COMMUNICATIONS TO THE EDITOR

EQUILIBRIA IN THE FIBRINOGEN–FIBRIN CONVERSION[1]

Sir:

In the previous work on the clotting of fibrinogen it has been assumed or at least implied that the reaction is irreversible and proceeds to completion. Some results in this laboratory led us to believe that this reaction can be treated as a reversible process. On the basis of information available at present about the clotting process three fundamental steps have been recognized.[2,3,4,5] We have postulated that these three reactions are reversible, involving the equilibria

$$(1)\quad F \overset{\text{thrombin}}{\rightleftharpoons} \Phi + P \qquad K_1 = (\Phi)(P)/(F)$$

$$(2)\quad n\Phi \rightleftharpoons \Phi_n \qquad K_2 = (\Phi_n)/(\Phi)^n$$

$$(3)\quad m\Phi_n \rightleftharpoons \text{Fibrin} \qquad K_3 = (\Phi_n)$$

where F is fibrinogen, Φ is activated fibrinogen,[3,5] P is fibrino-peptide,[4] n is a number between 5 and 15, Φ_n is the intermediate product described by Ferry and Shulman,[2,6] m is a very large number, and the parentheses indicate activities of the given components.[7] It is, of course, recognized that this reaction scheme is further complicated by the adsorption of the fibrino-peptide upon the fibrin clot,[4] by the non-activating fibrinogen–fibrin–thrombin interactions described by Waugh and Livingstone,[5] and by the effects of water and *p*H. It is also very probable that various polymeric intermediate products other than Φ_n may exist.

The following experiments[8] give evidence in support of this reaction scheme.

(1) Fibrin clots were washed for 24–48 hours in a large volume of 0.3 *M* KCl solution with at least ten changes of the wash solution. Such washed clots will not clot a 1% fibrinogen solution over a period of three days. Since thrombin is more strongly occluded on fibrin clots than any other protein studied[9] the fact that these clots were free of thrombin indicates that they were also free of other occluded proteins.

Washed clots were placed in salt solutions at *p*H 6.5, ionic strength 0.3 to 1.0 and were allowed to come to equilibrium at 2, 25.0 and 37.0°. In all cases a small amount of soluble protein material was detected in the supernatant and a very pronounced temperature dependence of this concentration was observed. This, in conjunction with experiment (3), tends to indicate that step 3 is reversible.

(2) Both viscosity[6] and flow birefringence[10] results point to the fact that in hexamethylene glycol-inhibited systems the polymerized intermediate Φ_n dissociates on dilution. This tends to support the reversibility of step 2.

(3) Several samples of bovine fibrinogen have been clotted at room temperature. The clots were removed and the supernatants placed at 37.0° for a period of 24 hours. No further clotting took place. The samples were then cooled to 2° and small but definite clots appeared after two hours. These clots redissolved almost completely, however, after 24 more hours at 37.0° and reformed[11] again at 2°.

An attempt has also been made to shift the equilibrium in reaction 1 to the left by adding fibrino-peptide and thrombin to washed, thrombin-free fibrin clots suspended in buffer solutions and then measuring the concentration of soluble proteins in the supernatant at equilibrium. While the precision in these measurements was not high due to experimental difficulties, nevertheless, in all cases, in solutions containing additional fibrino-peptide more soluble protein material was found than in those with no peptide added. These experiments tend to indicate the reversibility of step 1 and of the reaction as a whole.

Quantitative work is now in progress to characterize the postulated equilibria and to determine their respective thermodynamic parameters.

(1) This work was supported by contract N6-onr-26414 between Cornell University and the Office of Naval Research.

(2) J. D. Ferry, Am. Chem. Soc. Meeting Abstracts, New York, Sept., 1951, p. 9C.

(3) K. Laki, *Arch. Biochem. Biophys.*, **32**, 317 (1951).

(4) L. Lorand, *Nature*, **167**, 992 (1951).

(5) D. F. Waugh and B. J. Livingstone, *J. Phys. Colloid Chem.*, **55**, 1206 (1951).

(6) S. Shulman and J. D. Ferry, *ibid.*, **55**, 135 (1951).

(7) The activity of fibrin is assumed to be unity.

(8) In all of these experiments Laki purified fibrinogen[3] (95% clottable) and Seegers purified thrombin were used. We wish to thank Dr. W. H. Seegers for his generous gift of purified thrombin, and Dr. L. Lorand for sending us the details of his unpublished method for the preparation of the fibrino-peptide. The experimental details will be described elsewhere.

(9) P. R. Morrison, THIS JOURNAL, **69**, 2723 (1947).

(10) H. A. Scheraga and J. K. Backus, *ibid.*, in press.

(11) Formation and dissolution of these clots cannot be due to the precipitation of "cold insoluble globulin" since *purified* fibrinogen solutions stored at 2° under similar conditions gave no precipitate.

DEPARTMENT OF CHEMISTRY
CORNELL UNIVERSITY
ITHACA, NEW YORK

M. LASKOWSKI, JR.
D. H. RAKOWITZ
H. A. SCHERAGA

RECEIVED NOVEMBER 16, 1951

COMPONENTS OF PODOPHYLLIN. VI. ISOLATION OF TWO NEW COMPOUNDS FROM *PODOPHYLLUM EMODI* WALL

Sir:

In continuation of our search for tumor-necrotizing components of the resin, podophyllin, from different species of *Podophyllum*, we have investigated the resin from *P. emodi* Wall.[1] using chromatography with activated alumina. Three colorless crystalline compounds were isolated. In the order of increasing strength of adsorption these were: podophyllotoxin (36–39% yield),[2] demethylpodophyllotoxin (I) (1.7%), and 1-*O*-(β-D-glucopyran-

(1) We wish to thank Dr. W. G. Bywater of S. B. Penick and Co. for the procurement of the roots from India and the extraction of the resin therefrom.

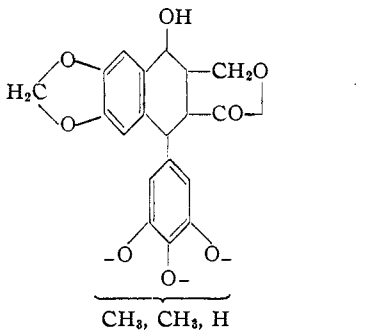
(2) In addition, a small amount of picropodophyllin (about 0.4%) appeared with the podophyllotoxin. This may have been an artifact.

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

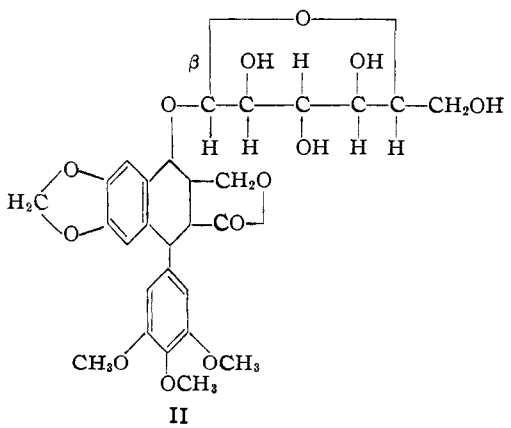
I crystallized in colorless transparent prisms from ethanol or as plates from 50% ethanol and had m.p. 250.0–251.6° cor. and $[\alpha]^{20}D$ −130° (*c*, 0.75, $CHCl_3$) (*Anal.* Calcd. for $C_{21}H_{20}O_8$: C, 63.0; H, 5.0; 2-OCH_3, 15.5. Found: C, 63.1; H, 5.1; OCH_3, 16.0). Methylation with diazomethane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



I



II

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.

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

LABORATORY OF CHEMICAL PHARMACOLOGY
NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH M. V. NADKARNI[4]
U. S. PUBLIC HEALTH SERVICE PRISCILLA B. MAURY
FEDERAL SECURITY AGENCY JONATHAN L. HARTWELL
BETHESDA, MARYLAND

RECEIVED NOVEMBER 21, 1951

(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.