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Research Article

Triplet-Sensitized Photooxygenation of Therapeutic Retinoids

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The triplet-sensitized photooxygenation of retinoic acid in hydroorganic buffer, methyl retinoate in a variety of solvents, and methyl 13-*cis*-retinoate and etretinate in ethanol has been investigated. By high-performance liquid chromatographic analysis, one major peroxide product was formed from each retinoid substrate under all conditions investigated. The structures of these peroxides have been assigned relying on high-field nuclear magnetic resonance and mass and ultraviolet spectroscopy. While product structures were not influenced, the rate of product formation was found to vary with solvent, substrate, and perhaps the nature of the sensitizer. The retinoid peroxides isolated are stable toward nucleophiles and weakly acidic and basic conditions. Possible reasons for rate variations in the photooxygenations are discussed.

KEY WORDS: retinoid; photooxygenation; high-performance liquid chromatography; nuclear magnetic resonance; peroxides.

INTRODUCTION

Retinoic acid (**1**) (Fig. 1) has emerged as a popular agent for the treatment of a variety of dermatological conditions such as acne and psoriasis (1). This metabolite of retinol and its analogues (retinoids) have also generated considerable interest because of their potential utility as cancer chemopreventive or chemotherapeutic agents (2). In these treatment areas the metabolite 13-*cis*-retinoic acid (**2**) and analogue etretinate (**3**) have become important therapeutic agents.

The metabolites of **1** have been extensively studied for their potential contribution to retinoid bioactivity. At this time only the metabolites retinoyl-β-glucuronide and **2** have been shown to retain the activity of **1** in assays for retinoid activity (3–5). Some evidence suggests, however, that even relatively inactive metabolites may still contribute to retinoid teratogenicity (6). As of yet, there have been fewer studies of the metabolism of **3** and the resulting impact on activity and teratogenicity (7).

The natural and synthetic retinoids are playing an increasing role in clinical trials for the treatment or prevention of cutaneous preneoplastic and neoplastic conditions (8). Perhaps because this activity requires concentration in cutaneous tissues, retinoid-induced skin toxicities including scaling, erythema, epidermal hyperplasia, and alopecia have been reported (9). For virtually all of the drugs in this class, there have been recent reports of drug-induced photosensitivity and phototoxicity (10).

As a class, the retinoids are strong absorbers of ultraviolet light, particularly of the UVA type. Thus, these compounds may cause photosensitization/phototoxicity through the formation of light-induced retinoid free radicals (11) or active oxygen species. This possibility for **1–3** has been receiving increased study (12).

An alternative potential source of retinoid dermatotoxicity is that of the generation of reactive retinoid "metabolites" through their own triplet-sensitized photooxygenation, via physiologically relevant sensitizers which absorb long-wavelength visible light. A number of years ago, Mousseron-Canet and Mani (13) and Lerner *et al.* (14) showed that both retinal (**4**) and retinyl acetate (**5**) undergo triplet-sensitized photooxygenation in methanol in the presence of oxygen, light, and rose bengal to give the endoperoxides **6** and **7**. While unexplored for **1**, the possibility that products from **1** analogous to **6** and **7** might be reactive species is suggested by the known reactivity and short half-life of other physiologic lipid endoperoxides, such as PGG_2 and PGH_2, found in the metabolism of the prostaglandins (15).

Because of our continued interest in the photochemistry and metabolism of retinoids (16,17) and the role this may play in the actions and toxicities of retinoids, we have investigated the triplet-sensitized photooxygenation of **1** in physiologic-like solvents. In addition, we have explored the effect of solvent environment and sensitizer on the rate and nature of the photooxygenation product mixture formed upon photooxygenation of **1** as its methyl ester, the methyl ester of **2**, and **3**. Finally, we have determined the reactivity of the principal photooxygenation products toward reagents thought to mimic weak acids, bases, and nucleophiles found *in vivo*.

MATERIALS AND METHODS

Materials and General Procedures. All-*trans*-retinoic

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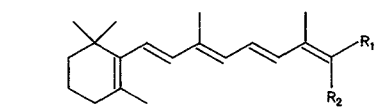
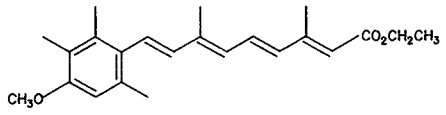


1 R_1 = COOH, R_2 = H

2 R_1 = H, R_2 =COOH

4 R_1 = CHO, R_2 = H

5 R_1 = CH_2OAc, R_2 = H



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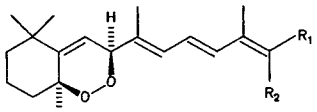

6 R_1 = CHO, R_2 = H

7 R_1 = CH_2O_2Ac, R_2 = H

8 R_1 = COOH, R_2 = H

9 R_1 = $COOCH_3$, R_2 = H

10 R_1 = H, R_2 = $COOCH_3$

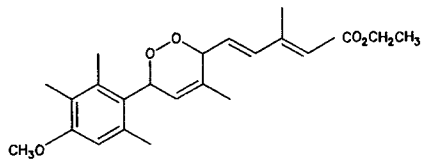

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Fig. 1. Structures of retinoids.

acid and 5,10,15,20-tetraphenyl-21H,23H-porphine (TPP)[3] were obtained from Aldrich Chemical Co. (Milwaukee, WI). Methylene blue chloride was from EM Science (Cherry Hill, NJ). Etretinate was extracted by ethyl acetate from 25-mg capsules manufactured by Hoffman–LaRoche (Nutley, NJ). Methyl retinoate (Me-1) and methyl 13-*cis*-retinoate (Me-2) were prepared by the reaction of retinoic acid with diazomethane generated in the usual manner from *N*-methyl-*N*-nitroso-*p*-toluensulfonamide (Aldrich). High-performance liquid chromatography (HPLC)-grade hexane and ethyl acetate were obtained from Fisher Scientific (Pittsburgh, PA). All manipulations were performed under yellow light (Sylvania F40 gold fluorescent lamps).

Chromatographic Methods. HPLC analyses were carried out on a Beckman Instruments (San Ramon, CA) Model 332 gradient liquid chromatograph system equipped with a Beckman Model 164 variable-wavelength ultraviolet detector.

Retinoic acid and etretinate photooxygenation was monitored on a 5-μm, spherical-particle octadecylsilane column (Zorbax-ODS, 250 × 4.6 or 250 × 9.4 mm; DuPont Instruments, Wilmington, DE). Other retinoid photooxygenation product mixtures were analyzed on 5-μm, spherical-particle silica gel columns (Zorbax-Sil, 250 × 4.6 or 250 × 9.4 mm; DuPont Instruments). Mobile phase compositions and flow rates employed in specific separations are indicated in the appropriate figure legends. All chromatography was performed at ambient temperature.

Photooxygenation Reactions. Approximately 2–50 m*M* solutions of **1**, Me-1, Me-2, and **3** containing 3×10^{-3} equivalents (for **1**), 3×10^{-2} equivalents (for **1**, Me-1, and Me-2), or 3×10^{-1} equivalents (for **3**) of sensitizer were photooxygenated in an NSG (NSG Precision Cells Inc., Farmingdale, NY) Type 50 jacketed quartz UV cell having a light path of 1.0 cm and a capacity of 1.1 ml. Samples were irradiated at a distance of 8.0 cm by a 60-W gold incandescent lamp (significant emission from 600 to 900 n*M*) and continuously oxygenated by bubbling oxygen through the solution. The cell temperature was maintained at 23 ± 2°C by passing cool water through the cell jacket. At regular intervals, 10-μl aliquots of the irradiated solutions were removed and analyzed by HPLC to determine the extent of reaction.

Photooxygenation of **1** was studied in 1:1 ethanol/10 m*M* phosphate buffer, pH 7.2, with methylene blue chloride as sensitizer. Preparative-scale irradiations of **1** were performed as above and also in 100% ethanol, which yielded the same product. Because of the sparing solubility of TPP in hexane and ethanol, photooxygenation of Me-1 with TPP as sensitizer was conducted in chloroform, 10:1 hexane/chloroform, and 10:1 ethanol/chloroform. Reaction rates for the photooxygenation of **1** and Me-1 were evaluated by periodic analysis of reaction mixture aliquots by HPLC. First-order rate constants were estimated according to the expression

$$K = (-1/t_2 - t_1) \ln P_{(t_2)}/P_{(t_1)}$$

where $P_{(tx)}$ is the chromatographic peak height for remaining **1** and Me-1 at time = x. Preparative-scale irradiations of Me-1 and Me-2 were conducted in ethanol with methylene blue chloride as sensitizer. As for **1**, this facilitated isolation and structure elucidation of the products derived from Me-1 and Me-2 since methylene blue was removed by passing the product mixture through a short silica gel or glass-wool column. Because of the modest solubility of **3** in ethanol, its photooxygenation was performed in 10:1 ethanol/ethyl acetate with methylene blue as sensitizer. Control photooxygenation reactions were performed for Me-1 in the absence of light ("dark controls") and also in the absence of photosensitizer.

Spectroscopic Measurements. UV spectra were recorded on a Beckman Instruments DU-40 spectrophotometer. Fast atom bombardment (FAB) mass spectra were determined on a Kratos MS-30 mass spectrometer. The FAB mass spectra were obtained using dithiothreitol:dithioerythritol (3:1 or 5:1), with up to 20% methanol in chloroform, as the solvent (18). Fourier transform nuclear magnetic reso-

[3] Abbreviations used: TPP, 5,10,15,20-tetraphenyl-21H,23H-porphine; HPLC, high-performance liquid chromatography; FAB, fast atom bombardment; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect.

nance (NMR) spectra were obtained at ambient temperature on samples dissolved in deuteriochloroform in amber 5 × 175-mm sample tubes (Wilmad Glass Co., Buena, NJ). ^{1}H-NMR and ^{13}C-NMR spectra were obtained on an IBM AF250 spectrometer operating at 250.1 and 62.9 MHz, respectively.

Characterization of Photooxygenation Product of 1. A 17-ml suspension of 51 mg (163 mmol) of 1 and 4 mg (10.8 mmol) of methylene blue chloride in 1:1 ethanol/10 m*M* phosphate buffer, pH 7.2, was photooxygenated as described above. By 9 hr all suspended 1 had dissolved and HPLC analysis showed that no unreacted 1 remained. The solution was evaporated under reduced pressure and the residue dissolved in ether and extracted repeatedly with water. The ether layer was separated and evaporated to yield a yellow residue assigned structure 8. The 5,8-epidioxy-5,8-dihydroretinoate (8) isolated showed spectroscopic properties essentially identical to those of the more extensively characterized methyl ester analogue 9 (see below).

Characterization of Photooxygenation Product of Me-1. A 1.0-ml ethanol solution containing 10 mg (32 mmol) of Me-1 and 1 mg (2.7 mmol) of methylene blue chloride was photooxygenated as described above. HPLC analysis showed that Me-1 had completely reacted within 2 hr to give one major product assigned structure 9. Methylene blue was removed by passing the resulting product mixture in ethyl acetate through a short column of silica gel. The methyl 5,8-epidioxy-5,8-dihydroretinoate (9) isolated showed the following: UV (ethanol) λ_{max} 309 nm (ϵ 2.9 × 10^4); ^{1}H-NMR ($CDCl_3$) δ 1.11 and 1.17 (2s, 6H, 1,1′-CH_3), 1.50 (m, 2H, 2-CH_2), 1.59 (s, 3H, 5-CH_3), 1.65 (m, 4H, 3,4-CH_2), 1.92 (s, 3H, 9-CH_3), 2.31 (s, 3H, 13-CH_3), 3.70 (s, 3H, OCH_3), 4.63 (d, 1H, H_8, $J_{7,8}$ = 3.9 Hz), 5.54 (d, 1H, H_7), 5.76 (s, 1H, H_{14}), 6.11 (d, 1H, H_{10}, $J_{10,11}$ = 11.1 Hz), 6.25 (d, 1H, H_{12}, $J_{11,12}$ = 15.2 Hz), 6.85 (dd, 1H, H_{11}); ^{13}C-NMR ($CDCl_3$) δ 13.77 (13-CH_3), 14.88 (9-CH_3), 18.77 (C_3), 25.49 (5-CH_3), 27.66, 30.60 (1,1′-CH_3), 35.27 (C_1), 35.52 (C_4), 41.04 (C_2), 50.75 (OCH_3), 79.73 (C_5), 82.56 (C_8), 115.53 (C_7), 118.52 (C_{14}), 127.32 (C_{10}), 130.24 (C_{11}), 135.96 (C_{12}), 141.40 (C_9), 149.61 (C_6), 152.51 (C_{13}), 167.31 (C_{15}); FAB MS, *m/z* (relative intensity) 369 (M + Na$^+$, 2), 347 (MH$^+$, 10), 329 (MH$^+$-H_2O, 23) 315 (MH$^+$-O_2, 22), 313 (MH$^+$-HOOH, 68).

Characterization of Photooxygenation Product of Me-2. A 5-ml ethanol solution 32 m*M* in Me-2 and 2.7 m*M* in methylene blue was photooxygenated as described above. HPLC analysis showed that Me-2 had nearly completely reacted within 2.5 hr to give one major product assigned structure 10. Methylene blue was removed by concentration of the reaction mixture, extraction of the residue with ether, and passage of the ethereal extract through glass wool. After concentration, the methyl 13-*cis*-5,8-epidioxy-5,8-dihydroretinoate (10) isolated showed the following: UV(CH_2Cl_2) λ_{max} 316 nm (ϵ 2.9 × 10^4); ^{1}H-NMR ($CDCl_3$) δ 1.11 and 1.18 (2s, 6H, 1,1′-CH_3), 1.51 (m, 2H, 2-CH_2), 1.58 (s, 3H, 5-CH_3), 1.63 (m, 4H, 3,4-CH_2), 1.93 (s, 3H, 9-CH_3), 2.04 (s, 3H, 13-CH_3), 3.68 (s, 3H, OCH_3), 4.63 (d, 1H, H_8, $J_{7,8}$ = 3.9 Hz), 5.54 (d, 1H, H_7), 5.64 (s, 1H, H_{14}), 6.19 (d, 1H, H_{10}, $J_{10,11}$ = 11.3 Hz), 6.85 (dd, 1H, H_{11}, $J_{11,12}$ = 15.5 Hz), 7.72 (d, 1H, H_{12}); FAB MS, *m/z* (relative intensity) 347 (MH$^+$, 7), 329 (MH$^+$-H_2O, 16), 315 (MH$^+$-O_2, 47), 313 (MH$^+$-HOOH, 22).

Characterization of Major Photooxygenation Product of 3. A 10:1 ethanol/ethyl acetate solution, 8.3 m*M* in 3 and 1 m*M* in methylene blue, was photooxygenated as above for 12 hr. HPLC analysis showed partial conversion of 3 to a number of products including a single prominent photooxygenation product which was isolated by semipreparative HPLC (95:5 methanol/H_2O). This material has been assigned the 7,10-epidioxide structure 11 based on the following: UV(ethanol) λ_{max} 258; ^{1}H-NMR ($CDCl_3$) δ 1.27 (t, 3H, OCH_2CH_3), 1.78 (s, 3H, 9-CH_3), 2.08 (s, 3H, 1-CH_3), 2.25 (s, 3H, 2-CH_3), 2.28 (s, 3H, 13-CH_3), 2.36 (s, 3H, 5-CH_3), 3.77 (s, 3H, OCH_3), 4.16 (q, 2H, OCH_2), 4.71 (br s, 1H, H_{10}), 5.75 (br s, 1H, H_7), 5.81 (s, 1H, H_{14}), 6.16 (br s, 1H, H_8), 6.39 (br s, 2H, H_{11} and H_{12}), 6.50 (s, 1H, Ar-H); FAB MS, *m/z* (relative intensity) 409 (M + Na$^+$, 12.9), 387 (MH$^+$, 8.0), 369 (MH$^+$-H_2O, 16.2), 355 (MH$^+$-O_2, 5.9).

Stability Studies. For 8, 1.8 m*M* methanol solutions were treated with 1 equivalent of trifluoroacetic acid, triethylamine, or 2-mercaptoethanol. Portions of 1:1 hexane/ethyl acetate solutions of 9 and 11 were treated with excess trifluoroacetic acid, triethylamine, 2-mercaptoethanol, or 1:1 triethylamine/2-mercaptoethanol. Aliquots of all solutions were removed and analyzed periodically by HPLC for the formation of new materials for up to 12 hr post reagent addition.

RESULTS

Photooxygenation

Aliquots were withdrawn at regular intervals from irradiated solutions of 1 and methylene blue chloride and analyzed by HPLC. A representative chromatogram from an intermediate reaction time point is shown in Fig. 2. Formation of a single major product (8) was observed for both solvents in which 1 was photooxygenated. The rate of photooxygenation appeared to be proportional to the concentration of sensitizer as shown by the change in apparent first-order rate constant from $K \simeq 2.7 \times 10^{-4}$ to $K \simeq 2.9 \times 10^{-3}$ sec^{-1} upon increase of sensitizer concentration from 3 × 10^{-3} to 3 × 10^{-2} equivalents relative to 1. The structure of the resulting 8 was assigned by comparison of its spectro-

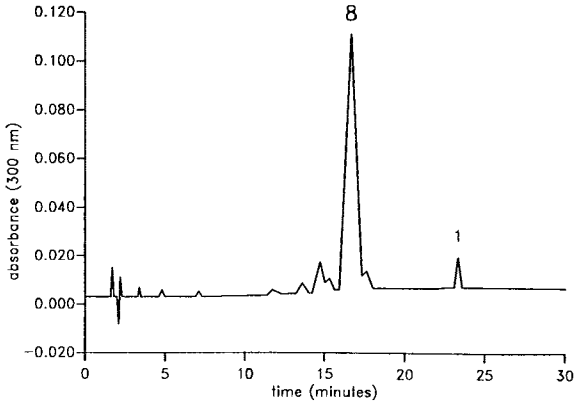

Fig. 2. Reversed-phase HPLC of retinoic acid (1) after 25 min of photooxygenation in ethanol-phosphate buffer (1:1). Analysis employed a linear gradient of methanol–water (65:45) to 100% methanol from time = 0 to time = 18 min at a flow rate of 1.2 ml/min.

scopic properties with the more extensively characterized methyl ester analogue **9** (see below).

Aliquots were also withdrawn at regular intervals from irradiated solutions of Me-**1** with a representative chromatogram from an intermediate time point of the reaction in 10:1 ethanol/chloroform shown in Fig. 3. Formation of a single major product (**9**) was observed for all solvents in which Me-**1** was photooxygenated. However, the reaction rate (Fig. 4) appeared to be much greater in chloroform ($K \simeq 1.6 \times 10^{-3}$ sec^{-1}) than in 10:1 hexane/chloroform ($K \simeq 2.8 \times 10^{-5}$ sec^{-1}) and 10:1 ethanol/chloroform ($K \simeq 4.5 \times 10^{-5}$ sec^{-1}).

When performed on a preparative scale as described under Materials and Methods, photooxygenation of Me-**1** to the major product was complete within 2 hr. The structure of the resulting material, **9**, was determined by MS, UV, and NMR analysis. Complete NMR chemical shift assignments were determined by a combination of one- and two-dimensional NMR techniques, especially proton decoupling, ^1H–^{13}C shift correlation spectra (19), and COLOC (COrrelation by LOng range Coupling) (20). A nuclear Overhauser effect (NOE) difference experiment showed the presence of a measurable (0.54%) enhancement at H_8 upon irradiation of the 5-CH_3 group.

Analysis by HPLC of control photooxygenations of Me-**1** under the conditions above, in the absence of either light or photosensitizer, shows that no reaction occurs during the length of time in which the experimental photooxygenations proceed to completion. Back addition of photosensitizer or initiation of irradiation of these controls permits these reactions to proceed normally to the single major product observed. That a single major product is formed in these above photooxygenations is supported by spectroscopic inspection of the crude product mixtures which show only the major product. In addition, HPLC analysis of the product mixture formed from Me-**1** shows no change in the peak height of **9** relative to the minor unidentified products seen in Fig. 3 when analyzed at multiple detection wavelengths (280, 300, and 320 nm).

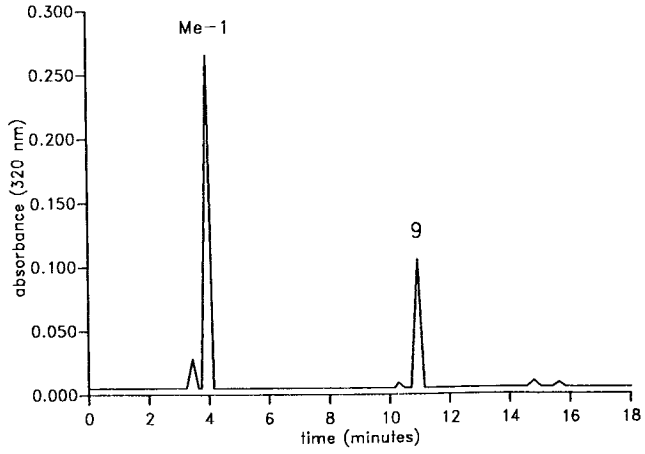

Fig. 3. Normal-phase HPLC of methyl retinoate (Me-**1**) after 25 min of photooxygenation in ethanol–chloroform (10:1). Analysis employed a linear gradient of ethyl acetate–hexane, 2:98, to ethyl acetate–hexane, 7:93, from time = 4 min to time = 10 min at a flow rate of 2.3 ml/min.

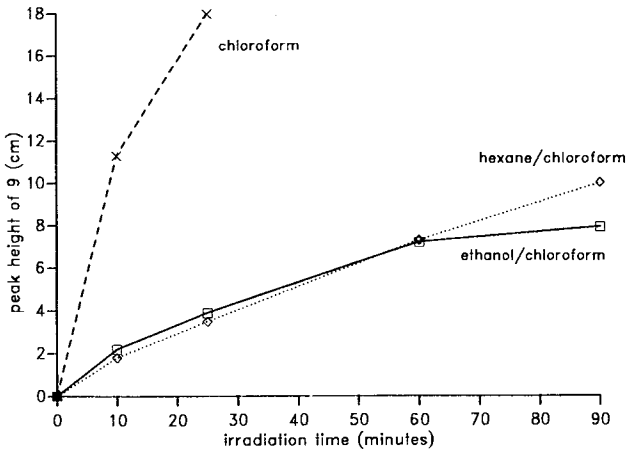

Fig. 4. Plot of peak height of **9** formed from photooxygenation of Me-**1** vs. time in chloroform (X), 10:1 hexane–chloroform (·· ◇ ··), and 10:1 ethanol–chloroform (—□—). HPLC analysis performed as in the legend to Fig. 3.

Preparative-scale photooxygenation of Me-**2**, as for Me-**1**, produced a single major product and was essentially complete within 2.5 hr. The structure of the resulting material was assigned as **10** based on its MS, UV, and ^1H-NMR spectra and comparison of these results with those for **9**.

Due to the insensitivity of the chemical outcome of these reactions to the nature of the solvents and sensitizers investigated, because of solubility considerations, and because of ease of purification, **3** was irradiated in 10:1 ethanol/ethyl acetate with methylene blue as sensitizer. Even with an additional 10-fold increase in sensitizer concentration, formation of the major product of this photooxygenation occurred much more slowly than **8**–**10**, being incomplete at up to 30-hr reaction times. A representative chromatogram of this reaction, at 12 hr of irradiation, is shown in Fig. 5.

Perhaps because of the prolonged reaction times required for significant photoconversion of **3**, a substantial number of nonminor products were produced (Fig. 5), many of which could be isolated by semipreparative HPLC for

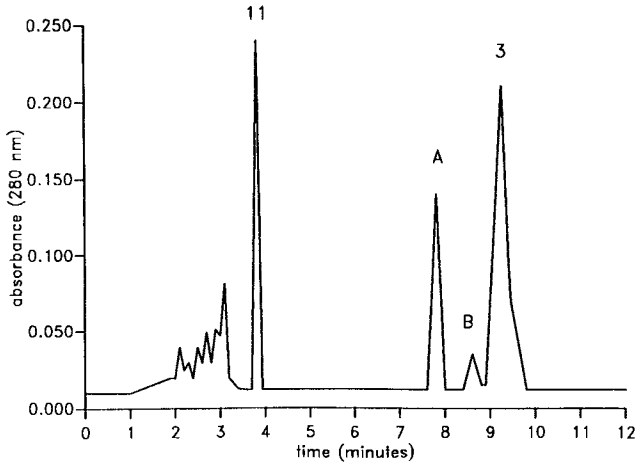

Fig. 5. Reverse-phase HPLC of etretinate (**3**) after 12 hr of photooxygenation in 95:5 methanol–water. Analysis was performed at a mobile-phase flow rate of 1.5 ml/min.

spectroscopic analysis. The major photooxygenation product was assigned the epidioxide structure **11** based on the MS, UV, ^{1}H-NMR, and COSY (21) spectra obtained. In particular, **11** shows a UV maximum at 258 nm which is nearly identical to that for ethyl 2,4-hexadienoate and a mass spectrum showing the addition of a molecule of oxygen. ^{1}H-NMR analysis of the product also shows the expected loss of a doublet-of-doublets for H_{11} and a pronounced and lesser upfield shift for resonances assigned H_{10} and H_7, respectively, by analogy with **9**. In addition, COSY spectra of this material show long-range couplings consistent with the assigned structure.

The less polar products eluting from the column around the region of unreacted **3** appear to be predominantly photoisomers of **3**. Based on our earlier NMR studies of the photoisomers of Me-1 (22), the components labeled A and B in Fig. 5 have been identified as 13-*cis*- and 9,13-*dicis*-etretinate respectively.

Stability Studies

Aliquots of solutions of **8**, **9**, and **11** containing the described amounts of trifluoroacetic acid, triethylamine, 2-mercaptoethanol, or triethylamine/2-mercaptoethanol were removed and analyzed for the formation of possible reaction products by HPLC. For none of these peroxides, exposed to these mildly acidic, basic, or nucleophilic conditions, was any apparent reaction observed at up to 12 hr post addition of the reagents.

DISCUSSION

The photodegradation of retinol has long been recognized because of its negative impact on the biological activity of this essential micronutrient. As early as the 1930s, one postulated source for changes in tissue and serum levels of retinol was the UV irradiation of skin by sunlight (23). Subsequently, Mousseron-Canet and co-workers observed that retinol-like compounds will undergo photooxygenation in the presence of oxygen, light, and sensitizer (13,14). Metabolites of this vitamin, such as **1** and its analogues, are now being widely used as dermatological agents. However, despite limited reports of retinoid-induced photosensitization (12), few studies have addressed the possibility that photochemically induced changes in skin retinoids may influence their activity/toxicity.

In the present studies, photooxygenation of **1** in physiologic-like solvents, as well as that of Me-1 in organic solvents of varying polarity chosen to mimic a variety of compartments into which these compounds might partition, has been investigated. Regardless of the sensitizer, solvent, and substrate employed, HPLC analysis showed the formation of a single major product found by NMR analysis to be the similar cyclic peroxide analogues **8** and **9**. The analogous peroxide **10** is also the major product formed from photooxygenation of Me-2 in ethanol.

Interestingly, the rate of formation of **8** and **9** appears to depend on both the sensitizer concentration and the nature of the solvent employed. For Me-1, the considerably more rapid formation of **9** in chloroform than in ethanol and hexane may be related to the lifetime of singlet oxygen in these solvents, which is known to be from 10 to 20 times longer in the former solvent than in the latter two (24). Surprisingly, however, the formation of **8** from **1** in hydroorganic buffer is virtually as rapid as the formation of **9** from Me-1 in chloroform. This does not appear to be consistent with the shorter lifetime of singlet oxygen in water and ethanol, as noted above, but may be due to the presence of the buffer salts and/or an altered "structure" for the dissolved carboxylate analogue versus its methyl ester. In addition, methylene blue chloride was employed as sensitizer in the photooxygenation of **1** because of the insolubility of TPP in aqueous ethanol. Thus, the change in sensitizer may have influenced the rate of singlet oxygen formation, particularly since these two sensitizers do differ in absorption maximum and molar absorptivity (25).

Detailed high-field NMR experiments were employed to permit the assignment of the cyclic peroxide structures **8** and **9**. The relative stereochemistry of these products has been assigned based on both results of the NOE experiment with **9** and the mechanism of singlet oxygen addition to *cisoid* dienes, which has long been known to occur in a *syn* [4 + 2]cycloaddition manner (26). The observation of a measurable NOE between the 8-proton and the 5-methyl group seems to support this assignment since the strong inverse dependence of the magnitude of the NOE on distance (27) and internuclear distance estimates derived from molecular modeling make an observable NOE for a *trans* relationship between these two groups unlikely.

When **3** was utilized as the reaction substrate, much slower formation of a major product, assigned structure **11**, was observed. This much slower reaction rate is not too surprising for a number of reasons. Our recent theoretical and spectroscopic studies (R. Curley and J. Fowble, unpublished), as well as earlier such studies (28) and crystallographic analyses (29), suggest that **1** prefers a conformation in which the ring is slightly twisted relative to the planar side chain. Nonetheless, the cyclohexenyl ring in **1** maintains a 6-s-*cis* relationship with this side chain, as shown in Fig. 1. This type of *cisoid* diene conformation is mechanistically required for the [4 + 2]cycloaddition, hence the rapid reaction of **1**. For the analogous reaction to occur in **3**, interruption of ring aromaticity would be required, presumably a less favored process. We have also found that the conjugated side chains in these molecules prefer the extended s-*trans* conformation. Thus, formation of **11** requires assumption of the sterically demanding s-*cis* conformation between the 7- and the 9-double bonds, perhaps also contributing to the reduced rate of product formation. Because of the reduced reaction rate of **3**, the production of cyclic dioxetanes formed across one of the side-chain double bonds might be anticipated. This type of product has just recently been observed in the autooxidation of **1** (30), although unactivated olefins of this type do not normally form stable dioxetanes readily (31,32) and we find no evidence here for formation of significant amounts of these from **3**.

Perhaps most interesting, while these peroxides are known to be transformed under strongly acidic or basic conditions (25), under the weakly acidic, basic, and nucleophilic conditions to which peroxides **8**, **9**, and **11** were subjected, no significant decomposition or reaction was observed by HPLC analysis. Thus, in contrast to the lipid endoperoxides formed in the prostaglandin metabolism cascade, **8**, **9**, and **11**

do not appear to represent particularly reactive species. Therefore, if formed in skin in therapeutic situations, these peroxides may not contribute to retinoid dermatotoxicity simply by behaving as chemically reactive intermediates. Consistent with this conclusion is the recent observation by Franck *et al.* (33) that, when fed to chickens and mice, the peroxide analogue of 8 formed from retinyl acetate was nontoxic.

In summary, the triplet-sensitized photooxygenation of 1, its methyl ester, methyl 13-*cis*-retinoate, and 3 has been investigated under a variety of conditions. Regardless of whether a physiologic-like porphyrin or methylene blue is used as sensitizer, the reaction products are identical. However, among the conditions investigated the rate of peroxide formation is greatest for 1 in hydroorganic buffer and Me-1 in chloroform. The aromatic analogue 3 reacts much more slowly, perhaps because of the preference for non-*cisoid* diene conformations. None of the peroxide products that form from these retinoids appear to be particularly reactive species. However, since 8–11 can be formed under physiologic-like conditions, it may now be of interest to determine if these types of compounds do form in the skin of patients utilizing retinoids and whether they retain any biological activity relative to the parent molecules.

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