204. The Fluorescence of Biliverdin Dimethyl Ester[1])

Preliminary Communication

by **Silvia E. Braslavsky, Alfred R. Holzwarth, Harald Lehner,** and **Kurt Schaffner**

Institut für Strahlenchemie im *Max-Planck*-Institut für Kohlenforschung, D-4330 Mülheim a.d. Ruhr

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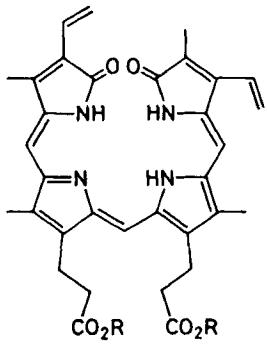
Summary

Freshly prepared solutions of biliverdin dimethyl ester (**2**) in ethanol showed fluorescence maxima at 710 and 770 nm [$\Phi_F = 1.1 \cdot 10^{-4}$ (room temperature) and $5.0 \cdot 10^{-4}$ (77 K)]. The maxima of monoprotonated **2** at 77 K were shifted to 725 and 806 nm and the quantum yield was increased to $2.6 \cdot 10^{-2}$. This acid effect was reversible by neutralization with base. When a neutral solution was kept standing in the dark at room temperature, or when an acidic solution was neutralized by base, an additional fluorescence maximum at 500 nm with a mirror image excitation spectrum with $\lambda_{max} = 470$ nm developed, which disappeared on addition of acid and which is attributed to a chemical change of **2**.

Biliverdin (**1**)[1]) is an intermediate in the heme catabolism of the bile pigment bilirubin[1]) [1], and it appears to be related to the chromophore of phytochrome, a morphogenically active plant chromoprotein [2].



1 R = H

2 R = CH_3

[1]) In accord with IUPAC/IUB recommendations on nomenclature, the names bilirubin and biliverdin represent the compounds with the IX*a* constitution. We thank Professor *R. Bonnett* for this communication prior to publication.

We now report on the fluorescence of biliverdin dimethyl ester (**2**) in ethanol and on the variations of this emission under specific conditions. Our results clarify and enlarge previous findings [3–5] which in part have been contradictory [4] [5]. Furthermore, our investigation has been carried out with a sample of **2** of unequivocally established homogeneity, an aspect which apparently may not have been assessed with sufficient scrutiny in previous spectroscopic studies[2–4]).

Fluorescence in Ethanol. – Degassed solutions of **2** in carefully purified ethanol exhibited fluorescence maxima at 710 and 770 nm when irradiated within 10 min after their preparation *(Fig. 1b)*[5]). The quantum yield was temperature-dependent, ranging from $\Phi_F = 1.1 \cdot 10^{-4}$ at room temperature to $5.0 \cdot 10^{-4}$ at 77 K (λ_{exc} = 377 nm).

When the conditions other than temperature alone were altered, two striking changes occurred selectively. One was an acid-induced red shift of the fluorescence *(Fig. 1a)*, and the other was an aging effect of **2** in solution giving rise to new 'blue fluorescence' and excitation bands *(Fig. 1c)*.

The Acid-induced Fluorescence. – Hydrochloric acid was added to freshly prepared solutions of **2**, $pK_a = 4.3 \pm 0.1$. The fluorescence maxima shifted to 725 and 806 nm, and at 77 K the quantum yield increased to a constant value of $\Phi_F = 2.6 \cdot 10^{-2}$ at [monobasic acid]/[**2**] ≥ 1.5. In analogy to this behaviour the visible band in the absorption spectrum had also undergone the already known [3] [8] bathochromic shift upon acidification.

The 'Blue Fluorescence'. – This additional emission showed a maximum at 500 nm with an excitation band at $\lambda_{max} = 470$ nm. It appeared when a degassed solution of **2** was kept standing in the dark at room temperature. Its development was strongly retarded in the cold (225 K), and it was both accelerated and accompanied by the appearance of still more fluorescence peaks when the solution was exposed to light. While base in a fresh solution did not affect the initial fluorescence of **2**, a blue emission as described above, with the same excitation spectrum, appeared when a previously acidified solution was treated with base in amounts equivalent to acid or in slight excess. Addition of acid to any solution

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[2]) The standard preparation from bilirubin invariably affords a mixture of **2** and the III*a* and XIII*a* structural isomers [6]. We have found that **2** (m.p. 201–203°) and the XIII*a* isomer (m.p. 229–231°) can be separated on a preparative scale by column chromatography, and that the two compounds form solid solutions (with sharp melting points over the entire range of isomeric composition) upon 'fractionate' crystallization.

[3]) The homogeneity of **2** was >99% by high-pressure liquid chromatography. Moreover, the fluorescence remained unchanged when the sample was further recrystallized several times from various solvents. Details on the separation, purity control and structural characterization by high-frequency ^{1}H-NMR. and induced CD. studies will be reported in a full paper by *H. Lehner, S.E. Braslavsky & K. Schaffner.*

[4]) *E.g.*, the absorption of biliverdin XIII*a* dimethyl ester in ethanol differs from that of **2** with regard to both band positions and intensities.

[5]) Fluorescence spectra were measured on a *Spex* fluorolog spectrometer. Quantum yields at 77 K were measured relative to 9,10-diphenylanthracene in ethanol as a standard, assuming $\Phi_F = 1$ (as determined at 77 K in ether/isopentane/ethanol 5:5:2 for this compound [7]).

exhibiting 'blue fluorescence' resulted in the disappearance of this emission and the generation of the acid-induced fluorescence.

If we accept the plausible assumption that the electronic configuration of the emitting lowest excited state of **2** does not change upon protonation, the increase of the red fluorescence yield with the addition of acid would suggest that proton exchange between the N-atoms of the two central rings is one of the important energy wasting steps in **2**, which lower the quantum yield in neutral solution. In the presence of acid, the intramolecular proton exchange is retarded in the evidently

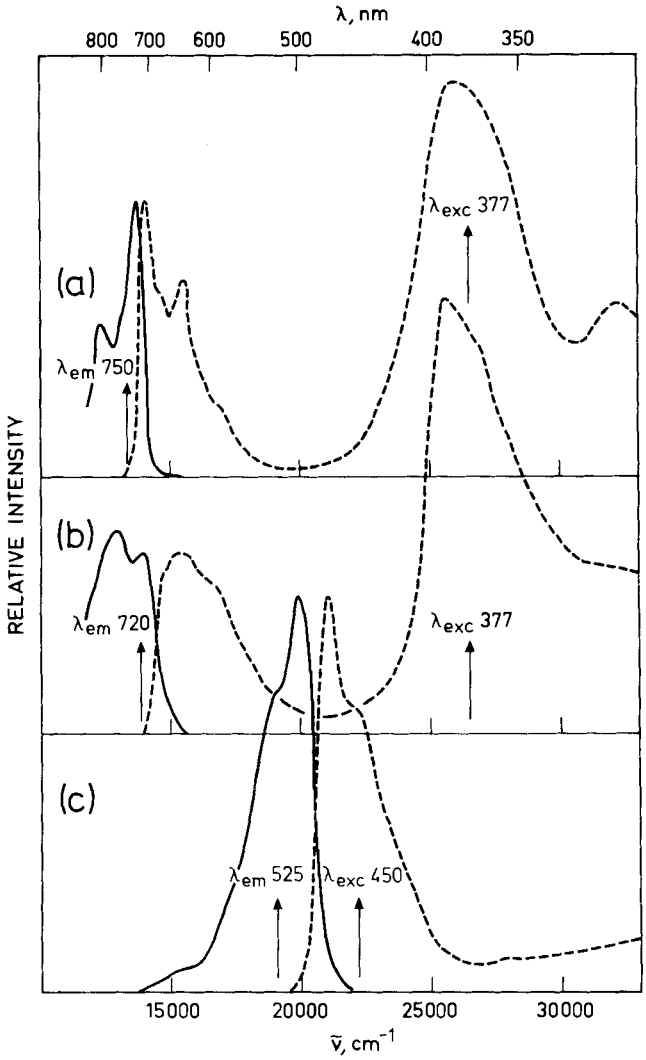

Fig. 1. *Corrected fluorescence* (——) *and excitation* (– – –) *spectra of* **2** ($3.3 \cdot 10^{-5}$M) *at 77 K.* (a) Ethanol + HCl ($8 \cdot 10^{-3}$M) solution (acid-induced fluorescence); (b) fresh ethanol solution; (c) aged ethanol solution ('blue fluorescence').

monoprotonated species and the emission efficiency would consequently increase. A similar phenomenon has been observed with pyrromethenes [9–11].

Furthermore, the 'blue fluorescence' appears to originate from a strongly emitting source of still unknown nature and concentration. Its induction rules out as possible origins an initial impurity, which might have escaped our purity control[3]), or a higher excited state of **2**. Rather, a chemical reaction, *e.g.*, nucleophilic addition of solvent at C(10), appears as a likely cause.

The considerable sensitivity towards acid and the change of the fluorescence of dissolved **2** as a function of time finally may clarify some hitherto contradictory reports on the emission of biliverdin esters [4] [5]. The absorption and emission described for **2** by *Song et al.* [4] are in fact reminiscent of those of a slightly acidic solution ([acid]/[**2**] < 1) in our hands which was kept standing at room temperature for about two hours after preparation and which still emitted 'blue fluorescence'. Furthermore, if we accept a similarity of the emissive properties of **2** and biliverdin IXγ dimethyl ester as proposed [5], the spectrum reported for the purified sample by *Gautron et al.* [5] would correspond to that in either a fresh and slightly acidic or an aged and strongly acidic solution.

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[11] Unpublished results in our laboratory on protonated pyrromethenes.