Photo-oxidation of Bilirubin in Hydroxylic Solvents: Propentdyopent Adducts as Major Products

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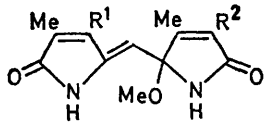
Summary Irradiation (>300 nm) of an oxygen-flushed solution of bilirubin in dilute methanolic ammonia gives a complex mixture including methanol–propentdyopent adducts as major products; one of these, and methylvinylmaleimide have been isolated.

PHOTOTHERAPY is employed in the treatment of neonatal jaundice:[1] although the bilirubin is destroyed the nature of the colourless products is not clear. Recently, the isolation of two propentdyopents from the photo-oxidation of bilirubin in chloroform was reported.[2] These compounds were identified from the pentdyopent test, and on the similarity of their electronic spectra to those reported by von Dobeneck[3] for a series of propentdyopent adducts, and they were formulated not at the propentdyopent but at the dihydroxydipyrromethene (dihydrogen–propentdyopent ad-

duct) oxidation level.[4] No analyses or confirmatory spectroscopic data were reported.

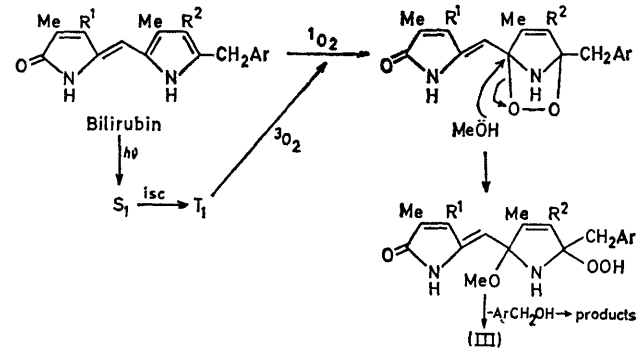
Our results, which refer to short term (<24 h) irradiations, are different. Oxygen was bubbled through a solution of bilirubin ($0{\cdot}68 \times 10^{-3}$M) in 0·2% methanolic ammonia irradiated at 15 °C in a Pyrex vessel with visible light (2×500 W). Electronic spectroscopy showed that bilirubin (λ_{max} 448 nm) rapidly disappeared, that very little biliverdin (λ_{max} *ca.* 650 nm) was produced, but that a new band appeared at *ca.* 280 nm. T.l.c. revealed a complex mixture.



(III) R^1 = -CH=CH$_2$
R^2 = -CH$_2$·CH$_2$·CO$_2$H

A minor product (6%) of high mobility was isolated by column chromatography followed by sublimation[5] and was shown to be methylvinylmaleimide, $M^+ = 137{\cdot}048$ (see also ref. 2). The major products (*ca.* 60%), incompletely resolved on t.l.c., gave a positive pentdyopent test. One of these products was obtained from methanol as colourless microcrystals which decomposed above 175 °C. It is formulated as a methanol–propentdyopent adduct (III) or one of three other positional isomers] on the following evidence: (i) $M^+ = 332{\cdot}137$, the mass spectrum showed the expected loss of methanol at *m/e* 300·111, (ii) the n.m.r. spectrum [220 MHz, $(CD_3)_2SO$] was sharp: τ 0·90 (NH, exchangeable), 1·49 (NH, exchangeable), 3·44 (–CH= vinyl), 4·48 (=CH$_2$), 5·06 (*meso*–CH=, 7·02 (OMe), and 8·10 and 8·18 (Me, Me), (iii) the electronic spectrum [λ_{max} (MeOH) 271 nm (ϵ 12,000)] was comparable with spectra of closely related adducts,[3] and (iv) the compound was identical (mixed t.l.c. in two systems) with a sample prepared by methanolysis of the water–propentdyopent adduct described by von Dobeneck.[6]

The course of the photoreaction is highly dependent on the nature of the solvent. In 0·2% aqueous ammonia a reaction analogous to that described above occurs, the water–propentdyopent adduct being formed among other



SCHEME. *A rationalisation of the formation of colourless methanol–propentdyopent adducts on photo-oxidation of bilirubin in methanol.*

products. In anhydrous freshly-distilled chloroform the formation of biliverdin IXα (mixed t.l.c. of dimethyl ester; two other minor verdins are also detected) is important. It is likely that alternative pathways are available in the photo-oxidation of this complex system. Biliverdin formation may involve radical abstraction at the central "benzylic" methylene bridge (Type I process[7]) while the formation of propentdyopent adducts may be rationalised in terms of the photosensitised generation of singlet oxygen, followed by its cycloaddition to the system and solvolysis as shown in the Scheme. McDonagh[8] has presented evidence which suggests that bilirubin can behave as a photosensitiser in singlet oxygen reactions, and the photo-oxidation of pyrroles in a manner analogous to that shown in the Scheme is well known.[9]

Propentdyopent adducts, in marked contrast to bilirubin, are very water soluble which may well be an important factor in determining the effectiveness of phototherapy.

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