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THE INTERACTION OF BILIVERDIN AND ITS DIMETHYL ESTER WITH SUPEROXIDE ION

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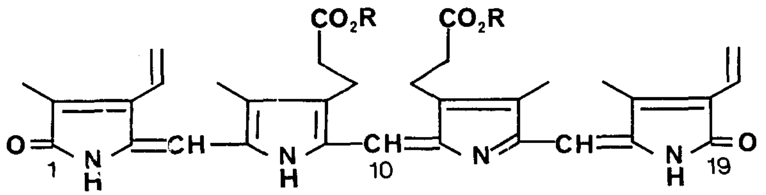
Summary: Bilin-1,19(21H,24H)-diones interact with $O_2^{\overline{\cdot}}$ in DMSO giving rise to adducts showing charge-transfer character. This reaction can be reversed by addition of $O_2^{\overline{\cdot}}$-consuming compounds. The $O_2^{\overline{\cdot}}$-biliverdin dimethyl ester adduct collapses partially to 10-oxobilirubin dimethyl ester when treated with thiourea and 2-mercaptoethanol.

The reactivity of superoxide ion ($O_2^{\overline{\cdot}}$)[1] toward biological compounds has become subject of considerable interest since the discovery that $O_2^{\overline{\cdot}}$ is a respiratory intermediate of aerobic organisms[2]. In this paper we report spectroscopic as well as chemical evidence that $O_2^{\overline{\cdot}}$ interacts with biliverdin (BV; **1a**)[3] and its dimethyl ester (BVDME; **1b**)[3] in DMSO giving rise to a reversible adduct[4]. Biliverdin is the first isolable product of heme catabolism[5].

When an excess of KO_2 in anhydrous DMSO was added to a solution of BVDME[6] in the same solvent at r.t., an instantaneous change of the colour from green to yellow was observed. Changes in UV-visible spectra corresponding to increasing amounts of added KO_2 are shown in Fig. 1. The absorption spectrum resulting from the addition of an excess of KO_2 to BV in DMSO is also reported in Fig. 1. It was observed that the spectrum of the BVDME-KO_2 mixture turns slowly (in 120 min. at r.t.) to that typical of BV in the presence of KO_2. This fact is likely due to an $O_2^{\overline{\cdot}}$-catalysed hydrolysis of the ester groups of BVDME[7]. For this reason, using BVDME as a substrate for studying the specific reaction between $O_2^{\overline{\cdot}}$ and the bilatriene skeleton only experiments sufficiently short to preclude hydrolysis were taken into account.



a : R=H
b : R=Me

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By adding methanol or a few drops of conc. HCl to a DMSO solution of BVDME and KO_2 in excess, the absorption spectrum of the verdin reappears at once in agreement with a quantitative recovery of the starting BVDME[8]. Since superoxide ion disproportionates to dioxygen and hydrogen peroxide in the presence of protic compounds[1], the occurrence of a rapidly shifting equilibrium, such as (1), could be inferred:

$$\underset{\mathbf{1b}}{BVDME + O_2^{\bar{\cdot}}} \rightleftharpoons \underset{\mathbf{2}}{[BVDME....O_2]^{\bar{\cdot}}} \qquad (1)$$

This was confirmed by experiments carried out to calculate equilibrium constants at different temperatures (see Table). Values were found to be consistent with a 1:1 stoichiometry[9] and $\Delta H° \cong -20$ kcal mol^{-1}. Concerning the nature of the adduct **2**, a charge-transfer character can reasonably be assumed on the basis of the following facts: i) BVDME and $O_2^{\bar{\cdot}}$ in aprotic solvents act as an electron acceptor (at C-10 position)[10] and an electron donor respectively; ii) the absorption maxima of **2** in DMSO $[\lambda_{max}$:440 nm($\epsilon_M \sim$ 21,000 when estimated by adding KO_2 in excess to a BVDME solution until constant absorbance) and 745 nm ($\epsilon_M \sim$ 20,000)$]$ are strongly reminiscent of those of the radical anion $BV^{3\bar{\cdot}}$ in H_2O $[\lambda_{max}$:400 nm ($\epsilon_M \sim$ 27,000) and 730 nm ($\epsilon_M \sim$ 17,000)$]$[11].

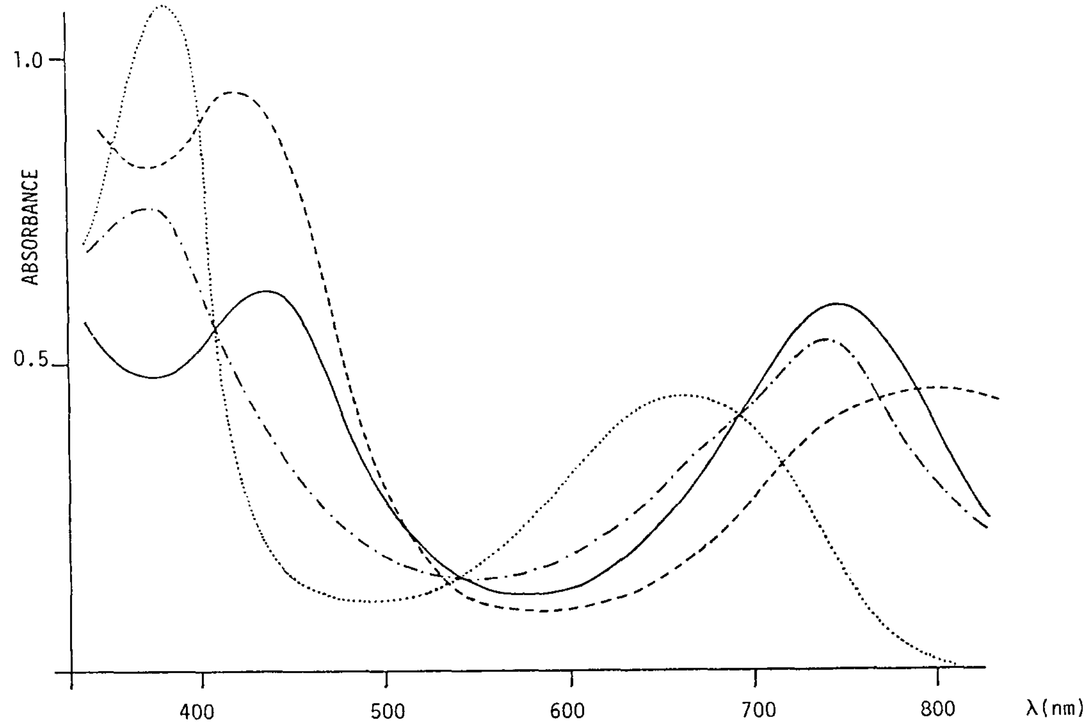


Fig.1 Electronic absorption spectra in DMSO: ...BVDME $3x10^{-5}$M; -·-·-·BVDME $3x10^{-5}$M + KO_2 2.6x10^{-4}M; —— BVDME $3x10^{-5}$M + KO_2 $6x10^{-4}$M; ------BV $2x10^{-5}$M + KO_2 $6x10^{-4}$M.

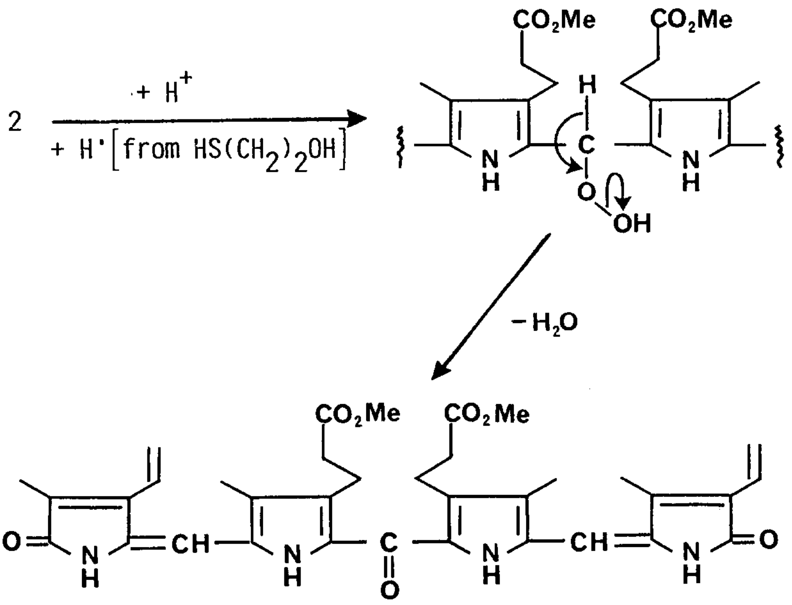
TABLE

Equilibrium Constants for Reaction (1) in DMSO[(a)]

T(°C)	$K \times 10^{-3} (M^{-1})$
28.0	31
37.0	13
42.0	6.6

(a) Absorbance at 745 nm was chosen to monitor the above condition. At fixed $[BVDME]_o$ the concentration $[O_2^{\overline{\cdot}}]_o$ was increased by gradual addition of KO_2 (titrated iodometrically)[12] to reach the condition $[BVDME] = [2]$ for which $K=2/(2[O_2^{\overline{\cdot}}]_o - [BVDME]_o)$. Measurements repeated at several $[BVDME]_o$ gave consistent values for K (which were averaged).

To gain an insight into the structure of **2** several attempts were made to induce it to collapse in a way different from the reverse of reaction (1). The best results were obtained by treating a solution of **2** in DMSO (100 ml, $\sim 10^{-3}$M, from BVDME and KO_2) with, in the order, an excess of thiourea (200 mg) and of 2-mercaptoethanol (0.3 ml). Thiourea was used to destroy H_2O_2[13], which could be formed as a by-product, and $HS(CH_2)_2OH$ served as a donor of hydrogen. The mixture was then treated with phosphate buffer (pH 7.6) and extracted with $CHCl_3$. After washing the organic phase with the above buffer several times, drying and evaporating the solvent under vacuum, a crude greenish material was obtained. It appeared to contain, together with minor verdinoid products, two yellow substances (TLC, Rf=0.2 and 0.5, C_6H_6-$CHCl_3$-MeOH 5:3:1) which were separated by prep. TLC. The more polar yellow compound was shown to be the BVDME-mercaptoethanol adduct by comparison with a sample prepared according to ref. 10. Structure **3**, i.e. 10-oxobilirubin dimethyl ester, was attributed to the second yellow product on the basis of spectral evidence: UV: $\lambda_{max}^{CHCl_3}$ = 408 nm and sh. at 446 nm; MS(FAB): m/z 627(M+1,30%), 329 (100%, likely due to 5-formylpyrromethenone fragments); ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) exhibiting signals consistent with a BVDME-type molecule[3a,6] lacking C-10 proton. **3** was unreactive toward diazo-reagents[10] and gave bilirubin dimethyl ester (**3** with CH_2 as central bridge) by reduction with $NaBH_4$[14]. It appeared to be unstable in solution, thus explaining, at least in part, its very low yields (~5%) after isolation. The formation of 10-oxobilirubin dimethyl ester can be explained as shown in the following Scheme:



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