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The Diels-Alder Adduct of Phycocyanobilin Dimethyl Ester and 4-Phenyl-1,2,4-triazolin-3,5-dione: A Model Intermediate for Chromatic Adaption of Biliprotein Chromophores?

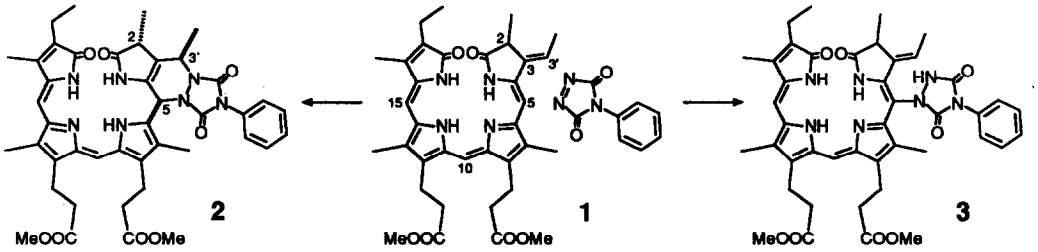
Thomas Koini, Hugo Stumpe, Rainer Gagstädter, and Karl Grubmayr*

Institut für Chemie, Johannes Kepler Universität Linz, Altenbergerstraße 69, A-4040 Linz, Austria

Abstract: Addition of 4-phenyl-1,2,4-triazolin-3,5-dione (PTAD) to the outer ring diene of phycocyanobilin dimethyl ester **1** results in the formation of the [4+2] cycloadduct **2**, which is the first representative of 2,5-dihydrobilindiones isolated so far. Thermal rearrangement of **2** yields the blue colored 2,3-dihydrobilindiones **4** and **5**, whereas acid catalyzed tautomerization results in the formation of the three diastereomeric, red colored 4,5-dihydrobilindiones **6**, **7**, and **8**.

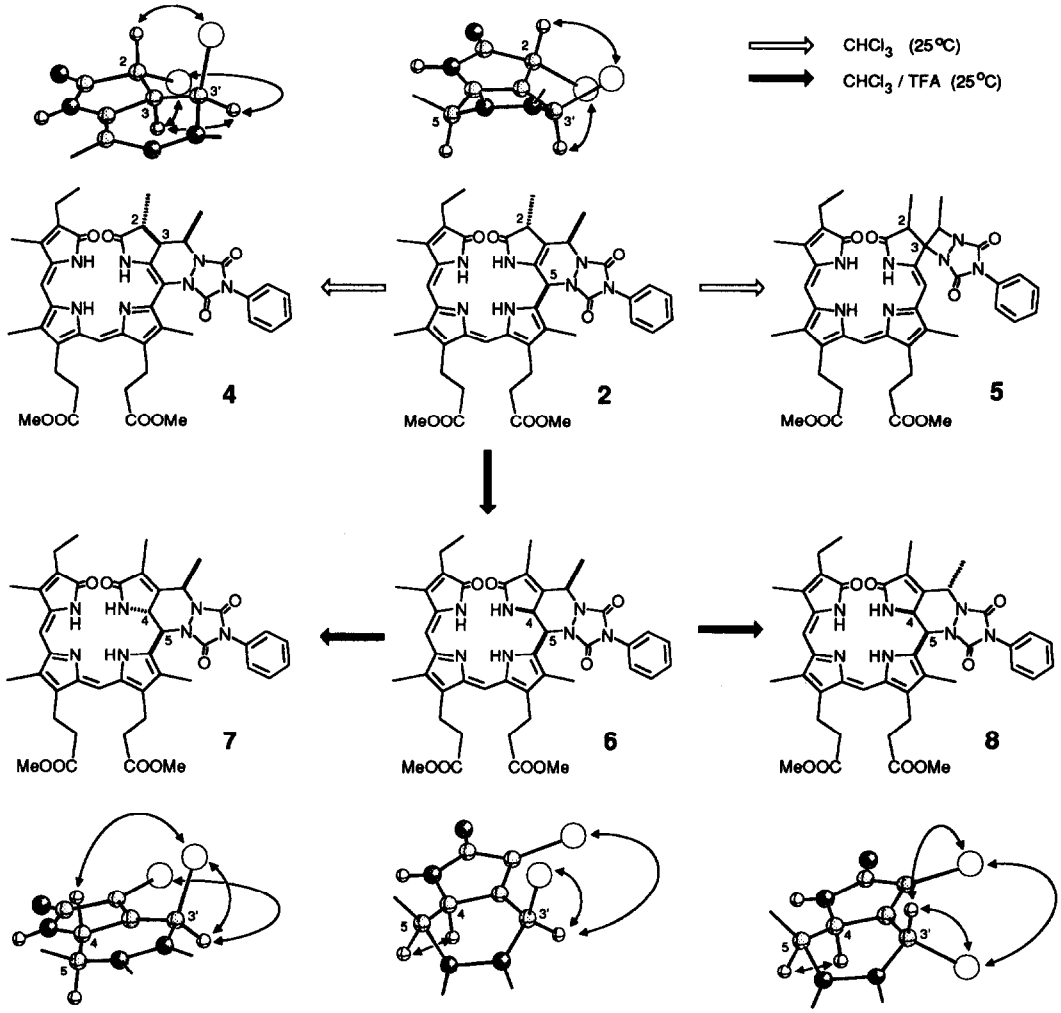
In the preceding communication[1] we presented the nucleophilic addition of thiols to racemic phycocyanobilin dimethyl ester **1** as model reaction for biliprotein biosynthesis. In this letter we report on the reactivity of **1** towards the electrophilic 4-phenyl-1,2,4-triazolin-3,5-dione (PTAD) showing the capability of **1** to act as a diene in a [4+2] cycloaddition. Furthermore, the reactivity of the cycloadduct is discussed in context with biomimetic model reactions for PXB chromophore biosynthesis in phycoerythrocyanin.

Reaction of PTAD (17.1 mg; 98 µmol) and **1** (50.0 mg; 81 µmol) in chloroform (100 ml) at -70 °C was complete within two minutes yielding the products **2** and **3**, which could be separated immediately by chromatography on silica (chloroform / acetone / methanol = 100 / 5 / 1; **2**[2] (25 mg; 39%), **3** (25 mg; 39%); R_f: **2** > **3**). Compound **2** is the *Diels-Alder* adduct of PTAD to **1**, whereas **3** is the *Michael* adduct of **1** to PTAD. **3** is not a precursor of **2**, or vice versa, because both compounds show different reactions on further treatment as



described in the following examples. Accordingly, carbon atom 5 appears to be the most nucleophilic carbon atom of the chromophore. In principle, its reactivity can be traced back to the chemistry of the enamidic partial structure of 2,3-dihydrodipyrrinones[3]. This is also in good accordance with the chemistry of PTAD and structurally related nitrogen heterocycles, like indolo-2,3-quinodimethanes[4]. However, differences arise in comparison with the oxidative dimerization of **1** by singlet oxygen[5], where carbon atom 4 is attacked by the electrophile.

Relative stereochemistry of cycloadduct **2** could be assigned mainly by N*O*E-differences (CH_3-(C2) ↔ H-(C3'); CH_3-(C3') ↔ H-(C2)) reflecting kinetic control of the cycloaddition. Approach of PTAD to the less sterically congested face of the outer ring diene moiety of **1**, opposite to the (C2)-methyl group, becomes



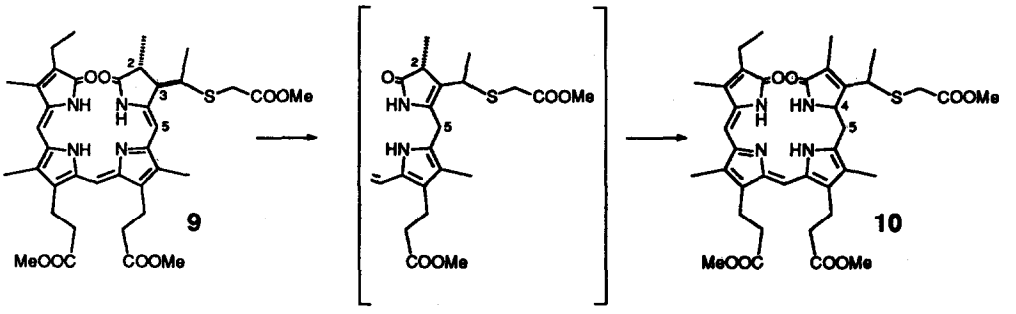
evident. To our knowledge cycloadduct **2** is the first representative of 2,5-dihydrobilindiones, which has been isolated so far. Normally **2** is a rather unstable compound, sensitive to temperature, acids, and oxygen. Stabilization by rearrangement or tautomerization forming 2,3- or 4,5-dihydrobilindiones is preferred.

Thermal rearrangement of **2** (5.0 mg; 6.3 μmol) could be achieved even at room temperature in chloroform (5 ml) within 4 days yielding the 2,3-dihydrobilindiones **4** and **5**, which were separated by thin layer chromatography (silica; chloroform/acetone/methanol = 100/5/3; **4**[6] (2.6 mg; 52%), **5** (1.5 mg; 30%); R_f: **4** < **5**). Both compounds can be assumed to be formed by formal [1,3]-shift reactions. In the case of **4** a *suprafacial* shift seems reasonable due to the assignment of the relative configuration by NOE-difference measurements (CH_3-(C2) ↔H-(C3) ↔H-(C3')). In the case of **5** we were not able to determine relative stereochemistry unequivocally because of the small chemical shift difference of the (C2)- and (C3')-methyl dublets. In addition, quantitative formation of **4** could be achieved by thermal cyclization of **3** (5.0 mg; 6.3 μmol) in chloroform (0.5 ml) at 50 °C within two hours.

Acid catalyzed tautomerization of **2** (10.0 mg; 12.6 μmol) in chloroform (4 ml) and trifluoroacetic acid (40 μl) at 25 °C yielded a mixture of the three diastereomeric 4,5-dihydrobilindiones **6**, **7**, and **8**, which could be separated by thin layer chromatography (silica; chloroform/acetone/methanol = 100/5/2; **6** (1.5 mg; 15%),

7[7] (5.6 mg; 56%), **8** (1.5 mg; 15%); R_f: **7 > 8 > 6**). Compound **6** is the kinetically favored tautomer, which is easily transformed to the thermodynamically favored tautomers **7** and **8** by steric strain release. *Cis* arrangements of H-(C5) with H-(C4) and H-(C3') can be changed to *trans* geometry by de- and reprotonation of the chemically similar carbon atoms C-4 and C-3'.

Chemical reactivity of **2** suggests that 2,5-dihydrobilindiones may act as intermediates during the tautomerization reaction of 2,3- to 4,5-dihydrobilindiones. Concerning the biosynthesis of the PXB chromophore in phycoerythrocyanin[8] we were able to run such a reaction on preparative scale using model compounds. After treatment of 2,3-dihydrobilindiones **9**[1] (25 mg; 35 μmol (*l,u*) : (*l,l*) = 1 : 1.4) dissolved in chloroform (10 ml) and triflouroacetic acid (40 μl) at 50 °C for 10 days under argon we isolated 4,5-dihydrobilindiones **10** (8 mg; 32%; *l*:*u* = 1:1). This reaction is supposed to consist of two consecutive tautomerization steps. The first one



starts with protonation at C-5, forming N-acyliminium-ions, and ends with deprotonation of C-3 giving 2,5-dihydrobilindiones. These tautomers cannot be detected in acidic medium. Like **2**, they rearrange immediately during the second tautomerization step by proton loss at C-2 and proton uptake at C-4. A chemical reaction mechanism like this is in good accordance with the deuterium exchange of H-5 observed in bilipeptides of phycocyanin[9] and parallels the tautomerization chemistry of structurally related dihydrodipyrrinones[10].

Together with recent crystal structure analyses of biliproteins[11] these model studies may offer a simple explanation for the biosynthesis of red colored phycobiliviolin chromophores from blue colored phycocyanobilin precursors: covalent attachment of pycocyanobilin to the apoprotein by adding the α-84 cysteinic thiol is followed by acid catalyzed tautomerization to the phycobiliviolin chromophore PXB with distinct change of color but without significant change of geometry. In conclusion, a strategy of color adaption in biliproteins seems to be based on simple chemical transformations of their chromophores causing distinct change in light absorption, whereas fine tuning of their light absorptions seems to be controlled by modifications to their proteins[12].

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References and Notes:

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2. **2**: ^{1}H-NMR (360 MHz; $CDCl_3$): δ = 1.08 (t, 3H, J = 7.6 Hz, CH_3-(C18')); 1.39 (d, 3H, J = 7.7 Hz, CH_3-(C2)); 1.54 (d, 3H, J = 6.5 Hz, CH_3-(C3')); 2.04, 2.06, 2.07 (3s, 3x3H, CH_3-(C7),CH_3-(C13),CH_3-(C17)); 2.34 (q, 2H, J = 7.6 Hz, CH_2-(C18)); 2.53 (triplet like, 4H, CH_2-(C8') + CH_2-(C12')); 2.87 - 2.95 (m, 4H, CH_2-(C8) + CH_2-(C12)); 3.25 (dq, 1H, J (2,5) = 2.4 Hz, J (2,2') = 7.7 Hz, H-(C2)); 3.65, 3.66 (2s, 6H, CH_3O-(C8'") + CH_3O-(C12'")); 4.81 (dq, 1H, J (3',5) = 2.4 Hz, J (3',3") = 6.5 Hz, H-(C3')); 5.76 (dd, 1H, J (2,5) = 2.4 Hz, J (3',5) = 2.4 Hz, H-(C5)); 5.85 (s, 1H,

H-(C15); 6.83 (s, 1H, H-(C10)), 6.91 (s, H-N, 1H), 7.28 - 7.48 (m, 5H, phenyl). UV-Vis ($CHCl_3$): nm (ε) = 324 (33700); 546 (21700).

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6. **4**: ^{1}H-NMR (360 MHz; $CDCl_3$): δ = 1.08 (t, 3H, J = 7.4 Hz, CH_3-(C18')); 1.40 (d, 3H, J = 6.5 Hz, CH_3-(C3'); 1.42 (d, 3H, J = 7.1 Hz, CH_3-(C2)); 2.05, 2.08, 2.10 (3s, 3x3H, CH_3-(C7),CH_3-(C13),CH_3-(C17)); 2.32 (q, 2H, J = 7.4 Hz, CH_2-(C18)); 2.48 (dq, 1H, J (2,3) = 8.3 Hz, J (2,2') = 7.1 Hz, H-(C2)); 2.55 (triplet like, 4H, CH_2-(C8') + CH_2-(C12')); 2.89 - 2.96 (m, 4H, CH_2-(C8) + CH_2-(C12)); 3.67, 3.68 (2s, 6H, CH_3O-(C8''') + CH_3O-(C12''')); 3.73 (dd, 1H, J (2,3) = 8.3 Hz, J (3,3) = 5.9 Hz, H-(C3)); 5.08 (dq, 1H, J (3,3') = 5.9 Hz, J (3',3") = 6.5 Hz, H-(C3')); 5.88 (s, 1H, H-(C15); 6.83 (s, 1H, H-(C10)), 7.35 - 7.52 (m, 5H, phenyl). UV-Vis ($CHCl_3$): nm (ε) = 270 (15800); 330 (30000); 546 (10460); 612 (13700).
7. **7**: ^{1}H-NMR (360 MHz; $CDCl_3$): δ = 0.97 (t, 3H, J = 7.6 Hz, CH_3-(C18')); 1.42 (d, 3H, J = 6.7 Hz, CH_3-(C3'); 1.8 (s br, 3H, CH_3-(C2)); 1.98, 2.00, 2.01 (3s, 3x3H, CH_3-(C7),CH_3-(C13),CH_3-(C17)); 2.25 (q, 2H, J = 7.6 Hz, CH_2-(C18)); 2.48 (triplet like, 4H, CH_2-(C8') + CH_2-(C12')); 2.82 - 2.92 (m, 4H, CH_2-(C8) + CH_2-(C12)); 3.59, 3.60 (2s, 6H, CH_3O-(C8''') + CH_3O-(C12''')); 4.19 (d, 1H, J (4,5) = 9.8 Hz, H-(C5)); 4.60 (d br, 1H, J (4,5) = 9.8 Hz, H-(C4)); 5.35 (q, 1H, J = 6.7 Hz, H-(C3')), 5.77 (s br, 1H, H-(N21)), 5.80 (s, 1H, H-(C15); 6.82 (s, 1H, H-(C10)), 7.21 - 7.45 (m, 5H, phenyl). UV-Vis ($CHCl_3$): nm (ε) = 324 (34900); 548 (24000).
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