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The photooxygenation of z-3-vinyl-4-methyl-5-(phenylmethylidene)-3-pyrrolin-2-one (**1**) and z-5'-oxo-4'-vinyl-4-ethyl-3',3,5-trimethyl-1',5'-dihydro(2,2')dipyrromethene (**2**) reveals no reaction at the vinyl groups; hence, none is expected at the vinyl groups of bilirubin IXα, and none has been found.

In connection with the now widely employed photo-herapy for jaundice due to unconjugated hyperbilirubin-mia in newly born infants (1,2), we have been investigat-ng the photooxygenation of bilirubin IXα (BR IXα) (3-5) nd various model compounds, especially oxodipyrrometh-nes (6-8) which are structurally similar to the BR chromo-ιhore. Photooxygenation studies with such model sub-trates (3,6) allowed us to predict and demonstrate aspects ιf BR IXα photooxygenation, especially its photodegrada-ion to more water-soluble oxidized mono- and dipyrroles 3,4,6,9). The latter such findings have a direct bearing ιn understanding that aspect of bilirubin photochemistry vhich in the phototherapy might account for the excretion ιf bilirubin (photo) degradation products found in the ιile of jaundiced babies undergoing phototherapy (10,11), ιr in the bile of congenitally jaundiced (Gunn) rats upon ·xposure to light irradiation (11-13).
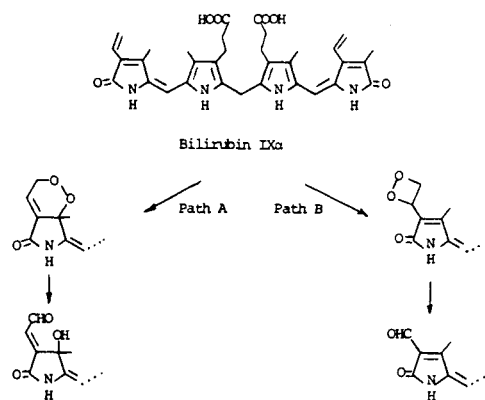
One of the first mechanisms postulated in the photo-legradation of BR IXα was 1,4-addition of singlet oxygen $^1O_2$] (14,15) to a diene involving a vinyl group and an ;ndocyclic ene-amide carbon-carbon double bond (13) as ·hown in Scheme 1 (Path A). The reference reaction for his postulated singlet oxygen 1,4-addition is the self-·ensitized photooxygenation reaction of photoporphyrin X (16). Although this reaction mechanism (14) has been· videly cited in connection with BR photodegradation 3,17,18), so far photoxidation products of the expected ype have not been isolated, and there have been no better nodels forthcoming than photoporphyrin on which to .ssess the mechanism. The possibility of an additional,

new type of reaction at the vinyl groups of bilirubin (Scheme 1, Path B) is raised by a recent report (19) on the 1,2-addition of singlet oxygen to the vinyl groups of photo-porphyrin IX leading to diformylporphyrins. In this note we report on the singlet oxygen reactivity of two model substrates (**1**) and (**2**) on which to examine reactions of singlet oxygen at the vinyl groups located in positions akin to those of bilirubin IXα.
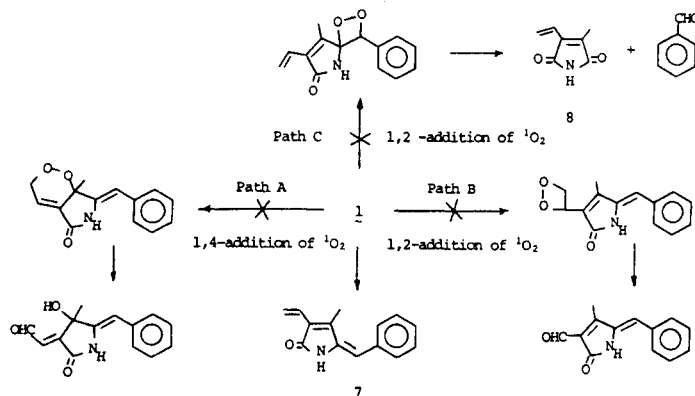
The preparations of **1** and **2** were straightforward. Compound **1** had been prepared earlier (20) from base-catalyzed condensation of benzaldehyde with 3-[β-tri-methylammonium ethyl]-4-methyl-Δ^3-pyrrolinone-(2) io-dide (**3**). Similarly, **2** could be prepared by condensation of kryptopyrrole aldehyde (**4**) (21) with **3**.
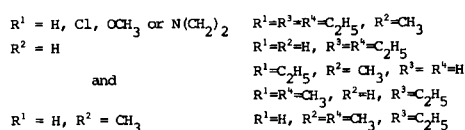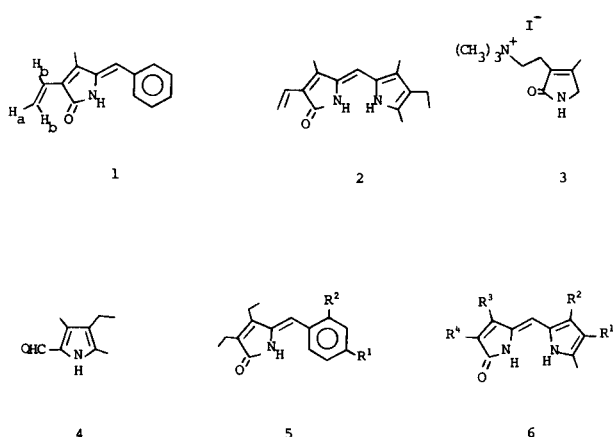
In earlier work (22) we showed that benzalpyrrolinones of general structure **5** do not undergo dye-(Rose Bengal) or self-sensitized photooxygenations. They do not react with singlet oxygen. On the other hand, oxodipyrro-methenes of type **6** do react with singlet oxygen (6,8,22), although their rate of reaction is dependent on the number and location of β-alkylsubstituents (21). Consequently, **1** would not be expected to undergo singlet oxygen reac-tion at the endocyclic ene-amide carbon-carbon double bond, and it becomes a very good test example for singlet oxygen reaction at the vinyl group (Scheme 2, Paths A and B). When **1** was subjected to Rose Bengal-sensitized photooxygenation for extended periods of time, no oxy-gen uptake could be measured. Work-up of the reaction led to a quantitative recovery of a mixture of **1** and its E-isomer (**7**). Thus, it would appear that the vinyl group

SCHEME 1

Bilirubin IXα

Path A   Path B

SCHEME 2

Path C   1,2 -addition of $^1O_2$

Path A   1   Path B

1,4-addition of $^1O_2$ 1,2-addition of $^1O_2$

7

1   2   3

4   5   6

R^1 = H, Cl, OCH$_3$ or N(CH$_2$)$_2$ R^1=R^3=R^4=C$_2$H$_5$, R^2=CH$_3$

R^2 = H R^1=R^2=H, R^3=R^4=C$_2$H$_5$

and R^1=C$_2$H$_5$, R^2= CH$_3$, R^3= R^4=H

                         R^1=R^4=CH$_3$, R^2=H, R^3=C$_2$H$_5$

R^1 = H, R^2 = CH$_3$ R^1=H, R^2=R^4=CH$_3$, R^3=C$_2$H$_5$

of **1** and probably bilirubin as well exhibits no tendency toward reaction with singlet oxygen. Also the presence of a vinyl group in **1** does not enhance the reactivity of the exocyclic ene-amide carbon-carbon double bond toward singlet oxygen (Scheme 2, Path C) over other non-reactive related systems (22).

Similarly, photooxygenation of oxodipyrrole **2** led to no recognizable products of reaction at the vinyl groups (similar to Paths A and B of Scheme 2 but to the expected types of products of Path C (Scheme 2), methylvinylmaleimide (**8**) and kryptopyrrole aldehyde (**4**). Thus, it would appear that the reactions shown in Scheme 1 are improbable based on the specific non-reactivity of **1** and **2**, and one is not likely to find such photooxygenation products with bilirubin itself (3,5,17).

Both **1** and **2** show the expected Z → E photoizomerization (5,8,22,23), which we believe is important in explaining the excretion of bilirubin during phototherapy (5,24). The mechanism can proceed both by a singlet or triplet pathway. Direct irradiation of **1** in THF or methanol with a mercury source or with 342 nm (10 nm bandpass) monochromatic light led to a mixture of Z and E isomers (**1** and **7**), 65:35 at photoequilibrium. The rate of photoisomerization was invariant in aerated and freeze-thaw degassed solutions. On the other hand, irradiation of a methanol solution of **1** with 557 nm (10 nm bandpass) monochromatic light led to formation of **7** when Rose Bengal was present (and no reaction in its absence) and a photoequilibrium of 83:17-**1**:**7**. There was a clear rate acceleration when oxygen (triplet quencher) was excluded (factor of 3). In order to avoid the competing photooxygenation reaction, the photoisomerization of **2** was best studied in degassed solution. As above, energy transfer from Rose Bengal led to some formation of the

E-isomer, but a competing (electron transfer, Type I ?) photooxidation reaction obscured the results and the determination of whether a triplet photoisomerism mechanism is tenable. Direct excitation of **2** at 438 nm in degassed solvents led to the formation of the E-isomer, which slowly reverted in the dark to **1** at room temperature.

In summary, Rose Bengal-sensitized photooxygenation of **1** and **2** reveals no reaction of singlet oxygen at the vinyl groups. On the basis of these models we conclude that reactions of the type shown in Scheme 1 are unlikely for bilirubin. The expected Z → E photoisomerizations of **1** and **2** are observed.

EXPERIMENTAL

General.

All melting points were determined on a Thomas-Hoover Uni-melt capillary apparatus and are uncorrected. All solvents used were reagent grade unless otherwise specified. Infrared spectra (ir) were run in chloroform or pyridine-d_5 on a Perkin-Elmer Infracord. Nmr spectra were measured in deuteriochloroform on a Perkin-Elmer R-24B spectrometer. Chemical shifts are reported in parts per million (δ) downfield from TMS as an internal standard with multiplicities: s = singlet, d = doublet, dd = doublet of doublets, br = broad and m = multiplet. Mass spectra were determined on a JEOL JMS-07 instrument at 70 ev. Uv spectra were recorded on a Cary 14 spectrophotometer. All liquid chromatography utilized silica gel 0.05-0.2 mm (M. Woelm, Eschwege) for column chromatography and silica gle F (M. Woelm, Eschwege) for analytical and preparative thin layer chromatography (tlc). Preparative photochemistry was carried out in a water-cooled pyrex immersion well apparatus (with circulating oxygen for photooxygenation experiments) using a Sylvania 500 Q/CL 500-W tungsten-halogen lamp. Photoisomerization studies were carried out in quartz cuvettes or tubes using a 10 nm bandpass monochromatic light from a Bausch and Lomb Model 33-86-07 monochromator equipped with either a B&L 200 W super pressure Hg source or a Sylvania 420 W tungsten-halogen lamp, or a Hanovia 100 W, 1.2 amp. high pressure mercury lamp, model SH. Freeze, pump, thaw degassings were carried out in special quartz cuvettes at 10^{-6} Torr. The methanol used in this work was Baker Analyzed anhydrous reagent, the tetrahydrofuran was Matheson distilled freshly from lithium aluminum hydride and the deuterated nmr solvents were obtained from E. Merck.

Table I

Percentage Change of **1** vs Irradiation Time with 557 nm Light

Irradiation Time (minutes)	Percent Disappearance of 342 λ max Absorbance of 1		
	Control, no Rose Bengal	Rose Bengal	Rose Bengal, Degassed
15	0	5.6	15.5
30	0	6.4	16.5
60	0	10.4	17.0
120	0	15.0	17.0
180	0	17.0	-----

Methyl γ-Carboethoxy-δ-oxocapric Acid Ester.

This compound was obtained according to the procedure of Plienger, et al. (20), in 75% yield, b.p. 94-96°/0.3 Torr [lit. (20) b.p. 105°/0.4 Torr].

Hydrogen Cyanide.

This compound was provided by the method of Ziegler (25).

Catalyst Raney Nickel, W-2.

This catalyst was prepared by the method of Mozingo (26). The nickel is very pyrophoric and so must be kept under liquid (e.g. methanol, ethanol) at all times.

3-(β-Carboxyethyl)-4-methyl-3-pyrrolin-2-one.

This compound was prepared by the method of Plieninger, et al. (20), in 51% yield, m.p. 167-169° [lit. (20) m.p. 166°, 56% yield]; nmr (pyridine-d_5): δ 1.87 (s, 3H, CH_3), 2.83 (s, 1H, CH_2 in pyrrolinone), 9.86 (br. s. 1H, NH) ppm.

Z-3-Vinyl-4-methyl-5-(phenylmethylidene)-3-pyrrolin-2-one (1).

This compound was prepared by the method of Plieninger, et al. (20), in 55% yield, m.p. 170-171° [lit. (20) m.p. 170-171°, 62%]; yellow-brown crystals but only one spot on analytical tlc with chloroform/ether (6:4); nmr (deuteriochloroform): δ 2.17 (s, 3H, CH_3), 5.43 (2d, 1H, J = 4 Hz, J = 8 Hz, vinyl proton a), 6.10 (s, 1H, exocyclic vinyl, CH=), 6.38 (m, 2H, 2H vinyl b protons), 7.33 (m, 5H, phenyl), 8.20 (br. s. 1H, NH); ms: m/e (relative intensity), 211 (M^+, 100%), 196 (16%), 182 (33%), 167 (33%) amu; uv (methanol): λ max = 342 nm, ε = 2.9 x 10^4; ir (chloroform): 3507 (free NH), 3257 (NH hydrogen bonded), 3057 (CH=), 3017 (CH=), 1693 (C=O), 1623 cm^{-1} (C=C).

5'-Oxo-4'-vinyl-4-ethyl-3',3,5-trimethyl-1',5'-dihydro-(2.2')-dipyr-romethene (2).

This compound was prepared by the method above in 27% yield using 3 and 4, m.p. 223-224° (dec.) (yellow crystals); nmr (deuteriochloroform): δ 1.03 (t, 3H, J = 7 Hz, CH_3), 2.10 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.43 (q, 2H, J = 7 Hz, CH_2), 5.27 (dd, 1H, J = 10 Hz, J = 3 Hz, vinyl H), 5.93 (dd, 1H, J = 18 Hz, J = 3 Hz, vinyl H), 6.17 (s, 1H, exocyclic vinyl H), 6.37 (d, 1H, J = 10 Hz), 10.2 (br. s. 1H, NH) ppm; ms: m/e (relative intensity), 256 (M^+, 100%) 241 (43%), 212 (23%) amu; uv (methanol): λ max = 438 nm, ε = 4.0 x 10^4; (chloroform) λ max = 443 nm, ε = 3.6 x 10^4; ir (chloroform): 3361 (NH) 1668 (C=O), 1628 cm^{-1} (C=C).

Anal. Calcd. for $C_{16}H_{20}N_2O$; C, 74.97; H, 7.86; N, 10.93. Found: C, 75.03; H, 7.80; N, 10.78.

Photooxygenation — Preparative.

A pyrex immersion well photolysis unit (400 ml. capacity), equipped for circulating and measuring oxygen uptake was used. A dilute methanolic solution of 1 or 2 (4 x 10^{-3} M) containing 3 mg./100 ml. of Rose Bengal was photolyzed with light from a 500 W tungsten-halogen lamp. The reaction was followed by monitoring oxygen uptake, by sampling for uv-visible spectro-photometry and by analytical tlc on silica glc. After irradiation, the methanol was removed by rotary evaporation at room temperature and the crude photolysate was separated by preparative tlc. The purified photoproduct(s) were characterized by nuclear magnetic resonance (nmr), infrared (ir) and mass spectrometry.

With 1, only starting material (1) and its E-isomer (7) were found to be present. With 2, methylvinylmaleimide (8) [R_f = 0.65 and 0.37 in chloroform/ether (6:4) and chloroform/ether (9:1) respectively] and kyrptopyrrole aldehyde (14) [R_f = 0.45 and

0.19 in chloroform/ether (6:4) and chloroform/ether (9:1) respectively] were found to be present. An authentic sample of methyl-vinylmaleimide was prepared according to the procedure of Bonnett and McDonagh (27), m.p. 83-84° [lit. (27) 86°], nmr (deuteriochloroform): δ 2.03 (s, 3H, CH_3), 5.63 (m, 1H, vinyl H), 6.40 (m, 2H, vinyl H), 7.30 (s, 1H, NH) ppm.

Photooxygenations — Analytical.

About 3 mg. of 1 were dissolved in 25 ml. of methanol. In a quartz cuvette 2.8 ml. of the solution were irradiated at 557 nm for appropriate times (control experiment). No change of the absorption maximum (342 nm) of the substrate 1 was observed within 3 hours. (The substrate does not absorb light at 557 nm).

The remainder of the stock solution (22.2 ml.) was diluted to 25 ml. again with 1 ml. of Rose Bengal solution and methanol ([RB] = 1.64 x 10^{-5} M). Again, 2.8 ml. of the solution were irradiated as described in the control.

Another 2.8 ml. of the solution were placed in an evacuable uv cell, degassed by freeze-degassed-thaw method at 10^{-6} Torr for several cycles and irradiated as described above.

The pertinent results are summarized in Table I.

Similarly, 2 was treated, and the results are shown in Table II.

Photoisomerizations.

A solution of 1 (8 mg., 0.039 mmole) was photoisomerized in tetrahydrofuran (0.5 ml.) in a quartz nmr tube with broad band uv light (mercury lamp). The characteristic vinyl peak at δ 6.1 in the nmr of 1 decreased in size while a peak at δ 6.5 increased in size, indicating that photoisomerization occurred (22). After photoequilibrium was reached, the solution was analyzed by analytical tlc on silica gle to give two bands (R_f = 0.65 and 0.42) in chloroform/ethanol (6:4). The band at R_f = 0.65 corresponds to starting material (1) and that at R_f = 0.42 corresponds to the E-isomer of 1.

Table II

Percentage Change of 2 vs Irradiation Time with 557 nm Light

Irradiation Time (minutes)	Percent Disappearance of 438 λ max Absorbance of 2		
	Control, no Rose Bengal	Rose Bengal	Rose Bengal, Degassed
4	0	80	18
30	0	-----	53

Table III

Percentage Change of 1 vs Irradiation Time with 342 nm Light

Irradiation Time (minutes)	Percent Disappearance of 342 λ max Absorbance of 1	
	Aerobic	Anaerobic
0.5	3.7	5.1
1.5	11.1	11.7
3	19.5	20.4
5	26.6	26.6
10	32.0	33.1
15	32.8	35.0

A solution of 1 in methanol (3.0 x $10^{-5}M$, 2 ml.) with Rose Bengal (5 x $10^{-6}M$) was irradiated at 557 nm (monochromatic light, tungsten lamp) for 5 hours. The maximum absorption maximum at 342 nm moved to shorter wavelength (333 nm). After evaporation of the solvent the residue was analyzed by analytical tlc or silica, which showed two spots, R_F = 0.66 and 0.42 in chloroform/ether (6:4), again indicative of Z → E photoisomerization.

For a time study, 1 (78 mg., 3.7 x 10^{-7} mmole) was dissolved in 10 ml. of methanol. The solution (2.8 ml.) in a uv cuvette (1 cm path) was irradiated at the wavelength of absorption maximum (342 nm). For oxygen-free photochemistry, the solution (2.8 ml.) was held in a evacuable uv cuvette (1 cm path), freeze-thaw degassed at 10^{-6} Torr and irradiated as above. The results are reported in Table III. After irradiation, the photoreaction mixture was placed in the dark at room temperature then monitored for the possible dark, reversible reactions, but no reversion could be observed.

Similarly, 2 showed conversion to its E-isomer, but careful analysis was prevented by Type I and Type II photooxidation reactions.

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