Tetrahedron Letters 56 (2015) 1060–1062

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

2-Amino-4,4α-dihydro-4α,7-dimethyl-3*H*-phenoxazin-3-one as an unexpected product from reduction of 5-methyl-2-nitrophenol

Suzanne M. Jansze [a,b], Vittorio Saggiomo [a], Antonius T. M. Marcelis [b], Martin Lutz [c], Aldrik H. Velders [a,*]

[a] *Laboratory of BioNanoTechnology, Wageningen University, Dreijenplein 6, 6703 HB Wageningen, The Netherlands*
[b] *Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands*
[c] *Crystal and Structural Chemistry, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands*

ARTICLE INFO

Article history:
Received 20 September 2014
Revised 17 December 2014
Accepted 13 January 2015
Available online 17 January 2015

Keywords:
2-Aminophenoxazin-3-one
Azobenzene
Reductive synthesis
5-Methyl-2-nitrophenol
Tricyclic compound

ABSTRACT

When attempting to synthesize symmetric 2,2′-dihydroxy-4,4′-dimethyl-azobenzene from 5-methyl-2-nitrophenol by reductive methods based on two literature procedures, an unexpected product was isolated in 30% yield. Full analysis by mass spectrometry, NMR spectroscopy, and single-crystal X-ray structure analysis, proved this product to be tricyclic 2-amino-4,4α-dihydro-4α,7-dimethyl-3*H*-phenoxazin-3-one. This Letter conveys a warning regarding reductive synthetic routes toward azobenzenes. We also present a novel reductive synthetic route for phenoxazines, an important class of tricyclic compounds.

Azobenzenes form an important class of compounds, which are useful as dyes and analytical reagents,[1] and as key components in photo-switchable and photo-responsive materials,[2] for example, microelectromechanical systems (MEMS).[3] In an attempt to synthesize 2,2′-dihydroxy-4,4′-dimethyl-azobenzene (**3**) via the mild reduction of 5-methyl-2-nitrophenol (**1**), we unexpectedly obtained 2-amino-4,4α-dihydro-4α,7-dimethyl-3*H*-phenoxazin-3-one (**2**) (Scheme 1).

A wide variety of synthetic methods are available for the synthesis of azobenzenes, typically using rather harsh conditions.[4] Milder routes have been described in which azobenzenes are prepared by reduction of nitrobenzenes. Radivoy and co-workers[5] obtained symmetrical azobenzenes from (substituted) nitrobenzenes by reduction with nanosized iron and lithium, while Gowda and co-workers[6] employed a method in which magnesium and triethylammonium formate are used. In both papers,[5,6] the methods were applied to a variety of differently substituted nitrobenzenes, and the synthetic methods appeared compatible with the presence of both hydroxyl and methyl groups.

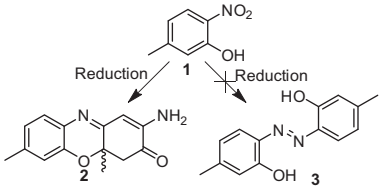
The synthesis of 2,2′-dihydroxy-4,4′-dimethyl-azobenzene (**3**) was described almost 60 years ago by White and co-workers,[7] starting from 5-methyl-2-aminophenol via a Cu(I)-catalyzed diazonium coupling after which the copper complex of the final product was obtained. In the search for an alternative, milder, and less elaborate synthetic route for **3**, reduction of 5-methyl-2-nitrophenol (**1**) was attempted following the method described by Gowda and co-workers[6,8] and that reported by Radivoy and co-workers.[5,9]

A deep orange/red solid compound (an indicator for azobenzenes) was isolated as the major product (30% yield) from both synthetic routes,[8,9] with an exact mass (ESI-MS) corresponding to the mass of the desired azobenzene **3**. However, the absorption maximum (λ_{max}) of 400 nm of the obtained product was at a higher wavelength than that of the characteristic azobenzene band around 360 nm (see Supplementary information S2). In addition, the product did not show the characteristic *cis–trans* photoswitching expected for an azobenzene. Furthermore, the ^{1}H NMR spectrum (Fig. 1) appeared too complex for the expected symmetrical azobenzene **3**.

A detailed NMR study (^{1}H-, ^{13}C-, COSY, HSQC, HMBC), revealed that the isolated product was in fact 2-amino-4,4α-dihydro-4α,7-dimethyl-3*H*-phenoxazin-3-one (**2**) (see Scheme 1). Whereas starting material **1** and target product **3** would only show one methyl group and three aromatic proton signals, for compound **2** additional resonances were observed. The three protons of the aromatic ring of **2** are clearly recognizable in the ^{1}H NMR spectrum (Fig. 1: signals a, b, c). (Details of the NMR analysis can be found in the Supplementary information.) The two methyl groups gave two different signals (Fig. 1: signals g and h), which can be assigned

* Corresponding author. Tel.: +31 317485970.
E-mail address: aldrik.velders@wur.nl (A.H. Velders).

http://dx.doi.org/10.1016/j.tetlet.2015.01.089
0040-4039/



Scheme 1. Reduction of 5-methyl-2-nitrophenol (**1**) does not yield azobenzene **3**, but the 2-aminophenoxazin-3-one **2**.

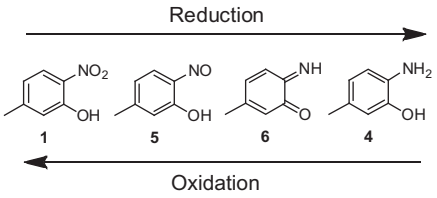


Figure 3. The intermediates that are formed upon reduction of 5-methyl-2-nitrophenol (**1**) and oxidation of 5-methyl-2-aminophenol (**4**).

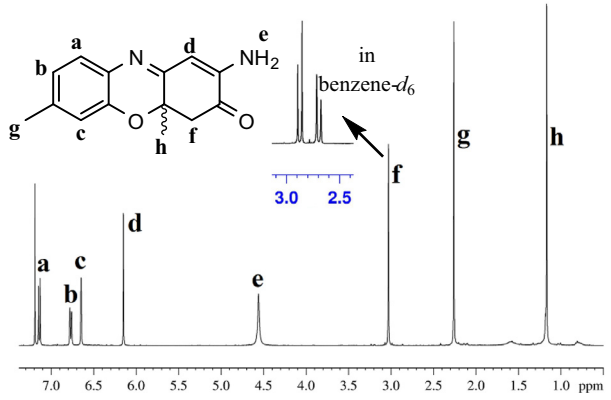


Figure 1. ^{1}H NMR spectrum of 2-amino-4,4α-dihydro-4α,7-dimethyl-3*H*-phenoxazin-3-one (**2**) in $CDCl_3$.

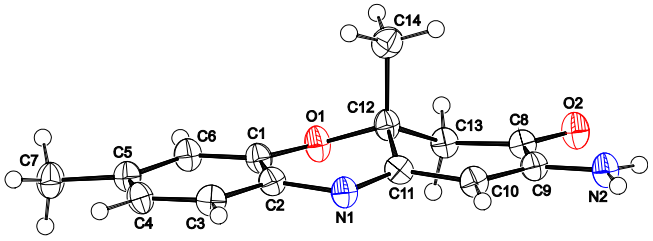


Figure 2. Displacement ellipsoid plot of 2-amino-4,4α-dihydro-4α,7-dimethyl-3*H*-phenoxazin-3-one (**2**), in the crystal (50% probability level).[10] Only one enantiomer of the racemic compound is shown. Selected bond lengths [Å], angles, and torsion angles [°]: C1–C2 1.398(2), C2–N1 1.407(2), N1–C11 1.300(2), C11–C12 1.524(2), C12–O1 1.4469(19), O1–C1 1.3798(19), C11-C10 1.432(2); C1-O1–C12 116.11(11), C2-N1–C11 117.39(13), O1–C12–C11 111.37(13); C2–C1–O1–C12-24.0(2), N1–C11–C12-O1-30.9(2), C1–O1–C12–C11 37.66(18). Angle sum at N2: 359(3)°.

through COSY spectra also revealing the 4-bond couplings. An enigmatic aliphatic signal (2H), present at 3.0 ppm in $CDCl_3$, split into two doublets when C_6D_6 was used as the solvent (Fig. 1: signal f, and insert), indicating non-equivalent protons as expected for the methylene group in the non-aromatic ring of the 4,4α-dihydrophenoxazine **2**. The signal at 4.5 ppm can be assigned to the amino group protons.

Single crystal X-ray diffraction analysis corroborated the molecular structure (Fig. 2). The compound crystallizes as a racemate in the centrosymmetric space group $P2_1/c$. As expected from the MS and NMR analyses, the structure of **2** is a condensation product from two molecules of the starting compound resulting in a three-ring structure. One ring is similar to the starting material, viz. an aromatic ring, while the other has lost its aromaticity, and the central ring is the typical phenoxazine six-membered ring with the oxygen and nitrogen atoms linking the other two rings. The extended conjugation over the two nitrogens and the carbonyl group explains the deep orange color of the molecule. Both nitrogen atoms have a planar geometry. The hydrogen atoms at N2 are involved in intra- and intermolecular hydrogen bonding forming a 2-dimensional network in the crystallographic b,c-plane. The amino group at N2 acts as donor of hydrogen bonds, with the carbonyl oxygen O2 and the ring nitrogen N1 as acceptors (for details see Supplementary information S10).

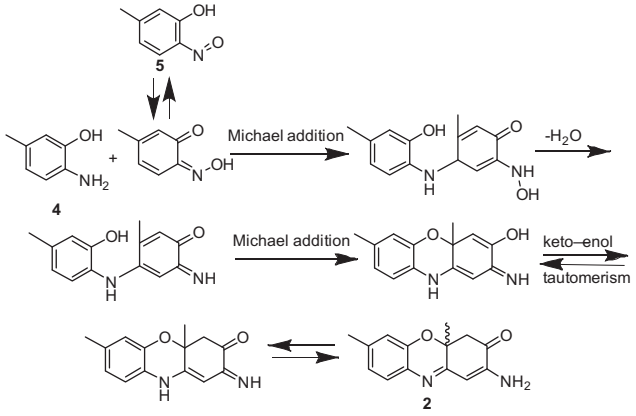
2-Aminophenoxazin-3-ones have a wide range of pharmaceutical properties and constitute the base skeleton of actinomycin, cinnabarinic acid, and questiomycin A. These compounds are usually prepared by enzymatic oxidation of *o*-aminophenols using laccases,[11] peroxidases, or hemolysates. Chemical synthesis also involves oxidation of *o*-aminophenols catalyzed by, for example, Co(salen)[12] or Mn-porphyrin complexes.[13] These known oxidative synthetic methods have an average yield of around 50%.

The 2-aminophenoxazin-3-one **2** has been actively investigated for its antitumor, antiviral, antibacterial, and antidiabetic effects, often under its code name Phx-1.[14] Only a few non-enzymatic methods for the synthesis of **2** are described. They all involve oxidative condensation of 5-methyl-2-aminophenol.[12,15,16] To our knowledge, for the synthesis of 2-aminophenoxazin-3-one compounds, no synthetic methods involving reduction of nitrophenols have been published.

There are different mechanisms proposed for the enzymatic and non-enzymatic oxidative synthetic routes toward **2** and related 2-aminophenoxazin-3-ones starting from (substituted) aminophenols.[12,17,18] The proposed non-radical enzymatic oxidative mechanisms involve the formation of the quinone imine (**6**) from the oxidation of the aminophenol and subsequent coupling to aminophenol.[17] This intermediate is then converted into the final product by successive oxidation steps. As under the described reductive reaction conditions[5,6] radicals will be quenched and oxidation is not expected, we propose another mechanism for the synthesis of the final product **2**. Reduction of the *o*-nitrophenol eventually produces *o*-aminophenol, but also other reduced species will be formed: *o*-nitrosophenol (**5**) and *o*-quinoimine (**6**),[19] as presented in Figure 3.

In theory, similar to the oxidative reaction mechanism discussed above, the reaction between the *o*-aminophenol and the *o*-quinone imine could still be possible in the reductive reaction mixture, but then the first intermediate cannot be oxidized to the final product, because of the reductive environment. Another reactive species that can react with the *o*-aminophenol is the *o*-nitrosophenol **5**. It is known that *o*-nitrosophenol **5** can chelate different metal ions[20,21] increasing its stability in solution. We therefore speculate that a double Michael addition from a fully reduced aminophenol **4**, present in solution, to the nitroso intermediate **5** gives the final tricyclic product **2** without the need of an oxidation step (Scheme 2).

In conclusion, the characterization of tricyclic 2-amino-4,4α-dihydro-4α,7-dimethyl-3*H*-phenoxazin-3-one (**2**) is described, which was obtained as an unexpected product, while using reductive synthesis methods starting from 5-methyl-*o*-nitrophenol. The results presented here indicate that reductive methods[5,6] for preparing azobenzenes from nitrobenzenes are not generally applicable for all types of (substituted) nitrobenzenes. On the other



Scheme 2. Proposed reaction mechanism from the *o*-nitrosophenol (**5**) and 2-aminophenol producing 2-amino-4α,7-dimethyl-4,4α-dihydro-3*H*-phenoxazin-3-one (**2**).

hand, a possible new reductive synthetic route toward the pharmaceutically interesting class of 2-aminophenoxazin-3-ones from nitrobenzenes has been elaborated, which is less laborious than the commonly used enzymatic methods of preparation.

Supplementary data

Supplementary data (these data include extensive spectral analysis (NMR (^{1}H-, ^{13}C-, COSY, HSQC, HMBC), exact mass, UV–vis, melting point) of 2-amino-4,4α-dihydro-4α,7-dimethyl-3*H*-phenoxazin-3-one (**2**)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.01.089. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Zollinger, H. *Color Chemistry: Syntheses, Properties and Applications of Organic Dyes and Pigments*; VCH Verslagsgesellschaft mbH: Weinheim, 1987.
2. Garcia-Amoros, J.; Velasco, D. *Light-triggered Azobenzenes: From Molecular Architecture to Functional Materials*; John Wiley & Sons: USA, 2014; p 27.
3. Merino, E.; Ribagorda, M. *Beilstein J. Org. Chem.* **2012**, *1071*, 8.
4. Merino, E. *Chem. Soc. Rev.* **2011**, *40*, 3835.
5. Moglie, Y.; Vitale, C.; Radivoy, G. *Tetrahedron Lett.* **2008**, *49*, 1828.
6. Srinivasa, G. R.; Abiraj, K.; Gowda, D. C. *Aust. J. Chem.* **2004**, *57*, 609.
7. Freeman, J. D. C.; White, C. E. *J. Org. Chem.* **1956**, *21*, 379.
8. 5-Methyl-2-nitrophenol (**1**) (1.0 g, 6.5 mmol) was dissolved in 10 mL of MeOH. Mg powder (2 g, 82.3 mmol) and 4 mL of triethylammonium formate (equimolar mixture of triethylamine and formic acid) were added. The mixture was first stirred for 3 h at room temperature and then for 3 h at 60 °C. After cooling, the mixture was filtered over Celite. Washing with MeOH and concentration of the filtrate by evaporation under reduced pressure gave a crude dark residue that was dissolved in 20 mL of CH_2Cl_2 and washed with brine (2 × 20 mL). After drying with $MgSO_4$ and concentration, the residue was purified by silica gel column chromatography using EtOAc as the eluent. The pure (red-coloured) fractions were concentrated. Yield was 30%. Single crystals for structure determination were grown by slow diffusion of hexane into a saturated $CHCl_3$ solution of the compound. The full characterization of the product can be found in the Supplementary information. Mp 178 °C, decomposes, literature value[12] 178.5–179.5 °C. ESI-MS pos. mode: 243.1132 (*m*/*z*) $[M+H]^+$; theoretical: $[M+H]^+$ = 243.1134. ^{1}H NMR (400 MHz, d_6-benzene) δ (ppm): 7.6 (d, *J* = 7.6 Hz ,1H), 6.7 (s, 1H), 6.6 (d, *J* = 7.6 Hz ,1H), 5.9 (s, 1H), 3.6 (s, 2H), 2.9 (d, *J* = 16 Hz, 1H), 2.7 (d, *J* = 16 Hz, 1H) 2.1 (s, 3H), 0.9 (s, 3H). ^{13}C NMR (100 MHz, d_6-benzene) δ (ppm): 190.4 (1C), 160.0 (1C), 145.6 (1C), 143.9 (1C), 137.9 (1C), 133.2 (1C), 126.7 (1C), 123.3 (1C), 117.02 (1C), 109.07 (1C), 71.2 (1C), 49.2 (1C), 21.6 (1C), 21.0 (1C).
9. A solution of 300 mg (2.0 mmol) of 5-methyl-2-nitrophenol (**1**) in 10 mL of THF was added to a mixture of $FeCl_2{\cdot}4H_2O$ (400 mg, 2.0 mmol), Li powder (110 mg, 16 mmol), and 4,4′-di-*tert*-butylbiphenyl (50 mg, 0.2 mmol) under nitrogen. The initially dark green coloured reaction mixture changed to black indicating the formation of Fe(0). Next, the reaction flask was transferred to a preheated oil bath (80 °C). When the reaction was complete as monitored by TLC (EtOAc), by the disappearance of the starting material, the resulting suspension was diluted with butanone (20 mL) and H_2O (30 mL) was carefully added. Extraction with butanone (2 × 20 mL), drying over $MgSO_4$, and evaporation of the solvent gave a residue that by NMR was shown to contain the tricyclic product (**2**) as the main product.
10. $C_{14}H_{14}N_2O_2$, Fw = 242.27, orange plates, 0.15 × 0.15 × 0.02 mm^3, monoclinic, *P*21/*c* (no. 14), *a* = 10.4501(3), *b* = 9.9726(3), *c* = 11.4446(3) Å, β = 98.691(2)°, *V* = 1179.00(5) Å^3, *Z* = 4.6309 measured reflections/1846 unique reflections/173 parameters/0 restraints. *R*1: 0.0376, *wR*2: 0.1059. *S* = 1.061. CCDC 1021075 contains the supplementary crystallographic data for this Letter. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
11. Bruyneel, F.; Dive, G.; Marchand-Brynaert, J. *Org. Biomol. Chem.* **1834**, *2012*, 10.
12. Maruyama, K.; Moriguchi, T.; Mashino, T.; Nishinaga, A. *Chem. Lett.* **1996**, 819
13. Liu, J.-M.; Peng, X.-G.; Liu, J.-H.; Zheng, S.-Z.; Sun, W.; Xia, C.-G. *Tetrahedron Lett.* **2007**, *48*, 929.
14. Nakachi, T.; Tabuchi, T.; Takasaki, A.; Arai, S.; Miyazawa, K.; Tomoda, A. *Oncol. Rep.* **2010**, *23*, 1517.
15. Zhao, J.-S.; Singh, A.; Huang, X.-D.; Ward, O. P. *Appl. Environ. Microbiol.* **2000**, *66*, 2336.
16. Giurg, M.; Piekielska, K.; Gebala, M.; Ditkowski, B.; Wolanski, M.; Peczynska-Czoch, W.; Mlochowski, J. *Synth. Commun.* **2007**, *37*, 1779.
17. Barry, C. E.; Nayar, P. G.; Begley, T. P. *Biochemistry* **1989**, *28*, 6323.
18. Simandi, L. I.; Barna, T.; Nemeth, S. *J. Chem. Soc., Dalton Trans.* **1996**, 473
19. Boateng, A.; Brajter-Toth, A. *Analyst* **2012**, *137*, 4531.
20. Baudisch, O. *Science (Washington, DC, U. S.)* **1940**, *92*, 336.
21. Cronheim, G. *J. Org. Chem.* **1947**, *12*, 7.