Tetrahedron 68 (2012) 8942–8944

Contents lists available at SciVerse ScienceDirect

Tetrahedron

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

Transferring oxygen isotopes to 1,2,4-benzotriazine 1-oxides forming the corresponding 1,4-dioxides by using the HOF·CH_3CN complex

Julia Gatenyo [a,†], Kevin Johnson [b], Anuruddha Rajapakse [b], Kent S. Gates [b], Shlomo Rozen [a,*]

[a] School of Chemistry, Tel-Aviv University, Tel-Aviv 69978, Israel
[b] Departments of Chemistry and Biochemistry, University of Missouri, Columbia, MO 65211, USA

ARTICLE INFO

Article history:
Received 11 May 2012
Received in revised form 20 July 2012
Accepted 7 August 2012
Available online 14 August 2012

Keywords:
Oxygen transfer
^{18}O isotope
Tirapazamine
HOF·CH_3CN
F_2/N_2
N-oxide
$H_2{}^{18}O$

ABSTRACT

Heterocyclic benzotriazine *N*-oxides are an interesting class of experimental anticancer and antibacterial agents. Analogs with ^{18}O incorporated into the *N*-oxide group may offer useful mechanistic tools. We describe the use of $H_2{}^{18}OF\cdot CH_3CN$ in a fast, readily executed and high-yielding preparation of 1,2,4-benzotriazine 1,4-dioxides containing an ^{18}O-label at the 4-oxide position.

1. Introduction

Heterocyclic benzotriazine *N*-oxides are an interesting class of experimental anticancer[1] and antibacterial therapeutic agents.[2] One of their important features is their ability to capitalize on the low oxygen (hypoxic) environment found in many solid tumors. The lead compound in this class of potential drugs is tirapazamine: 3-amino-1,2,4-benzotriazine 1,4-dioxide **2a** (x=16).[3] Tirapazamine and related *N*-oxides undergo intracellular one-electron reduction catalyzed by enzymes, such as NADPH:cytochrome P450 reductase.[4] In normally-oxygenated tissue, the resulting drug radical primarily undergoes relatively harmless back-oxidation to give the starting compound. In contrast, under hypoxic conditions the lifetime of the drug radical is extended, enabling a decomposition reaction that yields a highly reactive DNA-damaging radical.[5] The nature of the key DNA-damaging radical generated by the bioreductively-activated *N*-oxides remains a subject of ongoing investigation.[6]

Analogs bearing isotopic-labeled oxygens in the *N*-oxide functional groups may provide powerful tools for elucidating the mechanism(s), by which these potential drugs generate DNA-damaging radicals. For example, careful metabolic studies of **2** (x=18) could shed light on whether the 4-oxide of tirapazamine is released in the form of water or hydroxyl radical.[6] The 4-*N*-oxide in 1,2,4-benzotriazine 1,4-dioxides compounds are generally installed via reaction of the parent heterocyclic with H_2O_2–acetic acid mixtures or peracids, such as *m*-chloroperbenzoic acid. These reactions can be sluggish and low yielding.[1e–g,4c,7] Furthermore, introduction of ^{18}O via those routes generally necessitates preparation of $H_2{}^{18}O_2$ from $^{18}O_2$, a tedious, expensive, and inefficient procedure.[8] Here we describe the use of $H_2{}^{18}OF\cdot CH_3CN$ in an easy and high-yielding preparation of 1,2,4-benzotriazine 1,4-dioxides containing an ^{18}O-label oxide at the 4-position (Scheme 1).

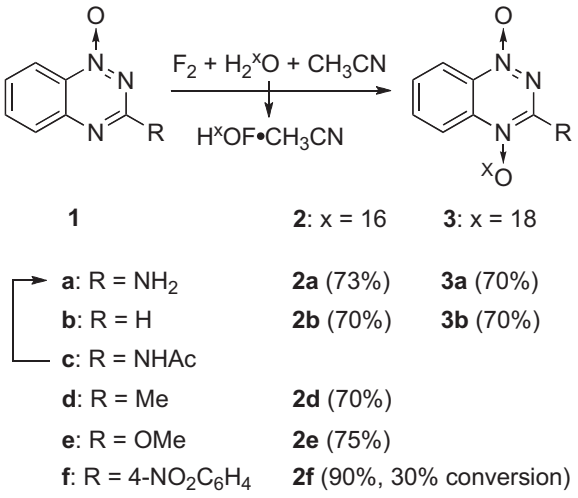
The acetonitrile complex of the hypofluorous acid HOF·CH_3CN, is readily prepared by passing dilute fluorine through aqueous acetonitrile,[9] and is considered today as one of the best oxygen-transfer agents available to organic chemists.[10] The oxygen atom is a potent electrophile because it is weakly bonded to the most electronegative element—fluorine. The complex is able to transfer an oxygen atom to very deactivated double bonds[11] and to very weak nucleophilic centers under mild conditions.[12] Following our work on the oxidation of the quinoxaline system with HOF·CH_3CN,[13] we felt that this reagent could be useful for N-oxidation of the benzotriazine ring system as well.

2. Results and discussion

Indeed we found that HOF·CH_3CN generated the desired 1,4-dioxides (**2**) from the 1-oxide precursors **1** rapidly and in good

* Corresponding author. Tel.: +972 3 640 8378; fax: +972 3 640 9293; e-mail address: rozens@post.tau.ac.il (S. Rozen).
† Tel.: +972 3 640 8378; fax: +972 3 640 9293.

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2012.08.017

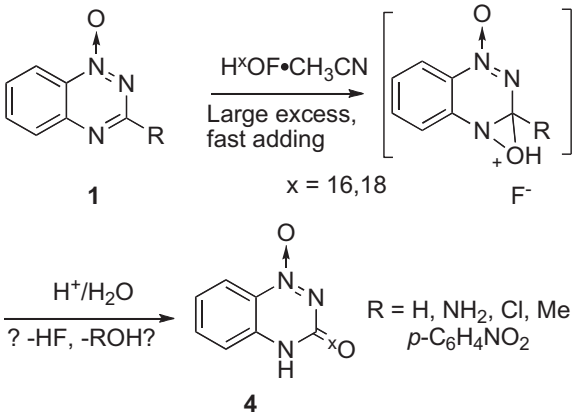


Scheme 1. Synthesis of 1,2,4-benzotriazine 4-dioxides with ^{16}O and ^{18}O isotopes.

yield. Thus 3-amino-1,2,4-benzotriazine 1-oxide (**1a**) was converted to tirapazamine (TPZ) (**2a**)[7] in 60% yield and 1,2,4-benzotriazine 1-oxide (**1b**) produced 1,2,4-benzotriazine 1,4-dioxide (**2b**)[6e] in 70% yield. Somewhat improved yield of **2a** (73%) could be achieved if the reaction was performed on the acetylated amine **1c** followed by spontaneous hydrolysis (Scheme 1). It should be noted that with reaction times of about 2 min, the *N*-oxidations with $HOF\cdot CH_3CN$ are much faster than any other method that has been reported.[7]

The electrophilic oxygen in the $HOF\cdot CH_3CN$ complex originates from water. This should enable preparation of $H^{18}OF\cdot CH_3CN$ from the most readily available and easy to handle precursor of ^{18}O isotope—$H_2^{18}O$.[14] Thus, passing dilute fluorine through a solution of acetonitrile and $H_2^{18}O$, resulted in the labeled $H^{18}OF\cdot CH_3CN$, which was then reacted with **1a** or **1c**. The product, identified by HRMS (APPI) m/z=181.0609 (M+1) (calcd for $C_7H_6N_4^{16}O^{18}O$, 181.0611) clearly showed that **3a**, labeled with ^{18}O isotope, was obtained for the first time in comparable yield as with the common ^{16}O isotope. The heavy oxygen in the N—^{18}O moiety is not readily exchanged with the common ^{16}O isotope when treated with regular water or exposed to air, enabling it to serve as a probe for metabolic studies. Reaction of 1,2,4-benzotriazine 1-oxide (**1b**) with $H^{18}OF\cdot CH_3CN$ produced **3b** having a molecular ion in HRMS (APPI) m/z=166.0500 (M+1) (calcd for $C_7H_5N_3^{16}O^{18}O$, 166.0502).

The above reactions were conducted at 0 °C using solutions containing 2 mol-equivalent of the oxidizing complex slowly added to the various substrates in small portions. However, when a large excess of $HOF\cdot CH_3CN$ solution was added in one portion to 1,2,4-benzotriazine 1-oxide (**1b**) at room temperature, only 3-oxo-3,4-dihydrobenzo-1,2,4-triazine 1-oxide (**4**) was obtained in almost quantitative yield. Among the common analytical methods, which supported this structure, one could observe a strong IR absorption at 1663 cm^{-1} characteristic of an amide. The same outcome was observed when **1b** was reacted with $H^{18}OF\cdot CH_3CN$ leading to the 3-oxo derivative (**4**) having the ^{18}O isotope. When other substrates were treated with an excess of the reagent, the presence of the triazolone derivative **4** was also observed. Thus with 3-chloro-1,2,4-benzotriazine 1-oxide (**1** R=Cl) the transformation to **4** took place in quantitative yield. In case of 3-amino, 3-methyl and 3-(4-nitrophenyl)-1,2,4-benzotriazine 1-oxide (**1**R=NH_2, Me, *p*-$C_6H_4NO_2$) the 3-oxo derivative was obtained as a byproduct with increasing yield proportionally to the excess of the reagent added. These results suggest that while low temperature and addition of small portions of oxidizing solution promoted the N-oxidation, large excess of the reagent probably encourages epoxidation reactions that eventually yield the triazolone derivative **4** (Scheme 2). It should be noted that this mechanism is only a plausible speculation as we have not isolated the oxaziridine byproduct (Scheme 2), which is anticipated to be very unstable.



Scheme 2. Amidation of 1,2,4-benzotriazine 1-oxide.

The described reaction is of general nature. Treating 3-methyl-1,2,4-benzotriazine 1-oxide (**1d**) with the oxidizing solution produced 3-methyl-1,2,4-benzotriazine 1,4-dioxide (**2d**)[15] in 70% yield. The strong electron donating methoxy substituent in 3-methoxy-1,2,4-benzotriazine 1-oxide (**1e**) provided 75% yield of 3-methoxy-1,2,4-benzotriazine 1,4-dioxide (**2e**).[15] Electron-withdrawing moieties, such as the one found in 3-(4-nitrophenyl)-1,2,4-benzotriazine 1-oxide (**1f**) deactivate the molecule as far as electrophilic oxidation is concerned and while the yield of 3-(4-nitrophenyl)-1,2,4-benzotriazine 1,4-dioxide (**2f**) was higher than 90% the conversion was only 30%. The remaining starting material could be easily separated by flash-column chromatography and recycled (Scheme 1).

3. Conclusion

There are many cases where it is highly desirable to have ^{18}O isotope attached to an organic molecule. It has been shown here that the best, easiest, and most efficient way to achieve such goal is through $H^{18}OF\cdot$ complex made readily from commercial diluted fluorine, the best and cheapest source of ^{18}O:$H_2^{18}O$ and acetonitrile.

4. Experimental

4.1. General

^1H NMR spectra were recorded using a 200 MHz spectrometer with $CDCl_3$ as a solvent and Me_4Si as an internal standard. The proton broad-band decoupled ^{13}C NMR spectra were recorded at 100.5 MHz. Here too, CD_3OD or $CDCl_3$ served as a solvent and Me_4Si as an internal standard. MS was measured under EI, or APPI conditions.

Preparation of 3-substituted 1,2,4-benzotriazine 1-oxides. Compounds **1a**,[7] **1b**, **1d**, **1e**,[15] and **1f**[16] were synthesized as previously described.

4.2. General procedure for working with fluorine

Fluorine is a strong oxidant and a corrosive material. It should be used with an appropriate vacuum line. For the occasional user, however, various premixed mixtures of F_2 in inert gases are

commercially available, thereby simplifying the process. Unreacted fluorine should be captured by a simple trap containing a solid base, such as soda-lime located at the outlet of the glass reactor. A detailed setup for working with F_2 could be found in the literature.[17] If elementary precautions are taken, work with fluorine is relatively simple and we have never experienced any difficulties or unpleasant situations.

4.3. General procedure for producing HOF·CH$_3$CN

A mixture of 10–20% F_2 in nitrogen was used throughout this work. The gas mixture was prepared in a secondary container prior to the reaction and passed at a rate of about 400 mL per minute through a cold (−15 °C) mixture of 100 mL of CH_3CN and 10 mL of H_2O (or 10 mL CH_3CN and 1mL $H_2{}^{18}O$) in a regular glass reactor. The development of the oxidizing power was monitored by reacting aliquots with an acidic aqueous solution of KI. The liberated iodine was then titrated with thiosulfate. Typical concentrations of the oxidizing reagent were around 0.4–0.6 M.

4.4. General procedure for working with HOF·CH$_3$CN

A mono oxide derivative **1** was dissolved in DCM, and the mixture was cooled to 0 °C. The solution containing the oxidizing agent was than added slowly to the reaction vessel. The reaction was stopped after a few minutes by evaporation of the solvent almost to dryness. The products were isolated and purified by flash chromatography, eluting with a gradient (0–15%) of MeOH/DCM.

4.4.1. Synthesis of 3-substituted 1,2,4-benzotriazine 1,4-dioxides. Compounds **2a**,[7] **2b**,[6e] **2d**, and **2e**[15] were prepared from the corresponding 1,2,4-benzotriazine 1-oxides as described above, using 2 equiv of the oxidizing agent. The physical and spectral properties of all products completely matched those appearing in the literature.

*4.4.2. Synthesis of 3-(4-nitrophenyl)-1,2,4-benzotriazine 1,4-dioxide (**2f**).* Compound **2f** was prepared from 3-(4-nitrophenyl)-1,2,4-benzotriazine 1-oxide (**1f**) (0.15 g, 0.56 mmol) as described above, using 2 equiv of the oxidizing agent. A pale beige solid (0.05 g, 95% yield, 30% conversion) was obtained: mp>300 °C; R_f (DCM) 0.38; ^{1}H NMR ($CDCl_3$) 8.84 (2H, d, J=8.8 Hz), 8.67 (1H, d, J=8.6 Hz), 8.57 (1H, d, J=8.4 Hz), 8.39 (2H, d, J=8.8 Hz), 8.09 (1H, t, J=8.6 Hz), 7.95 (1H, t, J=8.4 Hz); ^{13}C NMR 120.5, 124.1, 129.6, 129.8, 131.3, 134.0, 136.3, 140.0, 147.6, 150.1 158.8; HRMS (APPI) m/z calcd for $C_{13}H_8N_4O_4$ 285.0624 $(M+H)^+$, found 285.0629.

4.5. Formation of the triazolone 4

A large excess of HOF·CH_3CN solution was added in one portion to the mono oxide derivative **1** (0.09 g, 0.6 mmol) in DCM at room temperature. The reaction was stopped after a few minutes by evaporation of the solvent almost to dryness. The residue was purified by chromatography, eluting with a gradient (0–15%) of MeOH/DCM to give the 3-oxo-3,4-dihydrobenzo-1,2,4-triazine 1-oxide (**4**) as a pale yellow solid: mp 228–229 °C; R_f (E.A) 0.64; IR 1663 cm^{-1}; H NMR (CD_3OD) 8.23 (1H, dd, J=8.2, 1.2 Hz), 7.83 (1H, ddd, J=8.7, 7.8, 1.3 Hz), 7.36–7.42 (2H, m); ^{13}C NMR (DMSO) 153.3, 136.9, 136.8, 129.6, 124.2, 121.2, 116.6; HRMS (APPI) m/z calcd for $C_7H_5N_3O_2$ 164.0460 $(M+H)^+$, found 164.0463. Anal. Calcd for $C_7H_5N_3O_2$: C, 51.54; H, 3.09; N, 25.76. Found: C, 51.29; H, 2.80; N, 26.00.

References and notes

1. (a) Xia, Q.; Zhang, L.; Zhang, J.; Sheng, R.; Yang, B.; He, Q.; Hu, Y. *Eur. J. Med. Chem.* **2011**, *46*, 919–926; (b) Ismail, M. M. F.; Amin, K. M.; Naoman, E.; Soliman, D. H.; Ammar, Y. A. *Eur. J. Med. Chem.* **2010**, *45*, 2733–2738; (c) Lavaggi, M. L.; Cabrera, M.; González, M.; Cerecetto, H. *Chem. Res. Toxicol.* **2008**, *21*, 1900–1906; (d) Hicks, K. O.; Siim, B. G.; Jaiswal, J. K.; Pruijin, F. B.; Fraser, A. M.; Patel, R.; Hogg, A.; Liyanage, H. D. S.; Dorie, M. J.; Brown, J. M.; Denny, W. A.; Hay, M. P.; Wilson, W. R. *Clin. Cancer Res.* **2010**, *16*, 4946–4957; (e) Hay, M. P.; Gamage, S. A.; Kovacs, M. S.; Pruijn, F. B.; Anderson, R. F.; Patterson, A. V.; Wilson, W. R.; Brown, J. M.; Denny, W. A. *J. Med. Chem.* **2003**, *46*, 169–182; (f) Hay, M. P.; Hicks, K. O.; Pchalek, K.; Lee, H. H.; Blaser, A.; Pruijn, F. B.; Anderson, R. F.; Shinde, S. S.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **2008**, *51*, 6853–6865; (g) Solano, B.; Junnotula, V.; Marin, A.; Villar, R.; Burguete, A.; Vicente, E.; Perez-Silanes, S.; Monge, A.; Dutta, S.; Sarkar, U.; Gates, K. S. *J. Med. Chem.* **2007**, *50*, 5485–5492.
2. (a) Vicente, E.; Villar, R.; Burguete, A.; Solano, B.; Pérez-Silanes, S.; Aldana, I.; Maddry, J. A.; Lenaerts, A. J.; Franzblau, S. G.; Cho, S. h.; Monge, A.; Goldman, R. C. *Antimicrob. Agents Chemother.* **2008**, *52*, 3321–3326; (b) Ganley, B.; Chowdhury, G.; Bhansali, J.; Daniels, J. S.; Gates, K. S. *Bioorg. Med. Chem.* **2001**, *9*, 2395–2401; (c) Suter, W.; Rosselet, A.; Knusel, F. *Antimicrob. Agents Chemother.* **1978**, *13*, 770–783.
3. (a) Brown, J. M. *Br. J. Cancer* **1993**, *67*, 1163–1170; (b) Wilson, W. R.; Hay, M. P. *Nat. Rev. Cancer* **2011**, *11*, 393–410.
4. (a) Fitzsimmons, S. A.; Lewis, A. D.; Riley, R. J.; Workman, P. *Carcinogenesis* **1994**, *15*, 1503–1510; (b) Patterson, A. V.; Saunders, M. P.; Chinje, E. C.; Patterson, L. H.; Stratford, I. J. *Anti-Cancer Drug Des.* **1998**, *13*, 541–573; (c) Fuchs, T.; Chowdhary, G.; Barnes, C. L.; Gates, K. S. *J. Org. Chem.* **2001**, *66*, 107–114.
5. Laderoute, K. L.; Wardman, P.; Rauth, M. *Biochem. Pharmacol.* **1988**, *37*, 1487–1495.
6. (a) Zeman, E. M.; Brown, J. M.; Lemmon, M. J.; Hirst, V. K.; Lee, W. W. *Int. J. Radiat. Oncol., Biol., Phys.* **1986**, *12*, 1239–1242; (b) Zeman, E. M.; Baker, M. A.; Lemmon, M. J.; Pearson, B. A.; Adams, J. A.; Brown, J. M.; Lee, W. W.; Tracy, M. *Int. J. Radiat. Oncol., Biol., Phys.* **1989**, *16*, 977–981; (c) Junnotula, V.; Sarkar, U.; Sinha, S.; Gates, K. S. *J. Am. Chem. Soc.* **2009**, *131*, 1015–1024; (d) Chowdhury, G.; Junnutula, V.; Daniels, J. S.; Greenberg, M. M.; Gates, K. S. *J. Am. Chem. Soc.* **2007**, *129*, 12870–12877; (e) Shinde, S. S.; Maroz, A.; Hay, M. P.; Patterson, A. V.; Denny, W. A.; Anderson, R. F. *J. Am. Chem. Soc.* **2010**, *132*, 2591–2599.
7. Mason, J. C.; Tennant, G. *J. Chem. Soc. B* **1970**, 911–916.
8. (a) Jankowski, S.; Rafal, K. *J. Labelled Compd. Radiopharm.* **1995**, *36*, 373–376; (b) Sitter, A. J.; Terner, J. *J. Labelled Compd. Radiopharm.* **1985**, *22*, 461–465.
9. Rozen, S.; Bareket, Y.; Kol, M. *Tetrahedron* **1993**, *49*, 8169–8178.
10. (a) Rozen, S.; Bar-Haim, A.; Mishani, E. *J. Org. Chem.* **1994**, *59*, 1208–1209; (b) Rozen, S.; Carmeli, M. *J. Am. Chem. Soc.* **2003**, *125*, 8118–8119; (c) Rozen, S.; Dayan, S. *Angew. Chem., Int. Ed.* **1999**, *38*, 3471–3473.
11. (a) Rozen, S.; Golan, E. *Eur. J. Org. Chem.* **2003**, *10*, 1915–1917; (b) Hung, M. H.; Smart, B. E.; Feiring, A. E.; Rozen, S. *J. Org. Chem.* **1991**, *56*, 3187–3189.
12. Rozen, S.; Bareket, Y. *J. Org. Chem.* **1997**, *62*, 1457–1462.
13. Carmeli, M.; Rozen, S. *J. Org. Chem.* **2006**, *71*, 5761–5765.
14. (a) Rozen, S.; Kol, M. *J. Org. Chem.* **1992**, *57*, 7342–7344; (b) Kol, M.; Rozen, S. *J. Chem. Soc., Chem. Commun.* **1991**, 567–568.
15. Hay, M. P.; Pchalek, K.; Pruijn, F. B.; Hicks, K. O.; Siim, B. G.; Anderson, R. F.; Shinde, S. S.; Phillips, V.; Denny, W. A.; Wilson, W. R. *J. Med. Chem.* **2007**, *50*, 6654–6664.
16. Robbins, R. F.; Schofield, K. *J. Chem. Soc.* **1957**, 3186–3194.
17. Dayan, S.; Kol, M.; Rozen, S. *Synthesis* **1999**, 1427–1930.