alcohol[4] for 18 hours at room temperature, and then acidification of the reaction mixture with dilute HCl and extraction, yielded a neutral product III-C^{14} (R_F = 1.05 in ethylene dichloride–formamide; = 1.02 in toluene: ethyl acetate 9:1, methanol–water 1:1).

Tritium-labeled III was prepared by the sequence of reactions described by Ham, *et al.*[5] *d*-Aldosterone-7-H^3 [6] was diluted with carrier *dl*-aldosterone and oxidized with HIO_4 to aldosterone etiolactone IV[7] (R_B = 4.2 in methylcyclohexane: toluene 1:1-formamide) m.p.[8] 295–300°. Lactone IV was treated with $NaBH_4$ under the same conditions used for the reduction of II. Acidification and extraction of the reaction mixture yielded III-H^3 in the lactone form.

III-H^3 and III-C^{14} were combined and the mixture was converted to two derivatives. Ac_2O in pyridine yielded IIIAc (R_{DOCA} = 1.1 in methylcyclohexane–dimethylformamide). Oxidation of III with CrO_3-pyridine complex[9] gave the diketo lactone V, which was chromatographed first on paper (R_B = 2.5 in methylcyclohexane: toluene (1:1)-formamide), then on neutral alumina, and eluted with 0.5% ethanol in benzene; m.p. 239–244°, λ_{max}^{EtOH} 238 mμ, $\lambda_{max}^{CHCl_3}$ 5.60, 5.81, 5.97 and 6.14 μ. The properties of lactone V were in agreement with those previously reported.[5,10,11] H^3:C^{14} ratios[12] were determined before and after chromatographic purification of compound III and after the preparation and purification of the acetylated and oxidized derivatives (Table I).

The agreement of all values for H^3:C^{14}, within the precision of measurement, was interpreted to indicate that compound III-C^{14} of adrenal origin and synthetic compound III-H^3 were chemically identical.

The conversion of II and the known etiolactone IV to the same reduction product (III) and the identification of III in turn by oxidation to V, established that II was a 4-etienic acid lactone (20,-18) with additional oxygen functions at C-3 and C-11. Of the possible structures which could have been reduced to III, only II, IV and V did not possess an acetylatable hydroxyl group. Compounds IV and V were prepared and excluded on the basis of their chromatographic properties. This permitted the assignment of the structure 11β,18-dihydroxy-3-keto-4-etienic acid lactone to II and the formulation of I as 18-hydroxycorticosterone.[13]

These findings support the view[14,15] that hydroxylation of corticosterone at C-18 is an intermediate step in the biological oxidation of the angular methyl group to an aldehyde in the biosynthesis of aldosterone.[16]

TABLE I

COMPARISON OF ISOLATED (C^{14}) AND SYNTHETIC (H^3) SAMPLES OF COMPOUND III FOLLOWING CHROMATOGRAPHIC PURIFICATION AND THE PREPARATION OF DERIVATIVES IIIAc AND V

Substance	Chromatographic system	H^3:C^{14}
III	Before chromatography	16.9 ± 0.5
III	Ethylene dichloride-formamide	16.7 ± .7
III	Toluene-ethyl acetate (9:1)-MeOH:H_2O (1:1)	17.9 ± .5
IIIAc	Methylcyclohexane-dimethylformamide	17.4 ± .1
V	Methylcyclohexane:toluene, (1:1)-formamide	18.2 ± .8
V	Al_2O_3 column	17.6 ± .2
	Mean	17.4 ± .6

(4) J. I. Appleby, G. Gibson, J. K. Norymberski and R. D. Stubbs, *Biochem. J.*, **60**, 453 (1955).

(5) E. A. Ham, R. E. Harman, N. G. Brink and L. H. Sarett, THIS JOURNAL, **77**, 1637 (1955).

(6) Dr. James F. Tait kindly provided *d*-aldosterone-7-H^3. We wish to thank Dr. C. H. Sullivan, Ciba Co., for unlabeled aldosterone.

(7) S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler and T. Reichstein, *Helv. Chim. Acta*, **37**, 1200 (1954).

(8) Melting points (uncorrected) were determined on a Kofler type hot stage. The infrared spectrum was recorded on a Perkin Elmer Model 21 spectrophotometer.

(9) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(10) J. Schmidlin and A. Wettstein, *Helv. Chim. Acta*, **43**, 973 (1960).

(11) R. Neher and A. Wettstein, *ibid.*, **43**, 623 (1960). These authors also report some chromatographic properties of compounds II and V.

(12) Tritium and C^{14} were determined simultaneously in a Tricarb liquid scintillation spectrometer. Counting efficiencies were approximately 18% for H^3 and 73% for C^{14}. See reference 1 for discriminator and photomultiplier voltage settings.

(13) 18-Hydroxycorticosterone has not been described previously although its 3,20-diethylene ketal derivative was prepared in the course of the synthesis of 11-keto-18-hydroxy-cortexone.[10] Recently, some chromatographic properties of 18-hydroxycorticosterone have been recorded: R. Neher and A. Wettstein, *Helv. Chim. Acta*, **43**, 1171 (1960).

(14) In reference 11, although 18-hydroxycorticosterone itself was not found, the authors were able to isolate lactone II from hog adrenal extracts. This lactone may have been formed from 18-hydroxycorticosterone as an artifact.

(15) P. J. Ayres, J. Eichhorn, O. Hechter, N. Saba, J. F. Tait and S. A. S. Tait, *Acta Endocrinologica*, **33**, 27 (1960).

(16) The extension of results in the amphibian adrenal to higher vertebrates would appear to be justified since the secretory product of the bullfrog adrenal is similar to that of the *sona glomerulosa* of the mammalian adrenal cortex. See footnote 2, also H. Carstensen, A. C. J. Burgers and C. H. Li, THIS JOURNAL, **81**, 4109 (1959).

VETERANS ADMINISTRATION HOSPITAL
BRONX 68, NEW YORK
STANLEY ULICK
KATHRYN KUSCH

RECEIVED OCTOBER 31, 1960

AN EXPLANATION OF AN ANOMALOUS ANTIPODAL SPECIFICITY OF CHYMOTRYPSIN

Sir:

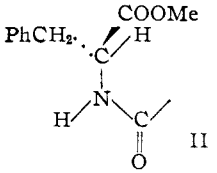
Recently Hein, McGriff and Niemann[1] reported the anomalous finding that α chymotrypsin hydrolyzes the D isomer of 1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline (I) much more rapidly than it hydrolyzes the L isomer. These authors suggested several lines of thought along which explanations might be sought. We wish to show here how this observation can be explained simply and plausibly within the framework of the polyaffinity concept without the necessity of invoking new principles.

In the polyaffinity theory it is recognized that although a substrate might exist in a very large number of conformations in solution, when it is bound to the enzyme most of the molecules will assume a well-defined conformation with the contributing groups and the functional group in definite positions in space. This conformation (which we will call the "correct" conformation) is, of course, determined by the reacting sites of the

(1) G. Hein, R. B. McGriff and C. Niemann, THIS JOURNAL, **82**, 1830 (1960).

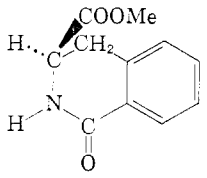
enzyme. To explain the finding of Hein, McGriff and Niemann, all that need be demonstrated is that there are conformations accessible to all the usual substrates of chymotrypsin and to the D isomer of I but not to the L isomer.

We start with the fact that N-acyl-L-phenylalanine esters, notably N-benzoyl-L-phenylalanine ethyl ester, are excellent substrates for chymotrypsin.[2] Sizable and distinct contributions to the activity of these substrates are made by the phenyl, the amino, and the acyl groups as judged by the poorer activity of substrates lacking one or more of these features.[3] We will consider the pertinent features of the "correct" conformation of an N-acyl-L-phenylalanine ester to be as shown in II.

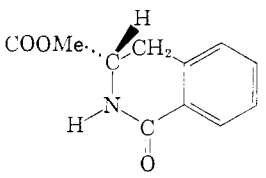


II

If we now examine the L and D isomers of substrate I, it is apparent that the D antipode has



I-D



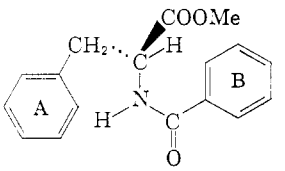
I-L

the correct conformation and that the L-isomer does not. We thus have demonstrated that an explanation exists within the framework of the polyaffinity theory.

(2) S. Kaufman and H. Neurath, *Arch. Biochem. Biophys.*, **21**, 437 (1949).

(3) N. M. Green and H. Neurath, "The Proteins," Vol. IIB, Academic Press, Inc., New York, N. Y., 1954, p. 1057.

On the basis of the above demonstration, we can formulate the "correct" conformation of N-benzoyl-L-phenylalanine methyl ester



It is pertinent to note that ring B makes a positive contribution to binding as judged by the lower K_m values of substrates and lower K_i values of inhibitors containing an acyl group with a cyclic function. Also, reactivators of diethylphosphoryl-chymotrypsin containing two aromatic rings are far superior to those containing only one.[4]

According to this conformation the aromatic nucleus of compound I interacts with the same part of the active site as the aromatic function of the benzoyl group. The chosen spatial position of ring A is based upon the reactivation studies[4] but its validity does not affect our argument.

The important finding of Hein, McGriff and Niemann can be interpreted within the framework of the polyaffinity theory. When so interpreted, a large number of *a priori* conformations of substrates are eliminated and we thus gain important information concerning the topology of the active site of chymotrypsin.

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DEPARTMENTS OF BIOCHEMISTRY IRWIN B. WILSON
NEUROLOGY AND MICROBIOLOGY BERNARD F. ERLANGER
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY
NEW YORK 32, N. Y.

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(4) W. Cohen and B. F. Erlanger, THIS JOURNAL, **82**, 3928 (1960).

BOOK REVIEWS

Rheology. Theory and Applications. Volume III. Edited by FREDERICK R. EIRICH, Polytechnic Institute of Brooklyn, New York. Academic Press Inc., 111 Fifth Avenue, New York 3, N. Y. 1960. xvi + 680 pp. 16 × 23.5 cm. Price, $21.00.

This third volume treats the more applied aspects of Rheology as contrasted with the first two volumes. This was intended to be the final volume but a fourth volume may be required to round out the field. The organization of material parallels the earlier treatments.

The sixteen chapters with authors are: 1. The Normal-Coordinate Method for Polymer Chains in Dilute Solution by B. H. Zimm; 2. The Principles of Rheometry by S. Oka; 3. Viscosity of Suspensions of Electrically Charged Particles and Solutions of Polymeric Electrolytes by B. E. Conway and A. Dobry-Duclaux; 4. The Rheology of Latex by Samuel H. Maron and Irvin M. Krieger; 5. The Rheology of Printing Inks by A. C. Zettlemoyer and Raymond R. Myers; 6. Rheology of Pastes and Paints by Ruth N. Weltmann; 7. Atomistic Approach to the Rheology of Sand–Water and of Clay–Water Mixtures by W. A. Weyl and W. C. Ormsby; 8. The Rheology of Inorganic Glasses by W. A. Weyl; 9. The Rheology of Concrete by M. Reiner; 10. The Deformation of Crystalline and Cross-Linked Polymers by I. L. Hopkins and W. O. Baker; 11. The Viscosity and Elasticity of Interfaces by Dean W. Criddle; 12. Rheology of Lubrication and Lubricants by A. Bondi; 13. The Rheology of Adhesion by J. J. Bikerman; 14. Rheology in Molding by C. E. Beyer and R. S. Spencer; 15. Rheology of Spinning by Bruno R. Roberts; 16. Theory of Screw Extruders by W. L. Gore and James M. McKelvey.

B. H. Zimm has discussed the use of the normal coördinate method of treating a polymeric molecule acted on by the forces of a flowing viscous solvent. Some interesting results have been obtained. S. Oka's chapter discusses various instruments for measuring viscosity and different models of flow. Viscosity is conditioned by all the bonds that impede molecules from sliding past each other. Ionic attractions form an especially interesting chapter discussed by