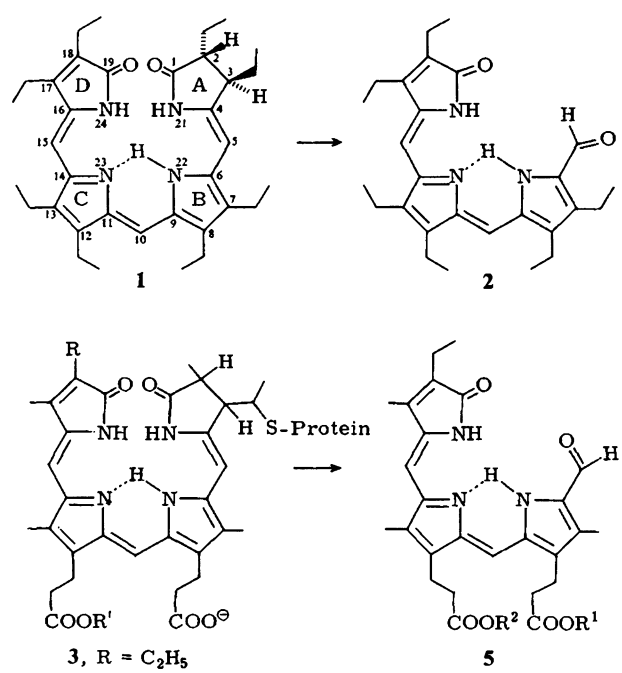
Two Mild, Regioselective Methods of Degrading Biliprotein Chromophores**

By *Werner Kufer, Corinna Krauss,* and *Hugo Scheer**

Biliproteins—receptor proteins in photosynthesis and photomorphogenesis—consist of bile pigments, which are covalently bound *via* a thioether bond at ring A to a protein; a second bond is disputed (see Discussion in [2]). We describe here two mild degradation precedures for free and protein-bound bile pigments. These methods, unlike chromic acid degradation[5], result in the retention of substantial information on the substituents at the α-pyrrolic and methine positions.



1 2 3, R = C_2H_5 5

Scheme I. (Part). 3: R' = H or protein, 5a: $R^1 = R^2 = H$; 5d: R^1, R^2 = H, protein.

[*] Prof. Dr. H. Scheer, Dr. W. Kufer, Dr. C. Krauss
Botanisches Institut der Universität
Menzinger Strasse 67, D-8000 München (Germany)

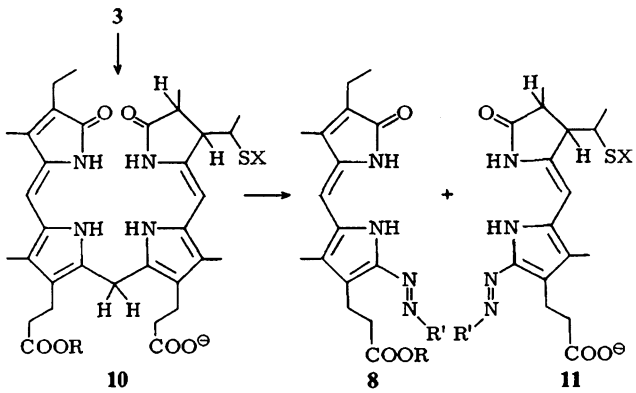
[**] Studies of Plant Bile Pigments, Part 10. This work was supported by the Deutsche Forschungsgemeinschaft. Prof. C. T. Soeder, Jülich, is thanked for a sample of *Spirulina platensis*. Part 9: C. Krauss, H. Scheer, *Photochem. Photobiol. 34* (1981) 385.

  0570-0833/82/0606-0446 $ 02.50/0

In the first method (Scheme 1), regioselective cleavage of the C-5 methine bridge occurs. Under mildly basic conditions the zinc complex of A-dihydrobilindione **1** loses the hydrogenated ring A to form **2**[6]. The biliprotein phycocyanin **3** is cleaved in an analogous manner.

3, obtained from *Spirulina platensis* (0.05 mM) was unfolded with urea (8 M). Addition of zinc acetate (0.05 mM) and titration with NaOH to pH 9.5 yielded the characteristic intermediate[6] ($\lambda_{max} = 720$ nm), which afforded the zinc complex of **5a** and/or **5d** within 8 min. Using **3**, which had been degraded by trypsin, the free tripyrinone **5a** could, in addition, be isolated.

Since trypsin is not only able to cleave proteins but also ester or amide bonds to the chromophore, this result would be evidence for the existence of one of these bonds to rings B, C, or D, whereby, however, possible artifacts (partial hydrolysis, complex binding[10] *via* Zn^{++}) make an unequivocal statement difficult.



Scheme 2. (Part). R' = 2-ethoxycarbonylphenyl, X = protein; **8a**: R = H; **10**: R = H or protein.

In the second method (Scheme 2) regioselective cleavage between rings B and C occurs. The denatured phycocyanin **3** was reduced with $NaBH_4$ to afford the rubin **10**[13], which after gel filtration was treated with a diazonium salt *e.g.* diazotized ethyl anthranilate in 5- to 10-fold excess[11]. By means of gel filtration or extraction with organic solvents the product mixture can be separated into two fractions, of which the low molecular and less polar ($\lambda_{max} = 522$ nm) is identical with authentic **8a** (rings C and D of **3**).

The high molecular and more polar fraction ($\lambda_{max} = 480$ nm) should contain **11**.

A preliminary quantitative analysis of the reaction mixture indicated that approximately 14% of the extinction at 520 nm in the crude mixture disappears after extraction with organic solvents or gel filtration. Consequently, apparently at least a part of the chromophore does not contain a protein binding site at rings C or D.

Received: February 27, 1981,
revised: April 21, 1982 [Z 107 IE]
German version: *Angew. Chem. 94* (1982) 455
The complete manuscript of this communication appears in:
Angew. Chem. Suppl. 1982, 1050-1060

[2] H. Scheer, *Angew. Chem. 93* (1981) 230; *Angew. Chem. Int. Ed. Engl. 20* (1981) 241.
[5] W. Rüdiger, *Hoppe-Seyler's Z. Physiol. Chem. 350* (1969) 1291.
[6] H. Scheer, U. Linsenmeier, C. Krauss, *Hoppe-Seyler's Z. Physiol. Chem. 358* (1977) 185.
[10] H. Plieninger, K. Stumpf, *Chem. Ber. 103* (1970) 2562.
[11] P. Ehrlich, *Zbl. Klin. Med. 45* (1883) 721; K. P. M. Heirwegh, J. Fevery, J. A. T. P. Meuwissen, F. Compernolle, V. Desment, F. P. van Roy, *Meth. Biochem. Anal. 22* (1974) 205.
[13] W. Kufer, H. Scheer, *Z. Naturforsch. C37* (1982) 179.

  0570-0833/82/0606-0447 $ 02.50/0