HEMATINIC ACID AND PROPENTDYOPENTS FROM BILIRUBIN PHOTO-OXIDATION *IN VITRO*

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1. Introduction

The structures of the photo-oxidation products [1] of bilirubin IXα (**1**) are of special interest to those concerned with a widely used clinic photo-therapy for hyperbilirubinemia (jaundice) in the newborn [2, 3]. Untreated neonatal jaundice may lead to cerebral palsy or even death. In the photo-therapy treatment, lipophilic bilirubin is removed by its conversion to excretable, water soluble substances [4] whose structures and toxicities are largely unknown as yet [1, 5]. Considerable data have accrued on the visible-ultraviolet spectral changes accompanying the photo-destruction of **1**, and paper chromatographic separations of the photo-products have been achieved [3, 5, 6] with an indication of possible structures [5–7]. Very recently a methanol propentdyopent adduct was determined to be a photoproduct, but the precise structure was not proved unambiguously [7]. McDonagh [8] has shown that **1** is a singlet oxygen ($^1O_2$) [9] sensitizer; apparently it sensitizes its own photo-destruction. In our earlier work [1] we first isolated and proved that methylvinylmaleimide is an *in vitro* photo-oxidation product of **1**. In the following we report on the first isolation and positive structure identification of hematinic acid (**2**) and two isomeric propentdyopent derivatives (**3** and **4**) as important products from *in vitro* photo-oxidation.

2. Materials and methods

A 1.5 mM methanolic solution of **1** (and 18 mM in NH_4OH to dissolve the bilirubin (Matheson, Coleman and Bell)) was irradiated for 36 hr with a tungsten-halogen lamp (500 W, Sylvania 500 Q/CL) as an internal source while a slow stream of oxygen was bubbled through the reaction vessel. After evaporation of 90% of the methanol and subsequent addition of 10% methanolic HCl (to neutralize the NH_4OH) in the cold, the mixture was treated with excess diazomethane in ether. Evaporation of solvent and preparative thin-layer chromatography (silica gel F, M. Woelm, Eschwege, 1 mm, ethyl acetate, 26°) afforded the methyl esters of **2** (R_f 0.65), **3a** (R_f 0.38) and **4a** (R_f 0.51) in yields of 7, 31, and 37%, respectively. When the photolysis was carried out with 3 mg percent added Rose Bengal ($^1O_2$ sensitizer) for 4 hr, the yields of **2**, **3a** and **4a** were 3–6, 31 and 32%, respectively.

All mass spectra (MS) were measured on an AEI MS-9 mass spectrometer, nuclear magnetic resonance (NMR) spectra were run on a Varian T-60 instrument, using tetramethylsilane as internal standard and ultraviolet (UV) spectra were determined on a Cary 14 spectrometer.

3. Results and discussion

Hematinic acid (**2**), was isolated as its methyl ester, whose structure was proved by its MS, *m/e* (relative intensity): *197.0681* (4%) [M^+, $C_9H_{11}NO_4$],

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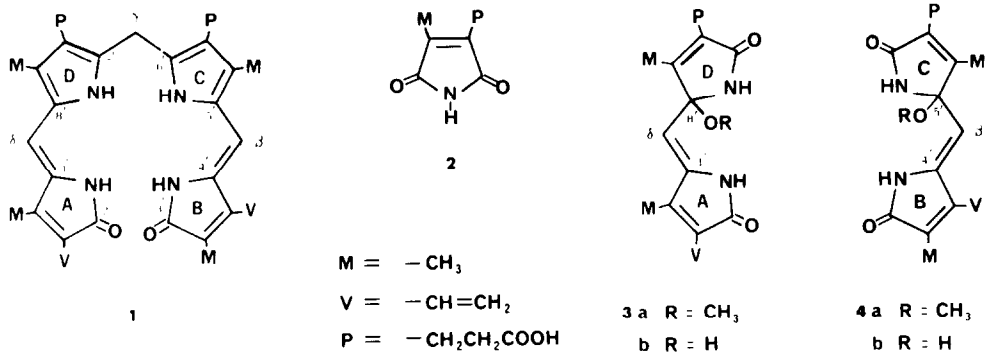

Fig. 1.

165 (100%), and *137* (85%); and its NMR spectrum: δ ($CDCl_3$) 1.98 (3H, singlet, =C–CH_3), 2.64 (4H, singlet, –CH_2CH_2–), and 3.63 (3H, singlet, OCH_3) ppm. This compound was identical with that prepared in quantitative yield by treating authentic **2** [10] with excess ethereal diazomethane.

The structure of the methyl ester of **3**α, mp 148° dec., was assigned by its MS, *m/e* (relative intensity): *346.1526* (19%) [M^+, $C_{18}H_{22}N_2O_5$], *327* (5%), 315 (9%) [M-OCH_3], *259* (17%), *255* (21%), and *212* (51%); NMR spectrum: δ ($CDCl_3$) 1.96 (6H, singlet, =C–CH_3 of rings A and D), [resolved in d_6-dimethylsulfoxide: 1.84 (3H, singlet) and 1.92 (3H, singlet)], 2.59 (4H, singlet, –CH_2CH_2–), 3.13 (3H, singlet, OCH_3), 3.62 (3H, singlet, ester OCH_3), 4.80 (1H, singlet = (δ) C–H), 5.53, 1H, multiplet, vinyl =C–H), 5.82 (1H, singlet, N–H), 6.35 (2H, multiplet, vinyl =CH_2), 8.37 (1H, singlet, N–H) ppm; and UV maximum; $\epsilon_{266\,nm}$ 12,000 (methanol). The structure of **4a**, mp 127°, dec., was determined by its MS, *m/e* (relative intensity): *346.1523* (15%) [M^+, $C_{18}H_{22}N_2O_5$], fragmentation pattern same as for **3a**; NMR spectrum: δ ($CDCl_3$) 1.82 (3H, singlet, =C–CH_3 of ring B), 2.01 (3H, singlet, =C–CH_3 of ring C), remaining NMR signals are essentially identical with those of **3a**; and UV maximum; $\epsilon_{293\,nm}$ 14,000 (methanol). The assignment of the orientation of M and V on rings A and B which distinguishes **3** from **4** is based on our observation [11] that a methyl group on the α-carbon of an α,β-unsaturated pyrrolin-2-one lies ~0.2 ppm upfield from a methyl group on a β-carbon in the $CDCl_3$ NMR spectrum. The assignment (M and V of rings A and B) of the structures of **3a** and **4a** allows us to suggest that Bonnett and Stewart's methoxy-propentdyopent isomer [7] is in fact the free carboxylic acid of **3a**. Both **3a** and **4a** gave a positive pentdyopent reaction [12]; for **3a** λ_{max} = 527 nm, for **4a** λ_{max} = 524 nm. Structures like **3** and **4** apparently account for the new peak which appears and grows near 300 nm in the visible-UV spectra of bilirubin during the early stages of its photodestruction [5].

When **1** is photo-oxidized under aqueous conditions and the work-up is also carried out under aqueous conditions (H_2O is removed by lyophilization) the yield of **2** was 10% but the isolated yields of the expected water-propentdyopent adducts (**3b** and **4b**) were only 3–6%. We attribute these low yields to the strong propensity of **3b** and **4b** to decompose under the acidic conditions of our isolation procedure. Additional evidence for the direct formation of **3b** and **4b** as well as their lability may be observed in the following. When **1** is photo-oxidized under aqueous conditions and the work-up is carried out under the *methanolic* conditions cited earlier, **3a** and **4a** are obtained (in yields of 12 and 9%, respectively), and under *ethanolic* conditions the corresponding ethanol–propentdyopent adducts are isolated (both in 8% yield). Presumably an extremely facile S_N1 solvolysis of the expectedly labile α-amino (and allylic) OH groups is achieved. These observations raise the spectre that **3** and **4** might have their C-8′ and 5′ OCH_3 (OH) groups located at the allylic (C-1′ and 4′) positions to some extent as a result of solvolysis reactions and allylic rearrangement in the work-up. Nonetheless, as best we can determine, **3a** and **4a** are isomerically

pure, and we have located the OCH_3 (OH) groups at C-8′ and 5′ based on the mechanistic considerations below.

We believe that $^1O_2$ adds in a 1,4-manner to rings C (at 5′, 6′), or D (at 7′, 8′) of **1**, and the resulting endoperoxide decomposes by di-dealkylation to give **2** and mono-dealkylation plus addition of solvent to yield **3** and **4**. Evidence for these types of reactions may be found in our work on the photooxidation of 3,4-diethyl-2,5-dimethylpyrrole which yields the analogous diethylmaleimide and 3,4-diethyl-5-methoxy-5-methyl-Δ^3-pyrrolin-2-one [13]. We have already addressed ourselves to the generality of another mode of photodegradation of **1** and related substances involving cleavage of double bonds at the β and δ carbons with the elision of imide products [14, 15]. Thus, we have shown evidence for the two likely, general modes of photo-oxygenation of bilirubin: i) by 1,2-addition of $^1O_2$ to and cleavage of the enamine-like β and δ bridges with formation of methylvinylmaleimide from rings A and B [1, 14] and ii) by 1,4-addition of $^1O_2$ to pyrrole rings C and D and decomposition of the resultant endoperoxide to give **2** (from rings C and D) and propentdyopent adducts **4** (from rings B and C) and **3** (from rings A and D). These reactions do not occur under our reaction conditions in the absence of light [1], and the products are the same with and without addition of known singlet oxygen sensitizers. Therefore, we expect the *in vivo* products formed during jaundice phototherapy to consist of water soluble methylvinylmaleimide, **2**, **3b** and **4b** as well as other products whose structures are presently being determined in our laboratories.

Neither methylvinylmaleimide nor hematinic acid (2) exhibit the usual enzyme toxicity associated with maleimide and *N*-alkylmaleimides [1, 16, 17]. Thus, should they be formed *in vivo*, we assume they would be non-toxic.

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