Mass Spectra of Naphthoquinones. Vitamin $K_{1(20)}$[1]

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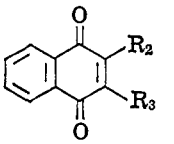
Abstract: In order to establish a firm basis for the mass spectral analysis of naphthoquinones of the vitamin K type, especially isotopically substituted analogs, a detailed examination has been made of the mode of fragmentation of 2-methyl-3-phytyl-1,4-naphthoquinone (vitamin $K_{1(20)}$) upon electron impact. To aid in the interpretation of the spectrum, nine model quinones were studied. These included four deuterium-labeled compounds (2-methyl-d_3-1,4-naphthoquinone, 2-methyl-d_3-3-phytyl-1,4-naphthoquinone, 2-methyl-3-phytyl-α-d_2-1,4-naphthoquinone, and 2-methyl-3-phytyl-1,4-naphthoquinone-5,6,7,8-d_4) prepared by application of known synthetic procedures. Through correlation of the spectra obtained from these models, structures for the major fragments found in the spectrum of vitamin $K_{1(20)}$ have been proposed.

Interest in the quinone as an important entity in oxidative and photosynthetic phosphorylation[3] has stimulated investigation into the distribution of this compound class in biological materials. To keep pace with this broadening search for new natural quinones, several diagnostic tools designed to detect and identify them have been applied and refined, including paper chromatography,[4] thin layer chromatography,[5] and ultraviolet,[6] infrared,[7] and nuclear magnetic resonance[8] spectroscopy. However, considering the severe limitation on the quantity of material usually available and the extent of structural information desired, the ideal tool, mass spectrometry, has been largely neglected. In part, this neglect stems from the high molecular weights and the complexity of the spectra of these vitamin K type quinones.

Since the exploratory work on the fragmentation of simple benzo- and polynuclear quinones,[9] the use of mass spectrometry for quinone structure analysis has been restricted largely to simply alkylated compounds such as piloquinone[10] and harungarin.[11] A quinone more representative of the polyisoprenoid-substituted varieties present in most organisms has also been subjected to mass spectral analysis;[12] in this instance, however, the mass spectrum was used merely as supplementary data, yielding mainly the molecular weight; no attempt was made to deduce structural information from the fragmentation pattern.

Our study was undertaken in an attempt to extend the utility of mass spectral analysis to the elucidation of quinone structure through the characterization of the fragments formed upon electron impact. The initial quinone chosen for this study was 2-methyl-3-phytyl-1,4-naphthoquinone (vitamin $K_{1(20)}$) both for its common occurrence in natural materials and for its relative simplicity of structure, facilitating the synthesis of isotopically labeled and unlabeled analogs.

Syntheses. To aid in the structural rationalizations for the various fragments found in the mass spectrum of vitamin $K_{1(20)}$, a series of ten compounds in addition to $K_{1(20)}$ itself (VIII) was prepared and subjected to mass spectral analyses. These consisted of mono- (I–IV) and di- (V–XI) alkyl and alkenyl 1,4-naphthoquinones. The compounds chosen for study offered useful structural variations and specific labeling with deuterium for fragment identification, and included three deuterio analogs (IX, X, XI) of vitamin $K_{1(20)}$ (VIII).



R_3 = H

I, R_2 = CH_3
II, R_2 = CD_3
III, R_2 = phytyl ($C_{20}H_{39}$)
IV, R_3 = D; R_2 = CH_3; 5, 6, 7, 8,-d_4

R_2 = CH_3

V, R_3 = CH_2—CH=$C(CH_3)_2$
VI, R_3 = CH_3
VII, R_3 = CH=CH_2

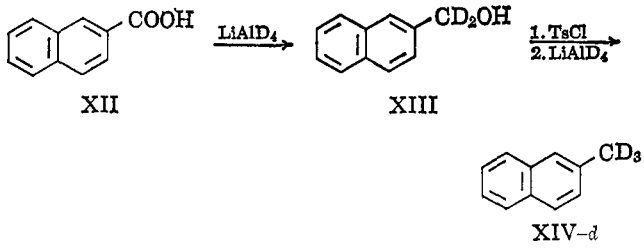
vitamins $K_{1(20)}$

VIII, R_2 = CH_3; R_3 = phytyl
IX, R_2 = CD_3; R_3 = phytyl
X, R_2 = CH_3; R_3 = phytyl; 5,6,7,8-d_4
XI, R_2 = CH_3; R_3 = phytyl-α-d_2

2-Methyl-1,4-naphthoquinone (I) and 2-methyl-3-phytyl-1,4-naphthoquinone (VIII) were commercial samples which were rigorously purified. 2-Phytyl-1,4-naphthoquinone (demethyl vitamin $K_{1(20)}$) (III), 2-methyl-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (V), 2,3-dimethyl-1,4-naphthoquinone (VI), and 2-methyl-3-vinyl-1,4-naphthoquinone (VII) are known compounds and were prepared by the reported procedures. The five deuterium analogs, II, IV, IX, X, and XI, are new, and their syntheses are now described.

All compounds containing the CD_3 group were prepared by the sequence starting with naphthoic acid (XII). The initial reduction to 2-naphthalenemethanol-α-d_2 differs from the normal hydride reduction[13,14]

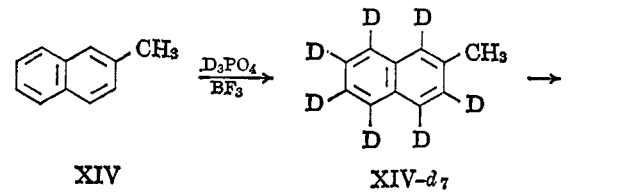
(1) This research was supported in part by Grant AI-04888 from the National Institutes of Health, U. S. Public Health Serivce.
(2) National Institutes of Health Predoctoral Fellow (1964–1966).
(3) C. Martius and D. Nitz-Litzow, *Biochim. Biophys. Acta*, 13, 152 (1954); A. F. Brodie and J. Ballantine, *J. Biol. Chem.*, 235, 232 (1960); J. S. C. Wessels, *Rec. Trav. Chim.*, 73, 18 (1954).
(4) J. P. Green and H. Dam, *Acta Chem. Scand.*, 8, 1341 (1954); H. K. Lichtenthaler, *J. Chromatog.*, 13, 166 (1964).
(5) R. A. Dilley, *Anal. Biochem.*, 7, 240 (1964); R. Ruegg and O. Isler, *Planta Med.*, 9, 386 (1961).
(6) D. F. Ewing, F. S. Tomkins, and O. Kamm, *J. Biol. Chem.*, 147, 233 (1943).
(7) H. Noll, *ibid.*, 235, 2207 (1960).
(8) C. V. Planta, E. Billeter, and M. Kofler, *Helv. Chim. Acta*, 42, 1278 (1959); B. Frydman and H. Rapoport, *J. Am. Chem. Soc.*, 85, 823 (1963); P. H. Gale, B. H. Arison, N. R. Trenner, A. C. Page, Jr., and K. Folkers, *Biochemistry*, 2, 196 (1963).
(9) J. H. Beynon and A. E. Williams, *Appl. Spectry.*, 14, 156 (1960).
(10) B. C. Johnson, P. Cohen, J. Polonsky, and E. Lederer, *Nature*, 199, 285 (1963).
(11) E. Ritchie, W. C. Taylor, and J. S. Shannon, *Tetrahedron Letters*, 1437 (1964).
(12) D. Mitisi, H. W. Moore, and K. Folkers, *J. Am. Chem. Soc.*, 87, 1402 (1965).
(13) R. F. Nystrom and W. G. Brown, *ibid.*, 69, 2548 (1947).
(14) R. V. Phillips, L. W. Trevoy, L. B. Jaques, and J. W. T. Spinks, *Can. J. Chem.*, 30, 844 (1952).



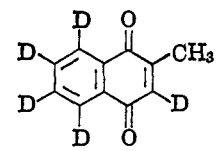
XII XIII XIV-*d*

only in that the rate of reduction with lithium aluminum deuteride was much slower and a 70-hr reflux in tetrahydrofuran was necessary to increase the yield to 70%. Several procedures were tried for replacement of the hydroxyl group of XIII by deuterium, including that *via* the bromide.[14] By far the best process was through the unstable tosylate which was directly reduced to 2-methyl-d_3-naphthalene (XIV-*d*). Both oxidation to 2-methyl-d_3-1,4-naphthoquinone (II) and condensation with phytol to the CD_3 analog of $K_{1(20)}$ (IX) were carried out by known procedures. In every case, the deuterium compounds were purified to the same standards as the corresponding protium compounds and differed from the latter only in the absence of that specific absorption in the nmr spectrum. The deuterium content is estimated as 98% or greater at the specified positions (except in the case of aromatic substitution), and it is interesting to note that no exchange occurred during either oxidation with chromic acid or boron trifluoride condensation with phytol (as established by mass spectral and nmr examination).

Introduction of deuterium into the aromatic ring of 1,4-naphthoquinones was achieved by extended treatment of 2-methylnaphthalene (XIV) with the phosphoric



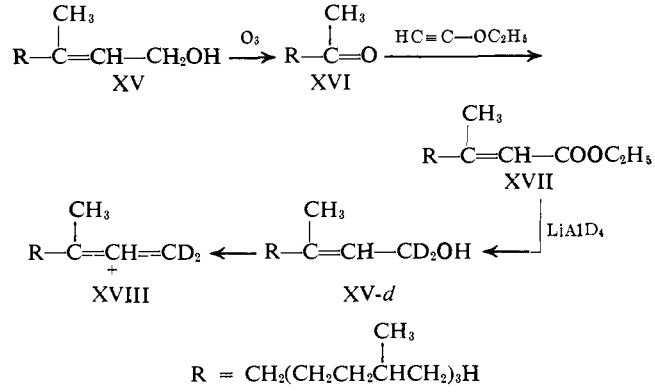
XIV XIV-d_7



IV

acid–boron trifluoride reagent previously used to introduce tritium at aromatic positions.[15] After 21 hr at 65°, XIV-d_7 was produced in which 93% of the aromatic hydrogens had been exchanged by deuterium while the CH_3 group remained intact as shown by nmr and mass spectral analyses. Chromic acid oxidation gave 2-methyl-1,4-naphthoquinone-3,5,6,7,8-d_5 (IV) which was condensed with phytol to give $K_{1(20)}$-5,6,7,8-d_4 (X). Subsequent nmr analyses of IV and X indicated that of the remaining aromatic protons in XIV-d_7, approximately half were at C-5, C-6, C-7, C-8, and half at C-3.

The synthesis of 2-methyl-3-phytyl-α-d_2-1,4-naphthoquinone (XI) required phytol-1-d_2 (XV-*d*). This was prepared by degradation of phytol (XV) to the C_{18} ketone (XVI), condensation of this ketone with ethoxyacetylene, and reduction of the resulting ester XVII. The procedure was adapted from a reported sequence,[16]



and the usual extension of reaction time with lithium aluminum deuteride over that with the hydride was necessary for a comparable yield. The unsaturated ester XVII was a mixture of *cis* and *trans* isomers in the ratio 1:2, as determined by measurement of the β-methyl absorption at δ 1.86, and 2.11, respectively.[16]

Since boron trifluoride catalyzed condensation of allylic alcohol XV-*d* with the naphthoquinone was presumed to proceed through a nonstereospecific carbonium ion XVIII, use of a *cis–trans* mixture should result in only the more stable *trans* isomer as product. Therefore the mixture of esters was reduced, and the resulting mixture of allylic alcohols was used in the reaction with 2-methyl-1,4-naphthoquinone (I) to form 2-methyl-3-phytyl-α-d_2-1,4-naphthoquinone (XI). Contrary to expectation, the $K_{1(20)}$ thus formed was a mixture of *cis–trans* isomers in the same proportion as in the starting ester XVII; therefore XVIII cannot be an intermediate in this reaction.[17] Attempted separation of the isomeric quinones on alumina and Decalso failed, and since the natural *trans* isomer predominated, this material was used directly as the mass spectral sample.

Mass Spectra. A. Procedure.[18] The spectra of the various 1,4-naphthoquinones were obtained in two ways, *viz.*, (1) normal operating procedure (*i.e.*, insertion of the sample into the spectrometer furnace followed by controlled introduction into the ion source), used for compounds having a substituent of C_5 or less at C-3, and (2) bypassing the initial high furnace temperature by insertion of a cold sample (4°) through the coverplate (temperature 80–100°) to the ion chamber, used for 2-methyl-3-phytyl-1,4-naphthoquinone and its deuterated analogs. The latter procedure stemmed from an early observation that when vitamin $K_{1(20)}$ was subjected to normal operating conditions, the relative intensities and positions of high molecular weight fragments (*m/e* 450–225) varied from spectrum to spectrum. When comparing the spectra of labeled and unlabeled vitamins $K_{1(20)}$ obtained under normal conditions, no correlation could be made, assuming a fragmentation pattern common to all of these com-

(15) P. M. Yavorsky and E. Gorin, *J. Am. Chem. Soc.*, **84**, 1071 (1962).

(16) J. W. Burrell, L. M. Jackman, and B. C. L. Weedon, *Proc. Chem. Soc.*, 263 (1959), and especially references therein.

(17) Similar retention of stereochemistry during this type of boron trifluoride catalyzed condensation has been observed by L. M. Jackman, R. Ruegg, G. Ryser, C. von Planta, U. Gloor, H. Mayer, P. Schudel, M. Kofler, and O. Isler, *Helv. Chim. Acta*, 48, 1332 (1965).

(18) A Consolidated Electrodynamics Corp. mass spectrometer was used (furnace temperature, 190°; ion chamber temperature, 250°; electron energy level, 70 ev).

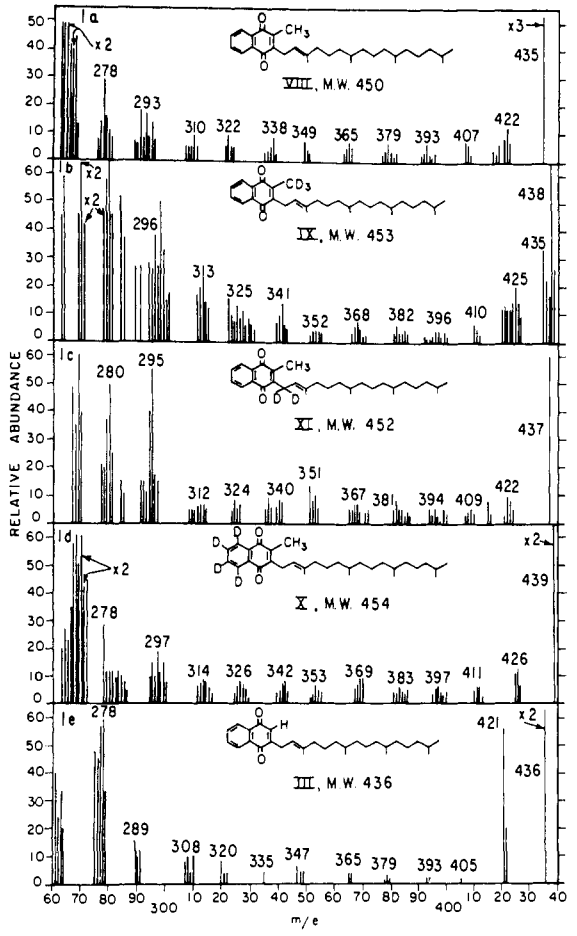


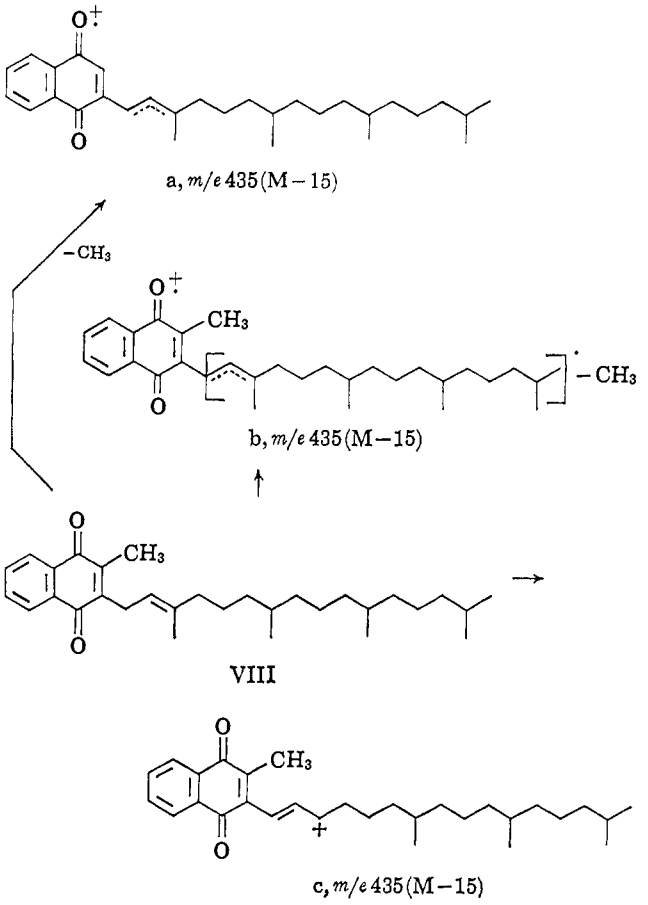
Figure 1. Mass spectra (high-mass region) of 2-methyl-3-phytyl-1,4-naphthoquinone (VIII), 2-methyl-d_3-3-phytyl-1,4-naphthoquinone (IX), 2-methyl-3-phytyl-α-d_2-1,4-naphthoquinone (XI), 2-methyl-3-phytyl-1,4-naphthoquinone-5,6,7,8-d_4 (X), and 2-phytyl-1,4-naphthoquinone (III) (intensity scale, 1% of Figures 2 and 3).

pounds. The mid-mass regions (*m/e* 225–104) were consistent with regard to position but varied mainly in the relative intensities of peaks in each set of spectra. These spectra were diagnosed as arising from partial thermal decomposition of the sample at the high temperature of the inlet system.[19] This difficulty was best surmounted by insertion of a cold sample of quinone through the coverplate of the instrument into the ion source, the use of a cold sample serving mainly to decrease the ready volatility of the quinone under these conditions and to thus partially regulate the sample pressure in the ion chamber.

B. Results and Discussion. The most intense peak found in the high-mass region of the spectrum of 2-methyl-3-phytyl-1,4-naphthoquinone (VIII; Figure 1a) is *m/e* 435 (M − 15) which corresponds to the loss of a methyl group. In this compound there are six methyl losses possible: one from the quinone nucleus and one of five from the phytyl side chain. The spectrum of 2-methyl-d_3-3-phytyl-1,4-naphthoquinone (IX; Figure 1b) contains, in addition to an M − 15 peak (*m/e* 438), an M − 18 peak (*m/e* 435), corresponding to the loss of CH_3 and CD_3, respectively, and indicating that two processes, loss of ring and side-chain methyls, are contributing to the M − 15 peak observed in VIII.

(19) R. Ryhage and E. V. Sydow, *Acta. Chem. Scand.*, 17, 2025 (1963).

As further evidence that the M − 18 peak observed with the CD_3 quinone proceeds directly from the molecular ion and is not an artifact arising from further decomposition of the M − 15 fragment, two discrete metastable peaks appear in spectrum 1b at approximately *m/e* 424.5 and 420.5 (calcd for M → M − 15, *m/e* 423.5, and for M → M − 18, *m/e* 417.7), indicating that, upon electron bombardment, both ring and side chain methyls are lost.

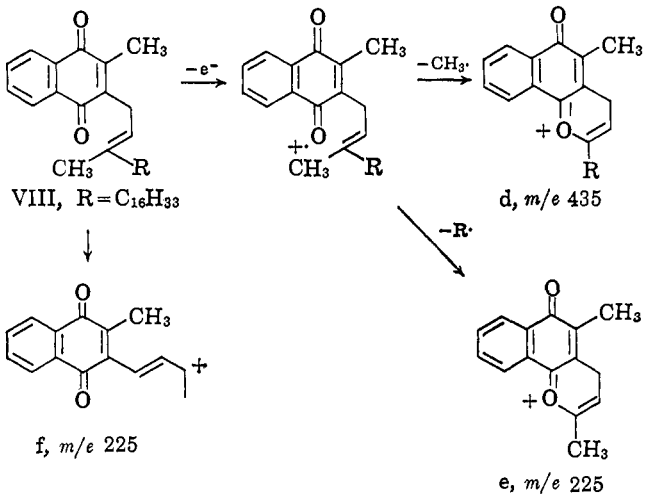


The portion of the *m/e* 435 peak reflecting the loss of methyl from the side chain b may represent one specific fragment or, more likely, may consist of several isomeric fragments arising from random loss of methyl at various positions along the chain. The most stable fragment which would result from such a loss would be ion c.[20] It is the enhanced stability of this ion which accounts for the side-chain contribution to the M − 15 peak being much greater than would be expected from the usual side-chain methyl loss.[21a]

For 1,4-naphthoquinone itself, the molecular ion is formed by the loss of one nonbonding oxygen electron.[21b] Assuming that in the highly alkylated analog VIII the molecular ion was also generated on the oxygen, one might consider both the *m/e* 435 (M − 15) and *m/e* 225 (M − 225, base peak) peaks as arising from such a species. The oxonium ion thus formed

(20) Throughout this study it has been assumed that the energy imparted to the molecule upon electron bombardment is sufficient to cause isomerization of the allylic double bond in the phytyl side chain to a vinylic position.

(21) (a) R. Ryhage and E. Stenhagen in "Mass Spectrometry of Organic Ions," F. W. McLafferty Ed., Academic Press Inc., New York, N. Y., 1963, Chapter 9; (b) J. H. Beynon, "Mass Spectrometry and Its Application to Organic Chemistry," Elsevier Publishing Co., New York, N. Y., 1960.



would lend sufficient stability to each of the two resulting fragments, d and e, to account for the observed high relative abundances of M − 15 and M − 225 fragments. Also, in the competitive loss of either methyl or long-chain, branched alkyl radical, loss of the latter would predominate in view of its greater stability. More will be said later on the possible identity of the *m/e* 225 fragment.

The remaining pattern in the high-mass region of vitamin $K_{1(20)}$ (VIII) (*m/e* 435–225) resembles those observed with long-chained aliphatic esters[21a] in that, at regular intervals approximately 14–15 mass units apart, are found low intensity peaks representing the loss of methylene and methyl units (with or without hydrogen rearrangement). The abundance of such fragments increases, as would be expected from the relative stabilities of the carbonium ion or radicals formed, as the point of fragmentation nears the site of unsaturation in the side chain. Also, as the chain becomes shorter, the probability of losing a smaller fragment must increase. Comparison of the spectra of 2-methyl-3-phytyl-1,4-naphthoquinone (VIII; Figure 1a), 2-methyl-d_3-3-phytyl-1,4-naphthoquinone (IX; Figure 1b), and 2-phytyl-1,4-naphthoquinone (III; Figure 1e) shows that this high-mass region is further complicated by minor fragmentation from the species which has undergone prior loss of the ring methyl.

To rationalize structures for fragments found in the mid-mass region of the spectrum (Figure 2a) several simple alkylated 1,4-naphthoquinones were used to supplement the information gained from the deuterated vitamins $K_{1(20)}$. The use of both deuterium-labeled and simple quinone models was necessary since the labeled models alone would show the presence or absence of the ring methyl group, the α-methylene of the phytyl side chain, or the benzenoid portion of the quinone nucleus, but would give little information concerning the presence of the quinone oxygens.

The base peak of the spectrum, *m/e* 225 (M − 225), is found in this mid-mass region, along with the corresponding peaks from the deuterium-labeled models (Table I). The previously proposed structure for this fragment, e, as well as f, fits the data of Table I. This fragment is also present in the spectrum of 2-methyl-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (V; Figure 3b). In this case, however, it is not the base peak of the spectrum. Since the same fragment is formed in both cases, the observed difference in intensities must be

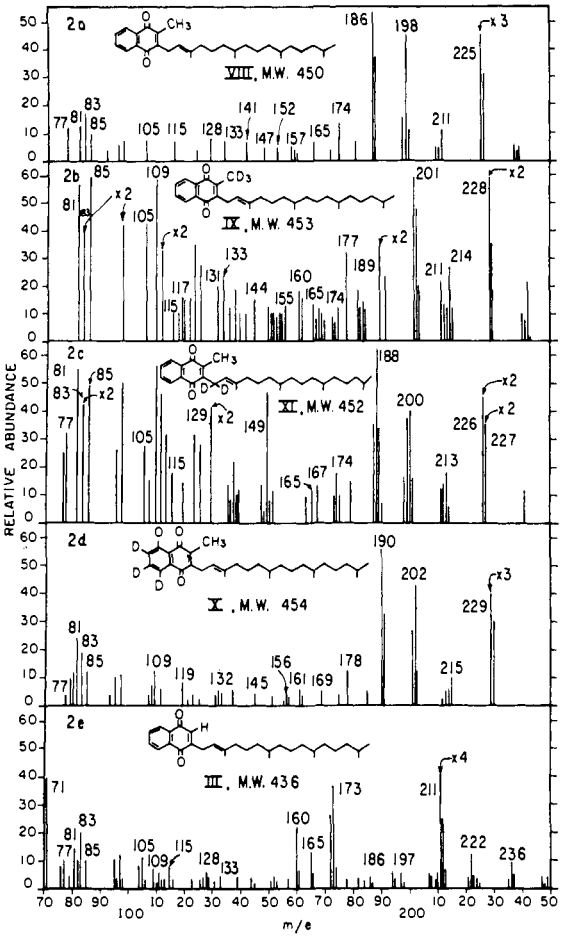
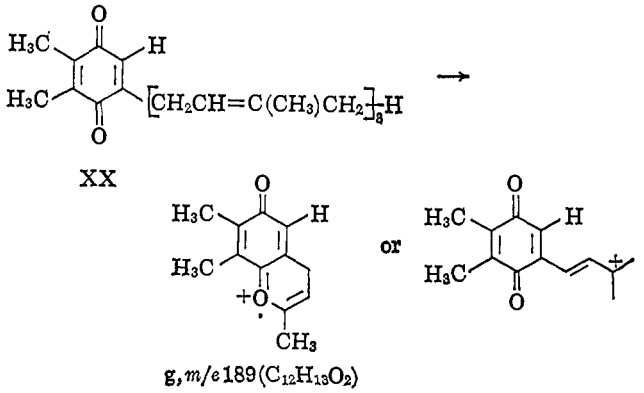


Figure 2. Mass spectra (mid-mass region) of compounds VIII (2a), IX (2b), XI (2c), X (2d), and III (2e).

related to the stability of the other portion expelled in its formation. In the case of vitamin $K_{1(20)}$ (VIII), assuming formation from the molecular ion, a C_{15}-alkyl radical is lost, whereas in the case of V only a methyl radical can be lost. Thus, the relative stabilities of the two expelled fragments could be prime factors in the driving force for formation of such an ion. Further support is given to the identity of this fragment by a report[12] that in the mass spectrum of plastoquinone-3 (XX), the base peak appears at *m/e* 189 and has the composition $C_{12}H_{13}O_2$. In view of what has been observed with vitamin $K_{1(20)}$, a probable structure for this fragment is g.



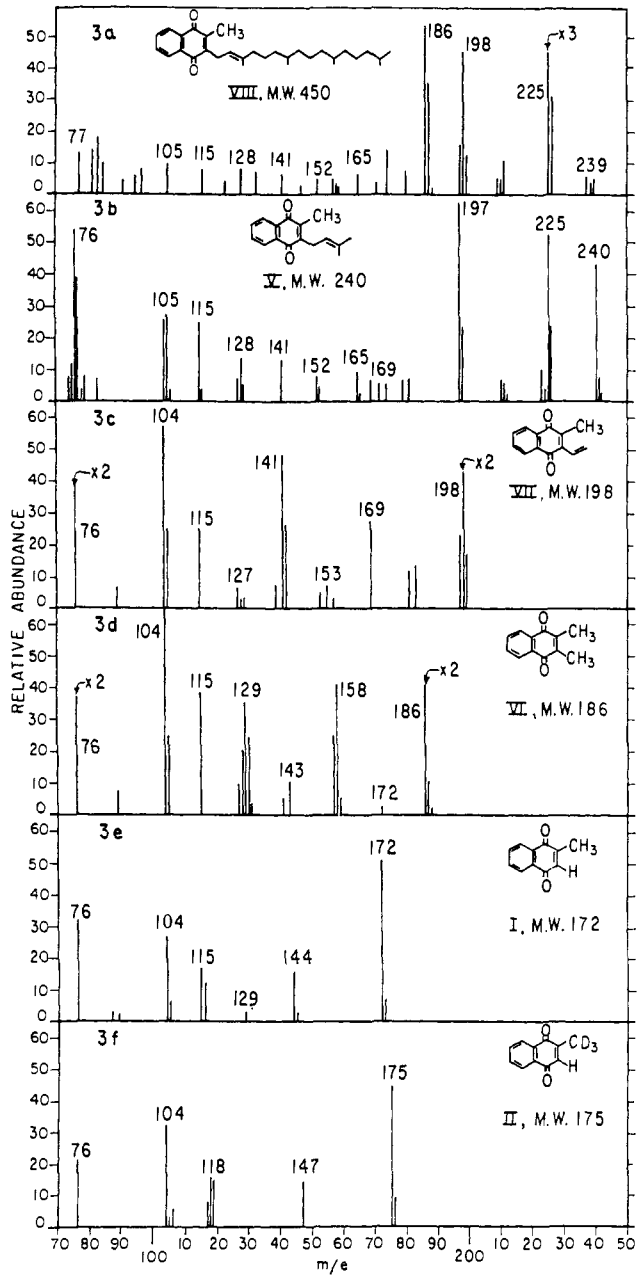
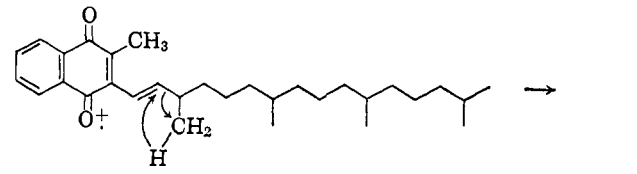


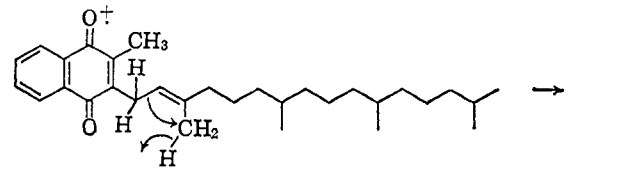
Figure 3. Mass spectra of 2-methyl-3-phytyl-1,4-naphthoquinone (VIII) (low-mass region), 2-methyl-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (V), 2-methyl-3-vinyl-1,4-naphthoquinone (VII), 2,3-dimethyl-1,4-naphthoquinone (VI), 2-methyl-1,4-naphthoquinone (I), and 2-methyl-d_3-1,4-naphthoquinone.

The next fragment of importance in the spectrum of vitamin $K_{1(20)}$ appears at *m/e* 198 (M − 252). By a comparison of the partial spectrum of vitamin $K_{1(20)}$ with that of 2-methyl-3-vinyl-1,4-naphthoquinone (VII; Figure 3c) and with those of the various deuterium-labeled vitamins $K_{1(20)}$ (Table I and Figure 2a–d), the best structure for this fragment appears to be the vinylquinone ion h. This fragment can be formed in several ways, and without the aid of metastable peaks in this area, the exact mode of formation is unknown. One can visualize its formation either through the molecular ion or through a fragment of higher molecular weight.



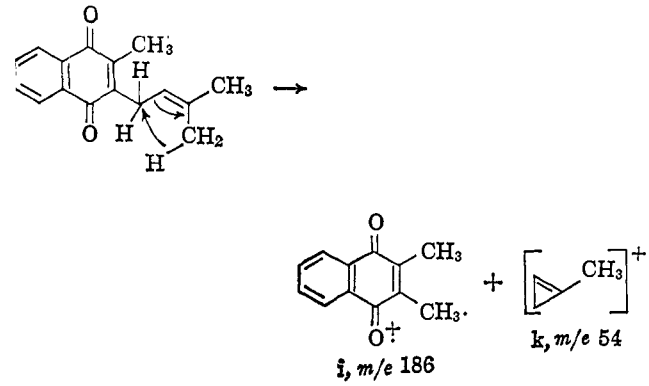
h, *m/e* 198

Also prevalent in this region is *m/e* 186 which appears to have the structure i. In view of the peak at *m/e* 188 found in the spectrum of 2-methyl-3-phytyl-α-d_2-1,4-naphthoquinone (XI; Figure 2c), this fragment appears to have been formed *via* cleavage of the α,β bond of the phytyl side chain, accompanied by hydrogen



i, *m/e* 186 j, *m/e* 264

migration. The absence of this fragment in the spectrum of 2-methyl-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (V; Figure 3b) may indicate that the fragment k is not as stable as fragment j, decreasing the driving force for such a fragmentation in the smaller molecule.

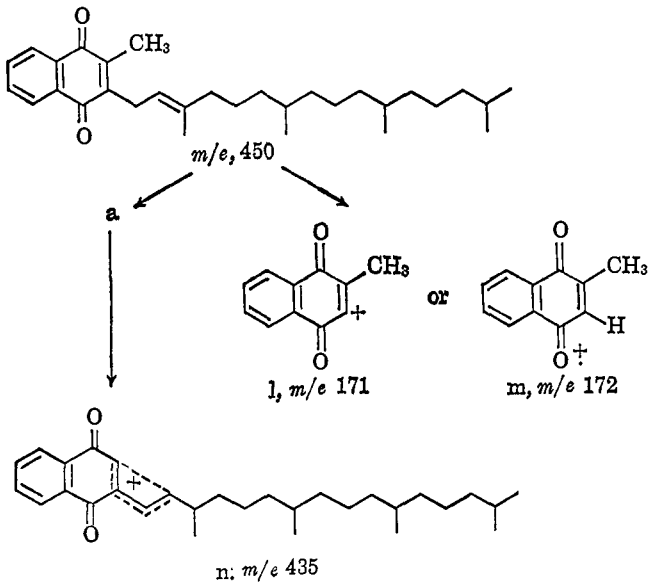


i, *m/e* 186 k, *m/e* 54

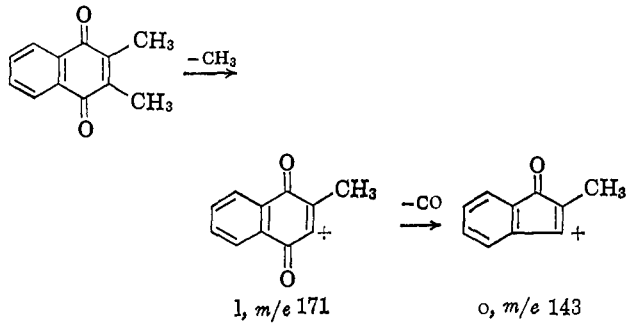
Obviously lacking from the spectrum of vitamin $K_{1(20)}$ is a fragment representing 2-methyl-1,4-naphthoquinone (*m/e* 171 or 172, l or m) arising from the loss

Table I. Principle Mass Spectral Peaks in the Mid-Mass Region of Vitamin $K_{1(20)}$ and Its Analogs

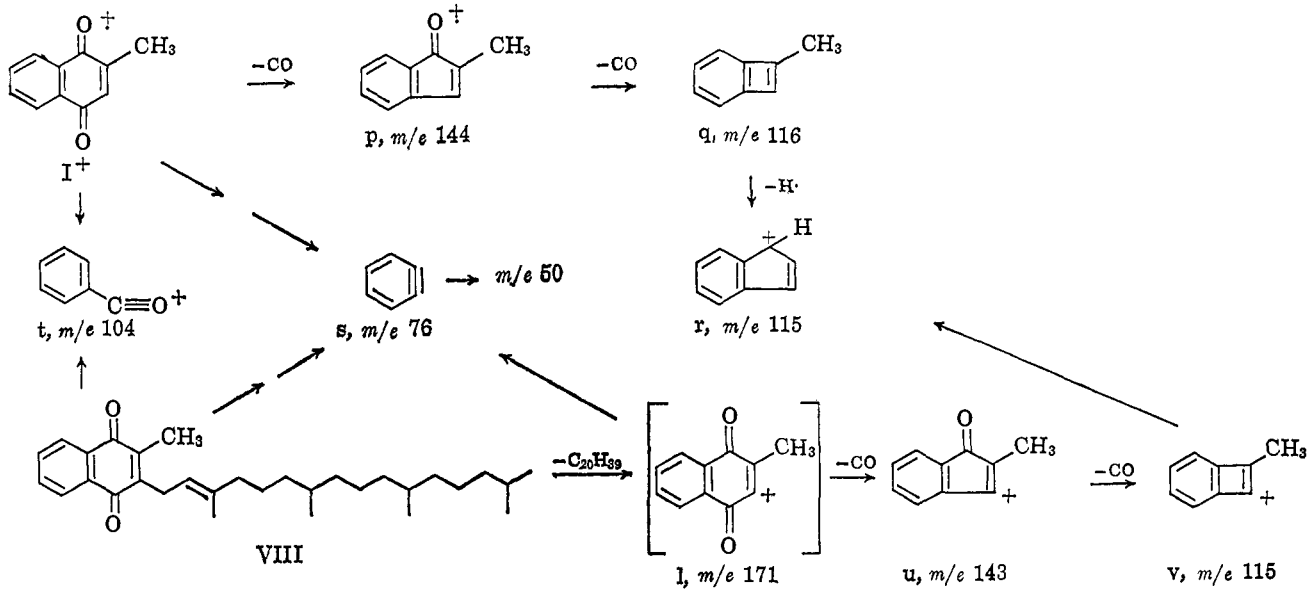
Compound	M	M − 225	M − 252	M − 264
VIII	450	225	198	186
IX	453	228	201	189
XI	452	226, 227	199	188
X	454	229	202	190
III	436	211	184	...



of the phytyl side chain as a complementary process to the observed loss of the ring methyl group. A possible



explanation for this might lie in the fact that expulsion of a methyl group from vitamin $K_{1(20)}$ results in a fragment in which the radical or charge is not localized on



a carbon adjacent to a carbonyl carbon but may be distributed throughout a diene-type system, n. Loss of the stable $C_{20}H_{39}$ fragment to produce l would result in a fragment in which the charge or radical is retained in an unfavorable position.

The instability of fragment l relative to i can be seen in the spectrum of 2,3-dimethyl-1,4-naphthoquinone (VI; Figure 3d) in which i is the molecular ion and l is the fragment which results on loss of methyl. The ratio *m/e* 171 to *m/e* 186 is 0.024. However, in the mass spectrum of 2-methyl-1,4-naphthoquinone (I; Figure 3e), the molecular ion is also the base peak. These observations indicate that generation of a radical or positive charge on the quinone nucleus of this molecule renders it very prone to further decomposition *via* loss of carbon monoxide to form o (*m/e* 143), thus gaining stability.

The fragmentation of 2-methyl-1,4-naphthoquinone (I) was interpreted with the aid of 2-methyl-d_3-1,4-naphthoquinone (II) to give the fragments p, q, r, s, and t[22] in accordance with the scheme proposed[21b] for 1,4-naphthoquinone. The fragments s and t have been designated in the above scheme as proceeding from the molecular ion although there are alternate ways in which they may be formed.[23] Unfortunately, no metastable peaks could be found which would indicate whether formation of either of these fragments proceeds from one discrete precursor or from a combination of several.

When comparing the spectrum of 2-methyl-1,4-naphthoquinone (I; Figure 3e) with those of the 2-methyl-3-phytyl-1,4-naphthoquinones (Figure 2) one can see that, although no substantial peak is present for the formation of I^+ (*m/e* 172), the peaks *m/e* 144, 116, and 115 are present, indicating the formation of an intermediate such as l at some phase in the fragmentation process from one or several possible sources. In the fragmentation of vitamin $K_{1(20)}$ (VIII), the formation of l is designated as directly arising from the molecular ion merely as a complement to the loss of the ring methyl group. As the degree of alkyl substitution in the 3 position of the quinone nucleus increases from $C_1 \rightarrow C_{20}$, one notices the gradual supplementation of *m/e* 104 by *m/e* 105. This latter fragment is believed to be merely a protonated form of t.

Although this work has been confined to the vitamin K_1 type of quinone, the fragments found are of a

(22) Essentially the same fragmentation has been postulated for this compound and for 2,3-dimethyl-1,4-naphthoquinone (VI) in a paper by J. H. Bowie, D. W. Cameron, and D. H. Williams, *J. Am. Chem. Soc.*, 87, 5094 (1965), which appeared after submission of our report.

(23) The specific carbonyl group lost to form t and the exact sequence of carbonyl loss in going to p and q is not known at this time.

general nature and will probably appear in the spectra of the vitamins K_2 and the related benzoquinones. Therefore, the structural assignments made should provide a basis for interpretation of the mass spectra of these quinone pigments as well.

Experimental Section[24]

The following four compounds were obtained from commercial sources and were purified as indicated. They served as authentic material with which subsequent synthetic samples were compared.

A. **2-Methylnaphthalene** (XIV) was distilled (bp 240°) and sublimed and was pure by vpc, mp 33–34°; δ 7.7–7.0 (m, 7 H), 2.27 (s, 3 H).

B. **2-Methyl-1,4-naphthoquinone** (I) was sublimed 50° at 0.1 mm) and chromatographed on Decalso (>250 mesh), eluting with petroleum ether–5% chloroform. It was pure by vpc, mp 104–105°; δ 8.14–7.52 (m, 4 H), 6.80 (q, 1 H), 2.18 (d, 3 H); λ_{max} 243, 248, 253, 261, 330 mμ.

C. **Phytol** (XV) was chromatographed on alumina (neutral, activity I), eluting with isooctane. It was pure by vpc; δ 5.42 (t, 1 H), 4.13 (d, 2 H), 2.00 (m, 2 H), 1.68 (s, 3 H); 1.20 (CH_2's), 1.00, 0.97, 0.81 (CH_3's).

D. **2-Methyl-3-phytyl-1,4-naphthoquinone (vitamin $K_{1(20)}$)** (VIII) was reduced to the hydroquinone with sodium hydrosulfite. The hydroquinone was crystallized four times from petroleum ether, and oxidized to the quinone with excess silver oxide in ether. Chromatography on >250 mesh Decalso (eluting with petroleum ether–5% chloroform) under nitrogen pressure gave quinone which produced one spot on thin layer (silica gel G, petroleum ether–5% chloroform) and on S_{288} alumina paper (petroleum ether–20% benzene); λ_{max} 245, 248, 262, 271, 330 mμ; δ 8.06–7.50 (m, 4 H, aromatic protons), 4.95 (t, 1 H, C—C*H*=C), 3.31 (d, 2 H, Ar—C*H*$_2$—C=), 2.15 (s, 3 H, Ar-C*H*$_3$), 1.95 (2 H, C—C*H*$_2$—C=), 1.77 (s, 3 H, —C(C*H*$_3$)=C), 1.20 (chain CH_2's), and 0.90, 0.87, 0.83 (chain CH_3's).

2-Naphthalenemethanol-α-d_2 (XIII). 2-Naphthoic acid (XII) was reduced with lithium aluminum deuteride following the usual procedure for the hydride[13] and using tetrahydrofuran as solvent. The reaction time was necessarily extended to 70 hr, yielding 70% of 2-naphthalenemethanol-α-d_2, mp 81.5–82° (lit.[25] mp 80° for the corresponding protium compound); δ 7.9–7.2 (m, 7 H), 3.20 (s, 1 H); absorption at δ 4.77 (s, 2 H), present in the protium compound, was absent.

2-Methyl-d_3-naphthalene (XIV-*d*). 2-Naphthalenemethanol-α-d_2 (XIII; 2 g, 13 mmoles) was converted to its *p*-toluenesulfonate[26] and the ether–benzene solution of the tosylate was filtered into a dropping funnel and slowly added, in a nitrogen atmosphere, to a stirred suspension of 2.0 g (50 mmoles) of lithium aluminum deuteride in 25 ml of ether at a rate sufficient to maintain steady reflux. The reaction mixture then was maintained at reflux for 72 hr. After excess deuteride was decomposed with water and the mixture was filtered, the filtrate was extracted with ether while the residue was digested with chloroform, and the combined extracts were dried over magnesium sulfate. Following evaporation of solvent, the residue was sublimed (25° at 50 mm) and 1.3 g (9 mmoles, 70% yield based on 2-naphthalenemethanol-α-d_2) of 2-methyl-d_3-naphthalene was obtained, identical with the authentic protium compound above except for the absence of the singlet at δ 2.27 (Ar–CH_3).

2-Methyl-d_3-1,4-naphthoquinone (II) was prepared *via* oxidation of 2-methyl-d_3-naphthalene (XIV-*d*) with chromic acid.[14] The resulting deuterium compound was identical with the authentic protium compound above except for the absence of the doublet at δ 2.18 representing the quinone methyl group and the presence of a singlet rather than a quartet at δ 6.80.

2-Methylnaphthalene-1,3,4,5,6,7,8-d_7 (XIV-d_7). 2-Methylnaphthalene (XIV) was treated with $D_3PO_4 \cdot BF_3$[15] in cyclohexane, and the effect of each treatment was measured by the ratio of methyl protons to the aromatic protons found in the nmr spectrum. The sample was subjected to five treatments, each of which yielded the indicated ratios of methyl to aromatic protons: (1) 25° for 20 hr (ratio, 3:6), (2) 25° for 20 hr followed by 65° for 1 hr (ratio, 3:4.5), (3) 65° for 4 hr (ratio 3:2.04), (4) 65° for 5 hr (ratio, 3:0.54), and (5) 65° for 21 hr (ratio, 3:0.49). After these treatments, 2-methylnaphthalene-1,3,4,5,6,7,8-d_7 (93% Ar-perdeuterio by nmr) was recovered in 61% yield; δ 7.7–7.0 (m, 0.49 H), 2.27 (s, 3 H).

2-Methyl-1,4-naphthoquinone-3,5,6,7,8-d_5 (IV), prepared from 2-methylnaphthalene-1,3,4,5,6,7,8-d_7 (XIV-d_7) by chromic acid oxidation,[14] was identical with authentic protium material except for very slight absorption in the aromatic proton region: δ 8.14–7.52 (0.34 H, H-5, H-6, H-7, H-8), 6.81 (0.3 H, H-3).

2-Phytyl-1,4-naphthoquinone (III) was prepared as described.[27]

2-Methyl-d_3-3-phytyl-1,4-naphthoquinone (IX) was prepared *via* the condensation of the hydroquinone of II with phytol under boron trifluoride catalysis.[28] The yellow oil obtained was distinguishable from vitamin $K_{1(20)}$ only in the absence of absorption at δ 2.15 indicative of the quinone methyl group.

2-Methyl-3-phytyl-1,4-naphthoquinone-5,6,7,8-d_4 (X) was prepared by the condensation of phytol with the hydroquinone from IV under the influence of boron trifluoride.[28] The resulting yellow oil was identical with authentic vitamin $K_{1(20)}$ except for the much decreased absorption in the aromatic proton region; δ 8.08–7.53 (0.4 H).

Phytol-1-d_2 (XV-*d*) was prepared by ozonolysis of phytol, reaction of the resulting ketone with ethoxyacetylene, and reduction of the ester thus formed by a 5-day reflux in ether with lithium aluminum deuteride. Purification as described above gave material identical with the authentic sample except for the absence of the doublet at δ 4.13 and the presence of a singlet at 5.42 instead of a triplet.

2-Methyl-3-phytyl-α-d_2-1,4-naphthoquinone (XI) was obtained *via* the condensation of phytol-1-d_2 (XV-*d*) with a large excess of 2-methyl-1,4-naphthohydroquinone monoacetate.[29] The oil obtained was identical with authentic vitamin $K_{1(20)}$ except for the absence of the doublet at δ 3.31 (ArCH_2—C=) and the presence of a singlet at δ 4.95 instead of the previous triplet.

2-Methyl-3-(3-methyl-2-butenyl)-1,4-naphthoquinone (V) was prepared by the condensation of 2-methyl-1,4-naphthohydroquinone with 2-methyl-3-buten-2-ol.[30]

2,3-Dimethyl-1,4-naphthoquinone (VI) was prepared from 2,3-dimethylnaphthalene by chromic acid oxidation,[14] mp 116–119° (lit.[31] mp 115–119°).

2-Methyl-3-vinyl-1,4-naphthoquinone (VIII) was prepared by conversion of 2-methyl-1,4-naphthoquinone to 2-methyl-3-(α-bromoethyl)-1,4-naphthoquinone and dehydrobromination of the latter, as reported.[32]

(24) Evaporations were made *in vacuo* using a rotary evaporator; petroleum ether refers to the fraction, bp 30–60°; nmr spectra were taken in deuteriochloroform with internal TMS as reference; ultraviolet absorptions were measured in isooctane.

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