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Photo-oxidations and photosensitized oxidations of vitamin A and its palmitate ester

George Crank, Mohammed S. Pardijanto

Department of Organic Chemistry, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Australia

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Abstract

Photo-oxidation of vitamin A with UV light gave retinal and a mixture of epoxy derivatives of the vitamin; however, vitamin A palmitate gave anhydrovitamin A and fragments derived from cleavage of the side-chain double bonds. The rate of oxidation and the type of oxidation products produced by photosensitized oxidation of vitamin A and its palmitate ester with visible light depended on the sensitizer. The vitamin gave epoxides, peroxides and cleavage products, while the palmitate gave mainly cleavage products. Some of the oxidation products were isolated and identified; many others were too unstable and decomposed during work-up. The photosensitized processes are considered to be initiated by singlet oxygen.

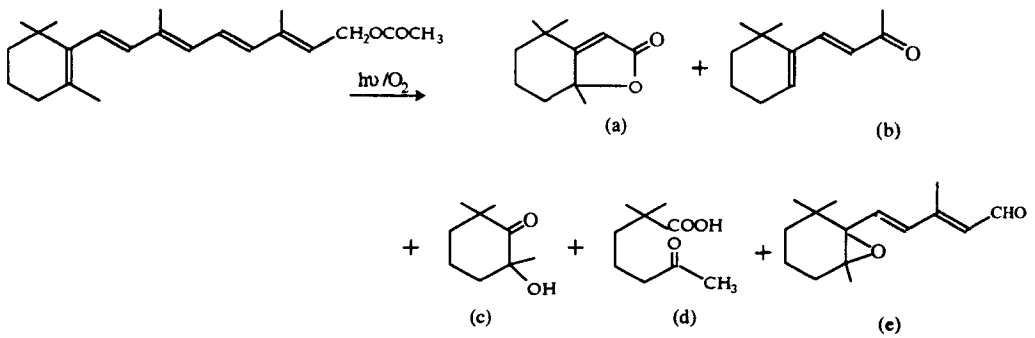
Keywords: Photo-oxidation; Photosensitized oxidation; Vitamin A

1. Introduction

The instability of vitamin A to oxidation has been well demonstrated, both in studies of its chemistry and in tests carried out on foods containing the free vitamin and its esters. Being a polyene alcohol, vitamin A is susceptible to oxidation at various sites, in particular the primary alcoholic group and the double bonds in the side-chain or the ring. The esters are expected to be more stable since one site of oxidation, the alcoholic group, is blocked. Studies on the oxidation of vitamin A have been complicated by the fact that initial oxidation products are often unstable and are susceptible to further oxidation.

Potassium permanganate [1] and manganese dioxide [2] have been shown to oxidize the alcoholic group to form the aldehyde (retinal), but other workers [3] have found additional products such as 3-hydroxyretinal and 3-oxoretinal. Peracids attack the double bonds and peracetic acid forms 11,12-epoxyretinal [4]. The auto-oxidation of vitamin A produces unidentified carbonyl compounds [5], but cobalt-catalysed auto-oxidation yields 11,12-epoxyretinol [6]. The auto-oxidation of vitamin A palmitate also produces a large number of volatile carbonyl decomposition products [7].

Vitamin A and its esters in milk are destroyed by exposure to sunlight [8], with ring opening [9] and photodimerization [10] occurring in model experiments. Photo-oxidation of vitamin A acetate with UV light



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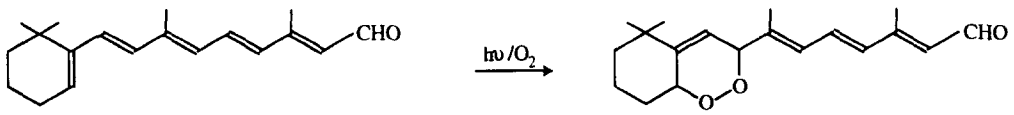


gives dihydroactinidiolide (a), β-ionone (b), 2-hydroxy-2,6,6-trimethylcyclohexanone (c), geronic acid (d) and desoxyxanthoxin (e) [11].

However, the photo-oxidation of retinal has been reported to give the 5,8-endoperoxide in low yield [12].

The reaction of singlet oxygen with vitamin A has been reported by Foote et al. [13] to be a singlet oxygen quenching process with no oxidation products of the vitamin detected. This reaction was reinvestigated by Smith [14] who showed that the singlet oxygen quenching rate was solvent dependent and faster in more polar solvents, but again no reaction products derived from vitamin A were observed.

In this paper, we report an investigation of the photo-oxidation and photosensitized oxidation of vitamin A and its palmitate ester with the aim of identifying the reaction products.

2. Experimental details

2.1. Materials and methods

Vitamin A and vitamin A palmitate (Roche) were purified before use by preparative layer chromatography (PLC). Experimental procedures and chromatographic separations were carried out in dim light and all equipment was flushed with nitrogen. All solvents were purified and distilled before use. Thin layer chromatography (TLC) and PLC were performed using Merck silica gel PF_{254} with light petrol–acetone (7 : 1) as developing solvent, except where indicated otherwise.

Nuclear magnetic resonance (NMR) spectra were recorded on a Brucker CXP300 or Brucker AM500 instrument. UV spectra were obtained using a Perkin–Elmer 124 double-beam spectrophotometer. Mass spectra (MS) were obtained using an AEI MS12 mass spectrometer.

2.2. Photo-oxidation of vitamin A (1)

Air was slowly bubbled (10 ml min^{-1}) into a quartz tube containing vitamin A (100 mg) in ethanol (25 ml), which was exposed to UV light (253.7 nm). After exposure for 24 h, TLC analysis showed that no vitamin A remained. Ethanol was removed by evaporation under vacuum on a rotary evaporator at a water bath temperature of 20 °C. The residue, in 1 ml of ethanol, was applied to a PLC plate. After development, the plate showed five bands, which were scraped off and extracted with ethyl acetate. Each extract was then concentrated under mild conditions to yield products which were subjected to spectroscopic examination.

Band 1 (R_f 0.55) (**2**). MS: 284 (M^+). UV: 235, 280, 370 nm. The NMR spectrum was identical to that of retinal.

Band 2 (R_f 0.33). MS: 286 (M^+). UV: 240, 270, 320 nm. NMR ($CDCl_3$): δ 0.91 (s, 6H, C-1 CH_3), 1.4–1.8 (m, 6H, ring CH_2), 1.66 (s, 3H, C-5 CH_3), 1.89 (s, 3H, C-13 CH_3), 2.13 (s, 3H, C-9 CH_3), 3.75 (s, 1H, exchanged with D_2O, OH), 4.20 (d, 2H, CH_2OH), 5.44 (t, 1H, H-14), 6.03 (m, 1H, H-7), 6.11 (m, 1H, H-12), 5.4–7.6 (m, 3H, H-8, H-10, H-11).

Band 3 (R_f 0.27) (**3**). MS: 302 (M^+). UV: 245, 310 nm. NMR ($CDCl_3$): δ 0.99 (s, 3H, C-5 CH_3), 1.1 (d, 6H, C-1 CH_3's), 1.4–1.8 (m, 6H, ring CH_2's), 1.86 (s, 3H, C-9 CH_3), 1.95 (s, 3H, C-13 CH_3), 4.30 (d, 2H, $-CH_2-O$), 5.7 (t, 1H, H-14), 5.9–6.7 (m, 5H, H-7, H-8, H-10, H-11, H-12).

Band 4 (R_f 0.19) (**4**). MS: 302 (M^+). UV: 260, 290 nm. NMR ($CDCl_3$): δ 1.1 (s, 3H, C-5 CH_3), 1.2 (d, 6H, C-1 CH_3's), 1.2–1.9 (m, 6H, ring CH_2's), 1.6 (s, 3H, C-9 CH_3), 3.7 (m, 1H, H-8), 4.3 (d, 2H, $-CH_2-O$), 5.6–7.5 (m, 5H, H-7, H-10, H-11, H-12, H-14).

Band 5 (R_f 0.14) (**5**). MS: 302 (M^+). UV: 240, 290 nm. NMR ($CDCl_3$): δ 1.01 (s, 3H, C-13 CH_3), 1.25 (d, 6H, C-1 CH_3's), 1.2–1.9 (m, 6H, ring CH_2's), 1.7 (s, 3H, C-9 CH_3), 1.94 (s, 3H, C-5 CH_3), 3.4 (m, 1H, H-14), 3.7 (m, 2H, $-CH_2OH$), 5.4–6.7 (m, 5H, H-7, H-8, H-10, H-11, H-12).

2.3. Photo-oxidation of vitamin A palmitate (6)

Air was slowly bubbled (10 ml min^{-1}) into a solution of vitamin A palmitate (260 mg) in ethanol (50 ml) in a quartz tube exposed to UV light. After 24 h, TLC analysis of the reaction mixture showed that none of the starting material remained. Ethanol was removed by vacuum evaporation as before and the residue was redissolved in 1 ml of ethanol and applied to a PLC plate. After development with light petrol–ethyl acetate (19 : 1), six bands were seen. Adsorbent from each band was scraped from the plate and extracted with ethyl acetate. Solvent extracts were concentrated under mild conditions and residues were analysed by spectroscopic means.

Band 1 (R_f 0.88) (**7**). MS: 496 (M^+). NMR ($CDCl_3$): δ 0.88 (t, $\underline{CH_3}$ CH_2–), 1.00 (m, $(CH_3)_2C$), 1.25 (m, $(CH_2)_n$).

Band 2 (R_f 0.75) (**8**). MS: 268 (M^+). UV: 355, 375, 393 nm. The NMR spectrum was identical to that of anhydrovitamin A.

Band 3 (R_f 0.57). MS: 286 (M^+). UV: 250, 323 nm. The NMR spectrum was identical to that of vitamin A.

Band 4 (R_f 0.46).

Band 5 (R_f 0.35) (**9**). MS: 310 (M^+). UV: 210 nm. NMR ($CDCl_3$): δ 0.88 (t, 3H, CH_3), 1.25 (m, 24H, $(CH_2)_{12}$), 1.6 (m, 2H, $\underline{CH_2}$–CH_2–CO), 2.0 (m, 3H, CH_3–CH=C–H–), 2.3 (m, 2H, CH_2–CO), 4.2 (m, 2H, CH_2–O), 5.3 (m, 2H, –CH=CH–).

Band 6 (R_f 0.19) (**10**). MS: 256 (M^+). The NMR spectrum was identical to that of palmitic acid.

2.4. Photosensitized oxidation of vitamin A (rose bengal)

A solution of vitamin A (100 mg) and rose bengal (2 mg) in ethanol (25 ml) was exposed to visible light with air bubbling. After 4 h, TLC analysis of the reaction mixture showed two products but no vitamin A. The solution was evaporated under vacuum and the residue was separated on a PLC plate. Products were recovered by removal of the adsorbent from the observed bands and extraction with ethyl acetate.

Band 1 (R_f 0.24) (**11**). MS: 224 (M^+). UV: 240, 280 nm. NMR ($CDCl_3$): δ 1.1 (s, 3H, C-5 CH_3), 1.15 (d, 6H, C-1, CH_3's), 1.5–1.7 (m, 6H, ring CH_2's), 2.13 (s, 3H, $COCH_3$), 4.19 (m, 1H, H-8), 5.45 (m, 1H, H-7).

Band 2 (R_f 0.18) (**3**). MS: 302 (M^+). UV: 245, 310 nm. NMR ($CDCl_3$): δ 1.00 (s, 3H, C-5 CH_3), 1.1 (d, 6H, C-1 CH_3's), 1.4–1.8 (m, 6H, ring CH_2's), 1.86 (s, 3H, C-9 CH_3), 1.95 (s, 3H, C-13 CH_3), 4.3 (d, 2H, –CH_2–O), 5.7 (t, 1H, H-14), 5.9–6.7 (m, 5H, H-7, H-8, H-10, H-11, H-12).

2.5. Photosensitized oxidation of vitamin A (riboflavin)

A solution of vitamin A (100 mg) in ethanol (25 ml) saturated with riboflavin was exposed to visible light with the passage of air. TLC analysis was performed at intervals and showed residual vitamin A at 30 h, when the reaction was terminated. After the usual work-up the reaction mixture was separated on a thick layer plate giving eight bands. Products were obtained as in previous experiments.

Band 1 (R_f 0.90) (**12**). UV: 225, 280 nm. IR: 1725 cm^{-1}. NMR ($CDCl_3$): δ 1.25 (s, 6H, C-1 CH_3's), 1.3 (t, 3H, $\underline{CH_3}CH_2$–), 1.4–1.7 (m, 6H, ring CH_2's), 2.1 (s, 3H, C-5 CH_3), 4.2 (q, 2H, $CH_3\underline{CH_2}$–O–CO).

Band 2 (R_f 0.81).

Band 3 (R_f 0.68). UV: 230, 280, 370 nm. MS: 284 (M^+). NMR: similar to that for retinal (**2**).

Band 4 (R_f 0.59).

Band 5 (R_f 0.54).

Band 6 (R_f 0.25). UV: 237, 280 nm. MS: 224 (M^+). NMR: identical to compound isolated from band 1 in Section 2.4 (**11**).

Band 7 (R_f 0.12) (**13**). UV: 270, 280 (s) nm. NMR ($CDCl_3$): δ 1.02 (s, 3H, C-5 CH_3), 1.25 (s, 6H, C-1 CH_3's), 1.2–1.9 (m, 6H, ring CH_2's), 1.80 (s, 3H, C-9 CH_3), 1.95 (s, 3H, C-13 CH_3), 3.5 (m, 1H, H-8), 3.8 (m, 2H, –CH_2–OH), 6.1–6.7 (m, 4H, H-10, H-11, H-12, H-14).

Band 8 (R_f 0.03) (**14**). UV: 235, 280 nm. MS: 300 (M^+). NMR spectrum similar to that of retinoic acid.

2.6 Photosensitized oxidation of vitamin A (chlorophyll)

A solution of vitamin A (100 mg) in ethanol (25 ml) containing chlorophyll (5 mg) was exposed to visible light with aeration. TLC analysis showed that no vitamin A remained after 90 min. After the usual work-up, the reaction mixture was separated on a thick layer plate. Originally eight bands were observed, but most of these products decomposed during subsequent work-up and only two provided data.

Band 6 (R_f 0.21). UV: 270 nm. NMR ($CDCl_3$): δ 1.1 (s, 6H, C-1 CH_3's), 1.25 (t, 3H, CH_3), 1.3–2.0 (m, 6H, ring CH_2's), 1.6 (s, 3H, C-5 CH_3), 2.05 (s, 3H, C-9 CH_3), 4.15 (q, 2H, CH_2), 5.2–6.5 (m, 3H, H-7, H-8, H-10).

Band 8 (R_f 0.00). UV: 270 nm. NMR ($CDCl_3$): δ 1.1 (s, 6H, C-1 CH_3's), 1.3–2.0 (m, 6H, ring CH_2's), 1.6 (s, 3H, C-5 CH_3), 2.08 (s, 3H, C-9 CH_3), 5.2–6.5 (m, 3H, H-7, H-8, H-10).

2.7. Photosensitized oxidation of vitamin A palmitate (rose bengal)

Vitamin A palmitate (300 mg) in ethanol (50 ml) containing rose bengal (6 mg) was exposed to visible light with aeration. After 6 h, TLC analysis showed no starting material and five product spots. The mixture was separated in the usual way on a thick layer plate using light petrol–ethyl acetate (85 : 15) as developing solvent. Products with R_f values of 0.70, 0.64 and 0.50 were trace constituents insufficient for identification.

Band 1 (R_f 0.79) (**17**). UV: 220, 282 nm. NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3), 1.25 (broad s, 24H, $(CH_2)_{12}$), 1.60 (d, 3H, CH_3–C=CH–), 2.0 (m, 2H, –$\underline{CH_2}$–CH_2–CO), 2.3 (t, 2H, $CH_2\underline{CH_2}$–CO), 4.20 (m, 2H, O–$\underline{CH_2}$–C=C), 5.3 (m, 1H, C=C–H), 9.7 (s, 1H, CHO).

Band 4 (R_f 0.59) (**17**). UV: 220, 280 nm. NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3), 1.25 (broad s, 24H, $(CH_2)_{12}$), 1.62 (d, 3H, $\underline{CH_3}$–C=CH), 2.0 (m, 2H, –$\underline{CH_2}$–CH_2–CO), 2.3 (t, 2H, –CH_2–$\underline{CH_2}$–CO), 4.15 (m, 2H, O–$\underline{CH_2}$–C=C–), 5.3 (m, 1H, –C=CH), 9.6 (s, 1H, CHO).

2.8. Photosensitized oxidation of vitamin A palmitate (riboflavin)

Vitamin A palmitate (300 mg) in ethanol (50 ml) saturated with riboflavin was exposed to visible light with aeration. After 30 h, TLC analysis showed no starting material and five product spots. The reaction mixture was separated as in Section 2.7 to give five products.

Band 1 (R_f 0.79). Spectroscopic data identical to band 1 in Section 2.7 (**17**).

Band 2 (R_f 0.66) (**19**). UV 212 nm. NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3), 1.25 (s, 24H, $(CH_2)_{12}$), 1.4 (t, 3H, CH_3), 1.60 (d, 3H, CH_3–C=CH), 2.3 (t, 2H, –CH_2CO–), 3.8 (q, 2H, –O–CH_2CH_3), 4.2 (m, 2H, O–CH_2–C=C), 5.3 (m, 1H, –C=CH).

Band 3 (R_f 0.55) (**20**). NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3), 1.25 (s, CH_2's), 1.6 (s, 3H, C=C–CH_3), 2.31 (t, 2H, CH_2CO), 4.2 (m, 2H, =C–CH_2–O), 5.3–7.0 (m, 3H, C=C–H), 9.60 (d, 1H, CHO).

Band 4 (R_f 0.26) (**18**). NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3), 1.25 (broad s, CH_2's), 2.3 (t, 2H, CH_2–CO), 4.3 (s, 2H, O–CH_2–CHO), 9.7 (t, 1H, CHO).

Band 5 (R_f 0.17) (**10**). NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3), 1.25 (m, CH_2's), 2.30 (m, CH_2CO).

2.9. Photosensitized oxidation of vitamin A palmitate (chlorophyll)

Vitamin A palmitate (300 mg) in ethanol (50 ml) containing chlorophyll (10 mg) was exposed to visible light with aeration for 2 h. The reaction mixture was separated in the usual way to give five products.

Band 1 (R_f 0.90) (**23**). NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3), 1.20 (broad s, CH_2's), 1.26 (t, 3H, CH_3CH_2–O), 2.30 (m, 2H, CH_2CO), 4.15 (q, 2H, –OCH_2CH_3).

Band 2 (R_f 0.81) (**24**). NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3), 1.20 (broad s, CH_2's), 1.25 (s, 6H, C-1 CH_3's), 1.30 (s, 3H, C-5 CH_3), 1.4–1.6 (m, 6H, ring CH_2's), 1.90 (s, 3H, C-9 CH_3), 1.95 (s, 3H, C-13 CH_3), 2.30 (m, 2H, CH_2–CO), 4.2 (m, 1H, H-8), 4.3 (m, 1H, H-7), 4.75 (d, 2H, CH_2–O), 5.6 (t, 1H, H-14), 6.25 (q, 2H, H-10, H-12), 6.50 (m, 1H, H-11).

Band 3 (R_f 0.72) (**21**). NMR ($CDCl_3$): δ 0.91 (t, 3H, CH_3), 1.25 (m, CH_2's), 1.3 (t, 3H, CH_3), 1.6 (s, 3H, CH_3–C=C), 4.2 (q, 2H, CH_3CH_2–O), 4.6 (dd, 2H, =CH–CH_2–O), 5.1–5.6 (m, 3H, =C–H's).

Band 4 (R_f 0.68) (**22**). NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3), 1.25 (broad s, CH_2's), 1.32 (t, 3H, CH_3CH_2), 2.31 (m, 2H, CH_2CO), 4.22 (q, 2H, CH_2–O), 4.7 (s, 2H, CO–CH_2–CO).

Band 5 (R_f 0.58) (**20**). NMR ($CDCl_3$): δ 0.90 (t, 3H, CH_3), 1.25 (broad s, CH_2's), 1.66 (d, 3H, C=C–CH_3), 2.3 (m, 2H, –CH_2CO), 4.17 (m, 2H, –O–CH_2–C=C), 5.3–7.0 (m, 3H, C=CH), 9.6 (d, 1H, CHO).

2.10. Effect of β-carotene on the photosensitized oxidation of vitamin A palmitate

Two solutions of vitamin A palmitate (300 mg) in ethanol (50 ml) containing rose bengal (5 mg) were prepared, and to one solution was added β-carotene (20 mg). Both solutions were exposed to visible light with aeration. TLC analysis showed that, in the solution without β-carotene, the starting material reacted within 6 h and yielded the same five product spots as observed in Section 2.7. The solution with added β-carotene showed only starting material after 6 h.

2.11. Photosensitized oxidation of β-ionone (rose bengal)

A solution of β-ionone (500 mg) in ethanol (50 ml) containing rose bengal (5 mg) was exposed to visible light with aeration. TLC monitoring of the reaction mixture showed that the starting material disappeared after 48 h and two product spots were noted. The products were separated on a thick chromatographic plate.

Band 1 (R_f 0.55) (**26**). UV: 208, 270 nm. MS: 208 (M^+). NMR ($CDCl_3$): δ 1.2 (d, 6H, C-1 CH_3's), 1.42 (s, 3H, C-5 CH_3), 1.4–1.9 (m, 6H, ring CH_2's), 2.25 (s, 3H, CH_3CO), 5.3 (d, 1H, H-7), 6.5 (m, 1H, H-8). The product gave a red colour and precipitate with 2,4-dinitrophenylhydrazine reagent.

Band 2 (R_f 0.21) (**27**). UV: 207, 230 nm. MS: 224 (M^+), 192 ($M-O_2$). NMR ($CDCl_3$): δ 1.00 (m, C-1 CH_3's), 1.50 (m, 3H, C-5 CH_3), 1.4–1.9 (m, 6H, ring CH_2's), 2.31 (d, 3H, CH_3CO), 5.3–6.5 (m, 3H, H-4, H-7, H-8). The product gave a red colour and precipitate with 2,4-dinitrophenylhydrazine reagent, and when treated with potassium iodide in dilute acetic acid iodine was liberated. The product also decomposed on standing.

3. Results and discussion

3.1. Photo-oxidation of vitamin A

A solution of vitamin A in ethanol was aerated and exposed to UV light. The progress of the reaction was monitored by TLC and, after 24 h, no vitamin A remained and five products were obtained. Small amounts of each product were obtained by PLC and, because of the instability of the products, identifications were based on spectroscopic examinations carried out as quickly as possible.

The products in the first two bands having the highest R_f values were identified as retinal and a geometric isomer of vitamin A; however, the quantity of the latter compound was too small to identify the isomer. The

other three products all had the same molecular weight (302), which is equivalent to the addition of one oxygen atom to the vitamin. On the basis of NMR analysis the three products were identified as the 5,6-epoxide, 5,8-epoxide and, tentatively, the 13,14-epoxide. Scheme 1 shows the reaction products.

Epoxides of vitamin A have been reported as products of chemical oxidation [4,6], but not previously as photochemical oxidation products.

3.2. Photo-oxidation of vitamin A palmitate

When the previous experiment was repeated for vitamin A palmitate, TLC analysis showed that most of the starting material reacted within 24 h and five products were formed. Separation was carried out on PLC plates and small amounts of the products were obtained. These products also proved to be rather unstable and their identification is based on spectroscopic studies carried out on freshly isolated material.

The material in the band with the highest R_f value had a molecular weight of 496, but there was insufficient material for identification. Bands 2 and 3 contained anhydrovitamin A and residual vitamin A respectively. The material in the fourth band could not be identified, but the fifth band contained a fragment of the vitamin A side-chain attached to palmitic acid. The last band contained palmitic acid. This reaction is illustrated in Scheme 2.

It was surprising that no epoxides were identified in this experiment. The formation of **8** and **10** suggests that hydrolysis occurs at some stage of the reaction.

3.3. Photosensitized oxidation of vitamin A

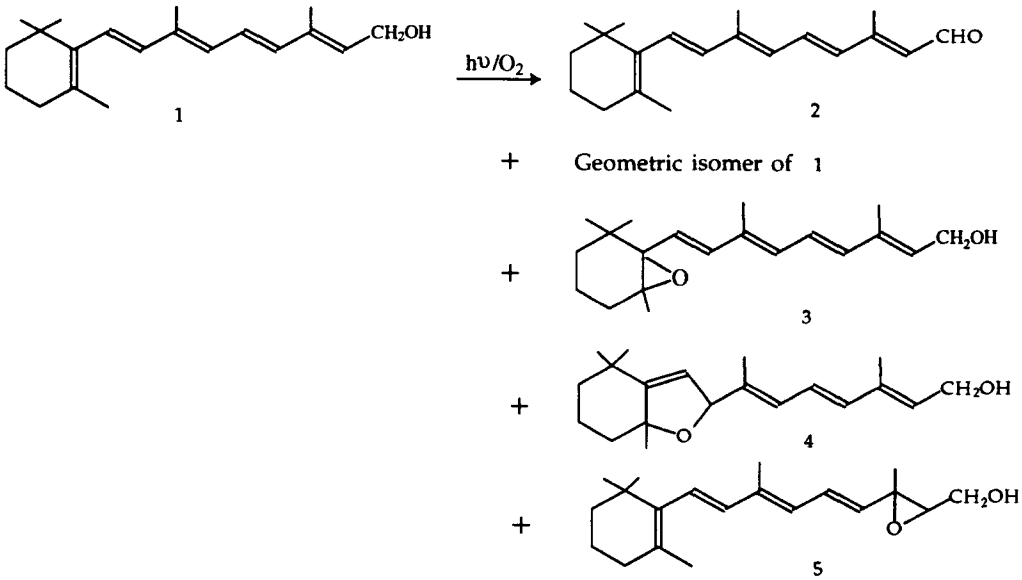
The photo-oxidation of vitamin A, sensitized by rose bengal, was completed within 4 h and produced only two products which were isolated on PLC plates. The first product had a molecular weight of 224 and gave a positive test with 2,4-dinitrophenylhydrazine. On the basis of the interpretation of the NMR spectrum it was identified as the 5,8-peroxide of β-ionone (**11**). The other product was identified as the 5,6-epoxide of vitamin A (**3**).

The reaction was slower when riboflavin was used as sensitizer, but a more complex mixture of products was obtained. When the reaction was stopped at 30 h, some vitamin A remained and eight product spots were noted.

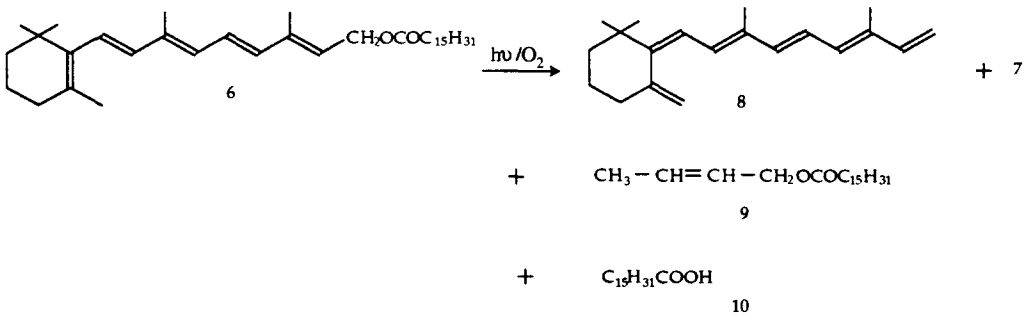
Band 1 contained an ester identified as ethyl-(2,6,6-trimethylcyclohex-1-ene)carboxylate (**12**). Material in band 2 was identified as retinal (**2**) and, in band 6, the 5,8-peroxide (**11**). The product in band 7 was identified as the 5,8-peroxide of vitamin A (**13**) based on its NMR spectrum. The last band contained retinoic acid (**14**).

In contrast with the above, the use of chlorophyll as sensitizer speeded up the reaction and all vitamin A was lost within 90 min. Originally eight bands were seen on the chromatogram, but six of the products were too reactive and decomposed during work-up. Only two products were identified as the carboxylic acid (**15**) and its ethyl ester (**16**).

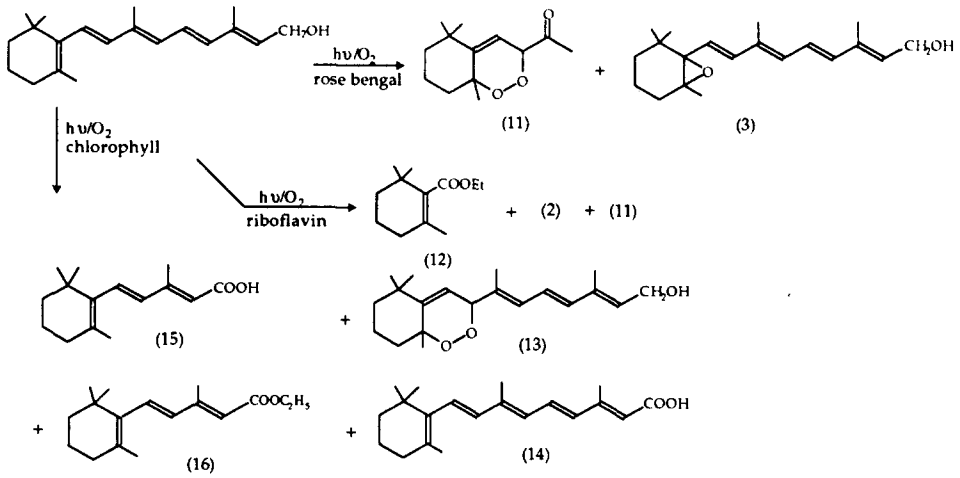
The photosensitized oxidation products of vitamin A are illustrated in Scheme 3.



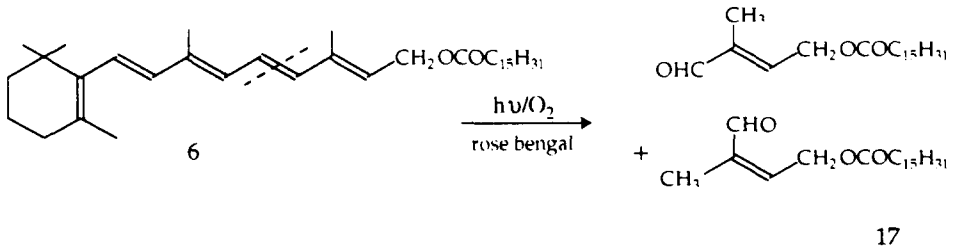
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

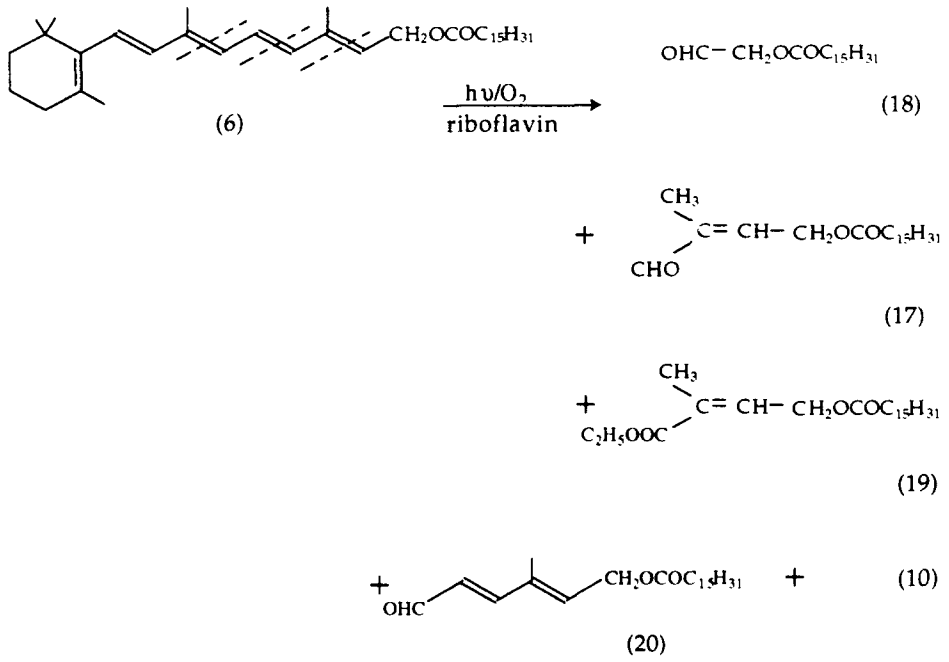
3.4. Photosensitized oxidation of vitamin A palmitate

The photosensitized oxidation of the palmitate ester, using rose bengal as sensitizer, was rapid and after 5 h no starting material remained. The five observed products were separated in the usual way, but only two were identified. The two products which had very similar spectra were considered to be geometric isomers (**17**) resulting from oxidative chain cleavage at the C-11–C-12 double bond (Scheme 4).

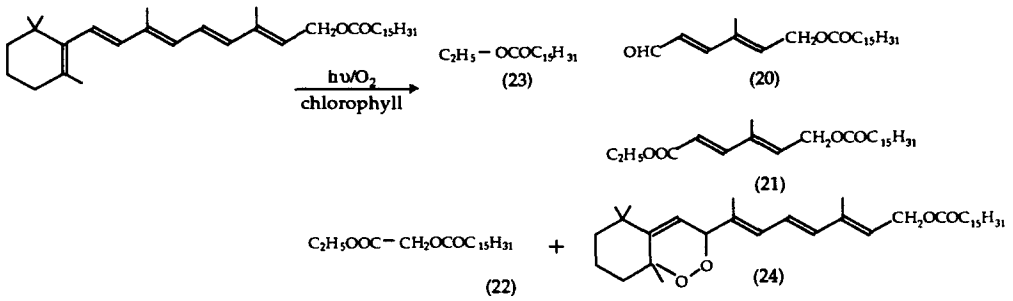
A similar reaction using riboflavin as sensitizer was slower, but gave a greater variety of products, which were formed by oxidative cleavage of the side-chain double bonds (Scheme 5). Aldehydes **17**, **18** and **20** were formed by cleavage of the double bonds at C-11, C-13 and C-9 respectively. The ester **19** is an over-oxidation product of **18** with esterification possibly occurring during work-up (Scheme 5).

In accord with the results for vitamin A, the sensitized photo-oxidation of the palmitate ester, using chlorophyll as sensitizer, was rapid. The chain cleavage products identified were aldehyde **20** and its corresponding ester (**21**), ester **22** and ethyl palmitate (**23**). The only product to retain the ring system was tentatively identified as the 5,8-peroxide of vitamin A palmitate (**24**) on the basis of spectroscopic analysis. The reaction is illustrated in Scheme 6.

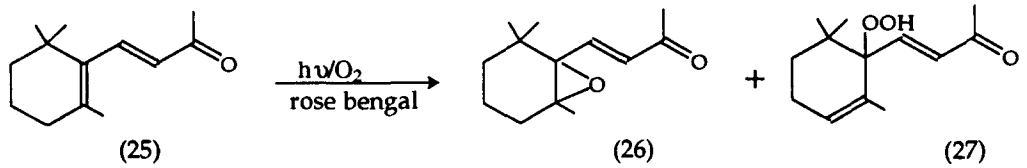
In a comparative experiment, two solutions of vitamin A palmitate were treated with rose bengal and one also with β-carotene. After 6 h of exposure to visible light, the solution containing β-carotene showed no evidence of products, whilst the other solution showed



Scheme 5.



Scheme 6.



Scheme 7.

virtually complete conversion of vitamin A palmitate to reaction products as observed previously. This supports the assumption that the reactions described in Sections 2.4–2.9 involve singlet oxygen as the initial oxidant.

It was suspected that β-ionone (**25**) might be an oxidation product of vitamin A by cleavage of the double bond at the C-9–C-10 position. Although it was not identified as an oxidation product in our experiments, it could have been further oxidized to smaller fragments. The sensitized photo-oxidation of β-ionone was therefore examined; however, it was found to react much slower than vitamin A and 48 h of exposure was required for the complete loss of starting material. Only two relatively unstable products were isolated and identified by chemical properties and spectral analysis. The proposed structures of these products are shown in Scheme 7.

Product **26** gave a reaction for a carbonyl group with 2,4-dinitrophenylhydrazine and showed UV and NMR spectra in accord with the epoxide structure given. Product **27** also gave a positive test with 2,4-dinitrophenylhydrazine, but it also demonstrated oxidizing action by converting iodide to iodine. The spectral properties are in accord with the hydroperoxide structure shown, and this product was probably formed by

the well-known "ene" reaction of singlet oxygen with olefins.

4. Conclusions

Our studies have shown that vitamin A and its ester undergo both photo-oxidation and photosensitized oxidation to give a variety of products which are dependent on the reaction conditions. The role of the sensitizer is particularly critical in affecting the rate of oxidation and the kinds of products formed.

In vitamin A, products were derived from oxidation of the alcoholic group and also from oxidation and oxidative cleavage of the side-chain. The ester gave only the latter types of product. Side-chain cleavage probably occurs via reactive oxygenated intermediates such as epoxides and peroxides. Further studies on these processes will be undertaken.

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