Heterocyclic ring cleavage upon collision-induced dissociation of deprotonated 3-hydroxy-1,2,5-oxadiazoles (3-hydroxyfurazans)

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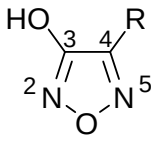
Abstract: A series of 4-substituted 3-hydroxyfurazans was subjected to electrospray ionization tandem mass spectrometry. At low collision energy, oxyisocyanate ([O=C=N–O]$^-$, *m*/*z* 58) was formed as the predominant product ion from each deprotonated 3-hydroxyfurazan, indicating cleavage of the heterocyclic ring. The facile energetics of this characteristic fragmentation process was confirmed by density functional computations.

Dear Editor,

Structure-specific fragmentation processes observed by tandem mass spectrometry (MS/MS) enable the rapid identification of trace quantities of drug metabolites,[1] [Prakash, 2007] pharmaceutical impurities,[2] [Gillespie, 2011] environmental contaminants,[3] [Budde, 2004] and numerous metabolomic constituents.[4,5] [Bowen, 2010; Neumann, 2010] Rings containing two or more heteroatoms are potential sites for cleavage initiated by collision-induced dissociation (CID), and the fragmentations of many members in the structurally diverse group of heterocycles are likely to be characteristic. Heterocyclic rings are common structural components of drugs and drug candidates, as well as their metabolites. For

example, the widely used drugs cimetidine, clotrimazole, esomeprazole and metronidazole have five-membered rings incorporating two nitrogen atoms. Despite the widespread occurrence of heterocycles, a relatively limited number of mass spectral studies have been documented for deprotonated and protonated heterocycles.[6,7,8] [Tian, 2013; Mamer, 2005; Adams, 1992]

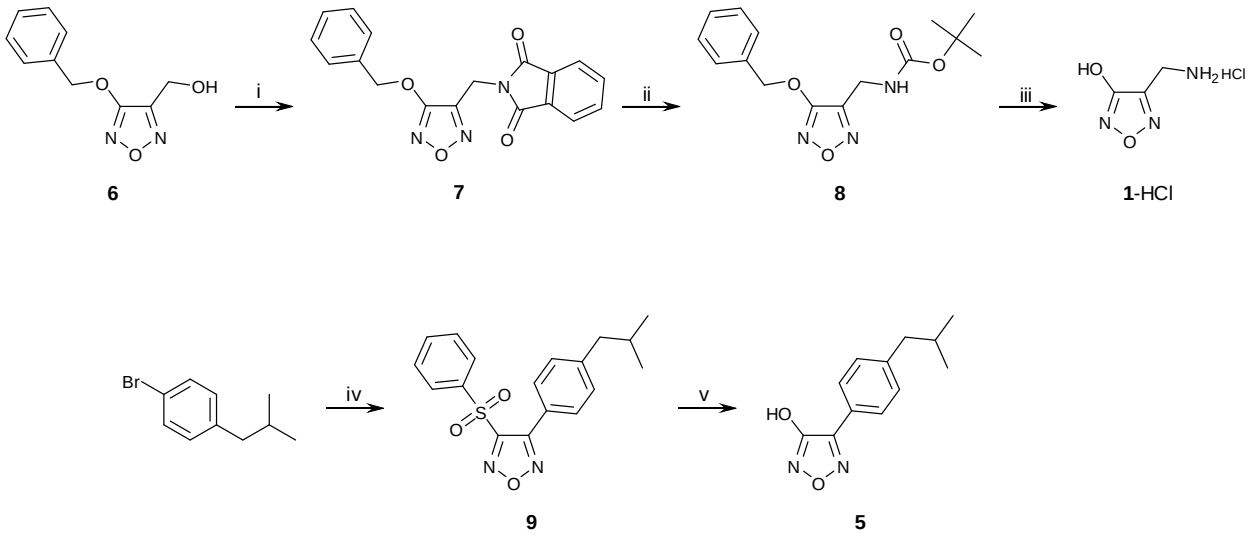
In recent medicinal chemistry studies, monohydroxytriazole[9] [Pippione, 2015] and monohydroxyfurazan[10,11] [Lolli, 2006; Lolli, 2010] (Fig. 1) heterocycles were introduced as a novel bioisosteres of carboxyl groups. Determinations of pK_a values by potentiometric titration,[10] [Lolli, 2006] demonstrated that the 3-hydroxyfurazans **2**–**4**, (pK_a 3.12–3.56) were more acidic than the typical aliphatic and aromatic carboxylic acids that are readily deprotonated when subjected to negative mode electrospray ionization (ESI). In the present mass spectral studies, deprotonation of the 3-hydroxyfurazans was confirmed, and the common fragmentation process observed upon CID of the $[M - H]^-$ ions **1a**–**5a** was readily interpreted as cleavage of the heterocyclic ring.



	R
1	CH_2NH_2
2	$(CH_2)_2NH_2$
3	$(CH_2)_3NH_2$
4	$(CH_2)_4NH_2$
5	$C_6H_4CH_2CH(CH_3)_2$

Figure 1. 4-Substituted 1,2,5-oxadiazol-3-ols (3-hydroxyfurazans). Compounds **1**–**4** were available as hydrochloride salts. The corresponding ions formed by deprotonation ($[M - H]^-$) are designated **1a**–**5a**.

The 4-aminoalkyl-3-hydroxyfurazans **2**–**4** were available from previous work,[10] [Lolli, 2006] while compounds **1** and **5** were synthesized as shown in Scheme 1. Briefly, Mitsunobu conditions were used to replace the hydroxyl group of [4-(benzyloxy)-1,2,5-oxadiazol-3-yl]methanol (**6**)[11] [Lolli, 2010] with the phthalimido moiety. While attempts to deprotect the amino functionality resulted in low yields of the amine corresponding to **7**, an 84% yield of the Boc derivative **8** was obtained by *in situ* trapping of the amine. Subsequent removal of the benzyl and Boc protecting groups by catalytic hydrogenation and ethereal hydrochloric acid treatment, respectively, gave **1**-HCl in 91% yield. For the preparation of **5**, Grignard reaction of [4-(2-methylpropyl)phenyl] magnesium bromide and 3,4-di(benzenesulfonyl)-1,2,5-oxadiazole[12] [Boschi, 1997] gave derivative **9** in 81% yield. Replacement of the benzenesulfonyl moiety with a hydroxyl group in the presence of NaOH and subsequent acidification gave **5** in 90% yield.



6 **7** **8** **1**-HCl

9 **5**

Scheme 1. Synthesis of 4-aminomethyl-1,2,5-oxadiazol-3-ol hydrochloride (**1**-HCl) and 4-[4-(2-methylpropyl)phenyl]-1,2,5-oxadiazol-3-ol (**5**). i) DIAD, Ph_3P, phthalimide, THF; ii) (a) CH_3NH_2, H_2O, THF; (b) Boc_2O, THF; iii) (a) H_2, Pd(C), EtOH; (b) HCl-diethyl ether; iv) (a) Mg, I_2, THF; (b) 3,4-di(benzenesulfonyl)-1,2,5-oxadiazole, THF; v) (a) NaOH, DMSO; (b) HCl, H_2O.

The hydrochloride salts of the 4-aminoalkyl-3-hydroxyfurazans (**1**–**4**) and compound **5** were dissolved in aqueous methanol (0.1 mg mL^{-1}) and introduced into a Waters Quattro LC triple quadrupole mass spectrometer by flow injection (H_2O:MeOH (1:1, v/v), 20 μL min^{-1}). Ions were generated by electrospray ionization (ESI, negative mode, 3.5 kV, 10–20 V cone). The CID experiments (10–20 eV, laboratory frame) employed argon as the collision and damping gas. Instrument settings for the Thermo-Finnigin LCQ Duo ion trap spectra are provided in the Supporting Information. Gas-phase acidities and the energetics of fragmentation processes were computed using density functional theory (DFT) within the Gaussian 09 suite of programs.[13] [Frisch, 2010] Geometry optimizations and frequency calculations were performed using the ωB97X-D/6-311+G(d) functional.[14] [Chai, 2008] Energy minima were characterized by having no imaginary vibrational frequencies, whereas saddle points had one such frequency. The latter were linked to minima on both sides of the energy surface by freezing atom motion towards both extremities of the imaginary frequency and reoptimizing the resulting geometry to an energy minimum. Single-point energy calculations were completed with the MP2/6-311++G(2d,p) level on the optimized geometries.

Thermochemical data are reported as combinations of single point MP2/6-311++G(2d,p) electronic energies and uncorrected entropies and thermal corrections from the ωB97X-D/6-311+G(d) calculations and are designated as MP2/6-311++G(2d,p)//ωB97X-D/6-311+G(d) free energies given in kJ mol^{-1}. Coordinates for the optimized structures are given in the Supporting Information.

The aminoalkyl (**1**–**4**) and isobutylphenyl (**5**) 3-hydroxylfurazans (Fig. 1) were readily ionized by ESI (negative mode), consistent with deprotonation of the acidic hydroxyl group (pK_a = 3.12–3.56).[10] [Lolli, 2006] In the ion trap mass spectrometer, ESI of compounds **1**–**4** yielded adduct ions ($[M + {}^{35}Cl]^-$, $[M + {}^{37}Cl]^-$ and $[2M - H]^-$) in greater abundance than the $[M - H]^-$ ions (Table S1 and Fig. S1, Supporting Information) in accord with a previous thermochemical analysis.[15] [Mansoori, 1997] Upon CID in the ion trap mass spectrometer, the collision energy needed for dissociation of each adduct ion to the corresponding $[M - H]^-$ ion as the sole product ion was less than that needed for fragmentation of **1a**–**4a** (Fig. S2). In the triple quadrupole mass spectrometer at a cone voltage of 20 V, the adduct anions had minor abundances.

Computations indicated that the gas-phase acidities of hydroxyfurazans **1**–**4** ($\Delta_r G°$ = 1412, 1426, 1421 and 1408 kJ mol^{-1}, respectively) were greater than those of other functional groups (e.g., phenols and carboxylic acids)[16] [NIST] that are readily deprotonated upon ESI.[17] [Kruve, 2014] A stabilizing interaction between ionized hydroxyl group and a hydrogen in the primary amino group was evident in each structure computed for the ions **1a**–**4a** (Fig. S3). The strength of this interaction was greatest for **2a** and **3a**, the ions with the side chains of intermediate length. The extended (staggered) conformations were of higher energy by some 18–23 kJ mol^{-1}.

When subjected to CID in the triple quadrupole mass spectrometer (Fig. 2), each deprotonated 3-hydroxyfurazan (i.e., **1a**–**5a**) yielded a predominant product ion at *m*/*z* 58 demonstrating that the fragmentation process was independent of the substituent variation at C4. The mass of the predominant product ion (58 u) was greater than the combined masses of the substituents in ion **1a**; thus, the product ion must contain atoms from the heterocyclic ring. Of the possible elemental compositions for an ion of mass 58 u, only CNO_2 corresponded to a contiguous arrangement of atoms present in the structures of the anions **1a**–**5a**. Moreover, this group of atoms was part of the common structural motif for compounds **1**–**5** and overlapped with the ionization site, suggesting a charge initiated fragmentation process leading to a neutral nitrile (R–CN; R = C4 substituent defined in Fig. 1) and the oxyisocyanate ion $[O=C=N–O]^-$ (*m*/*z* 58). The latter has been detected previously as a stable product ion formed upon CID of deprotonated *N*-hydroxycarbamates.[18] [Waugh, 1989] Interestingly, the assignment of ions in the electron ionization mass spectrum of 3,4-dimethylfurazan[19] [Ungnade, 1964] indicated analogous bond cleavages in the furazan ring and the loss of acetonitrile as a major fragmentation process.

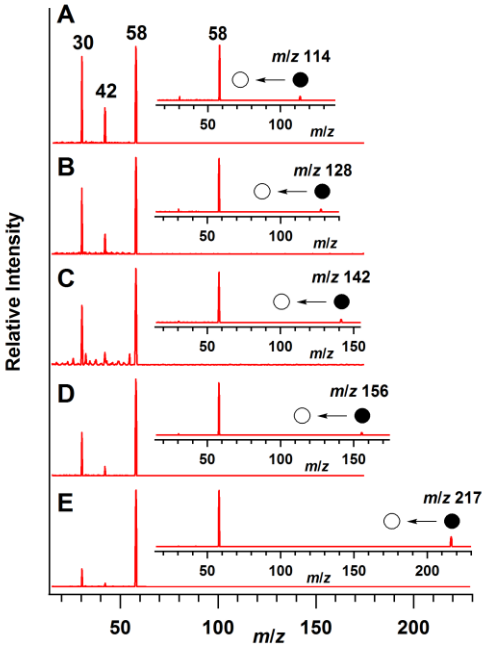


Figure 2. CID spectra of the deprotonated 4-aminoalkyl-1,2,5-oxadiazol-3-ols **1**–**5** (spectra **A**–**E**, respectively) collected on the triple quadrupole mass spectrometer (20 V cone) at 10 eV (inset spectra) and 20 eV. In the spectra obtained at higher collision energy, the *m*/*z* 15–55 region has been magnified by a factor of 12.

DFT computations (Fig. 3) indicated that cleavage of the heterocyclic ring in **1a** required only a modest input of energy (71 kJ mol^{-1}) to reach a transition structure (**$TS_{1a(N5–O)}$**) in which the N5–O and C–C bonds in the heterocyclic ring were lengthened to 2.27 and 2.00 Å, respectively, and the N2–O and N2–C3 bonds were shortened by about 0.1 Å, reflecting their bonding in the fragmentation products. With the extensive ring cleavage, the grouping of atoms was maintained by the hydrogen bonding between the oxygen substituent and the primary amino group (i.e., O - - H–N). The O – H distance decreased (2.32 → 2.17 → 1.97 Å) as the initial ion **1a** was sequentially

transformed into the transition structure (**$TS_{1a(N5–O)}$**) and the ion-neutral complex (**IN_{OCNO}**). Dissociation of **IN_{OCNO}** to aminoacetonitrile (H_2NCH_2CN) and the oxyisocyanate ion ($[O=C=N–O]^-$, *m*/*z* 58) required a smaller input of energy than restoration of the heterocyclic ring.

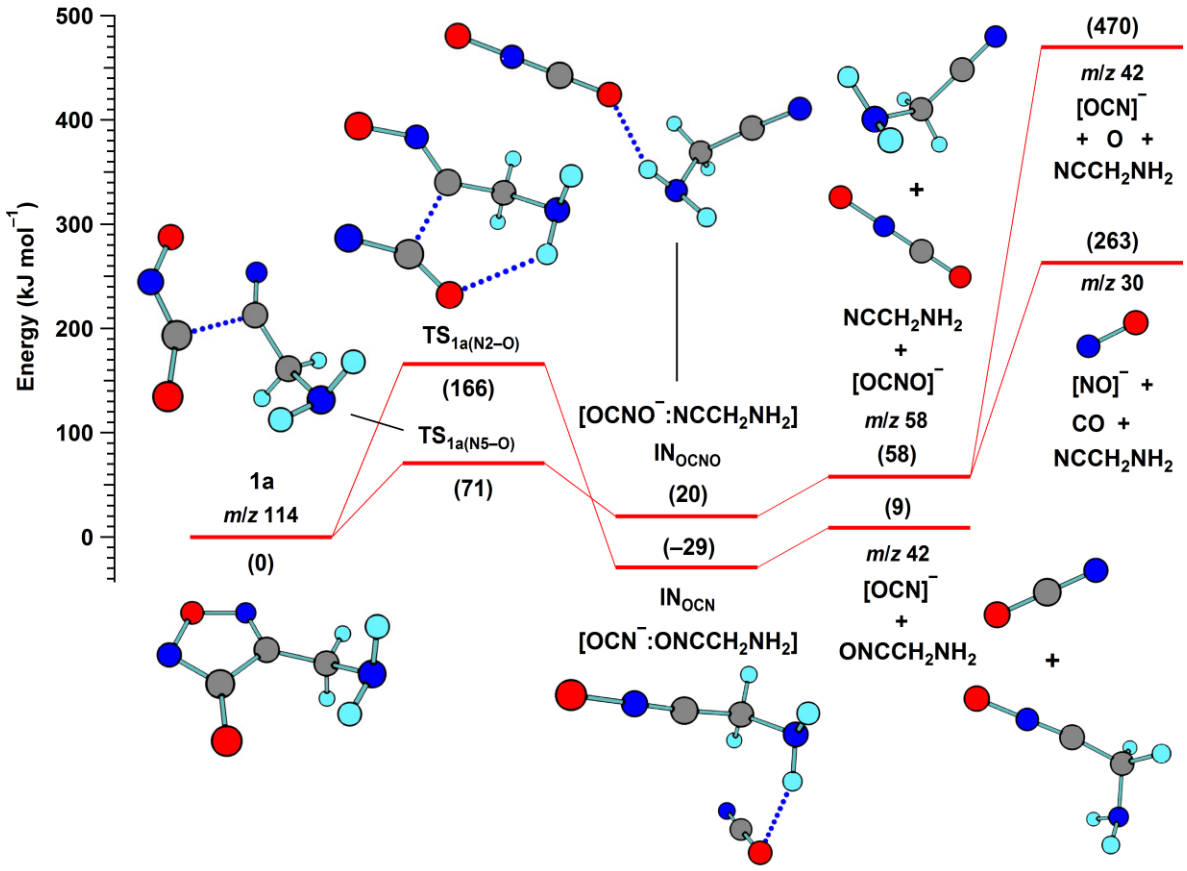


Figure 3. Potential energy profile for the fragmentation of deprotonated 4-aminomethyl-1,2,5-oxadiazol-3-ol (**1a**) to oxyisocyanate ($[O=C=N–O]^-$, *m*/*z* 58) and minor product ions at *m*/*z* 42 and 30. Numbers in parentheses are MP2/6-311++G(2d,p)//ωB97X-D/6-311+G(d) free energies given in kJ mol^{-1}.

At the higher collision energy, minor product ions at *m*/*z* 42 and 30 were also evident in the CID spectra of **1a**–**5a** (Fig. 2), suggesting fragmentation of the oxyisocyanate ion (*m*/*z* 58). The bond lengths computed for the oxyisocyanate ion (O–C, 1.23 Å; C–N, 1.16 Å; N–O, 1.30 Å) indicated significant multiple bond character for both the O–C and C–N bonds, in agreement with a previous computational study.[18] [Waugh, 1989] Nevertheless, the computations (Fig. 3) indicated feasible energetics for the formation of carbon monoxide and the nitric oxide anion ($[NO]^-$, *m*/*z* 30) by cleavage of the C–N bond in oxyisocyanate. This cleavage is consistent with the C–N bond stretches associated with several vibrational modes in the computed infrared spectrum of oxyisocyanate.

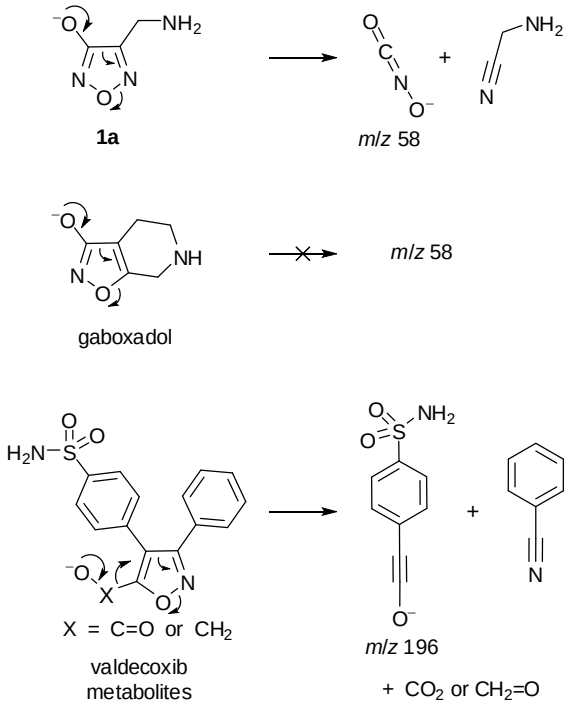
The formation of an ion at *m*/*z* 42 has been attributed to loss of an oxygen atom by cleavage of the longer N–O bond in oxyisocyanate (*m*/*z* 58),[18] [Waugh, 1989] but current computations (Fig. 3) predicted that a large input of energy was necessary for cleavage of the N–O bond with formation of isocyanate ion ($[O=C=N]^-$, *m*/*z* 42) and an oxygen atom. The alternative loss of an oxygen atom by cleavage of the O–C bond in oxyisocyanate required even more energy (761 kJ mol^{-1}). Given these unfavorable energetics, formation of isocyanate ion by ring cleavage of the deprotonated 3-hydroxyfurazans was considered. Accordingly, the computations predicted a barrier of 166 kJ mol^{-1} for cleavage of the N2–O and C3–C4 bonds in **1a** (Fig. 3) with formation of isocyanate (*m*/*z* 42) and a neutral nitrile oxide. While these energetics are less favorable than those associated with formation of oxyisocyanate (*m*/*z* 58), it is likely that isocyanate is formed from **1a**–**5a** by a few higher energy collisions in the triple quadrupole mass spectrometer.

The low intensity of the $[M - H]^-$ ions **1a**–**5a** upon CID at 10 eV (Fig. 2) and the predominance of the common product ion at *m*/*z* 58 (Figs. 2 and S2) are in full agreement with the computational prediction (Fig. 3) of a remarkably low barrier for generating oxyisocyanate by heterocyclic ring cleavage and the higher barriers for the fragmentation processes leading to the minor product ions. This facile cleavage of two bonds in the ring results in the formal placement of charge on the oxygen initially present in the ring and formation of a stable triple bond in the neutral product (Scheme 2). Each of the homologues **1a**–**4a** had a similar vibrational mode with symmetrical stretching of the N5–O and C3–C4 bonds at low frequency, as well as intramolecular hydrogen bonding between the ionized hydroxyl group and the primary amino group (Fig. S3). The analogous cleavage of **5a**, however, demonstrated that interaction of the amino group was not necessary for the characteristic fragmentation of the heterocyclic ring in deprotonated 3-hydroxyfurazans.

The contiguous OCNO grouping of atoms also is found in 3-hydroxyisoxazoles, such as gaboxazole, a $GABA_A$ agonist.[20] [Kall, 2007] In this bicyclic ring system, however, analogous cleavage to generate the oxyisocyanate ion (*m*/*z* 58) would unfavorably place a triple bond in a six-membered ring (Scheme 2), consistent with isotopic labeling experiments demonstrating loss of a neutral molecule from the six-membered ring of deprotonated gaboxazole.[20] [Kall, 2007] On the other hand, analogous cleavage of the isoxazole ring in valdecoxib metabolites triggered by decarboxylation or formation of formaldehyde (Scheme 2) would generate the previously proposed alkyne oxide and nitrile fragmentation products.[21] [Zhang, 2004] In general, the characteristic cleavage of C–C and N–O bonds in the heterocyclic ring can occur upon the build-up of negative

charge on an atom adjacent to the C–C bond and produce a stabilized anion and a neutral nitrile as products.

In conclusion, ESI deprotonation of the hydroxyl functionality was demonstrated for the 3-hydroxyfurazans. In addition, details of a very facile, characteristic fragmentation reaction of these hydroxy-substituted heterocycles have been documented.



Scheme 2. Analogous fragmentation processes of the deprotonated 3-hydroxyfurazan **1a** and isoxazole heterocycles.

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Yours,

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Supporting Information

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