

ZEITSCHRIFT FÜR NATURFORSCHUNG

Section c

B I O S C I E N C E S

Council

E. BÜNNING, Tübingen
A. BUTENANDT, München
M. EIGEN, Göttingen
W. GENTNER, Heidelberg
W. HEISENBERG, München

Editorial Board

A. HAGER, Tübingen
W. HASSELBACH, Heidelberg
E. HELMREICH, Würzburg
F. KAUDEWITZ, München
W. SCHÄFER, Tübingen

Advisory Editorial Board

P. BÖGER, Konstanz
K. G. GOTZ, Tübingen
G. GOTTSCHALK, Göttingen
H. HOFFMANN-BERLING, Heidelberg
R. JAENICKE, Regensburg
P. KARLSON, Marburg
G. F. MEYER, Tübingen
D. SCHULTE-FROHLINDE, Mülheim/R.

G. SCHWARZ, Basel
F. F. SEELIG, Tübingen
H. SIMON, München
W. STEGLICH, Bonn
W. TANNER, Regensburg
A. TREBST, Bochum
E. WECKER, Würzburg

EDITED IN COLLABORATION

WITH THE INSTITUTES OF THE MAX-PLANCK-GESELLSCHAFT

VOLUME 31 c

1976

VERLAG DER ZEITSCHRIFT FÜR NATURFORSCHUNG
TÜBINGEN



Anschrift des Verlages: 74 Tübingen, Postfach 2645
Satz und Druck: Konrad Triltsch, Würzburg

Nachdruck — auch auszugsweise — nur mit schriftlicher Genehmigung des Verlags

Lehrbuch

Section a

Physics, Physical Chemistry, Cosmic Physics

Section b

Inorganic and Organic Chemistry

Contents

Contents of Number 1/2

Original Communications

O. Sciacovelli, A. Dell'atti, A. De Giglio, and L. Casadei, Studies on Phenylpyruvic Acid. I. Keto-Enol Tautomerism
 S. Ramani and S. Kannan, Inhibition of Ca^{2+} and Zn^{2+} Uptake by Mn^{2+} in Excised Rice Roots
 K. Saito, A. Komamine, and S. Senoh, Further Studies on the Biosynthesis of Stizolobinic Acid and Stizolobic Acid in the Etiolated Seedlings of *Stizolobium hassjo* H. Wombacher and M. Reuter-Smerdka, Spectrometric and Biological Data of 1,N⁶-Ethenoadenosine 3',5'-Cyclic Monophosphate
 B. V. Burger, M. le Roux, C. F. Garbers, H. S. C. Spies, R. G. Bigalke, K. G. R. Pachler, P. L. Wessels, V. Christ, and K. H. Maurer, Studies on Mammalian Pheromones, I. Ketones from the Pedal Gland of the Bontebok (*Damaliscus dorcas dorcas*)
 H. Schmidt and P. Rosenkranz, On the Mechanism of the Acridine Orange Sensitized Photodynamic Inactivation of Lysozyme. I. Basic Kinetics
 G. G. Nöll, A Cell for Simultaneous Measurements of Optical and Electrical Properties of Black Lipid Membranes
 P. Chin and S. S. Brody, Mixed Monomolecular Films of Chlorophyll and Cytochromes
 W. Lockau and B. R. Selman, Correlation of the Photosynthetic Reduction of *p*-(Diazonium)-Benzene-sulfonic Acid with the Increased Binding of the Probe to the Thylakoid Membrane
 Hj. A. W. Schneider, Enzymic Capacities for Chlorophyll Biosynthesis. Activation and *de novo* Synthesis of Enzymes
 G. Döring, The Effect of Deoxycholate-Treatment to the Photoreactions of the Active Pigments in Photosynthesis
 H. Böhme, Photoreactions of Cytochrome b₆ and Cytochrome f in Chloroplast Photosystem I Fragments
 G. Döring, The Chlorophyll a₁₁ Reaction in Trypsin-Treated Spinach Chloroplasts in the Presence of Potassium Ferricyanide
 G. Kulandaivelu and D. O. Hall, Ultrastructural Changes in *in vitro* Ageing Spinach Chloroplasts
 K. Angermann, U. Grundmann, and H. Holz, Size Comparison of Poly(A)-RNA from Polysomes and from Nuclei of the Yeast *Saccharomyces cerevisiae* (In German)
 G. Reimer and D. Drahovsky, Chromosomal Structures of *Pseudomonas testosteroni*. I. Isolation and Characterization of the Chromosomal Complexes (In German)
 N. Kokolis, Variations in Melanin and Riboflavin Content of Amphibian Liver under the Influence of Reserpine and Amphetamine

Notes

R. Harcus, P. N. Preston, and J. S. Suffolk, Synthesis of Purine and Pyrimidine Substituted Nitroxides
 S. Reimer and A. Trebst, Reversal of Dibromothymoquinone Inhibition of Photosynthetic Electron Flow by Thiol Compounds
 H. Kern and J. Grübler, Redundant Nucleotide-Sequences in DNA from Wheat (In German)

A. Lüttke, U. Rahmsdorf, and R. Schmid, Heterogeneity in Chloroplasts of Siphonaceous Algae as Compared with Higher Plant Chloroplasts 108

Contents of Number 3/4

Original Communications

5 P. Bommer, H. Moser, W. Stichler, P. Trimborn, and W. Vetter, Determination of the Origin of Drugs by Measuring Natural Isotope Contents: D/H and ¹³C/¹²C Ratios of Some Diazepam Samples (In German) 111
 15 R. Kanne and H. Zähner, Metabolic Products of Microorganisms, 151. Comparative Studies on Intracellular Potassium- and Sodium Concentrations of Wild-Type and a Macrotetrolide Negative Mutant of *Streptomyces griseus* 115
 18 M. Roscigni, R. Endean, and A. Temperelli, New and Uncommon Indole- and Imidazole-Alkylamines in Skins of Amphibians from Australia and Papua New Guinea 118
 21 H. Kayser, Isolation of β,β -Caroten-2-ol from an Insect, *Cerura vinula* (Lepidoptera) 121
 29 M. G. Peter, K. H. Dahm, and H. Röller, The Juvenile Hormones in Blood of Larvae and Adults of *Manduca sexta* (Joh.) 129
 40 I. Kubota, M. Isobe, T. Goto, and K. Hasegawa, Molecular Size of the Diapause Hormone of the Silk-worm *Bombyx mori* 132
 44 H.-D. Lüdemann, E. Westhof, and I. Cuno, Ribose Conformations of 8-Azapurine Nucleosides in Solution 135
 48 G. Seibert and R. K. Zahn, Fractionated Precipitation of Acid Macropolyanions by Dialysis, a Symply Method for the Estimation of DNA in Complex Biological Samples 141
 55 C. Lesca, A. Moisand, and A. Puget, Characterization of Mitochondrial DNA from the Pika (*Ochotona rufescens rufescens*) 145
 64 D. Auer and G. Brandner, Loading of Human Red Blood Cells with DNA and RNA 149
 68 A. Trebst, Artificial Energy Conservation in Bacterial Photosynthetic Electron Transport 152
 78 G. F. Wildner, The Greening Process in *Euglena gracilis*, I. The Kinetics of Appearance of Chloroplast Proteins and the Effect of Cycloheximide and Chloramphenicol on Their Synthesis 157
 82 W. Oettmeier, J. R. Norris, and J. J. Katz, Photo-Induced Electron Transfer in Chlorophyll Containing Liposomes 163
 85 B. Schulz-Harder and E.-R. Lochmann, Initiation in a Polyribosome-Dependent Protein-Synthesizing Cell-Free System from *Saccharomyces* (In German) 169
 91 K. U. Berger and D. Schubert, Reversible Transformation of Precipitated and Nonprecipitated Lipoproteins Recombined from Proteins and Lipids of Erythrocyte Membranes 174
 98 H. P. Kulas and F. A. Anderer, Differences in the Lipid Distribution in Subcellular Fractions of Mouse Fibroblasts Derived from Logarithmic and Stationary Growth 179
 101 J. M. Amabis and K. K. Nair, Ultrastructure of Gene Transcription in Spermatocytes of *Trichosia pubescens* Morgante, 1969 (Diptera: Sciaridae) 186
 103 C. F. Bardele, Particle Movement in Heliozoan Axopods Associated with Lateral Displacement of Highly Ordered Membrane Domains 190
 104

Notes

S. Kannan and H. Keppel, Differential Migration of Foliar Applied Zinc in Maize Plants	195	F.-K. Lücke and J.-H. Klemme, Coupling Factor Adenosine-5'-triphosphatase from <i>Rhodospirillum rubrum</i> : A Simple and Rapid Procedure for Its Purification	272
W. Steglich and K.-F. Jendtke, Novel Anthraquinone Pigments from <i>Solorina crocea</i> (In German)	197	D. Müller-Enoch, E. Seidl, and H. Thomas, 6,7-Dihydroxycoumarin (Aesculetin) as a Substrate for Catechol-O-methyltransferase (In German)	280
P. D. Shirk, K. H. Dahm, and H. Röller, The Accessory Sex Glands as the Repository for Juvenile Hormone in Male <i>Cecropia</i> Moths	199	H. Rappold and A. Bacher, Nutritional Requirement for 4-Aminobenzoate Caused by Mutation of Dihydropteroate Synthetase	285
J. Ahlers, Th. Günther, and I. Schrandt, Kinetic Properties of Mg, Ca ATPase from Various <i>Escherichia coli</i> Mutants	201	B. Lotz, J. Betz, and L. Träger, Properties of an Antiserum Against <i>Streptomyces hydrogenans</i> 20 β -Hydroxysteroid Dehydrogenase	288
A. van de Vorst and Y. Lion, Indirect EPR Evidence for the Production of Singlet Oxygen in the Photosensitization of Nucleic Acid Constituents by Proflavine	203	R. Braun and H. Lange, Effect of N-Methyl-N- β -chloroethyl-hydrazine-HCl on the Growth and Multiplication of Yeast Cells (<i>Saccharomyces cerevisiae</i>) (In German)	292
G. Uhlenbrück, G. Steinhausen, and B. A. Baldo, Galactans and Anti-Galactans from Invertebrates	205	R. Braun, H. Lange, U. Mangold, and R. Mangold, Effect of N-Methyl-N- β -chloroethyl-hydrazine and Its Benzaldehydhydrazone on RNA- and Protein Synthesis as Soon as Metabolism of Synchronously Growing Yeast Cells (<i>Saccharomyces cerevisiae</i>) (In German)	298
H. Kern, Methylation of Transfer RNA in Plant Cells (In German)	207	G. H. Schmid, W. Menke, F. Koenig, and A. Radunz, Inhibition of Electron Transport on the Oxygen-Evolving Side of Photosystem II by an Antiserum to a Polypeptide Isolated from the Thylakoid Membrane	304
C. Reinke and G. Brandner, Estimation by Reassocation Assay of Viral DNA Copies in Three Polyoma Virus Transformed Cell Lines	209	A. Krauß and A. Hager, Growth Burst after Anaerobiosis: a "Stored-Growth" Effect in <i>Avena</i> Coleoptiles (In German)	312
G. Brandner and E. Koch, Failure of Inhibition of Polyoma Virus Replication by Distamycin A	212	R. Beiderbeck, Contact of <i>Agrobacterium</i> with Wound Cells as a Prerequisite of Tumor Induction (In German)	317
F.-C. Czygan, "Synthetical" <i>Aiptasia mutabilis</i> RAPP (Coelenterata) (In German)	214	A. Schüz, Pyramidal Cells with Different Densities of Dendritic Spines in the Cortex of the Mouse	319
E. Weber, The Influence of the Sample Size upon the Result of an Analysis of Variance in Long-Stemmed and Short-Stemmed Mutants of <i>Pisum sativum</i> (In German)	216	H. Stieve and M. Hanani, Light and Dark Adaptation of Crayfish Visual Cells Depending on Extracellular Calcium Concentration	324

Contents of Number 5/6

Original Communications

P. Schuster, K. Tortschanoff, and H. Winkler, Proton Transfer Reactions of Dibasic Acids in Aqueous Solution: 3-Hydroxypyridine (In German)	219	N. J. F. Dodd, R. G. Harcus, and P. N. Preston, Synthesis and Electron Spin Resonance Study of Spin-Labelled Compounds Related to Tumour-Growth Inhibitory Nitroarylaziridines	328
H. Zehner, W. Flossmann, and E. Westhof, Formation of H-Addition Radicals in Adenine Derivatives	225	J. L. Ingham, Isosativan: an Isoflavan Phytoalexin from <i>Trifolium hybridum</i> and other <i>Trifolium</i> Species	331
S. R. Nassif, C. Baumgartner, M. Sellers, and L. May, Studies on the Undecapeptide of Ferricytochrome c Using ESR, Mössbauer and Visible Spectroscopies	232	D. von Wachtendonk, Occurrence of an Acetylcholinesterase (E.C. 3.1.1.7) in the Haemolymph of the Mussel <i>Mytilus edulis</i> (In German)	333
J. Berger, I. Pilz, R. Witters, and R. Lontie, Small-Angle X-Ray and Sedimentation Studies on α -Haemocyanin <i>H. pomatia</i> (Heme Molecules) in Glycerol and Sucrose Solutions (In German)	238	K. Wolburg-Buchholz, The Dorsal Eye of <i>Cloeon dipteron</i> (Ephemeroptera) (A Light- and Electronmicroscopical Study)	335
H. A. B. Linke and C. A. Chang, Physiological Effects of Sucrose Substitutes and Artificial Sweeteners on Growth Pattern and Acid Production of Glucose-Grown <i>Streptococcus mutans</i> Strains <i>in vitro</i>	245	J.-P. Bourgin, Valine-Induced Inhibition of Growth of Haploid Tobacco Protoplasts and Its Reversal by Isoleucine	337
Th. Schmidt, C. Fedtke, and R. R. Schmidt, Studies on Optically Active Pesticides, I. Synthesis and Herbicidal Activity of d(+) and l(−) Methyl-2-chloro-3-(4-chlorophenyl)-propionate	252	R. Zander, The Distribution Space of Physically Dissolved Oxygen in Aqueous Solutions of Organic Substances (In German)	339
M. Moskophidis, C. M. Klotz, and W. Friedrich, Co α /Co β -Isomerism of Corrinoids. Partial Synthesis and <i>Escherichia coli</i> Activity of Further Isomer Pairs of the (Co-methyl)-Corrinoids (In German)	255	V. N. Babin, E. B. Zavelovich, and E. I. Fedin, 1 H and 13 C NMR Study of Proton Transfer in Azoles. Mechanism of Proton Transfer in Pyrazole in Solutions Containing bis-(Acetylacetoneato)Nickel(II)	353
F. Seela and F. Hansske, Polymer Linked N-(Hexyl)-5-azido-2-nitro-benzoic Acid Amide; a Photoreactive Resin for the Immobilization of Ligands (In German)	263	J. Sepiol, Z. Kazimierczuk, and D. Shugar, Tautomerism of Isoguanosine and Solvent-Induced Keto-Enol Equilibrium	361
G. F. Wildner, The Use of 1-Anilino-8-naphthalene Sulfonate as Fluorescent Probe for Conformational Studies on Ribulose-1,5-bis-phosphate Carboxylase	267	E. Westhof and M. van Rooten, MO Calculations of Some Thymine Radicals at the INDO Level	371

Contents of Number 7/8

Original Communications

G. Gundlach, E. L. Sattler, W. Trampisch, and U. Wagenbach, Direct Labeling of Amino Acids by Means of Hot ^{11}C -Atoms (In German)	377	J. Betz and L. Träger, Nucleoside Triphosphate Levels in <i>Streptomyces hydrogenans</i> during Growth and Induction of 20β -Hydroxysteroid Dehydrogenase	486	
M. Abdul Haleem and K. D. Parker, X-Ray Diffraction Studies on the Structure of α -Chitin	383	R. L. Brahmachary and D. Ghosal, Sulfated Macromolecules in Early Embryos of <i>Limnaea</i>	488	
F. Seela, Synthesis of 5-Azido-3-nitro- ω -bromo-acetophenone — a Photochemically Active Bifunctional Reagent for the Cross-Linking of Biopolymers (In German)	389	B. Bazin, G. Kümmel, and I. Zerbst-Boroffka, Studies on the Rectal Pads of the Honey Bee <i>Apis mellifera</i> (In German)	489	
S. Kannan and H. Keppel, Absorption and Transport of Pb^{2+} in Young Pea Seedlings	393	Contents of Number 9/10		
A. Nahrstedt, A New Cyanogenic Glycoside from <i>Sorbaria arborea</i> (Rosaceae) (In German)	397	<i>Original Communications</i>		
A. Matsuo, S. Uto, M. Nakayama, and S. Hayashi, Sesquiterpene Hydrocarbons of the Liverwort, <i>Dumortiera hirsuta</i>	401	F. Jüttner, β -Cyclocitral and Alkanes in <i>Microcystis</i> (Cyanophyceae)	491	
S. Imre and A. Özture, Further Anthraquinones from <i>Digitalis schischkinii</i> (In German)	403	J. Jacob and E. von Lehmann, Chemical Composition of the Nasal Gland Secretion from the Marsh Deer <i>Odocoileus (Dorcelaphus) dichotomus</i> (Illiger)	496	
B. Janistyn and M. Stocker, Enzymatic Degradation of [Ring B-U- ^{14}C]-5,7,3',4'-tetrahydroxyflavanone-7-O-glucoside to 5,7-Dihydroxychromone-7-O-glucoside and [Ring-U- ^{14}C]-1,2,4-trihydroxybenzene with a Cell Free System from <i>Mentha longifolia</i> (In German)	408	H. Arn, S. Rauscher, H.-R. Buser, and W. L. Roelofs, Sex Pheromone of <i>Eupoecilia ambiguella</i> : <i>cis</i> -9-Dodecetyl Acetate as a Major Component	499	
M. Stocker and B. Janistyn, Thallium-III-trifluoracetate Catalyzed Degradation of 5,7,3',4'-Tetrahydroxyflavanone to 5,7-Dihydroxychromone and 1,2,4-Trihydroxybenzene (In German)	411	J. L. Ingham, Induced Isoflavonoids from Fungus-Infected Stems of Pigeon Pea (<i>Cajanus cajan</i>)	504	
H. Scheer, Studies on Plant Bile Pigments: Characterization of a Model for the Phytochrome Pr Chromophor	413	D. Müller-Enoch, H. Thomas, W. Streng, W. Wildfeuer, and O. Haferkamp, O-Methylation of Epinephrine, 3,4-Dihydroxybenzoic Acid and 6,7-Dihydroxycoumarin in Yeasts (In German)	509	
A. Raschig and F. Schneider, Synthesis of a Fragment of the Active Center of the Streptococcal Proteinase (EC 3.4.22.10), IX (In German)	418	E. Kulikowska, B. Hingerty, W. Saenger, and D. Shugar, Unusual Photochemical Transformation of 5-Ethylorotate to 5-Ethylidenehydroorotate	514	
K. N. Shivaram, Purification and Properties of Potato Phosphorylase Isozymes	424	B. Hingerty, E. Kulikowska, W. Saenger, and D. Shugar, The Crystal Structure of the Pyrimidine Analogue Calcium-5-Ethylidenehydroorotate-1.5 Hydrate, a Photoproduct of Ethylorotate	518	
C. Döhler, Activity of Aminotransferases in the Blue Green Alga <i>Anacystis nidulans</i> (In German)	433	M. Overkamp, H. Twilfer, and K. Gersonde, Conformation-Controlled Trans-Effect of the Proximal Histidine in Haemoglobins. An Electron Spin Resonance Study of Monomeric Nitrosoyl- ^{57}Fe -Haemoglobins	524	
W. Menke, A. Radunz, G. H. Schmid, F. Koenig, and R.-D. Hirtz, Intermolecular Interactions of Polypeptides and Lipids in the Thylakoid Membrane	436	D. Petz and F. Schneider, Synthesis and Catalytic Properties of Peptidites with Hydrolytic Activity (In German)	534	
H. Hesse, R. Jank-Ladwig, and H. Strotmann, On the Reconstitution of Photophosphorylation in CF_1 -Extracted Chloroplasts	445	J.-H. Klemme, Regulation of Intracellular Pyrophosphatase-Activity and Conservation of the Phosphoanhydride-Energy of Inorganic Pyrophosphate in Microbial Metabolism	544	
G. Kulandaivelu and D. O. Hall, Stabilization of the Photosynthetic Activities of Isolated Spinach Chloroplasts during Prolonged Ageing	452	H.-J. Breter and R. K. Zahn, An Isocratic High-Pressure Liquid Chromatographic Purification Method for Radioactively Labeled Deoxyribonucleoside Triphosphates	551	
H.-G. Wuttke, Chromoplasts in <i>Rosa rugosa</i> : Development and Chemical Characterization of Tubular Elements	456	H.-J. Breter, F. Hundt, and R. K. Zahn, The Determination of the DNA Base Composition in 19 Species of Adriatic Sponges with High-Pressure Liquid Cation-Exchange Chromatography	554	
J.-P. Liautard and J. Liautard, On the Protein Composition of Ribonucleoprotein Particles before and after Fertilization of Sea Urchin Eggs (<i>Echinus esculentus</i>)	461	W. Gradmann-Rebel and V. Hemleben, Incorporation of T_4 Phage DNA into a Specific DNA Fraction from the Higher Plant <i>Matthiola incana</i>	558	
W. W. Fäth and M. Brendel, Isolation and Properties of Yeast Mutants with Highly Efficient Thymidylate Utilization	464	H. Spiller, G. Bookjans, and P. Böger, The Influence of Oxygen on Nitrite Reduction in a Reconstituted System	565	
<i>Notes</i>	468	S. S. Brody and N. F. Owens, Photosynthetic Electron Carriers at a Heptane-Water Interface	569	
H.-G. Reinhardt, Structure-Reactivity Relationships and Delayed Neurotoxicity of Organophosphorus Compounds (In German)	479	G. W. Pohl, Spectral Properties of Fluorescent Dyes in Lecithin Vesicles	575	
E.-R. Lochmann, C. Herrmann, I. Pietsch, and A. Micheler, Distribution of [^3H]Thiopyronine in Yeast Cells (<i>Saccharomyces cerevisiae</i>) (In German)	481	A. Radunz, Localization of the Tri- and Digalactosyl Diglyceride in the Thylakoid Membrane with Serological Methods	589	
D. Lindenau, U. Hagen, and W. Schnabel, Detachment of Segments from DNA Double Strands as Detected by Time Resolved Rayleigh Light Scattering	484	G. H. Schmid, G. Renger, M. Gläser, F. Koenig, A. Radunz, and W. Menke, Effect of an Antiserum to a Thylakoid Membrane Polypeptide on the Primary Photoreaction of Photosystem II	594	

G. Reimer and D. Drahovsky, Chromosomal Structure of *Pseudomonas testosteroni*. II. Activity of the Endogenous RNA-Polymerase (In German)
 H.-J. Lach and P. Böger, Variable Composition of Cytochromes b₆-f Particles
 W. Müller-Klieser and W. Kreutz, On the Cross-Section Structure of the Mitochondrial Cristae-Membrane as Revealed by X-Ray Diffraction

Notes

B. Stengel and H. Geiger, Kaempferole-3-(O-sinapoyl-sophoroside)-7-glucoside, a New Flavonoid from *Brassica napus* L. (Cruciferae) (In German)
 M. A. Alizade, K. Gaede, and K. Brendel, Chirality of the Hydrogen Transfer to NAD Catalyzed by *myo*-Inositol Dehydrogenase from *Klebsiella pneumoniae* . .
 G. H. Georgiew, E. J. Zöllner, and R. K. Zahn, Different Deoxyribonuclease-Activities in Bull Seminal Plasma (In German)
 R. Riehl, A Special Attaching-Mechanism between the Cortex radiatus externus and the Follicle Epithelium of the Oocytes of *Gobio gobio* (In German)
 K. Hausen, Functional Characterization and Anatomical Identification of Motion Sensitive Neurons in the Lobula plate of the Blowfly *Calliphora erythrocephala*

Contents of Number 11/12

Original Communications

H. Wawra, A Small-Angle-Scattering Camera of Medium Resolution without Slithigh-Error (In German)
 L. Cassidei, A. Dell'Atti, and O. Sciacovelli, Studies on Phenylpyruvic Acid, II. Keto-Enol Equilibrium in Water-Dimethylsulphoxide Mixtures
 H. Kayser, Identification of β,β -Caroten-2-ol and β,β -Carotene-2,2'-diol in the Stick Insect, *Carausius morosus* Br.; a Reinvestigation Study
 D. Urbach, M. Suchanka, and W. Urbach, Effect of Substituted Pyridazinone Herbicides and of Difunone (EMD-IT 5914) on Carotenoid Biosynthesis in Green Algae
 I. Zimmermann and H. W. Zimmermann, pK_a-Values of Ethidiumbromide and 7-Amino-9-phenyl-10-ethyl-phenanthridinium-bromide (In German)
 P. Dietsch, Th. Günther, and M. Röhnel, Dissociation Constants of Ethane-1-hydroxy-1,1-diphosphonate [EHDP] and Dichloromethylene-diphosphonate [Cl₂MDP] for H⁺, Ca²⁺, Mg²⁺ and Zn²⁺
 H. Twilfer and K. Gersonde, Non-Equivalence and Inverse Allosteric Response of the α and β Chains in Haemoglobins. An Electron Spin Resonance Study of NO-Ligated Hb Kansas
 D. Petz and F. Schneider, Kinetic Analysis of the Catalytic Properties of Peptides in Ester Hydrolysis . .
 P. Rosenkranz and H. Schmidt, On the Mechanism of the Acridine Orange Sensitized Photodynamic Inactivation of Lysozyme. II. Kinetics in Presence of N-Acetylglucosamine
 H. Wolburg, Evidence for the Involvement of the Na⁺-K⁺-ATPase in the Mechanism of Axonal Protein and Nucleoside Transport
 E. Göbel, R. Riessner, and P. Pohl, Influence of DCMU on the Formation of Lipids and Fatty Acids, and on the Ultrastructure of *Euglena gracilis* (In German)
 W. Löffelhardt and H. Kindl, Formation of Benzoic Acid and p-Hydroxybenzoic Acid in the Blue Green Alga *Anacystis nidulans*: A Thylakoid-Bound Enzyme Complex Analogous to the Chloroplast System

L. J. Anghileri, M. Heidbreder, and R. Dermietzel, On the Role of the Cell Coat Glycoproteins in the Permeability of the Cell 700
 601 M. Stromer and W. Hasselbach, Fusion of Isolated Sarcoplasmic Reticulum Membranes 703
 606 M. Stromer, R. The, and W. Hasselbach, The Effect of Ethylene Glycol and DMSO on Fusion of Isolated Sarcoplasmic Reticulum Membranes 708
 612 M. Gläser, Ch. Wolff, and G. Renger, Indirect Evidence for a Very Fast Recovery Kinetics of Chlorophyll-aII in Spinach Chloroplasts 712
 622 B. Schmidt and H. J. Rurainski, Light-Dependent Interactions of Phenazine Methosulfate with 3-(3,4-Dichlorophenyl)-1,1-dimethylurea-Poisoned Chloroplasts . . 722
 624 R. W. Wabnitz, A Dual Effect of Acetylcholine on Gastropod Smooth Muscle Preparations 730
 626 U. Zimmermann and G. Pilwat, Organ Specific Application of Drugs by Means of Cellular Capsule Systems (In German) 732
 628 R. T. C. Huang, Labeling of Animal Cells with Fluorescent Dansyl Cerebroside 737
 629 B. Ruth and F. A. Popp, Experimental Investigations on Ultraweak Photoemission from Biological Systems (In German) 741

Notes

W. Baumeister, M. Hahn, and U. P. Fringeli, Electron-Beam Induced Conformational Changes in Polypeptide Layers: an Infrared Study 746
 635 M. Bühring, W. Francke, and V. Heemann, Volatile Substances from *Formica rufa* L. and *F. polyctena* Först. (In German) 748
 641 H.-G. Reinhardt, Reactivity and Delayed Neurotoxicity of Organophosphates (In German) 750
 646 V. C. W. Chu and D. W. Gruenwedel, On the Reaction of Methylmercuric Hydroxide with Methylcobalamin . . 753
 652 F. Blobel, J. Eberspächer, S. Haug, and F. Lingens, Enzymatic Formation of a *cis,cis*-Muconic Acid Derivative Using Pyrazon-Degrading Bacteria (In German) 756
 656 F. Blobel, J. Eberspächer, and F. Lingens, Enzymatic Formation of 4-Hydroxy-2-Oxovalerate Using Pyrazon-Degrading Bacteria (In German) 757
 661 F. W. Tischendorf, M. M. Tischendorf, and B. Wittmann-Liebold, Immunoglobulin Diversity: Correlation of Non-Allelic Antigenic Markers with the Basic Sequences of the Variable Region of Human Lambda Chains 758
 664 R. Richl, The Yolk Nucleus in Young Oocytes of *Neomacheilus barbatulus* (L.) (Teleostei, Cobitidae) (In German) 761
 675 C. B. Boschek and K. Hamdorf, Rhodopsin Particles in the Photoreceptor Membrane of an Insect 763
 679 K. Kirschfeld and P. Wenk, The Dorsal Compound Eye of Simulid Flies: An Eye Specialized for the Detection of Small, Rapidly Moving Objects 764
 683 H. Beug, J. H. Peters, and T. Graf, Expression of Virus Specific Morphological Cell Transformation Induced in Enucleated Cells 766

Birthdays

Professor Erwin Bünning 70 Jahre 1

Erratum

To H. J. Rurainski (30c, 761 [1975]) 110

Subject Index 769
 Authors Index 785

Studies on Plant Bile Pigments: Characterization of a Model for the Phytochrome P_r Chromophor

Hugo Scheer

Institut für Botanik, Universität München

(Z. Naturforsch. 31 e, 413–417 [1976]; received February 23, 1976)

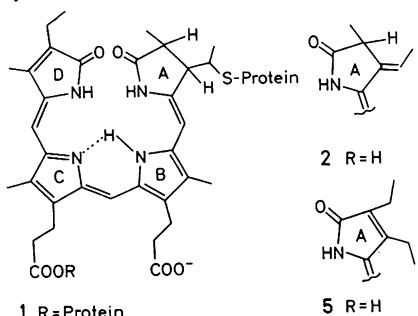
Dedicated to Prof. Dr. Dr. h. c. H. H. Inhoffen on the Occasion of His 70th Birthday

Bile Pigments, Phytochrome, Chromophor Model

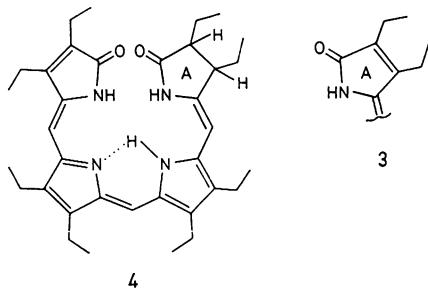
The common A-dihydrobobiliverdin chromophor proposed to be present in both phycocyanin and phytochrome P_r has been characterized by using octaethylbibiliverdin and its A-dihydro derivative as model compounds. The UV-vis spectra for the free bases, cations, anions and zinc complexes are reported, and the related protonation-deprotonation equilibria have been studied. The data support the postulated structures for the biliproteid chromophor, and at the same time they indicate A-dihydrooctaethylbibiliverdin to be a useful model.

Both the reaction center pigment of the photomorphogenetic phytochrome system, and the major photosynthetic antenna pigments in blue-green algae are biliproteids, in which the bile pigment chromophore is covalently linked by at least one bond to the protein¹. In the series of reactions leading from phytochrome P_r to P_{fr} and *vice versa*, it is probable that the genuine photo reaction(s) involve a transformation of the bile pigment chromophor². The detailed structure of this native chromophor and its reactivity and its interactions with the protein is yet unknown.

Structural studies on biliproteid chromophors involve mainly degradation experiments¹, and spectral studies in which the influence of the protein is assumed to be impeded^{1,3}. Hydrolytic cleavage of biliproteids yields a variety of structurally altered bile pigments. The only one of established structure, *viz.* phycocyanobilin (2) derived from phycocyanin (1), bears a 3-ethylidene group which is introduced during methanolysis^{3–8}. Degradation of phytochrome is even more ambiguous due to its poor accessibility.



Reprint requests should be sent to Dr. Hugo Scheer, Institut für Botanik, Menzinger Str. 67, D-8000 München 19.



Recently⁹, closely related structures with a common A-dihydrobobiliverdin chromophor have been advanced for the native chromophors of both phytochrome P_r ¹⁰ and phycocyanin on spectroscopic and reactivity arguments. In an attempt to characterize the salient properties of this A-dihydrobobiliverdin chromophor, a comparative study of octaethylbibiliverdin (3) and its A-dihydro analogue (4) is in progress. Here, the UV-vis spectra of the free bases, the cations, the anions and the Zn complexes, as well as the pK values of 3 and 4 are reported. The data support the A-dihydrobobilin structure postulated for native biliproteid chromophors and at the same time they prove 4 to be a useful compound for model investigations of the phycocyanin and phytochrome pigments.

Experimental

Octaethylbibiliverdin (3) and A-dihydrooctaethylbibiliverdin (4) were prepared from octaethylporphyrin¹¹, *via* *trans*-octaethylchlorin¹² by the method of Cavaleiro and Smith¹³. All reagents were reagent grade, guanidinium hydrochloride was recrystallized twice. UV-vis spectra were recorded under anaerobic conditions with a DMR-22 spectrophotometer

(Zeiss, W.Germany). Deoxygenated methanol containing sodium ascorbate (5×10^{-4} M) was used as solvent if not otherwise noted. The anions, cations and Zn-complexes of the bilins were prepared by adding small aliquots of concentrated solutions of KOH, HCl and Zn (OAc)₂ in MeOH under oxygen free conditions. All spectra are corrected for dilution. The pK determinations were carried out in the spectrophotometer cell under purified nitrogen by adding 1 N aqueous HCl or NaOH to solutions of the bilin (5×10^{-6} N) in a 1:1 mixture of MeOH and aqueous guanidinium-hydrochloride (6 M). The pH was monitored with a glass electrode and is uncorrected. The absorption changes of at least two bands were recorded independently. The reported pK values (averaged from three or more titrations) were determined from the first derivative of the E vs pH curves.

Results and Discussion

The UV-vis spectra (in dry methanol) of the two compounds **3** and **4**, and their cations, anions and Zn complexes, respectively, are listed in Table I. Band position and extinction coefficients of the free bases are markedly dependent on the solvent system used. These solvent induced shifts are comparable for **3** and **4**. There is a general red shift with increased solvent polarity according to the $\pi \rightarrow \pi^*$ type transitions involved, but pronounced shifts for the same solvent are frequently observed. In untreated reagent grade methylene chloride, extremes of $\lambda_{\text{max}} = 645$ and 670 nm, have been found for the red band of **3**. Comparably wide margins have been reported frequently for bile pigments and denaturated biliproteids^{1, 9}. At least one important factor for these variations is the easy cation formation of **3** and **4**, which can be suppressed by treatment with NaHCO₃, but aggregation seems to be important,

too. In methanol, both factors are reduced, and the absorption maxima found for **3** and **4** are reproducible within ± 2 nm.

The spectra of the free bases of **3** and **4** in methanol are very similar to each other, but that of the A-dihydrobilin **4** being shifted uniformly by about 1500 cm⁻¹ to the blue. Upon cation formation, the red band of **3** is shifted to longer wavelengths, and increased about twice in intensity. At the same time, the band is sharpened, and is accompanied by a pronounced shoulder at shorter wavelengths. The latter is present in the free base, too, but is obscured there by the increased width of the bands. By contrast, the blue band is almost unchanged except for a small red shift. The spectral changes of **4** upon cation formation are very similar, the cation of **4** exhibiting a uniform blue shift of about 500 cm⁻¹ as compared to that of **3**.

In the spectrum of the anion of **3**, the red band is shifted by 2280 cm⁻¹ to 770 nm. The intensity is only slightly increased, but again the asymmetry of this band is increased and a shortwavelength shoulder is apparent. The blue band is considerably more complex. Although the major maximum remains almost at the same position, the extinction coefficient is considerably reduced and intensity is transferred to a new, resolved band at 328 nm and a shoulder at longer wavelengths. At very high concentrations of KOH (~6 mol/l), this anion of **3** is further deprotonated. The product is unstable and transforms to a variety of compounds with ill defined absorptions in the visible range. The spectral changes of **4** upon addition of small amounts of methanolic KOH are similar, the spectrum of the anion of **4**, as compared to that of **3**, being shifted to shorter wavelengths (Fig. 1). However, the pure monoanion of **4** is difficult to prepare. Not only leads the presence

Table I. UV-vis spectra [$\lambda_{\text{max}} (\varepsilon \times 10^{-3})$ in methanol] of the free bases, cations, anions and zinc complexes of A-dihydro-octaethylbiliverdin (**4**) and octaethylbiliverdin (**3**). *, shoulder.

A-dihydrooctaethylbiliverdin (4)					Octaethylbiliverdin (3)		
free base	594 (17.6)	347 (39.4)	275 (20.4)	657 (15.6)	367 (51.2)	300 * (26.1)	275 * (21.6)
cation	665 (34.0)	351 (36.5)	279 (16.3)	693 (31.2)	357 (55.9)		
anion I	720 (19.2)	400 (27.1)	352 (32.6)	770 (16.6)	372 (39.6)	328 (29.0)	282 (23.5)
anion II	766 (19.8)	710 * (12.1)	406 (30.1)	357 (31.2)	810 (22.3)	400 * (41.2)	375 (28.3)
zinc complex	638 (21.3)	377 (28.3)	343 (30.0)	384 (19.7)	691 (22.3)	370 (28.3)	330 * (21.0)

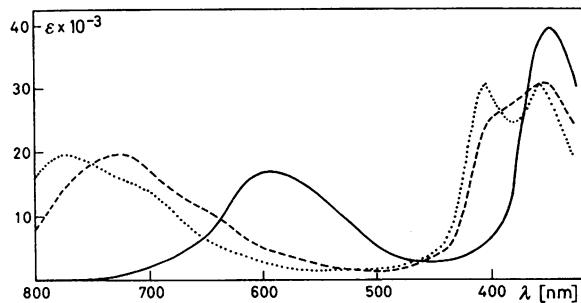


Fig. 1. UV-vis spectra of A-dihydrooctaethylbiliverdin (4) (—), its monoanion (---) and dianion (····). In methanol and KOH/methanol, respectively.

of equilibrium amounts of the dianion (see below) to a shoulder around 770 nm and 400 nm, but it is also readily oxidized to a purpurin anion giving rise to additional peaks at 625, 577, 540 and 328 nm.

The equilibrium between the two anions of **4** is shifted with increasing KOH concentrations, and above 1.5×10^{-3} mol/l only the second anion is present. Its formation is accompanied by an additional shift of the red band to longer wavelengths ($\lambda_{\text{max}} = 766$ nm) and by a distinct split of the Soret band ($\lambda_{\text{max}} = 406, 357$ nm) (Fig. 1). Like the monoanion, it is readily oxidized by traces of oxygen to a purpurin anion, while it decomposes slowly under anaerobic conditions to products without pronounced absorptions in the visible range. Although there are indications that the dianion of **4** can be further deprotonated under the conditions leading to the deprotonation of **3**, the product is too unstable under these conditions for a detailed characterization.

In the spectrum of the Zn-complex of **3**, as compared to the free base, the red band is increased in intensity, and it is shifted by 495 cm^{-1} to longer

wavelengths. In addition to the common blue shoulder, a red shoulder becomes apparent, too. The blue band of Zn-**3** is considerably broadened, without any apparent fine structure. While the spectral changes of **4** upon complexation with Zn are similar in the red region, the blue band shows increased fine structure. It is split and shows additional shoulders extending as far as 410 nm. Like the anion, the Zn complex of **4** is rather unstable in aerobic solution and is oxidized rapidly.

Bile pigments are generally characterized by two broad, poorly structured absorptions in the visible and near UV spectral range¹. The position of the red band has been correlated empirically with the size of the conjugated system by Köst *et al.*³. Roughly linear relationships have been found between the number of conjugated double bonds and the bathochromic shift of the red band for the free base bilins, as well as for their cations and zinc complexes. These results suggest a similar conformation for the non-rigid tetrapyrrolic system in the compounds investigated, because pronounced variations in both intensity and position of the red band are expected for conformation changes from theoretical calculations^{14–18}. The spectrum of the octaethyl-verdin **3** agrees well with that of mesobiliverdin IX α (**5**)³ to exclude a significant contribution of the different substituents in **3** and **5**. These similarities extend to the cations, anions and zinc complexes (Tables I, II). As the same is true for **4** as compared to denatured phycocyanin (**1**), respectively, these spectral similarities are good evidence for the A-dihydrobiliverdin structure for the native PC and P_r chromophors. In both cases the incremental shifts as well as the intensity changes of the red band are similar for the free bases, cations, and zinc complexes (Table II).

	Meso biliverdin IX α (5) ^a	Denatured Phytochrome P _r ⁹	Denatured C-Phycocyanin (1) (from <i>Pseudoanabena</i> spec. ²¹)	Δ [cm ⁻¹]
free base	630–655	620–625	590 [*]	610 ^b 590 ^c 583 ^a
cation	685	675–690	630 [*]	665–670 ^b 650 ^a
anion	—	765–770	—	675–770 ^b
zinc-complex	688	650	590 ^d	640 ^b 630 ^a
				590 [*] , d 583 [*] , d
				1090

Tab. II. Absorption (λ_{max} of the red band of mesobiliverdin IX α (**5**) in methanol, and of denatured phycocyanin and phytochrome P_r. Δ = incremental shift of **1** vs. **5** due to hydrogenation of ring A.

a. from ref. 3; b. in 1 M guanidinium hydrochloride pH 8, from ref. 9; c. heat denatured in N/15 phosphate buffer, pH 7.2; d. probably free base absorption due to incomplete complexation. *, shoulder.

The only significant deviation is that between the anions of **4** and **1**. The anion of **4** absorbs at 720 nm, as compared to 770 nm for the anion of **1**⁹. However, **4** can be deprotonated further easily (in contrast to **3**) to form a second anion absorbing at 766 nm, which agrees well with the data reported for the anion of **1** at a pH > 11⁹. From titration experiments, it is likely that **4** can be deprotonated easily in two stages leading subsequently to a monoanion ($\lambda_{\text{max}} = 720 \text{ nm}$), and dianion ($\lambda_{\text{max}} = 766 \text{ nm}$), and that denatured **1** was present as the dianion under these conditions. It should be noted that the pronounced red shift, shape and intensity of the anions makes them possible candidates for the P_{fr} chromophor. The long wavelength absorption has been accounted for by several authors to arise from an ionized chromophor^{1, 5, 9, 18, 19}, an idea which is especially attractive in view of the shorter conjugation system suggested recently for P_{fr} ⁹.

Both the easy abstraction of a second proton and the spectral similarities of the dianions of **4** (and **1**) and the monoanions of **3** (and **5**), respectively, can be rationalized in a straight-forward way. The biliverdin **3** contains formally one olefinic substituted 2H-pyrrole ring (C), one 1H-pyrrole ring (B), and two olefinic substituted 4-pyrrolin-2-one rings (A, D). The latter three bear NH-protons, of which the pyrrole ring B as expected to be deprotonated most easily, yielding the monoanion of **3**. The second deprotonation at the lactam-type nitrogens of rings A and D is considerably more difficult, the corresponding dianion is probably the species observed in concentrated methanolic KOH. In **4**, the first deprotonation is again expected to occur at the pyrrole type nitrogen of ring B, yielding a correspondingly shorter conjugation system than **3**, in agreement with the absorption at shorter wavelengths. However, the second deprotonation of **4** at ring A is facilitated for two reasons: The acidity of the hydrogenated pyrrolidin-2-one-ring A in **4** is expected to be higher than that of the pyrrolin-one-rings in **3**, if judged from the acidity of succinimide and maleimide, respectively, and the deprotonation of ring A leads to its conjugation with rings B, C and D. As a consequence, the conjugation system of the dianion of **4** is now similar to that of the monoanion of **3**, as reflected by its visible spectrum. Further deprotonation of both ions is difficult and proceeds only at high KOH concentrations. However, in spite of the spectral similarities of the anions of **3** and **4**, there

are pronounced differences in their reactivity. This increased reactivity of the anions of **4** is obviously a direct result of the hydrogenated ring A, and the involved reactions are currently studied.

To characterize the protonation-deprotonation equilibria of **3** and **4** further, their pK values were determined by acid-base titrations in a mixed solvent system of aqueous concentrated guanidinium hydrochloride buffer and methanol. The main reason for the use of this solvent was that both denatured biliproteids and the octaethylbilins are sufficiently soluble and stable in it, and that data for a variety biliproteids have been recorded in guanidinium-HCl buffered solutions^{1, 9, *}. An absolute pH scale for the system is yet to be defined.

For the cation formation of a series of bilins, increased pK values have been related empirically to a decreased extension of the conjugation system^{1, 2, 9, 20}. Thus, in the series mesobiliverdin (4 conjugated rings), mesobiliviolin (3 rings), urobilin (2 conjugated rings), the pK increases from 3.0 to 7.4. The pK values of denatured biliproteids do not correlate with this series in having pK values which are about 2 units too high, an increase which has been attributed to the hydrogenation of ring A^{1, 9}. The data obtained for the model compounds principally support this effect of the reduced double bond to increase the pK of the cation-free base transformation. For the biliverdin **3**, a pK of 5.11(±0.1) has been obtained, while the A-dihydroverdin (**4**) has a pK of 5.55. This difference is less than the one observed for denatured biliproteids. To separate levelling effects of the solvent system, and possible influences from the protein, a more detailed characterization of the acid-base transformation in bilins is under way.

The observed similarities between the octaethylbilins **3** and **4**, and the natural bilins with the IX α -substitution pattern, respectively, support the proposed structure of the chromophors of phycocyanin and phytochrome P_{r} as being A-dihydrobiliverdins. The results indicate, that the influence of the β -pyrrolic substituents on the properties of the tetrapyrrole-chromophor is small enough to render **4** a suitable model for a more extensive characterization of the free biliproteid chromophors.

* Although aqueous detergent solutions are principally suitable, too, the used Triton X-100 (10^{-4} M) gave less reproducible results and facilitated side reactions during the titration experiments.

¹ W. Rüdiger, *Fortschr. Chem. Org. Naturst.* **29**, 60 [1971]; *Ber. Dt. Bot. Ges.* **88**, 125 [1975].

² R. E. Kendrick and C. J. P. Spruit, *Photochem. Photobiol.* **18**, 153 [1973]; D. R. Cross, H. Linschitz, V. Kasche, and J. Tenenbaum, *Proc. Nat. Acad. Sci. U. S.* **61**, 1095 [1968]; L. H. Pratt and W. L. Butler, *Photochem. Photobiol.* **8**, 477 [1968]; W. R. Briggs and D. C. Fork, *Plant Physiol.* **44**, 1081 [1969].

³ H. P. Köst, W. Rüdiger, and D. J. Chapman, *Liebigs Ann. Chem.* **1975**, 1582.

⁴ C. O'hEocha, *Biochemistry* **2**, 375 [1963]; W. J. Cole, D. J. Chapman, and H. W. Siegelman, *Biochim. Biophys. Acta* **153**, 692 [1968].

⁵ H. L. Crespi, U. Smith, and J. J. Katz, *Biochemistry* **7**, 2232 [1968].

⁶ W. Rüdiger and P. O'Carra, *Eur. J. Biochem.* **7**, 509 [1969].

⁷ H. Brockmann, jr. and G. Knobloch, *Chem. Ber.* **106**, 803 [1973].

⁸ A. Gossauer and W. Hirsch, *Liebigs Ann. Chem.* **1974**, 1496.

⁹ S. Grombein, W. Rüdiger, and H. Zimmermann, *Hoppe-Seylers Z. Physiol. Chem.* **356**, 1709 [1975].

¹⁰ H. W. Siegelman, B. C. Turner, and S. B. Hendricks, *Plant Physiol.* **41**, 1289 [1966].

¹¹ We are indebted Prof. H. H. Inhoffen, Braunschweig, for a generous gift of octaethylporphyrin.

¹² H. W. Whitlock, R. Hanauer, M. Y. Oester, and B. K. Bower, *J. Amer. Chem. Soc.* **91**, 7485 [1969].

¹³ J. A. S. Cavaleiro and K. M. Smith, *J. C. S. Perkin I* **1973**, 2149.

¹⁴ R. Chae and P. S. Song, *J. Amer. Chem. Soc.* **97**, 4176 [1975].

¹⁵ G. Blauer and G. Wagnière, *J. Amer. Chem. Soc.* **97**, 1949 [1975].

¹⁶ J.-H. Fuhrhop, P. K. W. Wasser, J. Subramanian, and U. Schrader, *Liebigs Ann. Chem.* **1974**, 1450.

¹⁷ M. J. Burke, D. C. Pratt, and A. Moscowitz, *Biochemistry* **11**, 4025 [1975].

¹⁸ T. Sugimoto, K. Ishikawa, and H. Suzuki, *J. Phys. Soc. Jap.* **40**, 258 [1976].

¹⁹ W. Rüdiger and D. L. Correll, *Liebigs Ann. Chem.* **723**, 208 [1969].

²⁰ C. H. Gray, A. Kulczycka, and D. C. Nicholson, *J. Chem. Soc.* **1961**, 2276.

²¹ W. Rüdiger, G. Muckle, and R. Wagenmann, *Arch. Mikrobiol.*, in press.