BILIVERDIN PHOTO-OXIDATION. *IN VITRO* FORMATION OF METHYLVINYLMALEIMIDE

David A. LIGHTNER and Dave C. CRANDALL
Department of Chemistry, University of California, Los Angeles, Calif. 90024, USA

Received 4 November 1971

1. Introduction

The photo-oxidation of bilirubin IXα (fig. 3:1) is of current interest in the treatment of neonatal jaundice (hyperbilirubinemia) by phototherapy [1, 2]. Untreated neonatal hyperbilirubinemia may lead to cerebral palsy or even death. Since Cremer et al. [3] observed that exposing the jaundiced infant to intense light lowered elevated bilirubin levels in the newborn, phototherapy of the icteric infant has gained in popularity. However, it has also become highly controversial [1, 2] due mainly to 2 possible inherent dangers: i) the untoward effects of prolonged illumination and ii) uncertainty over the structures of the photo-products and their potential toxicities. Ostrow [4–6] and Schmid [6] have indicated, and we have since found [7], that the photo-destruction of bilirubin gives biliverdin IXα (fig. 3:3). Moreover, the photo-products from 1 and 3 (fig. 3) exhibit a similar thin layer chromatographic behavior [4, 7]. Since no firm structural information has yet appeared on the photo-degradation products from biliverdin, we wish to report here on the first isolation and structure proof of a biliverdin photo-product, methylvinylmaleimide (fig. 3:2).

2. Materials and methods

Crude biliverdin IXα was prepared in 90% yield by controlled oxidation of bilirubin IXα (100 mg, purchased from Matheson, Coleman and Bell) using benzoquinone (101 mg) in 50 ml of dimethyl sulfoxide–acetic acid (9:1 v/v) according to the procedure of Bonnett and McDonagh [9]. The pure biliverdin IXα used in this work was obtained in an overall 28% yield after column chromatography on silica gel (E. Merck, Darmstadt, 70–325 mesh) using 1:1 v/v $CHCl_3$–CH_3OH as eluate. On thin layer chromatography (TLC) (M. Woelm, Eschwege, CH_3Cl–CH_3OH 5:1 v/v) 1 moved as one green spot, R_f 0.50, with very slight traces of the IIIα and XIIIα isomers (R_f's 0.53 and 0.46, respectively) [8] which are the principal isomeric contaminants of commercial bilirubin IXα [10].

A 0.66 mmolar methanolic solution of biliverdin was irradiated for 132 hr in an immersion well apparatus using a 500 W Westinghouse tungsten halogen lamp (500 Q/CL) at 120 V while bubbling a rapid stream of oxygen through the solution. No known singlet oxygen sensitizers [11] were added. Changes in the visible ultraviolet spectrum (fig. 1) were recorded during the course of photolysis on a Cary 14 spectrophotometer. The formation of photo-degradation products was detected by TLC (fig. 2) as above for 3. Preparative TLC (M. Woelm, Eschwege, 1 mm, $CHCl_3$) of the crude photolysate after evaporation of the solvent in vacuo separated the products. All mass spectra were determined on a CEC-491 MS-21 mass spectrometer and nuclear magnetic resonance (NMR) spectra were run on a Varian T-60 instrument.

3. Results and discussion

The known [5] photo-destructibility of biliverdin in the presence of oxygen is fully evident in the spectral changes recorded in fig. 1. At our high con-

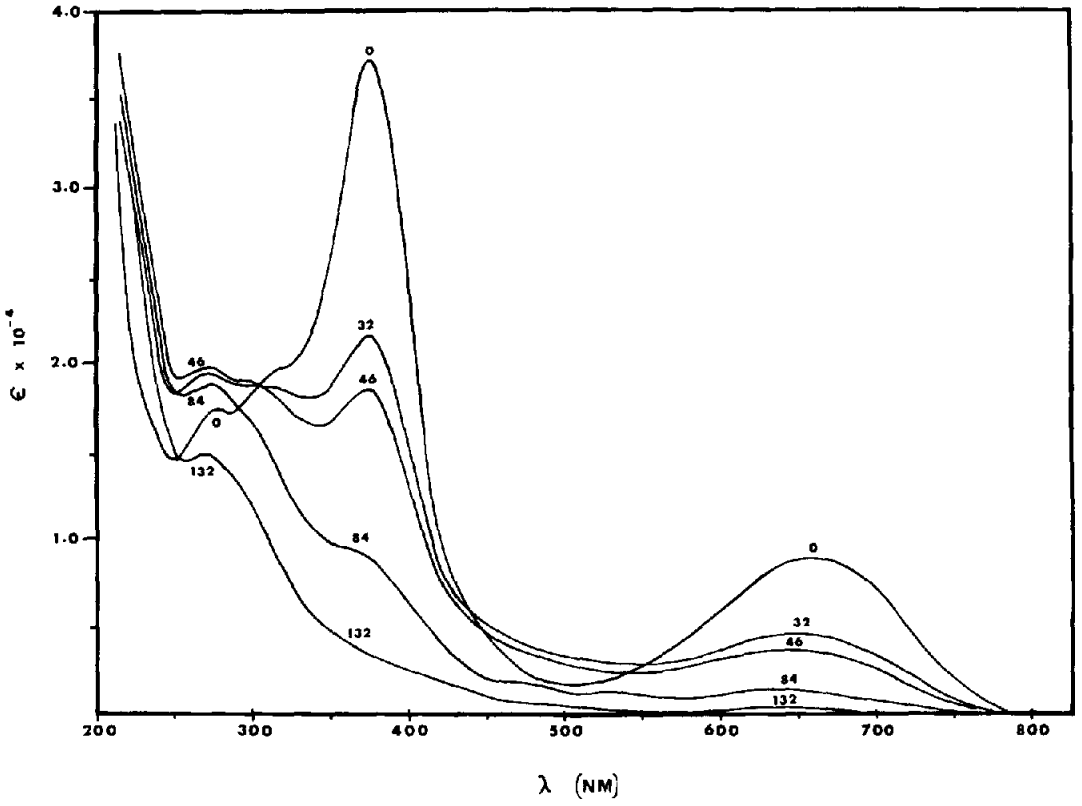

Fig. 1. Visible-ultraviolet spectral changes recorded in methanol solvent during the photo-oxidation of biliverdin. Curves are labelled with irradiation times.

centrations of 1, the intensity maxima in the vicinities of 660 and 375 nm gradually shrink (0–132 hr) leaving only the maximum near 275 nm (which may be found even at the onset of photolysis) and end absorption. Concomitant with these spectral changes, the appearance of new products can be shown by following the photo-destruction using TLC (fig. 2). At least 2 new colored substances become evident early (8 hr) in the photolysis. Eventually (46 hr) 7 or more new substances appear. As 1 is consumed (84 hr), even some of the initially formed photo-products ($R_f \sim 0.5$) begin to disappear until at the termination of the reaction (132 hr) 1 and the initially formed photo-products ($R_f \sim 0.5$) are only faintly present and there remain essentially non-polar ($R_f \sim 0.9$) and very polar ($R_f \sim 0.0$) products.

After 46 hr of irradiation, the characteristic pale blue fluorescence [12] of methylvinylmaleimide (*2*), R_f 0.88, becomes noticeable. On the basis of this visual method of detection, the concentration of *2* in the photolysis mixture increases through 132 hr of irradiation at which time the reaction was terminated. After evaporation of the solvent in vacuo and preparative TLC of the crude photolysate, a 10% yield of *2* was obtained. Its structure was proved by its mp 82–4° (mp 84–5° [11]); mass spectrum; *m/e* (relative intensity): *137* (39%) [M^+], *119* (2%), *109* (7%), *94* (5%) and *66* (100%); and its NMR spectrum (60 MHz, $CDCl_3$) δ 2.05 (s, 3H, CH_3), 5.70 (m, 1H, –CH=) and 6.45 (m, 2H, $=CH_2$) ppm. Methylvinylmaleimide is not formed during a control experiment omitting light.

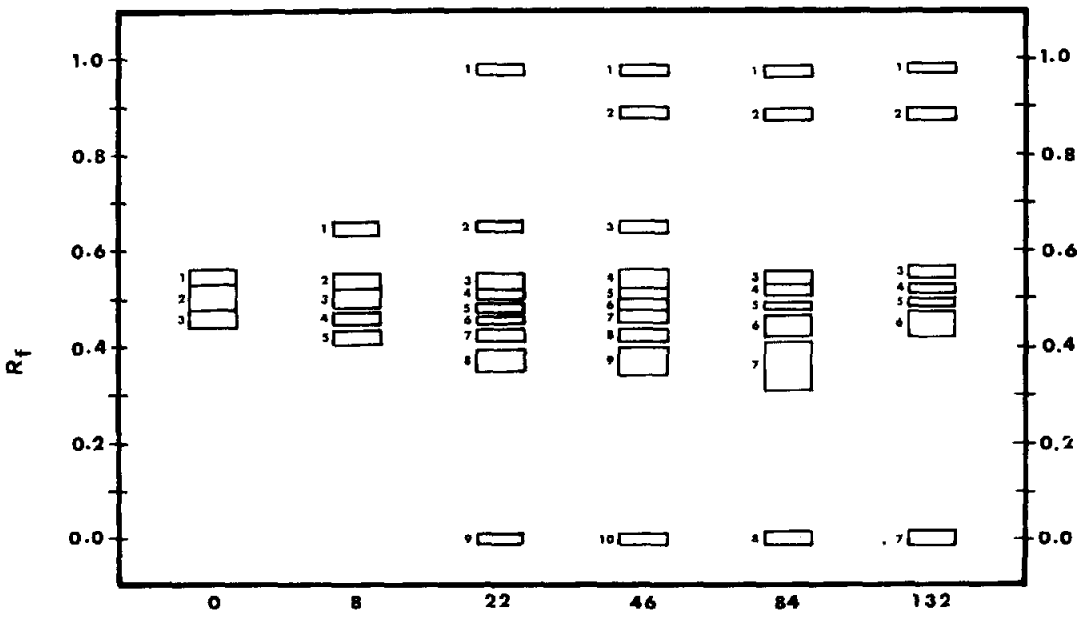

Fig. 2. Thin layer chromatogram ($CHCl_3$–CH_3OH, 5:1 v/v) of biliverdin and its photo-oxidation products during the course of irradiation. Colors and/or UV fluorescence (*) are as indicated: (0 hr), 1: pale green, 2: dark green, 3: pale green; (8 hr), 1: violet, 2: pale green, 3: dark green, 4: pale green, 5: yellow; (22 hr), 1: dark blue (*), 2: violet, 3: pale green, 4: dark green, 5: orange, 6: pale green, 7: violet, 8: yellow, 9: yellow; (46 hr), 1: dark blue (*), 2: pale blue (*), 3: violet, 4: pale green, 5: dark green, 6: orange, 7: olive, 8: violet, 9: yellow, 10: yellow; (84 hr), 1: dark blue (*), 2: pale blue (*), 3: yellow, 4: green, 5: orange, 6: brown, 7: yellow, 8: tan; (132 hr), 1: dark blue (*), 2: pale blue (*), 3: yellow, 4: green, 5: orange, 6: pale yellow, 7: tan. At time 0 hr spots 1, 2 and 3 are biliverdin IIIα, IXα and XIIIα in that order [7].

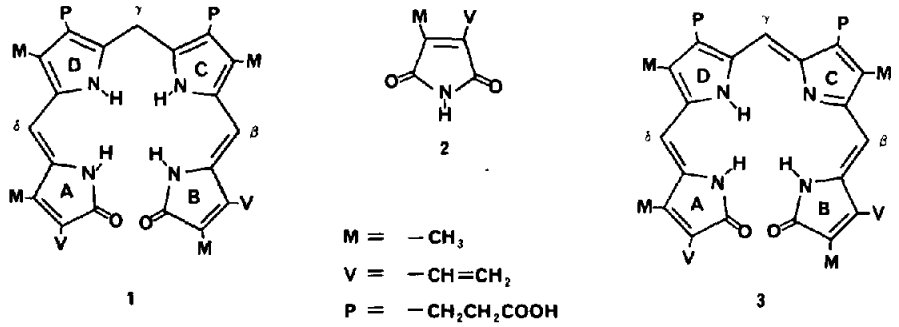

Fig. 3.

Methylvinylmaleimide is evidently formed by cleavage of rings A and B of *3*, presumably *via* photo-oxidation of the enamine-like β and δ bridges [13, 14]. Its isolation is remarkable on 2 grounds: i) the known photo-lability of the vinyl groups of protoporphyrin [15] and ii) the established tendency of *2* to polymerize [12, 16]. It is of special interest to note that *2* does not exhibit the usual enzyme toxicity associated with maleimides [17, 18]. This finding suggests that should *2* be formed *in vivo* during phototherapy it would not be toxic.

Acknowledgements

All mass spectra reported in this work were run by Miss Elizabeth Irwin and all NMR spectra were obtained through the assistance of Mr. Gary B. Quistad. We thank Dr. A.F. McDonagh for helpful discussions during the course of this work. This work was supported by Grant GP-9533 from the National Science Foundation and the donors of the Petroleum Research Fund of the American Chemical Society (4949-AC4). DCC thanks the National Science Foundation for an Undergraduate Research Fellowship.

References

[1] R. Schmid, New Eng. J. Med. 285 (1971) 520. Also, see leading articles in Brit. Med. J. 2 (1970) 5; Lancet, i (1970) 825; and J. Pediatrics 74 (1969) 989.

[2] For a recent summary, see: Bilirubin Metabolism of the Newborn, eds. D. Bergsma, D.Y.-Y. Hsia and C. Jackson (Williams and Wilkins Co., Baltimore, 1970).

[3] R.J. Cremer, P.W. Perryman and D.H. Richards, Lancet 1 (1958) 1094.

[4] J.D. Ostrow, in: Bilirubin Metabolism, eds. J.A.D. Bouchier and B.H. Billings (Blackwell Scientific, Oxford, 1967) p. 117.

[5] J.D. Ostrow and R.V. Branham, Gastroenterology 58 (1970) 15, and references therein.

[6] J.D. Ostrow, L. Hammaker and R. Schmid, J. Clin. Invest. 40 (1961) 1442.

[7] D.A. Lightner and G.B. Quistad, Science, in press.

[8] D.A. Lightner and D.C. Crandall, in preparation.

[10] A.F. McDonagh and F. Assisi, FEBS Letters 18 (1971) 315.

[11] C.S. Foote, Accts. Chem. Res. 1 (1968) 104.

[12] R. Bonnett and A.F. McDonagh, Chem. Ind. (London) (1969) 107.

[13] A.F. McDonagh, Biochem. Biophys. Res. Commun. 44 (1971) 1306.

[14] C.S. Foote and J.W.-P. Lin, Tetrahedron Lett. (1968) 3267.

[15] H.H. Inhoffen, H. Brockman, Jr. and K.-M. Bliesener, Justus Liebigs Ann. Chem. 730 (1969) 173.

[16] We have obtained methylvinylmaleimide from carefully controlled chromic acid oxidation of bilirubin. Small samples ($\sim$ 15 mg) polymerize upon standing overnight in an open container at room temp. and even somewhat more rapidly in $CDCl_3$ (NMR solvent); however, we have successfully stored methylvinylmaleimide for over a year in a sealed glass ampoule under nitrogen at 0°. G.B. Quistad, unpublished observation.

[17] M.A. Whitehouse, G.B. Quistad and D.A. Lightner, Biochem. Pharm., submitted.

[18] For a review of maleimide toxicities, see: Enzyme and Metabolic Inhibitors, Vol. III, Chapter 3, ed.J.L. Webb (Academic Press, New York, 1966).