Fragmentation Pattern of 1,4-Benzodiazepin-2-ones under Electron Impact[1]

Wolfgang Sadée

School of Pharmacy, University of California, San Francisco Medical Center, San Francisco, California 94122

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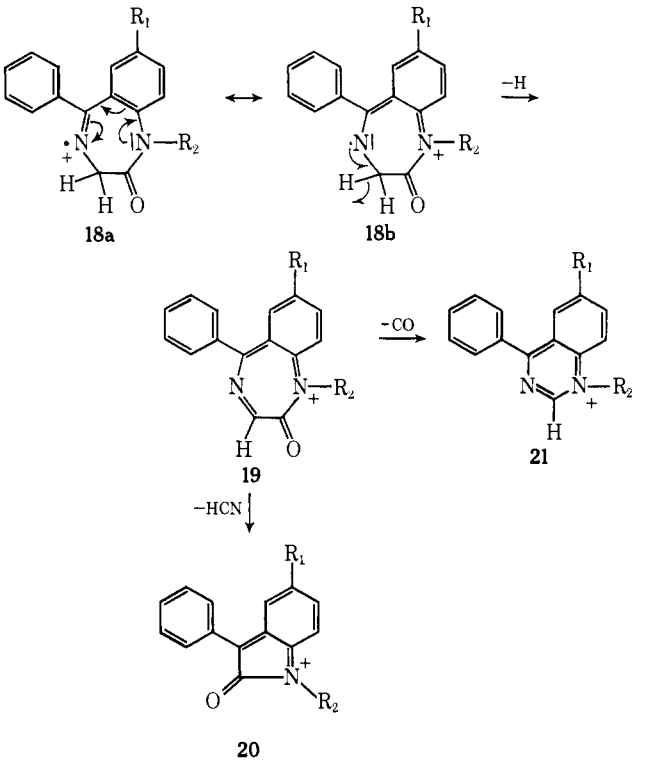
The mass spectra of a series of 5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-ones were determined, and the fragmentation pattern was elucidated by high resolution mass measurement of the major peaks and by metastable scanning. The parent ions degraded mainly by loss of H, HCN, CO, and HCO to smaller-membered ring systems, to which the structures of quinazolines, indolones, and indazoles have been assigned. By further rearrangements, condensed ring systems such as acridines and fluorenes were generated. Substitutions in positions 3 and 4 of the diazepine ring influenced the fragmentation markedly, and thus **1–17** could be classified into three groups, *i.e.*, 3,4-unsubstituted derivatives, 3-hydroxy derivatives, and N-4-oxides. The N-4-oxides rearranged to oxaziranes and then to 4-benzoylquinazolinones as intermediates. The latter species, which is the analog of a Beckmann rearrangement product, degraded to the benzoyl ion at *m/e* 105. The rearrangements, occurring under the conditions of electron impact, were compared to well known chemical rearrangements of benzodiazepines. The present investigation was designed for further studies of the metabolism of this pharmacologically interesting group of drugs.

Since the introduction of chlordiazepoxide[2a] as a potent tranquilizer, interest in the benzodiazepines as a class of pharmacologically active agents[2b] has rapidly increased. Five derivatives are now commercially used as tranquilizers, muscle relaxants, and anticonvulsants. These are chlordiazepoxide, diazepam, oxazepam, nitrazepam,[3] and medazepam.[3] The metabolism of these compounds has recently been studied thoroughly by several groups, but many problems remain to be solved. Structure elucidation of yet unknown metabolites requires mass spectrometry as an analytical tool. However, no comprehensive information on mass spectra of benzodiazepines has so far been available, and no structures have been assigned to the fragments. Since the chemistry of benzodiazepines is well known,[4,5] one may be able to draw analogies of the fragmentation, resulting from electron impact, to chemical rearrangements.

Compounds, of which mass spectra were taken, are listed in Table I. Chlordiazepoxide and medazepam were not included in this study, lacking the carbonyl function in position 2.

The 5-phenyl substituent could be detected by intense peaks at *m/e* 77 and *m/e* 51.[6] Furthermore, loss of Cl or NO_2 in position 7 occurred either from the parent ion or from some of the major fragments. All benzodiazepines gave rise to characteristic peaks at *m/e* 205, 193, 177, 165, and 151. However, substitution in the diazepine ring influenced the primary fragmentation markedly, so that **1–17** could be classified into three groups. Group A includes all those compounds which bear no substitution in position 3 and 4 (**1–5**). Compounds of group B bear hydroxy or acetoxy substitution in position 3 (**6–12**), and group C includes N-4-oxides (**13-17**).

3,4-Unsubstituted Benzodiazepines (Group A).—The mass spectrum of **1** is shown in Figure 1. It can be assumed that the fragmentation is directed by the two N-atoms in the diazepine ring. Thus, the parent ion,



from which the main degradations occur, can be formulated as **18a** ↔ **18b**. Losses of H, HCN, and CO have been reported to lead to the most intense peaks,[7,8] generated by fragmentation of the N-1-amide function (see references 9–11) and the N-4-imino function, which

(1) Part of a study on the mechanism of metabolic 3-hydroxylation of benzodiazepines.

(2) (a) L. H. Sternbach and E. Reeder, *J. Org. Chem.*, **26**, 1111 (1961). (b) L. H. Sternbach, L. O. Randall, R. Banzinger, and H. Lehr, in "Drugs Affecting the Central Nervous System," A. Burger, Ed., 1968, pp 237–264.

(3) Recommended as INN, nitrazepam (**2**), see Table I, medazepam for 7-chloro-2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepine.

(4) G. A. Archer and L. H. Sternbach, *Chem. Rev.* 747 (1968).

(5) S. J. Childress and M. I. Gluckman, *J. Pharm. Sci.*, **53**, 577 (1964).

(6) F. M. McLafferty, "Mass Spectrometry of Organic Ions," Academic Press, New York and London, 1963, p 475.

(7) M. A. Schwartz, P. Bommer, and F. M. Vane, *Arch. Biochem. Biophys.* **121**, 508 (1967).

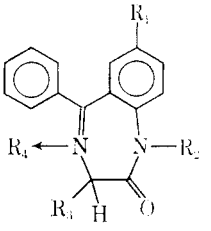
(8) M. A. Schwartz, F. M. Vane, and E. Postma, *J. Med. Chem.*, **11**, 770 (1968).

(9) J. L. Cotter, *J. Chem. Soc.*, 5477 (1964).

(10) K. G. Das, P. T. Funke, and A. K. Bose, *J. Amer. Chem. Soc.*, **86**, 3729 (1964).

(11) A. M. Duffield, H. Budzikiewicz, and C. Djerassi, *ibid.*, **86**, 5536 (1964).

TABLE I



	R_1	R_2	R_3	R_4	Mol wt
			Group A		
1[a]	Cl	CH_3	H		284
2[b]	NO_2	H	H		281
3	Cl	H	H		270
4	NO_2	CH_3	H		295
5	NH_2	H	H		251
			Group B		
6	H	H	OH		252
7[c]	Cl	H	OH		286
8	Cl	CH_3	OH		300
9	NO_2	H	OH		297
10	Cl	CH_3	$OCOCH_3$		342
11	NO_2	H	$OCOCH_3$		339
12	NO_2	CH_3	$OCOCH_3$		353
			Group C		
13	Cl	H	H	0	286
14	Cl	CH_3	H	0	300
15	NO_2	H	H	0	297
16	NO_2	CH_3	H	0	311
17	NO_2	H	CH_3	0	311

[a] Diazepam. [b] Nitrazepam. [c] Oxazepam.

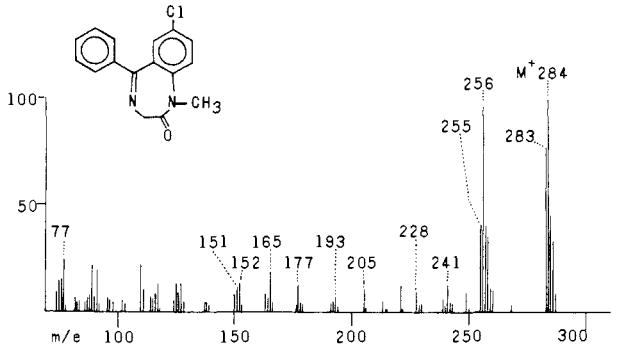
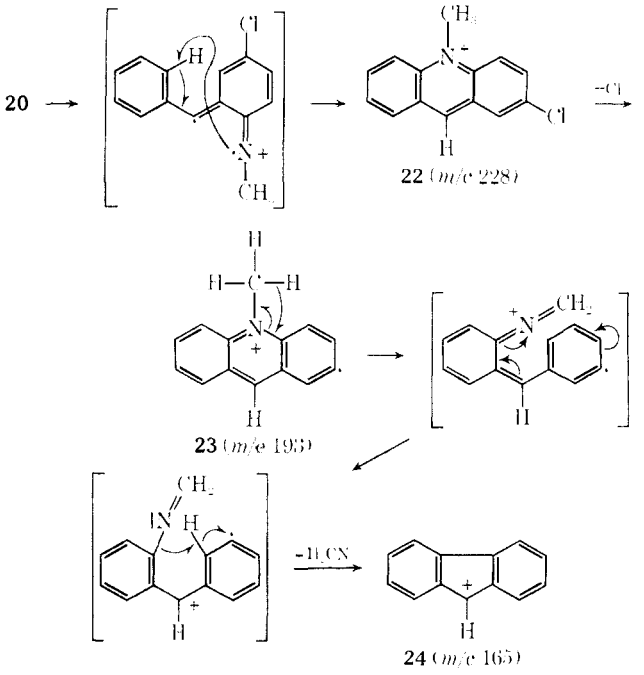


Figure 1.—Mass spectrum of compound 1 (diazepam).

represents the basic center of the molecule.[12] Species **18a** ↔ **18b** readily eliminated hydrogen to give $M^+ - 1$ peaks with relative abundances of 100% (**2**), 88% (**3**), and 35% (**5**). Since little change in the intensity of the $M^+ - 1$ peak was observed for the N-1-methylated congeners (80% for **1** and 93% for **4**), loss of the N-1 hydrogen accounted for only a small part of this peak. A likely loss of a C-3-CH_2 hydrogen occurred to about 7% as demonstrated by a dideutero label in position 3.[13] Possibly, loss of an aromatic H led to a rather stable fragment.

It has been reported repeatedly, that rearrangements of benzodiazepines are induced by abstraction of one proton in position 3, followed by ring contractions to smaller membered rings such as quinolines[14] and isoindoles.[15-17] It may be assumed that after elimination of a C-3-H similar ring contractions resulting from loss of CO and HCN led to the indolone **20** and the quinazoline **21**. Analogous chemical reaction products have been reported with various benzodiazepines.[18-21] The relative intensities of the peak at $M^+ - H$, CO (30–90%) were lower than $M^+ - H$, HCN (70–100%). The C-3-D_2 label gave rise to a very large peak at $M^+ - 30$ (100%),[13] which presumably consisted of the species $M^+ - D$, DCN and $M^+ - D$, CO, thus explaining the large abundance. This pattern supports strongly the proposed degradations. As can be seen from the metastable peaks derived from the transitions $M^+ - H$ and $M^+ - CO$, to a minor extent, first CO and then H were expelled. By accurate mass measurement of the peak at $M^+ - 28$ of **3**, a doublet could be detected consisting of $M^+ - CO$ and $M^+ - H$, HCN, with the latter ion being about 20 times as intense. A similar degradation by loss of CO has been discussed previously for benzodiazepine alkaloids.[22] The structure of a quinazolinone has been assigned to the generated fragment.

The indolone **20** eliminated CO, as reported for lactams.[23] This would lead to a four-membered ring system (*m/e* 228 for **1**). However, a rearrangement to the highly stabilized acridine **22** should be favored energetically. Similarly, acridine derivatives can be formed by chemical reaction from 2-aminobenzophenones.[23,24] The corresponding peaks were present

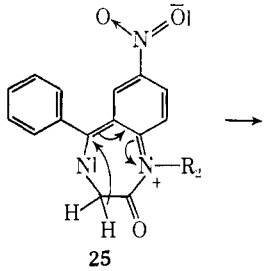


(12) K.-H. Beyer and W. Sadée, *Arch. Pharm.* (Weinheim), **300**, 667 (1967).
(13) F. M. Vane, personal communication.
(14) R. I. Fryer and L. H. Sternbach, *J. Org. Chem.*, **30**, 521 (1965).
(15) R. I. Fryer, B. Brust, J. V. Early, and L. H. Sternbach, *J. Chem. Soc. C*, 366 (1967).
(16) R. I. Fryer, J. V. Early, and L. H. Sternbach, *J. Amer. Chem. Soc.*, **88**, 3173 (1966).
(17) R. I. Fryer, J. V. Early, and L. H. Sternbach, *J. Org. Chem.*, **34**, 649 (1969).
(18) W. Merlesics, G. Silverman, and L. H. Sternbach, *ibid.*, **29**, 1621 (1964).
(19) L. H. Sternbach, E. Reeder, A. Stempel, and A. J. Rachlin, *ibid.*, **29**, 332 (1964).
(20) R. I. Fryer, J. V. Early, and L. H. Sternbach, *ibid.*, **32**, 3798 (1967).
(21) S. C. Bell and S. J. Childress, *ibid.*, **27**, 1691 (1962).
(22) M. Luckner, K. Winter, and L. Nover, *Tetrahedron*, **25**, 2575 (1969).
(23) R. I. Fryer, J. V. Early, and L. H. Sternbach, *J. Org. Chem.*, **30**, 521 (1965).
(24) R. I. Fryer, B. Brust, and L. H. Sternbach, *J. Chem. Soc. C*, 4977 (1963).

with relative intensities of about 10% and gave the correct empirical formulas (see also ref 7). The 7-NH_2 of **5** evidently stabilizes structure **22** by its electron-donating properties and increased the relative abundance to 70%. Species **22** eliminated R_1 and R_2 to give peaks at *m/e* 228 → 213 (−CH_3) and → 178 (−Cl) and 228 → 193 (−Cl) (**23**) and → 178 (−CH_3) in the spectrum of **1**. Compound **3**, which lacks the N-1 Me, degraded to *m/e* 214, 213, 179, and 178. Since the spectra were rather complex in this region, it was necessary to elucidate the fragmentation pattern by scanning the metastable transitions for each ion. This procedure confirmed the above sequence.

The formation of condensed ring systems as acridines was supported by the lack of peaks corresponding to fragments generated by loss of the 5-Ph substituent. Species **23** (*m/e* 193) also degraded to *m/e* 165 by loss of H, HCN. A rearrangement and H migration possibly formed the very stable fluorene **24** which was found in all spectra (*m/e* 165) and which was reported to occur also in the spectra of benzoxazepines.[25] However, in the case of 3-OH derivatives, **24** was generated in a different way as will be shown below. The peaks at *m/e* 151 and 152, which were present in all spectra, could be formed by degradation of the postulated acridines, *e.g.*, by loss of HCN from *m/e* 178 to *m/e* 151. The empirical formula of *m/e* 151 was found to be $C_{12}H_7$. In the spectrum of acridine itself, *m/e* 152 and 151 represent major peaks.[26]

Loss of HCN from the parent ion gave another intense peak for the NO_2 derivatives **2** (45%) and **4** (80%), while in the spectrum of the 7-NH_2 derivative **5** no such peak was present. The peaks at $M^+ - 27$ of **1** and **3** were due to ^{37}Cl and ^{13}C isotopes, as could be shown by high resolution measurement. Loss of HCN in the spectra of **2** and **4** may be explained by hydride migration from C-3 (**25**) to C-5 (**26**). This migration should



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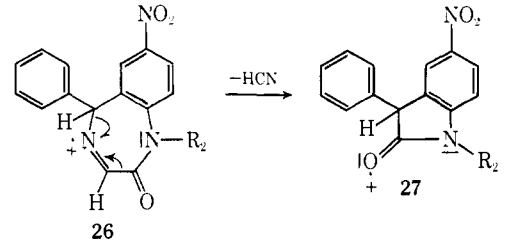
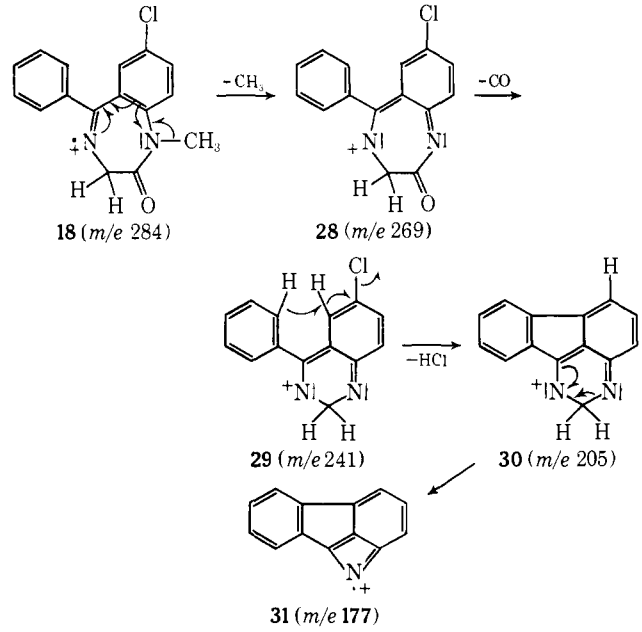


26 **27**

be favored by the 7-NO_2 which induced a positive charge in the quasi *para* position C-5. Such isomerizations of 1,3-dihydrobenzodiazepines to 1,5-dihydro derivatives and the reverse reaction were reported to occur readily.[21,27,28] Thus, the indolone radical ion **27** may be responsible for the peak at $M^+ - 27$. The metastable scanning of **1**, **7** and **14** (R_1 = Cl) revealed another degradation sequence, characteristic for 5-phenylbenzodiazepines, and found in all groups A-C. In this case the C-3 CH_2 stayed intact. The sequence was triggered by loss of the substituent R_2 in N-1 to **28**, followed by elimination of CO to give **29** at *m/e* 241 (10% relative abundance) in the spectrum of **1**. By accurate mass measurement a doublet was found for *m/e* 241 consisting of an ion with the correct empirical formula of **29** ($C_{14}H_{10}N_2Cl$) and of a smaller peak (1/6th of the intensity), which was generated by loss of Me from **20** (empirical formula $C_{14}H_8ClNO$). Species **29** degraded by loss of HCl to *m/e* 205. This is best rationalized by formation of the fluorene **30** which then eliminated H, HCN to yield **31** at *m/e* 177 with the correct empirical formula $C_{13}H_7N$. It was not possible



18 (*m/e* 284) **28** (*m/e* 269)

29 (*m/e* 241) **30** (*m/e* 205)

31 (*m/e* 177)

to decide which N was eliminated, and how species **31** would stabilize. Interestingly, loss of HCl could only be observed with an almost planar species and not with the intact benzodiazepines, in which the 5-Ph is in another plane than the condensed benzo ring.[29,30] If R_2 is H, the same degradation to *m/e* 177 can be formulated starting from **21**, which eliminated a C-3 H and CO. Since **21** gave a very large peak, the intensity of the peak at *m/e* 205 should increase. However, the relative abundance of *m/e* 205 in the spectra of **1** (R_2 = Me) and **3** (R_2 = H) was found to be equal (about 10%). Thus, species **21** seems to be of minor importance as an intermediate.

The metastable transitions led to the conclusion that the degradation sequence to *m/e* 177 occurred both stepwise or in a one step reaction starting from *m/e* 241 or directly from the parent ion. In the 7-NO_2 series loss of HNO_2 from the ion at *m/e* 252 yielded **30** in a similar reaction.

(25) O. Buchardt, A. M. Duffield, and C. Djerassi, *Acta Chem. Scand.*, **23**, 126 (1969).

(26) "Compilation of Mass Spectral Data," A. Cornu and R. Massot, Ed., Heyden and Son, Ltd., 1966.

(27) S. C. Bell, R. J. McCaully, and S. J. Childress, *J. Med. Chem.*, **11**, 172 (1968).

(28) R. I. Fryer, D. Winter, and L. H. Sternbach, *J. Heterocycl. Chem.*, **4**, 355 (1967).

(29) J. Karle and I. L. Karle, *J. Amer. Chem. Soc.*, **89**, 804 (1967).

(30) W. Sadée, *Arch. Pharm.* (Weinheim), **302**, 769 (1969).

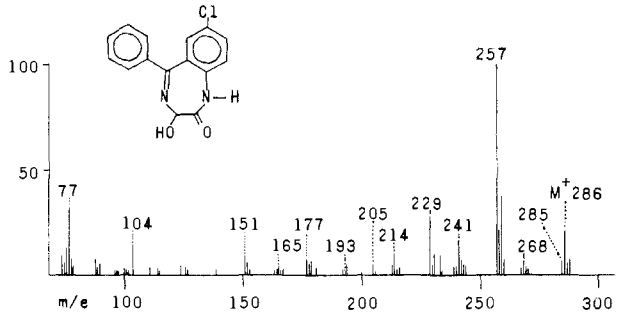


Figure 2.—Mass spectrum of compound **7** (oxazepam).

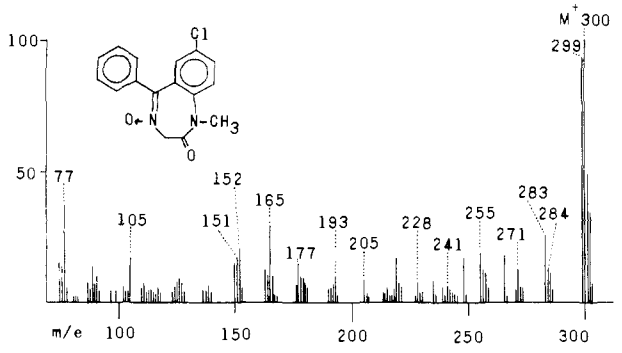
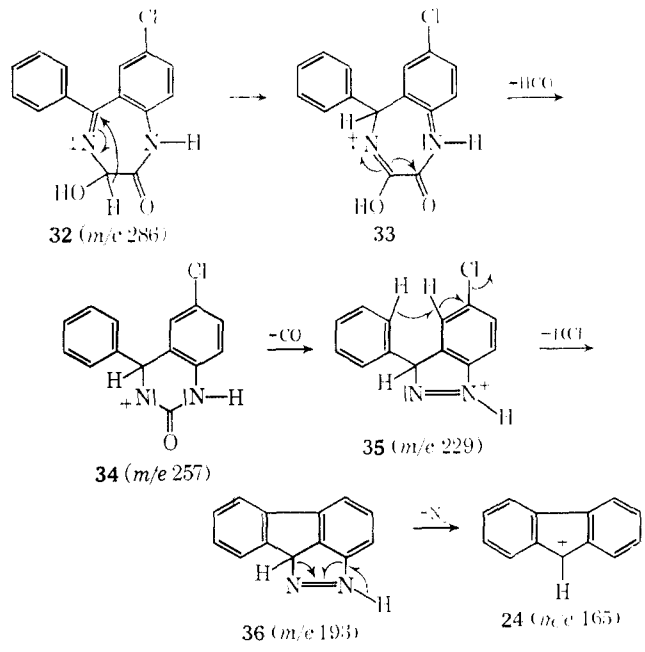


Figure 3.—Mass spectrum of compound **14** (diazepam *N*-oxide).

3-Hydroxy Derivatives (Group B).—The spectra of 3-OH and 3-AcO derivatives were closely related. The peaks of the parent ions of the 3-AcO derivatives possessed a very small abundance (less than 5%). The Ac ion at *m/e* 43 gave rise to the base peaks in the spectra of **10** and **12**. Loss of ketene yielded a similar species as the parent ion of the 3-OH series, since further fragmentation was almost identical. Starting from this species, losses of H, OH, and H_2O could be observed to some extent. The spectrum of **7** is shown in Figure 2. In most cases, the base peak was generated by loss of the formyl radical. Again a hydride migration from C-3 to C-5 was possibly involved before loss of HCO yielding **33** and the quinazolinone **34**. Analogous to the rearrangement **32** → **33**, treatment of 3-hydroxybenzodiazepines with alkali readily gave 4,5-dihydrobenzodiazepin-2,3-dione.[21] Additional loss of CO possibly generated the indazole **35**. This assignment is supported by metastable transitions and accurate mass measurement of the peak at *m/e* 229 of **7** ($C_{13}H_{10}ClN_2$). The indazole ring system seems to be fairly stable, since it could be synthesized starting from benzodiazepines[31] and benzoxadiazepines.[32] Species **35** eliminated Cl to give *m/e* 194. The metastable scan and high resolution measurement revealed a further degradation of **35** to **36** by loss of HCl (*m/e* 193) as discussed above, and to **24** (*m/e* 165) by loss of N_2. Thus, formation of the fluorene **24** occurred in a different way for compounds of class B as compared to class A.

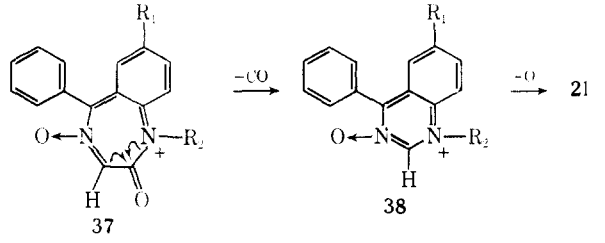
The peak at $M^+ - OH$ or $M^+ - CH_2{=}C{=}O$, OH, respectively, should be assigned to structure **19**.



Indeed, further fragmentation similar to class A could be detected. Also, analogous to class A, loss of H, HOCN, instead of HCN, was observed to a small extent for the 3-OH derivatives **6** (10%), **7** (6%), **8** (15%), and **9** (7%) (compare ref 7). The major fragmentation of 3-AcO derivatives was elimination of only HOCN after loss of ketene, and this indicates that again a hydride migration to C-5 might occur more readily as discussed for the 7-NO_2 derivatives **25**–**26**.

Compound **6** was the only derivative without substitution in position 7, of which mass spectra were determined. The peaks at *m/e* 205, 177, and 165, formed by loss of H and R_1 to yield condensed fluorenes, were not present. Elimination of OH, CO, and HCN generated the acridinium ion at *m/e* 180 in a similar mechanism which led to **22**, followed by loss of H to the acridinium ion at *m/e* 179. Again peaks at *m/e* 151 and 152 strongly supported formation of the acridine moiety.

N-4-Oxides (Group C). The mass spectrum of **14** is shown in Figure 3. The nitrone function in position 4 has been reported to influence the electron structure of the seven membered ring as shown by nmr spectroscopy.[30] Thus, the fragmentation pattern of the nitrone series should be expected to differ markedly from related benzodiazepines. Losses of H and CO led to **37** and **38** and were preferred to a possible loss of HCNO ($M^+ - H$, CO: 20% (**13**), 16% (**14**), 14% (**15**), 12% (**16**), and 6.5% (**17**).

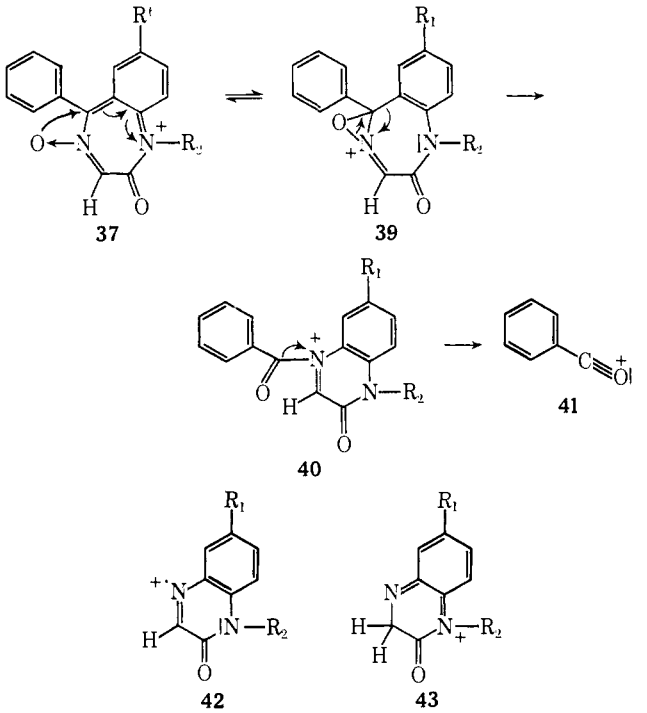


Species **38** underwent degradation by loss of O to the quinazoline **21**, which was generated in almost the same abundance as **38**. A strong peak was observed

(31) W. Metlesics, R. F. Tavares, and L. H. Sternbach, *J. Org. Chem.*, **30**, 1311 (1965).
(32) G. F. Field and L. H. Sternbach, *ibid.*, **33**, 4438 (1968).

in all spectra at $M^+ - 17$, whereas oxygen was eliminated only to a minor extent, in contrast to other N-oxides (compare ref 33). Intense metastable peaks supported the conclusion that the leaving group is an OH radical. Loss of OH would lead to structure **19**, which was discussed in group A. Further fragmentation was similar. However, the ratios of the peaks at $M^+ - H$, O, HCN and $M^+ - H$, O, CO were reversed with respect to group A, where the peak at $M^+ - H$, HCN was more abundant than $M^+ - H$, CO. This can be rationalized statistically, since $M^+ - H$, O, CO could be formed in two ways, *i.e.*, $M^+ - H$, $-CO$, $-O$ and $M^+ - OH$, $-CO$, whereas $M^+ - H$, O, HCN was generated only by $M^+ - OH$, $-HCN$.

Starting from species **37**, an orientation of the N-4 oxygen towards C-5 is feasible, yielding the oxazirane



39. The nitrone–oxazirane relation under the conditions of electron impact has been discussed in the case of benzoxazepines.[25] An analogous photoreaction of the nitrone chlordiazepoxide to an oxazirane was reported, which was reversible upon heating.[34] A simple rearrangement of species **39** would yield the **1**,4-quinazolinone **40**, which is the analog of a Beckmann rearrangement product. This type of rearrangement occurred upon prolonged irradiation of chlordiazepoxide[32] or by $POCl_3$ treatment of **13**.[35] Moreover, a fragmentation under the conditions of electron impact, similar to the Beckmann rearrangement, was suggested for benzophenone oxime giving rise to a benzoyl fragment (**41**) at *m/e* 105.[36] In fact, all spectra of the nitrones, including chlordiazepoxide, possessed a peak at *m/e* 105 with a relative abundance of 14–21%, having the empirical formula, C_7H_5O. This peak was lacking in the spectra of all other benzodiazepines. Homolytic cleavage seems to be unlikely in this case, and no peaks could be detected at mass units related to structure **42**. If the parent ion itself rearranged to a quinazolinone, homolytic cleavage to **43** would be reasonable. However, no evidence for this species was found. Thus it is conceivable that the rearrangement occurred only after loss of H from the parent ion.

In conclusion, it may be stated that the benzodiazepin-2-ones undergo several rearrangements to smaller ring systems under electron impact. Some of these rearrangements are analogous to regular chemical reactions described in the literature. All spectra showed characteristic peaks at *m/e* 205, 193, 177, 165, and 151. Differentiation of various substituents could be achieved following the primary fragmentation steps.

Experimental Section

Low and high resolution mass spectra were obtained with a A.E.I. MS-9 instrument at 70 eV using direct sample insertion. Empirical formulas of the fragment ions were determined by high resolution mass measurements using the peak matching technique. Mass measurements were accurate to within 3 ppm. Metastable transitions were determined by metastable scanning using the defocusing technique. The metastable scan was accurate within 0.3–1%, depending on the size and the shape of the metastable peaks. The benzodiazepines were prepared either by modification of **1**–**3** or by synthesis, *via* 2-aminobenzophenones and 1,3-quinazoline 3-oxides following known procedures.[4,37]

Acknowledgment.—I wish to thank Bill Garland for his valuable technical assistance.

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(34) L. H. Sternbach, B. A. Koechlin, and E. Reeder, *J. Org. Chem.*, **27**, 4671 (1962).
(35) S. C. Bell and S. J. Childress, *ibid.*, **29**, 506 (1964).
(36) P. Funke, K. G. Das, and A. K. Bose, *J. Amer. Chem. Soc.*, **86**, 2527 (1964).
(37) K.-H. Beyer and W. Sadée, *Arch. Pharm.* (Weinheim), **302**, 152 (1969).