Mass Spectra of Ubiquinones and Ubiquinols[1]

R. F. Muraca, J. S. Whittick, G. Doyle Daves, Jr., Palle Friis,[2] and Karl Folkers

Contribution from the Stanford Research Institute, Menlo Park, California.
Received November 21, 1966

Abstract: Mass spectra for eight ubiquinones ranging in molecular weight from 250 to 862 have been obtained. These spectra reveal a highly characteristic fragmentation pattern; the dominant feature is the appearance of an intense peak at *m/e* 235 due to the formation of a highly stabilized cyclohexadienone-1-pyrylium ion. The region between *m/e* 235 and the peak owing to the parent ion reveals a rhythmic pattern of low-intensity peaks characteristic of the breakdown of the polyisoprenoid side chain. An additional feature of each spectrum is the presence of peaks (most notably at *m/e* 197 and at M + 2) arising from the hydroquinone species.

The indispensable role of ubiquinone, Q_n (I), in biochemical electron transfer and coupled oxidative phosphorylation has been recognized,[3] and considerable effort[4-7] has been directed toward the isolation and structural elucidation of such naturally occurring quinones and their biosynthetic precursors. Limited stabilities and small quantities of isolated substances have often made structural determinations difficult. High molecular weights and low volatility have severely limited the role of mass spectrometry in these studies. Recently, advances in instrumentation have made possible the study of such high molecular weight polyisoprenoid quinones by mass spectrometry. Mass spectral data for plastoquinone-3 were reported by Misiti, *et al.*,[5] and data for plastoquinone-9 and plastoquinones B, C, and D have since been described.[8] A detailed investigation of the mass spectra of vitamin $K_{1(20)}$ and various derivatives has appeared.[9] Bohlmann and Kleine[6] reported mass spectral data for 2-(3′-methyl-2′-butenyl)-1,4-benzoquinone, a metabolite of *Phagnalon saxitale* Cass. Other studies are concerned with the mass spectra of nonisoprenoid benzoquinones[10-12] and naphthoquinones.[13]

The mass spectra of a series of eight ubiquinones have now been obtained.[13a] Examination of these spectra has revealed a characteristic fragmentation pattern. This knowledge has been significant in the structural elucidation of precursors in a complete biosynthetic sequence from *p*-hydroxybenzoic acid to ubiquinone.[14]

Results and Discussion

The mass spectra of each of the ubiquinones studied (I, n = 0, 1, 2, 4, 5, 6, 7, 9) are similar and relatively simple. The spectra of Q_3 and Q_{10}, shown in Figure 1, are representative. The characteristic feature in each spectrum is a prominent peak at *m/e* 235, and a surprising lack of intense peaks between *m/e* 235 and the parent mass (M). The region between *m/e* 235 and M reveals a characteristic rhythmic pattern of extremely weak peaks due to successive loss of isoprene units ($M - 69 - (68)_n$). Peak clusters are evident for each side-chain carbon atom; peak clusters corresponding to the loss of an entire isoprene group are more intense than peaks corresponding to fragments resulting from cleavage within an isoprene unit. This rhythmic pattern is evident in the spectra of the ubiquinones and the plastoquinones[8] and is particularly striking in the spectra of the polyisoprenoid alcohols.[15]

High-resolution mass measurement of the intense peak at *m/e* 235 indicated that the ion corresponds to an elemental composition of $C_{13}H_{15}O_4$ (Table I). This ion is assigned as the 7,8-dimethoxy-2,5-dimethyl-6-cyclohexadienone-1-pyrylium ion (II) which possesses exceptional stability owing to extensive charge delocalization.

Table I. High-Resolution Mass Measurement for Ubiquinone-6

m/e	Measured mass	Assignment
235	235.0982	$C_{13}H_{15}O_4$
197	197.0796	$C_{10}H_{13}O_4$
135	135.0465	$C_8H_7O_2$
	135.0823	$C_9H_{11}O$
	135.1200	$C_{10}H_{15}$
81	81.0698	C_6H_9
69	69.0704	C_5H_9
66	66.0475	C_5H_6

It has been noted[12] that the mass spectra of nonisoprenoid methoxybenzoquinones exhibit a diversity of fragmentation patterns. However, for polyisoprenoid quinones the preference for the formation of the py-

---

[1] (a) Coenzyme Q. LXXXIII. (b) The nomenclature used in this paper is based on a recommendation of an IUPAC-IUB Commission of Biochemical Nomenclature, *Biochim. Biophys. Acta*, **107**, 5 (1965).

[2] The Royal Veterinary and Agricultural College, Copenhagen, Denmark.

[3] See: "Quinones in Electron Transport," G. E. W. Wolstenholme and C. M. O'Connor, Ed., J. and A. Churchill, Ltd., London, 1961; "Biochemistry of Quinones," R. A. Morton, Ed., Academic Press Inc., New York, N. Y., 1965.

[4] D. Misiti, H. W. Moore, and K. Folkers, *Biochemistry*, **4**, 1156 (1965).

[5] D. Misiti, H. W. Moore, and K. Folkers, *J. Am. Chem. Soc.*, **87**, 1402 (1965).

[6] F. Bohlmann and K. M. Kleine, *Ber.*, **99**, 855 (1966).

[7] N. Eck and A. Trebst, *Z. Naturforsch.*, **18B**, 446 (1963).

[8] B. C. Das, M. Lounasmaa. C. Tendille, and E. Lederer, *Biochem. Biophys. Res. Commun.*, **21**, 318 (1965); W. T. Griffiths, *ibid.*, **25**, 596 (1966).

[9] S. J. Di Mari, J. H. Supple, and H. Rapoport, *J. Am. Chem. Soc.*, **88**, 1226 (1966).

[10] J. H. Beynon, G. R. Lester, and A. E. Williams, *J. Chem. Phys.*, **63**, 1861 (1959).

[11] J. H. Beynon and A. E. Williams, *Appl. Spectry.*, **14**, 156 (1960).

[12] J. H. Bowie, D. W. Cameron, R. G. F. Giles, and D. H. Williams, *J. Chem. Soc., Inorg. Sect.*, 335 (1966).

[13] J. H. Bowie, D. W. Cameron, and D. H. Williams, *J. Am. Chem. Soc.*, **87**, 5094 (1965).

[13a] NOTE ADDED IN PROOF. See also H. Morimoto, T. Shima, I. Imada, M. Sasaki, and A. Onchida, *Ann. Chem.*, in press. We thank Dr. Morimoto and his co-workers for a copy of this manuscript prior to publication.

[14] P. Friis, G. D. Daves, Jr., and K. Folkers, *J. Am. Chem. Soc.*, **88**, 4754 (1966).

[15] R. F. Muraca and J. S. Whittick, unpublished data.

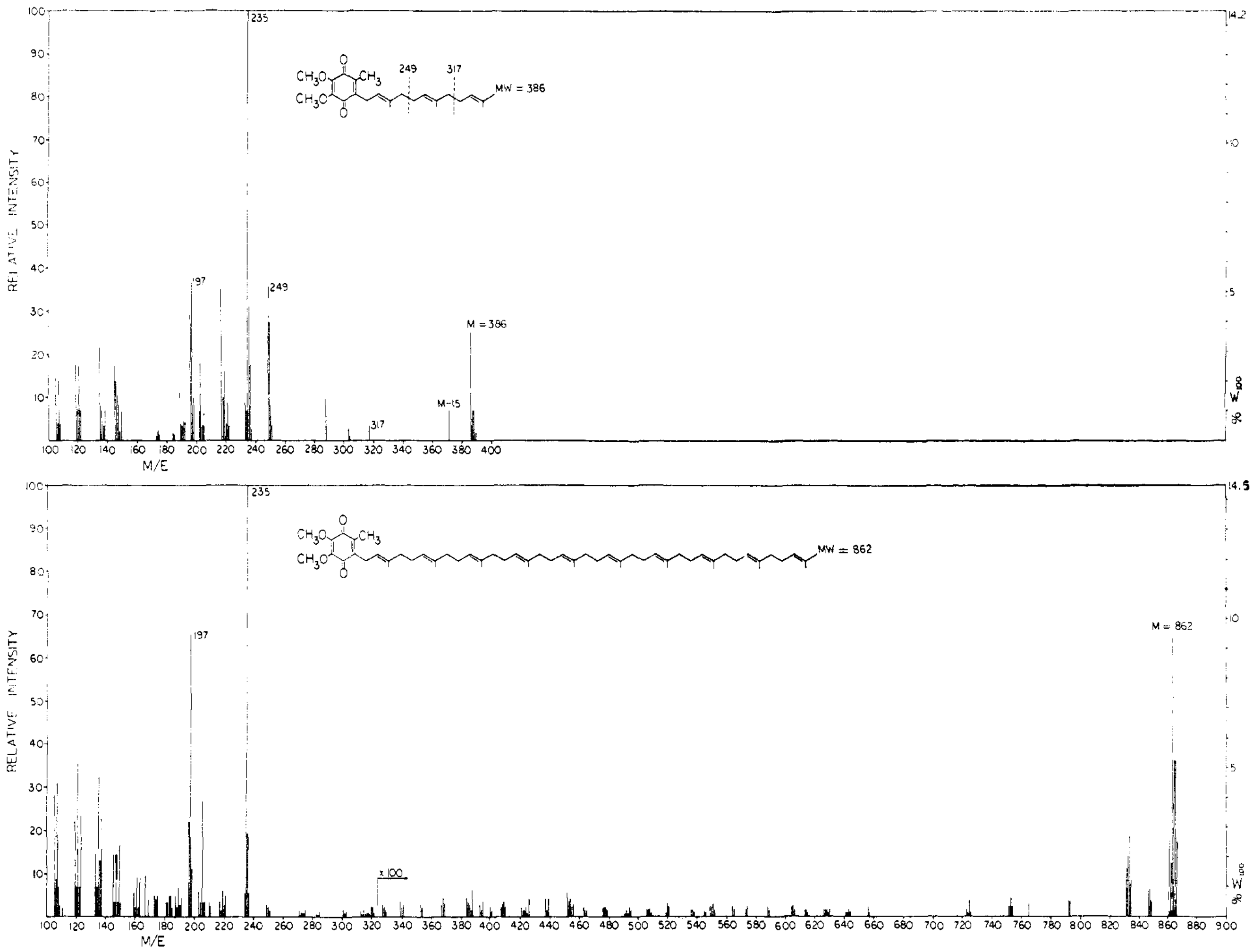
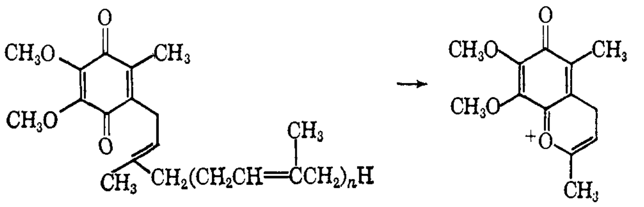


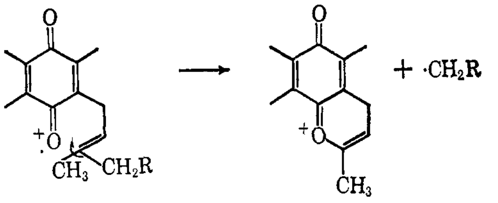
Figure 1. Mass spectra of ubiquinones 3 and 10.

rylium ion (III) is pronounced. A peak due to this species will presumably be a prominent feature of the mass spectra of all isoprenoid quinones. Accordingly,



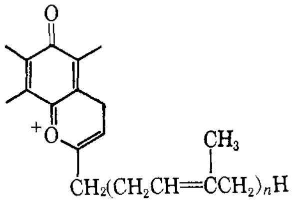
I II

the base peak in the spectrum of each of the compounds in the ubiquinone (I) and plastoquinone[5,8] series, of vitamin $K_1(_{20})$,[9] and of 2-(3′-methyl-2′-butenyl)-1,4-benzoquinone[6] arises from a common fragmentation in which the bond δ to the ring is cleaved with separation of the side chain and subsequent ring closure to form the generic pyrylium ion III.



III

Cleavage of the alternative δ bond with expulsion of a methyl radical would produce the pyrylium ion IV.[16] In the spectra of the ubiquinones, the peak at M − 15 is very small; the relative intensities of the peaks at *m/e* 235 and at M − 15 are clearly in accord with the known strong preference for expulsion of a primary alkyl radical rather than a methyl radical.[9,17]



IV (M−15)

The spectrum of ubiquinol-6 (V, n = 5) (Figure 2) is characterized by a single intense peak (*m/e* 197) due to a ring fragment and a lack of intense peaks at higher *m/e* values. The peak at *m/e* 197 corresponds to an ion ($C_{10}H_{13}O_4$, Table I), which can be represented as the benzylium ion VI.

---

[16] The observation[9] of an additional M − 18 peak in a study using 2-methyl-d_3-3-phytyl-1,4-naphthoquinone indicates that two processes, loss of ring and side-chain methyls, are contributing to the M − 15 peak in the spectrum of vitamin $K_{1(20)}$.

[17] F. D. Greene, M. C. Savitz, F. D. Osterholtz, H. H. Lau, W. N. Smith, and P. M. Zanet, *J. Org. Chem.*, **28**, 55 (1963).

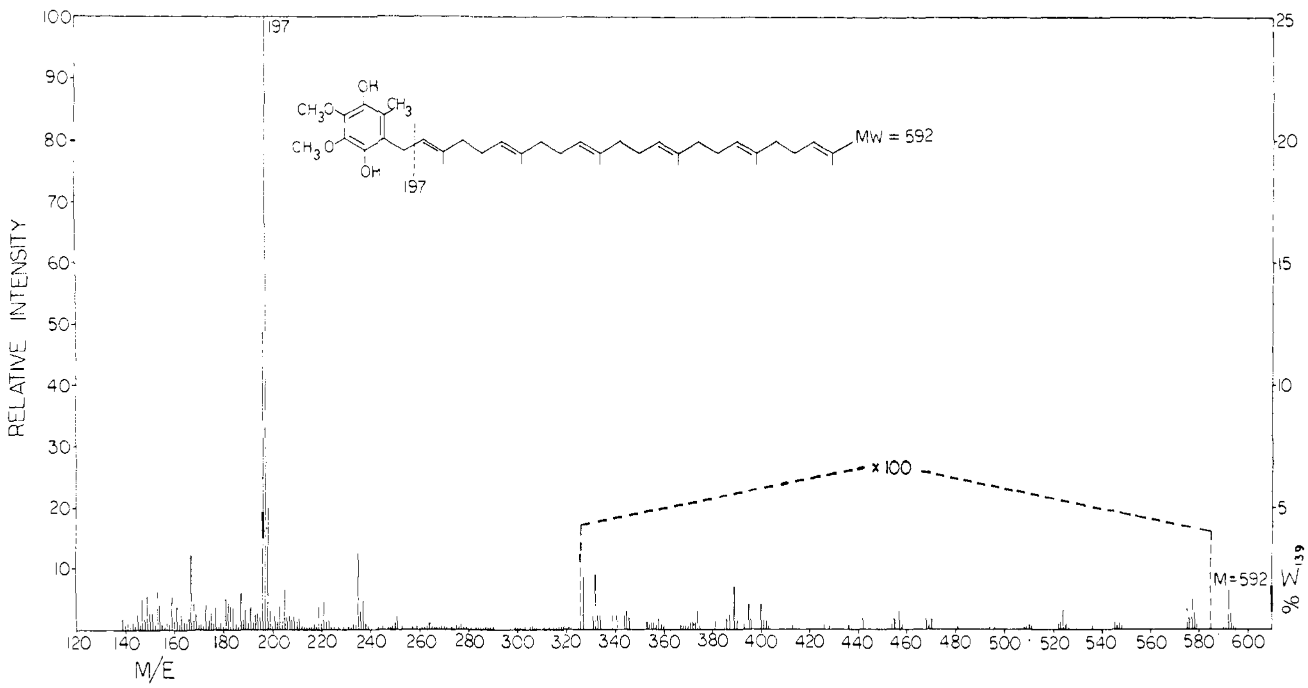
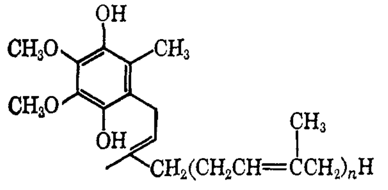
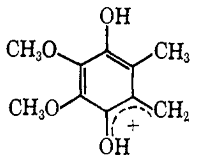


Figure 2. Mass spectrum of ubiquinol-6.

Comparison of the spectra in Figures 1 and 2 shows that none of the ubiquinones has been found to be free of their corresponding hydroquinones V when examined in the mass spectrometer. The mass spectra of the plastoquinones also exhibit peaks at M + 2, and because the intensities of these peaks increased with time, the suggestion was made[6] that the corresponding hydroquinones are present owing to dismutation reactions occurring in the mass spectrometer. In the present study, the peaks at M + 2 in the spectra of the ubiquinones showed little tendency to change with time.[15]

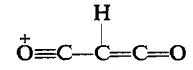


V



VI *m/e*197

Accurate masses were obtained for selected fragment ion peaks at *m/e* values below 235 (Table I). Absence of oxygen from ions of masses below 100 indicates that these ions are derived from the isoprenoid side chain. It is particularly pertinent that the peak at *m/e* 69 is due entirely to the hydrocarbon species C_5H_9; no contribution from ion VII was noted. Since fragmentation with the formation of ion VII is general for nonisoprenoid methoxybenzoquinones[12] it is evident that few correlations can be made between fragmentation patterns of the ubiquinones (I) and nonisoprenoid quinones.



$$\overset{+}{O}{\equiv}C{-}\overset{\overset{H}{|}}{C}{=}C{=}O$$

VII, *m/e* 69

Instrumentation. A Model 21-103C mass spectrometer of the Consolidated Electrodynamics Corp. (CEC) was used. It was modified to provide an increased magnetic field, an electron-multiplier–Wien-filter detection system, and 1-mm slits to permit unit resolution at *m/e* values as high as 1000. A special recording system permitted measurement of molecular ions to within ±0.005 amu. The sampling system was modified to permit direct sample introduction by means of a heated probe. A Model 110B mass spectrometer (CEC) was used with direct introduction of samples at a resolution of approximately 15,000–20,000 to measure the masses of fragment ions in the spectra. The probe temperature used for the ubiquinones are shown in Table II.

Table II. Mass Spectra of Ubiquinones

Ubiquinone	Mol wt	Probe temp, °C
Q_1	250	35
Q_2	318	75
Q_3	386	125
Q_5	522	145
Q_6	590	145
Q_7	658	170
Q_8	726	190
Q_{10}	862	300

Materials. Samples of Q_1, Q_2, and Q_3 were synthesized.[18] Purification was achieved by thin layer chromatography on silica gel G plates developed in chloroform–benzene (1:1) followed by thin layer chromatography on alumina plates developed in hexane–acetone (19:1).

Q_5 and Q_8 were isolated from *E. coli*,[19] Q_6 was iso-

---

[18] O. Isler, R. Rüegg, A. Langemann, P. Schudel, G. Ryser, and J. Würsch in "Ciba Foundation Symposium on Quinones in Electron Transport," G. E. W. Wolstenholme, and C. M. O'Connor, Ed., J. and A. Churchill, Ltd., London, 1961, pp 79–96.

[19] P. Friis, G. D. Daves, Jr., and K. Folkers, *Biochem. Biophys. Res. Commun.*, 24, 252 (1966).

lated from *Saccharomyces cerevisiae*,[20] Q_7 was isolated from Torula yeast,[21] and Q_{10} was isolated from *Rhodospirillum rubrum*.[14] The isolated samples were purified by thin layer chromatography as described previously.[19]

Ubiquinol-6 was prepared from rigorously purified Q_6 as follows. An ether solution of 10 mg of Q_6 was vigorously shaken with an aqueous solution of sodium hydrosulfite. When the color in the organic layer was completely discharged, this layer was carefully removed and evaporated under a stream of nitrogen. The sample was maintained under nitrogen until used, and was then introduced into the spectrometer with a minimum of handling.

Acknowledgment. This research was partially supported by funds from the Merck Sharp and Dohme Research Laboratories, Rahway, N. J. We also express our appreciation to Dr. Max Tishler.

[20] R. L. Lester, F. L. Crane, and Y. Hatefi, *J. Am. Chem. Soc.*, **80**, 4751 (1958).

[21] J. Stevenson, P. J. Hayward, F. W. Hemming, and R. A. Morton, *Nature*, 1291 (1962).

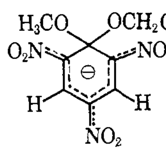
Nuclear Magnetic Resonance Studies of Meisenheimer Complexes

Kenneth L. Servis[1]

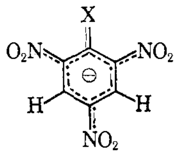
Contribution from the Department of Chemistry, University of Southern California, Los Angeles, California 90007. *Received September 14, 1966*

Abstract: The addition of methoxide to 1-substituted 2,4,6-trinitrobenzenes has been shown to occur both at the 1 and 3 positions in dimethyl sulfoxide solvent. Similarly, mono-N-substituted 2,4,6-trinitroanilines react both by NH proton loss and by addition at the 3 position. Diethylamine has been shown to react with either 2,4,6-trinitrobenzene or 2,4,6-trinitroanisole in a 2:1 molar ratio to give an ammonium salt of a Meisenheimer type complex. An unusual zwitterionic compound was prepared by the addition of triethylamine to 2,4,6-trinitroanisole.

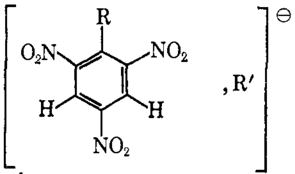
The structures of the highly colored complexes obtained from the reaction of aromatic nitro compounds with bases have been of interest to chemists for more than 80 years.[2] Despite numerous investigations the structures of many of these compounds remain unsettled.[3] The structure (**1**) proposed by Meisenheimer[4] has been extensively used as a model for the occasionally detectable intermediate[5] in nucleophilic aromatic substitution reactions.[6] In addition, many of the polynitroaromatics have been of interest as acidity indicators where anions of structure **2** are presumed to be formed.[7] Formation of charge-transfer complexes **3** have been proposed to account for the frequent observation of rapidly formed transient intermediates.[8] Direct ionization to form the phenyl anion, **4**, has been suggested to explain the erratically reported[9] deuterium exchange into nitrobenzenes upon treatment with base. Treatment of *m*-dinitrobenzene with base leads to the well-characterized radical anion **5**;[10] similar intermediates have been proposed in the reactions of trinitroaromatics.[11] Zwitterionic intermediates such as **6** have also been proposed to account for the reaction of polynitroaromatics with amine bases.[8a,12]



1

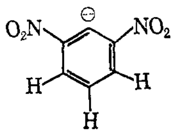


2

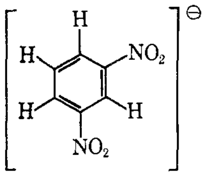


charge−transfer complex

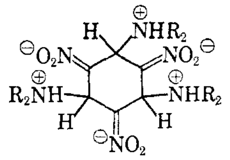
3



4



5



6

---

[1] The author gratefully acknowledges support by a grant from the Petroleum Research Fund of the American Chemical Society (PRF-563-G1).

[2] P. Hepp, *Ann. Chem.*, **215**, 345 (1882).

[3] For a review see J. Kapecki, University of Illinois Seminar Abstracts, 1965–1966, p 154; or R. Foster and C. H. Fyfe, *Rev. Pure Appl. Chem.*, **16**, 61 (1966).

[4] J. Meisenheimer, *Ann.*, 323, 205 (1902).

[5] J. F. Bunnett and J. J. Randall, *J. Am. Chem. Soc.*, **80**, 6020 (1958).

[6] For recent reviews see: (a) R. Huisgen and J. Sauer, *Angew. Chem.*, 72, 91, 294 (1960); (b) S. D. Ross, *Progr. Phys. Org. Chem.*, 1, 31 (1963).

[7] R. Stewart, J. P. O'Donnell, D. J. Cram, and B. Rickborn, *Tetrahedron*, **18**, 917 (1962); R. Stewart and J. P. O'Donnell, *J. Am. Chem. Soc.*, **84**, 493 (1962).

[8] (a) R. Foster, *J. Chem. Soc.*, 3508 (1959); (b) R. Foster and R. K. Mackie, *ibid.*, 3843 (1962); (c) R. E. Miller and W. F. K. Wynne-Jones, *ibid.*, 2375 (1959); (d) J. B. Ainscough and E. F. Caldin, *ibid.*, 2528, 2540, 2546 (1956); (e) ref 6b, p 44; (f) C. R. Allen, A. J. Brooks, and E. F. Caldin, *J. Chem. Soc.*, 2171 (1961).

[9] (a) R. J. Pollitt and B. C. Saunders, *Proc. Chem. Soc.*, 176 (1962); (b) V. Baliah and B. Ramakrishnan, *Rec. Trav. Chim.*, **87**, 783 (1959); (c) R. Foster and R. K. Mackie, *Tetrahedron* **19**, 691 (1963); (d) J. A. A. Ketelaar, A. Beer, and H. T. Vlaar, *Rec. Trav. Chim.*, 73, 37 (1954); (e) M. R. Crampton and V. Gold, *J. Chem. Soc., Phys. Org.*, 498 (1966).

[10] G. A. Russell, E. G. Janzen, and E. T. Strom, *J. Am. Chem. Soc.*, **86**, 1807 (1964); P. H. Rieger and G. K. Fraenkel, *J. Chem. Phys.*, **39**, 609 (1963).

[11] (a) Reference 8b; (b) R. Foster and R. K. Mackie, *Tetrahedron*, **16**, 119 (1961).

[12] G. N. Lewis and G. T. Seaborg, *J. Am. Chem. Soc.*, **62**, 2122 (1940); M. M. Labes and S. D. Ross, *J. Org. Chem.*, **21**, 1049 (1956).