Synthesis of Undecylprodigiosin. A new Route to 2, 2′-Bipyrrolyl-pyrromethylene Systems. *Synlett* **1996**, 513−514.

6. Bernasconi, C. F. *Relaxation Kinetics*; Academic: New York, 1976; Chapter 4.
7. Blake, A. J.; Hunter, G. A.; McNab, H. A Short Synthesis of Prodigiosin Analogues. *J. Chem. Soc., Chem. Commun.* **1990**, 734−736.
8. Stamnes, M. A.; Rutherford, S. L.; Zucker, C. S. Cyclophilins: A New Family of Proteins Involved in Intracellular Folding. *Trends Cell. Biol.* **1992**, *2*, 272−276.

JS980225W