large enhancement factors frequently occur in the secondary band of phenylalanine and derivatives. Little change in absorption intensities occurs in either primary or secondary bands upon incorporation into peptides or chelation by metal ions. In either free or complexed ligands the dissymmetry factors[16] of $g = \Delta\epsilon/\epsilon$ remain less than 0.01, conforming to the magnetic dipole forbidden nature of the primary and secondary band transitions in benzene. Results of an optical rotatory dispersion study have been interpreted as a major enhancement of optical activity of primary band aromatic transitions upon cupric ion chelation of ligands such as phenylalanine, tyrosine, and glycyltyrosine.[17] This measurement, however, exaggerates optical activity when Cotton effects of opposite sign appear appropriately spaced on the wavelength scale and has missed Cotton effects at longer wavelengths. The augmentations of CD magnitudes observed in aromatic transitions upon incorporation of aromatic amino acid residues into peptides or chelation by cupric ions seems consistent with more restricted rotation in these systems, and no special interactions need be invoked.

(16) S. F. Mason, *Proc. Chem. Soc.*, 137 (1962).

(17) J. E. Coleman, *Biochem. Biophys. Res. Commun.*, **24**, 208 (1966)

Photooxygenation of Phylloquinone and Menaquinones[1]

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Abstract: Phylloquinone was photolyzed aerobically in cyclohexane to yield a ketonic side-chain fragment, 6,10,14-trimethylpentadecan-2-one, *via* an intermediary hydroperoxide, *trans*-2-methyl-3-(3-hydroperoxy-3,7,11,15-tetramethyl-1-hexadecenyl)-1,4-naphthoquinone. The analogous hydroperoxide from the menaquinone MK-1, *trans*-2-methyl-3-(3-methyl-2-butenyl)-1,4-naphthoquinone, was also studied. Its structure was established by reduction of the hydroperoxide to *trans*-2-methyl-3-(3-hydroxy-3-methyl-1-butenyl)-1,4-naphthoquinone which was synthesized independently. The general photooxygenation reaction proceeded by attack on the quinone of singlet oxygen, which could be generated *via* methylene blue sensitized photolysis, to give the 3′-hydroperoxide. The hydroperoxide then photolytically rearranged to ketone. In the menaquinone series, this reaction is useful as a side-chain degradation procedure, particularly for those alkenyl naphthoquinones in which the second isoprenoid side-chain unit is saturated. When applied to the natural quinone of *Mycobacterium pheli*, MK-9 (II-H), photolysis in cyclohexane yielded a side-chain fragment which confirmed the structure previously assigned to this quinone.

Primary evidence for the involvement of quinone in oxidative phosphorylation has come from experiments in which the native quinone of crude bacterial extracts has been depleted thus permitting reconstitution of these extracts with structurally modified quinone analogs.[3] In this manner, the structural aspects of the naphthoquinone necessary for activity in oxidative phosphorylation have been deduced. In some cases depletion of the quinone has been effected by solvent extraction but for the most relevant studies the method of choice has been ultraviolet irradiation. Restoration of oxidative phosphorylation activity to near original levels by the addition only of exogenous quinone is illustrative of the selectivity of the irradiation method, the other components of the electron transport particle being little affected.

Although the photolability of quinones has been long recognized[4] and exploited, not until recently[5] has the reaction been studied in any way other than in a very qualitative manner. This is somewhat surprising in view of the biological significance of the photoreaction; for example, the light-induced structural modifications which cause the quinone to become inactive could have significance in determining its role in oxidative phosphorylation. Or perhaps the photoproducts could act during oxidative phosphorylation as inhibitors which have to be displaced by the addition of exogenous quinone. Questions like these have moved us to investigate the photolysis of some compounds of the vitamin K series.

Previous work[5a] has shown that exhaustive aerobic photolysis of phylloquinone in benzene solution gave phthiocol in unspecified yield. The presence of this photoproduct is interesting since phthiocol is incapable of restoring oxidative phosphorylation activity as determined with the *M. pheli* system,[3] but perhaps may

(1) Supported in part by Grant AI-04888 from the National Institutes of Health, U. S. Public Health Service.

(2) National Institutes of Health Predoctoral Fellow.

(3) A. F. Brodie, *Federation Proc.*, **20**, 995 (1961), and references therein. For reviews concerning the postulated role of naphthoquinone in oxidative phosphorylation see E. Lederer and M. Vilkas, *Vitamins Hormones*, **24**, 409 (1966), and A. F. Brodie in "Biochemistry of Quinones," R. A. Morton, Ed., Academic Press, New York, N. Y., 1965, Chapter 11.

(4) (a) H. J. Almquist, *J. Biol. Chem.*, **117**, 517 (1937); (b) D. T. Ewing, F. S. Tomkins, and O. Kamm, *ibid.*, **147**, 233 (1943); (c) J. P. Green and H. Dam, *Acta Chem. Scand.*, **8**, 1341 (1954).

(5) (a) G. Katsui and M. Ohmae, *J. Vitaminol.*, **12**, 117 (1966); (b) M. Ohmae and G. Katsui, *Vitamins* (Kyoto), **35**, 116 (1967); (c) S. Fugisawa, S. Kawabata, and R. Kamamoto, *J. Pharm. Soc. Japan*, **87**, 1451 (1967).

act as an inhibitor of phosphate fixation as does lapachol. Most recently[5c] the photolysis of phylloquinone in ethanol solution under anaerobic conditions has been investigated and the primary product was observed to be its chromenol. This was not unexpected in view of similar photolytic studies of ubiquinone which yielded ubichromenol and isounbiquinone in which the β,γ side-chain double bond had shifted into conjugation with the ring.[6] Interestingly enough the similar vinyl analog of phylloquinone is unreported.[5]

The system to which we ultimately intend to apply our results is the quinone MK-9 (II-H)–*Mycobacterium phlei* system which is the most biologically significant considering its active use in investigating quinone involvement in oxidation phosphorylation.[3] However, because of the difficulties involved in an immediate approach to the *in vivo* system, namely, that of identifying small quantities of high molecular weight, unknown photoproducts contaminated with large amounts of lipids, an alternate approach was first considered. This was to examine instead the *in vitro* photolysis of simple quinone analogs. Nevertheless, our photolysis conditions were chosen in an attempt to maximize the pertinence of the experiments with respect to the biological system.

An ideal solvent system for model studies would attempt to mimic the *in vivo* conditions as closely as possible. Since the electron transport particle is such a highly structured unit, the possibility exists, for example, that the quinone is encapsulated in a lipid–protein complex, being essentially insulated from oxygen and water. If this were the case, then the photolysis should be studied in an anhydrous, anaerobic hydrocarbon solvent. On the other hand, a lipid complex could solubilize the side chain leaving the polar naphthalenic end of the molecule available for hydration, thus perhaps creating a locally high activity of water about the naphthoquinone nucleus. An analogous system with a high water content, *e.g.*, (a) a detergent, quinone, and water or (b) dioxane, quinone, and water, would be a valid candidate for photolytic study. Finally, the possibility of a near neighbor interacting photolytically with the quinone is real and cannot be dismissed. Indeed another quinone molecule might be a near neighbor so that a system containing a fine emulsion of quinone in water might not be an irrelevant investigative system. Possible photolytic interaction with other members of the electron transport chain cannot be anticipated in model studies and must await *in vivo* study.

In view of all these possibilities, the system chosen was a dilute cyclohexane solution of quinone open to moisture and air, thus anticipating that the quinone in the natural system is most likely imbedded in an inert lipid layer with limited access at the chromophore to water and oxygen. Even if the system chosen is a distant approximation to the actual *in vivo* situation, hopefully the photoreaction will be insensitive to solvent modification and thus the results will have general applicability.

Photolysis of phylloquinone in cyclohexane yielded two major products as determined by thin layer chromatography; the minor (10%) and more polar constituent was a yellow hydroperoxide[7] as indicated by a potassium iodide test. The other product obtained in greater yield (50%) was colorless and of intermediate polarity between the starting quinone and hydroperoxide. Nuclear magnetic resonance spectroscopy, infrared spectroscopy, mass spectrometry, and thin layer chromatography indicated this latter material to be a ketone fragment from the side chain, 6,10,14-trimethylpentadecan-2-one.[8] Furthermore, isolation of the hydroperoxide and subsequent anaerobic photolysis of this material led to production of the same ketone in 91% yield, thereby proving the intermediacy of the hydroperoxide in the over-all reaction to the ketone. Involved then is a novel two-step photooxidation where the quinone acts as its own sensitizer followed by an equally interesting photodecomposition.[9]

Since the hydroperoxide is an intermediate in a two-step reaction where the second step is at least as fast as the first, yields of the hydroperoxide by direct ultraviolet irradiation were always low. Thus, an alternate method of synthesis was sought to improve its yield. Assuming initial attack is *via* singlet oxygen generated from excitation of quinone and oxygen, a reasonable alternative would be to generate dye-sensitized singlet oxygen. Advantageous would be the option of irradiating with long-wavelength light which would photolyze neither the quinone nor the product hydroperoxide. Indeed, using red light and methylene blue as a sensitizer, an excellent yield (75%) of the hydroperoxide of phylloquinone was obtained.

Proof of structure of the hydroperoxide was accomplished using the simpler quinone, MK-1, 2-methyl-3-(3-methyl-2-butenyl)-1,4-naphthoquinone, as a substrate. Both an elemental analysis and a molecular ion in the mass spectrum confirmed that the quinone had added the elements of molecular oxygen to form the hydroperoxide. Further spectral investigation lead to the conclusion that the material was *trans*-2-methyl-3-(3-hydroperoxy-3-methyl-1-butenyl)-1,4-naphthoquinone (IIa), IIb therefore corresponding to *trans*-2-methyl-3-(3-hydroperoxy-3,7,11,15-tetramethyl-1-hexadecenyl)-1,4-naphthoquinone.[7] Reduction of the hydroperoxide function with either hydrosulfite or borohydride and subsequent oxidation of the hydroquinone led to the product, *trans*-2-methyl-3-(3-hydroxy-3-methyl-1-butenyl)-1,4-naphthoquinone (III), which proved to be slightly more polar chromatographically but spectrally very similar to the starting hydroperoxide. Reaction with platinum oxide and

(6) (a) I. Imada, Y. Sanno, and H. Morimoto, *Chem. Pharm. Bull. (Tokyo)*, **12**, 1042 (1964); (b) I. Imada and H. Morimoto, *ibid.*, **12**, 1047 (1964); (c) I. Imada and H. Morimoto, *ibid.*, **12**, 1051 (1964).

(7) This hydroperoxide was assigned structure IIb in ref 5b which came to our attention after the present work was completed.

(8) H. Mayer, U. Gloor, O. Isler, R. Reugg, and O. Wiss, *Helv. Chim. Acta*, **47**, 221 (1964).

(9) Perepoxides and cyclic peroxides have been postulated as intermediates in various nitrogen-containing systems [F. McCapra and Y. C. Chang, *Chem. Commun.*, 522 (1966); C. S. Foote and J. W-P. Lin, *Tetrahedron Letters*, 3267 (1968); J. E. Huber, *ibid.*, 3271 (1968); T. Matsuura and I. Saito, *ibid.*, 3273 (1968)]. They are presumably obtained from the hydroperoxide *via* ionic intermediates (assisted and supported by the nitrogen) or by direct addition of O_2*. Decomposition then occurs to give the carbonyl components, as is the result in our quinone case. However, the latter differs in that we have established the hydroperoxide as an intermediate, and the previously postulated ionic and zwitterionic intermediates are not applicable. Thus hydroperoxide formation is being followed by photochemical cyclization and then decomposition.

Scheme I

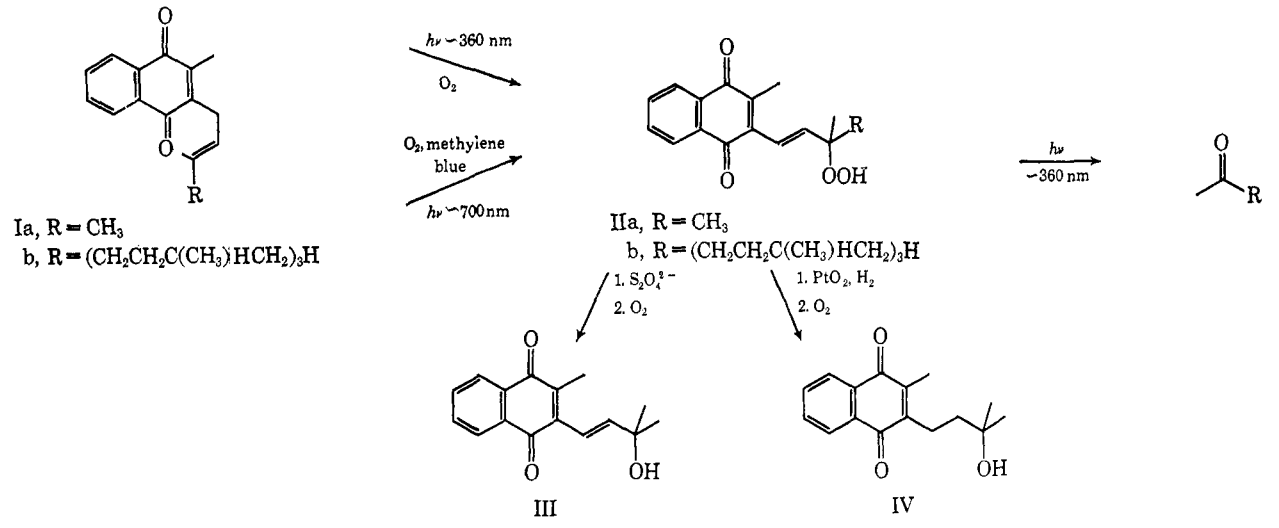
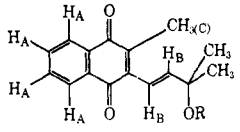
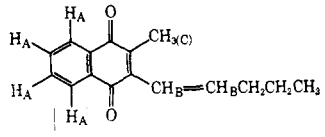


Table I. Nuclear Magnetic Resonance Assignments and Ultraviolet Absorptions of Some 3-Vinyl-2-methyl-1,4-naphthoquinones

Compound		Nmr[a] H_A	Nmr[a] H_B	Nmr[a] H_C	Uv[b]
	IIa, R = OH III, R = H	7.72, m, 4 7.70 m, 4	6.44, s, 2 6.53 s, 2	2.15, s, 3 2.17, s, 3	252 (22,000), 288 (7680), 335 (3320) 253 (22,800), 292 (7710), 335 (3300)
	V,[c] *cis*	7.78 m, 4	6.13, s, 1 6.01, t, 1 J = 7 Hz	2.10, d, 3 J = 1 Hz	249 (21,200), 286 (3700), 327 (3140)
	trans	7.78, m, 4	6.44, s, 1 6.48, t, 1 J = 7 Hz	2.22, s, 3	252 (23,000), 292 (7450), 333 (2760)

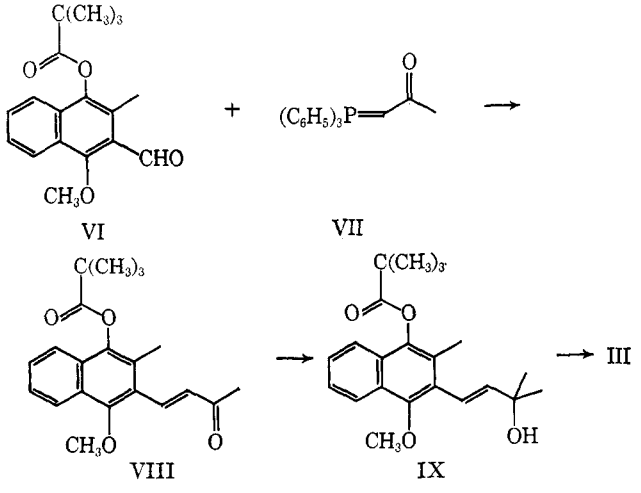
[a] δ values relative to internal TMS (0), followed by multiplicity and number of H's. [b] In ethanol, λ_{max} in nm (ϵ). [c] Material generously supplied by W. E. Bondinell; see footnote 11.

hydrogen effected reduction of the vinyl double bond in addition to reduction of the hydroperoxy function so that after silver oxide oxidation the known γ-hydroxy MK-1, 2-methyl-3-(3-hydroxy-3-methylbutyl)-1,4-naphthoquinone[10] (IV), was obtained. The above reactions are summarized in Scheme I.

Identification of the vinyl quinone chromophore was facilitated by the availability of various vinyl quinones synthesized in these laboratories. Ultraviolet spectra indicated both the chromophores of hydroperoxide IIa and carbinol III to be almost identical with the *trans* isomer of 2-methyl-3-(1-pentenyl)-1,4-naphthoquinone (V)[11] and distinctly different from *cis*-V,[11] clearly establishing the *trans* stereochemistry. In the nmr, the chemical shifts of the vinyl hydrogens also confirm *trans* stereochemistry for hydroperoxide IIa and carbinol III, although the appearance of the two vinyl absorptions as a singlet in IIa, IIb, and III is rather anomalous (Table I).

Presumably no rearrangement has occurred in the reduction of hydroperoxide IIa to carbinol III as evidenced by the constancy of ultraviolet and nmr absorptions. Therefore, as proof of the skeletal arrangement of IIa, the synthesis of III as outlined below was undertaken.

Before the synthesis was initiated several factors were considered. The naphthalenic starting material[11] (VI) was chosen primarily because of its established stability to Wittig, or, in general, strongly anionic conditions such as would be incurred in the conversion of the ketone to a tertiary alcohol. No difficulties were



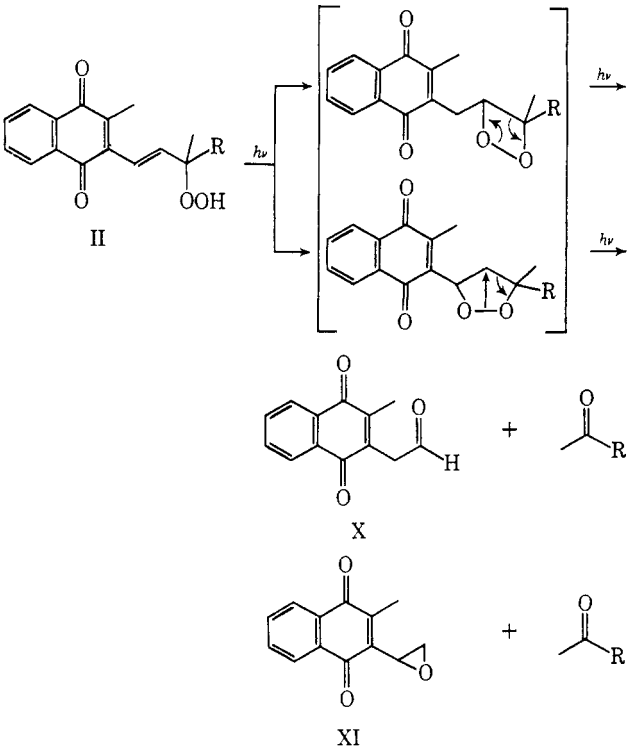
(10) P. Mamont, P. Cohen, R. Azerad, and M. Vilkas, *Bull. Soc. Chim. France*, 2513 (1965).
(11) W. E. Bondinell, S. J. Di Mari, B. Frydman, K. Matsumoto, and H. Rapoport, *J. Org. Chem.*, 33, 4351 (1968).

anticipated with the initial condensation since an analogous reaction proceeds with almost quantitative yield.[12] Methyllithium was preferred for conversion to the tertiary alcohol in order to minimize 1,4 addition which was feared to be a major side reaction. Also anticipated as complications were anionic polymerization of the vinyl–naphthyl system and aldol condensation, as well as anionic attack of the naphthalenic nucleus. Finally, the last step could be complicated by the suspected lability of the tertiary allylic alcohol system which one could envisage being reduced by the lithium aluminum hydride (used to remove the pivalyl group) or dehydrated by the mildly acidic conditions of oxidation (used to generate the quinone).

The stabilized ylide VII was prepared as described[13] and condensation with the naphthalenic aldehyde VI proceeded smoothly in refluxing dioxane to the unsaturated ketone VIII in 83% yield after chromatographic purification. The nmr spectrum of this compound was straightforward with the vinyl absorptions at δ 6.76 (d) and 7.67 (d), J = 16 Hz, confirming *trans* stereochemistry. Reaction with methyllithium lead to a very poor yield, but the addition of methylmagnesium bromide to the ketone resulted in improvement in product yield to 24%. The nmr spectrum again was consistent with that expected for ester carbinol IX showing vinyl protons at δ 6.20 (d) and 6.65 (d), J = 16 Hz. The ester carbinol IX was subjected directly to lithium aluminum hydride reduction, the hydroxy methoxy intermediate being isolated crude but not further purified. Thin layer chromatography indicated one compound while nmr showed the absence of the pivalate ester function. This crude material was then oxidized with *o*-chloranil in 50% aqueous acetonitrile and the yellow product purified. The material obtained in 53% yield proved to be identical with quinone carbinol III in all respects. Of particular interest was the collapse of the vinyl nmr absorptions to a singlet whereas until the quinone stage they had been separated into two distinct doublets. The possibility of conversion in the last stage of the synthesis to the *cis* isomer, which could possibly be stabilized by hydrogen bonding, seemed remote and was ruled out by its ultraviolet absorption (Table I).

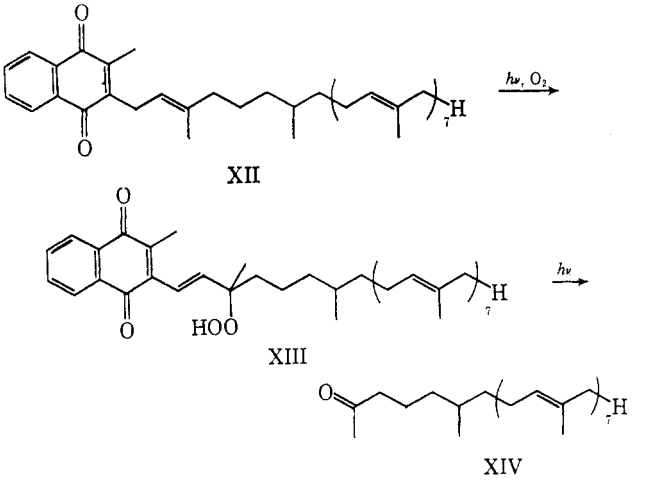
Undetermined in this study has been the fate of the naphthalenic fragment from the photodecomposition of the hydroperoxide. If one considers photocyclization of the hydroperoxide followed by electron rearrangement, two naphthalenic fragments are possible. Unfortunately neither the quinone aldehyde X nor the quinone epoxide XI could be detected by thin layer chromatography indicating either that they are not formed or that they themselves are very photolabile, perhaps especially so if they are released in an excited state.

The only naphthalenic fragment isolated was a yellow, amorphous solid which also fluoresced yellow under uv light; decomposition upon kieselgel chromatography was extensive. Its polymeric nature was indicated by its very limited solubility. Elemental analysis showed a higher oxygen content than would be expected for



$(C_{13}H_{10}O_3)_x$; however, in its mass spectrum the highest mass peak was at *m/e* 214, corresponding to this possible monomeric unit. Also, peaks for successive loss of one, two, and three CO's were also consistent with such a naphthoquinone unit.

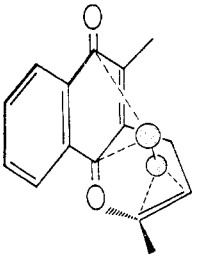
To continue the investigation of this photoreaction and its applicability to the *M. phlei* system, the MK-9 (II-H) quinone XII[14] was photolyzed in cyclohexane to yield the 3′-hydroperoxide XIII (8%) and side-chain ketone XIV (50%), perhaps somewhat unexpectedly since many unsaturated centers in the polyisoprenoid side chain are available for attack by singlet oxygen. Indeed, using the dye-sensitized system, none of the 3′-hydroperoxide was formed and instead extensive oxygenation of the side chain occurred. The deviation



of these two parallel reactions probably has some mechanistic significance. In the quinone-sensitized sys-

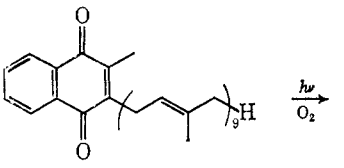
(12) H. Mayer, P. Schudel, R. Ruegg, and O. Isler, *Helv. Chim. Acta*, **46**, 650 (1963).
(13) F. Ramirez and S. Dershowitz, *J. Org. Chem.*, **22**, 41 (1957).
(14) M. Guerin, R. Azerad, and E. Lederer, *Bull. Soc. Chim. Biol.*, **46**, 2105 (1965).

tem, singlet oxygen is apparently generated in such a manner as not to permit its free circulation in solution. Since its generation must necessarily occur in the immediate vicinity of the quinone ring, the reactive β,γ position may be in a prime position to compete favorably with respect to the other unsaturated positions. The additional conjugation acquired by γ attack could be a driving force for the reaction though, in general, oxygenations are insensitive to this factor.[15a] On the other hand a quinone–oxygen complex appears attractive in that γ attack is assured.[14b]

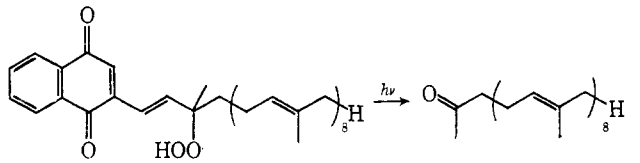


QUINONE-OXYGEN COMPLEX

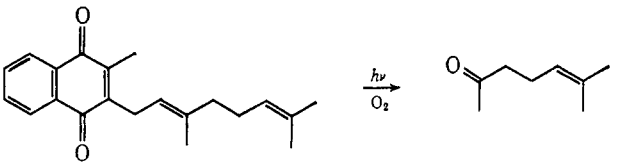
Anticipating a similar photoreaction, the fully isoprenoid, unsaturated quinone, MK-9, XV,[16] was photolyzed in cyclohexane to yield the 3-hydroperoxide XVI (8%) but surprisingly very little ketone XVII (3%). This was rather unexpected considering the distance of the additional double bond functionality from the reaction site. To investigate this disparity two comparison quinones, MK-2 (XVIII)[17] and 2-methyl-3-(3-methyl-2-nonenyl)-1,4-naphthoquinone (XIX), were synthesized in which the second isoprenoid double bond is correspondingly present and absent. Simultaneous estimation of the two product ketones, 2-methyl-2-hepten-6-one and 2-octanone, could be effected by gas–liquid partition chromatography. Upon photolyzing an equimolar mixture of the two quinones, the yields were 52% 2-octanone and 12% 2-methyl-2-hepten-6-one. Since hydroperoxide yields were nearly identical and since the 2-methyl-2-hepten-6-one was shown to be photostable, the discrimination must occur in the second step. The additional double bond could be in a position either to scavenge a radical intermediate from the decomposition of the hydroperoxide or to compete favorably for photoaddition of the hydroperoxide functionality.



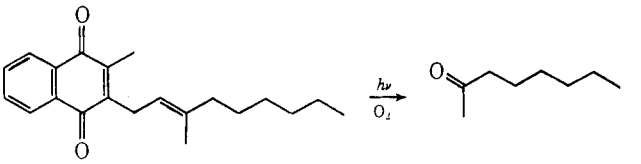
XV



XVI XVII

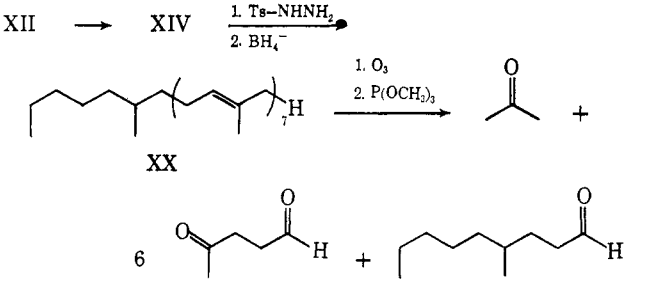


XVIII



XIX

Since the photoreaction appeared to be an effective degradation of the MK-9 (II-H) quinone, giving the ketonic side-chain fragment in good yield, a structural determination of this ketone was undertaken as a confirmation of the structure of the MK-9 (II-H) quinone. The structure of MK-9 (II-H), based upon independent chemical degradative evidence, is given as XII.[18] Photolytic degradation should yield ketone XIV, whereupon reduction to the alkene XX will then serve to label the end of the chain formerly attached adjacent to the naphthoquinone nucleus. The reported mild conditions[19] were chosen to reduce the carbonyl to methylene in order to minimize ambiguities resulting from possible double bond migrations. Ozonolysis followed by decomposition of the ozonides with trimethyl phosphite gave a mixture of keto aldehyde fragments which could then be converted to diagnostic 2,4-dinitrophenylhydrazones.



XII → XIV

XX

This series of reactions was accomplished as anticipated and a crude mixture of dinitrophenylhydrazones obtained. Thin layer chromatographic comparison with authentic material eliminated valeraldehyde dinitrophenylhydrazone as a possible component which would have been present had the second isoprene unit been unsaturated and the third, or any other, unit saturated. A less polar dinitrophenylhydrazone was isolated and examined by mass spectrometry. Strong peaks at *m/e* 206 and 224 were observed and are characteristic of aldehyde dinitrophenylhydrazones.[20] In addition a strong molecular

(15) (a) C. S. Foote, *Accounts Chem. Res.*, **1**, 104 (1968). (b) As emphasized in ref 15a the concept of a reactive sensitizer–oxygen complex is considered, in general, not to obtain; however, the quinone could be a special case.

(16) Generously supplied by Hoffmann-LaRoche Co., Basel, Switzerand.

(17) O. Isler, R. Ruegg, L. H. Chopard-dit-Jean, A. Winterstein, and O. Wiss, *Helv. Chim. Acta*, **41**, 786 (1958).

(18) R. Azerad, M.-O. Cyrot, and E. Lederer, *Biochem. Biophys. Res. Commun.*, **27**, 249 (1967).

(19) L. Caglioti and P. Grasselli, *Chem. Ind.* (London), 153 (1964).

(20) R. J. C. Kleipool and J. T. Heins, *Nature*, **203**, 1280 (1964).

ion at *m/e* 336 as well as a diagnostic peak at *m/e* 265 ($M^+ - C_5H_{11}$) marking the position of branching were sufficient evidence to identify the fragment as the expected 4-methylnonanal 2,4-dinitrophenylhydrazone, thus corroborating the structure of the *M. phlei* quinone, MK-9 (II-H).

This relatively simple method of (a) photooxygenation to cleave the side chain at the first isoprenoid unit as a ketone, (b) labeling the ketone end by reduction or some other reaction, and (c) ozonolysis followed by identification of the fragments may have application in the structure determination of other partially reduced menaquinones.

Experimental Section[21]

All quinones subjected to ultraviolet photolysis were exposed to the direct irradiation for 1 hr at room temperature of two 15-W General Electric blacklights (F15T8-BLB) at a distance of 20 cm.

Photolysis of Phylloquinone (Ib). Phylloquinone (Ib) (90 mg) was photolyzed in pure cyclohexane (100 ml). The solvent was removed and the photoproducts were chromatographed. 6,10,14-Trimethylpentadecan-2-one was eluted after residual starting quinone as a colorless oil (26 mg, 50% yield) and was spectrally identical with authentic material.[8] The hydroperoxide IIb, appearing as a deep yellow, uv dark band on the column, was eluted next to give 9 mg (10% yield) of a yellow oil after evaporation of solvent; nmr: 7.7 (m, ArH), 6.9 (s, C=CH), 2.14 (s, $ArCH_3$), and 1.39 (s, $\diagup\!\!\diagdown CH_3$).

Photolysis of Hydroperoxide IIb. The phylloquinone hydroperoxide IIb (40 mg) was photolyzed in 50 ml of cyclohexane. Chromatography as above gave 20 mg (91%) of 6,10,14-trimethylpentadecan-2-one.

Photolysis of 2-Methyl-3-(3-methyl-2-butenyl)-1,4-naphthoquinone, MK-1 (Ia). MK-1[22] (100 mg) was photolyzed in cyclohexane (200 ml). Chromatography of the photoproducts gave *trans*-2-methyl-3-(3-hydroperoxy-3-methyl-1-butenyl)-1,4-naphthoquinone (IIa) (12 mg) as a gummy yellow solid. An analytical sample was prepared by sublimation at 60°/μ; mp 90–94°; mass spectrum: *m/e* 272 (M^+, 0.1), 257 (1), 229 (30), 202 (40), 159 (100), 103 (50), 77 (70), and 43 (70).

Anal. Calcd for $C_{16}H_{16}O_4$: C, 70.1; H, 5.9. Found: C, 70.0; H, 5.8.

Dye-Sensitized Photolyses. Two crystallizing dishes (9 × 18 cm) were stacked; the top dish served as a red filter containing a solution of mineral oil and Sudan IV while the bottom dish, cooled in an ice bath, contained the solution (0.02 *M* in quinone, 0.02% in methylene blue in ethanol) to be photolyzed. A 300-W photoflood lamp provided illumination for the photolysis which proceeded for 1 hr, whereupon the ethanol solution was diluted with several volumes of water and extracted with petroleum ether to remove the photoproducts. After column chromatography, MK-1 (Ia) and phylloquinone (Ib) yielded hydroperoxides IIa and IIb as characterized above in 75% yield.

Reduction of *trans*-2-Methyl-3-(3-hydroperoxy-3-methyl-1-butenyl)-1,4-naphthoquinone (IIa). A. With Borohydride. Hydroperoxide IIa (26 mg) was dissolved in ethanol (3 ml) and a threefold excess of sodium borohydride added. The solution was diluted with several volumes of 0.1 *N* HCl and then extracted with ether. The product, *trans*-2-methyl-3-(3-hydroxy-3-methyl-1-butenyl)-1,4-naphthoquinone (III), was formed by air oxidation and yielded upon chromatographic purification a yellow solid (13 mg, 53%); mass spectrum: *m/e* 256 (M^+, 10), 241 (15), 214 (80), 198 (100), 76 (20), 59 (30), and 43 (20).

Anal. Calcd for $C_{16}H_{16}O_3$: C, 75.0; H, 6.3. Found: C, 74.7; H, 6.2.

B. With Hydrogen. Hydroperoxide IIa (25 mg) was dissolved in ethanol (5 ml), platinum oxide (5 mg) was added, and the mixture was shaken with hydrogen. After 2 mol of hydrogen had been taken up, the catalyst was removed by centrifugation and the solvent evaporated. The residue was dissolved in ether whereupon spontaneous air oxidation occurred. From column chromatography pure carbinol III (19 mg, 81%) was obtained; it was identical with authentic material.[10]

***trans*-4-Methoxy-2-methyl-3-(3-oxo-1-butenyl)-1-naphthol Pivalate (VIII).** 1-Hydroxy-4-methoxy-2-methyl-3-naphthaldehyde pivalate (VI)[11] (1.0 g) and acetonylidenetriphenylphosphorane[13] (VII) (1.15 g) were refluxed in dry dioxane (5.5 ml) for 20 hr. The solution was diluted with petroleum ether, the precipitated triphenylphosphine oxide was removed, the filtrate was washed with water and dried, and the solvent was evaporated. After chromatography (eluent: 40% ethyl ether in petroleum ether) the yield of unsaturated ketone, VIII was 0.95 g, 83%, mp 103–104°; uv: 350 nm (ϵ 3420), 305 (11,700), 278 (30,600), 271 (28,200), and 220 (30,500); nmr: 7.6 (m, 4, ArH), 6.76, 7.67 (d, C=CH, J = 16 Hz), 3.72 (s, 3, OCH_3), 2.24 (s, 3, $-(C{=}O)CH_3$), 2.18 (s, 3, $ArCH_3$), and 1.44 (s, 9, $C(CH_3)_3$); mass spectrum: *m/e* 340 (M^+, 100), 309 (90), 256 (60), 225 (100), 197 (30), 57 (40), and 43 (20).

Anal. Calcd for $C_{21}H_{24}O_4$: C, 74.1; H, 7.1. Found: C, 73.9; H, 7.1.

***trans*-4-Methoxy-2-methyl-3-(3-hydroxy-3-methyl-1-buteny)-1-naphthol Pivalate (IX).** Keto ester VIII (550 mg) was dissolved in tetrahydrofuran (3 ml) under nitrogen and then cooled in ice. An ethereal solution of methylmagnesium bromide (3 *M*, 0.55 ml) was added dropwise, keeping the temperature at 0° for 1 hr and then at room temperature for another hour. The mixture was treated with 1 *N* HCl and the product purified by chromatography (eluent: 70% ethyl ether in petroleum ether). After rechromatography (eluent: 30% ethyl ether in benzene) 130 mg (24% yield) of a colorless glass was obtained; nmr: 7.6 (m, 4, ArH), 6.20, 6.65 (d, 2, C=CH, J = 16 Hz), 3.67 (s, 3, OCH_3), 2.13 (s, 3, $ArCH_3$), and 1.45 (5 CH_3's); uv: 293 nm (ϵ 6190) and 248 (42,000); mass spectrum: *m/e* 356 (M^+, 100), 338 (5), 272 (100), 239 (10), 223 (8), 199 (5), and 57 (25).

Anal. Calcd for $C_{22}H_{28}O_4$: C, 74.1; H, 7.9. Found: C, 73.8; H, 8.0.

***trans*-2-Methyl-3-(3-hydroxy-3-methyl-1-butenyl)-1,4-naphthoquinone (III).** Alcohol ester IX (110 mg) was dissolved in tetrahydrofuran (2 ml) under nitrogen and a threefold excess of lithium aluminum hydride was added. The solution was allowed to stir for 15 min at room temperature; then excess hydride was decomposed with wet ether, 1 *N* hydrochloric acid was added, and the mixture was extracted with ether. The crude product (81 mg, 96%) left after evaporation of the ether was dissolved in 50% aqueous acetonitrile (10 ml) and 1 equiv of *o*-chloranil was added followed by immediate addition of ether and washing with water to remove the acetonitrile. The organic phase was evaporated and the residue was chromatographed (eluent: 50% ethyl ether in petroleum ether) to yield carbinol III (42 mg, 53%) which was identical in all respects with the material obtained *via* reduction of the hydroperoxide IIa.

Photolysis of MK-9 (II-H). The quinone MK-9 (II-H) (100 mg), isolated from *M. phlei*, was photolyzed in cyclohexane solution (100 ml). The residue after evaporation of the cyclohexane was chromatographed (eluent: 15% ethyl ether in petroleum ether) to obtain the 3′-hydroperoxide XIII (8 mg, 8% yield) and the ketone XIV (33 mg, 50% yield). The ketone was characterized by its mass spectrum: *m/e* 604 (M^+, 3), 535 (1), 467 (2), 409 (1), 399 (3), 341 (1), 331 (1), 273 (2), 263 (5), 205 (8), 137 (25), and 69 (100).

Photolysis of MK-9. The quinone MK-9[14] (330 mg) was photolyzed in cyclohexane (450 ml). The photoproducts were chromatographed on a kieselgel column (eluent: 15% ethyl ether in petroleum ether) giving 27 mg (8% yield) of hydroperoxide XVI; the products in the vicinity of the C_{45} ketone were collected (yield, 35 mg). This fraction was rechromatographed on another column (eluent: 50% chloroform in petroleum ether) to give 8 mg (3% yield) of pure ketone XVII; mass spectrum: *m/e* 602 (M^+).

Ethyl 3-Methyl-2-nonenoate. Sodium hydride (735 mg) was suspended in dry tetrahydrofuran (5 ml) and triethylphosphonoacetate (3.7 g) in tetrahydrofuran (6 ml) was added dropwise. After evolution of hydrogen had ceased, 2-octanone (1.5 g) in tetrahydrofuran (7 ml) was added dropwise and then the reaction was heated to 60° for 2.5 hr. It was cooled, acidified with 1 *N* HCl, and ex-

(21) Elemental analyses were performed by the Microanalytical Laboratory, University of California, Berkeley, Calif.; melting points are uncorrected. Ultraviolet spectra were recorded in isooctane with a Cary 14; a Varian M-66 was employed for mass spectrometry, and nmr spectra were recorded on a Varian A-60 using deuteriochloroform as a solvent; absorptions are reported as δ values relative to TMS. Column chromatographies were carried out on tlc grade kieselgel using petroleum ether (bp 30–60°) with increasing amounts of ether in 10% jumps as eluent. Analytical tlc plates employed a 250-μ layer of Camag kieselgel.

(22) O. Isler, R. Ruegg, A. Studer, and R. Jurgens, *Z. Physiol. Chem.*, **295**, 290 (1953).

tracted with ether and the dried ether solution evaporated *in vacuo*. The residual product, consisted of a mixture of *cis* and *trans* isomers [glpc: 10% SE-30 on 60–80 Firebrick, 135°; retention time (*cis*) 6.3 min, retention time (*trans*) 8 min; nmr: 5.57 (q, C=CH, J = 2 Hz), 2.13 (d, *trans* C=CCH$_3$, J = 2 Hz), 1.88 (d, *cis* C=CCH$_3$, J = 2 Hz)]. It was used in the next step without additional purification.

3-Methyl-2-nonen-1-ol. To a suspension of lithium aluminum hydride (1.03 g) and aluminum chloride (0.73 g) in absolute ether (75 ml), ethyl 3-methyl-2-nonenoate (2.14 g) in ether (20 ml) was added dropwise at −30°. After being stirred for 15 min at −30°, the mixture was hydrolyzed with wet ether and saturated aqueous ammonium chloride and then extracted with ether. Glpc (10% SE-30 on 60–80 Firebrick, 135°; retention time (*cis*) 6.2 min, retention time (*trans*) 6.5 min) showed a shorter retention time (4 min) impurity (~15%) which was probably the saturated alcohol 3-methylnonan-1-ol, an innocous impurity since it would be unreactive in the next condensation.

2-Methyl-3-(3-methyl-2-nonenyl)-1,4-naphthoquinone (XIX). Using crude 3-methyl-2-nonen-1-ol (above), boron trifluoride etherate, and 2-methyl-1,4-naphthoquinone under standard reaction conditions,[22] the allylic naphthoquinone XIX was prepared. Purification was effected by column chromatography to yield a mobile yellow oil; uv: 325 nm (ϵ 3050), 268 (16,690), 260 (17,000), 248 (17,990), and 243 (17,000); nmr: 7.8 (m, 4, ArH), 4.96 (t, 1, C=CH, J = 7 Hz), 3.27 (d, 2, CH_2CH=, J = 7 Hz), 2.11 (s, 3, ArCH$_3$), and 1.76 (s, 3, C=CCH$_3$); mass spectrum: m/e 310 (M^+, 80), 295 (10), 225 (90), 198 (100), and 186 (40).

Anal. Calcd for $C_{21}H_{26}O_2$: C, 81.3; H, 8.4. Found: C, 81.4; H, 8.4.

Comparative Photolysis of 2-Methyl-3-(3-methyl-2-nonenyl)-1,4-naphthoquinone (XIX) and MK-2 (XVIII). MK-2 (XVIII) (40 mg) and the allylic naphthoquinone XIX (40 mg) were photolyzed together in cyclohexane (100 ml). The solvent was carefully evaporated but not to complete dryness and the residue was diluted to 1 ml with ether. A 10-μl aliquot was assayed by glpc (6 ft × 0.25 in. column of 20% propylene glycol succinate on 60–80 Firebrick) and compared with a standard mixture containing 100 μg each of 2-octanone (retention time 4 min) and 2-methyl-2-hepten-6-one (retention time 5 min). The yields were: 2-octanone, 8.6 mg, 52%; 2-methyl-2-hepten-6-one, 1.9 mg, 12%.

Photostability of 2-Methyl-2-hepten-6-one. Two separate photolysis mixtures were made—one (A) containing MK-2 (40 mg) in 100 ml of cyclohexane and the other (B) containing, in addition, 2-methyl-2-hepten-6-one (25 mg); both were photolyzed for 1 hr after which 2-methyl-2-hepten-6-one (25 mg) was added to A. Both solutions were evaporated and each residue was diluted to 1 ml with ether. Glpc assay (above) showed the same ketone content in both A and B, indicating the ketone to be photostable.

Degradation of Ketone XIV from MK-9 (II-H). A. Reduction of Ketone XIV. The ketone (23 mg) was heated to reflux in absolute ethanol (3 ml) and tosyl hydrazide (11 mg) for 30 min, after which time tlc (solvent: 40% ethyl ether in petroleum ether) indicated the condensation to be complete. Four portions of sodium borohydride (40 mg each) were added while the solution was at reflux. After 1 hr the solution was cooled and acidified with dilute HCl, and the product was extracted into ether. Chromatography (eluent: petroleum ether) gave the colorless alkene XX as an oil (9 mg); mass spectrum: m/e 590 (M^+).

B. Ozonolysis of Alkene XX. Alkene XX (1 mg) was dissolved in methylene chloride (0.5 ml) and methanol (0.5 ml). The mixture was cooled in a Dry Ice–acetone bath and a stream of ozone enriched oxygen from a flow-through microozonizer[23] was bubbled into the solution until an excess of ozone was present. The solution was then flushed with nitrogen and trimethyl phosphite (5 mg) was added after which the solution was allowed to warm to room temperature. 2,4-Dinitrophenylhydrazine (7 mg), absolute ethanol (2 ml), and a drop of concentrated HCl were added, and the solution was refluxed for 5 min. The solvent was evaporated and the mixture chromatographed (eluent: 15% ethyl ether in petroleum ether), the 4-methylnonanal 2,4-dinitrophenylhydrazone being the first orange fraction; mass spectrum: m/e 336 (M^+, 60), 265 (30), 224 (70), 206 (100), and 83 (50).

(23) M. Beroza and B. A. Bierl, *Anal. Chem.*, **38**, 1976 (1966).

The Hydrolysis of Thioimidate Esters. II.[1] Evidence for the Formation of Three Species of the Tetrahedral Intermediate

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Abstract: The yield of amine formed on hydrolysis of the two thioimidate esters I and II varies with pH in a complex manner which has been interpreted in terms of two alternative mechanisms. The first involves three tetrahedral addition intermediates (cationic, neutral, and anionic) in acid–base equilibrium, each species partitioning to different ratios of amine to amide product. According to the second mechanism, hydrolysis proceeds *via* an anionic intermediate and two intermediates of zero net charge. Interconversion of the latter two species requires diffusion-controlled general acid–base catalysis. Buffer catalysis of amine formation with a tetrahedral intermediate generated from ethyl thioacetimidate (III) occurs with a Brønsted slope β = 0.94. This finding is, within experimental error, consistent with the value of β = 1.00 expected for a diffusion-controlled proton transfer and supports the second mechanism. Quantitative relationships are presented which allow the calculation of predicted rate–pH profiles for aminolysis reactions of esters and thiolesters which proceed *via* cationic, neutral, and anionic intermediates.

In 1959, Martin, *et al.*, showed that an unstable intermediate was formed during the hydrolysis of 2-methyl-Δ^2-thiazoline, and suggested that the same tetrahedral addition intermediate lay on the reaction pathway for the intramolecular aminolysis of the thiol ester S-acetylcysteamine.[2] A kinetic study of the latter reaction also gave evidence for an intermediate, although some features of the aminolysis reaction and of thiazoline hydrolysis could not be accommodated

(1) For a previous study in this series, see: R. K. Chaturvedi, A. E. MacMahon, and G. L. Schmir, *J. Am. Chem. Soc.*, **89**, 6984 (1967).

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