determine, except roughly, their melting points and other physical properties.

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PARTIAL PURIFICATION AND AMINO ACID CONTENT OF VASOPRESSIN FROM HOG POSTERIOR PITUITARY GLANDS

Sir:

A highly purified vasopressin preparation (400–500 pressor units per mg.) from beef posterior pituitary glands has recently been prepared[1] by countercurrent distribution of concentrates between *n*-butyl alcohol and 0.09 *M* *p*-toluenesulfonic acid. Analysis of hydrolysates by chromatography on starch columns[2] showed phenylalanine, tyrosine, proline, glutamic acid, aspartic acid, glycine, arginine and cystine in approximately equimolar amounts, plus three moles of ammonia per mole of any one amino acid. The preparation and the amino acid analysis have been verified on several batches of posterior pituitary material of bovine origin.

We wish to report here an unexpected result encountered when pressor concentrates from hog posterior pituitary lobes were used as starting material for vasopressin preparation. A mixture of pressor fractions obtained by a solvent fractionation procedure (fractions "e" and "f" of Kamm, *et al.*[3]) were subjected to a twenty-transfer countercurrent distribution at room temperature in an all-glass machine[4] in the system *s*-butyl alcohol and 0.1% acetic acid. The material from tubes 1–4 inclusive was then submitted to a fifty-transfer countercurrent distribution at 5–10° in the system *n*-butyl alcohol and 0.09 *M* *p*-toluenesulfonic acid. The peak of pressor activity seemed to be in the vicinity of tube 20, which indicated a distribution constant of 0.66. The vasopressin of bovine origin had a distribution constant of 1.25 in this solvent system.[1]

Material from tubes 10–24 inclusive was subjected to a 150-transfer distribution in the same solvent system. Analysis of the distribution pattern by quantitative ninhydrin reaction[5] on aliquots of the lower phase showed a peak at tube 59 (distribution constant 0.65) which corresponded to the peak of pressor activity. The combined material from tubes 54–65 inclusive had a potency of approximately 175 pressor units per mg. This potency probably does not represent the highest obtainable for this principle. We have reason to believe that some inactivation has occurred in the process of working up the material.

Analysis of a hydrolysate of this material by starch column chromatography showed a pattern similar to that of vasopressin of bovine origin except that arginine was absent and a peak occupying the position of lysine was present.[6] If calculated as lysine this peak represented approximately one mole per mole of each of the other amino acids. One-half mg. of this preparation gave a negative Sakaguchi test both before and after acid hydrolysis. The same amount of a purified beef vasopressin preparation or an equimolar amount of arginine gave a strong positive test. Two-dimensional paper chromatograms of a hydrolysate with arginine or lysine added showed clearly that the basic amino acid present was not arginine, and was inseparable from lysine under these conditions. Microbiological assay of a hydrolysate for L-lysine[7] gave a value in reasonable agreement with the value from the starch column analysis.

Efforts are being continued toward further purification of lysine-vasopressin. Additional efforts are being made to ascertain whether lysine-vasopressin can be found in beef glands and arginine-vasopressin in hog glands, or whether this interesting and unexpected result represents a qualitative species difference.

The authors wish to thank Dr. Dorothy S. Genghof for the microbiological determination and Miss Sachi Fujii and Miss Naida Das for their capable assistance.

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(1) R. A. Turner, J. G. Pierce and V. du Vigneaud, *J. Biol. Chem.*, **191**, 21 (1951).

(2) S. Moore and W. H. Stein, *ibid.*, **178**, 53 (1949).

(3) O. Kamm, T. B. Aldrich, I. W. Grote, L. W. Rowe and E. P. Bugbee, THIS JOURNAL, **50**, 573 (1928).

(4) L. C. Craig, *Anal. Chem.*, **22**, 1346 (1950).

(5) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

(6) It is of interest that *oxytocin* preparations from beef and hog sources had shown no difference in amino acid composition (Pierce, Gordon and du Vigneaud, manuscript in preparation).

(7) L. M. Henderson and E. E. Snell, *J. Biol. Chem.*, **172**, 15 (1948).

(8) Public Health Service Postdoctorate Research Fellow of the National Institutes of Health.

(9) Appreciation is expressed to Lederle Laboratories, American Cyanamid Company for a grant-in-aid and to Parke, Davis and Company and Armour and Company for gifts of material.

STREPTOLIN. THE STRUCTURE AND SYNTHESIS OF ISOLYSINE

Sir:

Hydrochloric–formic acid hydrolysis of the antibiotic streptolin[1] followed by chromatographic separation on Dowex-50 has given five major fractions; the last to be eluted possesses the empirical formula $C_6H_{14}O_2N_2$ for the free base and is designated as "*iso*-lysine." This substance, which is also a hydrolysis product of viomycin[2] and streptothricin,[3] we have characterized as the di-(*p*-hydroxyazobenzene-*p'*-sulfonate)[4] (I), dec. 243.5–244°, $[\alpha]^{25}D$ +6.5 ± 1 (alc.) (found: C, 50.91; H, 5.08; N, 12.00) and the dipicrate,[4] m.p. 200–201° (found: C, 35.38; H, 3.63; N, 18.06).

Isolysine gave a positive hydroxamic acid test[5]

(1) R. W. Rivett and W. H. Peterson, THIS JOURNAL, **69**, 3006 (1947).

(2) T. H. Haskell, S. A. Fusari, R. P. Frohardt and Q. R. Bartz, *ibid.*, **74**, 599 (1952).

(3) H. E. Carter, W. R. Hearn and W. R. Taylor, "Abstracts of Papers" 119th Meeting, American Chemical Society, Cleveland, Ohio, April 1951, p. 25A.

(4) These derivatives have been previously reported by Haskell *et al.* (ref. 2) and Carter, *et al.* (ref. 3).

(5) F. Feigl, "Qualitative Analysis by Spot Tests," Elsevier Publishing Co., Inc., New York, N. Y., 1946, p. 369.

but no cobalt(II) complex and no C-methyl in the Kuhn–Roth determination. The substance did not consume periodate; it yielded succinic acid upon permanganate oxidation. These observations, coupled with the absence of N-alkyl functions in streptolin itself, allow three structural possibilities: (i) β,ε-diaminocaproic acid, (ii) γ,ε-diaminocaproic acid, and (iii) α-aminomethyl-δ-aminovaleric acid.[6] Through synthesis we have confirmed the first possibility. L-Di-(N-phthaloyl)-ornithine, m.p. 187–188.5°, $[\alpha]^{25}D$ −31.5 ± 0.5° (alc.) (found: C, 63.95; H, 4.09) was homologated *via* the Arndt–Eistert sequence[7] to methyl β,ε-diphthalimido caproate (II), m.p. 156–157°, $[\alpha]^{25}D$ −13.5 ± 0.5° (chf.) (found: C, 65.33; H, 4.64). The melting point of II was not changed on admixture with the corresponding derivative (m.p. 155.5–156.5°) from iso-lysine; the infrared spectra of the two substances were identical. Hydrazinolysis[8] and subsequent acid hydrolysis of II afforded β,ε-diaminocaproic acid, which was purified and characterized as the di-(*p*-hydroxyazobenzene-*p'*-sulfonate) (III), dec. 242.2–243°, $[\alpha]^{25}D$ +6 ± 1° (alc.) (found: C, 51.31; H, 5.09). The infrared spectra of I and III were indistinguishable, as were the paperstrip chromatograms (R_f = 0.65; developed with phenol–water–formic acid).

Details of the present work as well as syntheses of (ii) and (iii) and the demonstration of their nonidentity with iso-lysine will be published shortly.

This research was supported by grants from The Parke-Davis Co. and The National Institute of Health.

(6) H. E. Carter and associates have independently arrived at similar conclusions (presented at the 120th meeting, American Chemical Society, New York, N. Y., September, 1951).

(7) K. Balenović and D. Fleš, *J. Org. Chem.*, **17**, 347 (1952).

(8) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **71**, 1856 (1949).

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RECEIVED MAY 22, 1952

BOOK REVIEWS

The Theory of Isotope Separation as Applied to the Large-scale Production of U^{235}. By KARL COHEN, Director, Atomic Energy Division, The H. K. Ferguson Company; formerly Director, Theoretical Division, SAM Laboratories. Edited by George M. Murphy, Washington Square College, New York University; formerly at SAM Laboratories, Columbia University. McGraw-Hill Book Co., Inc., 330 West 42nd Street, New York 18, N. Y. 1951. xviii + 165 pp. 16 × 23.5 cm. Price, $2.00.

This book gives a presentation of the theory of isotope separation by methods in which elementary processes are multiplied to reach large end results. The first five chapters develop the theory of cascades. Applications to centrifuges, two-phase separation, thermal diffusion, and the concentration of deuterium are included in the latter two chapters. The theory has been presented in clear and logical fashion and the numerous tables and very clearly drawn graphs present numerical data in usable form. A wide variety of problems in the design of efficient cascades has been treated. These include necessary deviations from ideality. A variety of types of operation is discussed.

It is probable that the book has suffered because of security restrictions in that examples of the use to which theoretical results can be put are virtually absent. The author has not attempted to compensate for this by elaborating the physical significance of the mathematical relations. It is not until one reaches Chapter 6 on centrifuges that any of the discussions relate to the characteristics of equipment itself. It may be of interest to note that the terms "barrier," "barrier diffusion," and "mass spectrograph" do not appear in the index.

In many respects the derivation of the theory of cascade processes is analogous to the study of thermodynamics since the theory does not depend on the nature of the element. It is based on a fundamental axiom—the conservation of matter—and the consequences are derived through mathematical treatment without reference to physical phenomena. The significance of the results is unfortunately not elaborated.

This book is useful for the engineer who needs specific numerical solutions as well as for the theorist who is interested in the mathematical formulations which have been developed. For the reader having the empirical approach, the book will bear fruit in proportion to the background on cascade processes which he brings to it.

W. R. KANNE

Starch Chemistry. By JIRO NIKUNI (Editor), Osaka University. Asakura Publishing Co., 1–10 Nishiki-Cho, Kanda, Chiyoda Ku, Tokyo, Japan. 1951. 540 pp. Price, regular 1000 Yen ($2.80); special 880 Yen.

The book is edited by Professor Jiro Nikuni of Osaka University and is divided into seven chapters which are contributed by ten men including the editor. The following are the titles of the various chapters: I. General Discussion on Starch Chemistry; II. Metabolism of D-Glucose in the Organism; III. X-Ray Diffraction of Starch; IV. Enzymatic Studies of Starch; V. Fundamental Experimental Methods; VI. Experimental Enzymatic Methods; and VII. Industrial Preparation.

As it stated in the preface, a part of the aim of this publication is to bring up to date the recent advances in starch chemistry which the Japanese missed during the war years. For this reason the material for the most part is probably familiar to Western students. The book is intended primarily for students and research men in Japan. However, the volume undoubtedly will be an excellent source of information and reference to all students in the field of starch chemistry.

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