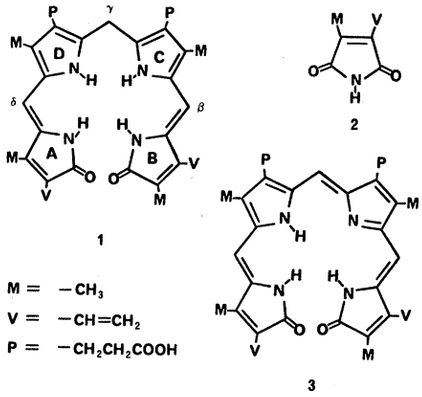
Methylvinylmaleimide from Bilirubin Photooxidation

Abstract. *Irradiation of an oxygenated methanolic solution of bilirubin IXα in the absence of known singlet oxygen sensitizers gave methylvinylmaleimide among other products.*

The photochemistry of bilirubin IXα (**1**), especially its photooxidation, is of interest in connection with a clinical treatment for jaundice (hyperbilirubinemia) of the newborn (*1*, *2*). Neonatal jaundice may lead to retarded motor development, cerebral palsy, or even death; and an increasingly common clinical remedy for the severe condition is a phototherapy method whereby the infant is exposed to blue light for long periods of time. By this procedure the lipophilic unconjugated bilirubin is photooxidized to water-soluble products (*3*). This phototherapy method for treatment of hyperbilirubinemia has inherent in it two possible dangers. Light might have other deleterious effects on the newborn and the photoproducts themselves might be toxic. At present neither the structures of the bilirubin photoproducts not their toxicities are known although the photodestruction of bilirubin in vivo was reported in 1958 (*4*) and has since been studied by Ostrow (*5–7*), Schmid (*3*, *7*), and others (*2*, *8*). These workers investigated principally the visible ultraviolet spectral changes and recorded paper chromatographic separations for comparison with the bile or urine of the congenitally jaundiced Gunn rat (*2*, *6*). More recently, McDonagh has shown that singlet oxygen is involved in the self-sensitized photodestruction of bilirubin (*9*). In view of the virtually complete absence of structural information on the bilirubin photoproducts, we report our findings for the photooxidation in vitro.

A 0.76 m*M* methanolic solution of **1** [a few drops of concentrated aqueous NH_4OH were added to dissolve the bilirubin (Matheson, Coleman, and Bell)] was irradiated for 12 hours with the use of a medium pressure mercury lamp (100 watt, Hanovia 8A36) as an internal source while a stream of oxygen was bubbled through the reaction vessel. Evaporation of the methanol at room temperature at reduced pressure gave a green solid. Preparative thin-layer chromatography (TLC) (silica gel F, M. Woelm Eschwege, 1 mm, diethyl ether, 26°C) yielded the characteristically fluorescent (*10*) methylvinylmaleimide (**2**), R_F 0.89, in 5 percent yield. Substantial amounts (~ 5 percent) of the dark green, bile pigment biliverdin (**3**) could also be isolated as the dimethyl ester by TLC. Moreover, by TLC analysis periodically during the photolysis (**3**) appears to be the major product formed in short-term irradiation (*11*). The complete structural identification of the remaining photoproducts remains to be determined.

The isolated and sublimed methylvinylmaleimide (**2**) was characterized by its melting point, 84° to 85°C [authentic m.p. 84° to 85°C (*10*)]; mass spectrum (*12*), *m/e* (relative intensity): *137* (35 percent) [M+], *119* (3 percent), *109* (8 percent), *94* (5 percent), and *66* (100 percent); and its nuclear magnetic resonance spectrum (*12*): δ ($CDCl_3$) 2.04 (3H, singlet, CH_3), 5.69 (1H, multiplet, =C—H), and 6.45 (2H, multiplet, $=CH_2$) ppm. It is apparently produced by elision of rings A and B of bilirubin (**1**) via photooxidation of the enamine-like β and δ bridges (*9*, *13*). The isolation of **2** is remarkable in light of its established tendency toward polymerization (*10*, *14*) and the known photooxidizability of vinyl groups in protoporphyrin (*15*). In view of these facts, our yield is doubtless a minimum.



1 2 3

As a control experiment to determine whether methylvinylmaleimide might be formed in the dark under the mildly basic conditions of the reaction, the "photolysis" reaction (including oxygenation) was repeated under the exact conditions as before, except that light was omitted and the solution was kept in the dark. No methylvinylmaleimide could be detected by very careful TLC during a 12-hour "reaction" period or even after 18 hours, and we conclude therefore that **2** is formed only during irradiation. It is worthwhile to note also that in this dark reaction 95 percent of the bilirubin remains intact after 18 hours.

It is pertinent to the clinical aspects of a potentially similar photoreaction in vivo of bilirubin that methylvinylmaleimide does not exhibit the usual enzyme toxicity exhibited by maleimides (*16*). This finding suggests that should **2** be formed in vivo, it would be nontoxic.

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11. The formation of biliverdin has also been noted by others (*5*, *7*); however, we have isolated the pigment as its dimethyl ester [D. A. Lightner and D. C. Crandall, in preparation].
12. Mass spectra were obtained with a CEC 491 MS-21 mass spectrometer at 70 ev; nuclear magnetic resonance spectra were determined on a Varian T-60 instrument.
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14. We have obtained methylvinylmaleimide from controlled chromic acid oxidation of bilirubin. Small samples (~ 15 mg) polymerize on standing overnight in an open container at room temperature; however, we have kept methylvinylmaleimide for more than a year sealed in a glass ampule under nitrogen and stored at 0°C.
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16. Residual adenosine triphosphate levels were tested in isolated lymphocytes. At 0.05 to 0.1 m*M* maleimide reduces the activity to zero, whereas methylvinylmaleimide at the same or even five times this concentration has no effect on the activity [M. A. Whitehouse, G. B. Quistad, D. A. Lightner, *Biochem. Pharmacol.*, in press]. For a review of maleimide toxicities see J. L. Webb, *Enzyme and Metabolic Inhibitors* (Academic Press, New York, 1966), vol. 3, chap. 3.
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