Reaction of Tetramethylallene with Singlet Oxygen.[13] Sodium hypochlorite (100 ml of a 4% solution) was added slowly to a vigorously stirred, ice-cold solution of 2.0 g of tetramethylallene and 6.1 g of 30% hydrogen peroxide in 150 ml of methanol. After 1.5 hr the solution was poured into 500 ml of water and extracted with pentane. The solvent was removed from the dried extracts and the crude product was shown to be recovered starting material by glpc and nmr analysis.

Photochemistry of Electron-Transport Quinones. I. Model Studies with 2-Methyl-1,4-naphthoquinone (Vitamin K_3)

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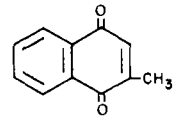
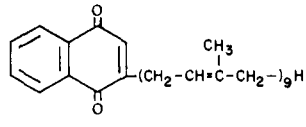
Abstract: The notion that near-ultraviolet radiation induces photochemical modification of electron-transport quinones in bacteria prompted us to study the effects of near-ultraviolet on 2-methyl-1,4-naphthoquinone (vitamin K_3), which can serve as a model for several naturally occurring quinones. Four near-ultraviolet cyclobutane photodimers of vitamin K_3 have been prepared and characterized by nmr, uv, and mass spectral analysis. Sunlight irradiation leads to the formation of the *syn* head-to-head and head-to-tail dimers, in contrast to the previously reported *anti* assignment. The *anti* dimers were prepared by near-ultraviolet irradiation of vitamin K_3 adsorbed on silica gel or dissolved in acetone. Two other photochemical products that were characterized were an oxetane dimer and an uncommon dimer of vitamin K_3 resulting from photochemical oxidation, 3,3′-bi(2-methyl-1,4-naphthoquinone).

In many recent studies concerned with the biological effects of radiation, the far-ultraviolet region of the spectrum (200–300 nm) has received preponderant attention. This was to be expected following the unraveling of deoxynucleic acid structure and the realization that this genetic material is the principal cellular target of far-ultraviolet radiation.[2] Because the irradiation of living organisms with near-ultraviolet light (300–400 nm) is considered to engender somatic rather than genetic damage, far fewer studies have been undertaken to uncover the harmful photomolecular events than has been the case with damage induced by far-ultraviolet illumination. Nevertheless, progress in this direction has been made. Brodie and coworkers[3] have studied extensively the effects of near-ultraviolet irradiation on bacteria and bacterial substructures that perform oxidative metabolism. Their work pinpoints the quinones that serve as electron carriers in electron transport as the principal uv targets and shows that photochemical modification of these quinones disrupts the oxidative and phosphorylative capacities of bacterial respiratory particles. Damage to bacterial quinones has been invoked also by Jagger[4] to explain the decreased sensitivity of *E. coli* B to far-ultraviolet radiation when the bacteria have received a prior or subsequent near-ultraviolet treatment.

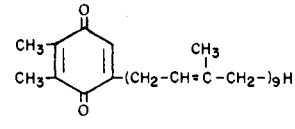
Although these studies leave little doubt that bacterial naphthoquinones and ubiquinones are the natural near-ultraviolet targets, as far as we are aware no reports have appeared on the separation and identification of quinone photoproducts arising *in situ*. Studies encompassing this objective were initiated, and in this publication we report on the near-ultraviolet modification of 2-methyl-1,4-naphthoquinone[5] (I) under several experimental conditions. This simple quinone can be considered as a model for two naturally occurring quinones: demethyl vitamin K_2 (II), which has been identified in *H. parainfluenzae*[6] and *S. faecalis*,[7] and plastoquinone[8] (III), present in plastids and serving as an intermediate in photosynthetic electron transport.[9]



I



II



III

Our studies have led to the isolation and characterization of the four possible cyclobutane photodimers of vitamin K_3 having the *syn* and *anti* head-to-head and head-to-tail configurations. The assignments of *syn* and *anti* isomers were made on the basis of infrared analysis and nmr shielding criteria while differentiation

(1) (a) Southwest Center for Advanced Studies, Dallas, Texas 75230. (b) Mobil Research and Development Corp., Dallas, Texas 75221.
(2) R. H. Haynes, *Rad. Res., Suppl*, 6, 1 (1966).
(3) A. F. Brodie in "Biochemistry of Quinones," R. A. Morton, Ed., Academic Press, New York, N. Y., 1965, p 384.
(4) J. Jagger, *Photochem. Photobiol.*, 3, 451 (1964).
(5) This quinone is also known as vitamin K_3 and menadione, and we will use the former designation.
(6) R. L. Lester, D. C. White, and S. L. Smith, *Biochemistry*, 3, 949 (1964).
(7) R. H. Baum and M. I. Dolin, *J. Biol. Chem.*, 240, 3425 (1965).
(8) F. L. Crane, *Plant Physiol.*, 34, 546 (1959).
(9) (a) E. R. Redfearn in ref 3, p 149; (b) D. I. Arnon and F. L. Crane in ref 3, p 433.

of head-to-head and head-to-tail isomers was accomplished by C^{13}–H analysis. This approach to the structural analysis of vitamin K_3 photodimers should facilitate the characterization of other cyclobutane photodimers that may be synthesized because of the recent interest in the photodimerization[10–14] and general photochemistry of conjugated cyclic enones.[15]

Results

Isolation of Four Near-Ultraviolet-Induced Cyclobutane Photodimers of Vitamin K_3 and Two Other Dimers. The two photoproducts which formed following sunlight irradiation of vitamin K_3 for 1 month were separated by Asahi[16] through fractional crystallization. One melted at 178° the other at 235°—the latter had already been described by Madinaveitia.[17] From elemental analysis, molecular weight determinations, infrared studies, and X-ray diffraction analysis, Asahi concluded that both photoproducts were cyclobutane dimers and intuitively, assuming that the higher melting one was more stable, he assigned to it the head-to-tail *anti* configuration; the lower melting one was assigned the head-to-head *anti* form.

We repeated Asahi's work and separated these two dimers by column chromatography on silicic acid–Celite (2:1) with ether–hexane as the eluting solvents. The separation was easily followed by thin layer chromatography (tlc) of the eluates on silica gel impregnated with a fluorescent indicator, 40% ether–hexane serving as the developing solvent. Dimer A,[18] mp 202–203° (lit.[16] mp 178°), was eluted first and pure dimer B, decomposing between 235 and 240° (lit.[16] mp 235°), was eluted after a mixture of the two dimers. We assigned the *syn* configuration to both dimers A and B in contrast to Asahi's *anti* description. The data upon which our assignments were made are presented below.

Dimers A and B were the major if not the sole photoproducts arising from a 15-min "black light"[19] irradiation of 25 μg of vitamin K_3 spread across a glass plate.

The two remaining cyclobutane photodimers of vitamin K_3 were prepared by black light irradiation of either the quinone adsorbed on silica gel, or a solution of the quinone in acetone. The new dimers, which are referred to as FD and SD,[18,20] were separated by preparative tlc (ptlc) with silica gel as sorbent and 1:1 ether–hexane as solvent. Each was scraped from 30 to 40 plates, eluted, and subjected to repetitive ptlc with ether–hexane as developing solvent until each one appeared on the chromatoplates as a single band. After elution and crystallization, FD melted at 238–239°. SD, in contrast to FD, separated into two bands on silica gel when methylene chloride was substituted for 1:1 ether–hexane. The compound in the lower band was identified as the fourth cyclobutane dimer, SD; it sublimed above 240°.

Elution and crystallization of the material in the upper band afforded an oxetane dimer which decomposed at 189°.

A yellow band just below vitamin K_3 was eluted, and the mixture was subjected to ptlc with 1:1 ether–hexane as solvent. Two minor fluorescent quenching bands and one major yellow band appeared on the chromatogram. The latter substance (J), after it was eluted and crystallized, decomposed at 242–245°.

Structural Assignments of the Four Cyclobutane Photodimers of Vitamin K_3. Although we have referred to A, B, FD, and SD as dimers, direct proof of the dimer structure was obtained by mass spectroscopic analysis. A, B, FD, and SD were subjected to low-voltage (~10 eV) electron impact. All four dimers showed a parent peak at *m/e* 344 and a monomer peak at *m/e* 172; at 70 eV, their cracking patterns were similar. A peak at *m/e* 299, corresponding to the loss of CO_2 and H, was apparent in all the mass spectra. This electron-impact-induced rearrangement was observed recently by Dekker and Venter[21] in the mass spectra of the cyclobutane photodimers of 1,4-naphthoquinone[22] and an unspecified photodimer of vitamin K_3.

The structure of the 1,4-naphthoquinone *anti* dimer[22] was assigned unequivocally on the basis of its conversion to the known *cis,trans,cis*-tetracarbomethoxycyclobutane. The *syn*-dimer assignment[22] was supported mainly by infrared and mass spectral analysis. The *anti* dimer migrates ahead of the *syn* dimer on silica gel in ptlc with methylene chloride as solvent. Similarly, the *anti*-cyclobutane photodimer of 1-nitroacenaphthalene[23] migrates faster on silica gel than the corresponding *syn* dimer. Thus the finding that dimers FD and SD migrate ahead of dimers A and B in ptlc with methylene chloride suggested that the former were the *anti* and the latter the *syn* dimers. These tentative assignments were corroborated by infrared and nmr analysis. The infrared spectra of the *syn*- and *anti*-1,4-naphthoquinone dimers in the 3000-, 1200–1300-, and 700–800-cm^{-1} regions were compared with the corresponding regions in the spectra of the four vitamin K_3 cyclobutane dimers. Dekker, *et al.*,[22] note that the out-of-plane aromatic CH wagging vibration of mono- and *ortho*-disubstituted benzenes in the 750-cm^{-1} region differ in the case of *cis* and *trans* isomers: the band is usually split in the former case.

(10) D. J. Trecker, A. A. Griswold, and O. L. Chapman, 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966, Abstract S-20.

(11) (a) P. E. Eaton, *J. Am. Chem. Soc.*, 84, 2344, 2454 (1962); (b) P. E. Eaton and W. S. Hurt, *ibid.*, 88, 5038 (1966).

(12) (a) P. Yates and M. J. Jorgenson, *ibid.*, 85, 2956 (1963); (b) P. Yates, S. E. Eğe, G. Büchi, and D. Knutsen, *Can. J. Chem.*, 45, 2929 (1967); (c) S. E. Eğe and P. Yates, *ibid.*, 45, 2933 (1967).

(13) (a) E. Y. Y. Lam, D. Valentine, and G. S. Hammond, *J. Am. Chem. Soc.*, 89, 3482 (1967); (b) D. Valentine, N. J. Turro, Jr., and G. S. Hammond, *ibid.*, 86, 5202 (1964).

(14) (a) J. L. Ruhlen and P. A. Leermakers, *ibid.*, 89, 4944 (1967); (b) J. L. Ruhlen and P. A. Leermakers, *ibid.*, 88, 5671 (1966).

(15) For a recent review see P. E. Eaton, *Accounts Chem. Res.*, 1, 50 (1968).

(16) Y. Asahi, *J. Pharm. Soc. Japan*, 76, 373 (1956).

(17) A. J. Madinaveitia, *Anales Soc. Españ. Fís. Quím.*, 31, 750 (1933).

(18) The correct nomenclature for the four cyclobutane photodimers of vitamin K_3 and the designations used in this paper follow: 5a,5bβ,-11a,11bβ-tetrahydro-5aβ,11aβ-dimethylcyclobuta[1,2-*b*:3,4-*b'*]dinaphthalene-5,6,11,12-tetrone (dimer A); 5a,5b,11aβ,11bβ-tetrahydro-5a,-β,5bβ-dimethylcyclobuta[1,2-*b*:3,4-*b'*]dinaphthalene-5,6,11,12-tetrone (dimer B); 5a,5b,11aα,11bβ-tetrahydro-5aβ,5bα-dimethylcyclobuta-(1,2-*b*:3,4-*b'*]dinaphthalene-5,6,11,12-tetrone (dimer FD); 5a,5bα,-11a,11bβ-tetrahydro-5aβ,11aα-dimethylcyclobuta[1,2*b*:3,4-*b'*]dinaphthalene-5,6,11,12-tetrone (dimer SD).

(19) "Black light" refers to near-ultraviolet (300–400 nm) emitted by 15-W, General Electric, F15T8-BLB lamps. The emissions at about 360 nm have the highest intensity.

(20) FD and SD refer to fast dimer and slow dimer; FD has a higher mobility on silica gel with 1:1 ether–hexane or methylene chloride as solvents.

(21) J. Dekker and D. P. Venter, *J. Am. Chem. Soc.*, 90, 1225 (1968).

(22) J. Dekker, P. J. van Vuuren, and D. P. Venter, *J. Org. Chem.*, 33, 464 (1968).

(23) T. S. Cantrell and H. Shechter, *ibid.*, 33, 114 (1968).

This difference is evident in the spectra of *cis*- and *trans*-stilbenes[24] and was observed by Dekker, *et al.*,[22] in the spectra of the *syn*- and *anti*-naphthoquinone dimers. This splitting appears in the spectra of dimers A and B but not in the spectra of FD and SD. The striking similarity between the spectra of dimer A and the *syn*-naphthoquinone dimer in the 3000-cm^{-1} region also supports the *syn* assignment made for dimer A. The *syn*-naphthoquinone dimer shows only two CH stretching vibrations in this region, while dimer A exhibits these two vibrations and two others attributable to the symmetrical and asymmetrical CH_3 stretching vibrations. Finally, bands in the 1200–1300-cm^{-1} region which may be due to the C–C stretch[25] of the phenyl–carboxyl group are generally more complex in the spectra of A and B compared to those of FD and SD. The same difference was observed in the spectra of the *syn*- and *anti*-naphthoquinones. These infrared data favor the *syn* assignment for dimers A and B and the *anti* one for FD and SD made on the basis of dimer mobility on silica gel.

Strong support for these assignments was gleaned from the nmr spectra of the dimers on the basis of the following arguments. Protons above the plane of a benzene ring should experience a shielding effect[26] as a result of the magnetic anisotropy of benzene. Examination of molecular models indicates that such an effect should be observed in the resonances of the aromatic protons of the *syn* dimers when compared to those of the *anti* ones and vitamin K_3.

A diamagnetic shielding is predicted for protons above the plane of a carbonyl group,[27] and such shieldings have been observed.[28] Examination of models indicates that the methyl groups of the *anti* isomers should be more shielded by the carbonyl function than those in the *syn* isomers and vitamin K_3. The data in Table I showing δ values for the CH_3, CH, and Ar–H

Table I. Chemical Shifts[a] (δ) of CH_3, CH, and Ar–H Protons in $CDCl_3$

Type of proton	Dimers A	B	FD	SD	Vitamin K_3
CH_3	1.68	1.60	1.39	1.32	2.19 (doublet)
CH	3.47	3.83	3.49	3.65	6.84 (quartet)
Ar–H[b]	7.60 (6)	7.62 (4)	7.88 (4)	7.83 (4)	7.73 (2)
	8.10 (2)	7.75 (4)	8.17 (4)	8.16 (4)	8.04 (2)

[a] Measured in ppm from TMS. [b] These numbers refer to centers of resonance of complex regions rather than true chemical shifts. The numbers in parentheses refer to the number of protons contributing to each complex resonance.

protons of vitamin K_3 and its four photodimers bear out these predictions. The tabulated chemical shifts indicate that dimers A and B, with the more highly shielded aromatic protons, have the *syn* configuration, and dimers FD and SD, with the more highly shielded methyl protons, have the *anti* configuration.

The nmr assignment of head-to-head (h-h) or head-to-tail (h-t) structure within each dimer pair was accomplished by measuring the C^{13}–H nmr spectra of the methine protons in dimers B and FD. This technique was used recently to distinguish between the h-h and h-t dimers of several cyclobutanes.[29-32] The C^{13}–H spectrum of a 6% solution of dimer B in $CDCl_3$ was obtained by time averaging 139 times over a 500-cycle region bracketing the methine resonance. Both high- and low-field C^{13}–H satellite resonances were observed with J = 142 cps. Both resonances were split into doublets with $J = 11 \pm 1$ cps. Thus, the h-h structure is assigned to dimer B. By similar time averaging (100 times) the spectrum of a 5.5% solution of dimer FD was obtained over a 250-cycle region bracketing the methine resonance. High- and low-field C^{13}–H satellites were observed with J = 140 cps. These resonances were also split into doublets with $J = 6.5 \pm 1$ cps. Therefore, dimer FD is also assigned the h-h structure.

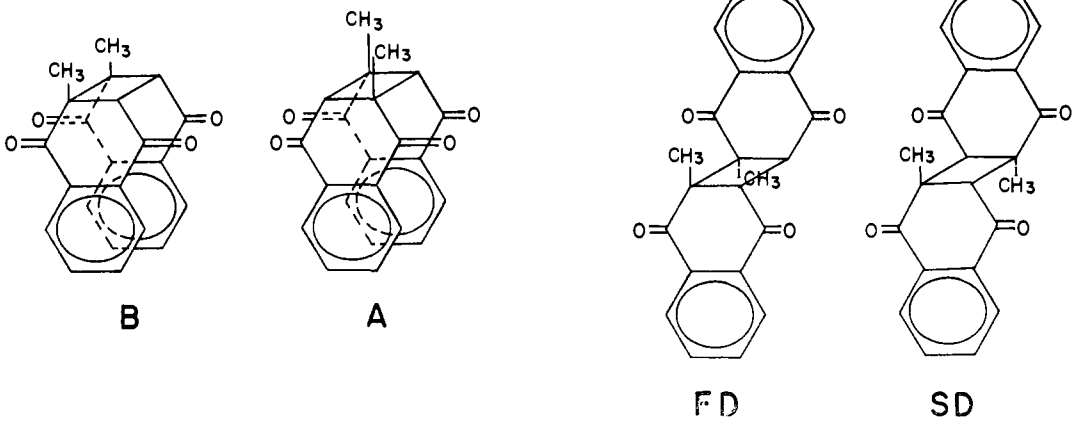
The positions of the resonances with respect to the h-h and h-t configurations can be readily rationalized on the basis of simple shielding arguments. Theoretical considerations suggest that a proton *cis* to a methyl group on a cyclobutane ring should be shielded.[33] The amount of this shielding has been empirically estimated as 0.496 ppm.[34] A smaller shielding effect would be expected between two *cis* neighboring methyl groups. The methine protons in the h-t *syn* isomer are more shielded than those in the h-h *syn* isomer because in the former each proton is shielded by two neighboring methyl groups. Similarly, the methyl protons in the h-h *syn* isomer are the more shielded (Table I). The effect of a methyl group on a neighboring *trans* proton in a cyclobutane ring should be to deshield the proton.[33] A similar but smaller deshielding effect should be felt by a neighboring *trans*-methyl group. In the h-t *anti* dimer, the shielding of the methine proton by the neighboring *cis*-methyl group is partially cancelled by the deshielding effect of the neighboring *trans*-methyl group. Thus, the methine proton is more deshielded in the h-t *anti* dimer as compared to the h-h *anti* dimer (Table I).

Our structural assignments of the four cyclobutane dimers of vitamin K_3 are presented in Chart I.

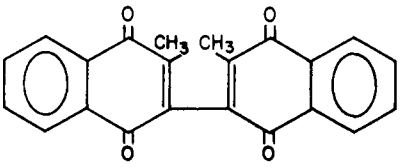
Structural Assignments for J and Oxetane Dimers. The nmr spectrum of compound J was decisive for elucidating its structure. Besides the resonances of the aromatic protons only a single resonance at 2.07 ppm (δ) was observed, ascribable to a methyl group vicinal to a double bond. Following integration, the calculated ratio for the areas occupied by the aromatic CH and the CH_3 resonances was 8:6. From these data, the structure for J was deduced as 3,3′-bi(2-methyl-1,4-naphthoquinone). The infrared and ultraviolet spectra of J are consistent with this assignment. The yellow color of J indicated

(24) Infrared Spectral Data, API Research Project No. 44, April 30, 1961, *cis* serial no. 2298, *trans* serial no. 2299.
(25) N. B. Colthup, L. H. Daly, and S. E. Wiberley "Introduction to Infrared and Raman Spectroscopy," Academic Press, New York, N. Y., 1964, p 244.
(26) C. E. Johnson, Jr., and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).
(27) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, pp 122–124.
(28) O. L. Chapman and H. G. Smith, *J. Am. Chem. Soc.*, **83**, 3914 (1961).
(29) R. Anet, *Tetrahedron Letters*, 3713 (1965).
(30) H. Ziffer, N. E. Sharpless, and R. O. Kan, *Tetrahedron*, 22, 3011 (1966).
(31) G. M. Blackburn and R. J. H. Davies, *J. Chem. Soc.*, 1342 (1966).
(32) H. Ziffer and J. R. Williams, *J. Org. Chem.*, 33, 920 (1968).
(33) Reference 27, pp 112–119.
(34) H. Weitkamp and F. Korte, *Tetrahedron Suppl.*, 7, 75 (1966).

Chart I



an intact 1,4-naphthoquinone nucleus. The similarity of the infrared spectra of J and vitamin K_3 in the 1350–1700-cm^{-1} region leaves no doubt that this is so (Figure 1). One would expect the ultraviolet absorption spectra



J

of J and vitamin K_3 to be similar and the extinction coefficients for the maxima in the spectrum of J to be about twice those of vitamin K_3. The data conform to this prediction: J, uv_{max}^{MeOH} 335 (5960), 252 (39,600), and 246 nm (ϵ 38,600); vitamin K_3, 330 (2900), 246 (20,600), 244 nm (ϵ 19,800). The mass spectrum of J showed a parent peak at 342.

The colorless photoproduct traveling ahead of SD in ptlc with methylene chloride was eluted and crystallized, mp 189°. A low-voltage mass spectral run demonstrated the presence of a monomer peak at 172 as well as peaks in the 340 range, but no well-defined parent peak was found. Again the nmr spectrum provided the most useful information for structure identification. The spectrum in $CDCl_3$ showed a complex aromatic region (8 H's), a quartet at δ 6.6 (1 H), a singlet at 4.25 (1 H), a singlet at 1.96 (3 H), and a doublet at 1.79 (3 H, J = 2 cps). These values would be expected for an oxetane dimer. The infrared data confirm the assignment. The carbonyl region was resolved into a triplet having frequencies at 1689, 1678, and 1672 cm^{-1}. A double bond conjugated to a keto group was observed at 1645 cm^{-1}, and bands at 1017 and 980 cm^{-1} are probably due to C–O–C vibrations. There are eight possible oxetane dimers of vitamin K_3. The chemical shift of the trimethylene oxide α-methylene protons in carbon tetrachloride is 4.63.[35] This suggests that the tertiary hydrogen in the oxetane dimer is α to the oxetane oxygen. There are four oxetane dimers having this structural feature, and the isolated oxetane dimer is no doubt one of these. However, in the absence of nmr spectra of suitable model compounds, it is not possible to decide which one.

(35) E. Lippert and H. Prigge, *Ber. Bunseges. Physik. Chem.*, 67, 415 (1963).

Discussion

In bacteria two types of quinones serve as electron-transport carriers; these are generally tetrasubstituted 1,4-benzoquinones (ubiquinones, referred to also as coenzyme Q's) and mono- and disubstituted naphthoquinones[36]—vitamins K_1, K_2, and demethyl vitamin K_2. The work of Brodie and coworkers[3] suggests that both types of quinones are photochemically altered *in situ* by near-ultraviolet irradiation of bacteria. What is the possibility that some of the photoproducts are cyclobutane dimers? Their formation would be controlled principally by steric and environmental factors. In the case of the ubiquinones and vitamins K_1 and K_2, the steric factor would no doubt predominate and preclude the formation of dimers since previous attempts to dimerize tetrasubstituted *p*-benzoquinones failed.[37] Since vitamin K_3 can be dimerized both in the solid state and in solution, it is possible that demethyl vitamin K_2 present in certain bacteria[6,7] may dimerize *in situ* when the microorganisms are treated

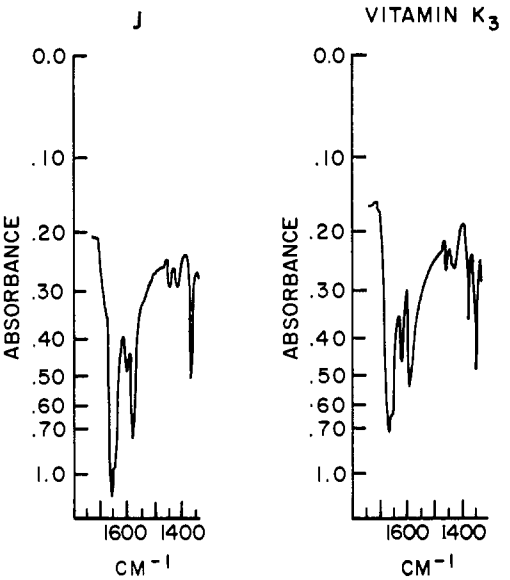


Figure 1. The 1350–1700-cm^{-1} region of the infrared spectra of compound J and vitamin K_3.

(36) The quinone in *M. phlei* has a vitamin K_2 structure but the second isoprene unit from the nucleus is saturated.

(37) C. Cookson, D. A. Cox, and J. Hudec, *J. Chem. Soc.*, 4499 (1961).

with near-ultraviolet light. However, whether dimerization actually occurs in these instances will depend upon the quinone milieu. In this connection it is of interest that Eck and Trebst[38] isolated dimers of plastoquinone, a trisubstituted *p*-benzoquinone, from chestnut leaves but they did not ascertain whether the dimers were actually present in the leaves or arose as photoproducts during the isolation. Further speculation at this time regarding the nature of quinone photoproducts arising in bacteria appears unwarranted.

Rabinovich and Schmidt[39] recently described the topochemical control of quinone autodimerization in the solid state and noted that vitamin K_3 is dimorphic. This certainly explains the formation of two *syn* cyclobutane dimers when the quinone is exposed to sunlight. These authors also noted that the large distance between the double bonds of 1,4-naphthoquinone in the crystal lattice[40] precluded the dimerization of this quinone. However, Dekker, *et al.*,[22] did obtain small yields of the *syn* dimer by a six-week exposure of the quinone to sunlight.

Although "black light" irradiation of solid vitamin K_3 yields two *syn* dimers, similar irradiation of the quinone adsorbed on silica gel or dissolved in acetone yields a plethora of photoproducts. Leermakers and coworkers[41] have directed attention to the altered electronic spectum of ketones and other molecules adsorbed on silica gel and have also noted differences in photochemical behavior of adsorbed molecules compared to those in solution. In the photochemical decomposition of tetramethyl-1,3-cyclobutanedione and azobisisobutyronitrile on silica gel, the adsorbent was observed to restrain the freedom of the radicals that were generated. In contrast, in our experiments with vitamin K_3 adsorbed on silica gel, the latter seems to allow greater freedom to the initial photochemical product enabling it to react in many ways. Thus we observed four different kinds of dimers: *syn* and *anti* cyclobutanes, an oxetane dimer, and a binaphthoquinone dimer.

The formation of 3,3′-bi(2-methyl-1,4-naphthoquinone) from vitamin K_3 is an example of an uncommon photooxidative coupling first noted by Hooker[42] in the preparation of 3,3′-bi(2-hydroxy-1,4,-naphthoquinone). A similarly coupled dimer, 5,5′-diuracil, was characterized by Ishihara and Wang[43] after far-ultraviolet irradiation of 5-bromuracil. The structure of a naturally occurring antibiotic, actinorhodin, is that of a biquinone, and one may speculate that it is formed *in situ* photochemically.[44]

Experimental Section

General. Infrared and ultraviolet spectra were recorded on a Perkin-Elmer Model 337 spectrometer using KBr disks and a Cary Model 14 spectrometer, respectively. For nmr analysis, a Varian H100 spectrometer was used, and frequencies were measured with a Hewlett-Packard 522-B electronic counter. The ^{13}C–H frequencies were accumulated on a Varian C-1024 time-averaging computer. Samples were dissolved in deuteriochloroform containing tetramethylsilane as an internal standard. Melting points are uncorrected and were obtained with a Fisher-Johns apparatus. Mass spectra were recorded on a Varian M-66 mass spectrometer. Microgram quantities of the four vitamin K_3 cyclobutane dimers were separated by tlc using Eastman chromatogram sheets (6060) with a 40% ether–hexane solvent mixture. The dimers appeared as fluorescent quenching areas when viewed with near-ultraviolet light. Ptlc was carried out on 20 × 20 cm glass plates coated with a 1-mm-thick layer of silica gel PF_{254}, Brinkmann Instruments, Westbury, N. Y. The plates were dried at room temperature overnight and were then activated for 0.5 hr at 120°. Vitamin K_3 was an Eastman Organic Chemical, No. 5185. All irradiations were performed with five General Electric 15-W F15T8-BLB "black light" lamps mounted in parallel. Practical grades of all solvents were distilled before use. The 1,4-naphthoquinone *anti* dimer precipitated after a 20-hr "black light" irradiation of an acetone solution of the quinone. It was filtered and recrystallized from chloroform.

Isolation of Dimers A and B.[45] Five grams of vitamin K_3 were left in an open petri dish on a windowsill facing south for 1 month. The yellow quinone turned amber. The photoproducts were separated on a 31 × 1.6 cm column packed in hexane with a mixture of 30 g of silicic acid, 100 mesh (Mallinckrodt 2847), and 15 g of Hyflo Super-Cel (Johns-Manville). Benzene (0.5 ml) containing 0.5 g of photoproduct was placed on the column, and it was eluted with 100 ml of hexane and 500 ml each of 20, 25, and 30% ether–hexane mixtures (v:v). Dimer A appeared in the 30% ether–hexane eluates. This was followed by a mixture of dimers A and B, and finally pure B appeared. The dimer composition of the eluates was analyzed by tlc. Eluates containing dimers A and B were pooled separately and evaporated, and each photoproduct was crystallized from 90% alcohol. Dimer A had mp 202–203°. Its mass spectra showed a parent peak at *m/e* 344 and a monomer peak at 172; ir (KBr) 1678, 1580, 1439, and 1361 cm^{-1}; λ^{MeOH} infl 311 (4200), max 302 (4490), sh 257 (22,800), max 227 nm (ϵ 60,100). Dimer B decomposes between 235 and 240°. Mass spectra showed a parent peak at *m/e* 344 and a monomer peak at 172; ir (KBr) 1689, 1678, 1595, 1467, 1445, 1395, and 1378 cm^{-1}; λ^{MeOH} sh 311 (3050), max 302 (3175), sh 260 (16,600), max 227 mm (ϵ 49,000).

Isolation of Dimers FD and SD. Although these dimers were prepared by two different procedures, the steps involving the separation and isolation of the dimers were identical in both cases. In the first preparation, 1 g of vitamin K_3 was dissolved in chloroform and 80 g of silica gel (Baker, AR 3405) was added to it. The chloroform was evaporated, and 2-g batches of the dry adsorbent were spread across 20 × 20 cm glass plates. The silica gel was irradiated for 7 min with black light lamps held 15 cm above it. The powder was collected, respread, and reirradiated for another 7 min. When all the silica gel had been irradiated, it was pooled and extracted with a 1:1 chloroform–acetone mixture. The solvent was removed under *vacuo*. Alternatively, FD and SD were prepared by irradiating for 20 hr a well-stirred solution of 1 g of vitamin K_3 in 30 ml of acetone. Then the acetone was removed under vacuum. The photoproducts from either preparation were dissolved in chloroform to yield a concentration of 0.1 g/0.5 ml, and 0.5 ml of the solution was applied across the starting line of a silica gel coated plate for ptlc. The plate was developed three times with a 1:1 ether–hexane solvent. FD and SD appeared as two poorly resolved fluorescent quenching bands running above dimer A which was used as a marker. Both bands were scraped off the plate with a razor blade and collected. These photoproducts from 30 to 40 plates were pooled and extracted with 1:1 chloroform–acetone. The solvent was removed under *vacuum* and the residue was dissolved in chloroform to yield a concentration of 10 mg/ml for ptlc. On each plate, 0.2 ml was run using 1:1 ether–hexane as solvent. After three to four runs using this ptlc system, FD and SD were isolated separately as single bands. FD purified to this step was scraped from 30 to 40 plates and eluted with 1:1 chloroform–acetone mixtures. It appeared as a single band in several ptlc runs with different developing solvents. The chloroform–acetone mixture was distilled under vacuum and the photoproduct was crystallized from 90% alcohol, mp 238–239°. The mass spectrum showed a peak at *m/e* 344 and a monomer peak at 172; ir (KBr) 1672, 1580, 1450, and 1361 cm^{-1}; λ^{MeOH} max 308 (3080), max 301 (3160), sh 253 (20,830), and max 231 mm (ϵ 58,600).

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(44) This speculation arose during conversations with Dr. W. Müller of the University of Göettingen who pointed out this feature of actinorhodin structure to us.
(45) The designation of dimers A and B (Abstracts, Fifth International Congress of Photobiology at Hanover, N. H., Aug 26–31, 1968, p 26) should be reversed.

Anal. Calcd for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.31; H, 4.39.

When SD, which ran as a single band in ptlc with 1:1 ether–hexane as solvent, was subjected to repetitive ptlc using methylene chloride as developer, two widely separated fluorescent quenching bands arose. The lower one, pure SD, was removed and collected from 30 to 40 runs. After crystallization from 90% alcohol the dimer was found to sublime at about 240°. The mass spectrum showed a parent peak at *m/e* 344 and a monomer peak at 172; ir (KBr) 1678, 1589, 1449, and 1367 cm^{-1}; λ^{MeOH} sh 307 (3550), max 301 (3630), max 253 (23,900), and max 230 nm (ϵ 68,600).

Anal. Calcd for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 77.00; H, 5.03.

Oxetane Dimer of Vitamin K_3. The silica gel containing the faster moving band from the methylene chloride runs described above was scraped from 20 to 30 plates. The photoproduct was eluted with a 1:1 chloroform–acetone mixture and the solvent evaporated. The residue was crystallized from 90% alcohol. The colorless crystals decomposed at 189°. The mass spectra showed a monomer peak at 172 but no apparent peak at *m/e* 344; ir (KBr) 1689, 1678, 1672, 1645, 1589, 1461, 1439, and 1378 cm^{-1}; λ^{MeOH} infl 314 (3000), infl 300 (4730), infl 260 (20,700), and max 232–235 nm (ϵ 44,700).

Anal. Calcd for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.57; H, 4.77.

3,3′-Bi(2-methyl-1,4-naphthoquinone). When the photoproduct (J) from 30 plates had been pooled, the material was eluted and rechromatographed by ptlc using 1:1 ether–hexane as solvent. Three fluorescent quenching bands were observed, two minor colorless ones and a major yellow one. The latter was eluted, the solvent evaporated, and the yellow material crystallized from 95% alcohol. The yellow crystals decomposed at 242–245°. The parent peak in the mass spectra was at *m/e* 342; ir (KBr) 1655, 1600, 1578, 1439, 1411, and 1366 cm^{-1}; λ^{MeOH} max 335 (5960), sh 263 (29,100), max 252 (39,600), and max 246 mm (ϵ 38,600).

Anal. Calcd for $C_{22}H_{14}O_4$: C, 77.18; H, 4.12. Found: C, 76.99; H, 4.33.

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