CHIMIA 42 (1988) Nr. 9 (September)

Bilindionostilbenoparacyclophanes Mimic the Spectroscopic Properties of Photoreceptors for Bacterial Oxygenic Photosynthesis

Khaled Abou-Hadeed, Petr Nesvadba, and Albert Gossauer*

Bilindionostilbenoparacyclophanes are a new type of photodynamic molecules capable of stabilizing, at convenience, helical-shaped or «stretched» conformations of bile pigment chromophores without changing the structure of the latter. – Why do «native» phycobiliproteins intensively fluoresce, but they do not after denaturation? Which is the reason for the enhanced absorption of visible light of biliprotein chromophores when compared with free bile pigment molecules? These and other related questions have to be answered by the study of the spectroscopic properties of bilindionostilbenoparacyclophanes, particularly of their E-isomers, in which the bile pigment chromophore is constrained in «stretched» conformations of adjustable strain, otherwise not accessible in model systems for biliproteins so far investigated. – Our present results prove, for the first time experimentally, the intimate relationship between the conformation of biliprotein chromophores and their light absorption. Until now, however, the origin of the fluorescence of native phycobiliproteins could not be elucidated. Most likely, the bile pigment chromophores are immobilized, in a particular conformation, within the apoprotein framework. In order to verify this hypothesis more sophisticated models are required, whose synthesis is a target of current work on this area.

Albert Gossauer: Born 1936 in Zürich. Studied chemistry at the University of Madrid, where he obtained his diploma in 1960 and his Ph. D. in 1962. Research collaborator at the Max-Planck Institute for Spectroscopy in Göttingen (1963–1964). From 1965 to 1978 at the Organic Chemistry Institute of the Technical University of Braunschweig (FRG), where he received the venia legendi for Organic Chemistry in 1974. In 1976 sabbatical at Yale University (New Haven, CT, USA). Assistant Professor (1977) and Associate Professor (1978) at the Technical University of Braunschweig. From 1979 to 1982 Full Professor at the Technical University in West-Berlin. Since September 1982 Full Professor and director of the Institute for Organic Chemistry of the University of Fribourg (Switzerland). – Research fields: preparative organic hetercyclic chemistry, bioorganic chemistry of plant photoreceptors (biliproteins, chlorophylls) and corrinoids.

Khaled Abou-Hadeed: Born 1951 in Cairo. Studied chemistry at the University of Cairo, where he obtained his M.Sc. in 1978. In 1985 Ph.D. at the University of Konstanz (FRG) under the guidance of Prof. Wolfgang Pfleiderer. Research assistant at the Egyptian Universities of Minia (1975–1978) and Suez Canal (1979–1980). Since 1985 Lecturer at the University of Suez Canal (Ismailia). Postdoctoral stays at the University of Konstanz, with W. Pfleiderer (1986), and since October 1986 at the University of Fribourg, with A. Gossauer.

Petr Nesvadba: Born 1954 in Tanwald (Czechoslovakia). Studied chemistry at the Institute of Chemical Technology at Prague, where he obtained his diploma in 1978. From 1979 to 1981 research assistant at the same Institute and in 1982, at the Institute of Molecular Biology of the Czech Academy of Sciences at Prague. From 1983 to 1986 he worked on his Ph.D. thesis at the University of Fribourg under the guidance of Prof. Albert Gossauer. Since 1987 he is employed by the Ciba-Geigy AG at Fribourg-Marly. Vigener Prize 1987 of the University of Fribourg.

1. The Role of Phycobiliproteins in Nature

The progressive incorporation of molecular oxygen, from biological origin, into the initially reducing earth atmosphere changed basically the environmental conditions under which already existing microorganisms further evoluted, and new living forms (e.g. the eukaryotes) appeared. The origin of the oldest so far known organisms capable of carrying out oxygenic photosynthesis – the cyanobacteria – traces back to about 3000 millions years[1]. Cyanobacteria differ from other photoautotrophic prokaryotes (i.e. green and purple bacteria) in containing chlorophyll a (instead of bacteriochlorophyll a) as photosynthesizing pigment and in utilizing two photosystems (I and II) in the photosynthetic process, which enable them, like green plants and algae, to oxidize water to molecular oxygen. In contrast to green plants, however, cyanobacteria do not contain chlorophyll b as an accessory photosynthesizing pigment but a system of photoreceptors – denominated collectively *phycobiliproteins* – which is also found in all red and some cryptomonad algae. On the basis of their coloration, phycobiliproteins are differentiated into two main groups: *phycoerythrins* are clear red by transmitted light and emit a brilliant orange-yellow fluorescence; *phycocyanins* and *allophycocyanins* are blue with a strong red fluorescence. *Phycoerythrocyanin* (which sometimes is considered erroneously as a synonym of phycoerythrin) is a phycobiliprotein whose occurrence is limited to certain genera of filamentous cyanobacteria[2]. The α subunit of this biliprotein (vide infra) carries a bile pigment chromophore of unknown structure.

Most species of cyanobacteria as well as red and cryptomonad algae contain both a phycoerythrin and one or more phycocyanins, although a single biliprotein usually predominates thus giving rise to the colour of the particular species. Depending on their origin, both phycoerythrins and phycocyanins are differentiated by capital letters set as prefixes, thus C, R, and B stand for Cyanobacteria, Rhodophyta, and Bangiophyceae (a subclass of the lower red algae), respectively. However, such a correspondence with the taxonomic pattern is now known to be only partly fulfilled.

* *Correspondence:* Prof. Dr. A. Gossauer
Institut für Organische Chemie
der Universität Freiburg i. Ue.
Route du Musée, Pérolles
CH-1700 Fribourg

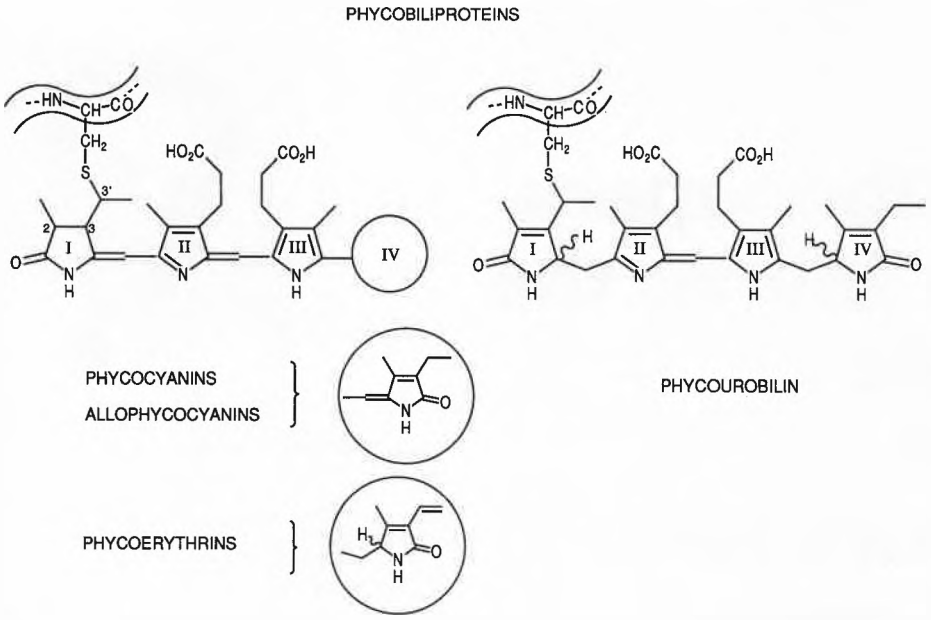
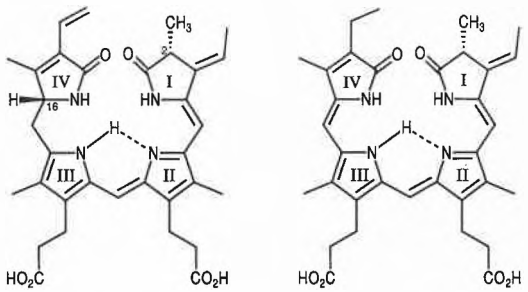


Fig. 1. Structural relationship between the different types of phycobiliprotein chromophores.



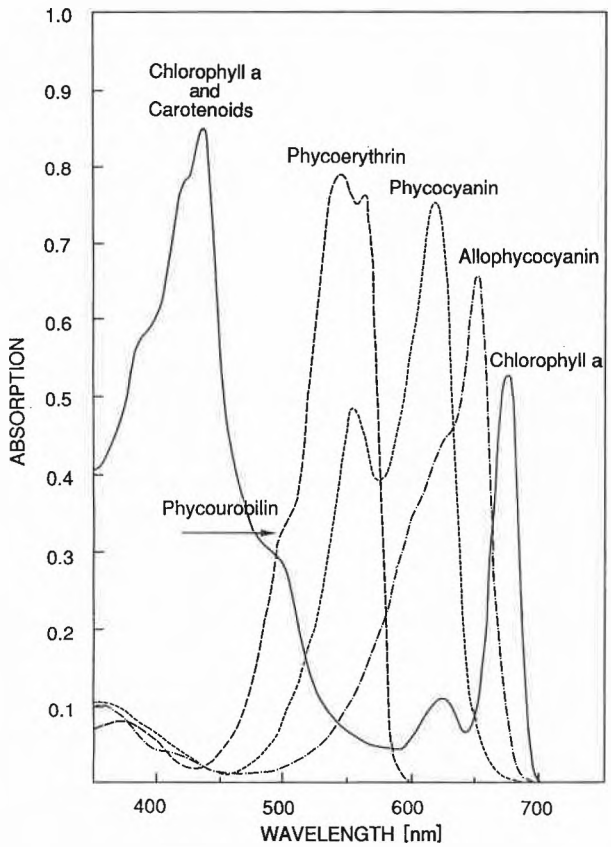


Fig. 2. UV/VIS absorption of phycobiliproteins, purified from phycobilisomes, superposed on the UV/VIS spectrum of chlorophyll a (after [18]).

The prosthetic group of the phycobiliproteins belongs to the same class of compounds as the bile pigments, i.e. they are bilindione derivatives (cf. Fig. 1). The chromophores which are covalently bound to the apoprotein via ring I can be released by boiling methanol, yielding two different types of bile pigments (termed *phycobilins*), namely *phycoerythrobilin* (PEB: **1**), from phycoerythrin, and *phycocyanobilin* (PCB: **2**) from phycocyanin and allophycocyanin. Phycocyanin and allophycocyanin differ only in the apoprotein moiety.

Besides PEB, phycoerythrins contain a third type of chromophore, named *phycourobilin* (PUB), which is not released by refluxing in neutral methanol. Its structure has been elucidated by high-resolution ^{1}H-NMR analysis of oligopeptide-bound derivatives obtained by enzymatic degradation of R-phycoerythrin[3]. In phycoerythrins, some PEB and PUB chromophores are attached to the apoprotein through two thioether bonds to rings I and IV[3].

The structures of PCB and PEB have been elucidated by means of spectroscopic analysis[4–7] and degradation methods[8,9], and confirmed by total syntheses[10–12]. The absolute configuration of PEB has been determined by synthesis[13] and recently corroborated by comparison with an urobilinoid model compound prepared from an optical active precursor whose absolute configuration was assigned by X-ray diffraction analysis[14]. The structures of the protein-bound chromophores of C-phycocyanin[15], B-phycoerythrin[16], and phycoerythrocyanin[17] have been investigated by high-field ^{1}H-NMR spectroscopy.

Phycobiliproteins play an important physiological role in bacterial and algal photosynthesis as photosensitizers of chlorophyll a in photosystem II. Because their light absorption range complements that of chlorophyll, cyanobacteria and red algae become able to absorb light of wavelengths which are not absorbed by chlorophyll (cf. Fig. 2). Thus, some red algae are able to grow up to 180 m depth in sea water, a property which possibly played a role during the evolution of these organisms before the ozone content in the earth stratosphere became sufficient to render possible live outside the oceans.

2. Supramolecular Structure of the Biliproteins

In the living organisms, the phycobiliproteins are organized in supramolecular assemblies – so-called *phycobilisomes* – which are attached to the outer surface of the thylakoid membrane which contains the chlorophyll-binding proteins and the reaction centre of the photosynthetic apparatus. In cyanobacteria, the free thylakoid membrane is situated near the periphery of the cytoplasm; in red algae, like in green plants, it is a component of the chloroplasts which actually seem to be adapted organelles descending from a cyanobacterium ancestor.

Depending on the species investigated, intact phycobilisomes, when observed by transmission electron microscopy, appear as bundle-shaped, hemidiscoidal, hemiellipsoidal, or block-shaped structures of 30–40 nm along the base (cf. Fig. 3a). Probably, the most common types are the hemidiscoidal and the hemiellipsoidal (viz. hemispherical-oblate) phycobilisomes, whereby the latter seems to be restricted to the red algae.

The development of improved isolation methods, using detergents for liberation of the phycobilisomes and high ionic strength buffers for their stabilization, combined with preparative ultracentrifugation techniques and electron microscopy analysis of negatively stained samples have furnished a more precise insight in the structure of the phycobilisomes. On the basis of these studies, a model of the biliprotein distribution in hemidiscoidal phycobilisomes has been proposed, which combines the aspects of fine structure and energy transfer[20–22]. Thus, hemidiscoidal phycobilisomes contain two major domaines of substructure: a «core» region consisting of three cyclindrical elements and «rods» or stacked discs, five to six of which radiate from the core in a hemidiscoidal arrangement (cf. Fig. 3b). In the phycobilisomes, the different phycobiliproteins are arranged following the frequencies of their visible absorption maximum (cf. Fig. 2). Thus, phycoerythrin is located at the tips of the rods, phycocyanin in the middle, and allophycocyanin resides in the core. The absorption maxima moves to longer wavelengths following the spacial order. Hence, excitation energy is transmitted down-hill from tips to core and, finally, to chlorophyll a, consistent with the energy transfer sequence established by spectroscopic studies[25].

In certain species, the phycobilisomes constitute up to 50% of the total protein content of the cell. The major portion of the protein content of phycobilisomes is represented by the phycobiliproteins: phycocyanin (75%), allophycocyanin (12%), and phycoerythrin. In addition, however, there are 10–15% colorless «linker» polypeptides which function is to mediate the ordered assembly of the biliproteins into multimeric complexes.

The most prominent building elements of the phycobilisomes are single discs, 10–12 nm in diameter and 6 nm high. In edge view, the discs have a central line dividing them into two layers, each 3 nm thick. The central line is also frequently seen in the discs comprising the «arms» of the phycobilisomes. An accumulation of stain is also visible at the centre of some subunits, suggesting that the discs have a ring-like structure.

As a morphological substructure, each disc is composed of oligomeric phycobiliproteins. Thus, at pH 5.3, C-phycocyanin from the thermophilic cyanobacterium *Mastigocladus laminosus* is a hexamer ($[\alpha\beta]_6$) of molecular weight 250000 dalton,

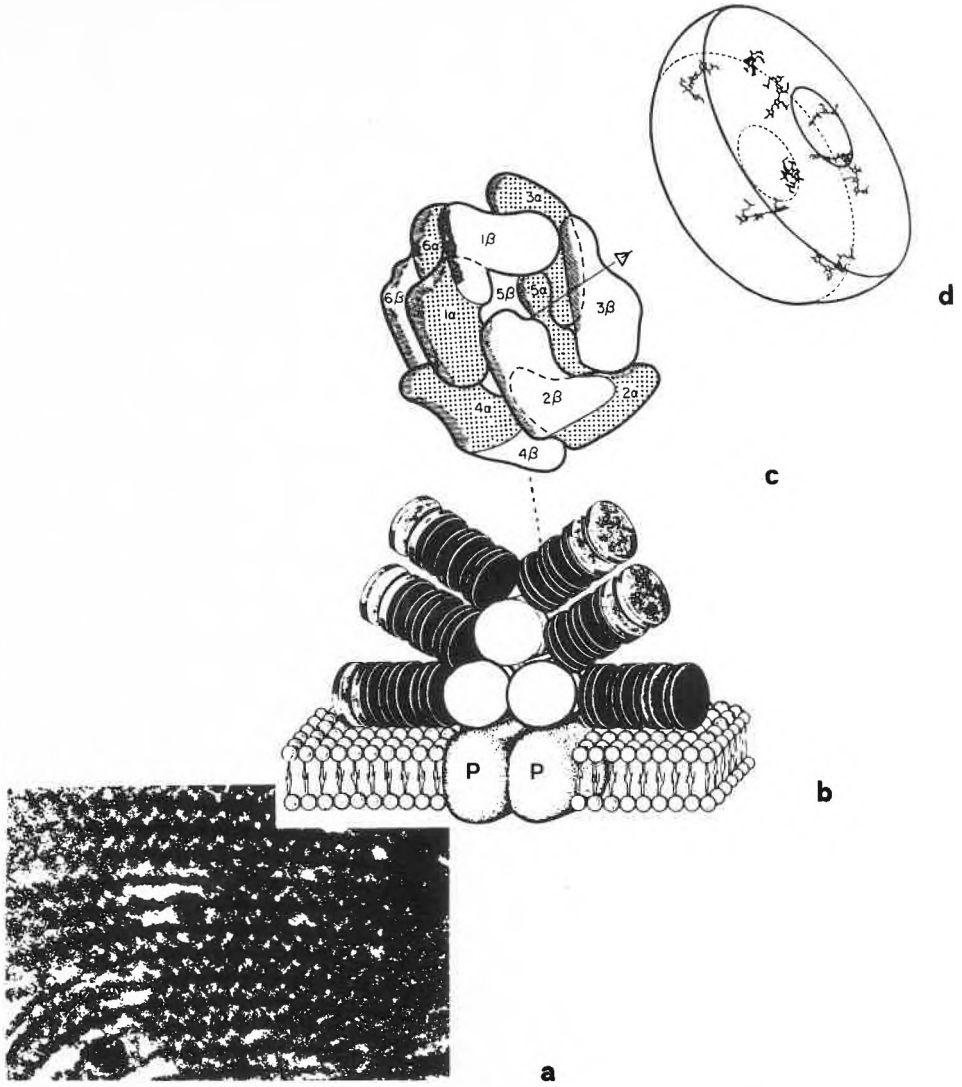


Fig. 3. Supramolecular structure of the phycobilisomes: a) Under the electron microscope, the phycobilisomes of the cyanobacterium Microcoleus vaginatus appear as small granules located in the stroma side of the photosynthetic lamellae (after [19]). – b) Schematic model of the supramolecular structure of the hemidiscoidal phycobilisome of the cyanobacterium Mastigocladus laminosus (after [20–22]); P = protein inserts in the thylakoid membrane containing linker polypeptides, chlorophyll a binding proteins, the reaction centre of photosynthesis, and the oxygen evolving complex. – c) Schematic representation of the C-phycocyanin hexamer from the cyanobacterium Agmenellum quadruplicatum, as determined by crystal structure analysis (after [24]); the 3fold symmetry axis of the complex is indicated. – d) Chromophore arrangement in the C-phycocyanin trimer from Mastigocladus laminosus (after [23]).

whose monomer ($\alpha\beta$) is made up of two distinctive subunits (termed α and β) differing both in the polypeptide chain, which contains 162 and 172 amino acids respectively, and in the number of covalently bound bile pigment chromophores (one to the α and two to the β subunit). The amino acid sequences of both α and β subunits of phycocyanin[26, 27] and allophycocyanin[28] are known. In C-phycocyanin, the bile pigment chromophores are linked covalently to the sulfur atom of cystein at α-84, β-82, and β-153. The monomer of R-phycoerythrin contains three subunits (α, β, and γ)[29].

Recently, the structure of C-phycocyanin from the cyanobacteria *Mastigocladus laminosus*[23] and *Agmenellum quadruplicatum*[24] has been determined at 300 and 250 pm resolution, respectively, by X-ray diffraction methods. These investigations confirmed that the above mentioned discoidal substructures are build up by two trimers ($[\alpha\beta]_3$) which are aggregated head-to-head to form the hexamer (cf. Fig. 3c). Both trimers fit complementarly and are held together by polar and ionic interactions. Each trimer consists of three identical ($\alpha\beta$)-units which are arranged around a three-fold symmetry axis to form a hollow cylinder of approximate dimensions 11 nm × 3 nm with a central channel of 3.5 nm in diameter. Chromophore β-82 is stretching into the central channel, α-84 is neighbouring, and β-153 is placed at the periphery of the disc (cf. Fig. 3d). Both subunits, α and β, exhibit a similar structure and are related by a local two-fold

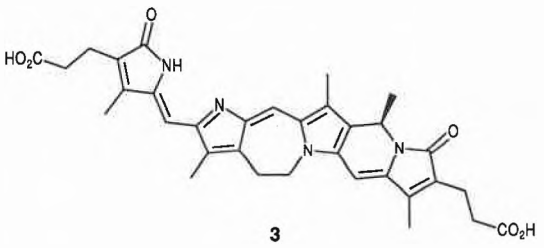
rotational axis. Each subunit is folded into eight helices and irregular loops. Six helices are arranged to form a globular part, whereas two helices stick out and mediate extensive contact between the subunits. Both tertiary structure and aggregation form of the crystalline C-phycocyanin are supposed to be the same as these found in the rods of native phycobilisomes.

As shown by the X-ray diffraction studies, the bile pigment chromophores of C-phycocyanin occur in «stretched» conformations rather than in the helical-shaped conformation which is characteristic for bile pigment molecules in solution. Both theoretical calculations[30, 31] and comparison with the UV/VIS spectrum of isophorcabilin (**3**, an almost rigid bile pigment chromophore isolated from the butterfly *Papilio phorcas*[32]) suggest that the «stretching» of the bile pigment chromophore conditions the enhanced extinction of the visible absorption band of phycobiliproteins with respect to the band in the near UV-range ($\varepsilon_{VIS}/\varepsilon_{UV} = 4.1$ for the native phycobiliprotein), a feature which is of essential physiological relevance for the microorganisms in order to utilize visible light for the ultimate excitation of the chlorophyll receptors (cf. Fig. 4).

As mentioned before, an additional characteristic spectroscopic property of the phycobiliproteins, namely their intense fluorescence, differentiate them from free bile pigment chromophores. Actually, phycobilisomes are very efficient transducers of light energy, which is transferred along phycobilisome rods with an overall efficiency of over 90%[25]. In aqueous solution (phosphate buffer, pH 6.5 – 7.0), native (i.e. not denatured) phycobiliproteins emit an intense fluorescence which is quenched when the protein is denatured with 8 M aqueous urea. On dilution with water, the fluorescence reappears (see Fig. 5).

Concerning their spectral properties, the phycobilisome chromophores can be divided into sensitizing («s») and fluorescing («t») bile pigment molecules[34–36]. The former absorb at the blue edge of the absorption band and transfer the excitation energy rapidly to the latter. However, a distinction between «s» and «t» chromophores is purely phenomenological, since it is not an inherent property of a chromophore, but rather of the aggregation state of the pigments. In all biliproteins and their functional aggregates, it is always the chromophore of lowest energy which fluoresces exclusively or, at least, predominantly. Moreover, although all bile pigment chromophores occur in «stretched» conformations, their geometries, which are enforced by the particular protein surroundings, are different, and correspondingly, their absorption and emission spectra are not identical. Thus, the α-84, β-82, and β-153 chromophores of C-phycocyanin from *Mastigocladus laminosus* absorb, in the visible range, at λ_{max} = 617, 623, and 599 nm, respectively[37].

The distances between the chromophore molecules in the hexamer subunits and in the columns of hexamers seem to be too large for strong excitonic coupling. Efficient energy transfer by inductive resonance (Förster mechanism), however, is possible[38]. As the transfer of the absorbed light energy within the phycobilisomes is initially dependent on the relative distances and orientations of the chromophore molecules, the X-ray diffraction studies on C-phycocyanin have contri-



3

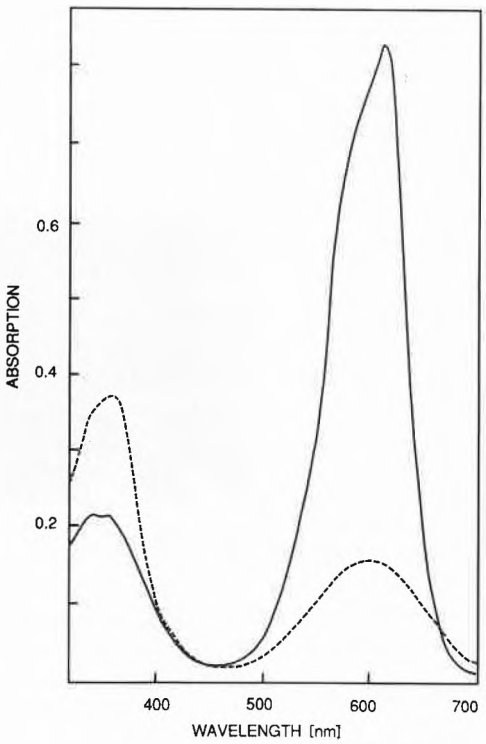


Fig. 4. Spectrum of «native» (—) and denatured C-phycocyanin (– – –) from the cyanobacterium Spirulina platensis (after[33]*).*

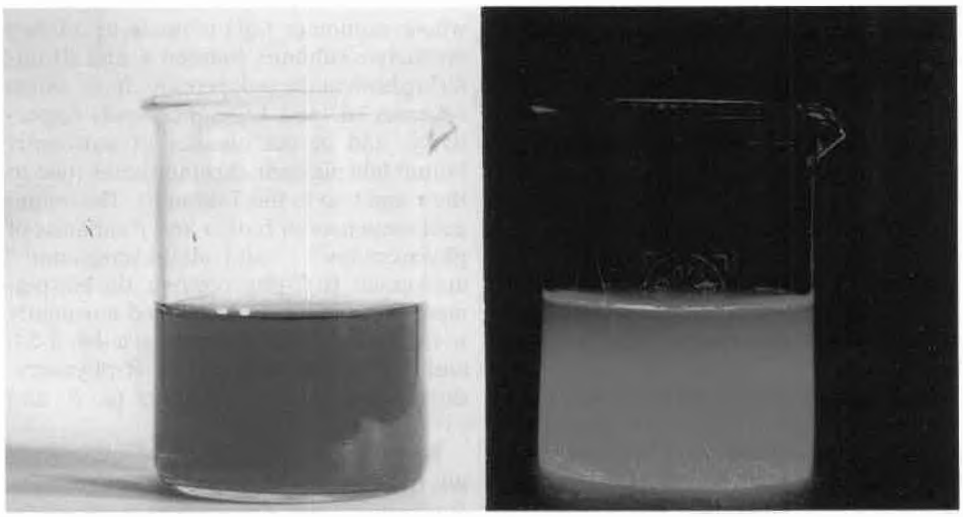


Fig. 5. Fluorescence of native C-phycocyanin in aqueous phosphate buffer (pH 6.5–7.0).

buted decisively to the knowledge of the preferential pathway for the energy transfer within the phycobilisomes. Thus, the crystal structure suggests that light energy absorbed by α-84 or β-153 pigments is transferred first to the β-82 chromophores within one hexamer and than transduced via β-82 chromophores along the phycobilisome rods. It is possible that chromophore β-153, located at the periphery of the hexamers, plays a role in lateral energy transfer between rods[24].

3. Bilindionostilbenoparacyclophanes as Model Compounds for the Chromophores of Phycobiliproteins

Despite of the enormous progress made during the last years in the study of the macromolecular structure of the phycobiliproteins, a precise knowledge of the relationship between conformation of bile pigment chromophores and their spectroscopic properties should be more directly accessible with the aid of models which mimic the features of the phycobiliproteins without changing the structure of the chromophore. Such a prerequisite is fulfilled by the macrocyclic compound **9f** (see Fig. 7), the synthesis of which has been reported recently[39]. The biliverdin chromophores of the (*E*)-bilindionostilbenoparacyclophane **9f***) and its (*Z*)-isomer **8f** are held in a «stretched» and helical-shaped conformation, respectively, by the rigid *p, p'*-diphenylstilbene moiety. On irradiation of benzene solutions of **8f** or **9f** with a high-pressure mercury lamp, a mixture of the two compounds in photostationary equilibrium is obtained (cf. Fig. 7), from which both isomers can be separated by chromatography. On the contrary, neither **8f** nor **9f** is photoisomerized by red light (λ_{max} = 525 nm). Thus, the conformational changes of the bile pigment chromophore brought about by isomerization of the stilbene double bond correspond presumably to the changes which are observed on denaturation and renaturation of the phycobiliproteins.

As a matter of fact, the light absorption of the *blue* biliverdin derivative **8f** and of its *magenta* isomer **9f** strikingly differs as in the natural pigments (see Fig. 6). Nevertheless, **9f** absorbs in the visible range at the same frequency as phycoerythrin, whose chromophore is three C=C bonds shorter. Thus it becomes apparent, that twisting of the chromophore in the «stretched» conformation equals a shortening of the conjugated system, a feature which accounts for the different absorption maxima of one and the same chromophore in natural phycobiliproteins, depending on the protein surroundings (vide supra). Moreover, whereas a well-resolved ^{1}H-NMR spectrum of **8f** could be obtained at room temperature, only broad signals are observed in the case of **9f** at + 25 to − 45 °C, in $CDCl_3$, thus indicating that rotation around the exocyclic formal σ-bonds of the bile pigment chromophore in the latter compound is strongly hindered by the strain imposed by the *p, p'*-diphenylstilbene spacer moiety. In order to investigate the influence of strain on the UV/VIS and NMR spectra of «stretched» bile pigment molecules, a series of (*E*)-bilindionostilbenoparacyclophanes (**9a–9c**) was synthesized as depicted in Fig. 7. As expected, the maximum of the absorption band of the biliverdin chromophore, in the visible range, is shifted to longer wavelengths on elongation of the aliphatic chains which link the bile pigment molecule to the spacer moiety. This bathochromic shift is more pronounced, however, when compounds **9c** and **9f** are compared, than within the homologous series **9a–9c** (cf. Fig. 6).

In agreement with theoretical calculations[31], the absorption of the «stretched» bile pigment chromophores, in the UV range, is extremely weak (cf. Fig. 8). On the other hand, the ratio of the absorption in the visible and UV range decreases regularly with increasing length of the connecting aliphatic chains (cf. Table 1).

As a matter of fact, the gain of energy caused by the transition of the bile pigment molecule from the energetically more favorable helical-shaped conformation in the *Z*-isomer [45] to a «stretched» conformation in the *E*-isomer seems to be balanced, to some extent, by the difference of energy between the *Z*- and *E*-isomers of the *p, p'*-diphenylstilbene moiety. Actually, photoisomerization experiments which were carried out with compounds **8d**, **8e**, and **8g** pointed out that, at least qualitatively, the above condition has to be met in order to «stretch» the bile pigment molecule. Thus, bilindionostilbenoparacyclophane **8g** in which two acetic (instead of propionic) acid residues link the bile pigment with the spacer moiety do not photoisomerize under the same conditions used for **8f** al-

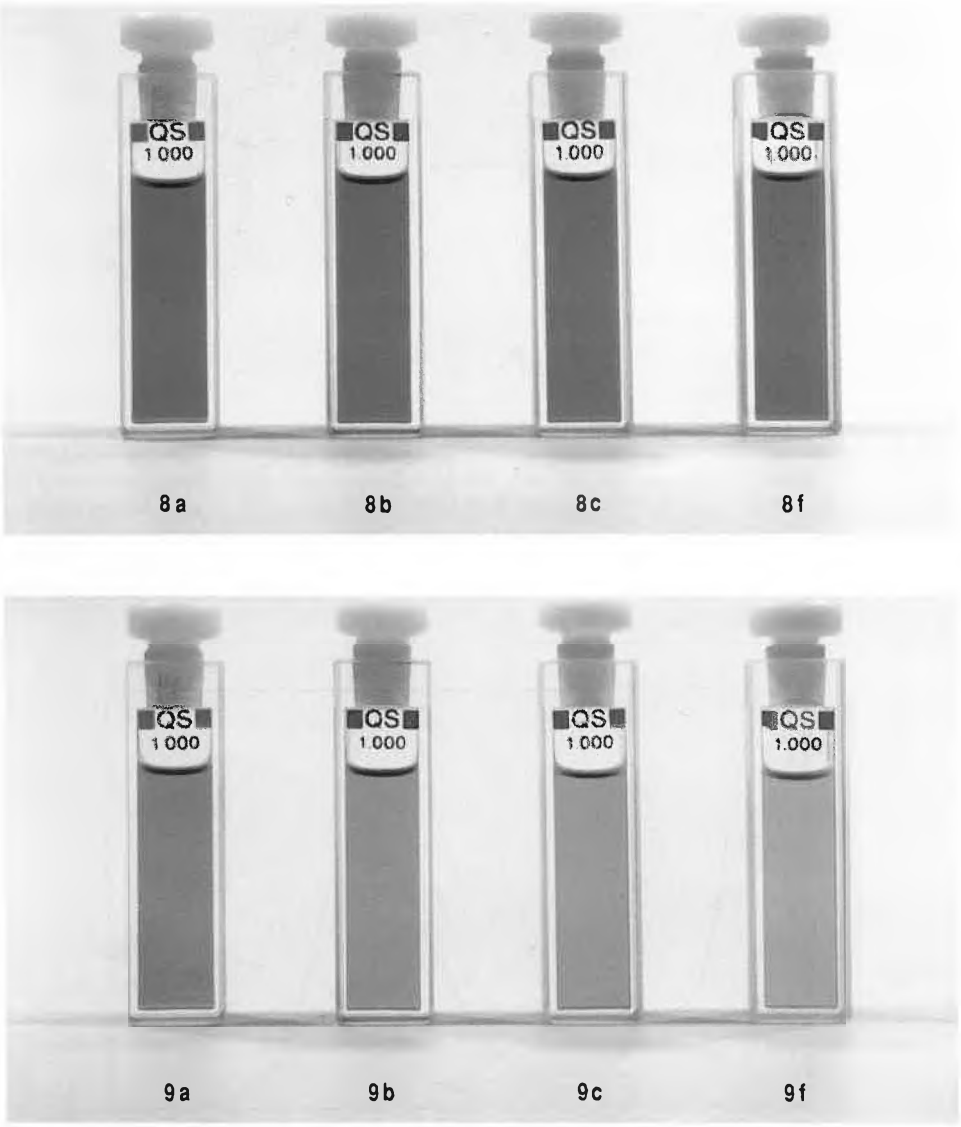


Fig. 6. Differences in coloration of (E)-bilindionostilbenoparacyclophanes **9a–c** *and* **9f** *in CH_2Cl_2 solution ($3 \cdot 10^{-5}$ M). Above, the corresponding (Z)-isomers.*

*) According to *Vögtle* et al.[40] the systematic name of **9f** is: 10,15,16,21,22,28-hexamethyl-46-octyl-2,32-dioxa-3,31-dioxo-[5](2,18)-1,19-[21*H*,23*H*,24*H*]bilindiono[5]paracyclo[0](4,4')-stilbeno[0]paracyclophane. Throughout this account the generic name bilindionostilbenoparacyclophane will be used.

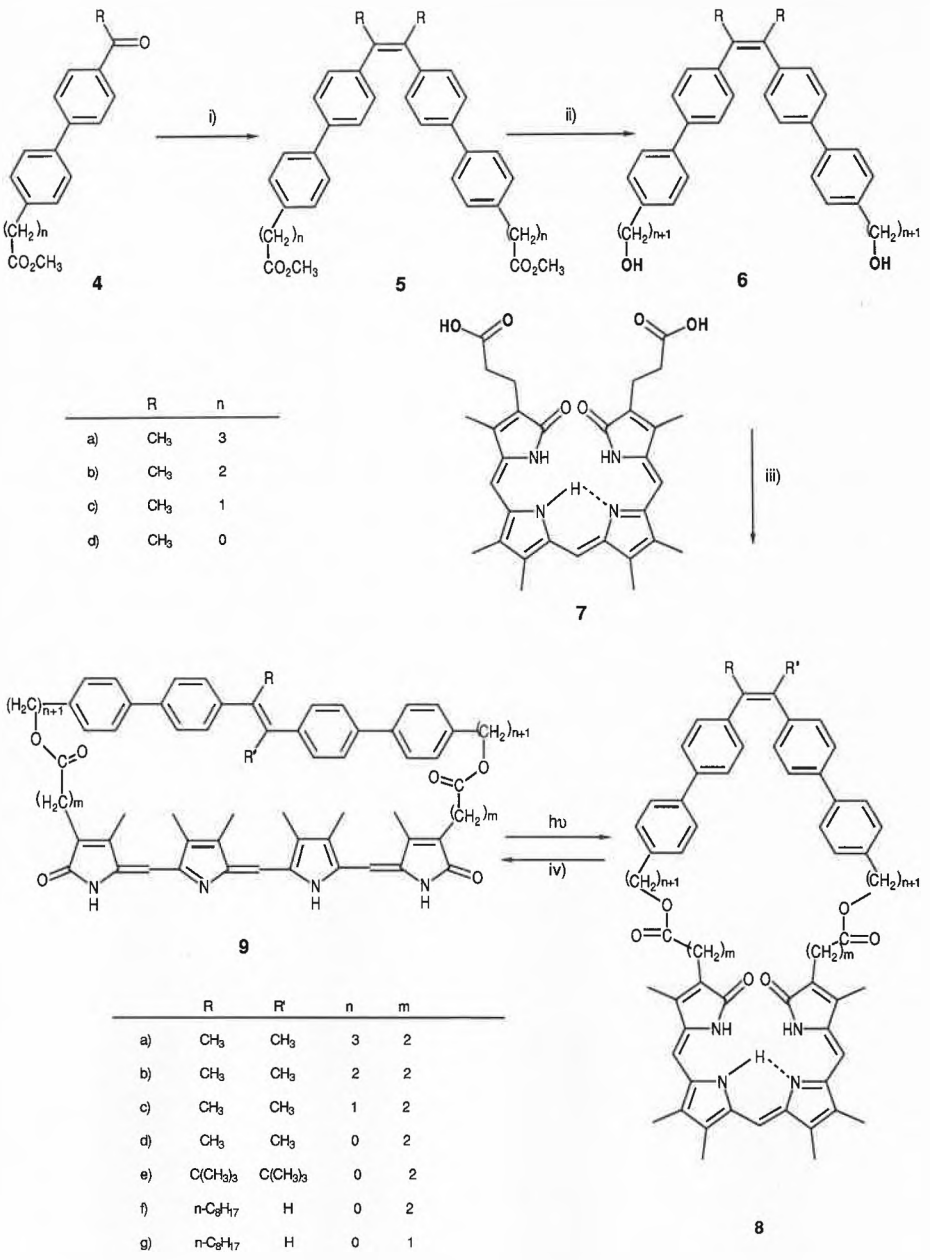


Fig. 7. A short synthesis of bilindionostilbenoparacyclophanes from biphenyl-ketone derivatives: i) $TiCl_4/Zn$ in dioxane, refl. for 6 h; ii) $LiAlH_4$ in tetrahydrofuran at room temperature; iii) dicyclohexylcarbodiimide and 4-dimethylaminopyridine in dichloromethane at room temperature for 72 h (cf. [41]*); iv) high-pressure Hg lamp in benzene ($8 \cdot 10^{-5}$ M) for 10 min. The Z- and E-isomers were separated by chromatography on Al_2O_3 (Woelm N, activity grade Super I) with ethyl acetate/dichloromethane (4:1), then gradient elution with dichloromethane containing isopropanol (2–5%).*

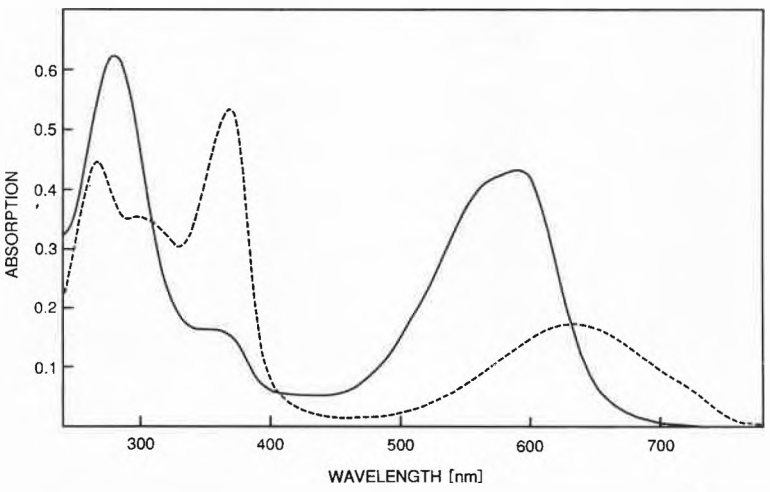


Fig. 8. UV/VIS spectra of **8c** *(---) and* **9c** *(—) in CH_2Cl_2 solution (10^{-5} M).*

though the stilbene moiety is the same in both compounds. Likewise, the yield of **9d**, isolated after photoisomerization of the *Z*-isomer **8d**, is less than 5%, whereas in the homologous series **8a–8c** the corresponding yields amount to 50–60%. As, under the same conditions, the photoisomerization of **8f**, in which the length of the connecting aliphatic chains is the same as in **8d**, yields a mixture of the *Z*- and *E*-isomers in the ratio of 2:1 [39], it may be inferred that the enthalpy for *Z*/*E* isomerization of the α, α′-dimethylstilbene moiety of **8d** is smaller than that of the octyl derivative in **8f**. This is in agreement with the free-enthalpy differences for the *E*- and *Z*-isomers of α-methylstilbene and α,α′-dimethylstilbene ($\Delta(\Delta G^0) = 0.83 \pm 0.02$ and 0.23 ± 0.2 kcal/mol, respectively) reported by *Fischer* et al. [42].

The above considerations do not take into account, however, the energy which is really necessary in order to «stretch» the biliverdin chromophore. Actually, as force field calculations show, the conversion of the energetically most favorable helical-shaped conformer, i.e. with 5*Z*,10*Z*,15*Z* geometry at the exocyclic double bonds and 5*sp*,10*sp*, 15*sp* *) conformations at the exocyclic formal single bonds, into a fairly linear conformer (e.g. *Z*,*Z*,*Z*; *ac*,*sp*,*ac*) requires about 11.4 kcal/mol [44], a figure which exceeds largely the energy which can be liberated by *Z*/*E* isomerization of the stilbene chromophore. Most likely, irradiation of the bile pigment chromophore during the photoisomerization of the stilbene double bond contributes to the «stretching» of the former. In this case, indeed, photoisomerization at the exocyclic double bonds of the bile pigment molecule is, in principle, possible, so that «stretched» conformations may be obtained with considerably less energy demand, or even with energy gaining (see also Ref. [45]). Accordingly to this hypothesis, monochromatic irradiation of **8c** at $\lambda_{max} = 290$ nm proved to be inefficient for its transformation into the *E*-isomer. As the ^{1}H-NMR spectra of derivatives **9a–9c** are well resolved, studies directed to the elucidation of the actual geometry of their bile pigment chromophores are, at present, in progress.

Particularly interesting in this connection proved to be compound **8e**. As both (*Z*)-α,α′-di-*tert*-butyl-stilbene [46] and its *p*,*p*′-diphenyl derivative isomerize nearly quantitatively to the corresponding (*E*)-isomers on heating at 140–160 °C in nitrobenzene, it was expected that bilindionoparacyclophane **8e** would likewise yield the corresponding *E*-isomer **9e** under similar conditions. Actually, however, no

*) According to the IUPAC rules (E-5.6) for the nomenclature of conformational isomers [43], conformations are described as synperiplanar (*sp*), synclinal (*sc*), anticlinal (*ac*), or antiperiplanar (*ap*) according as the torsion angle subtended by the two planes defined by the set of atoms N-C-C-N is within ±30°, ±60°, ±120°, or ±180°, respectively.

change in the UV/VIS spectrum of **8e** could be observed after heating in xylene, dimethylformamide or hexamethylphosphoric triamide (HMPTA) solutions for 12 h at 135°C. Apparently, the necessary energy to «stretch» the biliverdin chromophore of **8e** is not compensated by the gain of enthalpy for Z/E isomerization of the stilbene moiety, which may be estimated at about $\Delta(\Delta H^0) = 4.8$ kcal/mol according to the value determined experimentally for α, α′-di-*tert*-butylstilbene[46]. Under the same conditions, however, the E-isomer **9e** which was obtained by reaction of **7** with (*E*)-*p*,*p*′-bis(4-bromomethyl-phenyl)-α,α′-di-*tert*-butylstilbene in HMPTA solution at high dilution[47], was entirely transformed into **8e** within 15 min.

Table 1. UV/VIS spectra of bilindionostilbenoparacyclophanes in CH_2Cl_2 (10^{-5} M).

Compound	8 (Z)		9 (E)		Q ($\varepsilon_{VIS}^*/\varepsilon_{UV}^*$)	
	λ_{max} [a]	lg ε	λ_{max} [a]	lg ε	Z	E
a	632*	4.28	588*	4.58		
	366*	4.81	366*	4.31	0.30	1.84
	301	4.61	279	4.90		
	266	4.65				
b	626*	4.27	590*	4.66		
	367*	4.77	361*	3.98	0.32	4.84
	301	4.60	281	4.87		
	266	4.67				
c	626*	4.23	589*	4.63		
	367*	4.72	362*	3.87	0.32	5.88
	301	4.55	281	4.79		
	266	4.65				
d	610*	4.28				
	367*	4.72	–		0.36	–
	302	4.62				
	266	4.75				
e	623*	4.23	588*	4.69		
	367*	4.75	364*	4.16	0.30	3.53
	290	sh	286	4.79		
	259	4.74				
f	608*	4.32	559*	4.54		
	368*	4.62	(345)*[b]	(4.12)[b]	0.50	2.60[b]
	306	4.51	317	4.62		
	265	4.66				
g	607*	4.36				
	368*	4.67	–		0.49	–
	302	4.57				
	263	4.71				

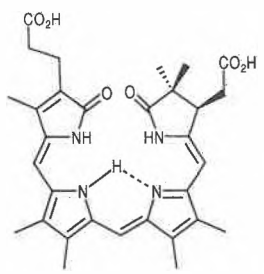
[a] The absorption of the bile pigment chromophore is indicated by an asterisk. [b] Calculated from the difference spectra (cf.[39]).

4. Conclusions and Outlook

Although not fluorescent, the bridged biliverdin derivatives so far investigated imitate the spectral properties of phycobiliproteins in solution: the Z-isomer allows the energetically more favorable helical-shaped conformation of the bile pigment chromophore, the E-isomer, on the contrary, forces the latter in a «stretched» conformation, which is characterized by a considerable enhancement of the extinction of its visible absorption band at the expense of the absorption in the near-UV range.

In contrast to the relative stabilization of «stretched» bile pigment molecules in some solvents (e.g. HMPTA[48]), the constraint imposed by the rigid spacer moiety of the bilindionostilbenoparacyclophanes enables to investigate the spectroscopic and chemical properties of a complete population of «stretched» bile pigment chromophores.

Moreover, bilindionostilbenoparacyclophanes are versatile models for the protein-bounded chromophores in native phycobiliproteins. The degree of strain of the «stretched» bile pigment molecules can be adjusted by the length of the aliphatic chains connecting the stilbene and bile pigment chromophores. Both the structures of the *p*, *p*′-diphenylstilbene and bilindione moieties can be widely varied, so that, particulary, weak interactions between both chromophores (e.g. via hydrogen bonding with suitable proton acceptor sites in the spacer moiety) may be investigated. At present, the syntheses of a series of bilindionostilbenoparacyclophanes bearing the bile pigment *rac*-**10**[49] as a more appropriate model for the 2,3-dihydrobilindione chromophore of C-phycocyanin is in progress.



10

Acknowledgement: Financial support of this work by the Swiss National Science Foundation (Project No. 2.278–0.86) is gratefully acknowledged.

Received: August 11, 1988 [FR 57]

[1] J.M. Olsen, *Science 168* (1970) 438.
[2] D.A. Bryant, *J. Gen. Microbiol. 128* (1982) 835.
[3] J.O. Nagy, J.E. Bishop, A.V. Klotz, A.N. Glazer, H. Rapoport, *J. Biol. Chem. 260* (1985) 4864, and references given therein.
[4] W.J. Cole, D.J. Chapman, H.W. Siegelman, *Biochemistry 7* (1968) 2929.
[5] H.L. Crespi, U. Smith, J.J. Katz, *Biochemistry 7* (1968) 2232.
[6] D.J. Chapman, W.J. Cole, H.W. Siegelman, *J. Am. Chem. Soc. 89* (1967) 5976.
[7] H.L. Crespi, J.J. Katz, *Phytochemistry 8* (1969) 759.
[8] W. Rüdiger, P.O'Carra, *Eur. J. Biochem. 7* (1979) 509.
[9] W. Rüdiger, P. O'Carra, C. O'h Eocha, *Nature (London) 215* (1967) 1477.
[10] A. Gossauer, W. Hirsch, *Justus Liebigs Ann. Chem.* (1974) 1496.
[11] A. Gossauer, R.-P. Hinze, *J. Org. Chem. 43* (1978) 283.
[12] A. Gossauer, E. Klahr, *Chem. Ber. 112* (1979) 2243.
[13] A. Gossauer, J.-P. Weller, *J. Am. Chem. Soc. 100* (1978) 5928.
[14] C. Pasquier, A. Gossauer, W. Keller, C. Kratky, *Helv. Chim. Acta 70* (1987) 2098.
[15] J.C. Lagarias, A.N. Glazer, H. Rapoport, *J. Am. Chem. Soc. 101* (1979) 5030.
[16] R.W. Schoenleber, S.-L. Leung, D.J. Lundell, A.N. Glazer, H. Rapoport, *J. Am. Chem. Soc. 105* (1983) 4072.
[17] J.E. Bishop, H. Rapoport, A.V. Klotz, C.F. Chan, A.N. Glazer, P. Füglistaller, H. Zuber, *J. Am. Chem. Soc. 109* (1987) 875.
[18] E. Gantt, *BioScience 25* (1975) 781.
[19] R.B. Wildman, C.C. Bowen, *J. Bacteriol. 117* (1974) 866.
[20] E. Mörschel, E. Koller, K.P. Wehrmeyer, H. Schneider, *Cytobiologie 16* (1977) 118.
[21] D.A. Bryant, G. Guglielmi, N. Tandeau de Marsac, A.-M. Castets, G. Cohen-Bazire, *Arch. Microbiol. 123* (1979) 113.
[22] M. Nies, W. Wehrmeyer, *Arch. Microbiol. 129* (1981) 374.
[23] T. Schirmer, W. Bode, R. Huber, W. Sidler, H. Zuber, *J. Mol. Biol. 184* (1985) 257.
[24] T. Schirmer, R. Huber, M. Schneider, W. Bode, M. Miller, M.L. Hackert, *J. Mol. Biol. 188* (1986) 651.
[25] A.N. Glazer, *Biochim. Biophys. Acta 768* (1984) 29.
[26] P. Freidenreich, G.S. Apell, A.N. Glazer, *J. Biol. Chem. 253* (1978) 212.
[27] G. Franck, W. Sidler, H. Widmer, H. Zuber, *Hoppe-Seyler's Z. Physiol. Chem. 359* (1978) 1491.
[28] W. Sidler, J. Gysi, E. Isker, H. Zuber, *Hoppe-Seyler's Z. Physiol. Chem. 362* (1981) 611.
[29] P. Füglistaller, F. Suter, H. Zuber, *Hoppe-Seyler's Z. Physiol. Chem. 364* (1983) 691.
[30] G. Blauer, G. Wagnière, *J. Am. Chem. Soc. 97* (1975) 1949.
[31] H. Falk, H. Gsaller, E. Hubauer, N. Müller, *Monatsh. Chem. 116* (1985) 939, and references given therein.
[32] M. Bois-Choussy, M. Barbier, *Heterocycles 9* (1978) 677.
[33] H. Scheer, W. Kufer, *Z. Naturforsch. C 32* (1977) 513.
[34] G.F.W. Searle, J. Barber, G. Porter, C.J. Tredwell, *Biochim. Biophys. Acta 501* (1978) 246.
[35] F.W.J. Teale, R.E. Dale, *Biochem. J. 116* (1970) 161.
[36] B. Zickendrath-Wendelstadt, J. Friedrich, W. Rüdiger, *Photochem. Photobiol. 31* (1980) 367.
[37] S. Siebzehnrübl, R. Fischer, H. Scheer, *Z. Naturforsch. C 42* (1987) 258.

[38] J. Grabowski, E. Gantt, *Photochem. Photobiol. 28* (1978) 39.
[39] P. Nesvadba, A. Gossauer, *J. Am. Chem. Soc. 109* (1987) 6545.
[40] F. Vögtle, P. Neumann, *Tetrahedron 26* (1970) 5847.
[41] A. Hassner, V. Alexanian, *Tetrahedron Lett. 19* (1978) 4475.
[42] G. Fischer, K.A. Muszkat, E. Fischer, *J. Chem. Soc. (B)* (1968) 1156.
[43] *Pure Appl. Chem. 45* (1976) 13.
[44] H. Falk, N. Müller, *Tetrahedron 39* (1983) 1875.
[45] H. Falk, N. Müller, *Monatsh. Chem. 112* (1981) 791.
[46] D. Lenoir, J.E. Gano, J. McTague, *Tetrahedron Lett. 27* (1986) 5339.
[47] P. Nesvadba, A. Gossauer, unpublished results.
[48] H. Falk, N. Müller, S. Wansch, *Monatsh. Chem. 116* (1985) 1087.
[49] H. Falk, K. Grubmayr, K. Magauer, N. Müller, U. Zrunek, *Isr. J. Chem. 23* (1983) 187.