the addition of 2 *N* potassium hydroxide. After removal of a small amount of an amorphous precipitate by filtration, 10% barium chloride solution (147 cc.) was added, and the resulting suspension of precipitated barium salts was heated on the steam-bath for 30 minutes. The precipitate was collected after cooling to room temperature, washed with water, and dried (9.67 g., 56.9% Ba).

The barium salts were dissolved in hot 3 *N* hydrochloric acid (100 cc.) and decomposed by the addition of the calculated amount of 3 *N* sulfuric acid (27.8 cc.). The filtrate and hot water washings from the barium sulfate were concentrated to 50 cc. and placed in the refrigerator. The oxalic acid crystals which were deposited overnight were removed by filtration (3.57 g.). The filtrate, after reduction of its volume to 10 cc., was again placed in the refrigerator. The resulting mixture of large oxalic acid crystals and very small short rods was separated by aspirating the latter together with the mother liquor with a rubber-capped pipet. The fine crystals were collected by centrifugation (180 mg.). The mother liquor was brought to dryness (418 mg.), and the residue triturated with absolute ethanol. The insoluble portion, consisting of inorganic material, was removed by filtration, and the filtrate brought to dryness. The semi-crystalline residue (375 mg.), as well as the original crop of fine rods, was treated separately with boiling concentrated nitric acid (3 cc.) under reflux till the evolution of brown nitrous gases ceased (*ca.* 1 hour). The solutions were concentrated on a hot-plate till crystallization commenced. Since the two batches of crystalline material collected after chilling did not differ materially in melting point and appearance, they were combined (185 mg.) and recrystallized again from boiling concentrated nitric acid, from which it formed small, clear-cut prisms melting with effervescence at 241–245° after softening beginning at 224°. The product did not depress the melting point of the reference specimen (244–248°, soft 224°) obtained from Professor Purves.[4] For analysis the compound was recrystallized from acetone–hexane (m.p. 235–238.5°, soft, 231°).

*Anal.* Calcd. for $C_{10}H_8O_8$ (254.1): C, 47.25; H, 2.38; neut. equiv., 63.5. Found: C, 47.43; H, 2.49; neut. equiv., 63.1.

**The tetramethyl ester IIa** was prepared in the usual manner by adding excess ethereal diazomethane to a solution of the acid in dry methanol. After recrystallization from acetone–hexane it melted at 127–130°, alone or in mixture with an authentic preparation of the same melting point.[4]

*Anal.* Calcd. for $C_{14}H_{14}O_8$ (310.3): C, 54.20; H, 4.55; $OCH_3$, 40.0. Found: C, 54.40; H, 4.77; $OCH_3$, 39.6.

The infrared spectrum (Nujol mull) of the ester was identical with that of the reference preparation[4]; 3.45 d, 3.87 ss, 5.76 d, 6.05 s, 6.27 ss, 6.36 ss, 10.48 ss, 10.56 m, 11.13 s, 11.54 m, 11.65 s, 12.79 s, 13.22 md, 13.62 sm, 14.30 s (d = deep, m = medium, s = small, ss = very small).

**The 1,4-dimethyl ester IIb** was prepared by refluxing a solution of the acid (48 mg.) in 3% methanolic hydrogen chloride (3 cc.) for 6 hours.[3] The esterified material was separated into acidic and neutral fractions in the usual manner. The acidic fraction (47.3 mg.) was recrystallized from acetone–hexane, from which it formed rosettes of platelets melting at 160–167.5°. Further recrystallization failed to raise or sharpen the melting point. In mixture with the reference specimen[4] (m.p. 171–175°) the preparation melted at 167–173°.

*Anal.* Calcd. for $C_{10}H_{10}O_8$ (282.2): $OCH_3$, 22.0; neut. equiv., 141. Found: $OCH_3$, 21.9; neut. equiv., 138.

**Ultraviolet Absorption Spectra of Acid II and its Esters.**—The ultraviolet absorption spectra of the acid II and its esters IIa and IIb in ethanol are practically identical. They are characterized by a single maximum at 288 mμ (II, IIa) or 290 mμ (IIb), with ε 1260–1300, a shallow minimum at 279 mμ (II) or 275 mμ (IIa, IIb), with ε 1130–1250, and end absorption with an inflection at about 240 mμ, ε 8000–8300, which probably corresponds to the high band at 226 mμ in the spectrum of phthalic acid (butyl phthalate, $\lambda_{max}^{alc.}$ 226 mμ (9500), 272 mμ (1500)). The spectrum of the isomeric ester, tetramethyl benzene-1,2,4,5-tetracarboxylate[8] in ethanol shows the same general pattern (maximum at 291 mμ (2370), minimum at 275 mμ (1780), end absorption with inflection at 240 mμ (9050)). It would thus appear that these features are associated with the number of carboxyl functions (and hence of possible resonance structures) rather than with their distribution over the benzene ring. On the other hand, the segregation in the isomeric ester of the carbomethoxy groups into two pairs insulated from each other by unsubstituted ring carbon atoms seems to impart on each of these groupings something like the character of a separate chromophore, if the nearly twice as high extinction of the maximum over that of the corresponding band of the 1,2,3,4-substituted ester IIa can be so interpreted.

The authors are indebted to Mr. Joseph A. Alicino and his associates for the microanalyses, and to Dr. Nettie Coy for the ultraviolet and infrared measurements.

(8) We are greatly indebted to Prof. R. T. Arnold of the University of Minnesota for making available to us a sample of this ester.

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH
NEW BRUNSWICK, NEW JERSEY

# COMMUNICATIONS TO THE EDITOR

## N-PANTOTHENYLCYSTEINE AS A PRECURSOR FOR PANTETHEINE AND COENZYME A

*Sir:*

Pantetheine is readily converted *in vivo* to coenzyme A (CoA), and the intermediate reactions involved are now known.[1] In contrast, nothing is known of the mechanisms by which pantetheine arises from pantothenic acid. A study of this transformation revealed that in the presence of adenosine triphosphate and cysteine, extracts of acetone-dried cells of *Proteus morganii* transform pantothenate to a compound (I) essentially inactive in replacing pantothenate for *Saccharomyces carlsbergensis*, *Lactobacillus arabinosus* and *Lactobacillus casei*.[2] Pantethine was not formed.[2] However, the product (I) formed was considerably more active in promoting growth of *Acetobacter suboxydans*[2] than an amount of pantothenate equal to that inactivated by the enzyme preparation.

Since cysteine could not be replaced by β-mercaptoethylamine in the inactivation reaction with *P. morganii*, it appeared that I might be N-pantothenylcysteine (II). The corresponding disulfide,

(1) G. D. Novelli and M. H. Hoagland. Abstract 26C, 123rd Meeting. Am. Chem. Soc., Los Angeles. March, 1953; G. D. Novelli, *Fed. Proc.*, in press.

(2) Free pantothenate was determined by assay with *S. carlsbergensis;* pantethine was determined with *Lactobacillus helveticus* 80 (J. Craig and E. E. Snell, *J. Bact.*, **61**, 283 (1951)) before and after digestion with intestinal phosphatase. Assays for pantothenate activity with *A. suboxidans* were conducted in the medium of L. A. Underkofler, A. C. Banz and W. H. Peterson (*J. Bact.*, **45**, 183 (1943)).

TABLE I

THE COMPARATIVE ACTIVITIES OF PANTOTHENIC ACID AND BIS-[N-(PANTOTHENYL)]-L-CYSTINE FOR *Acetobacter suboxydans*

| Compound tested | Amount required for half maximum growth, $\mu$M. $\times 10^3$ per 10 ml. |
|---|---|
| Calcium pantothenate | 11.2 |
| Calcium pantothenate + sulfhydryl compound[a] | 11.2 |
| Bis-[N-(pantothenyl)]-L-cystine (III) | 2.0 |
| Bis-[N-(pantothenyl)]- + sulfhydryl compound | 0.7 |

[a] Cysteine, $\beta$-mercaptoethylamine, thiomalic acid and sodium thioglycolate were equally effective. Each compound was added to III at a 200 to 1 molar ratio.

bis-[N-(pantothenyl)]-cystine (III),[3] corresponded closely in biological properties to I. Thus, III was less than 2% as active as pantothenate for *L. arabinosus*, *L. casei* and *S. carlsbergensis*, and less than 2% as active as pantethine for *L. helveticus*. For *A. suboxydans*, however, III was 2 to 3 times as active as pantothenate, and following treatment with any of several sulfhydryl compounds, was about 8 times as active (Table I). The latter did not alter the activity of pantothenate, and hence presumably acted by reducing the disulfide III to the more active thiol, II. Finally, dried cells of *A. suboxydans* convert II to pantetheine (Table II).

TABLE II

THE ENZYMATIC CONVERSION OF N-PANTOTHENYLCYSTEINE TO PANTETHEINE

The complete system contained per 4 ml.: 0.021 $\mu$mole of bis-[N-(pantothenyl)]-L-cystine, 35 $\mu$moles of –SH compound (cysteine or $\beta$-mercaptoethylamine), 80 mg. of vacuum-dried cells of *A. suboxydans*, and 1 ml. of 0.25 *M* phosphate buffer, *p*H 6.5; incubation was for 3 hours at 30°.

| System | Pantetheine formed, $\mu$M. $\times 10^3$ |
|---|---|
| Complete | 2.9 |
| Complete, heated[a] | 0.0 |
| Minus bis-[N-(pantothenyl)]-L-cystine | .0 |
| Minus –SH compound | .0 |
| Minus dried cells | .0 |

[a] Heated at 100° for 5 min. immediately after the addition of the dried cells.

These experiments indicate that pantothenylcysteine is an intermediate in the formation of pantetheine (and hence CoA) from pantothenic acid. The fact that both II and CoA have high activities for *A. suboxydans*, whereas pantethine is no more active than pantothenic acid, may indicate that pantothenylcysteine is phosphorylated before decarboxylation, thus yielding phosphopantetheine (which has high activity for *A. suboxydans*[4]) directly.

Under conditions that liberate the theoretical amount of pantothenate from pantethine, an extract of pigeon liver liberated only 7% of the bound pantothenate from III. If animals can utilize III, its natural occurrence could explain the observations of Lih, *et al.*,[5] that the pantothenate content of some natural materials as indicated by assay with rats was higher than when the material was subjected to enzyme treatment and pantothenate was measured microbiologically.

(3) We are indebted to Drs. J. F. Cavella and R. E. Bowman, Parke, Davis and Co., Ltd., Hounslow, England, for a sample of this compound.

(4) G. D. Novelli, private communication.

(5) H. Lih, T. E. King, H. Higgins, C. A. Baumann and F. M. Strong, *J. Nutrition*, **44**, 361 (1951).

THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH
AUSTIN, TEXAS

GENE M. BROWN
ESMOND E. SNELL

RECEIVED APRIL 30, 1953

## HELICAL POLYPEPTIDE CHAIN CONFIGURATION IN COLLAGEN[1]

*Sir:*

Methods for testing X-ray diffraction data for evidence of helical molecular chain configuration have recently been provided.[2] Applications have shown that helical structures hitherto proposed do not account for wide-angle diffraction by collagen.[3] We wish to describe specifications for a helix which promises to be satisfactory.

The table lists the commonly observed near-meridional diffractions of kangaroo tail tendon, as well as new ones photographed in a cylindrical camera with specimens tilted at appropriate angles to the incident Cu K$\alpha$ radiation. Assignment of layer indices ($k$) further supports the 20 Å. pseudo-period previously suggested.[4]

| Observed spacings, $d$, Å. | $k$ | $b_0 = kd$, Å. | $n$ |
|---|---|---|---|
| $\infty$ | 0 | ... | 0 |
| 9.55 | 2 | 19.1 | 1 |
| 5.0 | 4 | 20.0 | 2 |
| 3.97 | 5 | 19.9 | 1 |
| 2.86 | 7 | 20.0 | 0 |
| 2.27 | 9 | 20.4 | 1 |
| 1.83 | 11 | 20.1 | 2 |
| 1.64 | 12 | 19.7 | 1 |
| 1.45 | 14 | 20.3 | 0 |

A discontinuous helix with 2 turns and 7 roughly equivalent scattering groups per 20 Å. of axial projection should contribute to near-meridional diffraction according to Bessel functions whose orders, $n$, are shown. The observed layer lines are those for which $n$ is 2 or less (indeed often one or zero). For missing layer lines $n$ is 2 or greater. The higher layers observed with shorter wavelength radiation[5] would have zero $n$'s, and $k$ indices which are multiples of 7.

The approximately seven-fold screw axis does not permit exact hexagonal packing of these chains in fibrils, but there is no evidence requiring this. Pseudo-hexagonal packing, with about 12 Å. between chain centers (dry fibers), accounts for the

(1) Supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council. One of us (C. C.) gratefully acknowledges fellowship support by the National Science Foundation.

(2) W. Cochran, F. H. C. Crick and V. Vand, *Acta Cryst.*, **5**, 581 (1952).

(3) L. Pauling and R. B. Corey, *Proc. Roy. Soc.* (*London*), **B141**, 31 (1953).

(4) R. S. Bear, *Advances in Protein Chem.*, **7**, 115, 130 (1952).

(5) M. F. Perutz, *Ann. Repts., Chem. Soc., London*, **48**, 379 (1952).

principal equatorial reflection. Density requires about 21 amino-acid residues along 20 Å. of helix axis.[4] The 7 nearly equivalent groups should, therefore, comprise 3 residues each.

The above analysis does not determine uniquely the chemical connection of the residues. Primitive helical connection has the merit of allowing several-fold chain extensibility from the average 0.95 Å. of axial projection per residue.[4] Intensity relationships and stereochemical considerations are being used to derive detailed models.

DEPARTMENT OF BIOLOGY CAROLYN COHEN
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RECEIVED MAY 16, 1953

## REACTIONS OF ALIPHATIC AMINES WITH SUGARS*

*Sir:*

We have found that reactions of long-chain primary aliphatic amines with sugars can go far past the amine glycoside stage. In our work as many as five or six moles of amine have reacted with one mole of hexose sugar. In effect, these reactions are replacement of hydroxyl groups by alkylamino groups. The actual mechanism of the reaction, however, is probably a series of Amadori rearrangements, each followed by reaction of the carbonyl group so formed with another mole of amine.

Ketoses appear to be more reactive than aldoses toward amines. In general, formation of amine glycosides takes place very readily at room temperature. A solution of equimolar amounts of fructose and octadecylamine in aqueous isopropyl alcohol, however, reacts in a day or two to give a good yield of a white solid, m.p. 105.5–106.2° (dec.). Elementary analysis shows that this compound is formed from two moles of amine and one of fructose by loss of two moles of water. From this fact it is clear that the product is not an aldehyde-ammonia type of compound or a mixture of amine and amine glycoside.

*Anal.* Calcd. for $C_{42}H_{86}N_2O_4$: C, 73.81; H, 12.69; N, 4.10. Calcd. for $C_{42}H_{88}N_2O_5$: C, 71.92; H, 12.65; N, 4.00. Found: C, 74.36: H, 12.52; N, 4.29.

To obtain a similar product with glucose it is necessary to use an excess of amine and it is desirable to heat the mixture. By increasing the severity of the reaction conditions, one may introduce still more amino groups. A solution of six moles of octadecylamine and one mole of glucose in aqueous isopropyl alcohol, heated several hours at 60–70°, yields a yellow solid, m.p. 66.5–68°, derived from four moles of amine and one of sugar.

*Anal.* Calcd. for $C_{78}H_{160}N_4O_2$: C, 78.96; H, 13.60; N, 4.72. Found: C, 78.66; H, 13.37; N, 4.41.

This octadecylamine-glucose product forms a monopicrate of uncertain m.p. (*ca.* 50–95°).

*Anal.* Calcd. for $C_{84}H_{163}N_7O_9$: C, 71.29; H, 11.61; N, 6.93. Found: C, 71.83; H, 11.77; N, 6.70.

* Paper No. 151, Journal Series, Research Laboratories, General Mills, Inc.

Five moles of octadecylamine react with one mole of sorbose on extended heating in alcohol to give a tan solid, m.p. 67–69.5°.

*Anal.* Calcd. for $C_{96}H_{197}N_5O$: C, 80.19; H, 13.81; N, 4.87. Found: C, 80.24; H, 13.25; N, 4.79, 4.53.

This sorbose-octadecylamine product forms a yellow monopicrate, m.p. 45–47°.

*Anal.* Calcd. for $C_{102}H_{200}N_8O_8$: C, 73.50; H, 12.10; N, 6.72. Found: C, 72.95; H, 11.43; N, 6.73.

These findings suggest new approaches to the study of the browning reaction. A more detailed report on this work will be published later.

CHEMICAL LABORATORIES
GENERAL MILLS, INC. JOHN G. ERICKSON
MINNEAPOLIS, MINN.

RECEIVED APRIL 20, 1953

## THE FREE AMINO GROUPS OF γ-GLOBULINS OF DIFFERENT SPECIES

*Sir:*

Porter[1] has found that normal rabbit γ-globulin and the specific rabbit antibody to hen's ovalbumin possess the identical N-terminal peptide sequence (Ala.Leu.Val.Asp.Glu-). It has been suggested that this is in agreement with theories of antibody formation which ascribe specificity to specific surface configuration rather than to differences in amino acid sequence or composition. In contrast are earlier findings by many investigators[2] which have shown that human, bovine and equine γ-globulins are heterogeneous by a variety of criteria. We now wish to report a study of the free amino groups of these globulins by reaction with dinitrofluorobenzene to form the dinitrophenyl (DNP) derivatives by the procedure of Sanger. The present results indicate differences among preparations of human γ-globulins and considerable species variation (Table I).

TABLE I

NUMBER OF FREE AMINO GROUPS IN VARIOUS γ-GLOBULINS

| | Human II-1,2 | Human II-3 | Human cryoglobulin | Bovine A |
|---|---|---|---|---|
| Asp (60%)[a] | 1.06 | 1.01 | 1.3 | 0.13 |
| Ser (81%) | .10 | .17 | [c] | .09 |
| Glu (56%) | 1.82 | 1.06 | 1.2 | .15 |
| Ala (55%) | | | | .09 |
| Val (57%) | | | | .11 |
| Lys (90%) | 75 | 74 | 70 | 73 |
| Lys[b] | 79 | 69 | | 74 |

[a] Parentheses give recovery values for DNP amino acids after hydrolysis for 24 hours in a sealed tube at 105°. The tabulated values are based on these recoveries, and on an assumed molecular weight of 160,000 for all these proteins. Data on II-1,2 and II-3 globulins are the averages of five determinations each. The cryoglobulin values are averages of three determinations and the bovine, of four independent measurements. [b] Values calculated from microbiological assays.[2] [c] None detectable.

The results on the II-1,2 and II-3 fractions suggest the presence of two or more distinct molecules with different N-terminal residues. The "cryoglobulin" is a γ-globulin from a patient with multiple

(1) R. R. Porter, *Biochem. J.*, **46**, 473 (1950).

(2) E. L. Smith and B. V. Jager, *Ann. Rev. Microbiol.*, **6**, 207 (1952).

myeloma.[3] Results on the II-1,2 and II-3 fractions have been confirmed by Putnam,[4] who has analyzed other γ-globulin fractions and performed extensive studies on a series of myeloma proteins. His results, privately communicated to us, show an even more striking range of variations in the N-terminal amino acids of human γ-globulins.

Studies on a highly purified bovine γ-globulin (γ-globulin A[5]) show that all the N-terminal residues are present in less than molar quantities. This suggests that bovine γ-globulin, like the human, is a mixture of closely related proteins which differ in the nature of their N-terminal residues. A preparation of bovine γ-globulin (B)[6] from animals hyperimmunized to mixed antigens (vaccinia, *H. pertussis*, and diphtheria toxin) yields the same end groups as A but contains greater amounts of N-terminal valine and only traces of the other DNP amino acids. An equine γ-globulin[6] gives glutamic acid, aspartic acid, serine, threonine and leucine (or isoleucine) as N-terminal residues, each present in less than a molar quantity.

The DNP proteins were prepared and hydrolyzed by the methods of Sanger (see Porter[7]). The quantity of protein in the DNP protein was estimated from the amide $NH_3$ of the untreated and the DNP proteins. The ether-soluble DNP amino acids were separated on a series of buffered Celite columns[8] at *p*H 4.0, 5.6, 6.5 and 7.1, with water-saturated ethyl acetate, chloroform and various chloroform–ether and chloroform–butanol mixtures as developing solvents. Positive identification of the separated DNP amino acids was made on Whatman No. 1 and No. 4 papers, buffered with phthalate at *p*H 6.0. ε-DNP-lysine was separated from the acid fraction of the hydrolysate on 1 *M* HCl–Celite columns. Quantitative measurements were made according to the method described by Sanger. Because breakdown of the protein may occur during treatment with dinitrofluorobenzene, *e.g.*,[9] the mother liquors were examined for DNP amino acids. No liberation of DNP amino acids or peptides has been found during the preparation of DNP γ-globulins. Further study of these and other γ-globulins and of specific antibodies is in progress.[10]

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RECEIVED MAY 6, 1953

(3) The "cryoglobulin" spontaneously precipitated from the cooled serum of this patient. It was washed several times with cold water and further purified by separation in the electrophoresis cell. The electrophoretic mobility of this γ-globulin is $-1.4 \times 10^{-5}$ cm.$^2$ volt$^{-1}$ sec.$^{-1}$ at *p*H 8.5 in veronal buffer. This protein precipitates completely with rabbit antisera to human γ-globulin. We are indebted to Dr. B. V. Jager and Mr. D. M. Brown for their coöperation in these studies.

(4) F. W. Putnam, THIS JOURNAL, **75**, 2785 (1953).

(5) E. L. Smith, *J. Biol. Chem.*, **164**, 345 (1946).

(6) E. L. Smith and T. D. Gerlough, *ibid.*, **167**, 679 (1947).

(7) R. R. Porter, "Methods in Medical Research," Year Book Publishers, Inc., Chicago, Ill., **3**, 256 (1950).

(8) J. C. Perrone, *Nature*, **167**, 513 (1951).

(9) E. O. P. Thompson, *Biochim. et Biophys. Acta*, **10**, 633 (1953).

(10) Supported by grants from the National Institutes of Health, United States Public Health Service. We are indebted to Dr E. O. P. Thompson for his advice and help in these studies.

## N-TERMINAL GROUPS OF NORMAL HUMAN GAMMA GLOBULIN AND OF MYELOMA PROTEINS

*Sir:*

Although proteins of different isoelectric point and antibodies of unlike specificity have been demonstrated in normal human γ-globulin, there is yet no firm evidence for forms differing in molecular weight or chemical structure. To be sure, the electrophoretic inhomogeneity of γ-globulin is well known, variation in the amino acid content of subfractions has been reported, and a faster sedimenting component is observed after ethanol fractionation of pooled sera.[1] On the other hand, in support of the theory of the chemical identity of normal and antibody globulins, Porter[2] has found in the rabbit that both the normal γ-globulin and the antibody to egg albumin end in the same pentapeptide sequence with a single N-terminal group of alanine per molecule. The aberration in protein synthesis occurring in patients with multiple myeloma has prompted a similar study of the N-terminal groups of normal human γ-globulins and of the myeloma proteins produced by different individuals. This has led to the finding that normal human γ-globulins contain aspartic and glutamic acids as the major N-terminal groups, whereas the pathological γ-globulins we have so far investigated contain neither amino acid nor only aspartici n this position.[3]

Five well-characterized preparations of human γ-globulin obtained by ethanol fractionation of pooled plasma were received from various sources.[4,5] All contained 15 to 25% of a second component with a sedimentation constant of 9S and migrated with a diffuse boundary in electrophoresis. These were compared with myeloma globulins prepared by salt fractionation of the serum of five patients.[6] Electrophoretically four of the myeloma proteins were of the gamma type, one with a mobility of $-0.7$ *u* at *p*H 8.6 in Veronal buffer, and three with a mobility of $-1.1$ *u*. These proteins migrated with a sharp single boundary in electrophoresis; they had an $s_{20}$ of 6.6*S* and exhibited only 0 to 5% of a heavy component in the ultracentrifuge. A fifth myeloma protein was of the "beta" type; it had a mobility of $-3.4$ *u* at *p*H 8.6, was 90% homogeneous in electrophoresis, but contained two major components on ultracentrifugation ($s_{20}$ = 6.2 and 8.8*S*). The N-terminal amino acids were determined by Sanger's method with use of a buffered silica gel column for separation of the dinitrophenyl- (DNP) amino acids and paper chromatography for their identification.[7,8]

(1) E. L. Smith and B. V. Jager, *Ann. Rev. Microbiol.*, **6**, 207 (1952).

(2) R. R. Porter, *Biochem. J.*, **46**, 479 (1950).

(3) McFadden and Smith[4] report that a "cryoglobulin" from a patient with multiple myeloma contained 1.2 and 1.3 moles, respectively, of N-terminal glutamic and aspartic acids. One of our proteins of lower mobility ($-1.1$ *u* at *p*H 8.6) was also a cryoglobulin; it yielded 1.8 moles of N-terminal aspartic but only 0.14 mole of N-terminal glutamic acid per mole protein. The other myeloma globulins of the same mobility were devoid of detectable N-terminal serine and glutamic acid.

(4) M. L. McFadden and E. L. Smith, THIS JOURNAL, **75**, 2784 (1953).

(5) Two lots of Fraction II kindly supplied by Dr. John T. Edsall, $\gamma_2$ globulin by Dr. R. A. Alberty, and Fractions II-1,2 and II-3 by Dr. E. L. Smith.

(6) F. W. Putnam and B. Udin, *J. Biol. Chem.*, in press.

(7) F. Sanger, *Advances in Protein Chem.*, **7**, 1 (1952).

(8) S. Blackburn and A. G. Lowther, *Biochem. J.*, **48**, 126 (1951).

Unlike rabbit γ-globulin, normal human γ-globulin (Fraction II or $\gamma_2$) contains approximately one mole each of N-terminal aspartic and glutamic acids per 160,000 g. It also yields 0.1 to 0.2 mole of N-terminal serine and small amounts of an unidentified DNP-derivative. In contrast, the three myeloma globulins of mobility −1.1 *u* had two moles of N-terminal aspartic acid per 160,000 g., and in accord with their greater physical homogeneity, were entirely free or nearly so of other N-terminal groups. The myeloma globulin of mobility −0.7 *u* was essentially devoid of N-terminal glutamic or aspartic acids, whereas these occurred in an almost equimolar ratio in the heterogeneous β-globulin.

This appears to be the first demonstration of a difference from normal in the chemical structure of serum proteins elaborated in any disease. It may, however, be interpreted in terms of the physiological occurrence of three (or more) gamma globulins only one of which is synthesized profusely by a given patient with multiple myeloma. Of these proteins one may contain at least two peptide chains both terminating in glutamic acid, another two chains with only aspartic acid as the amino end-group, and the third has neither amino acid in the N-terminal position. This hypothesis is supported by results communicated to us by McFadden and Smith[4] and confirmed in this laboratory, in which it was found that human γ-globulin subfraction II-1,2 has 1.7 times as many moles of N-terminal glutamic acid as of aspartic, whereas subfraction II-3 has one mole of each. There is no correlation of N-terminal groups with the isoelectric point, for in three proteins with a *p*I of 7.3 to 7.5, the molar ratio of N-terminal glutamic to aspartic ranged over twenty-fold. Since physicochemical analysis has indicated great variation in the nature of myeloma globulins,[6] further terminal group analysis may reveal new end-groups and other information of interest to the study of normal serum proteins and of antibody globulins.[9]

(9) Supported by grants of the Lasdon Foundation and The National Cancer Institute, National Institutes of Health, United States Public Health Service. The aid and encouragement of Dr. Fred Sanger is gratefully acknowledged.

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CAMBRIDGE AND
DEPARTMENT OF BIOCHEMISTRY FRANK W. PUTNAM
UNIVERSITY OF CHICAGO

RECEIVED MAY 6, 1953

## REVERSIBLE CATALYTIC CLEAVAGE OF HYDROXY-AMINO ACIDS BY PYRIDOXAL AND METAL SALTS

*Sir:*

Pyridoxal and salts of copper, iron and aluminum catalyze the deamination of serine to pyruvate.[1] We have now found that while threonine and allothreonine undergo similar reactions they are much more rapidly cleaved to glycine and acetaldehyde throughout the *p*H range 3–12 in the presence of pyridoxal and copper, iron or aluminum salts. The reactions are readily reversible. Some analyses on reaction mixtures heated at 100° are given in Tables I and II. Similar results were obtained by allowing the reaction to proceed at room temperature for a few days. The amino acid concentrations were determined microbiologically or by the ninhydrin color reaction after separation on a column of cation exchange resin.

The cleavage of serine to glycine and formaldehyde can be demonstrated readily at *p*H 7 where its deamination is slow as can the conversion of formaldehyde and glycine to serine and pyruvate.

Other aldehydes such as propionaldehyde, glyoxylic acid, and pyridoxal can be condensed with glycine under the same conditions to yield a variety of β-hydroxy amino acids.

The presence in animal livers of an enzyme which splits threonine, allothreonine, β-hydroxyvaline, β-hydroxynorvaline and β-phenylserine to glycine and carbonyl compounds has been reported.[2] The reactions are inhibited by carbonyl reagents and despite the lack of decrease in activity in livers of vitamin $B_6$-deficient rats we predict that this will be found to be a pyridoxal-containing enzyme. A similar enzyme which splits serine is stated to require a cofactor related to folic acid and not to be inhibited by carbonyl reagents. However, vitamin $B_6$ is also required by both bacteria[3] and chicks[4] for synthesis of serine from glycine.

The combination of the following three vitamin $B_6$-catalyzed reactions provides an attractive mechanism of amino acid biosynthesis.

TABLE I

CLEAVAGE OF THREONINE TO GLYCINE AND ACETALDEHYDE

Reaction mixtures 20 mM. in threonine, *p*H 5, were heated 30 min. at 100°.

| Additions, millimoles per l. | | Products, millimoles per l. | | | |
|---|---|---|---|---|---|
| Pyridoxal | Alum[a] | Threonine | Glycine | Acetaldehyde | Ammonia |
| 0 | 2 | 19.2 | 0.0 | | |
| 10 | 0 | 17.5 | 1.2 | | |
| 10 | 2 | 3.7[b] | 12.5 | 14.0 | 1.3 |

[a] $KAl(SO_4)_2 \cdot 12H_2O$. [b] A small amount of allothreonine was also produced.

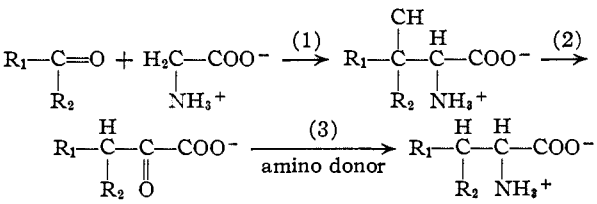
TABLE II

THE REVERSIBILITY OF THREONINE CLEAVAGE

Reaction mixtures containing 10 mM. pyridoxal and 2 mM. alum at *p*H 5 were heated at 100°.

| Reactants, millimoles per l. | | | Heating time, hr. | Threonine, found millimoles per l. |
|---|---|---|---|---|
| Threonine | Glycine | Acetaldehyde | | |
| 20 | 20 | 80 | 0.25 | 11.7 |
| 20 | 20 | 80 | 2.0 | 4.4 |
| 0 | 40 | 100 | 0.25 | 2.6[a] |
| 0 | 40 | 100 | 2.0 | 3.7[a] |

[a] Allothreonine was also produced in similar amounts.




The non-enzymatic reactions catalyzed by pyridoxal and metal salts have been carried out for

(1) D. E. Metzler and E. E. Snell, *J. Biol. Chem.*, **198**, 353 (1952).

(2) G. Ya. Vilenkina, *Doklady Akad. Nauk S.S.S.R.*, **84**, 559 (1952), from *C. A.*, **46**, 10227 (1952), and preceding papers.

(3) J. Lascelles and D. D. Woods, *Nature*, **166**, 649 (1950).

(4) S. Deodhar and W. Sakami, *Fed. Proc.*, **12**, 195 (1953).

each of the steps leading to the formation of aminobutyric acid from acetaldehyde and animal livers appears to contain enzymes which can catalyze these same reactions. The enzyme reported by Vilenkina[2] should catalyze reactions (1) and Lien and Greenberg[5] have reported conversion of threonine to aminobutyric acid, apparently by reactions (2) and (3) in rat livers. Though this synthetic pathway may not be used by animals it may be of importance in some organisms.

(5) O. G. Lien, Jr., and D. M. Greenberg, *J. Biol. Chem.*, **200**, 367 (1953).

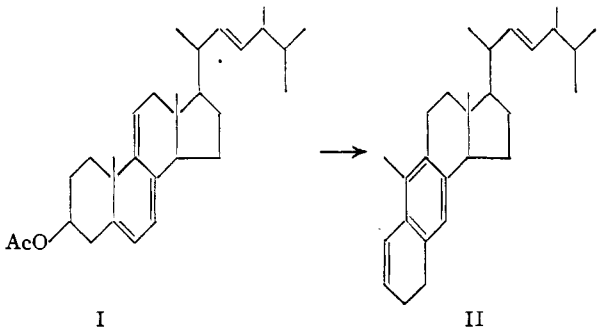
THE BIOCHEMICAL INSTITUTE AND THE
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF TEXAS, AND THE DAVID E. METZLER
CLAYTON FOUNDATION FOR RESEARCH J. B. LONGENECKER
AUSTIN, TEXAS ESMOND E. SNELL

RECEIVED MAY 8, 1953

## THE REARRANGEMENT OF DEHYDROERGOSTERYL ACETATE TO A *s*-OCTAHYDROANTHRACENE DERIVATIVE

*Sir:*

On treatment of a chloroform solution of dehydroergosteryl acetate (I) with catalytic amounts of hydrogen chloride at room temperature a skeletal rearrangement of the steroid takes place. The pure product (II) obtained in a yield of about 30% lacks an oxygen function and shows an ultraviolet absorption spectrum characteristic of an aromatic ring with one conjugated double bond, $\lambda_{max.}$ (isooctane) 222, 227, 266, 296, and 308 mμ. (ε 26,100, 27,100, 18,600, 2,760, 2,220, respectively); $\lambda_{max.}$ ($CS_2$) 968 cm.$^{-1}$; m.p. 105–107°; $[\alpha]^{20}D$ −70° ($CHCl_3$); *Anal.* Calcd. for $C_{28}H_{40}$: C, 89.29; H, 10.70. Found: C, 88.96; H, 10.74. It is proposed that, by the rupture of the $C_1$–$C_{10}$ bond and reattachment of $C_1$ to $C_6$, 1,2,3,4,7,8-hexahydro-3′-(5,6-dimethyl-3-heptenyl-2)-2,10-dimethyl-1,2-cyclopentanthracene (II) is formed. (Positions 7,8 and 3,4 for the conjugated double bond have not been ruled out experimentally.) Kinetic measurements by ultraviolet spectrophotometry show that this rearrangement is first order in steroid and approximately second order (1.85) in hydrogen chloride. The reaction rate constant is equal to 0.146 ± 0.003 liter$^2$ moles$^{-2}$ sec.$^{-1}$ at 20°.




I II

By catalytic hydrogenation ($PtO_2$, ethyl acetate–acetic acid) the double bond in the side chain and the conjugated olefinic double bond are saturated to give the corresponding *s*-octahydroanthracene derivative (III), m.p. 106–107°; $[\alpha]^{20}D$ +21° ($CHCl_3$); $\lambda_{max.}$ (isoöctane) 273, 278 and 282 mμ (ε 670, 550 and 695 respectively), $\lambda_{min.}$ 247 mμ (ε 95); *Anal.* Calcd. for $C_{28}H_{44}$: C, 88.34; H, 11.65. Found: C, 88.42; H, 11.47. Oxidation of II with 70% nitric acid and subsequent esterification of the resulting compound with diazomethane leads to 1-methyl-2,3,5,6-tetracarbomethoxybenzene (IV), m.p. 121–123°; *Anal.* Calcd. for $C_{15}H_{16}O_8$: C, 55.55; H, 4.97. Found: C, 55.43; H, 5.06. The structure of IV was confirmed by its comparison with a sample obtained by an analogous oxidation of 9-methyl-*s*-octahydroanthracene. Compound IV, incidentally, was found to be identical with the methyl tetracarbomethoxybenzene obtainable by the nitric acid oxidation of various steroids.[1] From the analogous oxidation of 9-methyl-*s*-octahydrophenanthrene we obtained pentacarbomethoxybenzene instead of the expected, unknown 1-methyl-2,3,4,5-tetracarbomethoxybenzene (V).

We are considering the possibility that this type of facile rearrangement, *i.e.*, the transformation of steroids into anthracene derivatives, is involved in spontaneous carcinogenesis.

(1) (a) H. H. Inhoffen, *Ann.*, **494**, 122 (1932); (b) A. Windaus and G. Zühlsdorff, *ibid.*, **536**, 204 (1938); (c) M. Müller, *Z. physiol. Chem.*, **233**, 223 (1935).

NATIONAL INSTITUTE OF ARTHRITIS AND
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RECEIVED APRIL 30, 1953

## ENZYMES OF THE FATTY ACID CYCLE. II. ETHYLENE REDUCTASE[1]

*Sirs:*

We have recently reported on the identification and isolation of β-keto thiolase and β-keto reductase.[2] Similar results have been obtained in other laboratories.[3,4,5] Through the combined action of these two enzymes the cell elongates the chain of the CoA thioester derivatives of fatty acids by the addition of a $C_2$ carbon chain from acetyl-S-CoA forming the corresponding β-hydroxy-CoA-thioester derivatives. In this way β-hydroxy-butyryl-S-CoA is formed from acetyl-S-CoA (Reactions 1 and 2).

$$2CH_3—CO—S—CoA \rightleftharpoons CH_3—CO—CH_2—CO—S—CoA + HS—CoA \quad (1)$$

$$CH_3—CO—CH_2—CO—S—CoA + DPNH + H^+ \rightleftharpoons CH_3—CHOH—CH_2—CO—S—CoA + DPN^+ \quad (2)$$

$$CH_3—CHOH—CH_2—CO—S—CoA \rightleftharpoons CH_3—CH=CH—CO—S—CoA + H_2O \quad (3)$$

$$\text{Leuco-safranine} + CH_3—CH=CH—CO—S—CoA \longrightarrow \text{Safranine} + CH_3—CH_2—CH_2—CO—S—CoA \quad (4)$$

(1) This work was supported in part by a grant from the Research Foundation of Germany. The following abbreviations are used: Coenzyme A, CoA–SH; acyl coenzyme A derivatives, acyl-S-CoA; oxidized and reduced diphosphopyridine nucleotide, DPN$^+$ and DPNH; reduced triphosphopyridine nucleotide, TPNH; flavinadenine dinucleotide, FAD; micromoles, μM.

(2) F. Lynen, L. Wessely, O. Wieland and L. Rueff, *Angew. Chem.*, **64**, 687 (1952).

(3) J. R. Stern, M. J. Coon and A. del Campillo, THIS JOURNAL, **75**, 1517 (1953).

(4) A. L. Lehninger and G. D. Greville, *ibid.*, **75**, 1515 (1953).

(5) D. E. Green and S. Mii, *Federation Proc.*, **12**, 211 (1953).

The remaining two enzymes of the cycle have been recently characterized. One of them, which may be referred to as crotonase (Reaction 3), is the subject of a preceding note.[6] The other enzyme, ethylene reductase, catalyzes Reaction 4. Our method of replacing the naturally occurring CoA compounds by the readily synthesized analogs of N-acetylthioethanolamine again proved useful in this case. We found that in place of crotonyl-S-CoA the simpler compound S-crotonyl-N-acetyl thioethanolamine is reduced through the action of ethylene reductase.

S-Crotonyl-N-acetylthioethanolamine was obtained through reaction of crotonyl chloride with the lead salt of N-acetyl thioethanolamine, m.p. 61.5–62°. In aqueous solution it shows two characteristic absorption bands with peaks at 224 mμ ($\epsilon$ = 11500) and 262 mμ ($\epsilon$ = 6750). The method used by Fischer and Eysenbach[7] to study fumarate reductase, namely, the oxidation of a leuco dye, such as leucosafranin, was used to assay ethylene reductase as shown in Reaction 4.

In this reaction crotonic acid cannot replace the thioester derivative. The enzyme assay, in which the appearance of color from the leuco dye is followed, is illustrated in Fig. 1. By the use of this assay ethylene reductase was purified about 50-fold from sheep liver extracts through steps involving acetone fractionation, adsorption and elution from calcium phosphate gel and ammonium sulfate fractionation. The solution of the purified enzyme is yellow. A colorless, almost inactive protein can be precipitated from the above solution with ammonium sulfate at *p*H 3.6. Activity of this protein can be partially restored by addition of yeast Kochsaft or crude preparations of FAD. This suggests that, like fumarate reductase, ethylene reductase may be a flavoprotein. The two enzymes, however, are not identical. DPNH or TPNH cannot substitute for the leuco dye.

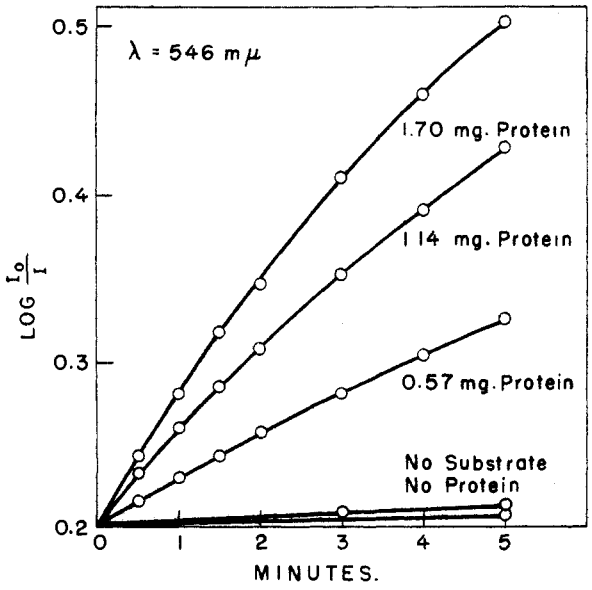


Fig. 1.—2.6 μM. S-crotonyl-N-acetylthioethanolamine and 0.5 μM. leucosafranin T in 2.1 ml. of 0.066 *M* phosphate buffer, *p*H 7.1; enzyme as indicated; temp. 17° (*d*, 0.5 cm.).

With cruder preparations of the enzyme the crotonyl-thioethanolamine derivative can be replaced by β-hydroxybutyryl-S-CoA (prepared either enzymatically[2] or synthetically[8]) indicating that the preparations also contain crotonase,[6] the enzyme catalyzing Reaction 3. These observations prove that ethylene reductase reacts with crotonyl CoA.

(6) J. R. Stern and A. del Campillo, THIS JOURNAL, **75**, 2277 (1953). Joint work on this enzyme is being carried out in the New York University and Munich laboratories.

(7) F. G. Fischer and H. Eysenbach, *Ann. Chem.*, **530**, 99 (1953).

(8) T. Wieland and L. Rueff, *Angew. Chem.*, in press.

BIOCHEMICAL DIVISION
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WERNER SEUBERT
FEODOR LYNEN

RECEIVED APRIL 3, 1953

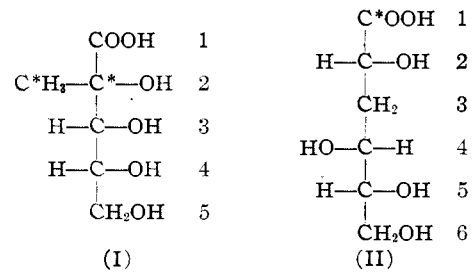
## CONCERNING THE MECHANISM OF FORMATION OF SACCHARINIC ACIDS

*Sir:*

Since the discovery of the saccharinic acids some seventy years ago, the mechanism by which they arise through the action of alkali on reducing sugars has remained obscure. Nef[1] first suggested as the crucial step in their formation an intramolecular isomerization and hydration similar to the benzilic acid rearrangement. This proposal was later modified and modernized by Isbell,[2] who interpreted the reaction sequence in terms of consecutive electron displacements. An alternative mechanism, involving the intermolecular recombination of fragments of the original sugar has been largely disregarded on account of the failure to observe formation of higher-carbon saccharinic acids from the action of alkali on lower-carbon sugars.

We now have examined, by means of $C^{14}$-labeling experiments, the formation of two saccharinic acids, "D-glucosaccharinic" acid (I) and "D-galacto-α-metasaccharinic" acid (II). Our results indicate that the branched-chain and the straight-chain acid studied are formed by *different* general mechanisms.

2a




1-$C^{14}$-D-Mannose[3] was converted by the action of saturated lime-water at room temperature[4] to $C^{14}$-"D-glucosaccharinic acid." The latter was degraded, by oxidation with sodium metaperiodate, to carbon dioxide (C-1), acetic acid (C-2a, C-2), formic acid (C-3, C-4) and formaldehyde (C-5). Over 95% of the original radioactivity was found in the acetic acid fragment and degradation of the latter showed that the labeling was distributed approximately in the ratio C-2a:C-2, 2:3.

(1) J. U. Nef, *Ann.*, **357**, 294 (1907); **376**, 1 (1910).

(2) H. S. Isbell, *J. Research Natl. Bur. Standards*, **32**, 45 (1944).

(3) J. C. Sowden, *J. Biol. Chem.*, **180**, 55 (1949).

(4) H. Kiliani, *Ber.*, **15**, 701, 2953 (1882).

To confirm this result, the branched-chain saccharin was condensed with *o*-phenylenediamine to give an anhydro-saccharin benzimidazole (m.p. 240–241°; C, 61.3, H, 6.00) and the latter was oxidized to benzimidazole carboxylic acid (C-1, C-2). Decarboxylation of the benzimidazole carboxylic acid confirmed that nearly 60% of the original radioactivity had been located in the tertiary carbon (C-2). This result is incompatible with the benzilic acid rearrangement mechanism as postulated by Nef and Isbell, but is compatible with a recombination mechanism involving sugar fragments whose identity is not yet known with certainty.

1-$C^{14}$-D-Galactose[5] was converted by the action of saturated lime-water at room temperature to $C^{14}$-"D-galacto-$\alpha$-metasaccharinic acid."[6] A Ruff degradation of the latter[6] and radio-assay of the resulting D-*threo*-2-deoxypentose as the benzylphenylhydrazone[7] showed that less than 5% of the original radioactivity was located in this 5-carbon fragment (C-2, C-3, C-4, C-5, C-6). To confirm this result, the straight-chain saccharin was converted to its benzimidazole derivative (m.p. 186–187°; C, 57.1, H, 6.53) and the latter converted, by oxidation followed by decarboxylation, to benzimidazole (C-1). Approximately 95% of the original radioactivity was found in this derivative. This result is compatible with the benzilic acid rearrangement mechanism of Nef and Isbell.

Further work is in progress with other saccharinic acids to generalize the results reported here and to clarify the mechanism of formation of the branched-chain "D-glucosaccharin."

We wish to thank Anheuser–Busch, Inc., St. Louis, Mo., for their generous support during the course of this work.

RADIOCHEMISTRY LABORATORY
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SAINT LOUIS, MISSOURI

RECEIVED APRIL 18, 1953

(5) Obtained from the National Bureau of Standards through the courtesy of Dr. H. S. Isbell.

(6) H. Kiliani and H. Naegell, *Ber.*, **35**, 3528 (1902).

(7) P. A. Levene and T. Mori, *J. Biol. Chem.*, **83**, 803 (1929).

# BOOK REVIEWS

**Charbons Actives (Adsorption des Gaz et des Vapeurs).** By C. COURTY, Professeur de Chimie Physique, à la Faculté des Sciences de Lyon. Gauthier-Villars, 55 Quai des Grands-Augustins, Paris (6e), France. 1952. ix + 534 pp. 16.5 × 25 cm. Price, 4,500 fr.

This rather long book is essentially an expansion of some notes that related to war-time work on gas masks. The theoretical parts have been expanded to the limit, but the experimental data that are used as examples remain confined to charcoal; even carbon black is excluded. Now as Professor Duclaux points out in the preface, most adsorption theories have little to do with the chemical nature of the adsorbent, and so in the reviewer's opinion the scheme of the book is unsound.

Most of the theoretical material presented is rather old; for example the 1943 paper of Emmett and de Witt is a high water mark for the work of this school. However, the 563 references, and the exhaustive treatment of semi-practical rate problems will make this book very interesting to people working directly in the field of active carbons.

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**Die Physik der Hochpolymeren. Volume I. Die Struktur des Freien Molekuls—Allgemeine Physikalische Methoden zur Bestimmung der Struktur von Molekulen und ihre Wichtigsten Ergebnisse.** By H. A. STUART, Früher O. Professor der Physik an der Technischen Hochschule Dresden, Z. 'Zt. Hannover. Springer-Verlag, Reichpietschufer 20, Berlin W 35, Germany. 1952. xxi + 609 pp. 16.5 × 23.5 cm. Price, DM 69.

This volume should be very useful as a reference work to everyone, such as the writer, who is interested in the structure of molecules and the methods of molecular structure investigation, as well as to the high polymer chemist. It is the most comprehensive, critical and up-to-date review of this subject with which this writer is acquainted.

According to the preface it was undertaken because of the dependence of structural investigations of polymers on purely physical methods. Consequently, Prof. Stuart has undertaken to collect the available structural information which these methods have yielded on small molecules and to examine critically the available methods for studying the structure of molecules. Various sections of the book have been written by G. Scheibe, W. Maier and J. Juilfs. The extent of the survey is indicated by the chapter headings: I. Valence and Molecular Forces, (79 pp.); II. The Size and Shape of Molecules, (23 pp.); III. The Nuclear Framework of Molecules, (80 pp.); IV. The Internal Mobility of Molecules and Their Statistical Shape, (63 pp.); V. Dielectric Constants, Electric Moment and Molecular Structure, (89 pp.); VI. Light Scattering, Polarizability, and Molecular Structure, (78 pp.); VII. Electrical Double-Refraction, Optical Anisotropy and Molecular Structure, (49 pp.); VIII. Characteristic Vibrations of the Nuclear Framework, (109 pp.); IX. Light Absorption and Constitution, (13 pp.).

The thoroughness with which the literature on these numerous topics is covered is impressive; in general the references seem quite complete up to about 1950 and some are as recent as 1951. For example, there is an excellent short summary of the results obtained in the very current field, microwave spectroscopy. The results are conveniently compiled in 129 tables, many of them available elsewhere, but some of them unique. In the section entitled "Stability of Internuclear Distances and Valence Angles," for example, the energy necessary to deform molecules is tabulated for a number of characteristic linkages. These energies are calculated straightforwardly from force constants (also given), to be sure, but this reviewer found it most enlightening to realize that the energy necessary to deform many bond angles by 5.73° was less than 500 cal./mole.

Naturally, as in any such compilation, occasional minor errors have crept in. (Rollefson's determinations of the dipole moments of HCl and DCl from infrared dispersion are referred to as Stark-Effect measurements in Table 57.) The number of such errors appears to be remarkably small; this,