Two Mild, Regioselective Methods of Degrading Biliprotein Chromophores**

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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 procedures for free and protein-bound bile pigments. These methods, unlike chromic acid degradation[1], result in the retention of substantial information on the substituents at the α-pyrrolic and methine positions.

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

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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. The biliprotein phycoerythrin 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 10 ($\lambda_{\text{max}} = 720$ 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 via Zn$^{+2}$) make an unequivocal statement difficult.

In the second method (Scheme 2) regioselective cleavage between rings B and C occurs. The denatured phycoerythrin 3 was reduced with NaBH$_4$ to afford the rubin 10, which after gel filtration was treated with a diazonium salt e.g. diazotized ethyl anthranilate in 5- to 10-fold excess. 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 ($\lambda_{\text{max}} = 522$ nm) is identical with authentic 8a (rings C and D of 3).

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