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# THE PHOTOOXYGENATION OF BILIVERDIN*

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The extensively used phototherapy for neonatal jaundice has been widely discussed[1,2], and its success presumably depends at least in part on the photodegradation of bilirubin IXα (1) to excretable, water-soluble products. McDonagh has shown that bilirubin is a singlet oxygen ($^1O_2$) sensitizer[3], and Bonnett and Stewart have recently reinforced this belief[4]. Some of the water soluble mono- and dipyrroles from in vitro photooxidation of 1 have been identified[5,6,7]. Ostrow[8,9,10] and Schmid[10] have also indicated that the in vitro photo-destruction of 1 gives biliverdin (2a), and Ostrow[9] has postulated that 2a is an intermediate in the photooxidation of 1. In our work on the photooxidation of 1 we have identified 2a as a photoproduct[11], but we do not believe that 2a is the precursor to photoproducts of 1, for 2a is photodestroyed at a considerably slower rate than is 1[12]. Furthermore, McDonagh has shown that 2 is a singlet oxygen quencher[13]. Despite these findings, very little is know of the photooxidation products of 2a[14]. We wish to report here on the first isolation and structure proof of hematinic acid methyl ester (3) and of a methanol propentdyopent adduct (4) from photooxidation of biliverdin dimethyl ester (2b).

Crude biliverdin IXα (2a) was prepared in 90% yield by controlled oxidation of bilirubin IXα (1) [100 mg, Matheson, Coleman and Bell] using benzoquinone (101 mg) in 50 ml of dimethylsulfoxide-acetic acid (9:1 v/v) according to the procedure of Bonnett and McDonagh[15]. The pure dimethyl ester (2b) was prepared from 2 (22% overall yield from 1) by esterification using $BF_3$-methanol[15] followed by preparative thin layer chromatography (Silica Gel F, M. Woelm,

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Eschwege, 1mm, 12% acetone in $CHCl_3$ v/v). Pure 2b moved as a dark blue-green band, $R_f$ 0.46, with only slight traces of the IIIα and XIIIα isomers.[16]

A dilute (1.88 mM) methanolic solution of biliverdin IXα dimethyl ester (2b) containing 4.3 mg % Rose Bengal ($^1O_2$ sensitizer) was irradiated in an immersion well apparatus using 500 watt Westinghouse tungsten-halogen lamp (500 Q/CL) at 100V while bubbling a slow stream of oxygen through the solution. After a photooxidation period of 32 hours (followed in the ultraviolet-visible spectrum by the disappearance of the 670 and 380 nm maxima of 2b and the emergence of new maxima at 570, 310 and 280 nm), the photoproducts were separated by a combination of gradient elution column chromatography on silica gel (E. Merck, Darmstadt, 70-325 mesh ASTM) using ether → ethyl acetate → acetone → methanol and preparative tlc (Silica Gel F, M. Woelm, Eschwege, 1mm, ether). Two new substances were isolated from a polar mixture. The structure of the methanol propentdyopent adduct methyl ester (3) isolated in 10% yield, was determined by its mass spectrum[17] m/e (relative intensity) 346.1526 (10%) [$M^+$, $C_{18}H_{22}N_2O_5$], 315 (9%) [M-$OCH_3$], 314 (17%) [M-$HOCH_3$], 259 (14%) [M-$CH_2CH_2CO_2CH_3$], 256 (44%) [M-$CO_2CH_3$-$OCH_3$], 212 (75%) and 152 (100%); its nmr spectrum: δ 1.80 ($CH_3$/s), 1.95 ($CH_3$/s), 2.59 (-$CH_2CH_2$-/s), 3.12 ($OCH_3$/s), 3.62 ($CO_2CH_3$/s), 4.76 (-CH=, meso/s), 5.59 (-CH=, vinyl/m), 6.30 (=$CH_2$/m), and 8.30 (NH/br. s) ppm; and its ultraviolet spectrum: $\varepsilon_{293nm}$ 14,000 methanol. A positive pentdyopent reaction, $\lambda_{max}$ = 524 nm, was obtained for 3.[18] The expected isomeric compound (4)[5] was not found. We presume it suffers more rapid (than 3) non-photochemical alterations, as we have shown that the hydroxy isomer of 4 is much more labile than the hydroxy isomer of 3 upon standing in methanol at room temperature[19]. Hematinic acid methyl ester (5) was isolated in 5% yield. Its structure was proved by its mass spectrum, m/e (relative intensity): 197.0681 (4%) [$M^+$, $C_9H_{11}NO_4$], 165 (100%) [M-$CH_3OH$], and 137 (85%) [M-$CO_2CH_3$-H]; and its nmr spectrum: δ 1.99 ($CH_3$/s), 2.66 (-$CH_2$-$CH_2$, s), 3.65 ($OCH_3$/s), and 7.52 (NH/br. s) ppm.

As shown in Scheme 1, we believe that both 3 and 5 are formed from an endoperoxide intermediate 4 (Path I) involving the inner rings of 2b with subsequent cleavages reminiscent of those observed for 3,4-diethyl-2,5-dimethylpyrrole[20]. These findings may be coupled with the earlier report of isolating methylvinylmaleimide (6) from unsensitized photooxygenation of 2a, presumably via a dioxetane intermediate (β) formed (Path II) by addition of $^1O_2$ to the enamine-like bridges a and c as originally suggested by McDonagh[3] based on the earlier observations of Foote and Lin[21] for enamines. Thus, two different modes of attack by $^1O_2$ on the biliverdin skeleton are clearly implicated (Routes I and II), just as they are for bilirubin[5]. We are currently looking for additional photoproducts from 2a and 2b.

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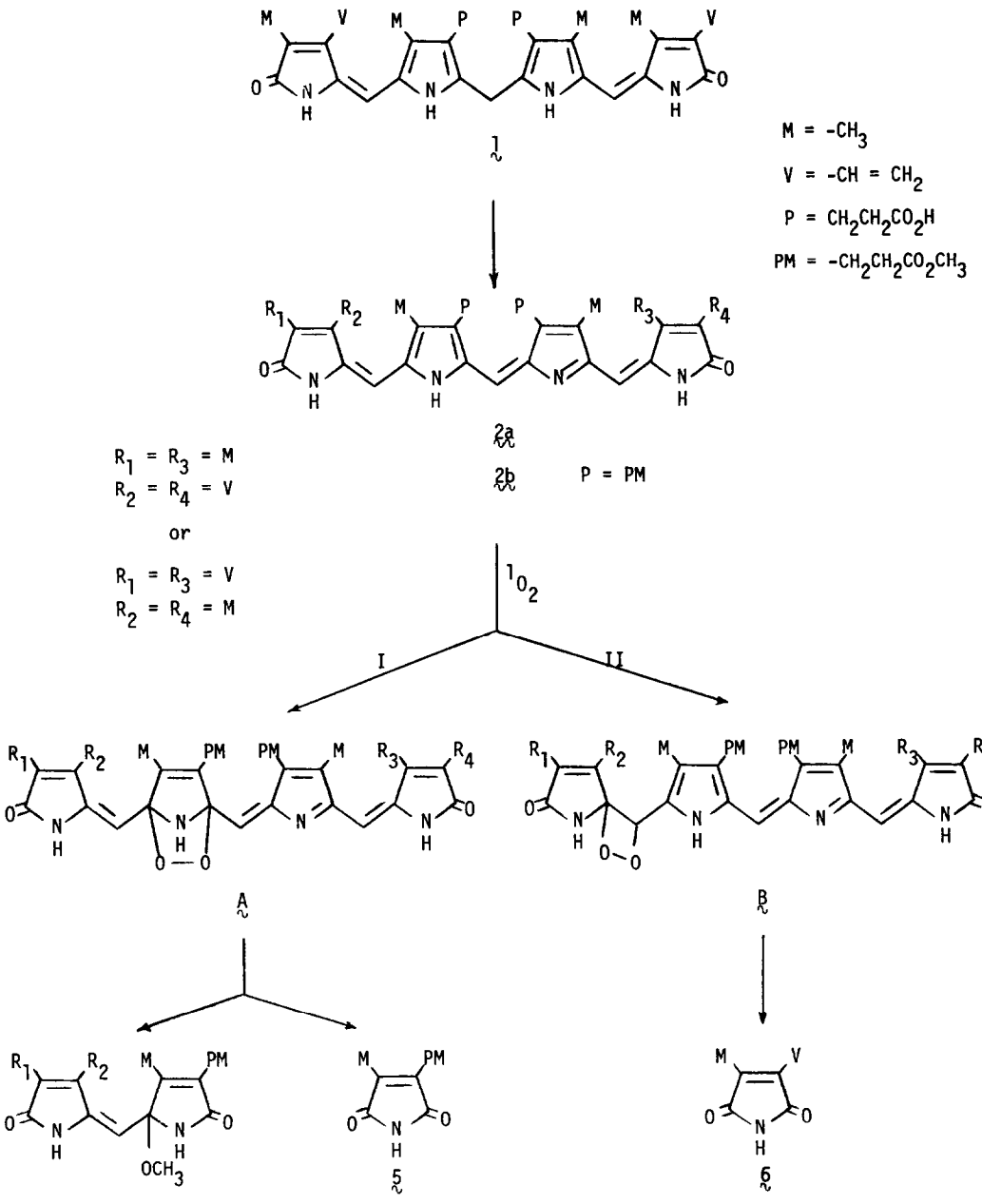
Scheme 1


M = -CH3
V = -CH = CH2
P = CH2CH2CO2H
PM = -CH2CH2CO2CH3
1
2a
2b
P = PM
R1 = R3 = M
R2 = R4 = V
or
R1 = R3 = V
R2 = R4 = M
1O2
I
II
A
B
OCH3
3 R1 = M, R2 = V
4 R1 = V, R2 = M
5
6

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