[CONTRIBUTION FROM THE WEIZMANN INSTITUTE OF SCIENCE]

Poly-DL-arginine

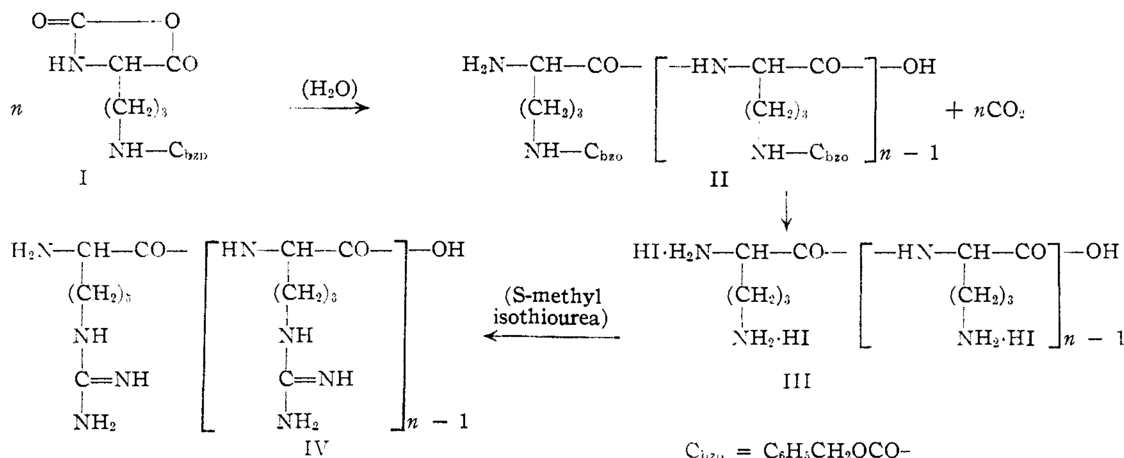
BY EPHRAIM KATCHALSKI AND PNINA SPITNIK[1]

δ-N-Carbobenzoxy-α-N-carboxy-DL-ornithine anhydride (I) yielded by polymerization in bulk or in solution polycarbobenzoxy-DL-ornithine (II). On reduction with phosphonium iodide, II gave poly-DL-ornithine hydriodide (III). III was guanized with S-methylisothiourea to yield the required poly-DL-arginine (IV).

Our general interest in water-soluble poly-α-amino acids led us to extend our previous work[2] to the synthesis of polyarginine.[3] This polymer is of interest in view of the high content of arginine residues in protamines (salmine, for example, contains 85–88% arginine[4]) and in view of the relatively low molecular weight of these strongly basic proteins.[5]

Because of the strongly basic character of the guanido group of arginine and the absence of a suitable means to block this group reversibly,[6] it was decided to use ornithine as the starting material for the synthesis of the required polymer and to postpone the introduction of the guanido group to the last step. The synthesis of poly-DL-arginine from δ-N-carbobenzoxy-α-N-carboxy-DL-ornithine anhydride (I),[7] which is summarized in the following scheme, was facilitated by our experience with polylysine[2] and by the elaboration of quantitative guanidization methods of free amino groups of proteins and peptides.[8]

to give polycarbobenzoxy-DL-ornithine (II). The number average degree of polymerization (n = 7 to 80) of the various polymeric preparations obtained, was calculated from amino N (Van Slyke)[9] end group analysis. On reduction with phosphonium iodide[10] II (n average = 80) yielded the water-soluble poly-DL-ornithine hydriodide (III) which gave positive ninhydrin and biuret reactions. The negative picric acid test indicated the absence of ornithine anhydride[7] in the reduced polymer. The constitution of III was ascertained by elementary analysis (C, H, N, amino N, I) and by total acid hydrolysis which yielded ornithine quantitatively. The amount of the latter was determined by the microbiological assay[11] as well as by the amino N[9] and carboxyl N[12] content of the hydrolysate. No attempt was made to determine the average molecular weight of III; it may be assumed, however, that in analogy with the case of polylysine,[2] no change in degree of polymerization occurs during the reduction under the experimental conditions extant. III was found to contain a negligible amount of ornithine monomer.



I polymerized on heating in bulk or in acetophenone solution with evolution of carbon dioxide,

III yielded on guanidization with S-methylisothiourea, according to Christensen,[8] poly-DL-arginine (IV). The course of guanidization was followed by determining the decrease in amino N (Van Slyke),[9] increase in total N (Dumas) and increase in arginine content (microbiological assay)[13] in flavianates precipitated from samples

(1) This paper is part of a thesis presented by Pnina Spitnik to the Hebrew University, Jerusalem, in partial fulfillment of the requirements of the degree of Ph.D.

(2) E. Katchalski, I. Grossfeld and M. Frankel, THIS JOURNAL, **70**, 2094 (1948); E. Katchalski, *Advances in Protein Chemistry*, **6**, in press.

(3) Preliminary note *cf.* E. Katchalski and P. Spitnik, *Nature*, **164**, 1092 (1949).

(4) G. R. Tristram, *ibid.*, **160**, 637 (1947); R. Block and D. Bolling, *Arch. Biochem.*, **6**, 419 (1945).

(5) E. Waldschmidt-Leitz, *Monatsh.*, **66**, 357 (1935); K. Linderstrøm-Lang, *Trans. Faraday Soc.*, **31**, 324 (1935).

(6) M. Bergmann, L. Zervas and H. Rinke (*Z. physiol. Chem.*, **224**, 40 (1934)) showed that nitro-L-arginine could be converted to L-arginine by reduction; however, attempts to use this compound for the synthesis of arginyl-peptides were unsuccessful (*cf.* J. S. Fruton, *Advances in Protein Chemistry*, **5**, 64 (1949)).

(7) E. Katchalski and P. Spitnik, THIS JOURNAL, **73**, 2946 (1951).

(8) E. Schutte, *Z. physiol. Chem.*, **279**, 52 (1943); W. L. Hughes, H. A. Saroff and A. L. Carney, THIS JOURNAL, **71**, 2476 (1949); H. N. Christensen, *J. Biol. Chem.*, **160**, 75 (1945).

(9) D. D. Van Slyke, *ibid.*, **83**, 425 (1929).

(10) C. R. Harington and T. H. Mead, *Biochem. J.*, **29**, 1602 (1935).

(11) L-Ornithine-requiring mutant of *Escherichia coli* (M 39A-23) grown in the minimal medium described by B. D. Davis and E. S. Mingioli (*J. Bact.*, **60**, 17 (1950)) was used.

(12) D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen and P. Hamilton, *J. Biol. Chem.*, **141**, 627 (1941).

(13) L-Arginine-requiring mutant of *Escherichia coli* (M 45A-25) grown in the minimal medium described by Davis and Mingioli[11] was used.

withdrawn from the guanidization mixture, at various time intervals. Guanidization was practically complete within 24 hours (*cf.* Table II).

Crude poly-DL-arginine flavianate, gave on treatment with sulfuric acid a water-soluble preparation to which the formula of poly-DL-arginine sulfate ($IV \cdot n/2H_2SO_4$) is attributed. The elucidation of the constitution of the water-soluble sulfate was based on its elementary analysis (C, H, N, amino N, S) and arginine content. The amount of arginine present in an acid hydrolysate of the polymeric sulfate was determined by enzymatic,[14] microbiological[13] and colorimetric[15] methods. The first two methods were found to determine L-arginine exclusively. Since a hydrolysate of poly-DL-arginine sulfate contains equal amounts of D- and L-arginine, both methods were expected to yield half of the total amount of arginine present. By the colorimetric method on the other hand, the total amount of racemic arginine was determined. The analytical data obtained by the three analytical methods mentioned, ascertained the formula of poly-DL-arginine sulfate ($IV \cdot n/2H_2SO_4$) attributed to the water-soluble sulfate.

Poly-DL-arginine sulfate gives positive Sakaguchi, ninhydrin and biuret reactions and a negative picric acid test.

From poly-DL-arginine sulfate, poly-DL-arginine flavianate was prepared. On acid hydrolysis the latter also gave DL-arginine in practically quantitative yield.

The relatively high amino N values of the sulfate and the flavianate of poly-DL-arginine (0.9 and 0.5%, respectively), seem to indicate, in view of the practically quantitative guanidization of the free δ-amino groups of III (n average = 80), that the guanido groups of polyarginine react with nitrous acid, under the experimental conditions used in the Van Slyke manometric method, considerably more rapidly than that of the arginine monomer. This conclusion is in accord with the findings of Lieben and Loo[16] concerning the rate of interaction of the guanido groups of salmine with nitrous acid. The hydrolysis of the peptide bonds of III during the guanidization procedure applied seems most unlikely.[8]

No final conclusion can as yet be drawn as to the presence of a free α-amino group in IV, as this group may also be guanized on treatment of III with methylisothiourea. It should be noted, however, that the α-amino group of lysylglutamic acid is not guanized under conditions leading to the guanidization of the free ε-amino group.[17]

Experimental

Polycarbobenzoxy-DL-ornithine (II) (a) **By Bulk Polymerization.**—Twice recrystallized, freshly prepared δ-N-carbobenzoxy-α-N-carboxy-DL-ornithine anhydride (I)[7] was dried *in alto vacuo* (10^{-4} mm.) over phosphorus pentoxide at room temperature for 2 hours. The temperature was then raised to 120°, whereupon the anhydride melted with vigorous carbon dioxide evolution. The hard spongy residue obtained after heating for another three hours (120°) was dissolved in a small amount of hot dimethylformamide, the solution filtered and poured into water. A colorless spongy precipitate formed: precipitation may be facilitated by adding a few drops of concentrated hydrochloric acid to the mixture. The precipitate was filtered, after standing overnight at room temperature, and dried *in vacuo* over sulfuric acid; yield quantitative.

Polycarbobenzoxy-DL-ornithine (II) readily dissolves in hot dimethylformamide, glacial acetic acid and acetophenone. It is insoluble in water, ethyl acetate, alcohol and ether. On heating an aqueous suspension of II with ninhydrin for 30 minutes, the polymer turns deep blue while the water remains colorless.

Anal. Calcd. for II (n average = 80): C, 62.7; H, 6.5; N, 11.3, amino N, 0.07. Found: C, 62.3; H, 6.5; N, 11.6; amino N, 0.07.[9]

In a preliminary experiment, it was found that 1.238 g. of I yields on heating to 120° at normal pressure 1.054 g. of II. The amount of carbon dioxide evolved was 0.184 g. (98.6% of the theoretical).

(b) **By Polymerization in Solution.**—A solution of I (4 g.) in acetophenone (200 ml.) (purified according to Waley and Watson[18]), was heated at 80° for 12 hours. The solution was concentrated *in vacuo* (2 mm.) to 40 ml. and methanol (200 ml.) was added. After standing overnight at room temperature, the precipitate which had formed was filtered, dissolved in a small amount of dimethylformamide and reprecipitated by water. The number average molecular weight of the dried polymer (2.0 g.) was calculated by amino N end-group analysis.

Anal. Calcd. for II (n average = 62): C, 62.7; H, 6.5; N, 11.3; amino N, 0.09. Found: C, 62.0; H, 6.8; N, 11.4; amino N, 0.09.[9]

The analytical data and calculated average degree of polymerizations (n) of preparations obtained on heating I under conditions differing from those specified above are summarized in Table I.

TABLE I

ANALYTICAL DATA ON PREPARATIONS OBTAINED ON HEATING δ-N-CARBOBENZOXY-α-N-CARBOXY-DL-ORNITHINE ANHYDRIDE UNDER VARIOUS CONDITIONS

Prepn.	Mg. of element (or group) per 100 mg. of preparation: Amino N	Total N	C	H	Average degree of polymn., n	Remarks
[a]	0.83	11.4	61.5	7.0	7	Soluble in cold glacial acetic acid
[b]	.21	11.5	61.0	6.6	27	Soluble in hot glacial acetic acid. Transparent films obtained from solutions of the polymer in dimethylformamide
[c]	.18	11.6	62.1	6.7	32	

[a] Obtained from I recrystallized once from ethyl acetate and petroleum ether and dried over sulfuric acid. The anhydride was left to polymerize in an open vessel at 120° for half an hour. [b] Obtained from I treated as in *a* and polymerized at 120° (atmospheric pressure) for two hours. [c] Obtained from I twice recrystallized from boiling benzene. Polymerization carried out in high vacuum (10^{-4} mm.) at 120° for two hours.

Poly-DL-ornithine hydriodide (III) (n average = 80) was obtained in almost quantitative yield by reduction of polycarbobenzoxy-DL-ornithine (n average = 80) with phosphonium iodide in glacial acetic acid (*cf.* reduction of polycarbobenzoxylysine[2]). Poly-DL-ornithine hydriodide readily dissolves in water and its aqueous solution gives a positive ninhydrin reaction and a strong violet biuret reaction. A negative Abderhalden test, obtained on heating III with an alkaline solution of picric acid, indicates the absence of ornithine anhydride.[7]

Anal. Calcd. for III (n average = 80): C, 24.6; H, 4.6; N, 11.5; amino N, 5.8; I, 52.7. Found: C, 24.5; H, 4.2; N, 11.1; amino N, 5.5[9]; I, 53.0 (Volhard).

The amount of free ornithine in the polyornithine hydriodide described was determined by the ninhydrin–carbon

(14) A. Hunter and J. B. Pettigrew, *Enzymologia*, **1**, 341 (1936).
(15) H. T. Macpherson, *Biochem. J.*, **36**, 59 (1942).
(16) F. Lieben and Y. C. Loo, *J. Biol. Chem.*, **145**, 223 (1942).
(17) J. P. Greenstein, *ibid.*, **109**, 541 (1935).
(18) S. G. Waley and J. Watson, *Proc. Roy. Soc. (London)*, **A199**, 501 (1949).

dioxide method[12] at pH 2.5. One hundred mg. of polymer contained 0.3 mg. of free ornithine.

Total Hydrolysis of Poly-DL-ornithine Hydriodide (*n* average = 80).—Dried poly-DL-ornithine hydriodide (*n* average = 80) (13.6 mg.) was dissolved in 20% hydrochloric acid (4 ml.) and the solution refluxed for 24 hours. The acid hydrolysate was neutralized with sodium hydroxide and brought to a volume of 15 ml. In a 2-ml. aliquot of the final solution the amount of carboxyl N[12] was determined; in another 2-ml. aliquot the total free amino N (Van Slyke manometric method[9] upon shaking 30 minutes with nitrous acid) was determined. From the data obtained, the total amounts of carboxyl N and amino N in the hydrolysate were calculated.

Anal. Calcd. for hydrolysate of 100 mg. III (*n* average = 80): carboxyl N, 5.7 mg.; amino N, 11.5 mg. Found: carboxyl N, 5.3 mg.[12]; amino N, 11.1 mg.[9]

The analytical data show that III (*n* average = 80) is made up exclusively of ornithine units. An independent proof for the constitution of III was obtained by the microbiological determination of L-ornithine[11] present in the hydrolysate described above.

Anal. Calcd. for hydrolysate of 100 mg. poly-DL-ornithine hydriodide (*n* average = 80): L-ornithine, 27.1 mg. Found: L-ornithine, 26.5 mg.

The picrate of poly-DL-ornithine (*n* average = 80) precipitated from a methanolic solution of poly-DL-ornithine hydriodide on adding a saturated methanolic solution of picric acid; m.p. 170° (dec.).

Anal. Calcd. for poly-DL-ornithine picrate (*n* average = 80): C, 38.5; H, 3.8; N, 20.4. Found: C, 38.3; H, 3.9; N, 20.0.

Poly-DL-ornithine hydrochloride (*n* average = 80).—Poly-DL-ornithine picrate (150 mg.) was suspended in water (5 ml.), 1 *N* hydrochloric acid (0.7 ml.) was added and the mixture shaken several times with ether to remove the picric acid liberated. The colorless aqueous solution was poured into absolute ethanol (20 ml.) and the white flocculent precipitate (35 mg.) centrifuged, washed with ethanol and dried *in vacuo* over phosphorus pentoxide and sodium hydroxide.

Anal. Calcd. for poly-DL-ornithine hydrochloride (*n* average = 80): C, 39.7; H, 7.4; N, 18.5; amino N, 9.3; Cl, 23.7. Found: C, 39.7; H, 7.2; N, 18.8; amino N, 9.1; Cl, 24.1.

The hydrochloride readily dissolves in water and the aqueous solution gives a positive ninhydrin reaction, a strong violet biuret reaction and a negative picric acid test.

Poly-DL-ornithine flavianate (*n* average = 80) was prepared in 80% yield from poly-DL-ornithine hydriodide in methanol; m.p. 235° (dec.), after drying *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for poly-DL-ornithine flavianate (*n* average = 80): C, 42.1; H, 3.8; N, 13.1; S, 7.5. Found: C, 42.2; H, 4.2; N, 13.1; S, 7.1.

The flavianate dissolves in boiling absolute methanol and in boiling water. It is sparingly soluble in ethanol and insoluble in ether.

Determination of Arginine in DL-Arginine Flavianate by the Enzymatic and Microbiological Methods.—DL-Arginine flavianate was prepared from L-arginine hydrochloride according to Heinsen.[19] The arginine content of the racemic flavianate, as well as that of the L-isomer was determined enzymatically[14] and microbiologically.[13]

Anal. Calcd. for 100 mg. L-arginine flavianate: arginine, 35.7 mg. Found: arginine, 35.7 mg. (enzymatic method)[14]; 35.3 mg. (microbiological method).[13]

Anal. Calcd. for 100 mg. DL-arginine flavianate: arginine, 35.7 mg. Found: arginine, 17.7 mg. (enzymatic method)[14]; 17.0 mg. (microbiological method).[13]

The analytical data show that under the experimental conditions specified for both analytical methods, only the optically active L-arginine isomer is determined.

Course of Guanidization of Poly-DL-ornithine (*n* average = 80).—A methanolic guanidization mixture (100 ml.) of polyornithine hydriodide (2 g.) and S-methylisothiourea (derived from 10 g. of S-methylisothiourea sulfate) was prepared as described below (*cf.* preparation of poly-DL-arginine sulfate). The methanolic solution was divided into six equal portions. The first aliquot was immediately treated with flavianic acid (1 g.) in methanol (5 ml.) while the other aliquots were treated with the same amount of flavianic acid after 3, 6, 10, 24 and 48 hours, respectively. Each one of the precipitates formed was purified by conversion into the corresponding sulfate, by a procedure analogous to that given for the preparation of poly-DL-arginine sulfate, and by reconversion into the corresponding flavianate which was purified as described below (*cf.* preparation of poly-DL-arginine flavianate). The analyses (total N, amino N and arginine content) of the various purified flavianates are summarized in Table II.

(19) H. A. Heinsen, *Z. physiol. Chem.*, **239**, 162 (1936).

TABLE II

COURSE OF GUANIDIZATION OF POLY-DL-ORNITHINE

Time of guanidization, hours	Weight of flavianate, mg.[a]	Total N	Amino N	L-Arginine[b]
		mg. per 100 mg. flavianate		
0	300	13.1	3.3	0
3	320	14.5	3.0	2.9
6	400	15.6	2.3	7.4
10	420	16.3	1.6	11.5
24	430	17.8	1.0	17.5
48	480	17.9	0.5	18.0

[a] Weight of crude flavianate before purification. [b] Determined by the microbiological assay[13] after total acid hydrolysis. Calcd. for 100 mg. poly-DL-arginine flavianate: L-arginine, 18.5 mg.

Poly-DL-arginine Sulfate ($IV \cdot n/2H_2SO_4$) **(a) Methanolic S-Methylisothiourea.**—Ice-cold *N* methanolic sodium methoxide (72 ml.) was added to an ice-cold suspension of S-methylisothiourea sulfate[20] (10 g.) in absolute methanol (20 ml.). The mixture was shaken mechanically for 45 minutes at room temperature, the sodium sulfate filtered off and the filtrate (Solution A) used for the guanidization of poly-DL-ornithine.

(b) **Guanidization of Poly-DL-ornithine** (*n* average = 80).—To poly-DL-ornithine hydriodide (2 g.) in methanol (5 ml.) was added an equivalent amount of 1 *N* methanolic sodium methoxide (8.25 ml.) and solution A. The volume was brought to 100 ml. with absolute methanol and the mixture allowed to stand at room temperature for 48 hours.

(c) **Isolation of Poly-DL-arginine Sulfate.**—Poly-DL-arginine was isolated from the above mixture as the flavianate by adding a solution of flavianic acid (5 g.) in methanol (10 ml.). The precipitate (2.88 g.) was centrifuged, washed several times with boiling absolute ethanol (5-ml. portions) and ether. The crude flavianate was suspended in ice-cold water (6 ml.) to which sulfuric acid (0.5 ml.) was added; the liberated flavianic acid was extracted with butanol, the aqueous phase filtered and poured into absolute ethanol (125 ml.). The mixture was left overnight in the refrigerator and the colorless flocculent sulfate centrifuged and washed with absolute ethanol. The sulfate was further purified by dissolving in a small amount of water and precipitation with absolute ethanol. The final product was dried *in vacuo* (0.1 mm.) over phosphorus pentoxide at 100° to constant weight (1.00 g.).

Anal. Calcd. for poly-DL-arginine sulfate (*n* average = 80) ($IV \cdot n/2H_2SO_4$): C, 35.1; H, 6.3; N, 27.3; amino N, 0.09; S, 7.8. Found: C, 35.3; H, 6.7; N, 27.9; amino N, 0.9; S, 7.6.

The hygroscopic poly-DL-arginine sulfate readily dissolves in water and is insoluble in ethanol. The aqueous solution gives positive biuret, Sakaguchi and ninhydrin reactions.

Total Hydrolysis of Poly-DL-arginine Sulfate.—Poly-DL-arginine sulfate was hydrolyzed with 10% hydrochloric acid at 120° for 6 hours. The amount of arginine in the neutralized hydrolysate was determined colorimetrically,[15] enzymatically[14] and microbiologically.[13]

Anal. Calcd. for 100 mg. poly-DL-arginine sulfate ($IV \cdot n/2H_2SO_4$, n = 80): DL-arginine, 84.8 mg.; L-arginine, 42.4 mg. Found: DL-arginine, 81.2 mg. (colorimetric method)[15]; L-arginine, 40.3 mg. (enzymatic method),[14] 42.0 mg. (microbiological method).[13]

(20) P. R. Shildneck and W. Windus, "Organic Syntheses," Coll. Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 411.

Poly-DL-arginine Flavianate.—A solution of flavianic acid (5 g.) in water (5 ml.) was added to poly-DL-arginine sulfate (1 g.) in water (2 ml.) and the mixture left in the refrigerator for 48 hours. The supernatant liquid was decanted and the residue washed with cold water and alcohol and dried *in vacuo* over sulfuric acid. It was dissolved in hot dimethyl formamide (2 ml.), the solution filtered and poured into absolute ethanol (75 ml.). The yellow flocculent precipitate of poly-DL-arginine flavianate which separated out was centrifuged, washed several times with absolute ethanol and ether and dried *in vacuo* over sulfuric acid; yield quantitative, m.p. 214–220° (dec.).

Anal. Calcd. for poly-DL-arginine flavianate (n average = 80): C, 40.8; H, 3.8; N, 17.9; amino N, 0.04; S, 6.8. Found: C, 40.3; H, 4.1; N, 17.9; amino N, 0.5; S, 7.2.

Total Hydrolysis of Poly-DL-arginine Flavianate.—Poly-DL-arginine flavianate was hydrolyzed with 10% hydrochloric acid at 120° for 6 hours and the arginine content of the neutralized hydrolysate determined enzymatically and microbiologically.

Anal. Calcd. for 100 mg. poly-DL-arginine flavianate (n = 80): L-arginine, 18.5 mg. Found: L-arginine, 19.6 mg. (enzymatic method),[14] 18.0 mg. (microbiological method).[13]

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The Chemistry of Allergens. XI. Properties and Composition of Natural Proteoses Isolated from Oilseeds and Nuts by the CS-1A Procedure[1,2]

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The procedure used to isolate the principal allergen of cottonseed, CS-1A, has been applied to the following oilseeds and nuts: Almonds, Brazil nuts, castor beans, coconuts, Barcelona and DuChilly filbert nuts, flaxseed, kapok seed, black mustard seed, peanuts, pecan nuts, soybeans, tung nuts and black walnuts and English walnuts. Typical natural proteoses possessing similar chemical, allergenic and antigenic properties were obtained from almond nuts, Brazil nuts, castor beans, cottonseed, Barcelona and DuChilly filbert nuts, flaxseed, kapok seed and mustard seed. Soybeans and peanuts yielded immunologically atypical fractions, and black walnuts yielded a fraction having immunological properties intermediate between those of the typical and atypical groups. The chemical composition, allergenic and antigenic properties of these substances are described. It is suggested that these substances be recognized as a class of native proteins and that they be designated by the term natural proteose proposed by Wells and Osborne, or the term natural proteone to show that, while these substances have chemical properties similar to those of non-antigenic derived proteoses or peptones they are highly antigenic preformed components of the seeds.

It has long been known that multiple sensitivity to cottonseed, nuts and other oilseeds is a frequently encountered clinical grouping.[3] Consideration of this clinical classification, after isolation of the principal allergen of cottonseed, CS-1[4] or CS-1A,[5] led to the assumption that allergens of other oilseeds and nuts might be similar enough to be isolated by a general procedure.[6,7] The validity of this speculation was shown by the isolation of the ricin-free allergenic fraction CB-1A from castor beans[7] by the same procedure originally developed for the isolation of CS-1A from botanically unrelated cottonseed. CB-1A was remarkably similar in composition and chemical and immunological properties to CS-1A, although differing in allergenic specificity. Kapok seed, which is botanically related to cottonseed, also yielded a similar allergen, KS-1A.[8] CS-1A and CB-1A, although not homogeneous, are important because they contain the most potent allergens of respective seeds, completely freed from other allergens and antigens present in the seeds. This has been conclusively shown by extensive passive transfer and cutaneous tests on sensitive human subjects[9,10] and by gross anaphylaxis and Schultz–Dale tests with guinea pigs.[11–14]

CS-1A and CB-1A provided relatively abundant and highly concentrated source materials for further studies designed to obtain homogeneous allergens or allergens sufficiently purified to permit determination of the chemical nature of the active components with reasonable certainty. The essentially carbohydrate-free natural proteoses[15] CS-60C[16] and CB-65A,[17] possessing full allergenic activity, were isolated from CS-1A and CB-1A and all evidence adduced showed that the allergenic and immunological specificities of CS-1A and CB-1A were inherent in the type of substance represented by CS-60C and CB-65A, respectively.

To determine the distribution of allergenic natural proteoses, several more oilseeds and nuts were subjected to the CS-1A procedure. The present paper describes results of this study with almond nuts, Brazil nuts, castor beans, cottonseed, coconuts, filbert nuts, flaxseed, kapok seed, mustard seed, pecan nuts, peanuts, soybeans, tung nuts, black walnuts and English walnuts.

(1) Not subject to copyright.

(2) Paper X, Spies, Coulson and Stevens, THIS JOURNAL, **66**, 1798 (1944).

(3) Bowman and Walzer, "Asthma and Hay Fever in Theory and Practice," Charles C. Thomas, Baltimore, Maryland, 1931, p. 394.

(4) Spies, Bernton and Stevens, *J. Allergy*, **10**, 113 (1939).

(5) Spies, Coulson, Bernton and Stevens, THIS JOURNAL, **62**, 1420 (1940).

(6) Spies, *ibid.*, **63**, 2994 (1941).

(7) Spies and Coulson, *ibid.*, **65**, 1720 (1943).

(8) Coulson, Spies and Stevens, *J. Immunol.*, **49**, 99 (1944). KS-1A contained a minor component that was serologically identical with CS-1A from cottonseed.

(9) Bernton, Spies and Stevens, *J. Allergy*, **13**, 289 (1942).

(10) Spies, Chambers, Bernton and Stevens, *ibid.*, **14**, 7 (1942).

(11) Coulson, Spies and Stevens, *J. Immunol.*, **41**, 375 (1941).

(12) Coulson, Spies and Stevens, *ibid.*, **46**, 347 (1943).

(13) Coulson and Spies, *ibid.*, **46**, 367 (1943).

(14) Coulson, Spies and Stevens, *J. Allergy*, **21**, 34 (1950).

(15) Term proposed by Wells and Osborne, *J. Infectious Diseases*, **17**, 259 (1915).

(16) Spies and Umberger, THIS JOURNAL, **64**, 1889 (1942).

(17) Spies, Coulson, Chambers, Bernton and Stevens, *ibid.*, **66**, 748 (1944).