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Short Report

The Preparation of a 10-Membered Ring Macrolactone by Selective Ozonolysis and the Role of the Dihydropyran-Substituent on the MCPBA-Oxidation Reaction Profile of β-Lapachone Phenazines

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A benzofenazina derivada da β-lapachona foi submetida a ozonólise em condições que forneceram seletivamente a lactona macrocíclica 7,7-dimetil-7,8,9,10-tetrahidro-5*H*-benzo[3,4]oxecino[5,6-b]quinoxalina-5,10-diona, com rendimento de 52%. O efeito de substituintes localizados no anel diidropirânico das fenazinas, no caso C2'–OH, C2'–Br e C2'–I, em reações de oxidação com MCPBA, foi também investigado.

The benzophenazine from β-lapachone was submitted to ozonolysis under conditions that selectively furnished the corresponding rigid macrocyclic lactone 7,7-dimethyl-7,8,9,10-tetrahydro-5*H*-benzo[3,4]oxecino[5,6-b]quinoxaline-5,10-dione in an yield of 52%. The effect of substituents located at the dihydropyrane moiety of the phenazines, namely C2'-OH, C2'-Br and C2'-I, in the oxidation with MCPBA, was also investigated.

Keywords: β-lapachone, ozonolysis, 10-membered ring macrolide, selectivity, effect of substituent

Introduction

In general, natural and synthetic phenazines have attracted considerable attention because of their interesting biological activities,[1] including broad-spectrum antibiotic,[2] antimalarial,[3,4] trypanocidal[5] along with anti hepatitis C viral replication activities.[6] Benzo[a]phenazines are also efficient DNA intercalating ligands, exhibiting antitumor activity in leukaemia and solid tumors.[7] They have also been described as dual inhibitors of topoisomerase I and II.[8]

In our search for preparing new derivatives, for synthetic purposes and biological screening, we recently reported[9] that peroxidation of the b-lapachone-derived benzophenazine, named lapazine (**1**), with *m*-$ClC_6H_4CO_2H/CH_2Cl_2$ generated, unexpectedly, a macrocyclic lactone **2** with a rigid 10-membered ring (27% yield), the corresponding N-oxide **3** (13% yield), and the dihydrobenzophenazine-5-one **4** (35% yield) (Figure 1). Macrocyclic compounds **2** and **3** were formed *via* oxidative cleavage of the aromatic double bond at the site of fusion of the dihydropyran moiety with the phenazine component. This region (rings C/E, in **1**) (Figure 1) would behave as a conjugated enol ether, which, after the expected epoxide formation and fragmentation would lead to **2** and **3** (further oxidation). An acid-catalysed epoxide-acetal or α-hydroxy hemiacetal would furnish **4**.[9]

Compounds **2** and **3** belong to the class of macrolides, an important group of biologically active compounds, especially recognized as antibiotics.[10] Among 10-membered macrolactones, the antibiotic activity of apicularen A and B was reported.[11]

In the present work, considering **2** and **3**, the presence of diaza heterocyclic groups close to the ion-binding macrolactone moiety constitutes attractive molecular features.

In searching for a more general application of the oxidative cleavage by MCPBA,[9] we have analysed the role of substituent, at C-2', in the dihydropyrane moiety of lapazine, namely -OH (**5**), -Br (**6**) and -I (**7**) (Figure 1).

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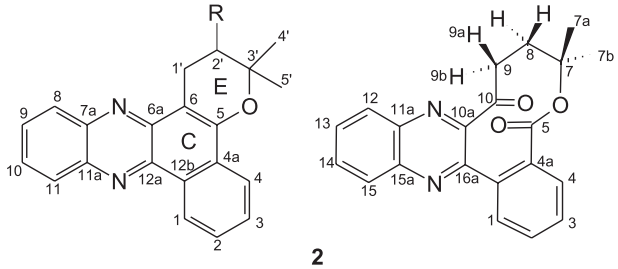

1 R = H, **5** R = OH

6 R = Br, **7** R = I

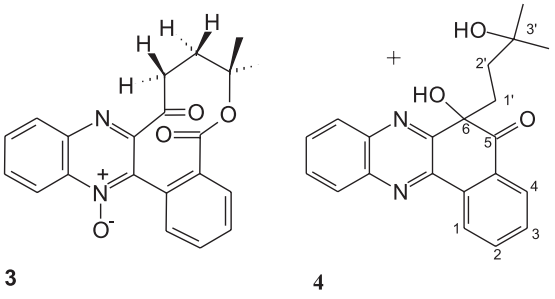

Figure 1. Lapazine (**1**), its derivatives (**5**, **6**, **7**) and oxidation products (**2**-**4**).

Additionally, in an attempt to develop a more selective and simpler method by which to bring about this oxidative cleavage,[9] we have evaluated the use of ozone, a versatile reagent capable of oxidizing both cyclic and acyclic olefins.[12,13] Since double bonds connected to electron-donating groups react with ozone many times faster than those connected to electron-withdrawing groups,[14] this gaseous reagent possesses significant selective reaction potential. Ozone has been used for the selective cleavage of double bonds in alicyclic compounds, a strategy that gave rise to the expanded C-15 macrocyclic system in the synthesis of muscone.[15] Aromatic bonds can also be cleaved through ozonolysis as shown, for example, in the formation of biphenyl compounds from benzo[c]phenanthrene and phenanthrene.[16,17] A number of studies have concerned the ozonolysis of heterocyclic molecules,[18-22] but none has focused on the reaction with phenazines.

Results and Discussion

As described before,[9] the oxidation with MCPBA caused the oxidative cleavage of the aromatic double bond at the site of fusion of the dihydropyran moiety with the phenazine component. To know the role of the substituent on the reaction profile, we have studied the phenazines (**5**-**7**) derived respectively from 3-hydroxyl, 3-bromo and 3-iodo-β-lapachone.

The phenazine **5** reacted with MCPBA to produce the macrolide **8**, as the sole product, in 52% yield, along with unchanged **5** (Figure 2). This hydroxymacrolide constitutes a versatile intermediate for the synthesis of new derivatives.

In the case of **6**, a complex mixture of products was obtained, from which it was possible to isolate **9**, as the corresponding N-oxide bromomacrolide, in low (10%) yield (Figure 2). Supplementary crystallographic data is given.[23]

The structure was solved by direct methods and refined by full-matrix least squares calculations (Figure 3). The refinement was conducted until all atomic parameters shifts were smaller than their standard deviations. All H atoms were located by geometric considerations. In the final difference Fourier map there are no peaks greater than 0.34 Å^{-3}. Bond lengths and angles are in good agreement, to within experimental accuracy, with the values found in the literature.[24]

The oxidation of compound **7** with MCPBA, due to the liability of the C-I bonding, was unsuccessful.

From these results, it is possible to envisage that the method would be valuable for the synthesis of substituted macrolides, specially for substituents, which are not easily broken in the oxidation conditions.

Concerning oxidation of phenazines, a natural development would be to try ozonolysis. A solution of lapazine (**1**) in methylene dichloride was, then, subjected to ozonolysis, and the ozonides thus formed were reduced with Zn/water.[25] The progress of the reaction was followed by TLC using pure samples of **2**, **3** and **4** as reference standards. The macrocyclic lactone **2** was formed as the major product of the reaction, together with some minor polar by-products that were not identified. Neither **3** nor **4** could be detected in the crude reaction mixture by TLC analysis. Compound **2** was isolated from the reaction mixture by column chromatography as pure white crystals in an overall yield of 52% (Figure 2).

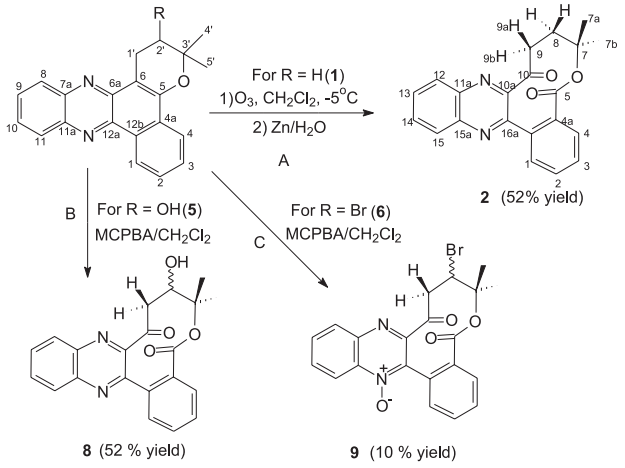

Figure 2. Oxidation of phenazines. A: ozonization of lapazine **1** furnishing **2;** B: oxidation of **5** furnishing **8;** C: oxidation of **6** furnishing **9**.

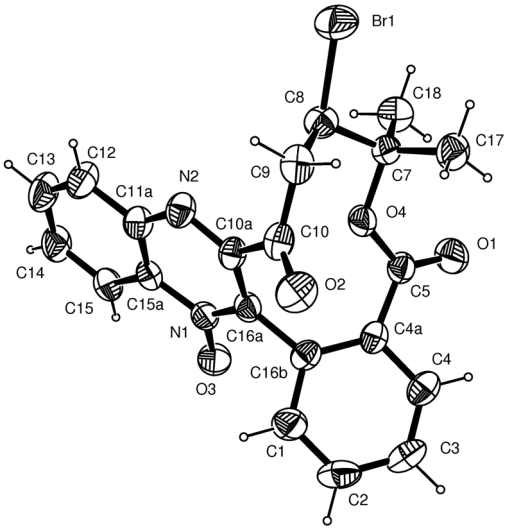

Figure 3. Perspective view of **9** showing the atom-labelling scheme. Displacement of ellipsoids plotted at the 50% probability level, are shown for the non-H atoms.

In addition to providing an easier protocol and cleaner conditions for the preparation and isolation of **2**, the reaction of **1** with ozone was also more selective and efficient than the reported direct formation of **2**.[9]

To the best of our knowledge, this is the first example of the ozone-mediated formation of a rigid macrocyclic lactone. Although a more general view of the synthetic aspects of the use of ozone is not addressed in the present communication, the formation of **2** suggests the possible use of this reagent to cleave phenanthrene rings linked face-to-face with a heterocyclic moiety. Experiments along this line are in progress.

Experimental

NMR experiments were performed in deuteriochloroform with TMS as the internal standard with a Bruker *AVANCE* DRX-400 or Gemini-200 MHz instruments; chemical shifts are given on the δ scale, and J values are given in Hz. For elemental analysis, we used Perkin-Elmer CHN 2400 and for IR spectra, a Perkin-Elmer 783. The ozonolysis was performed in a T-408 Welsbach, with ozone pressure of 2 Lb in^{-2}.

*Preparation of 7,7-dimethyl-7,8,9,10-tetrahydro-5H-benzo[3,4]oxecino[5,6-b]quinoxaline-5,10-dione (**2**).* Yellow solution of lapazine, was prepared as described[4,9] (**1**; 314 mg; 1.0 mmol) in 20 mL of methylene chloride, treated with ozone at –5.0 °C until all substrate had reacted (1h). The colourless reaction product was reduced with Zn/water and, after the usual workup, was submitted to silica gel column chromatography using hexane/ethyl acetate (95:5) as eluent. Pure **2** (179 mg; 0.52 mmol, 52% yield) was obtained as a colourless crystalline product (melting point 162-163 °C) that was shown to be identical to an authentic sample of 7,7-dimethyl-7,8,9,10-tetrahydro-5*H*-benzo[3,4]oxecino[5,6-b]quinoxaline-5,10-dione (**2**) by mixed melting point and TLC (co-chromatography). All of the physico-chemical data of **2** were in full accordance with those previously published.[9,26]

*Preparation of 7,7-dimethyl-8-hydroxy-7,8,9,10-tetrahydro-5H-benzo[3,4]oxecino[5,6-b]quinoxaline-5,10-dione (**8**).* The hydroxyphenazine **5** (0.330 g, 1 mmol) in a solution of 10 mL of methylene chloride, at room temperature, was treated with *m*-chloroperbenzoic acid (0.690 g, 4 mmol) in small portions until total consumption. The reaction was followed by TLC. After 24 hours, the analysis by silica gel TLC revealed the presence of a sole spot, together with remaining substrate. After the usual work up, with sodium carbonate (10%), the reaction residue was submitted to chromathography in a silica gel column, using hexane/ethyl acetate, in increasing polarity, as eluents. **8** (0.188 g, 0.52 mmol, 52%) was eluted with a mixture of hexane/ethyl acetate (7:3) and recrystallized from this fraction, as light yellow needles, mp 195°C, with decomposition. δ H [200 MHz, $CDCl_3$; J(Hz)]: 1.35 (3H, *s*, C7-Me); 1.45 (3H, *s*, C7-Me); 2.60 (1H, dd, *J* 13; 2 Hz, C9-H); 2.80 (1H, dd, *J* 2; 13 Hz, C9-H), 4.90 (1H, m, C8-H); 5.80 (1H, bs, -OH, exchangeable with D_2O); 7.70 (3H, m, ArH); 8.0 (3H, m, ArH); 8.25 (1H, m, ArH); 8.50 (1H, m, ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ C: 17.3 (CH_3); 24.5 (CH_3); 45.0 (CH_2); 73.8 (CHOH); 86.4 (C-O); 119.7 (CH); 129.7 (C); 129.8 (C); 130.2 (CH); 131.5 (CH); 131.7 (CH); 133.1 (CH); 132.3 (CH); 132.4 (CH); 132.7 (CH); 137.6 (C); 138.9 (C); 142.2 (C); 153.6(C); 163.9 (COO); 196.0 (C=O). IR (KBr) ν_{max}/cm^{-1}: 3407, 2926, 1723, 1597, 1574, 1368, 1350, 1257, 1038, 766, 754. Found C 69.50 H 5.13 N 7.60. Calc. for $C_{21}H_{18}N_2O_4$, C 69.60 H 5.01 N 7.73.

*Preparation of 8-bromo-7,7-dimethyl-5,10-dioxo-7,8,9,10-tetrahydro-5H-benzo[3,4]oxecino[5,6-b]quinoxaline-16-N-oxide (**9**).* The bromophenazine **6** (0.393 g, 1 mmol) in a solution of 10 mL of methylene chloride, at room temperature, was treated with *m*-chloroperbenzoic acid (MCPBA) (0.690 g, 4 mmol) in small portions until total consumption. The reaction was followed by TLC. After 24 hours, the reaction was complete. After the usual work up, with sodium carbonate (10%), the reaction residue, a complex mixture of products, was submitted to chromathography in a silica gel column, using hexane/ethyl acetate, in increasing polarity, as

eluents. **9** (0.044 g, 0.1 mmol) was eluted with a mixture of hexane/ethyl acetate (95:5) and recrystallized from this fraction, as colourless needles, mp 194-198°C, with decomposition. Any other product was isolated in a pure form. δ H [400 MHz, $CDCl_3$; *J*(Hz)]: 1.65 (3 H, *s,* C7-Me); 1.71 (3H, *s*, C7-Me); 3.14 (1H, dd, *J* 14.8; 4.0 Hz, H-9); 4.17 (1H, dd, *J* 14.8; 12.8 Hz, C9-H), 4.38 (1H, dd, *J* 12.8; 4.0 Hz, *C*8-H); 7.60 (2H, m, C1-H and C3-H); 7.73 (1H, td, *J* 8.0; 1.6 Hz, C2-H); 7.85 (2H, td, *J* 7.2, 1.6 Hz, C14-H); 7.93 (1H, td, *J* 7.2; 1.6 Hz, C13-H); 8.15 (1H, dd, *J* 8.0, 1.6 Hz, C4-H); 8.22 (1H, dd, *J* 8.0, 1.2 Hz, C12-H); 8.60 (1H, dd, *J* 8.0, 1.2 Hz, C15-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ C: 18.2 (C7-Me); 25.4 (C7-Me); 47.1 (C-9); 52.5 (C-8); 87.2 (C-7); 119.7 (C-15); 128.9 (C-1a); 129.2 (C-4a); 129.9 (C-1 or C-3); 130.4 (C-12); 131.6 (C-14); 132.0 (C-4); 132.2 (C-13); 132.7 (C-2); 132.8 (C-3); 137.7 (C-15a); 138.8 (C-16a); 142.3 (C-11a); 152.9 (C-10a); 163.7 (C-5); 195.1 (C-10). IR (KBr) ν_{max} / cm^{-1}: 3096, 2987, 2963, 1716, 1638, 1597, 1574, 1480, 1346, 1291, 1256, 1121, 768, 762, 703, 661, 624, 587. Found C 57.10 H 4.06 N 6.17. Calc. for $C_{21}H_{17}BrN_2O_4$, C 57.16 H 3.88 N 6.35.

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