Electron-Impact Induced Fragmentation of 3-Hydroxy Quinazoline-2,4(1*H*,3*H*)dione, Pyridopyrimidine-2,4(1*H*,3*H*)diones, Lumazine and Alloxazine

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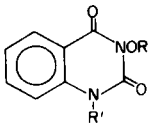
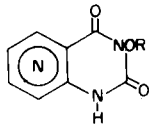
Electron-bombardment of the *N*-3-hydroxy derivatives of the above-mentioned condensed uracils revealed that the major fragmentations involved the heterocyclic ring. The most intense ion proved to be the M-32 ion which was created by the loss of the NHOH· radical from the molecular ion. Mechanisms for this transition are presented. Other fragmentations common to these systems are discussed and compared with those reported for the corresponding N-3 deoxy analogs of the title compounds. The mass spectral fragmentations of the *O*-methyl-, *N*-methyl- and *O,N*-dimethyl derivatives of 3-hydroxyquinazoline-2,4(1*H*,3*H*)dione were analyzed and were consistent with those expected from these structures. Electron bombardment of the 3-benzenesulfonyloxy derivatives of the title compounds resulted primarily in the scission of the sulfonate group in preference to that of the heterocyclic dione ring. These sulfonates also showed ions which indicated that a Lossen rearrangement had taken place in the mass spectrometer.

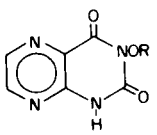
Interest in the electron-bombardment induced fragmentation of quinazolinones (1,3), pyridopyrimidinones (4,5), pteridinones (6-8), cyclic hydroxamic acids (9,10) and aromatic *N*-oxides (11), prompted us to examine the mass spectra of a number of closely related heterocycles. Of specific interest was the correlation of the primary fragmentation patterns of 3-hydroxyquinazoline-2,4(1*H*,3*H*)-dione, **1a**, 3-hydroxypyridopyrimidine-2,4(1*H*,3*H*)diones, **2a**, 3-hydroxylumazine, **3a**, and 3-hydroxyalloxazine, **4a**, with the fragmentation patterns reported for the structurally related compounds listed above (1-11).



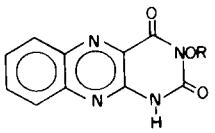
1a, R = R′ = H
b, R = R′ = D
c, R = $C_6H_5SO_2$, R′ = H



2a, R = H
b, R = $C_6H_5SO_2$



3a, R = H
b, R = $C_6H_5SO_2$



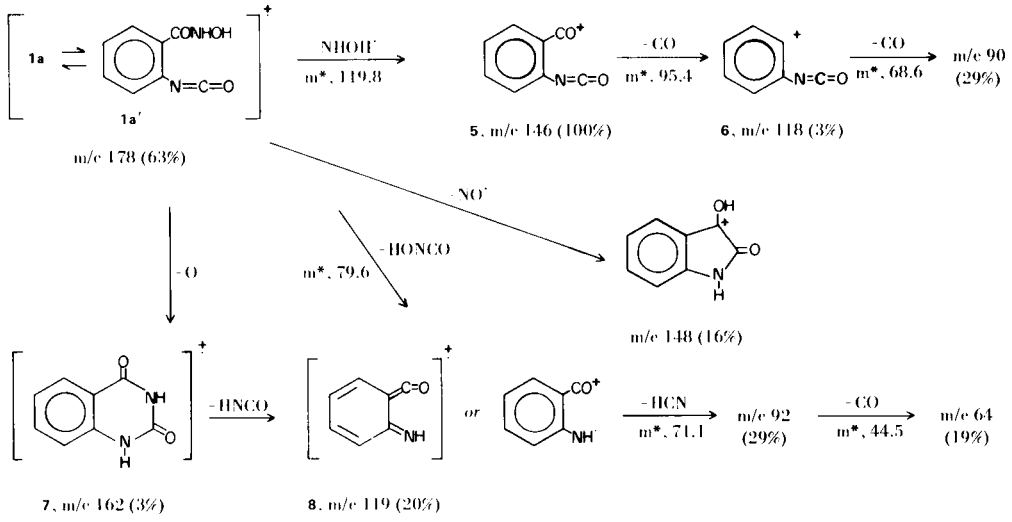
4a, R = H
b, R = $C_6H_5SO_2$

It was found that electron bombardment of these fused uracil systems resulted in similar fragmentation patterns which involved a number of concurrent processes. The principle fragmentation pathways all commenced with the disruption of the pyrimidinedione ring system. The most noteworthy transition from these molecular ions gave rise to an [M-32] ion. A portion of this work was devoted to establishing the nature of this transition and to establish the structure of the [M-32] ion, particularly since a comparable loss was absent from the molecular ions of the N-3 deoxy analogs of **1a-4a** (3,5,6).

3-Hydroxyquinazoline-2,4(1*H*,3*H*)dione, **1a**.

The mass spectrum and fragmentation pathway for the 3*N*-deoxy analog of this compound, 2,4-quinazoline-2,4-(1*H*,3*H*)dione, **7**, had been published (3) and agreed with the recently obtained spectrum which gave the molecular ion at m/e 162 (95%) and the most intense fragmentations at m/e 119 (100%), 92 (60%), 64 (33%) and 63 (30%) corresponding to the consecutive losses of HNCO, HCN and CO from **7**. A comparison of this result with the mass spectrum of **1a** (Scheme I) revealed the same fragment ions present but with significantly lower abundances. The salient difference between the mass spectra of **1a** and **7** was the decomposition of the molecular ion of **1a** to give a [M-32] ion as the base peak at m/e 146. A metastable ion observed at m/e 119.8 established the origin of this ion and

Scheme I



with the molecular formula obtained by high resolution mass spectrometry (12) of $C_8H_4NO_2$ (Calcd., 146.0242; Found, 146.0244) proved the loss of NHOH' from the molecular ion of **1a**. This fragmentation was further substantiated by the spectrum of the dideutero derivative, **1b** (m/e 180, 47%) which showed a loss of the corresponding NDOD' radical to produce the same base peak at m/e 146 with m* 118.4.

The loss of 32 mass units as NHOH' from a molecular ion is apparently of rare occurrence although Bowie *et al.*, (9) reported that the molecular ion of benzohydroxamic acid lost an NHOH' radical according to the equation:

$$C_6H_5CONHOH]^{+\cdot} \rightarrow C_6H_5CO^{+} + NHOH'$$

In order to accommodate a similar loss in the present system, a ring opening of **1a** to **1a'** was proposed from which NHOH' could be cleaved readily to give the base peak fragment ion, **5**. Subsequent losses from **5** and other processes are summarized in Scheme I.

The occurrence of an ion at m/e 162 in the spectrum of **1a**, and the frequent appearance of other [M-16] ions in the related compounds studied, was of particular concern since its relative abundance varied pronouncedly (3-20%) with different samples of the compound **1a**. The apparent loss of atomic oxygen in the spectra of other *N*-hydroxy systems is common and has been reported for benzohydroxamic acid (9), most cyclic hydroxamic acids (10), *N*-hydroxyphthalimide (9), several 3-hydroxypyrido[3,4-*d*]-pyrimidine-5(3*H*)ones (5) and 3-hydroxypteridine-4(3*H*)-one (7). However, only recently did a definitive study on the spectra of quinoline *N*-oxides show that the observed loss of oxygen was actually thermally induced (11).

In the present study time-temperature analyses in which spectra were obtained periodically as the sample temperature was increased refuted a thermal origin for the [M-16] ions since their abundance *decreased* markedly and rapidly to as little as 3% relative abundance in **1a** as the temperature was raised. These results indicated the cause of the pronounced [M-16] ion was sample contamination with a small amount of the corresponding *N*-deoxy compounds. Initial evidence to support this conclusion was found in the low ionizing voltage spectra in which both the molecular ion and the [M-16] ion persisted at 10 eV. Further evidence was supplied when purification of **1a** by repeated recrystallizations from water produced samples giving spectra which exhibited decreasing amounts of the m/e 162 ion.

Even though different methods of preparation of **1a** were used (13,14) and careful purification steps taken, it was not possible to obtain a sample whose spectrum was completely devoid of the [M-16] ion indicating a *small* contribution to this species from electron bombardment. For **7**, the supposed impurity in **1a**, the loss of HNCO gives ion **8** at m/e 119. The partial removal of the impurity as evidenced by a decrease in the M-16 ion abundance also resulted in a decrease of the m/e 119 and m/e 92 ions consistent with the known relative abundances of these ions in the spectrum of pure **7**. However, it was evident that most of the m/e 119 species arose from **1a** itself with the elimination of HONCO and was supported by a metastable ion at m/e 79.6.

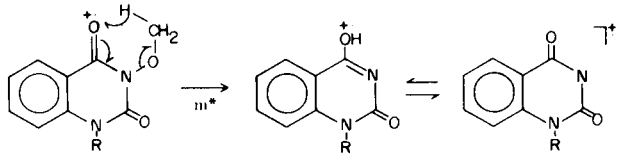
It is interesting to note that in none of the other *N*-hydroxy systems mentioned above where an [M-16] ion was reported, was this loss substantiated with an accompanying metastable transition or other evidence attesting to the origin of the [M-16] ion and leads to the speculation that these ions could be due to similar impurities or are actually thermally induced.

The spectrum of **1a** also exhibited an [M-30] ion at

m/e 148 (16%) (Scheme I). The deuterated analog, **1b**, showed this same loss and it is suggested that NO· was cleaved from the molecular ion. A loss of NO· was reported in the fragmentation of *N*-hydroxyphthalimide (9).

O and *N*-Methyl Derivatives of **1a** (15).

In the decomposition of the molecular ion of 3-methoxy-2,4-quinazoline-2,4(1*H*,3*H*)dione, **9**, the loss of 30 mass units was attributed to a McLafferty type rearrangement in which CH_2O is lost initially to produce the *N*-deoxy analog, **11a**, (equivalent to **7** in Scheme I) which then accounted for most of the prominent ions in the spectrum. The [M-30] ion in the mass spectrum of **10** was intense.



9, R = H, m/e 192 (31%)
10, R = CH_3, m/e 206 (82%)

11a, R = H, m/e 162 (41%), m* 136.7
b, R = CH_3, m/e 176 (51%), m* 150.4

Both transitions (**9** → **11a** and **10** → **11b**) were supported by metastable ions. In addition, *N*-methoxysuccinimide (9) and 3-methoxypteridine-4(3*H*)one (8) were reported to fragment in this manner. While the base peak in the spectrum of **9** could be accounted for by the loss of HCNO from **11a**, a retro-Diels-Alder cleavage of CH_3ONCO directly from **9** would also yield the m/e 119 ion.

The remaining significant peak in the spectrum of **9** at m/e 146 was assigned to structure **5** (Scheme I) and arose by the same transition as shown for **1a** to **5** except that for **9** the species lost was CH_3ONH·.

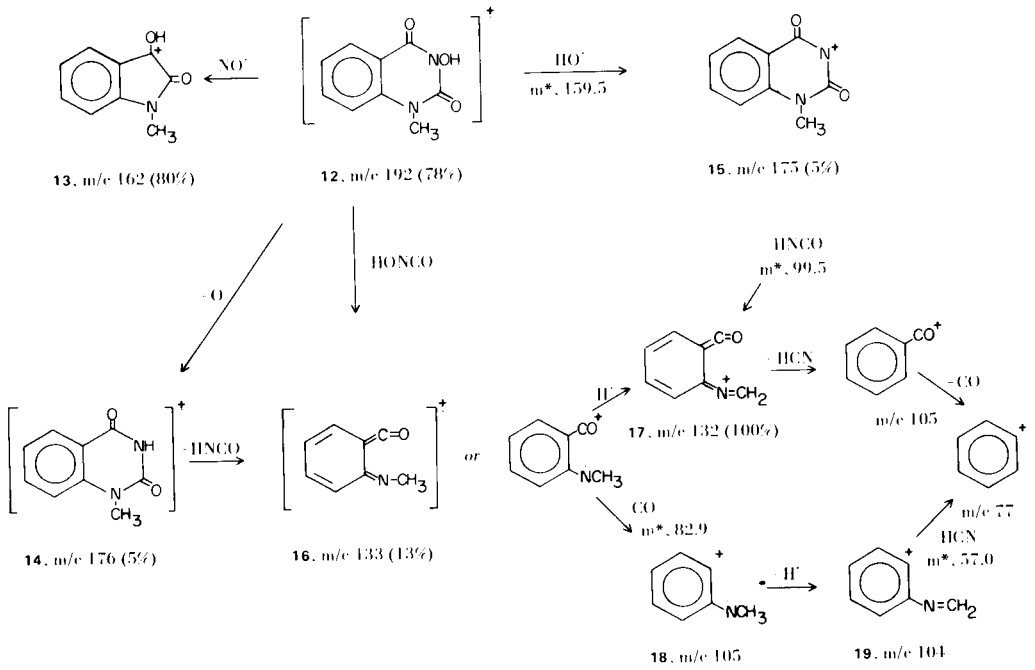
The isomeric *N*-methyl derivative, **12**, presented a different fragmentation pattern from that for **9** and the origin proposed for the prominent fragments is given in Scheme II. For this isomer, the [M-30] ion at m/e 162 was believed to involve the loss of NO· to create **13** in a process similar to that reported for *N*-hydroxyphthalimide (9), 3-hydroxypyridopyrimidones (5) and 3-hydroxypteridine-4(3*H*)ones (7).

As expected, the presence of a methyl group at *N*-1 prevented ring opening of the type depicted in Scheme I (**1a** → **5**) and the analogous subsequent sequence of ions did not appear in the spectrum of **12**. An [M-16] ion of low abundance did appear m/e 176, **14**, however it again was of questionable origin.

A retro-Diels-Alder fragmentation of either **12** or **14** would yield **16** at m/e 133 which then could lose a proton to give the base peak **17** at m/e 132. Another route to this most abundant ion is through the initial loss of the OH group of **12** to produce **15** at m/e 175 which subsequently loses HNCO to produce **17**. Although the m/e 175 ion is of low abundance, (*ca.* 5%) both steps involved in its formation and its decomposition were accompanied by an appropriate metastable ion. Other decompositions are postulated in Scheme II to account for most other prominent ions.

To lend further support to the fragmentation route proposed for **12**, the spectrum of the *O*,*N*-dimethyl derivative, **10**, exhibited most of the same fragment ions with

Scheme II



corroborating metastable ions. The molecular ion of **10** at m/e 206 (82%) decomposed by loss of CH_2O to generate **11b** (see also **14**), with a 51% abundance (m*, 150.5). The retro-Diels-Alder loss of HNCO produced **16** at m/e 133 (29%) (m*, 100.5). Cleavage of H˙ from **16** to form **17** at m/e 132 (34%) was also verified by a metastable ion at m/e 131.0. In addition, **16** was the source of the most abundant ion *via* expulsion of CO to yield **18** at m/e 105 (100%) and this was substantiated by a metastable ion at m/e 83.0. The transition of **18** to **19** was also supported by a metastable ion in the spectrum of this dimethyl derivative.

Pyrido and Pyrazino Analogs of **1a**.

The major fragmentations of **2a** and **3a** revealed identical patterns to that discussed for **1a** in Scheme I. Either the molecular ion or the [M-NHOH] ion were the base peak, with ions of considerable abundance due to [M-NHOH-CO], [M-O] and [M-HONCO] processes. The spectra for the four isomers represented by **2a** have been published (16) and conformed to this pattern. There are discrepancies in the pattern reported for one of these isomers, *viz.*, 3-hydroxypyrido[3,2-*d*]pyrimidine-2,4(1*H*, 3*H*)dione (16) since the [M-16] ion was shown as the base peak. In view of our recent discovery that the [M-16] ion was due largely to the corresponding deoxy compound, purified samples were examined and revealed the molecular ion to be the base peak with the relative abundance of the [M-16] ion considerably diminished. This new spectrum is recorded in the Experimental Section.

The mass spectrum of **3a** was interpreted also within the routes proposed in Scheme I. Apparently, the presence of the sp^2-hybridized nitrogen atoms in the ring attached to the *N*-hydroxy uracil ring did not influence the overall pattern, nor was the pattern changed in the multifused-ring compound **4a**. Except for a less intense [M-32] ion and a more intense [M-HONCO] ion, there was little departure from the overall type of decomposition (18).

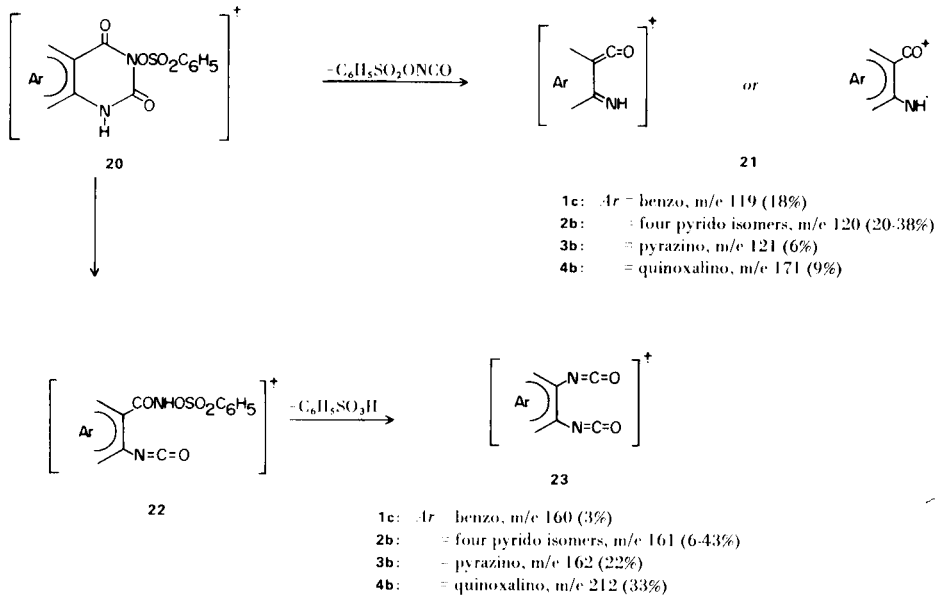
Benzensulfonates, **1c** (15), **2a** (16), **3b** (17), **4b** (18).

With few exceptions, the 3-benzensulfonyloxy derivatives of the heterocyclic systems **1-4** exhibited similar molecular ions (10-20% of the base peak) and very similar fragmentation patterns. At the lowest direct inlet temperature, the base peak was consistantly, m/e 77 ($C_6H_5^+$), and there was also present an intense m/e 141 ion ($C_6H_5SO_2^+$).

The dominant process during electron bombardment involved scission of the O-S bond of sulfonate group to form the stable $C_6H_5SO_2^+$ ion with the release of the corresponding heterocyclic moiety bearing a 3-oxy radical. The mass spectrum of **1c** exhibited few other ions with an intensity over 25% of the base peak which could be correlated with fragmentations discussed for the *N*-hydroxy compounds or the simple methyl derivatives of **1a**. One analogous process involved a retro-Diels-Alder type of loss of $C_6H_5SO_2ONCO$ shown by the abbreviated formulas **20** to **21** in Scheme III. The expected loss of $NHOSO_2C_6H_5$˙ from the sulfonates did not occur.

An ion equivalent to a Lossen rearrangement product of **22**, namely the bisisocyanate, **23** was observed and was not surprising in view of the facile chemical Lossen rearrangements of **20** (15,18). Evidence was obtained showing that the Lossen rearrangement was both electron-impact and thermally induced, however most of the seven sulfonates studied were stable to 300°. The pyrido isomers and, in

Scheme III



particular, 3-benzenesulfonyloxypyrido[3,2-*d*]pyrimidine-2,4(1*H*,3*H*)dione decomposed thermally at an inlet temperature of 325° to give a spectrum with the Lossen product at m/e 161 as the base peak. The anticipated $C_6H_5SO_2^+$ ion at m/e 141 was absent. In addition, the molecular ion of benzenesulfonic acid (m/e 158) was evident, all indicating that thermal changes had occurred.

At the lower inlet temperature of 290°, the spectrum showed the ion at m/e 161 at only 9% while the expected ion at m/e 141 was indeed prominent at 68%.

The previously reported spectrum (16) was recorded at 325° and shows the thermal decomposition products therefore the lower temperature spectrum is included in the Experimental.

EXPERIMENTAL

Mass spectra were obtained by Mr. Richard Dvorak using an Hitachi-Perkin Elmer RMU-6D single-focusing mass spectrometer operating at an ionizing voltage of 70 eV. The samples were introduced by means of a direct insertion probe at the lowest effective inlet temperature (dit) (250-340°). Mass spectra were usually obtained over a range of temperatures to ascertain if changes would occur and if changes occurred, additional spectra were run at 2-5 minute intervals until an unchanging spectrum was obtained.

The spectra recorded below were not previously reported. Usually, ions over 5% of base peak are recorded over m/e 60.

3-Hydroxyquinazoline-2,4(1*H*,3*H*)dione **1a**.

The major fragments (dit = 340°) were m/e (rel intensity; all fragments over 2%), 180 (1), 179 (8), 178 (63), 162 (3), 148 (16), 147 (10), 146 (100), 134 (3), 130 (3), 119 (20), 118 (3), 105 (4), 104 (2), 103 (4), 101 (5), 92 (29), 91 (9), 90 (29), 78 (4), 77 (6), 75 (3), 65 (10), 64 (19), 63 (17).

Several crystallizations from deuterium oxide produced a deuterated sample, (O, N-d_2), **1b** (dit = 290°) m/e (rel intensity) 181 (3), 180 (47), 179 (19), 178 (3), 164 (2), 150 (11), 149 (4), 148 (1), 147 (10), 146 (100), 120 (15), 92 (26), 91 (5), 90 (21), 64 (12), 63 (13).

1-Methyl-3-hydroxyquinazoline-2,4(1*H*,3*H*)diones.

Although the mass spectrum of a highly purified sample (dit, 215°) gave all the peaks published previously their relative intensity changed slightly: m/e (rel intensity) 193 (10), 192 (78), 176 (5), 175 (5), 163 (9), 162 (80), 148 (7), 133 (13), 132 (100), 131 (7), 119 (10), 118 (8), 116 (7), 106 (7), 105 (42), 104 (58), 92 (14), 91 (7), 90 (18), 78 (24), 77 (51), 76 (15), 75 (7), 74 (7), 64 (20), 63 (21), 62 (7). A sample crystallized from deuterium oxide had the following ions, (dit. 60°), 194 (7), 193 (64), 192 (25), 177 (7), 176 (6), 175 (3), 163 (51), 162 (22), 133 (14), 132 (100), 120 (6), 119 (8), 118 (5), 116 (6), 106 (6), 105 (41), 104 (49), 92 (14).

3-Benzenesulfonyloxyquinazoline-2,4(1*H*,3*H*)dione.

(Dit, 310°); m/e (rel intensity) 319 (2), 318 (13), 303 (8), 254 (4), 160 (3), 146 (7), 143 (6), 142 (8), 141 (98), 119 (18), 104 (26), 92 (10), 90 (10), 78 (8), 77 (100), 76 (21), 65 (9).

3-Hydroxypyrido[3,2-*d*]pyrimidine-2,4(1*H*,3*H*)dione.

The reported spectrum possessed a strong [M-16] ion at m/e 163. The revised spectrum (dit, 345°) is as follows: m/e (rel intensity) 180 (10), 179 (100), 163 (12), 147 (27), 121 (29), 120 (9), 119 (39), 106 (7), 93 (7), 92 (40), 91 (20), 78 (30), 70 (12), 66 (16), 65 (30), 64 (26).

3-Benzenesulfonyloxypyrido[3,2-*d*]pyrimidine-2,4(1*H*,3*H*)dione.

(Dit, 290°); 319 (21), 255 (4), 163 (10), 161 (9), 141 (68), 121 (5), 120 (24), 119 (6), 93 (25), 92 (24), 91 (12), 89 (12), 77 (100), 65 (20), 64 (13).

3-Hydroxylumazine (17).

(Dit, 310°); 181 (10), 180 (100), 164 (20), 149 (7), 148 (76), 122 (10), 121 (25), 120 (54), 95 (3), 94 (7), 93 (28), 79 (10), 70 (15), 69 (8), 68 (5), 67 (15), 66 (24).

3-Benzenesulfonyloxylumazine.

(Dit, 300°); 320 (10), 256 (4), 164 (5), 162 (10), 158 (5), 141 (51), 122 (11), 121 (6), 94 (16), 93 (24), 78 (12), 77 (100), 70 (12), 69 (7), 67 (6), 66 (23), 65 (15).

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