KURZE MITTEILUNGEN

Bis am 20. des Monats bei der Redaktion eingehende Kurze Mitteilungen werden in der Regel am 15. des folgenden Monats veröffentlicht
Es werden auch Manuskripte aus dem Ausland angenommen

Polymerization-Depolymerization of Tobacco Mosaic Virus Protein: Refinement of a Model*

Summary

Detailed analysis of the difference between "low temperature," *p*H insensitive, and "high temperature," *p*H sensitive polymerization of TMV A protein has led to a revision of the model originally proposed by LAUFFER. All of the basic postulates are retained, but the positions of the various types of water-interacting center on the surface of the monomeric unit have been changed. In the new model, lateral polymerization will take place at "low temperatures" and will lead to the formation of double discs. At "high temperatures," diagonal polymerization will take place leading to open helices, possibly like those shown by the PM 2 strain. At still higher temperatures, the open helices polymerize side by side to form closed cylindrical helices similar to TMV. A consequence of this theory is that polymerization at *p*H 7.5 should result primarily in the formation of double discs and polymerization at *p*H values below 6.5 should form open helices and complete helical cylinders. Preliminary optical rotatory dispersion measurements and electron microscope results are consistent with this prediction.

Two models have been proposed to explain the endothermic polymerization of TMV A protein. The earlier identifies the positive enthalpies in excess of 100,000 cal/mole of "bond" and the correspondingly high positive entropies for polymerization with the stacking of double discs or the end-to-end joining of helical cylinders.[1] A more recent model associates these enthalpies and entropies with the formation of double discs.[2] Several recent developments demonstrate that neither identification is correct.

It is now clear[5] that low temperature polymerization in 0.1 ionic strength phosphate buffer, *p*H 6.5, exemplified by the osmotic pressure results at temperatures below 14°,[3] is a radically different process from high temperature polymerization in the same buffer, as exemplified by the results obtained at temperatures above 14°C.[4] Not only does the low temperature polymerization have a ΔH^0 of +30,000 cal/mole and a ΔS^0 of +126 e.u. as contrasted with values of +206,000 cal and 739 e.u. for high temperature polymerization, but also low temperature polymerization is accompanied by small volume changes[5] and no change in charge[6] in contrast with large volume changes[5] and a charge change of about 1 ion per chemical subunit[6] for the high temperature process. By comparing results obtained at concentrations of about 1 mg/ml[4,7,8] with results obtained at higher concentrations,[3] it is clear that the high temperature process covers the weight average molecular weight range from ca 100,000 or less to ca 5,000,000 or more. We have repeated such experiments over a wider range of concentrations on a single sample of TMV A protein with similar results. Since double discs have a molecular weight of ca 560,000, one should expect different values of ΔH^0 and ΔS^0 for data obtained at molecular weights below and above 560,000, if the actual process involves formation of double discs followed by stacking. No such result is observed. Finally, SIEGEL *et al.*[9] have shown that the protein from the PM 2 strain of TMV polymerizes in several forms, the simplest of which is an open "single" helix with a pitch of 280 to 290 Å and a diameter of 120 Å. We have found in a preliminary experiment that the high temperature polymerization of this protein has positive ΔH^0 and ΔS^0 values comparable to those for the high temperature polymerization of the protein of the common strain of TMV.

It is proposed, therefore, that high temperature polymerization of normal strains of TMV A protein follows approximately the pattern illustrated in Figure 1, which

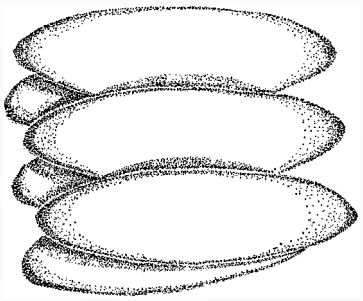


Figure 1. Diagonal polymerization. All ellipsoids point toward a common vertical axis on the right

* Received July 6, 1967.

1 D.L.D. CASPAR, *Advances Protein Chem. 18* (1963) 37.
2 M.A. LAUFFER, *Chimia 20* (1966) 89.
3 K. BANERJEE and M.A. LAUFFER, *Biochemistry 5* (1966) 1957.
4 C.E. SMITH and M.A. LAUFFER, *Biochemistry 6* (1967) in press.
5 M.A. LAUFFER and C.L. STEVENS, *Advances in Virus Res. 13* (1967) in press.
6 R.B. SCHEELE and M.A. LAUFFER, *Biochemistry 6* (1967) in press.
7 R.A. SHALABY and M.A. LAUFFER, *Biochemistry 6* (1967) in press.
8 M.T.M. KHALIL and M.A. LAUFFER, *Biochemistry 6* (1967) in press.

we will call "diagonal polymerization," forming open helices like the "single" helices of SIEGEL *et al.*,[9] while low temperature polymerization follows a pattern which leads to double discs and which we will call "lateral polymerization," as illustrated in Figure 2. The pitch and outside diameter in solution of the open helices might be 375 Å and 190 Å, respectively, instead of the somewhat lower values observed for dried preparations.[9] Diagonal polymerization could proceed from very low to very high molecular weights without change in ΔH^0 and ΔS^0, consistent with observations. Furthermore, such open helices of normal TMV protein could later polymerize side by side to form the closed cylindrical helices normally found as the end product of extensive polymerization. Protein from the PM2 strain seems to be unable to participate readily in this final step.

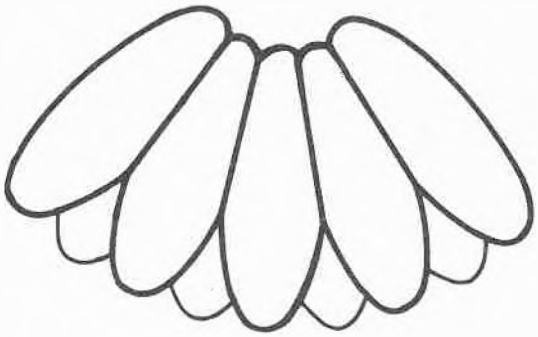


Figure 2. Lateral polymerization

To accommodate this changed point of view, LAUFFER'S model must be revised slightly, even though the principles originally set forth[2,10] can be preserved. The idea that there are four types of water interacting center with "melting temperatures" ranging from lowest Type I to highest Type IV is retained. However, the locations of these centers must be changed. (The reader should consult reference[2] for a detailed description of the model, omitted here for the sake of brevity; only changes are here described.)

Figure 3A illustrates a possible arrangement. It is now assumed that when the temperature is above the melting temperature of Type II but below the melting temperature of Types III and IV centers, polymerization proceeds as in Figure 2 and in Figure 3B to the formation of double discs. It is also assumed that Type II centers can be isolated from water without change in hydrogen ion dissociation. In the double disc at the temperature under consideration, all hydrophobic centers will be out of contact with water and all hydrophilic centers will be in contact with water. Double discs can stack only when the temperature is high enough to make both Type III and Type IV centers hydrophobic.

[9] A. SIEGEL, G. J. HILLS and R. MARKHAM, *J. Molec. Biol. 19* (1966) 140.

[10] M. A. LAUFFER, *Biochemistry 5* (1966) 2440.

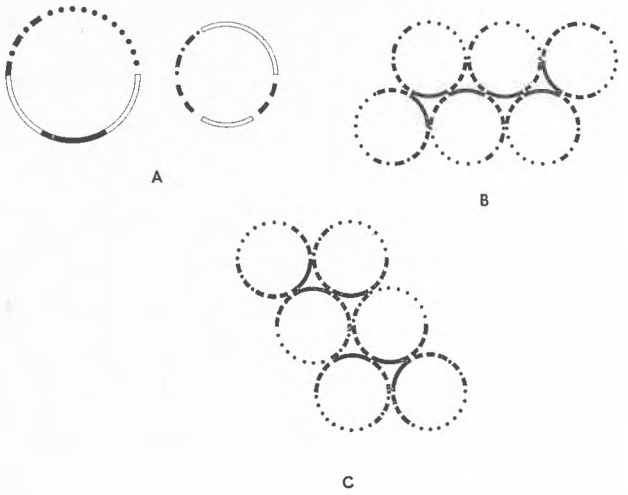


Figure 3. Diagram showing positions of water-interacting centers on ellipsoid of revolution. Type I ——; Type II — — —; Type III ·—·—·; Type IV ···

A Large circle represents a band near the center or maximum diameter of ellipsoid of revolution. Smaller circle represents a band near the inner end of ellipsoid.
B represents lateral polymerization.
C represents diagonal polymerization.

In both B and C, centers shown on the large and on the small circles in A are shown on a single circle. In all cases, the long axis of the ellipsoid of revolution is approximately perpendicular to the plane of the diagram

Type III centers are located in the positions illustrated in Figure 3. When the temperature is raised above the melting temperature of Type III centers but still below that of Type IV centers, existing polymers will rearrange by simple rotation of a few ellipsoids into the form illustrated by Figure 3C, and subsequent polymerization will be diagonal. It is observed that in this arrangement some Type II centers must be exposed to water. However, what determines whether or not a side of an ellipsoid can be exposed to water in our hypothesis is the net free energy of interaction with water. As long as Type IV centers are sufficiently hydrophilic to overcome the hydrophobic tendency of Type II centers, the arrangement shown in Figure 3C will be thermodynamically stable. An interesting feature of both the lateral and the diagonal modes of polymerization is that individual ellipsoids, except at open ends, are all oriented either bottom-side down or bottom-side up. Type III centers are assumed to contain groups which must bind H^+ ions before they can be isolated from water.

According to our present view, the formation of the ultimate helical cylinders (with pitch ca 23 Å) comes about by the lateral polymerization of the open helices. This should happen when the temperature exceeds the melting temperature of Type IV centers. It is necessary to assume that half of the ellipsoids will rotate about their long axes so that all are oriented bottom-side down in the final cylindrical helix. Since the protein of the PM2 strain does not form the cylindrical helix, it is assumed that the known chemical differences between

the PM2 and common strain proteins occur at Type IV centers.

If the present model is correct, one would predict that raising the *p*H would suppress the formation of open helices but would not affect materially the formation of double discs. Thus, whatever polymerization takes place at high *p*H should be in the double disc form. Optical rotatory dispersion measurements, to be reported in detail elsewhere,[11] indicate that at *p*H values of 6 and 6.2, there is an increase in helicity correlated with polymerization as temperature is raised from 7 to 25 °C. However, at *p*H 6.8, no remarkable change in helicity is indicated between 12 and 34 °C, even though solutions were clear at 12 and turbid at temperatures above 25 °C. This result is consistent with our model.

Finally, electron micrographs obtained after spraying at 20 °C solutions of TMV A protein at concentrations of 6.5 and 9 mg/ml in 0.1 ionic strength phosphate-ammonium acetate buffer at *p*H 7.5 and staining negatively with PTA showed discs in abundance and a few stacked discs. In contrast, similar electron micrographs obtained after spraying at 20 °C from solutions at a concentration of 0.2 mg/ml in 0.1 ionic strength phosphate-ammonium acetate buffer at *p*H 5.25 showed fully formed TMV-like rods and also definitely thinner rods reminiscent of the open helices obtained by SIEGEL *et al.* for the PM2 protein. The original micrograph reproduced as Figure 1 in LAUFFER *et al.*[12] shows occasional thin rods, heretofore unnoticed, as well as the usual ones. These results confirm our prediction.

[11] M.T.M. KHALIL, unpublished results.

Acknowledgments

The authors are indebted to Dr. ALBERT SIEGEL for the samples of PM2 protein and to Mrs. ALICE P. ESTES for the preparation of electron micrographs and for drawing Figure 1.

MAX A. LAUFFER, RAGAA A. SHALABY and M. THANAA M. KHALIL

Department of Biophysics and Microbiology*
University of Pittsburgh
Pittsburgh (Pennsylvania) 15213 (U.S.A.)

* Publication No. 132 of the Department of Biophysics and Microbiology. Work was supported by a U.S. Public Health Service Grant No. GM 10403.

[12] M. A. LAUFFER, A. T. ANSEVIN, T. E. CARTWRIGHT and C. C. BRINTON jr., *Nature 181* (1958) 1338.

Solubilization of a Tissue-Bound Activator of Plasminogen under Physiological Conditions*

Summary

The extraction of a tissue-bound activator of plasminogen under physiological conditions is effected by an autolytic release mechanism. Solubilization of activator is achieved by incubating pre-washed organ tissue homogenates (porcine lung or heart) for 6 h at room temperature, in phosphate-saline buffer, *p*H 7.3 to 7.4. The clear extracts which are devoid of cells or cell debris, exhibit plasminogen activating properties. The influence of extraction duration, *p*H and temperature on the yield of activator and spontaneous proteolytic activity is discussed. This procedure gives activator preparations comparable with those of previously published methods.

Organ tissue is known to contain a substance capable of activating the fibrinolytic system by converting plasminogen to plasmin.[1] The plasminogen activating properties, ascribed to the action of so-called tissue activator, were shown to reside mainly in the microsomal cell fraction.[2] Tissue activator is firmly bound to the particulate cell material[3] and can therefore not be solubilized under physiological conditions. Extraction from tissue homogenates was brought about under relatively harsh conditions, such as in the presence of concentrated salt (2 M KCNS)[4] or urea[5] or at acidic *p*H.[6]

We wish to report a method which permits the extraction of tissue activator at physiological *p*H and ionic strength, involving an autolytic release of the plasminogen activator from organ tissue homogenates.**

* Received July 7, 1967.

[1] T. ASTRUP and P. M. PERMIN, *Nature 159* (1947) 681.

[2] H. J. TAGNON and L. E. PALADE, *J. Clin. Invest. 29* (1950) 317.

[3] P. M. PERMIN, *Nature 160* (1947) 571.

Experimental

1. Phosphate-saline buffer

0.01 M phosphate buffer (according to SOERENSEN), *p*H 7.3 to 7.4, containing 0.9% NaCl. To prevent bacterial growth, merthiolate was added to a concentration of 0.01%.

2. Plasminogen

We originally used an euglobulin preparation obtained after dialyzing human serum and acidifying to *p*H 5.3. The precipitate was isolated, dissolved in phosphate-saline buffer and reprecipitated at *p*H 7.3 by dilution with 19 parts of cold,

** Part of the experimental work is described in detail in [7].

[4] T. ASTRUP and A. STAGE, *Nature 170* (1952) 929.

[5] D. C. MCCALL and D. L. KLINE, *Thromb. Diath. Haemorrhag. 14* (1965) 116.

[6] F. BACHMANN, A. P. FLETCHER, N. ALKJAERSIG and S. SHERRY, *Biochemistry 3* (1964) 1578.

[7] I. BAUNOK, *Versuche zur Isolierung eines Gewebe-Aktivators für Plasminogen*, Dissertation, Bern 1965.

deionized water. This preparation contains considerably less inhibitory material as compared to the *p*H 5.3 precipitate.In order to exclude inhibitors entirely and to possess a better defined product, we later preferred to use a more extensively purified plasminogen. It was prepared from a plasminogen rich globulin fraction (supplied by the Central Laboratory of the Blood Transfusion Service of the Swiss Red Cross, Berne) as described by NITSCHMANN *et al.*[8] After adjusting the *p*H to 4.0, the final solution was stored at −20 °C. Immediately before use the *p*H was brought to 7.8 to 8.0 by adding a few crystals of solid Tris (tris [hydroxymethyl] aminomethane) and the protein content was adjusted to 0.4 mg/ml.

3. *Determination of activator activity*

Activator activity was assayed quantitatively by activating a fixed amount of plasminogen and the plasmin activity thus generated was determined with a fluorescence method according to LÜSCHER and KÄSER-GLANZMANN,[9] using fibrinogen labeled with Lissamine-Rhodamine B. In order to assay separately for activator and nonspecific proteolytic activity, the original method had to be modified as follows: small round bottom centrifuge tubes were rinsed with acetone and heated to 120 to 130 °C for abour 30 min. Unless the vessels are not specially cleaned, the fibrin clot will not adhere properly to the glass wall. Then, 0.4 ml of fluorescence labeled fibrinogen (5 mg protein/ml) and 0.1 ml of thrombin (Hoffmann-La Roche, as an 0.1% solution in saline, containing 60 to 70 NIH units per mg protein) were pipetted into the tubes. After clot formation the tubes were stoppered and heated to 85 °C for 35 min to inactivate plasminogen. The assay mixture (usually 0.1 ml of plasminogen and 0.4 ml of tissue extract) was given on top of the fibrin gel and incubated for 20 h at 37 °C. The reaction was stopped in an ice-bath, undigested fibrin was broken up with a glass rod and the mixture diluted with water to 5.0 ml. Residual fibrin particles were removed by centrifugation and the fluorescence of the clear supernatant was measured in an Eppendorf fluorimeter.

The use of heated fibrin substrate permitted the separate determination of 1) total activity (A) of the tissue extract, 2) residual plasmin activity (B) of the plaminogen preparation, 3) nonspecific, spontaneous proteolytic activity (C) of the tissue extract, and 4) reagent blank (D) by chosing the proper combination of incubation mixtures. Activator activity was calculated according to:

$$\text{activator activity} = (A - B) - (C - D)$$

Activity is expressed in terms of scale units of the fluorimeter.

4. *Solubilization of tissue activator*

In our extraction experiments we used procine lung and heart tissue. Fresh organ material, obtained from the slaughterhouse and collected in ice, was washed externally with water. Connective tissue, fat, large blood vessels or trachea were removed and discarded. The tissue was then cut up into small pieces. They were frozen at −5 °C and ground in a pre-chilled meat grinder. 60 g of ground tissue, together with about an equal amount of dry ice was pulverized in a mixer for 2 min at full speed. After evaporation of the CO_2 the tissue residue was suspended in 300 ml of cold phosphate–saline buffer. The suspension was treated at 1 to 2 °C in the mixer twice for 1 min at full speed, and was then centrifuged for 1 h at 50,000 x g in a Spinco Model L centrifuge. This procedure serves to eliminate a considerable amount of soluble, inert protein. Under these conditions, the tissue activator remains bound to the tissue fragments and is therefore recovered quantitatively in the precipitate. The clear supernatant was discarded. The tissue sediment was suspended in a 150 ml portion of cold phosphate-saline buffer and broken up in the mixer at medium speed. After adding another 150 ml portion of phosphate-saline buffer the suspension was agitated in the mixer three times for 1 min at full speed. Homogenisation was subsequently effected in a homogenizer with a glass pestle. The tissue activator was then solubilized by incubating the homogenate during 6 h at room temperature in a stoppered flask. The mixture was kept in constant motion on a rolling machine. After extraction, the cell debris were removed by high-speed centrifugation (1 h at 50,000 x g), and the clear, reddish supernatant was recovered and assayed for activity. The solution can be kept at −20 °C for two weeks without any detectable loss of activity.

We investigated the influence of increased temperature and prolonged extraction time on the yield of activator activity and on the content of spontaneous activity of the extracts. Extraction at room temperature was found to be optimal. On extending the duration of the extraction to 20 h or increasing the temperature to 37 °C the yield of activator activity from porcine lung tissue remains practically constant; however, these extracts contained almost four times as much spontaneous activity as compared to the 6 h extraction at room temperature.

An investigation of the influence of *p*H upon the extraction clearly showed that the liberation of activator activity is optimal in the range between *p*H 6.9 and 8.6. At acidic *p*H values (*p*H 4.7) only trace amounts of activator activity went into solution. At alkaline *p*H (9.3) still considerable solubilization of the plasminogen activator occurs. The highest amounts of spontaneous activity were extracted between *p*H 7.5 and 8.0. Our extraction *p*H of 7.3 to 7.4 favors optimal yields of activator activity, yet, at this *p*H, the solubilization of spontaneous activity is still somewhat reduced as compared to higher *p*H values.

We also compared our method with those developed by BACHMANN *et al.*[6] and by MCCALL and KLINE.[5] For this purpose, equal amounts of the same organ (porcine heart) were extracted according to the three procedures. The extracts were dialyzed against 0.01 M Tris buffer, *p*H 7.4, in 0.9% NaCl solution. Activities were measured with the fluorescence method; they are expressed in terms of scale units per unit weight of fresh organ tissue. The results are given in Table 1.

Table 1

Method		Total activity	Spontaneous activity	Activator activity
BACHMANN *et al.*[6]	I	6.9	5.0	1.9
MCCALL and KLINE[5]	II	5.8	2.0	3.8
Our method	III	3.6	1.5	2.1

The amount of total activity extracted by methods I and II is not very different. In comparison, the activity obtained by our method (III) amounts to 52 to 62% of

[8] H. NITSCHMANN, U. P. SCHLUNEGGER and C. H. SCHNEIDER, *Vox Sanguinis* 7 (1962) 641.

[9] E. F. LÜSCHER and R. KÄSER-GLANZMANN, *Vox Sanguinis 6* (1961) 116.

methods I and II. The content of spontaneous activity of the extracts varies considerably. In method I it accounts for 73% of the total activity; in method II the corresponding figure is 35% and in our method 42%. The highest yield of activator activity is obtained with method II. The figures for methods I and III are lower, namely 50 and 55%, respectively, of method II.

We have demonstrated that under physiological conditions an activator of plasminogen is solubilized from organ tissue homogenates. The liberation involves an autolytic release, most likely under the influence of enzymatic action. The activator is obtained in yields comparable to those of previously published methods, and the content of spontaneous activity is relatively low. Work presently in progress has indicated that activator solubilized by the three methods is probably identical. Details of this investigation will be published later.

We gratefully acknowledge the financial support of the Swiss National Science Foundation (grant No. 3704), of the «Kommission zur Förderung der Eiweißforschung an der Universität Bern» and of the Central Laboratory of the Blood Transfusion Service of the Swiss Red Cross, Berne.

E. E. Rickli*, I. Baunok, H. Zaugg and Hs. Nitschmann

Theodor Kocher Institute and Institute of Organic Chemistry. University of Berne, Berne (Switzerland)

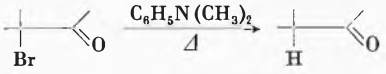
* Author's address: Dr. Egon E. Rickli, Theodor-Kocher-Institut, Freiestrasse 1, 3000 Bern (Switzerland).

Reductive Debromination of α-Bromo- and α,α-Dibromoketones with N,N-dimethylaniline*

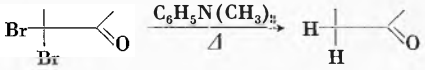
Common means for the replacement of an α-halogen of a ketone with hydrogen are catalytic hydrogenation and reactions with electropositive metals like zinc and sodium amalgam in a suitable protic solvent. We wish to report now an alternative method for the reductive debromination of ketones making use of N,N-dimethylaniline.

In a typical experiment α-bromo-*d*-camphor (7.5 g) was refluxed with 60 cm³ of N,N-dimethylaniline (free from monomethylaniline) during 24 hours in an oil bath kept at 220 °C. The solution turned subsequently yellow, blue, violet and finally red-brown, while a small amount of sublimate appeared at the bottom of the condenser. After cooling to room temperature, the solution was diluted with 100 cm³ of diethyl ether. The ether solution was chilled to about 0 °C, extracted with 80 cm³ of chilled concentrated hydrochloric acid, washed with water and dried with anhydrous sodium sulfate. Evaporation of the solvent afforded 3.63 g of a white crystalline solid melting at 169–173 °C. A thin layer chromatogram revealed the presence of only one major component with the R_f value of *d*-camphor. Recrystallization from cyclohexane raised the m.p. to 178 °C[1]. A mixed m.p. with authentic *d*-camphor did not depress. The spectral characteristics of the product matched those of *d*-camphor, thus confirming the attribution. Yield: 75%. The aqueous acidic solution from the extraction of the reaction mixture, showed the qualitative colour changes with *p*H associated with the presence of a species of the crystal violet type. The material sublimed in the condenser was washed with boiling ether. The colourless solid[2] thus obtained was dissolved in water, the aqueous solution was made strongly alkaline with potassium hydroxide and extracted with ether. This ether extract, washed with water and dried over sodium sulfate, upon evaporation of the solvent, yielded a liquid containing N-methylaniline and N,N-dimethylaniline according to the spectral (i.r. and p.m.r.) evidences.

The reductive debromination of a ketone according to the schema



has been found to be a rather general reaction. α,α-Dibromoketones, in fact, are also reduced to the parent ketones by the action of hot N,N-dimethylaniline. The experiments made on a number of compounds are reported in Table 1.



In most of the cases the isolation of the pure debrominated ketone from the reaction mixture was straightforward. In table 1 are also reported the two sole precedents recorded in the literature.[3,4]

Tests with other amines, namely triethylamine, N-methylaniline and N,N-diethylaniline on α-bromo-*d*-camphor failed to give appreciable amounts of dehalogenated products. These results point to a high steric and electronic selectivity favouring N,N-dimethylaniline. On this basis it is attractive to picture the reductive debromination of ketones as going through the following sequence:

* Received July 10, 1967.

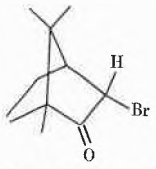
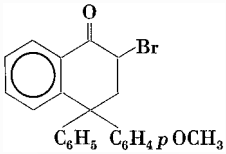
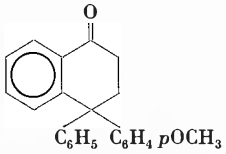
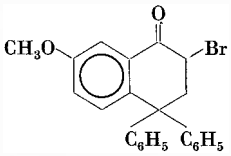
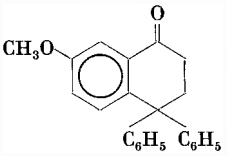
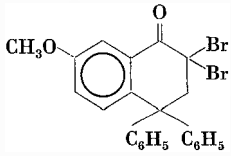
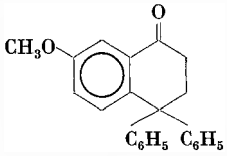
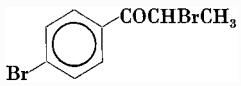
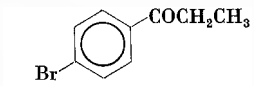
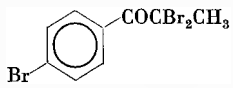
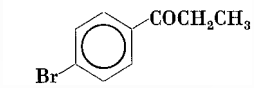
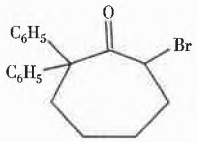
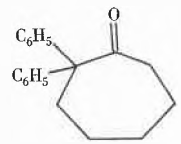
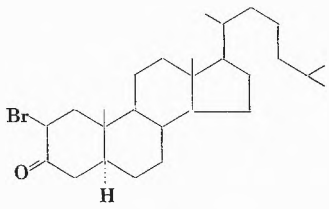
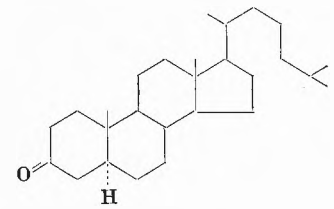
[1] Lit.: 178 °C.

[2] The ether washed solid did not exhibit any carbonyl absorption in the infrared (KBr).

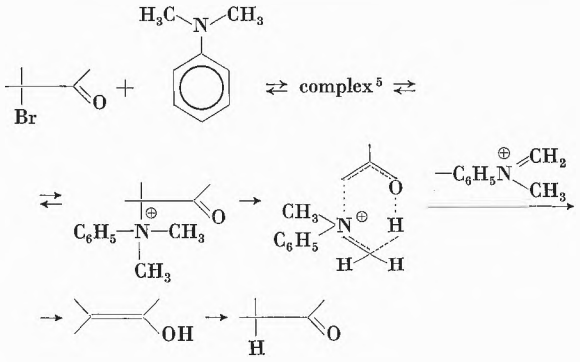
[3] E. Schwenk and B. Whitman, *J. Amer. Chem. Soc.* *59* (1937) 949.

[4] R. E. Lyle and R. A. Covey, *J. Amer. Chem. Soc.* *75* (1953) 4973.

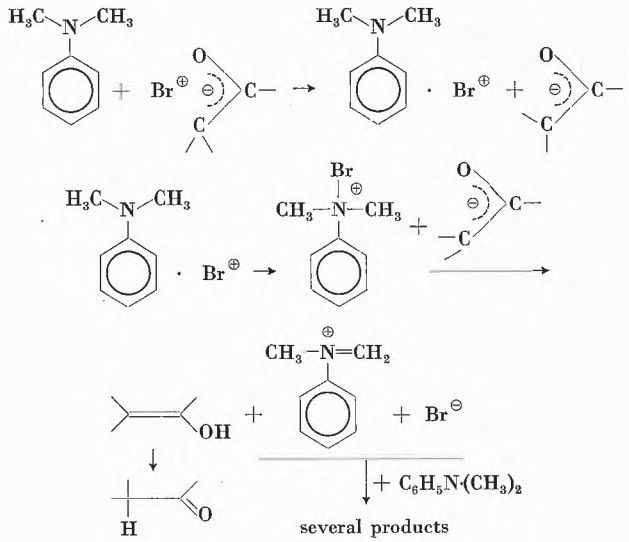
Table 1. Reaction of α-Bromo and α,α-Dibromoketones with N,N-dimethylaniline. Yields of Parent Ketones

Bromoketone	Reaction Time (h)	Parent Ketone	Yield (%)[a]
 α-Bromo-*d*-camphor	24	 *d*-Camphor	76[b]
 (±, ±) 2-Bromo-4-(*p*-anisyl)-4-phenyl-α-tetralone	8	 (±) 4-(*p*-Anisyl)-4-phenyl-α-tetralone	79
 (±) 2-Bromo-4,4-diphenyl-7-methoxy-α-tetralone	2	 4,4-Diphenyl-7-methoxy-α-tetralone	93
 2,2-Dibromo-4,4-diphenyl-7-methoxy-α-tetralone	12[c]	 4,4-Diphenyl-7-methoxy-α-tetralone	36
 α,*p*-Dibromopropiophenone	2	 *p*-Bromopropiophenone	56
 α,α,*p*-Tribromopropiophenone	14	 *p*-Bromopropiophenone	16[d]
 (±) 7-Bromo-2,2-diphenylcycloheptanone	10	 2,2-Diphenylcycloheptanone	87[e]
 α-Bromocholestanone	8	 Cholestanone	f

[a] In separated purified product. – [b] Average of two experiments. – [c] Reaction temperature: 120°C, then 1 hour reflux. – [d] The product was identified by proton magnetic resonance and its yield was determined standardizing the reaction mixture with acetone. – [e] From reference [4]. – [f] From reference [3]; the yields were not specified.



The qualitative and quantitative analysis of the nitrogen containing products has not yet been completed and it is difficult to evaluate the merits of the hypothetical mechanism here advanced. The presence of the crystal violet type product can be rationalized in terms of successive condensations and rearrangements of the iminium salt initially produced in the above scheme. N-methylaniline is usually found when N,N-dimethylaniline hydrobromide is heated at high temperature.[6] An alternative mechanism invokes a reaction of the «positive»[7] bromine atom with N,N-dimethylaniline and successive transfer of a proton from the N-bromoammonium cation to the anion according to the scheme:[8]



Angelo G. Giumanini

Istituto di Chimica Generale ed Inorganica, Facoltà di Scienze dell'Università di Milano, Italy and Department of Chemistry, University of Wisconsin** Madison (Wisconsin, USA).

[5] A yellow colour often appears as soon as the bromoketone and the amine are mixed at room temperature.

[6] W. Staedel, *Ber. dtsch. Chem. Ges. 19* (1886) 1947. E. W. Warnhoff and P. NaNonggai, *J. Org. Chem. 27* (1962) 1186.

[7] H. H. Jaffé and M. Orchin, *Theory and Applications of Ultraviolet Spectroscopy*, J. Wiley & Son, London 1962, p. 181.

** Present address of the author.

[8] A similar mechanism was suggested by G. Stork for explaining the reductive deiodination of α-iodoketonse with collidine; see: G. Rosenkranz, O. Mancera, J. Gatica and C. Djerassi, *J. Amer. Chem. Soc. 72* (1950) 4077, note 5. The author is indebted to a referee for pointing out this reference to him.

Beitrag zur Charakterisierung der Chlorophyllase*

Summary

The chlorophyllase discovered first by Willstätter and Stoll hydrolyses the phytylesterbond in chlorophylls. The enzyme was solubilized by homogenisation of *Chlorella pyrenoidosa* following the experimental procedure described by Böger. It was then purified by ammoniumsulphate precipitation and gelfiltration over sephadex G-200. Some properties of the purified product were investigated. Also the products of the enzymic reaction were analized.

Im Jahre 1910 berichteten Willstätter und Stoll[1] über eine «Chlorophyllase», die *in vitro* die hydrolytische Abspaltung der Phytylseitenkette vom Chlorophyllmolekül katalysierte. Da die Chlorophyllase sehr stark strukturgebunden ist, wurde die nähere Untersuchung des Enzyms außerordentlich erschwert, indem die älteren Autoren stets nur mit Blattpulvern arbeiten konnten. Diese Verwendung von Blattpulvern anstelle löslicher, gereinigter Enzympräparate, die erst vor kurzem teilweise erhalten wurden[2,3,4], führte naturgemäß zu zahlreichen Widersprüchen in den experimentellen Befunden. In einigen Fällen wurde z. B. beobachtet, daß unter Einwirkung der Chlorophyllase sich Chlorophyllid bzw. Phäophorbid und Phytol zu Chlorophyll bzw. Phäophytin vereinigten, während die Mehrzahl der Autoren diese Wirkung nicht beobachten konnte oder sie gar nicht untersuchte.

Auf Grund dieser Widersprüche schien es uns wünschbar, im Rahmen unserer Arbeiten über die Biochemie der Chloroplasten die Chlorophyllase zu isolieren, zu reinigen und zu charakterisieren.

Zunächst untersuchten wir mit Aceton extrahierte und getrocknete Blattpulver von *Herakleum sphondylium* (Bärenklau) und *Beta vulgaris* var. *saccharifera* (Zuckerrübe) auf ihre enzymatische Aktivität. Als Substrat wählten wir zuerst dünnschichtchromatographisch ge-

* Vorläufige Mitteilung. Eingegangen am 19. Juli 1967.

[1] R. Willstätter und A. Stoll, *Ann. Chem. 378* (1910) 18.

[2] A. C. Klein und W. Vishniac, *J. Biol. Chem. 236* (1961) 2544.

[3] M. Holden, *Photochem. Photobiol. 2* (1963) 175.

[4] S. Shimizu und E. Tamaki, *Arch. Biochem. Biophys. 102* (1963) 152.

reinigtes Chlorophyll a in einer Lösung, die 60% Aceton enthielt. Nach 3 bis 48 Stunden wurde der Umsatz durch Verteilung der Pigmente zwischen Petroläther und wäßrigem Aceton ermittelt, wobei die Oberphase das unveränderte Chlorophyll, die Unterphase das Chlorophyllid enthalten soll. Dabei stellte sich heraus, daß Aussagen über Chlorophyllaseaktivitäten, die nur auf Grund dieser Verteilung gemacht werden, mit Vorsicht aufzunehmen sind. Die dünnschichtchromatographische Trennung der Pigmente aus der wäßrig-acetonischen Unterphase ergab eine mit der Versuchsdauer steigende Anzahl von grünen Banden, die – von wenigen Ausnahmen abgesehen – alle gebundenes Phytol enthielten. Analog zu diesem Resultat enthielten die Oberphasen der Verteilung kein freies Phytol. Ferner beobachteten wir eine krasse Verkleinerung der Reaktionsgeschwindigkeit, wenn die Luft im Reaktionsgefäß durch Stickstoff verdrängt wurde. Diese Ergebnisse weisen auf die Anwesenheit einer hochaktiven Oxydase hin, deren Existenz auch von anderen Autoren bestätigt wird[5]. Aus diesem Grunde verwendeten wir für unsere weiteren Versuche das wesentlich oxydationsbeständigere Phäophytin als Substrat und kontrollierten ferner den Verlauf der enzymatischen Reaktion auf der Dünnschicht. Modifiziert wurde ebenfalls die Nachweisreaktion für den enzymatischen Umsatz, indem wir die Verteilung nunmehr zwischen Äther und dem mit 1 bis 2 ml 0,02 N Ammoniak alkalisch gemachten wäßrig-acetonischen Reaktionsmedium durchführten.

Es ergab sich schließlich, daß an der Existenz einer spezifischen Chlorophyllase nicht gezweifelt werden kann, denn:

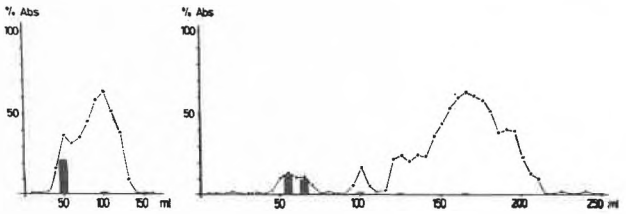
a) in der ätherischen Oberphase ließ sich freies Phytol nachweisen;
b) die Pigmente der Unterphase enthielten kein (gebundenes) Phytol;
c) die Pigmente der Unterphase ließen sich mit Methanol/HCl verestern zu einem Produkt, das von reinem Methylphäophorbid auf der Dünnschicht nicht zu trennen war.

Zur weiteren Untersuchung verwendeten wir lösliche Chlorophyllasepräparate, die nach Angaben von BÖGER[6] aus *Chlorella pyrenoidosa* gewonnen wurden. Das Enzym wurde aus diesen Grünalgen durch Homogenisation bei *p*H-Werten über 7 in Lösung gebracht. Die hellgrüne Lösung enthielt das Enzym in einer inaktiven Vorstufe, die durch Behandlung mit Trypsin in die aktive Form übergeführt wird.

Es wurde nun versucht, das Enzym von seinen Begleitstoffen (andere Proteine, Lipide, Salze) zu befreien und es so möglichst weitgehend zu reinigen. Dazu war das Vorgehen folgendes:

Die Algen wurden in einem Polyäthylenglykol enthaltenden Milieu suspendiert und anschließend homogenisiert. Das Homogenat wurde zentrifugiert und der Überstand 24 h gegen Wasser dialysiert. Die dialysierte Lösung wurde dann einer fraktionierten Ammonsulfatfällung unterworfen. Der Niederschlag zwischen 30 und 80% Ammonsulfat wurde gesammelt und in Wasser aufgenommen. Diese Lösung wurde etwas angesäuert und mit Äther erschöpfend extrahiert, dann wurde das *p*H wieder auf schwach alkalische Werte gebracht. Nach dem Zusatz von 0,1% Trypsin wurde die Lösung zur Aktivierung 2 Stunden bei etwa 30° stehen gelassen.

Der nächste Reinigungsschritt bestand in einer Gelfiltration durch eine Säule von Sephadex G-200. Es wurden Fraktionen von je 10 ml aufgefangen und die Proteinverteilung durch Messung der Lichtabsorption bei 280 nm bestimmt, wobei eine Elutionskurve entstand, wie sie in Abb. 1a dargestellt ist. Die Chlorophyllaseaktivität wurde in den Fraktionen mit Absorptionsmaxima untersucht. Dann wurde eine zweite Gelfiltration angeschlossen, wobei der Reihe nach die Fraktionen der ersten Sephadex-Trennung auf die Säule aufgegeben wurden. Damit wurde eine weitgehende Auftrennung in die einzelnen Komponenten erreicht, wie Abb. 1b zeigt. Auch nach dieser Trennung wurde wiederum an verschiedenen Stellen der Elutionskurve die Chlorophyllaseaktivität untersucht. Anschließend wurden die Fraktionen von 45 bis 55 ml Eluatvolumen zusammengefaßt und gefriergetrocknet.



a) 1. Gelfiltration b) 2. Gelfiltration

▬ Chlorophyllaseaktivität in % Absorption (gemessen bei 660 nm) der Subphase nach Ausäthern der Reaktionsmischung

Abb. 1. Elutionsdiagramme und Aktivitätsverteilung

Die Reinheit dieses Präparates wurde in der analytischen Ultrazentrifuge geprüft. Es zeigte sich, daß in diesem Proteinpulver noch mindestens zwei Komponenten vorhanden sind, wie dies ja eigentlich aus dem Elutionsdiagramm bereits hervorgeht. Aus den Ultrazentrifugenaufnahmen (s. Abb. 2) ließen sich die Sedimentationskonstanten berechnen. Die Sedimentationskonstante der Hauptkomponente beträgt 4,1 Svedberg-

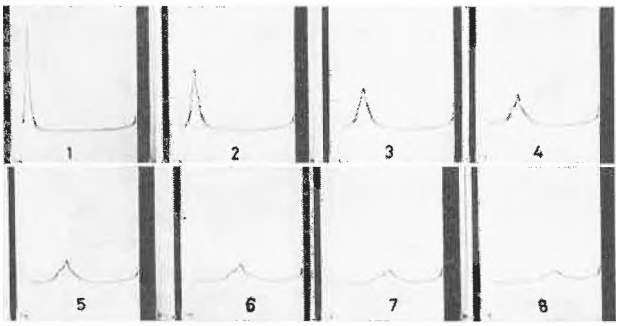


Abb. 2. Ultrazentrifugenaufnahmen der aktiven Proteinkomponente (1% Protein in 0,9% NaCl-Lösung, 20°, 59780 rpm, Aufnahmeabstand 16')

[5] F. C. PENNINGTON, H. H. STRAIN, W. A. SWEC und J. J. KATZ, *J. Amer. Chem. Soc. 86* (1964) 1418.
[6] P. BÖGER, *Phytochem. 4* (1964) 435.

Einheiten, diejenige der später erscheinenden Nebenkomponente 3,4 Sv.

An diesem gereinigten Produkt wurden bereits einige Untersuchungen zur Enzymspezifität durchgeführt. Wir konnten feststellen, daß weder ein niedermolekularer Säureester (Isoamylbenzoat) noch ein Porphyrinester (Phäoporphyrin-a_5-Dimethylester) von der Chlorophyllase hydrolytisch gespalten wird. Danach scheint sich die erstaunlich hohe Spezifität der Chlorophyllase zu bestätigen.

Die vollständige Reinigung und Charakterisierung des Enzyms bildet den Gegenstand unserer weitern Untersuchungen.

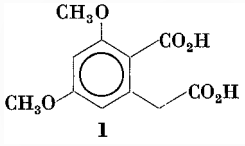
Diese Arbeiten wurden mit Unterstützung des Schweizerischen Nationalfonds durchgeführt.

E. C. Grob und J. Seiler
Institut für Organische Chemie der Universität Bern

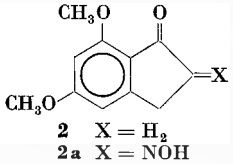
3,5-Dimethoxyhomophthalic Acid and its Transformation to Intermediates in Mold Metabolite Syntheses*

An efficient synthesis of 3,5-dimethoxyhomophthalic acid **1**, an important intermediate in the synthesis of various mold metabolites, is described. Heretofore, **1** was available synthetically only by the erratic and low yield sequence originating from the self-condensation of acetone dicarboxylic ester[1]. In the present synthesis of **1** and its transformation products **3**, **4**, **5** and **6**, all steps proceeded in 90% yield or better.

5,1-Dimethoxyindanone[2], which is readily available in high yield by cyclization of 3,5-dimethoxyphenylpropionic acid, was converted *via* the oximino ketone **2a** (m.p. 239–243 °d, found: C 59.69, H 4.95, N 6.43) by exchange-hydrolysis with formaldehyde and hydrochloric acid[3] to the yellow indandione **2b** (m.p. 148–151 °, found: C 63.77, H 4.85). Oxidation of **2b** with 10% hydrogen peroxide in acetic acid provided 3,5-dimethoxyhomophthalic acid **1** directly in pure form (m.p. 169 to 172 °, reported[1] 172–173 °, found: C 55.04, H 5.36).



1

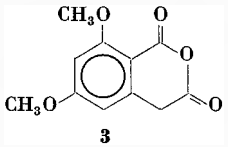


2 X = H_2
2a X = NOH
2b X = O

Treatment of **1** with acetic anhydride at 50 ° gave the anhydride **3** (m.p. 159–162 °, reported[1] m.p. 160–162 °); whereas in the presence of pyridine the diacetyl isocoumarin **4** was produced (m.p. 156–160 °d, found: C 59.08, H 4.70). Hydrolysis of **4** with 2% aqueous potassium hyroxide at 80 ° afforded the dimethyl ether of C-acetyl orsellinic acid **5** (m.p. 137–139 ° reported[1b] 139–140 °). Formation of the isocoumarin **6** (m.p. 155–156 ° reported[1a] 155.5 °) was best effected with acetic anhydride and a catalytic amount of perchloric acid.[4]

Ozonolysis of 6 in ethyl acetate solution at −60 °C yielded 2-formyl-4,6-dimethoxybenzoic acid 7 (m. p. 193–196 °), the aromatic component in the synthesis of zeralenone.[5]

Hydrogenation of the isocoumarin 6 over 10% Pd on charcoal catalyst in ethyl acetate solution yielded the known (±)3-methyl-6,8-dimethoxy-3,4-dihydroisocoumarin 8.[6] The latter was cleaved with boron trichloride in methylene chloride at 0 ° to give (±)3-methyl-6-methoxy-8-hydroxy-3,4-dihydro isocoumarin **8a** (m.p. 95–97 °, found: C 64.99, H 6.48) which did not respond to resolution *via* its 8-(1-menthoxy acetate) derivative. The optically active (−) form of **8a** was isolated from mouldy carrots.[7]



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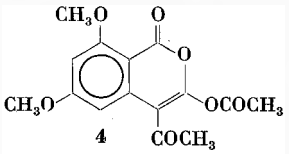

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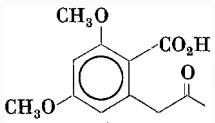

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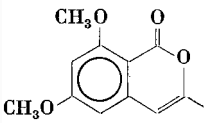

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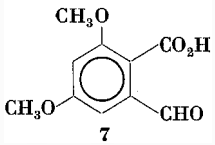

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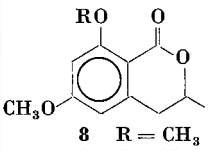


8 R = CH_3
8a R = H

H. L. Slates, S. Weber and N. L. Wendler
Merck Sharp & Dohme Research Laboratories,
Merck & Co., Inc., Rahway (New Jersey, U.S.A.)

* Received July 26, 1967.

1 a) H. Nogami, *J. Pharmac. Soc. Japan 61* (1941) 56. b) E. Hardegger, W. Rieder, A. Walser and F. Kugler, *Helv. Chim. Acta 49* (1966) 1283 and references therein. The authors are indebted to Professor Hardegger for informative correspondence.

2 R. Huisgen, G. Seidl and I. Wimmer, *Ann. Chem. 677* (1964) 21.

3 Cf. Method of S. N. Chakravorti and M. Swaminathan, *J. Indian Chem. Soc. 11* (1934) 101.

4 Method of B. E. Edwards and P. M. Rao, *J. Org. Chem. 31* (1965) 324.

5 D. Taub, N. N. Girotra, R. D. Hoffsommer, C. H. Kuo, H. Slates, S. Weber and N. L. Wendler, *Chem. Comm. 1967*, 225.

6 W. R. Logan and G. T. Newbold, *Chem. & Ind.* (London) *1957*, 1485.

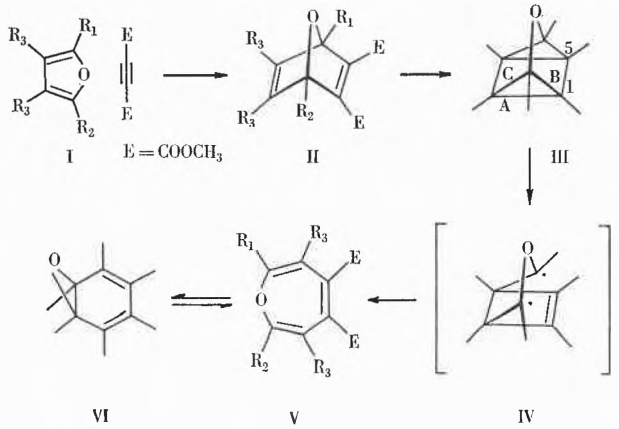
7 E. Sondheimer, *J. Amer. Chem. Soc. 79* (1957) 5036.

Oxépines à partir d'oxaquadricyclènes*

Summary

Several substituted oxepines have been synthesized by pyrolysing the corresponding oxaquadricyclanes which are easily obtainable from furane- and acetylene-derivates. Some limitations of the method are briefly discussed.

La 4.5-diméthoxycarbonyl-oxépine Va a permis de montrer[2] récemment qu'il est possible de synthétiser des oxépines monocycliques substituées selon la séquence réactionnelle I → II → III → V. Les exemples suivants démontrent l'utilité du procédé et quelques-unes de ses limites**.



a: R_1 = R_2 = R_3 = H
b: R_1 = R_2 = CH_3 R_3 = H
c: R_1 = CH_3 R_2 = R_3 = H
d: R_1 = R_2 = R_3 = CH_3
e: R_1 = R_2 = H R_3 = $COOCH_3$

2.7-diméthyl-4.5-diméthoxycarbonyl-oxépine (Vb)

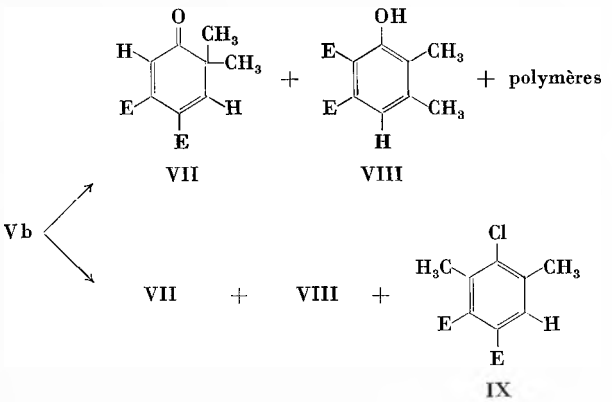
Le 2.5-diméthylfurane donne avec l'acétylène-dicarboxylate de diméthyle (ADM) dans un rendement supérieur à 90% et sous forme d'une huile légèrement jaune un composé d'addition IIb, que les données spectroscopiques caractérisent sans équivoque (Table 2). Exposé à basse température (−30° à −40°) et en solution éthérée dégazée environ $6 \cdot 10^{-2}$ molaire au rayonnement d'une lampe Hanau Q81 en présence d'un filtre de pyrex IIb s'isomérise quantitativement en IIIb. Les preuves structurales de l'oxaquadricyclène IIIb, obtenu sous forme de cristaux incolores, s'appuyent sur ses données physiques figurant dans la table 2. Un bref chauffage de IIIb dans le bromobenzène (170°) par exemple le transforme complètement; par chromatographie d'élution sur gel de silice (benzène) on isole l'oxépine Vb (cristaux jaunes, rendement 65 à 75%). Compte tenu de l'influence des substituants, la position des signaux de RMN et les absorptions UV et IR sont en bon accord avec les valeurs publiées pour Va et pour la 2.7-diméthyl-oxépine par VOGEL et collaborateurs[4]. Le spectre de RMN de Vb dépend beaucoup moins de la température que celui de Va:

Table 1

T (°C)	105	69	30	25	− 25	− 80
τ_H	4.31	4.28	4.27	4.37	4.25	4.12
Solvant	hexachlorobutadiène (PCB)			CD_3COCD_3		

De ce fait, ici aussi (cf. *l. c.*[4]), les substituants en position 2 et 7 déplacent l'équilibre V ⇄ VI en faveur de la forme triénique qui est stériquement la moins empêchée.

Vb est stable jusqu'à 100°, mais un chauffage à 160° (PCB, traces possibles de HCl) le transforme rapidement en la cétone VII (32%) et le phénol VIII (60%)*. VII (18%) et VIII (25%) apparaissent aussi, à côté de IX (57%), lorsqu'on fait barboter lentement HCl gazeux sec dans une solution de Vb dans CCl_4**.



* Recu par la rédaction le 26 juillet 1967. Publication n° X de la série «La Photochimie des cyclohexadiènes». Pour n° IX voir réf.[1].

** Il doit être en principe possible, avec des substituants appropriés tout au moins, d'obtenir d'autres hétérotropilidènes par ce procédé; les premiers résultats obtenus avec les hétéroatomes N, Si et P seront traités ailleurs[3].

[1] H. PRINZBACH et J. RIVIER, *Tetrahedron Letters 1967*, sous presse.

[2] H. PRINZBACH, M. ARGUËLLES et E. DRUCKREY, *Angew. Chem. 78* (1966) 1057.

[3] H. PRINZBACH, R. KITZING, R. FUCHS et P. WÜRSCH, en préparation.

* Par un traitement analogue Va fournit le 3-hydroxy et le 4-hydroxyphtalate de diméthyle[5]. Cf. aussi l'isomérisation acide d'oxépines substituées par des groupes alcoyles[4].

** Les structures de tous les hétérocycles III et V traités ici ont été confirmées par les composés d'addition obtenus avec des diénophiles différents[6].

[4] E. VOGEL et H. GÜNTHER, *Angew. Chem. 79* (1967) 429.

[5] M. ARGUËLLES, travail de diplôme, Université de Lausanne 1966.

[6] H. PRINZBACH, M. ARGUËLLES, W. AUGE, W. EBERBACH et P. VOGEL, en préparation.

Table 2

	PF ou PE °C	UV λ_{max}, nm(ε) (é = épaule)	IR ν C=C, cm^{-1}	RMN (τ, TMS = 10)	Spectre de masse: «base peak» (b.p.) m/e pic moléculaire (% b.p.)
IIb	104–105 (1 Torr)	284 (1150)[a] 222 (é, 5500)	1636[b] 1562	3.08 (2,s)[b] 6.27 (6,s) 8.31 (6,s)	96 238 (0,5%)
IIIb	38	abs. finale $\varepsilon_{230} = 1200$[a]		6.24 (6,s)[b] 7.45 (2,s) 8.26 (6,s)	164 238 (2%)
Vb	76	325 (2200)[a] 265 (1820)	1655[c] 1560	4.30 (2,s)[b] 6.24 (6,s) 8.02 (6,s)	91 238 (30%) perte de 0: 222 (30%)
IIc	100 (0,5 Torr)	287 (1260)[a] 222 (é, 4400)	1645[b] 1563	2.67 (1,q,5.5; 2.0)[b] 2.90 (1,d,5.5) 4.38 (1,d,2.0) 6.13 (3,s) 6.18 (3,s) 8.24 (3,s)	82 224 (1%)
IIIc				5.17 (1,d,3.8)[b] 7.37 (1,m,3.8; 2.8) 7.49 (1,d,2.8) 6.30 (3,s) 6.33 (3,s) 8.31 (3,s)	
Vc				3.94 (1,d,5.3)[b] 4.14 (1,s) 4.44 (1.d,5.3) 6.27 (6,s) 8.09 (3,s)	
IId	116 (0,6 Torr)	283 (1660)[a] 222 (é, 4700)	1634[b] 1555	6.23 (6,s)[b] 8.26 (6,s) 8.38 (6,s)	124 266 (5%)
IIId		abs. finale $\varepsilon_{230} = 2450$[a]		6.32 (6,s)[b] 8.39 (6,s) 8.70 (6,s)	192 266 (4%)
VII		300 (2880)[d] 229 (é, 3300)	1572 (C=C) 1678 (C=O)	2.84 (1,s)[b] 3.69 (1.s) 6.12 (3,s) 6.16 (3,s) 8.70 (6,s)	206 238 (34%)
VIII	132	264 (10700)[d]		2.23 (1,s)[e] 3.56 (1,s) 5.99 (3,s) 6.11 (3,s) 7.75 (3,s) 7.82 (3,s)	31 238 (11%)
IX	111	293 (1200)[d] 284 (1260) 243 (10250)		2.28 (1,s)[b] 6.08 (3,s) 6.13 (3,s) 7.65 (6,s)	225/227 (3/1) 256/258 (3/1) (17%)
IIe	108–109	260 (3360)[g] 224 (15750)	1645[c]	3.97 (2,s)[f] 6.09 (12,s)	
IIIe	111–112			4.59 (2,s)[f] 6.17 (12,s)	
Ve		364 (1820)[d] 266 (8890)	1610 1575	4.00 (2,s)[h] 6.29 (6,s) 6.40 (6,s)	
XII	201	286 (é, 1300)[i] 226 (4860) 200 (é, 6300)		1.17 (2,m)[j] 2.10 (2,m) 2.57 (2,t,1) 4.17 (2,t,1)	68 180 (20%)
XIII	147–149 (déc.)			1.85 (2,m)[j] 2.70 (2,m) 4.95 (2,d,3.5) 7.10 (2,d,3.5)	
XIV	194	299 (é, 1920)[g] 270 (2060) 231 (2740)	1635[c] 1575	2.35 (2,m)[j] 2.80 (2,m) 3.33 (2,q,3.5; 1) 5.47 (2,q,3.5; 1)	

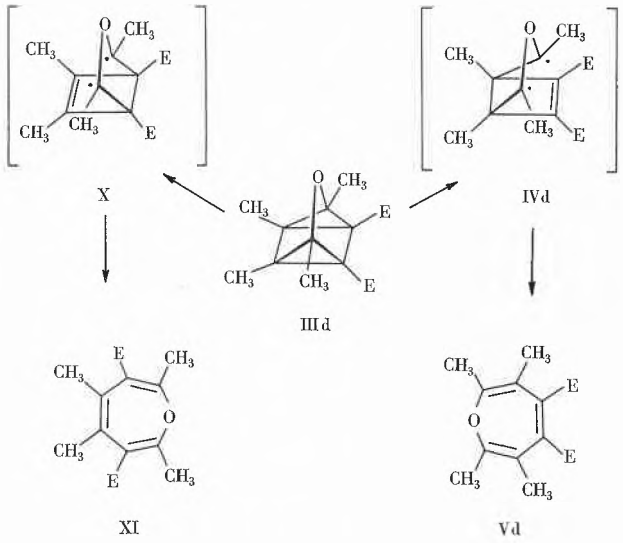
a) = isooctane, b) = CCl_4, c) = KBr, d) = éthanol, e) = $CDCl_3$, f) = $CHCl_3$, g) = éther, h) = bromobenzène, i) = H_2O, j) = DMSO-d_6

2-méthyl-4.5-diméthoxycarbonyl-oxépine (Vc)

Dans les mêmes conditions optimales d'isomérisation de valence IIb → IIIb, on obtient aussi avec IIc → IIIc un rendement quantitatif *. Les preuves structurales de IIIc se basent sur des constatations analogues à IIIb (Table 2). Par chauffage (solution à 10% dans le bromobenzène, température du bain 170°) IIIc se transforme deux fois plus vite que IIIa et deux fois plus lentement que IIIb. Par chromatographie d'élution sur gel de silice (benzène) on isole dans un rendement de 60 à 70% l'oxépine Vc sous forme d'une huile jaune-orange qui polymérise lentement à 20°. C'est pourquoi il n'est pas encore possible de donner les valeurs exactes d'absorption UV.

2.3.6.7-tétraméthyl-4.5-diméthoxycarbonyl-oxépine (Vd)

La photoisomérisation de l'oxanorbornadiène IId, obtenu dans un très bon rendement, fournit quantitativement IIId sous la forme d'une huile incolore qui cristallise lentement à −20°. Les nombreux essais d'isomérisation thermique de IIId dans des conditions différentes ont donné des résultats peu concordants: à chaque fois on obtient au moins 3 produits en quantités comparables, dont la séparation et la purification posent de grosses difficultés et dont les structures ne sont pas déterminées avec une certitude suffisante. C'est pourquoi il n'est pas encore possible de savoir si l'on obtient des produits de transformation de l'oxépine Vd, ou s'il y a concurrence entre les réactions IIId → IVd → Vd et X → XI par exemple.



* La synthèse de IIIc à partir de IIc sous des conditions semblables a été publiée entretemps par PAYO *et al.*[7]

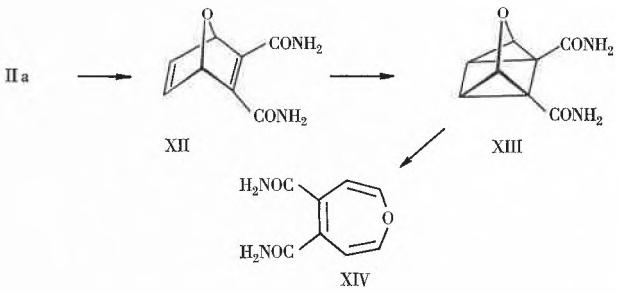
[7] E. PAYO, L. CORTÉS, J. MANTECON, C. RIVAS et G. DE PINTO, *Tetrahedron Letters 1967*, 2415.

3.4.5.6-tétraméthoxycarbonyl-oxépine (Ve)

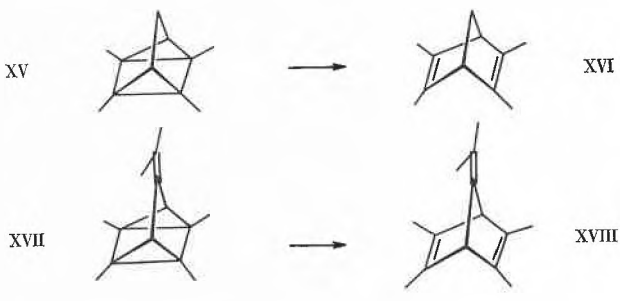
La photoisomérisation de IIe obtenu à partir du 3.4-diméthoxycarbonylfurane et ADM en tube scellé à 90° (3 jours) donne des résultats peu concordants. A côté de IIIe (cristaux incolores qui en fondant se transforment en Ve; rendement de 50 à 60%) on obtient en quantité réduite un produit secondaire remarquable par son maximum d'absorption UV à 328 nm (éther) mais de structure encore inconnue. Un court chauffage dans le benzène (10 min, température du bain 105°) suffit à la transformation IIIe → Ve; l'oxépine visqueuse est séparée et purifiée sur une colonne de SiO_2 ($CHCl_3$) (rendement provisoire: 60 à 65%). Les mesures de RMN n'ont pas pu prouver à côté de Ve l'existence de IIe et d'isomères de Ve.

4.5-diamido-oxépine (XIV)

La diamide XII obtenue par traitement du diester IIa en solution aqueuse de NH_3 (−10°) et recristallisée dans l'acétone fournit par irradiation dans l'eau l'oxaquadricyclène XIII isolé sous forme d'un produit solide incolore. La pyrolyse de XIII est effectuée dans le dioxane (température du bain 120°). La stabilité de XIV est comparable à celle du diester Va.



L'excitation thermique isomérise en oxépines par homolyse des liaisons B – de préférence au moins – tous les éthers tétracycliques III que nous avons examinés, à l'exception du composé tétraméthylé IIId. Au contraire, les composés homocycliques XV, respectivement XVII, substitués de manière analogue donnent les diènes de départ XVI, respectivement XVIII, par rupture des liaisons A[1].



Encore doit-on déterminer si les groupes accepteurs fixés en position 1 et 5 sont essentiels dans la préférence du mode III → V au mode III → II, ou si l'activation des liaisons B par l'oxygène seulement suffit à faciliter cette homolyse aux dépens de celle des liaisons A observée dans les cas XV → XVI et XVII → XVIII.

L'oxaquadricyclène non substitué ou ses dérivés substitués par des groupes alcoyles exclusivement devraient apporter des éclaircissements à ce problème. Cependant leur synthèse présente de grosses difficultés: soit par manque de réactivité des dérivés de l'acétylène (p.ex. butyne-2) vis-à-vis du furane, soit par addition rapide d'une deuxième molécule de diène sur le composé d'addition primaire[8]. Il semble toutefois, à la suite de récents résultats, que les dérivés III dialcoylés soient accessibles à partir de III a.

H. Prinzbach, P. Vogel et W. Auge*

Laboratoire de Chimie organique
Université de Lausanne (Suisse)

* Chemisches Laboratorium der Universität Freiburg im Breisgau (Allemagne).

[8] C. J. Weis, *J. Org. Chem. 28* (1963) 74.

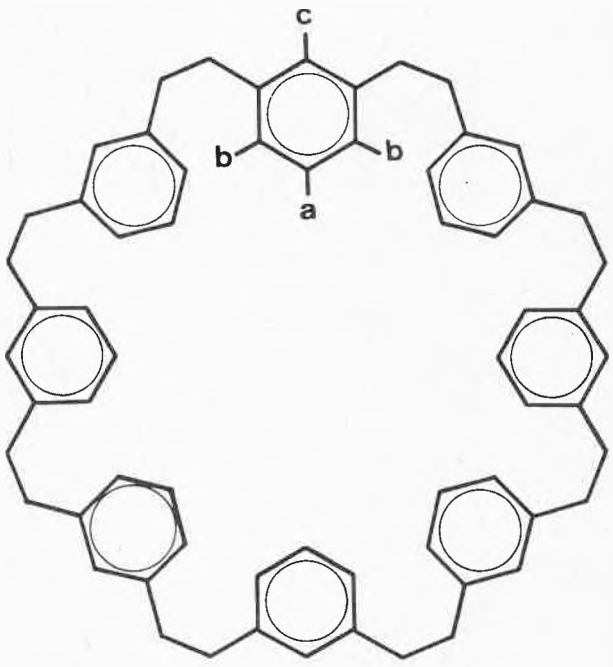
Ein 40gliedriges Ringsystem der Metacyclophanreihe*

Summary

From the crude product of a Wurtz reaction with α,α'-dibromo-*m*-xylene a further ring system, [2.2.2.2.2.2.2.2] metacyclophane was isolated and its structure confirmed by UV, mass and NMR spectra.

Die Chemie der überbrückten, zyklischen Kohlenwasserstoffe[1] ist in den letzten zehn Jahren besonders durch eine große Zahl von Arbeiten in der Paracyclophanreihe bereichert worden. Die Gruppe der vernachlässigten, aber theoretisch, besonders stereochemisch, ebenso interessanten Metacyclophane konnte kürzlich durch die Synthese einer lückenlosen homologen Reihe mit 2 bis 6 *m*-Xylyleneinheiten ausgebaut werden[2,3,4].

Eine weitere chromatographische Auftrennung des durch Einwirkung von Natrium auf α,α'-Dibrom-*m*-xylol erhaltenen Reaktionsproduktes hat nun zur Isolierung von [2.2.2.2.2.2.2.2] Metacyclophan geführt. Zur Herstellung des neuen Ringsystems wird das Rohprodukt, wie bereits beschrieben[2], säulenchromatographisch in seine Komponenten zerlegt. Durch Eluieren der Alox-Säule (Camag, Akt. II, neutral) mit *n*-Hexan werden der Reihe nach [2.2] Metacyclophan, Tri-*m*-xylylen[3] vermischt mit etwas Tetraphenyläthylen und schließlich Tetra-*m*-xylylen[4] vermischt mit Tetraphenyläthan erhalten. *n*-Hexan/Äthylacetat (200 : 1) eluiert darauf Penta-[2], dann Hexa-[2] und anschließend in 0,9% Ausbeute Okta-*m*-xylylen ([2.2.2.2.2.2.2.2] Metacyclophan) als weißes, kristallines Pulver. Nach Umkristallisieren aus *n*-Hexan, dann *n*-Pentan und zuletzt aus Methanol fällt die Verbindung analysenrein in weißen Nadeln vom Smp. 100 bis 102° an. Schmelzpunkt, Elementaranalyse (für $C_{64}H_{64}$ ber. C 92,26, H 7,74; gef. C 92,26, H 7,85%), UV-, NMR- und Massenspektren sind im Einklang mit der angenommenen Struktur.



[2.2.2.2.2.2.2.2] Metacyclophan

Die Schmelzpunkte der [2.2. ...] Metacyclophane zeigen eine gesetzmäßige Abhängigkeit von der Anzahl der in den Ringsystemen enthaltenen *m*-Xylyleneinheiten

* Eingegangen am 17. August 1967.

[1] Zusammenfassende Darstellung: B. H. Smith, *Bridged Aromatic Compounds, Organic Chemistry*, A Series of Monographs, Vol. 2, Academic Press, 1964.

[2] W. Jenny und K. Burri, *Chimia 21* (1967) 186–7.

[3] W. Jenny und K. Burri, *Chimia 20* (1966) 436–7. Die Ausbeute an [2.2.2] Metacyclophan konnte nachträglich durch Variation der Versuchsbedingungen (Arbeiten bei −80° in Tetrahydrofuran bei Gegenwart von Tetraphenyläthylen) von 1% auf 7,5% gesteigert werden.

[4] K. Burri und W. Jenny, *Chimia 20* (1966) 403–4.

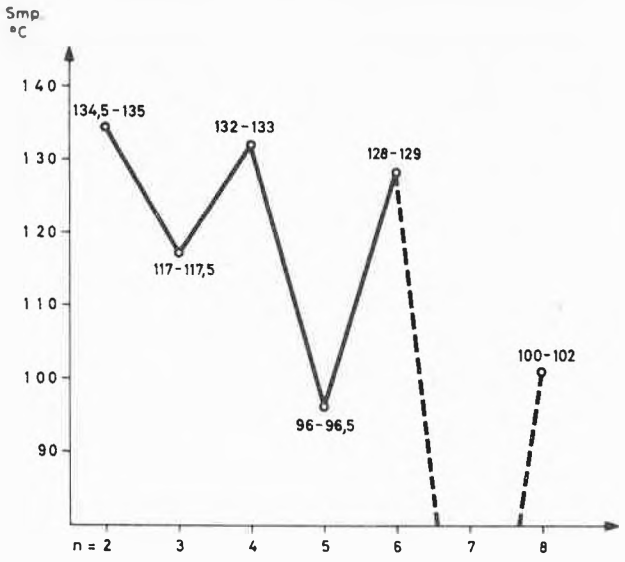


Abb. 1. Schmelzpunkte der [2.2. ...] Metacyclophane in Abhängigkeit von der Ringgröße. n = Anzahl m-Xylyleneinheiten

(vgl. Abb. 1). Sie nehmen sowohl in der geradzahligen als auch in der ungeradzahligen Reihe mit zunehmender Ringgröße ab. Die Schmelzpunkte der ungeradzahligen Glieder liegen immer tiefer als diejenigen der benachbarten geradzahligen. Wir haben darum schon aus dem gefundenen Schmelzpunkt des hier beschriebenen, neuen Ringsystems auf das wahrscheinliche Vorliegen des Oktameren schließen können, da das Heptamere nach der angeführten Gesetzmäßigkeit bei wesentlich tieferer Temperatur (40 bis 50°) schmelzen sollte. Einen eindeutigen Hinweis auf die Struktur eines 40gliedrigen Ringes ergab aber erst das Massenspektrum.

Die massenspektrometrische Untersuchung ergab bei Tiegeltemperaturen oberhalb 400°C einen intensiven Molekül-Ionenpeak bei der berechneten Massenzahl m/e 832. Diese hohe Massenzahl wurde unter Zuhilfenahme von Perfluorkerosan als Eichsubstanz sichergestellt. Das Mutterion zerfällt wie bei allen früher beschriebenen [2.2. ...] Metacyclophanen in Fragment-Ionen der Massenzahl $M-(91+n\cdot 104)$, $M-(105+n\cdot 104)$ und $M-(119+n\cdot 104)$, $n=0, 1, 2\ldots$

Eine überraschende Beobachtung konnten wir bei Massenspektren machen, welche bei tieferer Temperatur (Tiegeltemperatur $\sim$ 180°) durchgeführt wurden. Hier kann im unteren Massenbereich der intensive Molekül-Ionenpeak des [2.2] Metacyclophans (m/e 208) nachgewiesen werden. Dieser ist mit einer charakteristischen, bei den höheren [2.2. ...] Metacyclophanen nicht beobachtbaren Fragmentierung $M-C_2H_4$ verbunden. Wir konnten in dem gleichen Präparat weder dünnschichtchromatographisch noch spektroskopisch (NMR) das Vorhandensein von [2.2] Metacyclophan nachweisen. Die Frage, ob und unter welchen Bedingungen das Oktamere in Dimeres zerfallen könnte, ist momentan Gegenstand einer weiteren Untersuchung.

Interessant ist auch die Tatsache, daß – im Gegensatz zum Tetra-, Penta- und Hexameren – neben der oben skizzierten ringförmigen Konformation des [2.2.2.2.2.2.2.2] Metacyclophans auch eventuell wahrscheinlichere schlaufenförmige Molekelmodelle aufgebaut werden können. Ein Vergleich der UV-Spektren (vgl. Tabelle 1) des zyklischen Oktameren mit denjenigen des Tri- und Hexameren erlaubt keine Rückschlüsse auf einen unterschiedlichen sterischen Aufbau.

Tabelle 1. UV-Spektren von [2.2. ...] Metacyclophanen in Isooctan

	λ_1 mμ (log ε)	λ_2 mμ (log ε)	λ_3 mμ (log ε)	λ_4 mμ (log ε)
Di-	264* (2,59)	272 (2,64)	275* (2,59)	277* (2,55)
Tri-	258 (2,71)	264 (2,81)	270 (2,72)	273 (2,63)
Hexa-	259* (3,07)	265 (3.17)	268 (3,06)	273 (3,08)
Okta-	258 (3,24)	265 (3,34)	269 (3,24)	273 (3,29)

* Inflexionen

Gegenüber [2.2] Metacyclophan ist bei allen höheren Ringsystemen eine deutliche Verschiebung der vier beobachteten Banden nach kürzeren Wellenlängen festzustellen. Es ist dies eine weitere Bestätigung der wiederholt gemachten Beobachtung, daß nichtebene Benzolringe ([2.2] Metacyclophan!) die Absorption nach längeren Wellen verschieben[5].

Das NMR-Spektrum des neuen Ringsystems bietet keine wesentlich neuen Erkenntnisse. Sein Spektrum ähnelt sehr stark demjenigen des [2.2.2.2.2]- und des [2.2.2.2.2.2] Metacyclophans[2]. Die a-, b- und c-Protonen bilden ein AB_2C-Spektrum, wobei die Signale (in $CDCl_3$) bei δ 7,14 (H_a), 6,94 (H_b) und 6,83 (H_c) erscheinen. Die aliphatisch gebundenen d-Protonen geben Anlaß zur Ausbildung eines Singletts, welches bei δ 2,78 beobachtbar ist.

Eine ausführliche Publikation dieser Arbeit wird in den *Helv. Chim. Acta* erscheinen.

W. Jenny und K. Burri

Forschungslaboratorien der
CIBA Aktiengesellschaft, Basel, Farbstoffabteilung
Institut für organische Chemie der Universität Bern

[5] Vgl. dazu auch N. L. Allinger *et al.*, *J. Amer. Chem. Soc. 83* (1961) 1975.