Reaction of Phthalaldehyde with Aminoethanol under Different Conditions: Products and Mechanisms of Their Formation

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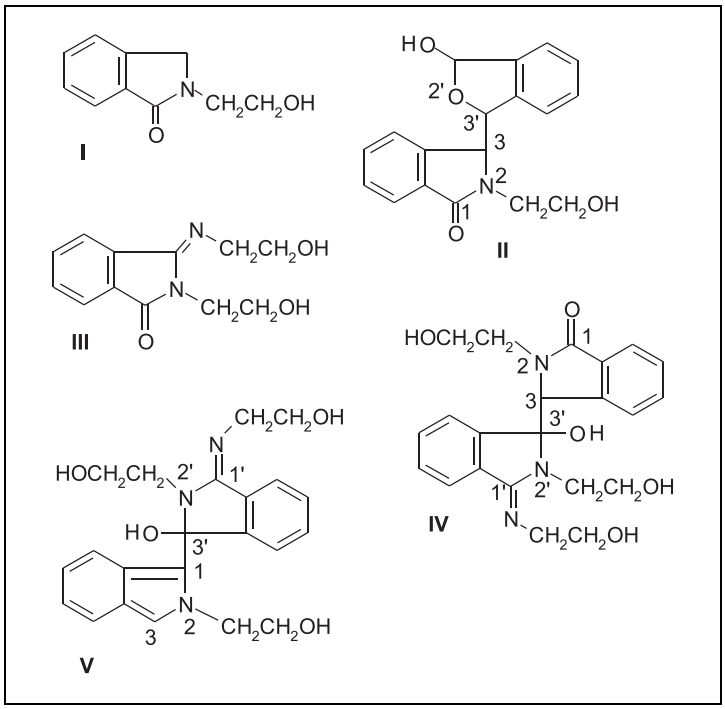
Received May 20, 2011

DOI 10.1002/jhet.1121

Published online 29 October 2012 in Wiley Online Library (wileyonlinelibrary.com).



Analysis of amino acids and very efficient disinfection procedures are based on the reaction of phthalaldehyde (OPA) with primary amines. In this contribution, aminoethanol (= kolamin) as a nitrogen-containing nucleophile was used for the investigation of its reaction mechanism with OPA performed in basic aqueous buffered media (pH 9.5), where an influence of hydration or solvation is expected, and in anhydrous acetonitrile. Depending on the detailed reaction conditions, seven products were isolated and their structures determined by NMR, mass spectrometry, and X-ray structure analysis. Reaction mechanisms are proposed, which involve hydride transfers to OPA (Cannizzaro-type reaction).

J. Hetercycl Chem., **49**, 1202 (2012).

INTRODUCTION

The reaction of phthalaldehyde [= *ortho*-phthalaldehyde (OPA)] with amines was generally studied several times already [1–4]. This reaction is significant among others from the analytical point of view because the analysis of amino acids in biological samples (e.g., in food) is based on the reaction of a primary amine group with OPA to form fluorescent species [1,5–7]. In addition to this, OPA is used also as a disinfection agent for sterilization of surgery instruments and materials (e.g., made from plastics) that cannot undergo a high-temperature treatment. The activity is based on the reaction of OPA with primary amino and thio groups of peptides, leading to their cross-linking and final denaturation [8–11]. The detailed reaction mechanism is also complicated by the existence of three forms of OPA being in equilibrium in aqueous media: unhydrated dialdehyde form, monohydrated monoaldehyde form, and the cyclic 1,3-phthalandiol [12,13].

The complex mechanism involving parallel reactions of species being in equilibrium is not known. The first attempt to elucidate this process including its kinetics is based on the detailed polarographic and spectroscopic investigation of the reaction of OPA with the most simple amine-ammonia [14]. In the unpublished preliminary experiments with other nucleophiles, we have found that the number of (even relatively) stable intermediates and products is high and depends on the concentration of reactants, their stoichiometry, the

sequence and rate of their mixing, and the composition of the media (Klíma, Urban, and Rulíšková, unpublished results).

In this article, the reaction of OPA with 2-aminoethanol (kolamin) has been studied under conditions close to the analytical ones: at laboratory temperature; at concentrations 10^{-2} to 10^{-3} mol l^{-1}; in aqueous buffered solutions pH 9.5; and, for comparison in acetonitrile, where the hydration of OPA does not take place. The main stress was given to the isolation and identification of stable intermediates and products that give indices concerning the understanding of the reaction mechanism.

RESULTS AND DISCUSSION

In acetonitrile, where OPA exists completely in its most reactive unhydrated dialdehyde form, the reaction of OPA with a slight excess of kolamin (molar ratio 1:1.25) leads to a mixture of products. Some of them are polar, and on the TLC they do not move from the start. Two main organic products, however, were isolated and identified (see EXPERIMENTAL) as 2-(2-hydroxyethyl)-2,3-dihydro-1H-benzo[c]pyrrol-1-on (**I**) and (3*R**,1′*S**,3′*R**)-3-(3′-hydroxy-1′H,3′H-benzo[c]furan-1′-yl)-2-(2″-hydroxyethyl)-2,3-dihydro-1H-benzo[c]pyrrol-1-one (**II**) (Fig. 1). Their structures were confirmed using X-ray structure analysis [15,16]. The mechanism of their formation will be discussed below.

In aqueous media, different reaction conditions were used in order to bind up the previous study [14]: (a) in borate buffer pH 9.5, OPA and kolamin were mixed in the ratio 1:1.7 (again, a slight excess of kolamin), where a second-order kinetics is expected; (b) in a OPA : kolamin : HCl mixture 1:10:5 (a fivefold excess of kolamin in a kolamin buffer pH 9.5), where the reaction should follow first-order kinetics; and (c) in borate buffer pH 9.5, OPA and kolamin were mixed in the ratio 2:1 and 4:1 (an excess of OPA).

From the borate buffer (reaction a), two compounds were isolated using column chromatography and identified: compound **I** and 2-(2-hydroxyethyl)-3-[(2-hydroxyethyl) imino]-2,3-dihydro-1H-benzo[c]pyrrol-1-on (**III**). From the kolamin buffer (reaction b), compound **III** and 1′-[(2-hydroxyethyl)imino]-2′-(2-hydroxyethyl)-3′-hydroxy-3′-[2-(2-hydroxyethyl)-2,3-dihydro-1H-benzo[c]pyrrol-1-on-3-yl]-2′,3′-dihydro-1′H-benzo[c]pyrrol (**IV**) were isolated and identified. As a side product, 2-hydroxymethyl benzaldehyde was isolated. In a repeated experiment under condition (b), when the mixing of components was performed more slowly, a new compound 1-[(2-hydroxyethyl)imino]-2-(2-hydroxyethyl)-3-hydroxy-3-[2′-(2-hydroxyethyl)isoindol-1′-yl]

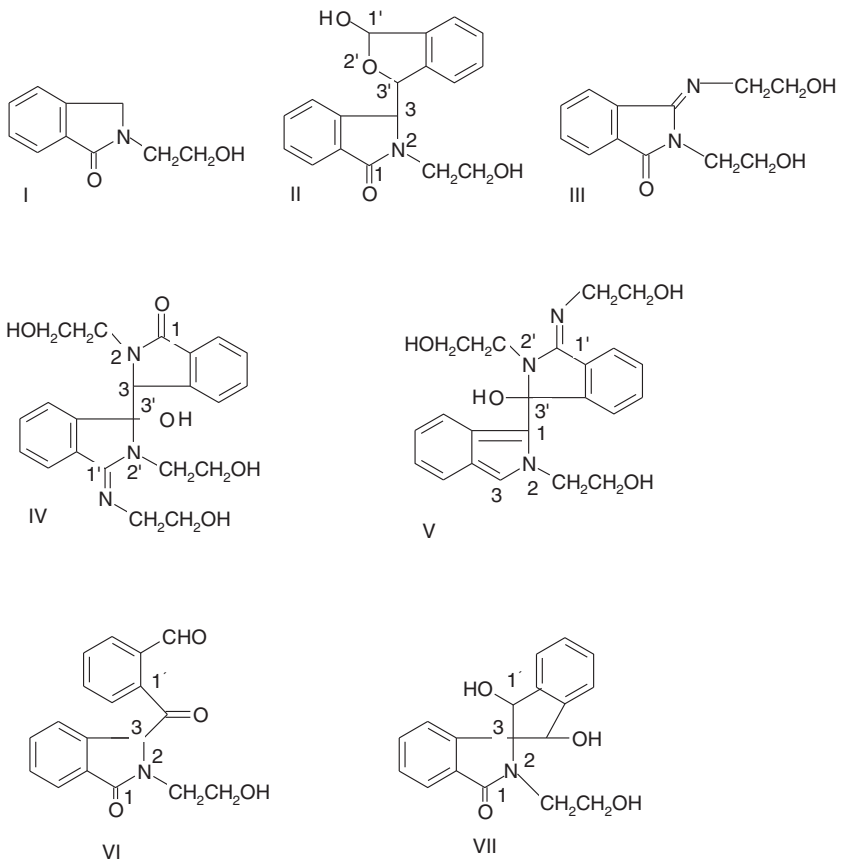


Figure 1. Structures of isolated products **I–VII**.

(**V**) was isolated as yellowish crystals. Besides that, unisolable species were formed, most probably of macromolecular nature.

Under excess of OPA (condition c), the mixture after the reaction contains also some unreacted starting material. The main product at millimolar concentration is compound **I**. The next product is 2-(2-hydroxyethyl)-3-(2-formylbenzoyl)-2,3-dihydro-1H-benzo[c]pyrrol-1-one (**VI**), isolated only under an excess of OPA. When kolamin is present in excess, compound **VI** reacts readily with kolamin, giving rise to the product **IV** (cf. Scheme 3). At higher concentration of OPA (0.1 *M*), besides compound **I**, three diastereoisomers of spiro [2-(2-hydroxyethyl)-2,3-dihydro-1H-benzo[c]pyrrol-1-on-3, 2′-indan-1′,3′-diol] (**VII**) were separated and isolated.

The structure of compound **III** was unambiguously confirmed using X-ray analysis [17]. The structure of compound **IV** was determined from the analysis of its ^{1}H and ^{13}C NMR spectra and confirmed using a comparison with the spectra of compounds **I–III**. Compound **IV** is not fully stable. In acidic media, it decomposes yielding compounds **I** and **III**. However, the reverse reaction has not been observed.

The structure of compound **V** was determined from the analysis of its ^{1}H and ^{13}C NMR spectra. They exhibit signals both of isoindol and isoindoline, the latter being consistent with those of compounds **III** and **IV**. The interpretation and spectral data are in agreement with the compound having the same skeleton (but different substitution) prepared from OPA and an amine in a different way [3].

The structure of compounds **VI** and **VII** was determined from the analysis of their ^{1}H and ^{13}C NMR spectra and from their mass spectra. In the case of compound **VII**, the isolation of all three possible diastereoisomers (two meso-forms, one DL pair) was helpful because the meso-forms exhibit a typical symmetry in the NMR spectra.

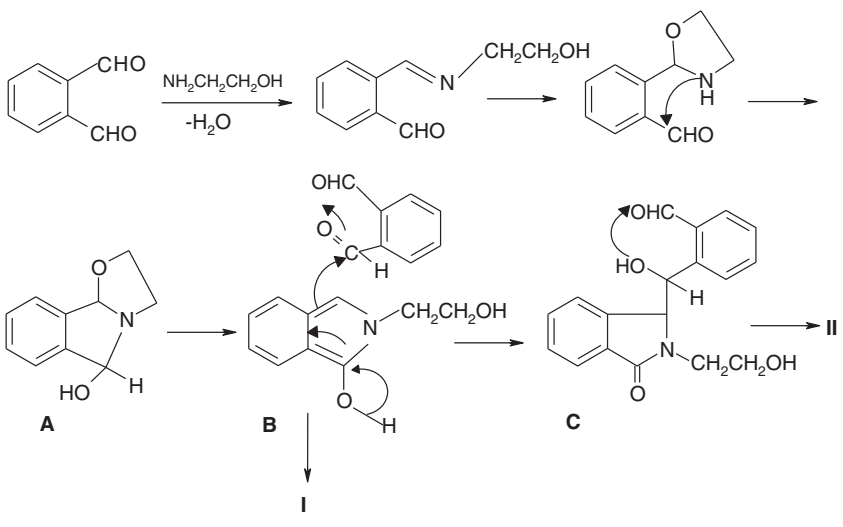
Suggested reaction pathways. The different pathways for the formation of compounds **I–V** are shown in Schemes 1 (formation of **I** and **II**), 2 (formation of **III**), 3 (formation of **IV** and **VI**), 4 (formation of **V**), and 5 (formation of **VII**). Our hypothesis is that these compounds can be generated through intermediates **A**, **B**, and **C** shown in Scheme 1: intermediate **A** would lead to compound **III** (Scheme 2); intermediate **B** would be the precursor of compound **I** (Scheme 1), and intermediate **C** would give compounds **II** (Scheme 1), **IV** and **VI** (Scheme 3), **V** (Scheme 4), and **VII** (Scheme 5).

In acetonitrile, compound **II** is formed together with compound **I**. According to our hypothesis, intermediate **A** would be converted to intermediate **B**, which would give compound **I** by proton transfer (proton tautomerism) (Scheme 1) [4]. Nucleophilic addition of intermediate **B** to a second molecule of OPA would give intermediate **C**, which would cyclize to afford compound **II** (Scheme 1). The ratio of compounds **I** and **II** depends namely on the OPA : kolamin ratio and on the overall concentration of both reactants.

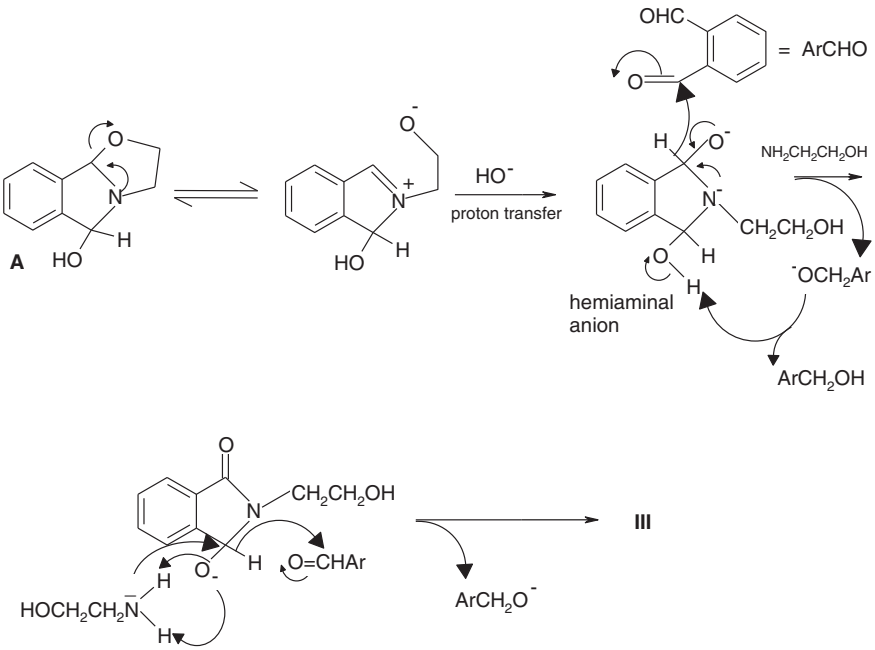
Compound **II** exhibits three chiral centers. Hence, eight stereoisomers are possible, and four diastereoisomeric couples could have been formed. According to the NMR analysis, one diastereoisomer largely predominates (90% pure). Its relative configuration $3R^{*}$, $1'S^{*}$, $3'R^{*}$ was determined by X-ray structure analysis.

In the formation of compounds **III** (Scheme 2), **IV**, and **VI** (Scheme 3), oxidations have occurred. As repeated experiments proved that all compounds were formed both in the presence of oxygen as well as under deoxygenated nitrogen, we propose that a Cannizzaro-type reaction intervenes in the formation of these compounds, the

Scheme 1



Scheme 2



oxidative agent (hydride acceptor) being OPA. Thus, OPA has a dual role, electrophilic substrate and oxidative agent. The isolation of 2-hydroxybenzaldehyde supports this hypothesis (cf. Schemes 2, 3, and 4). The reaction would certainly proceed also by effect of atmospheric oxygen; however, the absence of oxygen in our conditions (due to the inert nitrogen atmosphere) supports the possibility of the Cannizzaro-type oxidation.

In aqueous media in borate buffer pH 9.5, OPA and kolamin were mixed in the ratio 1:1.7, and compounds **I** and **III** were formed in the molar ratio 7:5 (yield 30%). Compound **III** can be obtained from intermediate **A** as shown in Scheme 2, and its formation must involve two oxidation steps with OPA as hydride acceptor as already mentioned. A hemiaminal anion is a very good hydride donor because the hydride transfer is assisted by electron pairs, both on the oxygen anion and on the nitrogen. Furthermore, in such buffered solution, the base-catalyzed proton transfers are fast.

Under excess of OPA in the borate buffer, intermediate **VI** was isolated, whereas in the presence of free kolamin, it reacts further to form compound **IV** as shown in Scheme 3. The identification of compound **VI** supports the suggested reaction pathway. This compound can be obtained directly from intermediate **C** (Scheme 1) by oxidation with OPA as depicted in Scheme 3.

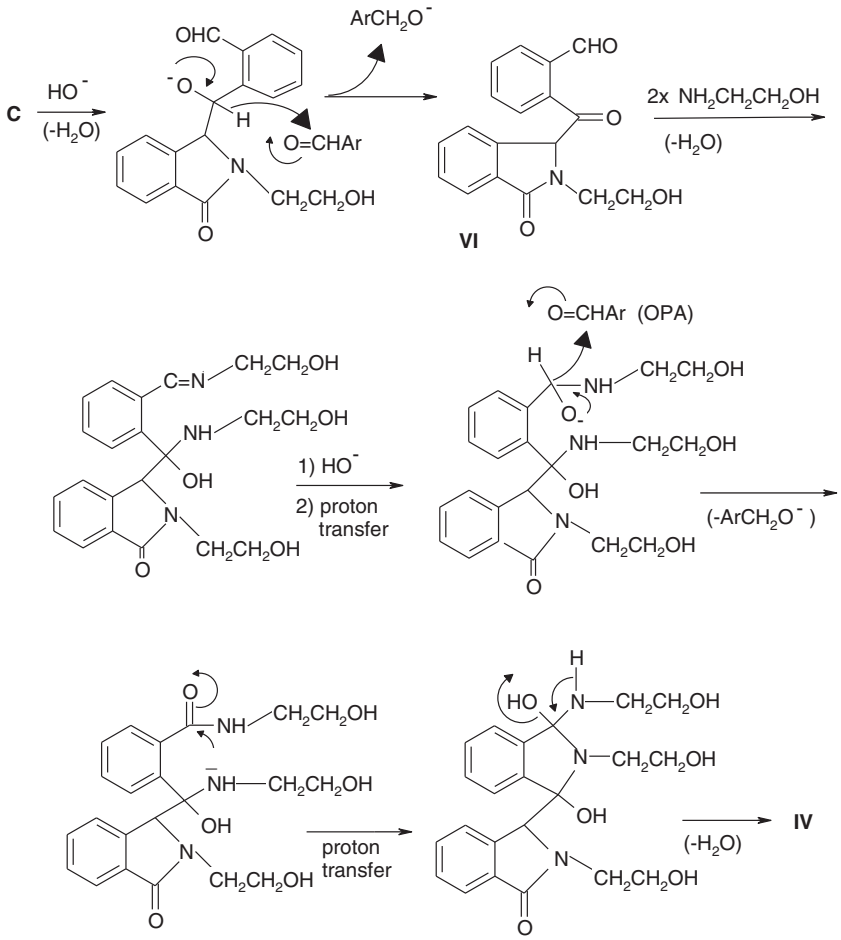
The mechanism of the formation of the product **V** is shown in Scheme 4 starting from intermediate **C** (Scheme 1) in equilibrium with the tautomers **D** and **E**. Dehydration of the latter followed by five subsequent steps gives compound **V**. This product is formed (yield 15%) when kolamin is added to the concentrated solution of OPA (0.164 *M*) very slowly. Compounds **III**, **IV**, and **V** are formed only in aqueous basic solutions (borate or kolamin buffer).

Under excess of OPA and at higher OPA concentration (0.1 *M*) in the buffered aqueous solution, the three diastereoisomers of the spiro compound **VII** were isolated (yield 22%). The precursor (cf. Scheme 5) is the same as in Scheme 4. Under these conditions, the cyclization of tautomer **D** to compound **VII** competes with its tautomerization to **E** and dehydration of the latter (cf. Scheme 4).

CONCLUSIONS

The reaction of OPA with kolamin was performed in basic aqueous buffered media (pH 9.5), where an influence of hydration or solvation is expected, and in nonaqueous acetonitrile. This reaction has a broad number of variations, very sensitive to reaction conditions. Depending on the medium, molar ratio of the reacting compounds, total concentration of reactants, the order and rate of their mixing, and so forth, seven new products were isolated and their structure determined by NMR, mass spectrometry, and X-ray structure analysis. Reaction mechanisms for their formation were suggested. Because of the fact that all isolated compounds were formed both in the presence of oxygen and under a nitrogen atmosphere, the observed

Scheme 3



oxidation processes most probably involve OPA as oxidant (as hydride acceptor) and follow a variation of the Cannizzaro reaction. This conclusion is supported also by the isolation of 2-hydroxymethylbenzaldehyde. OPA acts here simultaneously as a substrate and an oxidation agent.

EXPERIMENTAL

Chemicals. Phthalaldehyde (Sigma), min.97%; kolamin (Lachema, Czech Republic), distilled; sodium borate·10H_2O (Lachema), p.a.; HCl (Lach-Ner, Czech Republic), 35%, p.a.; acetonitrile (Fluka), puriss. p.a.; and deionized water were used.

Instrumentation. The ^1H and ^{13}C NMR spectra (ppm, Hz) were measured on the spectrometer Varian 300 MHz (299.970 MHz for ^1H and 75.434 MHz for ^{13}C).

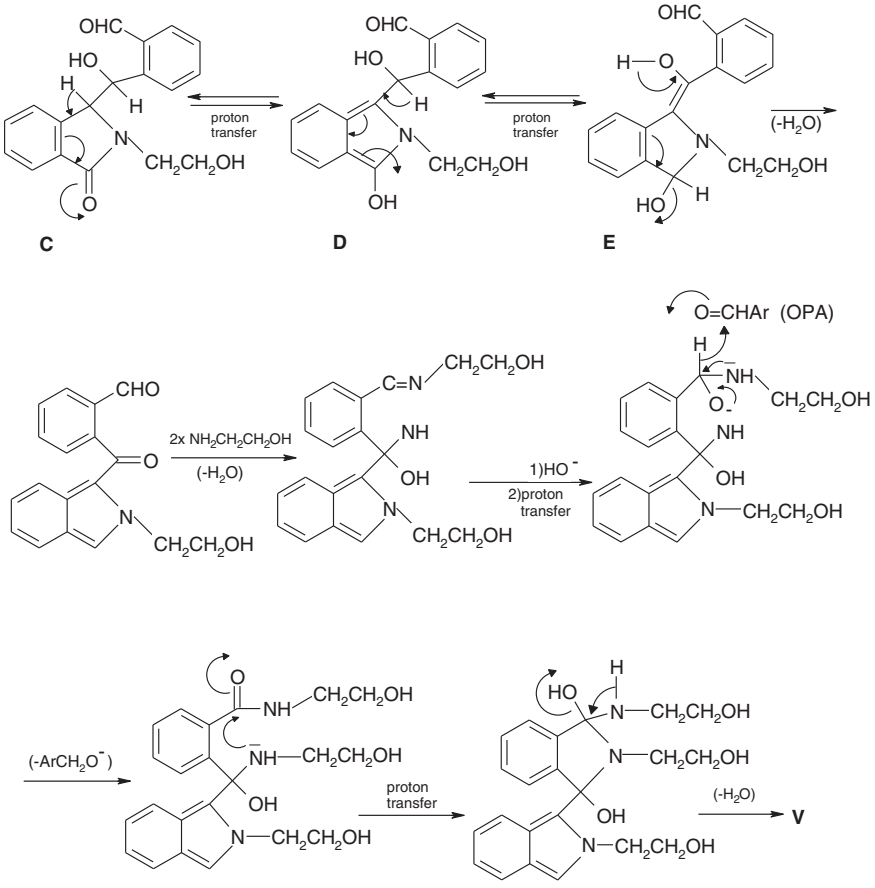
Mass spectra were measured using the Quattro Premiere XE tandem quadrupole mass spectrometer (Waters) consisting of two quadrupole mass analyzers and a T-wave collision cell. The samples were directly infused into the electrospray ion source using built-in syringe pump as approximately 5×10^{-5} mol l^{-1} solutions in 50% acetonitrile. Typical source conditions were as follows: ESI+, capillary voltage 3.8 kV; cone voltage, 30 V; source temperature, 100°C; desolvation temperature, 200°C; desolvation gas, N_2 (200 l h^{-1}). Syringe pump flow was typically 5 µl min^{-1}. Collision-induced dissociation (CID) mass spectra were obtained in a way in which the ions of interest (i.e., protonated molecules of analyte) were mass selected by the first quadrupole, activated by collisions with argon at 15 eV collision energy at a pressure of 1.02×10^{-2} mbar, and products of these collisions were analyzed by the second quadrupole.

The melting points were measured using the Kofler block and are not corrected.

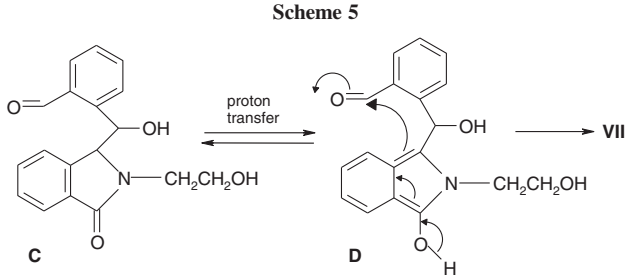
Synthetic procedures and characterization.

2-(2-Hydroxyethyl)-2,3-dihydro-1H-benzo[c]pyrrol-1-on (I) and (3R*,1′S*,3′R*)-3-(3′-hydroxy-1′H,3′H-benzo[c]furan-1′-yl)- 2-(2″-hydroxyethyl)-2,3-dihydro-1H-benzo[c]pyrrol-1-on (II). Phthalaldehyde (1.33 g) was dissolved in 80 mL of acetonitrile, and under stirring 0.76 g (0.75 mL) of kolamin was added. After 4 h at room temperature, acetonitrile was evaporated, and the n chromatography (silica gel, chloroform–ethanol 10%). Yield: 308 mg (16%) of compound **I** (recrystallized from toluene, mp 117–119°C) and 256 mg (13%) of compound **II** (recrystallized

Scheme 4



Scheme 5



from the mixture chloroform–heptane, mp 228–230°C). Compound **I**, ^{1}H NMR (deuteriochloroform): δ 3.678 (t, 2H, CH2–N, J = 5.1), 3.839 (t, 2H, CH2–O, J = 5.1), 4.215 (br s, 1H ,OH), 4.454 (s, 2H, 3-H), 7.358 (m, 2H, 4-,6-H), 7.453 (t, 1H, 5-H, J = 7.5), 7.708 (d, 1H, 7-H, J = 8.1).^{13}C NMR (deuteriochloroform): δ 45.52 (t, CH2–N), 51.42 (t, C-3), 60.95 (t, CH2–O), 122.46 (d, C-6 or 7), 123.24 (d, C-7 or 6), 127.73 (d, C-4), 131.18 (d, C-5), 132.27 (s, C-7a), 141.42 (s, C-3a), 169.34 (s, C-1). Compound **II**, ^{1}H NMR (dimethyl sulfoxide d_6): δ 3.288 (dt, 1H, CH2–N, J1 = 13.8, J2 = 6), 3.630 (m, 2H, CH2–O), 3.368 (s, 2H, 1′-OH, H2O), 3.879 (dt, 1H, CH2–N, J1 = 13.8, J2 = 6), 4.820 (t, 1H, J = 5.1 CH2–OH); 5.206 (d, 1H, 3-H, J = 7.2), 5.706 (d, 1H, 3′-H, J = 7.2), 6.007 (s, 1H, 1′-H), 6.874 (d, 1H, Ar–H, J = 6.6), 7.340 (m, 5H, Ar–H), 7.630 (d, 1H, 7-H, J = 7.2). ^{13}C NMR (dimethyl sulfoxide d_6): δ 42.79 (t, CH2–N), 58.88 (t, CH2–O), 62.96 (d, C-3), 80.17 (d, C-3′), 100.51 (d, C-1′), 121.34 (d, Ar–H), 122.63 (d, 2x Ar–H), 123.18 (d, Ar–H), 128.42 (d, Ar–H), 128.65 (d, Ar–H), 128.87 (d, Ar–H), 130.85 (d, Ar–H), 133.33 (s, C-7a), 138.31 (s, C-7′a), 141.10 and 141.68 (s, C-3a and 3′a), 167.81 (s, C-1).

2-(2-Hydroxyethyl)-3-[(2-hydroxyethyl)imino]-2,3-dihydro-1H-benzo[c]pyrrol-1-on (III). Phthalaldehyde (500 mg) is dissolved in 2500 mL of distilled water containing 3 g of sodium tetraborate (pH 9.5). Then, 405 mg (400 mL) of kolamin is added and stirred for 2 h under laboratory temperature. The reaction mixture was evaporated to dryness using reduced pressure at room temperature. The obtained solid was dissolved in the mixture ethylacetate–water and shaken. The organic phase was dried by Na_2SO_4 and evaporated to dryness. The obtained products were separated by column chromatography (silica gel, chloroform–ethanol 10%). Yield: 126 mg of compound **I** and 90 mg of compound **III** (recrystallized from the mixture chloroform–toluene, mp 110–112°C). Compound **III**, ^{1}H NMR (dimethyl sulfoxide d_6): δ 3.548 (t, 2H, CH_2–N, J = 6), 3.725 (m, 4H, 2x CH_2), 3.972 (t, 2H, CH_2–O, J = 6), 7.676 (m, 2H, 5,6-H), 7.761 (d, 1H, 4-H, J = 7.19), 8.037 (d, 1H, 7-H, J = 7.19). ^{13}C NMR (dimethyl sulfoxide d_6): δ 41.14 (t, CH_2–N), 53.03 (t, CH_2–N),

58.83 (t, CH_2–O), 62.61 (t, CH_2–O), 123.41 (d, C-4), 126.72 (d, C-7), 129.92 (s, C-3a), 132.43 (d, C-5), 132.77 (s, C-7a), 133.83 (d, C-6), 151.42 (s, C-3), 167.38 (s, C-1).

1′-[(2-Hydroxyethyl)imino]-2′-(2-hydroxyethyl)-3′-hydroxy-3′-[2-(2-hydroxyethyl)-2,3-dihydro-1H-benzo[c]pyrrol-1-on-3-yl]-2′, 3′-dihydro-1′H-benzo[c]pyrrol (IV). Kolamin [5.06 g (5 mL)] was mixed with 412.5 mL 0.1 *M* HCl giving rise to a kolamin buffer, pH 9.5. This solution was added for 10 s under stirring to the solution of 1.1 g OPA in 30 mL ethanol. After 2 h at room temperature, the reaction mixture was evaporated to dryness under reduced pressure. The solid was dissolved in the mixture chloroform–water and shaken. The aqueous phase was once more washed by chloroform; the two organic phases were combined, dried by Na_2SO_4, and evaporated to dryness. The obtained products were separated by column chromatography (silica gel, chloroform–ethanol 10%). Yield: 123 mg of compound **III** and 192 mg of compound **IV** (recrystallized from toluene, mp 199–201°C). Compound **IV** ^{1}H NMR (deuteriochloroform): δ 3.979 (m, 12H, CH_2–N and CH_2–O), 4.880 (s, 1H, 3-H), 5.245 (br s, 4H, OH), 7.083 (m, 2H, Ar–H), 7.243 (m, 4H, Ar–H), 7.702 (d, 1H, 7′-H, J = 6.3), 7.837 (d, 1H, 7-H, J = 7.5). ^{13}C NMR (deuteriochloroform): δ 38.71 (t, CH_2–N2′), 44.98 (t, CH_2–N2), 50.76 (t, CH_2–N═), 60.58 (d, C-3), 62.36 (t, CH_2–O), 63.09 (t, CH_2–O), 64.06 (t, CH_2–O), 91.84 (s, C-3′), 122.71 (d, Ar–H), 123.68 (d, Ar–H), 123.98 (d, Ar–H), 126.63 (d, Ar–H), 129.12 (d, Ar–H), 129.56 (s, C-7′a), 129.92 (d, Ar–H), 130.87 (d, Ar–H), 132.27 (d, Ar–H), 132.43 (s, C-7a), 140.51 (s, C-3a and 3′a), 159.71 (s, C-1′), 167.24 (s, C-1).

Decomposition of compound IV. Fifty milligrams of compound **IV** was dissolved in 15 mL of ethanol, and several drops of concentrated HCL was added. After 4 h, the solution was neutralized by addition of $NaHCO_3$, filtered and evaporated to dryness. The products were separated using TLC; the two obtained pure substances were identical with compounds **I** and **III** (checked by NMR).

1-[(2-Hydroxyethyl)imino]-2-(2-hydroxyethyl)-3-hydroxy-3-[2′-(2-hydroxyethyl)isoindol-1′-yl] (V). Kolamin [1.012 g (1 mL)] was mixed with 82.5 mL 0.1 *M* HCl. This solution was added very slowly (for 2 min) under stirring to the solution of 220 mg of OPA in 10 mL ethanol. After a night in a refrigerator, yellowish crystals were formed, isolated by filtration, washed with water, and dried. The recrystallization was not performed because of the expected decomposition. Compound **V**, ^{1}H NMR (deuteriochloroform): δ 3.446–3.876 (br s, 4H, 4xOH), 3.572 (m, 4H), (2xCH_2N), 3.894 (t, 2H, CH_2N, J = 5.14), 4.056 (t, 2H, CH_2O, J = 5.14), 4.458 (m, 4H, 2xCH_2), 6.71 (m, 1H, Ar–H), 6.895 (m, 1H, Ar–H), 7.150 (d, 1H, Ar–H, J = 7.5), 7.233 (s, 1H, 3′-H), 7.331–7.518 (m, 4H, Ar–H), 8.029 (d, 1H, Ar–H, J = 7.5).

^{13}C NMR (deuteriochloroform): δ 45.59 (t, CH_2N), 46.46 (t, CH_2N), 51.10 (t, CH_2N═), 61.81 (t, CH_2O), 63.59 (t, CH_2O), 63.86 (t, CH_2O), 92.64 (s, C-1′), 111.38 (d, Ar–H), 111.92 (s, C-3a), 118.36 (d, Ar–H), 118.76 (d, Ar–H), 121.12 (s, C-7a), 121.86 (d, 2xAr–H), 123.47 (d, Ar–H), 124.30 (s, C-3′a), 126.31 (d, Ar–H), 128.60 (s, C-7′a), 129.69 (d, Ar–H), 131.53 (d, Ar–H), 147.40 (s, C-1), 158.53 (s, C-3′).

2-(2-Hydroxyethyl)-3-(2-formylbenzoyl)-2,3-dihydro-1H-benzo[c]pyrrol-1-on (VI). Phthalaldehyde (300 mg) was dissolved in 8 mL of ethanol, and this solution was added to 500 mL of water with dissolved sodium tetraborate (600 mg). Kolamin [69 mg (68 μL)] was then slowly added under stirring to the solution. The reaction mixture (excess of OPA was 2:1) was then stirred for 4 h at laboratory temperature under nitrogen gas, then evaporated to dryness, extracted by ethylacetate, and the products separated by column chromatography (60 g of silica gel, chloroform as eluent). Isolated substances: 18 mg of OPA, 133 mg of **I**, and 42 mg of **VI**. The latter was recrystallized from the chloroform–toluene mixture, mp 158–160°C. ^{1}H NMR (deuteriochloroform): δ 3.715 (t, 2H, CH_2–N, J = 5.6), 3.862 (t, 2H, CH_2–O, J = 5.6), 4.435 (br s, 1H, OH), 4.960 (s, 1H, 3-H), 7.268 (m, 3H, Ar–H), 7.726 (m, 3H, Ar–H), 7,898 (d, 1H, 3′-H, J = 7.5), 8.097 (d, 1H, 6′-H, J = 7.5), 10.128 (s, 1H, CHO). ^{13}C NMR (deuteriochloroform): δ 43.54 (t, CH_2–N), 59.86 (t, CH_2–O), 66.52 (d, C-3), 122.61 (d, Ar–H), 123.48 (d, Ar–H), 127.34 (d, Ar–H), 128.66 (d, Ar–H), 129.45 (d, Ar–H), 130.92 (d, Ar–H), 132.78 (d, Ar–H), 133.52 (s, C-7a), 134.01 (d, Ar–H), 136.72 (s, Ar), 137.83 (s, Ar), 141.57 (s, C-3a), 168.26 (s, C-1), 190.89 (d, CHO), 196.11 (s, CO). The analogous reaction where the molar excess of OPA was 4:1 (400 mg of OPA and 45 μL of kolamin) was performed in the same way. Using TLC, the same substances were found in the reaction mixture; only the amount of the unreacted OPA was higher.

Spiro[2-(2-hydroxyethyl)-2,3-dihydro-1H-benzo[c]pyrrol-1-on-3,2′-indan-1′,3′-diol] (VII). Phthalaldehyde (600 mg) was dissolved in 40 mL of 50% ethanol containing 50 mg of sodium tetraborate. Kolamin [140 mg (138 μl)] was added and stirred for 4 h at laboratory temperature. The product was evaporated to dryness and extracted by ethylacetate. The solid was purified using column chromatography (100 g of silica gel, chloroform, and chloroform–ethanol 10% as eluent). The unreacted OPA was separated. Products: besides compound **I**, 36 mg of compound **VII-1**, 49 mg of **VII-2**, and 71 mg of **VII-3** were isolated (compounds **VII 1** and **3** are meso-forms, the product **VII-2** is a DL pair). Compound **VII-1** ^{1}H NMR (dimethyl sulfoxide d_6): δ 2.958 (t, 2H, CH_2–N, J = 5.7), 3.440 (t, 2H, CH_2–O, J = 5.7), 3.420 (br s, OH, H_2O), 5.447 (s, 2H, 1′, 3′-H), 6.145 (bs, 2H, 2xOH), 7.371 (s, 4H, 4′-7′-H), 7.491 (t, 1H, 6-H, J = 7.5), 7.621 (t, 1H, 5-H, J = 7.5), 7.657 (d, 1H, 4-H, J = 7.5), 7.922 (d, 1H, 7-H, J = 7.5). ^{13}C NMR (dimethyl sulfoxide d_6): δ 45.80 (t, CH_2–N), 59.19 (t, CH_2–O), 76.62 (d, C-1′, 3′), 83.26 (s, C-3), 122.50 (d, Ar–H), 122.62 (d, Ar–H), 123.40 (d, 2x Ar–H), 128.84 (d, Ar–H), 128.90 (d, 2x Ar–H), 132.28 (d, Ar–H), 133.16 (s, C-7a), 142.87 (s, C-3′a, 7′a), 147.80 (s, C-3a), 170.23 (s, C-1). CID ms (*m/z* (%)): 312($M + H^+$; 20), 294(8), 276(100), 258(2), 250(3), 248(23), 234(1), 233(8), 232(8), 219(6), 207(1), 205(1), 204(1), 176(3), 159(1).

Compound **VII-2** ^{1}H NMR (dimethyl sulfoxide d_6): δ 3.440 (m, N–CH_2CH_2–OH, H_2O), 5.017 (s, 1H, 3′-H)*, 5.470 (s, 1H, 1′-H)*, 5.673 (br s, 1H, OH), 6.084 (br s, 1H, OH), 7.400 (m, 7H, Ar–H), 7.637 (d, 1H, 7-H, J = 7.2). ^{13}C NMR (dimethyl sulfoxide): δ 45.04 (t, CH_2–N), 59.12 (t, CH_2–O), 75.66 (d, C-1′)*, 78.53 (s, C-3), 78.60 (d, C-3′)*, 122.72 (d, Ar–H), 124.00 (d, Ar–H), 125.33 (d, Ar–H), 125.38 (d, Ar–H), 128.65 (d, Ar–H), 129.31 (d, Ar–H), 129.73 (d, Ar–H), 131.58 (d, Ar–H), 132.50 (s, C-7a), 143.12 (s, C-7′a)*, 143.72 (s, C-3′a)*, 146.59 (s, C-3a), 169.56 (s, C-1). CID ms (*m/z* (%)): 312($M + H^+$; 22), 294(100), 276(67), 258(3), 250(34), 248(26), 234(7), 233(31), 232(22), 219(1), 207 (7), 205(3), 204(3), 176(90), 159(3).

Compound **VII-3** ^{1}H NMR (dimethyl sulfoxide d_6): δ 3.480 (br s, OH, H_2O), 3.720 (m, 4H, N–CH_2CH_2–O), 5.460 (s, 2H, 1′, 3′-H), 5.600 (br s, 2H, 2xOH), 7.154 (t,1H, Ar–H, J = 7.5), 7.310 (m, 6H, Ar–H), 7.586 (d, 1H, 7-H, J = 7.5). ^{13}C NMR (dimethyl sulfoxide d_6): δ 43.15 (t, CH_2–N), 59.29 (t, CH_2–O), 72.28 (d, C-1′,3′), 85.75 (s, C-3), 122.49 (d, Ar–H), 123.81 (d, 2xAr–H), 124.16 (d,

Ar–H), 128.20 (d, Ar–H), 128.62 (d, 2xAr–H), 130.60 (d, Ar–H), 133.47 (s, C-7a), 141.82 (s, C-3′a, 7′a), 144.65 (s, C-3a), 169.51 (s, C-1). CID ms (*m*/*z* (%)): 312($M+H^+$; 49), 294(100), 276(67), 258(3), 250(39), 248(26), 234(7), 233(34), 232(21), 219(2), 207 (7), 205(4), 204(4), 176(29), 159(2).

Acknowledgment. This work was supported by the grant KONTAKT ME-09002 (Ministry of Education, Youth and Sports of the Czech Republic).

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