Structural Characterization of a Benzoxazinophenoxazine Formed as a By-Product During the Synthesis of a 3-Amino-N,N-dimethylsalicylamide

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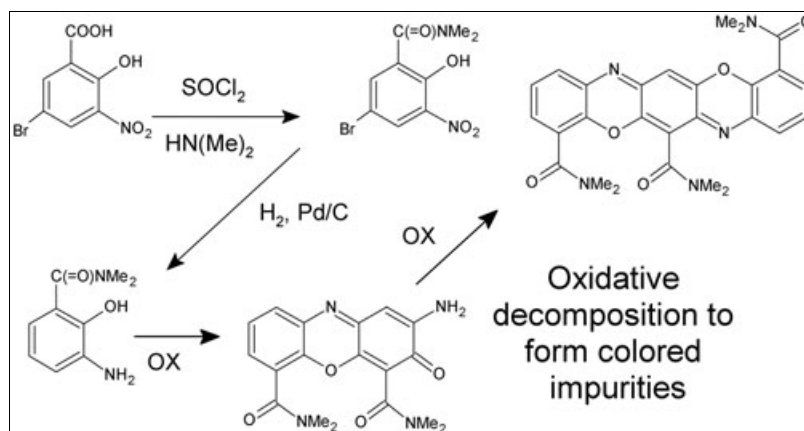[b]Merck Research Laboratories, Discovery and Preclinical Sciences, Process Chemistry, Rahway, New Jersey 07065
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Several highly colored by-products were observed chromatographically during the synthesis of 3-amino-N,N-dimethylsalicylamide. One of these, an oxidative dimerization products, bis-(N,N-dimethylcarboxamido)-aminophenoxazinone, was previously isolated and characterized. A tris-(N,N-dimethylcarboxamido)-benzoxazino[3,2-b]phenoxazine, presumably a higher oxidation or further reaction product of the previously isolated and characterized 2-amino-3H-phenoxazin-3-one, has also been isolated and characterized by mass spectrometry and multinuclear NMR methods. We now wish to communicate the results of that structure characterization effort.

It is known that electron-rich 2-aminophenol derivatives can undergo catalytic oxidative cyclocondensation to form 2-amino-3H-phenoxazin-3-one derivatives [1]. We previously reported the isolation and structure characterization of a highly colored bis-(N,N-dimethylcarboxamido)-2-amino-3H-phenoxazin-3-one (**4**), presumably formed as an oxidative by-product of the synthesis of 3-amino-N,N-dimethylsalicylamide (**3**) as shown in Scheme 1 [2]. The isolation of a stable and suitably pure sample of a somewhat less abundant, highly colored by-product also formed in that reaction, **5**, proved to be chromatographically challenging. A small sample of that material was, however, isolated and subsequently characterized as **5** through a combination of mass spectrometry and NMR spectroscopy methods, the latter relying heavily on cryoprobe technology at 600 MHz.

Isolation of the impurity that was subsequently identified as **5** began with the recrystallization of **3** (HCl salt) in methanol. The mother liquor was enriched with the

impurity to about 0.5%. The mother liquor was subsequently separated by prep HPLC using a YMC Pack pro C_{18} 5 μm, 20 × 150 mm, flow rate 15 mL/min, 40/60 methanol/0.1% TFA in water, monitored at 215 nm. The desired fractions were concentrated and repurified by prep HPLC using a YMC Pack-pro C_{18} column 20 × 150 mm, flow rate 15 mL/min, eluted with 36/64 methanol/0.1% TFA in water.

Following chromatographic isolation, the accurate mass of the impurity was determined and an MS/MS study was performed using a Thermo-Fisher LTQ Orbitrap™ mass spectrometer equipped with a Surveyor HPLC system. The measured accurate mass for the protonated molecule was determined to be m/z 500.19302 corresponding to an empirical formula of $C_{27}H_{26}N_5O_5$ [M+H]$^+$ with an error of 0.36 ppm. The MS/MS experiments were conducted in the linear trap portion of the instrument and helium was used as the collision gas. The accurate mass measurements of these fragment ions are performed in the Orbitrap. There

Scheme 1

are four major fragment ions observed in the MS/MS spectrum. The fragment ions at m/z 455.13550 ($C_{25}H_{19}N_4O_5^+$, error: 1.10 ppm) and 414.10904 ($C_{23}H_{16}N_3O_5^+$, error: 1.43 ppm) are the results of the consecutive loss of two dimethyl amine moieties. Consequentially, the fragment ions at m/z 386.11401 ($C_{22}H_{16}N_3O_4^+$, error: 1.25ppm) and 358.11911 ($C_{21}H_{16}N_3O_3^+$, error: 1.37 ppm) arose from the subsequent loss of two CO moieties. The MS/MS study showed the molecule could lose the components of two *N,N*-dimethylcarboxamido moieties. The nucleus of the molecule, however, did not undergo fragmentation under these low energy activation processes, which was consistent with the highly conjugated nature of the linear five ring system.

NMR data were recorded for the impurity isolated using a sample of <1 mg dissolved in d_5-pyridine (Cambridge Isotope Laboratories). The solid material was nearly black in color but afforded a cherry-red solution. The resulting solution was transferred to a 3 mm NMR tube (Wilmad) using a flexible Teflon™ needle and a Hamilton gas-tight syringe. NMR data were acquired using a Varian three channel 600 MHz NMR spectrometer equipped with a 5 mm gradient triple resonance ColdProbe™ operating at an rf coil temperature of 20 K. All experimental data were acquired with the sample temperature maintained at 25°C. The ensemble of NMR data consisted of ^{1}H and ^{13}C reference spectra; 2D NMR experiments consisted of GCOSY, 400 msec ROESY, adiabatic multiplicity-edited GHSQC, 8 and 5 Hz optimized ^{1}H-^{13}C GHMBCAD, and 6 Hz optimized ^{1}H-^{15}N GHMBCAD (the adiabatic version "AD" of the GHMBC was used for both GHMBC experiments).

Seven aromatic and six *N*-methyl resonances were observed in the 600 MHz ^{1}H NMR spectrum. The aromatic resonances could be subgrouped into two three-spin systems and an isolated singlet shifted upfield (6.70 ppm) that was directly bound to a carbon resonating at 106.9 ppm.

The upfield shift of the carbon to which the aromatic singlet was bound is consistent with a carbon that has multiple electron-donating substituents, (*e.g.,* oxygen or nitrogen) located β to the carbon. The chemical shift of this ^{1}H/^{13}C resonant pair is quite similar to that of the 1-position (6.53/99.9 ppm) of the previously characterized 4,6-bis-(*N,N*-dimethylcarboxamido)-2-amino-3*H*-phenoxazin-3-one (**4**) [2].

Long-range ^{1}H-^{15}N heteronuclear couplings were pivotal in establishing the structure of the isolated impurity. Two ^{15}N resonances were observed downfield at 296 and 301 ppm (relative to liq. NH_3 = 0 ppm). The nitrogen resonating at 301 ppm had long-range correlations to the 6.70 ppm singlet and a doublet resonating at 7.60 ppm that was part of one of the contiguous three-spin systems. The other nitrogen, resonating at 296 ppm, had only a single correlation to a doublet resonating at 7.58 ppm that was a part of the other three-spin aromatic system. The downfield shift of the two nitrogen resonances is consistent with the sp^2 nitrogen of **4** that resonates at 293 ppm [2] The ^{1}H-^{15}N long-range data suggested a benzoxazinophenoxazine structure for the impurity. Linear and angular five, fused-

Figure 1. ^{1}H, ^{13}C, and ^{15}N chemical shift assignments of **5**. Pertinent long-range ^{1}H-^{13}C and ^{1}H-^{15}N long-range correlations are designated by black arrows; weak long-range ^{1}H-^{13}C long-range heteronuclear correlations are designated by dashed black arrows. Key ROESY correlations are denoted by bold, double-headed black arrows.

ring systems were considered but the latter could be ruled out when chemical shift considerations, long‑range ^{1}H‑^{13}C, and ROESY correlations were taken into account. When the ensemble of NMR data, including the long‑range heteronuclear and ROESY correlation data shown in Figure 1 were interpreted in conjunction with the mass spectrometric data, the structure of the molecule was established as a benzoxazino[3,2-*b*]phenoxazine.

Mechanistically, it is believed that the formation of **5** occurs *via* the further oxidative addition of a molecule of **3** to **4**. This contention is supported by the observation that levels of both **4** and **5** increased when a solution of **3** in acetonitrile or ethanol was exposed to air over several days.

Impurity **5** was also observed at an even higher level when **3** was treated with hydrogen peroxide in the presence of a selenium catalyst using the reaction conditions used to prepare **4** [2]. However, attempts to prepare **5** directly from **3** in appreciable yield were unsuccessful.

REFERENCES AND NOTES

[1]   Giurg, M.; Piekielska, K.; Gebala, M.; Ditkowski, B.; Wolanski, M.; Peczynska‑Czoch, W.; Mlochowski, J. Syn Commun 2007, 1779.

[2]   Zhong, W.; Yang, J.; Hilton, B. D.; Martin, G. E.; Yin, J.; Muppalla, K.; Tong, W.; Fu, X. J Heterocycl Chem 2012, 49, 1163.