

Arch. Int. Pharmacodyn. Ther., **193**, 213(1971).

(29) S. Posner, *Aerosol Age*, **5**, 39(1972).

(30) W. S. Harris, S. M. Kilen, G. J. Taylor, and S. Levitsky, *Circulation*, **44**, 119(1972).

(31) S. D. Silver, *J. Lab. Clin. Med.*, **31**, 1153(1964).

(32) D. A. Fraser, R. E. Bales, M. Lippman, and H. E. Stroking-er, "Exposure Chambers for Research Animal Inhalation," Public Health Monograph No. 57, U.S. Government Printing Office, 1959, pp. 34-36.

(33) B. Carnow, *Nat. Tuber. Resp. Dis. Ass.*, **4**, 8(1972).

(34) H. E. Swann, *Modern Packag.*, **6**, 58(1972).

(35) D. A. McClure, *Toxicol. Appl. Pharmacol.*, **22**, 265(1971).

(36) R. M. Burgison, W. E. O'Malley, C. K. Heisse, J. W. Forrest, and J. C. Krantz, *J. Pharmacol. Exp. Ther.*, **114**, 470(1955).

(37) L. Schamroth, "An Introduction to Electrocardiography," 4th ed., Blackwell Scientific Publications, Oxford, England, 1971, pp. 155, 156.

(38) A. C. Guyton, "Textbook of Medical Physiology," 3rd ed., W. B. Saunders, Philadelphia, Pa., 1966, p. 226.

(39) L. Schamroth, "An Introduction to Electrocardiography," 4th ed., Blackwell Scientific Publications, Oxford, England, 1971, pp. 69-77.

(40) *Ibid.*, pp. 199-201.

(41) "Principles and Procedures for Evaluating the Toxicity of Household Substances," National Academy of Sciences, National Research Council, Washington, D.C., 1964, p. 4.

(42) J. W. Clayton, D. B. Hood, M. S. Nick, and R. S. Waritz, *Amer. Ind. Hyg. Ass. J.*, **27**, 234(1966).

(43) H. J. Horn, *Proc. Sci. Sect. Toilet Goods Ass.*, **29**, 36(1958).

(44) R. G. Marks, *Prod. Manag.*, **1**, 21(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 28, 1973, from the *Departments of Pharmaceutical Sciences and Allied Health and Industrial Sciences, St. John's University, College of Pharmacy and Allied Health Professions, Jamaica, NY 11439*

Accepted for publication June 24, 1974.

Presented to the Pharmacology Section, APhA Academy of Pharmaceutical Sciences, San Diego, Calif., November 15, 1973.

Abstracted in part from a dissertation submitted by M. A. Dorato to the Graduate Faculty, St. John's University, in partial fulfillment of the Master of Science degree requirements.

The authors acknowledge the assistance of Walter Swimm and Ben Wolyniec during this investigation.

* To whom inquiries should be directed.

Mass Spectral Analysis of Medicinal Pyrazolidinediones

R. A. LOCOCK*, R. E. MOSKALYK, L. G. CHATTEN, and L. M. LUNDY*

Abstract □ For the facilitation of mass spectral analysis of medicinal pyrazolidinediones, fragmentation pathways of phenylbutazone, oxyphenbutazone, and sulfinpyrazone were established by means of deuterium labeling, metastable peaks, and accurate mass determinations. The major pathways are the McLafferty rearrangement of the molecular ion and formation of azobenzene and substituted azobenzene ions. The mass spectra of 1,2-diphenyl-3,5-pyrazolidinedione and the 4-methyl and 4,4-dimethyl derivatives are also discussed.

Keyphrases □ Pyrazolidinediones, medicinal—mass spectra, fragmentation pathways, mechanisms □ Phenylbutazone—mass spectrum, fragmentation pathways, mechanisms □ Oxyphenbutazone—mass spectrum, fragmentation pathways, mechanisms □ Sulfinpyrazone—mass spectrum, fragmentation pathways, mechanisms □ Mass spectroscopy—medicinal pyrazolidinediones, fragmentation pathways

Although medicinal pyrazolidinediones such as phenylbutazone have received wide attention in the past 20 years, only a few reports of their mass spectra have appeared recently (1-3). This technique was used for the characterization of degradation products of phenylbutazone (1, 2), but no details of the fragmentation pathways of phenylbutazone or the degradation products were described.

The fragmentation patterns of phenylbutazone, oxyphenbutazone, and their *O*-methyl and *C*-methyl derivatives were described (3), but that investigation did not include verification by evidence of metastable

ions, accurate mass determinations, or use of labeled derivatives.

This paper presents the mass fragmentation patterns of 1,2-diphenyl-3,5-pyrazolidinediones of medicinal importance: phenylbutazone, oxyphenbutazone, and sulfinpyrazone, as well as some simpler model derivatives. The establishment of characteristic mass spectral patterns and fragmentation schemes for these compounds may facilitate further characterization of pharmaceutical degradation products, metabolic studies, and forensic analysis of this important class of medicinals.

EXPERIMENTAL¹

All mass spectra were recorded on a mass spectrometer² at an ionizing potential of 70 ev. The samples were introduced *via* the direct probe and were vaporized at temperatures between 150 and 200°. Accurate mass measurements were made by the peak matching technique.

Phenylbutazone³ (I), Oxyphenbutazone³ (II), and Sulfinpyrazone³ (III)—Compounds I-III had literature melting points and gave NMR and IR spectra consistent with their structures. Official assays gave values in excess of 98% for each compound. They were used without further purification.

¹ IR spectra were taken on a Unicam SP 1000 IR spectrophotometer, and NMR spectra were recorded on a Varian A-60D spectrophotometer using tetramethylsilane as the internal standard. UV spectra were obtained on a Unicam SP 1800 UV spectrophotometer. Elemental analyses were determined by Mr. W. Dylke.

² AEI MS-9.

³ Gift of Geigy Pharmaceuticals.

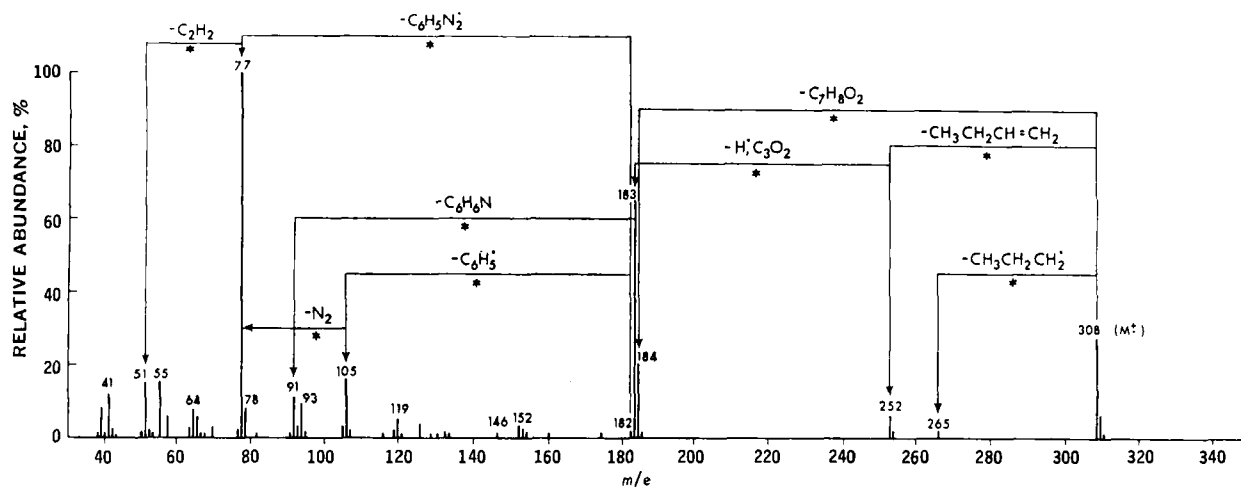


Figure 1—Mass spectrum of phenylbutazone.

1,2-Diphenyl-3,5-pyrazolidinedione (IV)—Compound IV was prepared following the method of Pesin *et al.* (4), mp 180–181° (ethanol) [lit. mp 173.5° (4) and 178° (5)]; IR (KBr): 1753 and 1722 ($\nu_{\text{C=O}}$) cm^{-1} ; NMR (CDCl_3): 2.68 (s, 10 p., phenyl) and 6.62 (s, 2 p., CH_2) τ .

4-Methyl-1,2-diphenyl-3,5-pyrazolidinedione (V)—Compound V was prepared by an adaptation of the method reported for phenylbutazone (6). Sodium metal (0.9 g) was dissolved in 30 ml of anhydrous ethanol, and 7.5 g of methyl diethylmalonate and 6.4 g of 1,2-diphenylhydrazine were added. The mixture was refluxed for 12 hr, the progress of the reaction being followed by TLC (hexane–ethyl acetate, 2:1). The alcohol was distilled off and the residue was heated at 130° overnight.

After cooling, 30 ml of water was added and the resulting mixture was filtered. The filtrate was made acidic with 10% HCl, and the resulting precipitate was collected and recrystallized from 10% ethanol, mp 115° [lit. (7) mp 114–116°]; IR (KBr): 1753 and 1725 ($\nu_{\text{C=O}}$) cm^{-1} ; NMR (CDCl_3): 2.65 (s, 10 p., phenyl), 6.61 (q, 1 p., C-4 H), and 8.43 (d, 3 p., C-4 methyl) τ .

4,4-Dimethyl-1,2-diphenyl-3,5-pyrazolidinedione (VI)—Compound VI was prepared by a modification of the method described for the methylation of phenylbutazone (6). To a solution of 1.5 g of V in 40 ml of 1% alcoholic potassium hydroxide solution was added 3.1 g of methyl iodide. The resulting solution was refluxed with stirring for 3 days, after which time the solution turned a dark brown and a precipitate had formed. The precipitate was

collected and recrystallized from 60% ethanol to give a white crystalline product, mp 134–135°; IR (KBr): 1755 and 1725 ($\nu_{\text{C=O}}$) cm^{-1} ; NMR (CDCl_3): 2.69 (s, 10 p., phenyl) and 8.52 (s, 6 p., C-4 methyl groups) τ .

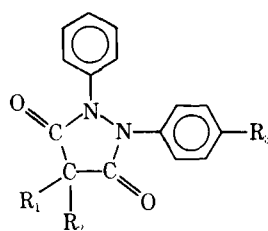
Anal.—Calc. for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.58; H, 6.02; N, 9.84.

The deuterated Compounds Ia and IVa were prepared by refluxing a solution of the parent compound in tetrahydrofuran containing deuterium oxide for 4 hr, followed by removal of the solvents *in vacuo*. The exchange was monitored by the disappearance of the C-4 proton absorption in the NMR.

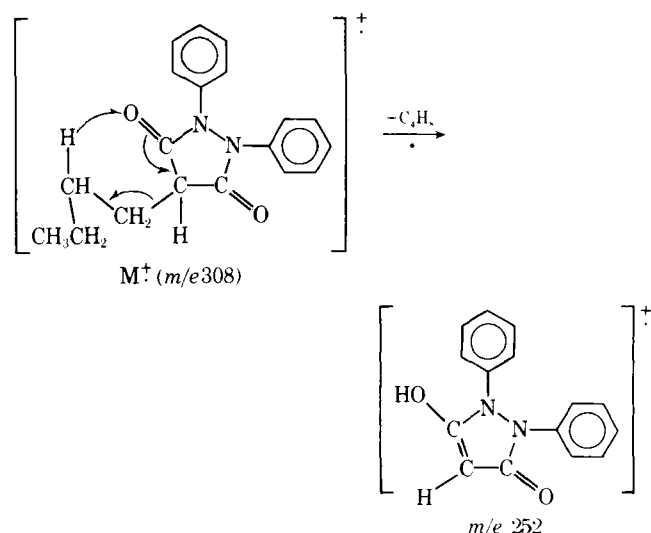
RESULTS AND DISCUSSION

The mass spectra of the pyrazolidinediones, I–VI, are reproduced in Figs. 1–6. As observed by Unterhalt (3), phenylbutazone, oxyphenbutazone, and sulfinpyrazone undergo the McLafferty rearrangement to give a radical ion at m/e 252 (m/e 268 in oxyphenbutazone). Metastable ions are present in all three spectra to indicate that this is a direct fragmentation from the molecular ion with the loss of the elements of butene or $\text{C}_6\text{H}_5\text{SOCHCH}_2$ for sulfinpyrazone. This fragmentation is presented in Scheme I for phenylbutazone.

A minor fragmentation pathway for the molecular ions of phenylbutazone and oxyphenbutazone, also substantiated by the presence of metastable ions, is the loss of a propyl radical from the butyl side chain of the molecular ion to give ions at m/e 265 (1.2%, phenylbutazone) or m/e 281 (3.2%, oxyphenbutazone). In the spectrum of sulfinpyrazone, strong peaks at m/e 279 (20.0%) and



compound	R ₁	R ₂	R ₃
phenylbutazone (I)	H	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2$	H
phenylbutazone deuterated (Ia)	D	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2$	H
oxyphenbutazone (II)	H	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2$	OH
sulfinpyrazone (III)	H	$\text{C}_6\text{H}_5\text{SOCH}_2\text{CH}_2$	H
1,2-diphenyl-3,5-pyrazolidinedione (IV)	H	H	H
1,2-diphenyl-3,5-pyrazolidinedione deuterated (IVa)	D	D	H
4-methyl-1,2-diphenyl-3,5-pyrazolidinedione (V)	H	CH_3	H
4,4-dimethyl-1,2-diphenyl-3,5-pyrazolidinedione (VI)	CH_3	CH_3	H



Scheme I—McLafferty rearrangement of phenylbutazone

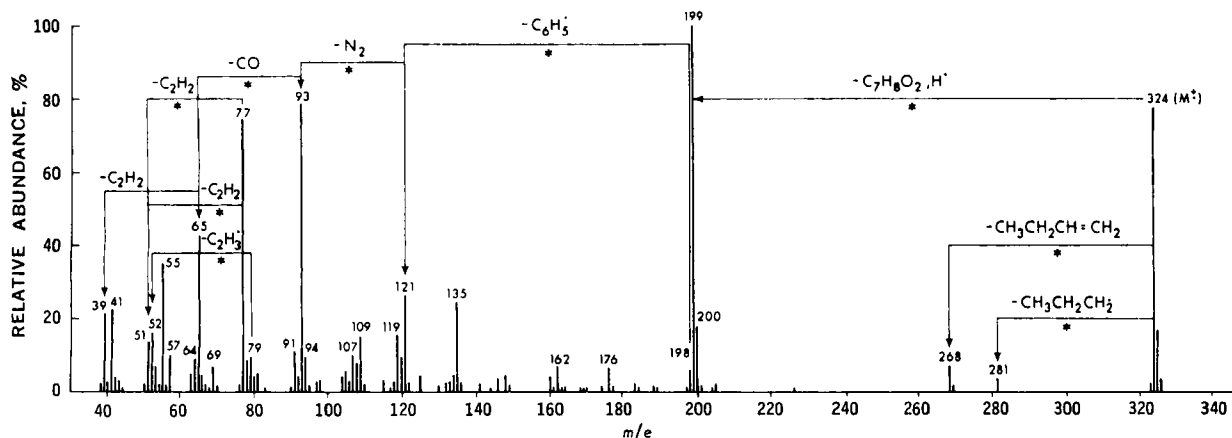


Figure 2—Mass spectrum of oxyphenbutazone.

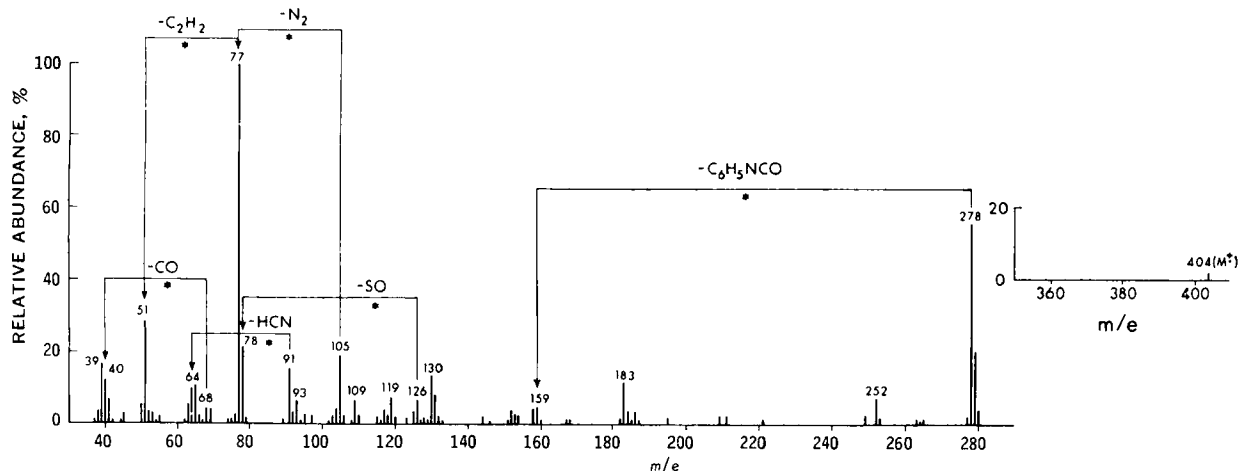


Figure 3—Mass spectrum of sulfinpyrazone.

at m/e 278 (55.4%) represent the loss of C_6H_5SO and C_6H_5SOH from the side chain of the sulfinpyrazone molecular ion. No metastable ion is present in the spectra for this fragmentation. An ion occurring at m/e 125 was identified as the phenylsulfoxide ion, $(C_6H_5SO)^+$, by accurate mass measurement (125.0041 measured, 125.0061 calculated).

The most characteristic fragments in the mass spectra of all pyrazolidinediones studied were a series of peaks at m/e 182, 183, and 184 (198, 199, and 200 in oxyphenbutazone). The relative abundances of these peaks are given in Table I.

The peak at m/e 182 can be attributed to the formation of the azobenzene radical ion, $(C_6H_5N_2C_6H_5)^+$. Metastable evidence for

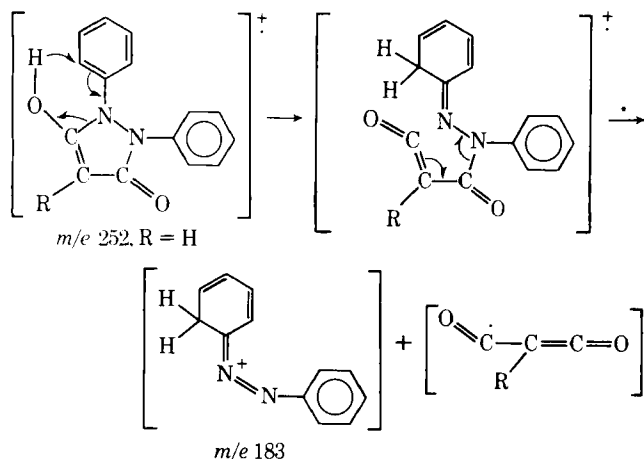
the origin of this ion could not be found in any spectra except that for Compound VI, in which the m/e 182 ion was formed from the molecular ion, M^+ (m/e 182) $\rightarrow$ m/e 182, (m^* observed, 118.4; m^* calculated, 118.3).

The ion at m/e 183 can originate from the molecular ion or the McLafferty rearrangement radical ion (m/e 252) by hydrogen transfer as depicted in Scheme II. Metastable ions at 133.0 (calculated 132.9) were present in the spectra of I and IV to indicate that the fragment ion at m/e 183 arose from the ion at m/e 252. In the spectrum of oxyphenbutazone, the equivalent peak, m/e 199, apparently is derived from the molecular ion, M^+ (m/e 324) $\rightarrow$ m/e 199 (m^* observed, 122.3; m^* calculated, 122.2). This is also the case in the spectrum of Compound V: M^+ (m/e 266) $\rightarrow$ m/e 183 (m^* observed, 126.0; m^* calculated, 125.9).

Table I—Relative Abundances (Percent) of Fragment Ions in the Range m/e 182–185

Compound	m/e 182 ^a	m/e 183 ^a	m/e 184 ^a	m/e 185
I	2.3	65.1	19.8	2.1
Ia	2.9	32.4	42.6	14.0
II	6.8	100.0	17.7	—
	(m/e 198)	(m/e 199)	(m/e 200)	—
III	1.9	11.2	3.2	—
IV	5.8	100.0	35.8	4.0
IVa ^b	1.1	7.2	70.4	13.7
V	4.1	50.0	6.8	—
		$C_{12}H_{11}N_2$	$C_{12}H_{12}N_2$	—
VI	14.4	3.7	—	—
	$C_{12}H_{10}N_2$	$C_{12}H_{11}N_2$		

^a Where formula followed percent relative abundance, the identity was established by accurate mass measurement. ^b This spectrum also contained an ion at m/e 186 (13.0%).



Scheme II—Mechanism for formation of ion at m/e 183

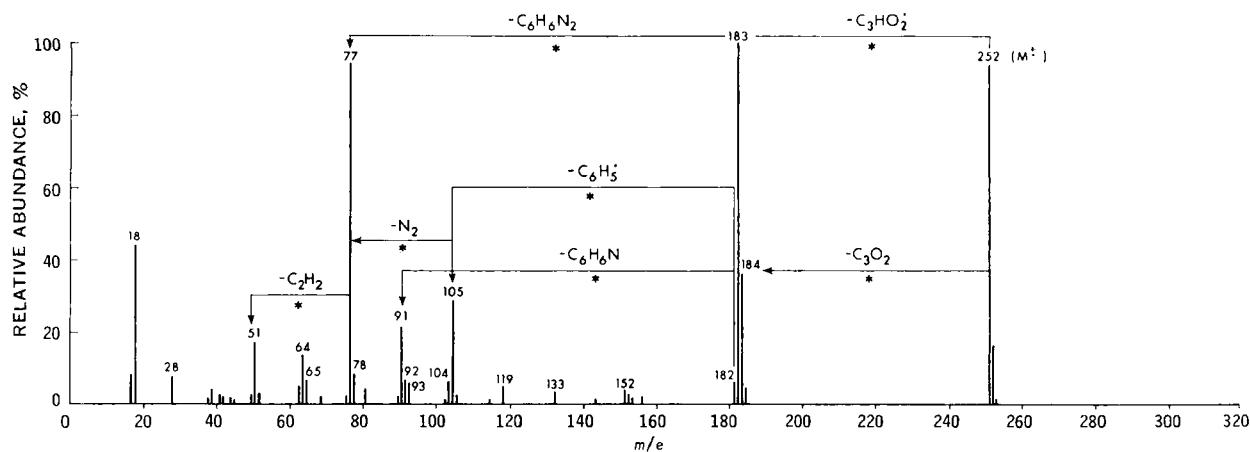


Figure 4—Mass spectrum of 1,2-diphenyl-3,5-pyrazolidinedione.

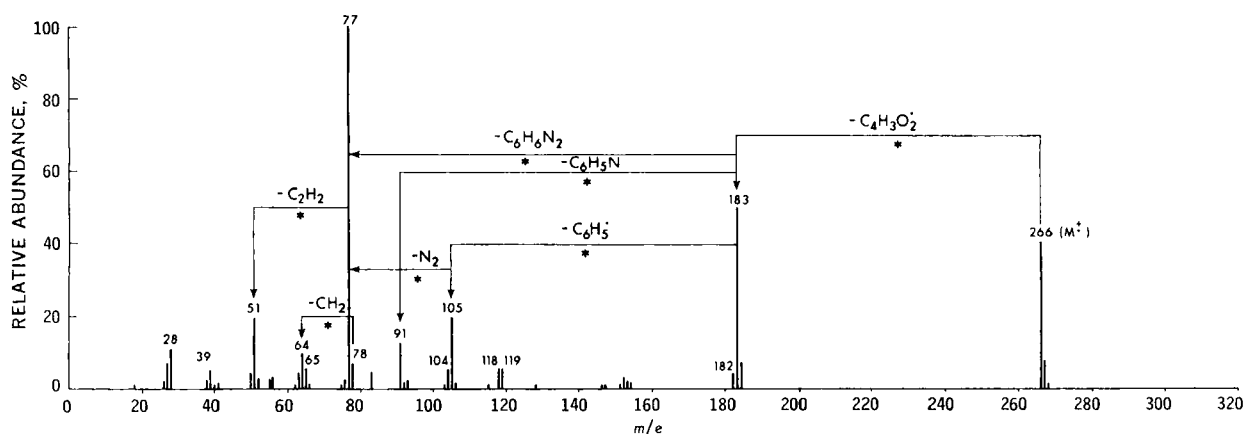
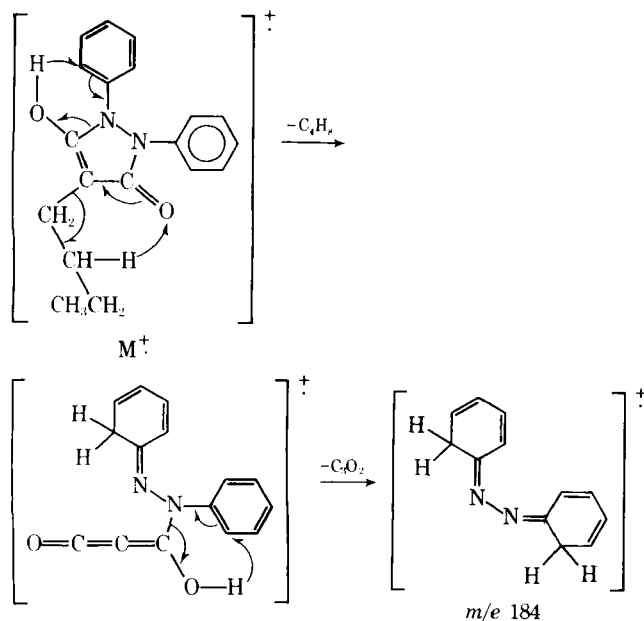


Figure 5—Mass spectrum of 4-methyl-1,2-diphenyl-3,5-pyrazolidinedione.

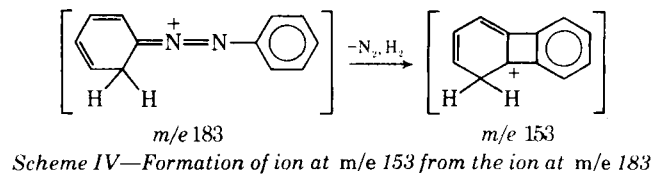
Compound VI was examined with the expectation that there would not be a peak at m/e 183, since neither enolization nor a McLafferty rearrangement is possible with this compound. Although it is only present to a small extent (3.7%), an accurate mass determination did establish that this ion had the composition $C_{12}H_{11}N_2$ and was not a simple isotope peak. It would appear that

intermolecular hydrogen transfer may be involved in the formation of this ion in the spectrum of VI.

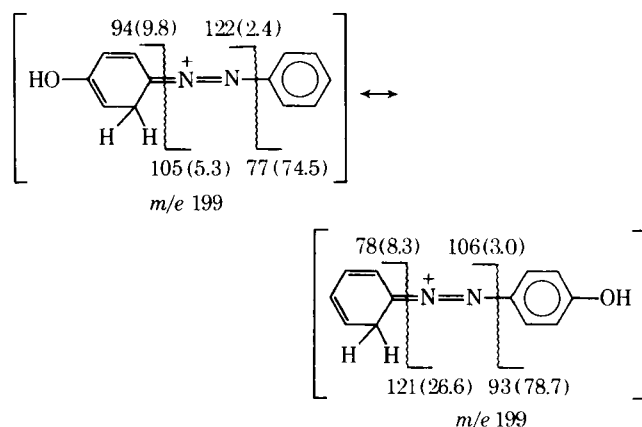
The ion at m/e 184 may arise from the transfer of two hydrogens to *ortho* positions of the aromatic rings (Scheme III). Metastable ions were present for the formation of this ion from the mo-



Scheme III—Formation of ion at m/e 184, $C_{12}H_{12}N_2$, from the molecular ion



Scheme IV—Formation of ion at m/e 153 from the ion at m/e 183



Scheme V—Ions arising from the m/e 199 ion

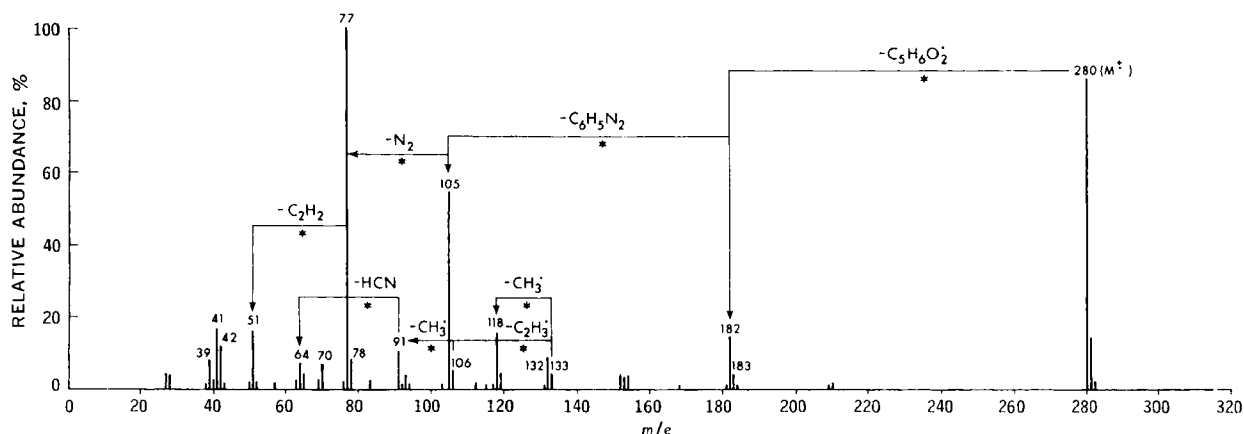


Figure 6—Mass spectrum of 4,4-dimethyl-1,2-diphenyl-3,5-pyrazolidinedione.

lecular ion in the spectra of I [M^+ , m/e 308 $\rightarrow$ m/e 184 (m^* observed, 110.0; m^* calculated, 109.9)] and IV [M^+ , m/e 252 $\rightarrow$ m/e 184 (m^* observed, 134.3; m^* calculated, 134.4)].

The peak at m/e 184 in the spectrum of V cannot be explained by Scheme III and, although of low relative abundance (6.8%), it did have the composition $C_{12}H_{12}N_2$. It may also arise from an intermolecular hydrogen transfer mechanism. There is no peak at m/e 184 in the spectrum of VI, as would be predicted, since there are no hydrogen atoms for transfer in the 4-position of the pyrazolidinedione nucleus.

Deuteration of phenylbutazone and 1,2-diphenyl-3,5-pyrazolidinedione yielded Ia and IVa, respectively. Peaks in the mass spectra of these deuterated derivatives are consistent with the mechanism given in Scheme III. Comparison of the peaks in the m/e 182–185 region in the spectra of I and Ia demonstrates that the peak at m/e 182 is almost unchanged in percent relative abundance upon deuteration. Deuteration decreases the intensity of the peak at m/e 183 and increases the intensity of the m/e 184 peak by almost exactly one-half, which may be explained by the observation that in the deuterated compound (Ia) there is now equal opportunity for the formation of $C_{12}H_{11}N_2$, m/e 183, and $C_{12}H_{10}DN_2$, m/e 184, from the McLafferty ion, m/e 252, or the enol form of the molecular ion. A significant peak at m/e 185 (14.0%) likewise corresponds to $C_{12}H_{11}DN_2$.

A similar comparison can be made for analogous peaks in the spectra of IV and IVa. A peak at m/e 186, 13.0% relative abundance, corresponding to $C_{12}H_{10}D_2N_2$, occurs in the mass spectrum of IVa.

Skeletal rearrangement ions of m/e 152, 153, and 154 were observed in all compounds studied. In oxyphenbutazone, these occur

Table II—Fragmentations Involving Loss of C_6H_5NCO as a Neutral Molecule

R_1	R_2	X	Compound (Relative Abundance, %)
H	H	133	IV (2.6)
D	D	135	IVa (2.2)
CH ₂	H	146	I (1.0)
		[162]	[II (6.8)]
CH ₂	D	147	Ia (0.7)
CH ₂ CH	H	159	III (4.2)
CH ₂ CH ₂	H	160	I (1.2), III (1.3)
		[176]	[II (3.7)]
CH ₂ CH ₂	D	161	Ia (0.6)

at m/e 168, 169, and 170. These ions may be assumed to be due to the loss of nitrogen and hydrogen from the azobenzene radical ion. Such rearrangement ions were described (8) in a study of mass spectra of azobenzenes. The peak at m/e 153 in all spectra probably arises from an equivalent rearrangement of the m/e 183 ion (Scheme IV).

Other ions in the spectra of the pyrazolidinediones that may arise from the azobenzene radical ion are the result of the loss of a phenyl radical, C_6H_5 , to give $C_6H_5N_2^+$, m/e 105, which subsequently may lose nitrogen to give $C_6H_5^+$, m/e 77. The azobenzene radical ion may also lose $C_6H_5N_2$ as a radical to give $C_6H_5^+$, m/e 77. These fragmentations were documented for azobenzene (8).

A series of analogous ions may be generated from the m/e 199 ion in the mass spectrum of II (Scheme V).

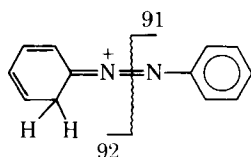
A fragmentation from the m/e 183 ion, which yields ions at m/e 92 and 91, may be due to the fission of the two nitrogen atoms (Scheme VI).

The ion of mass 91 (ionized phenyl nitrene) has been observed in the mass spectra of 3-phenyl-5-pyrazolone (9), substituted pyrazolone azomethine dyes (10), and 4-acyl-substituted 1-phenyl-3-methyl-5-pyrazolones (11). The $C_6H_5N^+$ ion, m/e 92, has also been observed in the mass spectra of substituted pyrazolones (11). In

Table III—Ions Resulting from Loss of CO after Loss of Neutral Phenyl Isocyanate

R_1	R_2	X (Relative Abundance, %)	Compound
H	H	105 (28.3) m^* obs. 82.9, calc. 82.9	IV
D	D	107 (3.0) m^* obs. 85.0, calc. 84.8	IVa
H	CH ₂	118 (2.5) m^* obs. 95.4, calc. 95.4	III
H	CH ₂	118 (5.4)	V
D	CH ₂	119 (5.4)	Ia
H	CH ₂ CH	131 (8.1)	III
H	CH ₂ CH ₂	148 (4.1)	II (p-OH)
H	CH ₂ CH ₂	132 (1.0)	I
D	CH ₂ CH ₂	133 (0.9)	Ia
CH ₃	CH ₃	133 (4.1)	VI ^a

^a m/e 133 in the spectrum of VI may lose H^+ to yield m/e 132 (8.7%), $C_9H_{10}N$ (132.0808 measured, 132.0813 calculated).



m/e 183

Scheme VI—Fragmentation of the *m/e* 183 ion

the spectrum of oxyphenbutazone, analogous peaks were seen at *m/e* 108 (7.6%), (C₆H₅NO)⁺, and at *m/e* 107 (11.2%), (C₆H₅NO)⁺.

A characteristic fragmentation of all pyrazolinediones studied was the loss of the elements of phenyl isocyanate either to form the radical ion (C₆H₅NCO)⁺, *m/e* 119 [(HOC₆H₅NCO)⁺, *m/e* 135 in the case of II] or as a neutral molecule. These pathways have been observed in the mass spectrum of aminopyrine (12). Fragmentations involving the loss of phenyl isocyanate as a neutral molecule are listed in Table II.

The ions (X, Table II) resulting from the loss of the elements of phenyl isocyanate as a neutral molecule may then lose CO to give ions C₆H₅NCR₁R₂ (Table III).

The remaining peaks in the low mass range, below *m/e* 100, are common to substituted aromatic systems, and the fragmentations observed are illustrated in Figs. 1–6.

REFERENCES

- (1) J. Slingsby and D. A. Zuck, *Can. J. Pharm. Sci.*, **7**, 115(1972).
- (2) D. V. C. Awang, A. Vincent, and F. Matsui, *J. Pharm. Sci.*, **62**, 1673(1973).

- (3) B. Unterhalt, *Arch. Pharm.*, **305**, 334(1972).
- (4) V. G. Pesin, A. M. Khaletskii, and T. Jun-Hsiang, *J. Gen. Chem. USSR*, **28**, 2841(1958).
- (5) J. Büchi, J. Ammann, R. Lieberherr, and E. Eichenberger, *Helv. Chim. Acta*, **36**, 75(1953).
- (6) A. M. Khaletskii, V. G. Pesin, and T. Jun-Hsiang, *J. Gen. Chem. USSR*, **28**, 2393(1958).
- (7) H. Stenzl, U.S. pat. 2,562,830; through *Chem. Abstr.*, **47**, 1191(1953).
- (8) J. H. Bowie, G. E. Lewis, and R. G. Cooks, *J. Chem. Soc., B*, **1967**, 621.
- (9) J. M. Desmarchelier and R. B. Johns, *Org. Mass. Spectrom.*, **2**, 697(1969).
- (10) D. P. Maier, G. P. Happ, and T. H. Regan, *ibid.*, **2**, 1289(1969).
- (11) E. Larsen, I. H. Qureshi, and J. Møller, *ibid.*, **7**, 89(1973).
- (12) W. Arnold and H. F. Grutzmacher, *Z. Anal. Chem.*, **247**, 179(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 30, 1974, from the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7

Accepted for publication June 21, 1974.

Presented at the 21st Annual Canadian Conference on Pharmaceutical Research, Ottawa, Canada, 1974.

Supported in part by Grant MA 3078 from the Medical Research Council of Canada.

* Recipient of a Medical Research Council of Canada Summer Scholarship.

* To whom inquiries should be directed.

Kinetics of Dehydration of Epitetracycline in Solution

BETTY-ANN HOENER, THEODORE D. SOKOLOSKI*, LESTER A. MITSCHER, and LOUIS MALSPEIS

Abstract □ The dehydration kinetics of epitetracycline in solution to form epianhydrotetracycline were studied using UV and visible spectrophotometry. The reaction was found to be first order with respect to epitetracycline and hydronium-ion concentrations. The activation energy for the reaction was 28.3 kcal/mole at pH 2.0 in 0.1 M potassium chloride solutions. Dehydration of epitetracycline at pH 2.0 and 70° was found to be slower than that for tetracycline under similar solution conditions, although the activation energy for both reactants is essentially the same. This result is explicable on the basis of conformational differences in the molecules. This paper represents a portion of studies of the rates of various degradation reactions of tetracycline that lead to the toxic material epianhydrotetracycline.

Keyphrases □ Epitetracycline—dehydration kinetics in solution, activation energy, UV and visible spectrophotometry □ Dehydration—epitetracycline to epianhydrotetracycline kinetics in solution, activation energy, UV and visible spectrophotometry □ Epianhydrotetracycline—formation from epitetracycline, dehydration kinetics in solution, activation energy, UV and visible spectrophotometry □ Kinetics, dehydration—epitetracycline in solution

Studies show that commercially available tetracycline products contain significant amounts of degradation products of the antibiotic (1–3). This might be expected because tetracyclines can degrade through

at least four different pathways: epimerization, dehydration, hydrolysis, and oxidation. Since the first two reactions are the most commonly encountered, they have been of specific interest for study. In solution at acid pH, two pathways connect tetracycline and 4-epianhydrotetracycline, as shown in Scheme I.

Epimerization about carbon-4 in tetracycline leads to inactive, nontoxic epitetracycline (4). The kinetics of this reaction were studied by other workers (4–7). Epimerization, which is a reversible first-order process, occurs most rapidly between pH 3 and 5. Dehydration and aromatization of the C-ring of tetracycline follow pseudo-first-order kinetics, leading to anhydrotetracycline, which is inactive *in vivo* and nontoxic. This reaction occurs in solution at very low pH (8) and in the solid state under thermal conditions (9).

There are two important steps in the overall degradation of tetracycline, whose kinetic characteristics have not as yet been studied in solution or in the solid state. These are the epimerization of anhydrotetracycline and the dehydration of epitetracycline. Both reactions lead to the inactive, but toxic, epi-