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Article

Chemoselective Oxidation of Benzophenazines by *m*-CPBA: *N*-Oxidation *vs.* Oxidative Cleavage

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Quimiosseletividade é observada, após reação de oxidação, utilizando-se *m*-CPBA, a partir da piranobenzo[a]fenazina e da furanobenzo[a]fenazina, derivadas, respectivamente da β-lapachona e da nor-β-lapachona. A fenazina pirânica forneceu macrolactonas, principalmente, enquanto a fenazina furânica conduziu a um único produto: o *N*-8 óxido da fenazina. Objetivando entender essa diferença de reatividade, uma nova fenazina furânica foi sintetizada, tendo como característica principal, a presença da ligação dupla em um sítio menos encoberto estericamente do que aquele presente no derivado nor-beta-lapachônico. Essa furanofenazina, após oxidação com *m*-CPBA, forneceu, principalmente, a macrolactona esperada. Esses resultados experimentais, aliados a cálculos mecânico-moleculares do estado fundamental dos substratos, permitiram sugerir que a quimiosseletividade observada pode ter controle estérico, relacionado à presença dos grupos substituintes metila geminados, que dificultam a aproximação do oxidante. Muitos dos compostos sintetizados são relatados, pela primeira vez, no presente artigo.

Chemoselectivity is observed when a pyran-benzo[a]phenazine and a furan-benzo[a]phenazine from β-lapachone and nor-β-lapachone, respectively, were submitted to oxidation by *m*-CPBA. The pyran phenazine furnished mainly macrolactones, while the furan one led exclusively to a phenazine *N*-8 oxide. To understand this difference in reactivity, we synthesized a new furan phenazine, with the reactive double bond site less hindered than that of the derivative from nor-beta-lapachone. This furan phenazine, upon oxidation with *m*-CPBA, furnished mainly the expected macrolactone. These experimental results, along with preliminary analysis based on mechanical molecular calculations of the ground state of the substrates, allowed us to suggest that the observed chemoselectivity has a steric oxidant approach control origin, related to the presence of the geminal methyl groups in the phenazine structure. Several of the synthesized compounds are, herein, reported for the first time.

Keywords: *m*-CPBA oxidation, phenazines, chemoselectivity, *N*-oxides, macrolactones, oxidative cleavage

Introduction

In previous communication,[1] we reported the unexpected reactivity of β-lapachone phenazine **1** upon oxidation by *m*-CPBA. The oxidative cleavage of the aromatic double bond at the site of ring junction was observed, leading to the formation of a 10-membered macrolactone **2** (27%) and its *N*-oxide **3** (13%), together with an α-hydroxy-dihydrobenzophenazinone derivative **4** (35%), resultant from the pyranic ring cleavage (Figure 1).[1] Hydroxy and bromo derivatives of **1**, respectively, **5** and **6**, were also submitted to oxidation in the same conditions and furnished substituted 10 membered macrolactones: **7** from **5** and **8** from **6**.[2]

Based on that initial study,[1] some papers dealing with modifications of the methodology and a more general view

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of the synthetic aspects have appeared in the literature, showing the large potential of this macrolide synthetic approach.[3,4]

Macrolides are an attractive class of compounds that have shown a wide variety of bioactivities,[5] especially recognized as antibiotics.[6] The presence of diaza heterocyclic groups close to the ion-binding macrolactone moiety constitutes attractive molecular features.[3] On the other hand, *N*-oxides were also designed and synthesized to behave as bioreducible drugs, being the anticancer hypoxic agent tirapazamine, one of the most successful example.[7]

These results and the relevance of the synthesized compounds led us to perform a more detailed study of this reaction. Two furan phenazines, **9** (Figure 2) and **10** (Figure 3), were synthesized and submitted to *m*-CPBA oxidation in an attempt to obtain 9-membered macrolactones.

It is worth mentioning that medium-sized heterocycles (with 8 to 10-membered rings) are often pharmaceutically important compounds and the production of such rings remains challenging.[1,8]

Results and Discussion

The new compound **9** (2-methyl-1,2-dihydro-benzo[a]furo[2,3-c]phenazine) is derived from *o*-naphthoquinone **9a**[9] (Figure 2) which was recently evaluated against *T. cruzi* with good results.[10] Its homologue**,** 1,2-dihydro-2,2-dimethylbenzo[a]furo[2,3-c]phenazine, compound **10,** is obtained from nor-β-lapachone **10a** which was synthesized following the methods described in the literature (Figure 3).[12-13]

The new monomethyl derived-phenazine **9** (Figure 2), submitted to *m*-CPBA oxidation has furnished two compounds: the 9-membered macrolactone *N*-oxide **11** and the α-hydroxy-ketone **12** (Figure 2). However, for **10**, analysis of the reactional mixture pointed to the almost exclusive formation of the mono-*N*-oxide product (**13**), without any evidence of the formation of the corresponding 9-membered macrolactone (Figure 3).

The product characterization was based on data from spectroscopic methods, mainly IR, ^{1}H NMR and ^{13}C NMR and by comparison with spectra of similar compounds.[1-4, 10, 14]

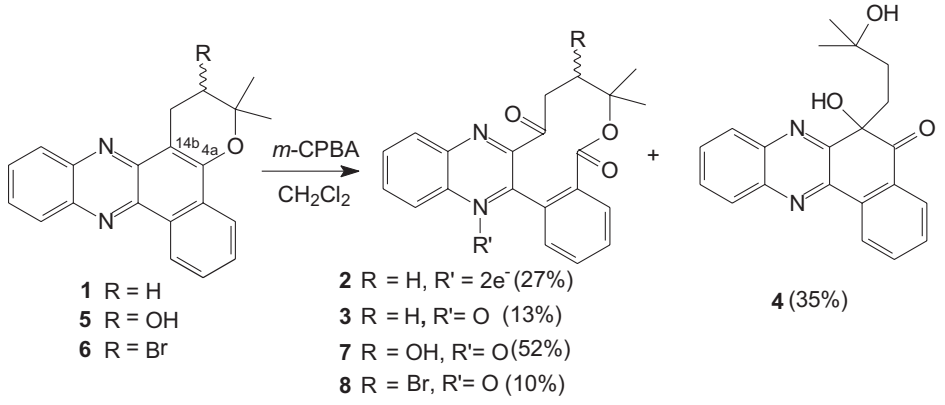

Figure 1. *m*-CPBA oxidation of selected pyran phenazines.

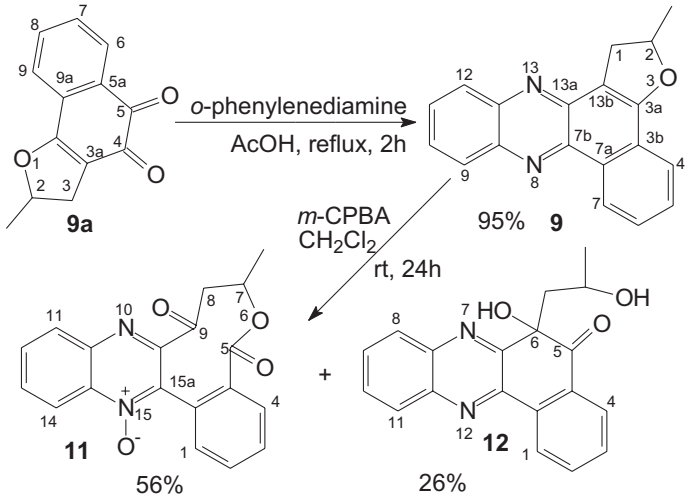

Figure 2. Preparation and reactivity of a furan phenazine toward *m*-CPBA.

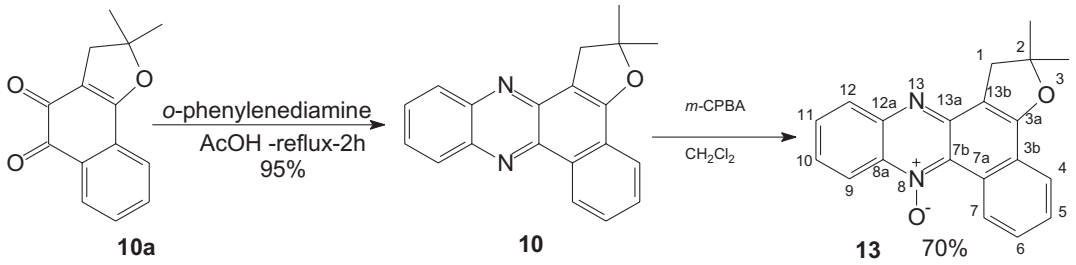

Figure 3. Preparation and reactivity of a sterically hindered furan phenazine toward *m*-CPBA.

The differences in reactivity of these phenazines toward *m*-CPBA oxidation (**1**, **5**, **6**, and **9**, Figures 1 and 2, which produced the corresponding macrolactones, *versus* **10**, Figure 3, which led only to *N*-8 oxide product) has prompted us to perform some preliminary theoretical calculations on these substrates.

Firstly, considering that the different reactivity might have an electronic origin, we performed electronic structure calculations at B3LYP/6-31G(d,p) level of theory,[15-17] on compounds **1**, **9**, and **10**. At this level, no appreciable differences were detected between the values of these compounds, either in charge distribution around the reactive double bond site or in HOMO and/or LUMO energy levels (Table 1)[18] which could have been responsible for such a dramatic difference in reactivity.

Nevertheless, during the theoretical conformational analysis of these compounds, we observed that while one of methyl groups in the half-chair pyranic ring of **1** occupied a *pseudo*-equatorial position, leaving a relatively accessible surface for *m*-CPBA approach, both faces of the planar furanic ring of phenazine **10** were sterically hindered by its methyl groups (Figure 4). On the other hand, phenazine **9** assumed a envelop conformation with one of its dihydrofurane faces relatively free for *m*-CPBA approach (Figure 4). So, it seems reasonable to suppose that a steric approach control based on the proposed spiro transition structure for epoxidation of double bonds with *m*-CPBA[19-21] could be responsible for the dramatic effect observed on reactivity. The two methyl groups in **10** would preclude the attack of *m*-CPBA on the enol double bond shared by aromatic and heterocyclic rings, leading exclusively to the N-8 oxidation and the production of **13**.

As a conclusion, both experimental results and theoretical evidences seem to indicate that the steric approach control would explain the different reactivities, especially if we consider that the attack of *m*-CPBA tends to be perpendicular to the plane of the rings (spiro-transition-structure). Additional theoretical study is currently being performed in order to find transition structures and barrier heights for these oxidations and to corroborate this initial guess.

This rationale could now be extended to reactions with other phenazines, with the goal of preparing 9- or 10-membered macrolactones or *N*-oxides. The phenazine **9**, the *N*-oxide **13** and the oxidized products from **10** are reported, herein, for the first time.

Experimental

NMR experiments were performed in deuteriochloroform with TMS as the internal standard with Gemini-200 MHz instrument: chemical shifts are given in δ (ppm), and *J* values are given in Hz. For elemental analysis, IR spectra and mass spectra we used Perkin-Elmer CHN 2400, Perkin-Elmer 783 and a Shimadzu GCMS-QP 5050-A, respectively.

*Preparation of 2-methyl-1,2-dihydrobenzo[a]furo [2,3-c]phenazine (**9**)*

A solution of glacial acetic acid (50 mL) containing quinone **9a** (2-methyl-2,3-dihydronaphtho[1,2-b]furan-4,5-dione), synthesized by the procedure described by Fieser[9] and adapted by Pinto and coworkers[10] (214 mg, 1.00 mmol), *o*-phenylenediamine (119 mg, 1.10 mmol) and sodium acetate (107 mg, 1.30 mmol) were kept under reflux for 2 h, and monitored by TLC. After

Table 1. Calculated HOMO/LUMO energies, HOMO-LUMO, energy gap (hartrees) and reactive double bond atomic charges (electrons)

Compound	HOMO	LUMO	GAP	CHARGE	CHARGE
1	-0.20247	-0.07454	0.12793	-0.26 C-14b	0.15 C-4a
9	-0.19963	-0.07683	0.12280	-0.20 C-13b	0.14 C-3a
10	-0.19876	-0.07607	0.12269	-0.21 C13b	0.15 C-3a

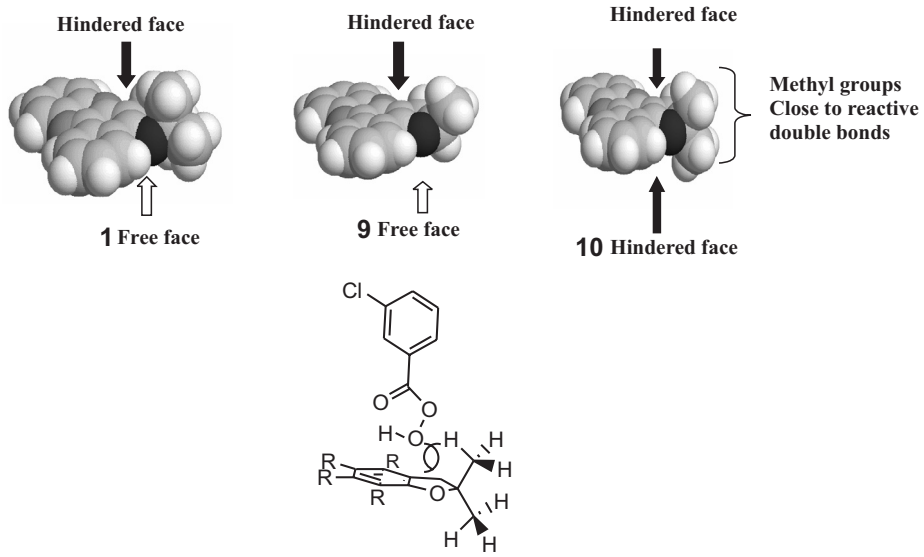

Figure 4. Proposed spiro transition structure for epoxidation of double bonds with *m*-CPBA.

reaction, the mixture was added into ice and allowed to stay overnight. The formed yellow precipitate was filtered off in Buchner funnel and washed with cold water (3 × 100 mL) and the phenazine **9** (273 mg, 0.95 mmol) was isolated with 95% yield.

Physical data, mp 134 °C, ^{1}H NMR 200 MHz ($CDCl_3$) δ 9.3 (m, H-7) 8.25 (m, H-4) 8.15 (m, H-12 or H-9), 8.0 (m, H-9 or H-12) 7.75 (m, H-6, H-5, H-11, H-10) 5.35 (m, H-2), 3.8 (dd, *J* 14.3 Hz, 7.5 Hz H-1a or H-1b) 3.3 (dd, *J* 14.3 Hz, 7.5 H-1a or H-1b) 1.65 (d, *J* 4 Hz, C-2-Me), ^{13}C NMR 50 MHz ($CDCl_3$) δ 158.01 (s, C-3a) 142.7 (s, C-13a) 142.4 (s, C-8a) 140.9 (s, C-7b), 139.7 (s, C-12a), 131.5 (s, C-7a) 129.65 (d, C-5), 129.62 (d, C-12), 129.4 (d, C-10), 129.4 (s, C-3b) 128.2 (d, C-11), 127.8 (d, C-9), 125.6 (d, C-6), 124.4 (d, C-7), 121.9 (d, C-4), 112.7 (s, C-13b), 81.9 (d, C-2), 35.6 (t, C-1), 22.2 (q, C-2-Me). Anal. Calc: $C_{19}H_{14}N_2O$, C 79.70, H 4.93, N 9.78, O 5.59; Found: C 79.62, H 5.03, N 9.75.

*Oxidation of phenazine **9***

Phenazine **9** (286 mg, 1.00 mmol) in a solution of dichloromethane (7 mL) was treated at room temperature with *m*-CPBA (860 mg, 5 mmol) added dropwise. After 24 h of contact under stirring and the usual work up, the reaction product residue was submitted to column chromatography over silica gel and eluted with mixtures of hexane/ethyl acetate of increasing gradient of ethyl acetate. The macrolactone *N*-oxide **11**, colourless solid, was eluted with a mixture of hexane/ethyl acetate (85:15), yielding 188 mg (56%, 0.56 mmol). The α-hydroxy-ketone, colourless solid **12** was eluted with a mixture of hexane/ethyl acetate (60:40) yielding 84 mg (0.26 mmol) with 26% yield.

*7-Methyl-7,8-dihydro-benzo[3,4]oxonino[6,7-b]quinoxalin-5,9-dione 15-N-oxide (**11**)*

Colourless solid, mp 145 °C, ^{1}H NMR 200 MHz ($CDCl_3$) δ 8.6 (dd, *J* 7.8 Hz, *J* 1.6 Hz, H-14), 8.2 (dd, *J* 7.8 Hz, *J* 1.6 Hz, H-11), 8.1 (dd, *J* 7.8 Hz, *J* 1.6 Hz, H-4), 7.9 (m, H-3 + H-13), 7.7 (dd, *J* 7.6 Hz, *J* 1.6 Hz, H-2), 7.6 (dd, *J* 7.6 Hz, 1.6 Hz, H-1), 5.6 (m, H-7), 3.7 (dd, *J* 14.8 Hz, *J* 7.6 Hz, H-8a or H-8b), 3.3 (dd, *J* 14.8 Hz, *J* 7.6 Hz, H-8a or H-8b), 1.3 (d, *J* 3,9 Hz, C-7-Me), ^{13}C NMR 50MHz ($CDCl_3$) δ 194.67 (s, C-9), 166.67 (s, C-5), 153.7 (s, C-9a), 143.9 (s, C-11a + C-15a), 133.80 (d, C-2), 133.7 (d, C-3), 133.45 (d, C-8), 132.55 (d, C-10), 131.1 (d, C-9), 130.8 (d, C-4) 130.2 (d, C-1) 129.6 (s, C-14a), 128.0 (s, C-15b), 127.6 (s, C-4a), 118.2 (d, C-14), 70.2 (d, C-7), 29.3 (t, C-8), 21.49 (q, C-7-Me), IR (KBr) ν_{max}/cm^{-1}: 3063, 2985, 2933, 1721, 1709, 1597, 1286, 1264, 1198, 760, 701. MS [70 eV, m/z (%)]: 319 (15), 318 (65), 291 (4), 290 (20), 274 (4), 249 (72), 248 (47), 234 (52), 220 (100), 204 (44), 177 (20), 102 (21), 76 (57), 50 (30). Anal. Calc: $C_{19}H_{14}N_2O_3$, C 68.26, H 4.22, N 8.38, O 19.14; Found: C 68.11. H 4.35, N 8.30.

*6-Hydroxy-6-(2-hydroxypropyl)-5,6-dihydrobenzo[a]phenazin-5-one **(12)***

Colorless crystals, mp 155 °C. ^{1}H NMR, 200 MHz ($CDCl_3$) δ 8.1 (m, H-1, H-4, H-9, H-10), 7.8 (m, H-2, H-11, H-5), 7.6 (dd, *J* 7.5 Hz, 1,4 Hz, H-3), 5.6 (bs, H-2'), 3.8 (dd, 14.1 Hz, 4.6 Hz, H-1'a or H-1'b, 1H), 3.3 (dd, 14.1 Hz, 4.6 Hz H-1'a or H-1'b, 1H), 1.2 (bs, H-3'), ^{13}C NMR, 50 MHz ($CDCl_3$) δ 200.5 (s, C-5), 153.7(s, C-6a), 143.9 (s,

C-12a), 139.1 (s, C-7a), 133.8 (s, C-11a), 133.6 (d, C-2), 133.5 (d, C-3), 131.2 (d, C-11), 131.0 (d, C-8), 130.6 (d, C-10), 129.8 (d, C-9), 129.5 (d, C-4), 128.7 (d, C-1), 126.6 (s, C-12b), 123.0 (s, C-4a), 85.6 (s, C-6), 69.4 (s, C-2'), 29.3 (t, C-1'), 21.6 (q, C-3'). IR (KBr) ν_{max}/cm^{-1}: 3063, 2985, 2933, 1721, 1597, 1286, 1264, 1198, 760, 701. MS [70 eV, m/z (%)]: 320 (3) 319 (11), 318 (6), 301 (3), 291 (4), 290 (20), 274 (4), 263 (27), 249 (59), 233 (53), 220 (100), 204 (15), 192 (13), 177 (9), 164 (6) 102 (25), 76 (30), 50 (16). Anal. calcd: $C_{19}H_{16}N_2O_3$, C 71.24, H 5.03, N 8.74, O 14.98; Found: C 71.05, H 5.12, N 8.60.

*Preparation of 2,2-dimethyl-1,2-dihydrobenzo[a]furo[2,3-c]phenazine-8-N-oxide (**13**)*

Phenazine **10** (300 mg, 1.00 mmol), in a solution of dichloromethane (7 mL), was treated at room temperature with a solution of *m*-CPBA (860 mg, 5.00 mmol) added dropwise. After 24 h of contact and the usual work up, the reaction product residue was submitted to column chromatography over silica gel and eluted with mixtures of hexane/ethyl acetate of increasing gradient of ethyl acetate up to 100% of ethyl acetate, followed by mixtures of ethyl acetate / methanol of increasing polarity. The *N*-8 oxide **13** was eluted with mixture of ethyl acetate/methanol (90:10) and isolated as a fluorescent yellow solid (221 mg, 0.70 mmol), with a yield of 70%. Yellow solid, mp 173 °C, ^{1}H NMR 200 MHz ($CDCl_3$) δ 9.3 (m, H-7) 8.7 (m, H-4) 8.3 (m, H-12), 8.1 (m, H-9) 7.8 (m, H-11, H-10, H-6, H-5) 4.1 (s, 2H, H-1), 1.7 (s, 6H, 2 x C-2 Me), ^{13}C NMR 50 MHz ($CDCl_3$) δ 157.2 (s), 143.4 (s), 141.6 (s), 135.4 (s), 133.6 (s), 131.8 (s), 131.4 (d) 129.5 (s), 129.4 (d) 129.2 (d), 128.3 (d), 125.8 (d), 124.3 (d), 122.2 (d), 117.9 (d), 107.6 (s), 89.8 (s), 45.5 (t), 29.3 (s), MS [70 eV, m/z (%)]**:** 316 (6.97), 300 (11.31), 299 (32.45), 283 (11.21), 257 (28.18), 233 (24.85), 142 (24.50), 102 (25.85), 77 (37.41), 43 (100.00). Anal. Calc: $C_{20}H_{16}N_2O_2$, C 75.93, H 5.10, N 8.86, O 10.11; Found: C 75.88, H 5.21, N 8.78.

Computational methods

Electronic calculations were performed with the Gaussian 98 package.[15] Geometries were fully optimized at the B3LYP/6-31G(d,p) level (Becke three parameter hybrid functional, combined with the Lee, Yang, and Parr (LYP) correlation functional),[16,17] and all of them were characterized as genuine local minima (no imaginary frequencies) by calculation of vibrational frequencies at the same level of theory. In order to search for additional local minima, rough conformational analysis of the furan and pyran rings were performed through small changes in ring torsion angles (from planar to twisted conformations and vice versa), followed by geometry re-optimization. Calculated charges are those that fit the electrostatic potential at points selected according to CHelpG scheme.[22]

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