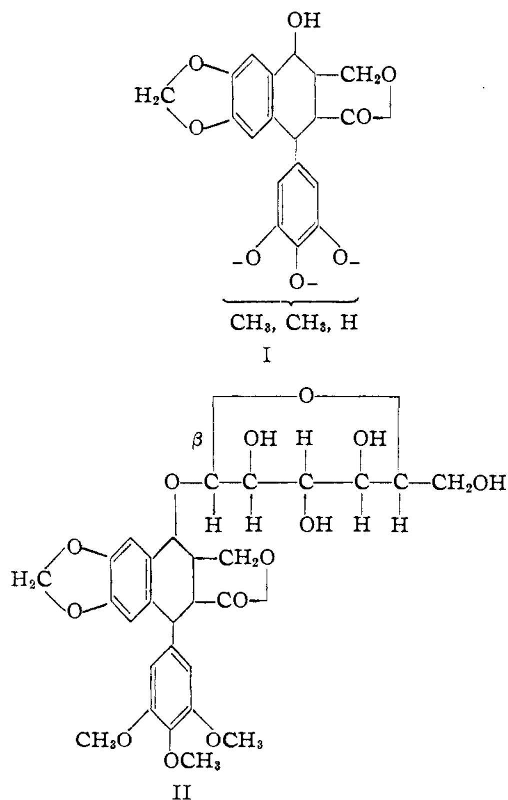
osyl)-picropodophyllin (II) (1.8%). The latter two compounds are new.

I crystallized in colorless transparent prisms from ethanol or as plates from 50% ethanol and had m.p. 250.0–251.6° cor. and $[\alpha]^{20}D$ −130° (*c*, 0.75, $CHCl_3$) (*Anal.* Calcd. for $C_{21}H_{20}O_8$: C, 63.0; H, 5.0; 2-OCH_3, 15.5. Found: C, 63.1; H, 5.1; OCH_3, 16.0). Methylation with diazomethane gave podophyllotoxin, identical with an authentic specimen by m.p., mixed m.p. and optical rotation, and forming an acetyl derivative identical with acetylpodophyllotoxin.

II crystallized in long, thin, colorless needles from 75% methanol or from water, and had m.p. 237.0–238.2° cor. and $[\alpha]^{20}D$ −11.5° (*c*, 0.5, pyridine) (*Anal.* Calcd. for $C_{28}H_{32}O_{13}.0.5H_2O$: C, 57.4; H, 5.7; 3-$OCH_3$, 15.9. Found: C, 57.4; H, 5.7; OCH_3, 15.2). Hydrolysis with dilute hydrochloric acid gave (a) D-glucose (Calcd. for one mole: 31%. Found: 28%), identified by m.p. and mixed m.p. of the phenylosazone and by m.p. and mixed m.p. of the aldobenzimidazole derivative, and (b) picropodophyllin, identified by m.p. and optical rotation, and by m.p., mixed m.p. and optical rotation of its acetate. The fact that the glucoside did not reduce Benedict solution and was hydrolyzed by emulsin (but not by maltase) indicated it to be a β-glucopyranoside.[3]

The structures of I and II are thus:



The location of the phenolic hydroxyl group in I is being determined.

According to the findings of Dr. J. Leiter, to be published elsewhere, I is active in producing hemorrhage and necrosis in Sarcoma 37 in mice, while II is inactive even in high doses.

LABORATORY OF CHEMICAL PHARMACOLOGY
NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH
U. S. PUBLIC HEALTH SERVICE
FEDERAL SECURITY AGENCY
BETHESDA, MARYLAND

M. V. NADKARNI[4]
PRISCILLA B. MAURY
JONATHAN L. HARTWELL

RECEIVED NOVEMBER 21, 1951

(3) E. Fischer, *Ber.*, **47**, 1980 (1914); W. N. Haworth, *et al.*, *J. Chem. Soc.*, 2254 (1932).

(4) Post-doctorate Research Fellow of the National Cancer Institute.

SEQUENCE OF FOUR AMINO ACIDS AT THE AMINO END OF THE SINGLE POLYPEPTIDE CHAIN OF LYSOZYME

Sir:

During the investigation of DNP-lysozyme in which the end group of lysozyme was found to be lysine,[1] a DNP-peptide (or peptides) was also isolated from certain hydrolysates. The fact that this DNP-peptide material yielded α,ε-di-DNP-lysine on complete hydrolysis lead to the conclusion that it is derived from the amino end of the lysosyme molecule. Further study has now been made of this peptide material and hydrolytic conditions have been found which cause DNP-lysozyme to yield some DNP-tetrapeptide as well as shorter DNP-peptides. Analysis of the tetrapeptide and of the smaller peptides has shown that the tetrapeptide has the composition α,ε-di-DNP-lysyl-valyl-phenylalanyl-glycine.

About 20% of the end group of DNP-lysozyme may be isolated in the form of the tetrapeptide from the ether and ethyl acetate extracts of a sample of DNP-lysozyme which has been partially hydrolyzed by refluxing for ten minutes in 6 *N* hydrochloric acid. The tripeptide accounts for about 60% of the end-group; most of the remainder is present as the end amino acid (α,ε-di-DNP-lysine) itself and little is to be found in the form of the dipeptide.

These three α,ε-di-DNP-lysyl peptides and α,ε-di-DNP-lysine may readily be separated from each other by adsorption chromatography on 2:1 silicic acid–Celite by methods which are an extension of the scheme for the separation of 16 ether-soluble DNP-amino acids which has recently been developed in these Laboratories.[2]

The three peptides so isolated were found to contain the following amino acids in addition to the end group which was α,ε-di-DNP-lysine

Most strongly adsorbed peptide	Valine, phenylalanine, glycine
Intermediately adsorbed peptide	Valine, phenylalanine
Least strongly adsorbed peptide	Valine

These results immediately suggest the sequence lysyl → valyl → phenylalanyl → glycine and this conclusion is further strengthened by the isolation of the tripeptide and dipeptide from partial hydrolysates of the tetrapeptide and of the dipeptide from partial hydolysates of the tripeptide. Quantitative analysis showed that the ratio of the amino acids in each peptide was unity.

(1) F. C. Green and W. A. Schroeder, THIS JOURNAL, **73**, 1385 (1951).

(2) F. C. Green and L. M. Kay, to be published.

Milder conditions of hydrolysis have been to no avail in producing a peptide longer than the tetrapeptide. The peptide bond between the fourth and fifth amino acids clearly is a labile one. The work of Synge[3] and of Desnuelle and Casal[4] indicates that if the fifth amino acid were serine or threonine the bond would indeed be labile and it may also be that tryptophan which is sensitive to acid would behave similarly.

Several authors[5,6] have concluded that lysozyme contains one or at most two peptide chains. Thompson[7] after applying corrections could account for little more than half an end-group per molecule. In previous work[1] and in the present study, the actual uncorrected amount of α,ϵ-di-DNP-lysine which was isolated accounted for more than 0.6 end-group per molecule. Our analyses of the peptides which were isolated from DNP-lysozyme and also of model synthetic peptides demonstrate that 25 to 40% of α,ϵ-di-DNP-lysine may be destroyed during hydrolysis. Our results agree with those of Thompson[7] that a complete hydrolysate of DNP-lysozyme contains no DNP-amino acids other than α,ϵ-di-DNP-lysine and ϵ-DNP-lysine. We may, therefore, conclude that lysozyme has a single polypeptide chain and, from the present work, that the sequence on the amino end of this chain is lysyl-valyl-phenylalanyl-glycyl—.

(3) R. L. M. Synge, *Biochem. J.*, **39**, 351 (1945).

(4) P. Desnuelle and A. Casal, *Biochim. Biophys. Acta*, **2**, 64 (1948).

(5) J. C. Lewis, N. S. Snell, D. J. Hirschmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **186**, 23 (1950).

(6) H. Fraenkel-Conrat, A. Mohammed, E. D. Ducay and D. K. Mecham, THIS JOURNAL, **73**, 625 (1951).

(7) A. R. Thompson, *Nature*, **168**, 390 (1951).

CONTRIBUTION NO. 1622
GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA 4, CALIFORNIA W. A. SCHROEDER

RECEIVED NOVEMBER 15, 1951

A SECOND CRYSTALLINE MODIFICATION OF POLYTHENE

Sir:

We have obtained conclusive evidence of the occurrence of a second crystalline modification of polythene in films of this polymer subjected to the mechanical process of redrawing.[1] The new modification is characterized by the appearance of two strong crystalline interferences in the X-ray pattern, corresponding to spacings of 4.23 and 4.55 Å. Four to seven successive redrawings were necessary to obtain a high degree of sharpness and intensity in the new diffraction peaks. The two strong peaks, 110 and 200, of the usual orthorhombic modification of polythene,[2] with spacings to 3.78 and 4.17 Å., remained strong, indicating only partial conversion to the new modification.

A polythene film[3] subjected to seven redrawings was mounted successively in each of three orientations with respect to the X-ray beam: (A) beam normal to the film plane; (B) beam parallel to the film plane but normal to the last direction of draw; (C) beam parallel to the film plane and to the last direction of draw. Flat camera photographs of moderate exposure were taken for each orientation. Orientation A showed only the two equatorial spacings 3.78 and 4.17 Å. characteristic of the well-known modification. Orientation B showed only the two equatorial spacings 4.23 and 4.55 Å., characteristic of the new modification, while orientation C showed all four spacings, with 3.78 and 4.17 Å. doubled on equatorial lines about 65° each side of the single equator of the 4.23 and 4.55 Å. spacings. This suggests that the familiar modification had undergone twinning by a glide process similar to that observed by other investigators[4,5]; in this case the glide was probably parallel to the two sets of 110 planes.

Additional spacings corresponding to the new modification have not yet been obtained; hence, it is not possible to determine either the shape or the dimensions of the unit cell. A more detailed report of our work will be submitted to THIS JOURNAL at the conclusion of the research.

(1) Cold drawing of the specimen along a direction 90° with respect to that of previous cold drawing has been termed redrawing: W. M. D. Bryant, *J. Polymer Sci.*, **2**, 558 (1947).

(2) C. W. Bunn, *Trans. Faraday Soc.*, **35**, 482 (1939)

(3) The specimen was coated on both sides with powdered sodium chloride to provide a simultaneous calibration.

(4) A. Brown, *J. Applied Phys.*, **20**, 552 (1949).

(5) I. L. Hopkins, W. O. Baker, J. B. Howard, *ibid.*, 206 (1950).

POLYCHEMICALS DEPARTMENT R. H. PIERCE, JR.
EXPERIMENTAL STATION LABORATORIES J. P. TORDELLA
E. I. DU PONT DE NEMOURS & COMPANY, INC.
WILMINGTON, DELAWARE W. M. D. BRYANT

RECEIVED NOVEMBER 7, 1951

ABSENCE OF DETECTABLE POLY-*CIS* FORMS FROM HEAT-ISOMERIZED LYCOPENE SOLUTIONS

Sir:

While poly-*cis* lycopenes, $C_{40}H_{56}$, are occasionally found in nature,[1] it has not been possible so far to obtain such forms *in vitro*, by submitting all-*trans* lycopene to any of the well known stereoisomerization methods.[2] Since, however, all pertinent experiments had been carried out with only small amounts of starting material so far, we endeavored to investigate the thermic *trans* → *cis* isomerization of lycopene on an unusually large scale, under conditions which would allow the recovery of even a trace of poly-*cis*-lycopene formed.

Thirty grams of analytically pure and chromatographically homogeneous lycopene was prepared from 180 kg. of commercial tomato paste.[3] Two-gram portions of this pigment were refluxed in 2 l. of benzene (per portion) in diffuse daylight for one half hour. A subsequent resolution on fifteen, slightly conical percolators (50 × 24 cm.) filled with lime–celite, yielded 30 l. of a weakly colored chromatographic filtrate that was free of all-*trans* or *neo* forms which were held strongly by the adsorbent. This filtrate underwent further resolutions on alu-

(1) L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Nat. Acad. Sci.*, **27**, 468 (1941); A. L. LeRosen and L. Zechmeister, THIS JOURNAL, **64**, 1075 (1942); L. Zechmeister and W. A. Schroeder, *J. Biol. Chem.*, **144**, 315 (1942); L. Zechmeister and J. H. Pinckard, THIS JOURNAL, **69**, 1930 (1947).

(2) L. Zechmeister, *Chem. Rev.*, **34**, (1944); *cf.* also L. Pauling, *Helv. Chim. Acta*, **32**, 2241 (1949).

(3) The following method was adapted for large-scale isolation work: A. Sandoval and L. Zechmeister, "Biochemical Preparations," J. Wiley and Sons, Inc., New York, N. Y., Vol. I, p. 57, and London: Chapman and Hall, 1949.