exceptions, pro-lys and gly-ser. On the other hand, small amounts of the rather unusual peptides, pro-ser and pro-thr, have been detected in our partial hydrolysates of gelatin. On the basis of such data, namely that proline and hydroxyproline are present in approximately equal amounts in gelatin, that both seem to be associated largely with glycine, and that a prolyl-hydroxyproline bond probably would be rather labile, one may speculate that sequences of the type -gly-pro-hypro-gly- or -gly-pro-hypro-gly-pro-hypro-gly- may be important in gelatin. Beyond question, a prolyl-hydroxyproline linkage is unusual but collagen and gelatin are unusual proteins because of their high content of proline and because of the mere presence of hydroxyproline. The use of models[34] shows no steric conflicts which would make such a sequence improbable; in fact, models of pro-hypro may be constructed with facility.

If the sequence -gly-pro-hypro-gly were to occur in proteins, it would have interesting structural implications. From molecular models it is clear that the planes of the proline and hydroxyproline rings must be approximately normal to one another because of steric hindrance: thus the sequence -pro-hypro- forces and maintains an approximately 90°-change in the direction of the peptide chain. Although the peptides which have been isolated from gelatin suggest that the sequence -gly-pro-hypro-gly may be present, the models show no reason to exclude the general sequence –R–P–P–R– where R may be any amino acid and P may be either proline or hydroxyproline. If the dark bands which collagen exhibits under the electron microscope represent regions of lesser organization,[35] this lesser organization or more open packing may perhaps be produced by the presence of the sequence –R–P–P–R– which occasions an approximately 90° deviation in the direction of the polypeptide chain.

Acknowledgment.—This investigation was supported in part by a grant from the Lederle Laboratories and in part by a grant-in-aid from E. I. du Pont de Nemours and Company.

(34) R. B. Corey and L. Pauling, *Rev. Sci. Instr.*, **24**, 621 (1953).

(35) An excellent review of the structure of collagen has been given by R. S. Bear, *Adv. Prot. Chem.*, **7**, 69 (1952).

PASADENA 4, CALIFORNIA

[CONTRIBUTION NO. 1882 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Chromatographic Separation and Identification of Some Peptides in Partial Hydrolysates of Silk Fibroin

BY LOIS M. KAY AND W. A. SCHROEDER

RECEIVED FEBRUARY 26, 1954

By means of chromatography, 12 peptides have been isolated from a partial acidic hydrolysate of silk fibroin, then identified and quantitatively estimated. These peptides account for 60% of the alanine, 50% of the glycine, 47% of the serine, 35% of the tyrosine, 15% of the threonine, 7% of the valine and 5% of the phenylalanine in silk fibroin: these quantities make up almost half of the protein molecule. Few definite conclusions can be drawn from these preliminary experiments about the structure of silk fibroin but they raise some question as to the significance of the minimum repeating sequence -gly-X-ala-gly-ala-gly-X- which has been proposed by Levy and Slobodian.

Synge[1] and Sanger[2] have reviewed the literature pertaining to the amino acid sequence in silk fibroin. There is unequivocal evidence for the presence of ala-gly,[3] gly-ala and gly-tyr in partial hydrolysates of silk fibroin. Other peptides also have been isolated or indirectly estimated[4,5] but their structure has not been demonstrated so convincingly.

In previous investigations in these laboratories, chromatographic methods have been used to separate, identify and estimate 34 peptides in partial hydrolysates of gelatin.[6,7] When these methods were used in small-scale exploratory chromatograms of complete and partial hydrolysates of silk fibroin, the results of Fig. 1 were obtained under the chromatographic conditions given in the legend. The zones which emerge with *p*H 3.42 buffer may with confidence be identified as the free amino acids because the conditions of chromatography are the same as those of Moore and Stein[8] during development with *p*H 3.42 buffer. Zones 2, 3, 4, 7 and 8 have no counterparts in the complete hydrolysate and must be peptides. Experience with gelatin suggested that zone 1 should contain a peptide. Smaller amounts of the amino acids in the corresponding zones of the complete hydrolysate may be present in zones 5 and 6. The partial hydrolysate thus contains a few well-separated zones of peptides in considerable amount. We have chromatographed the partial hydrolysate of silk fibroin on a large scale and shall describe the isolation, identification and estimation of 12 peptides.

Experimental

The silk fibroin was prepared from silk of *Bombyx mori* purchased from a commercial source.

The degumming procedure of Dunn, *et al.*,[9] was followed in detail.

(1) R. L. M. Synge, *Chem. Revs.*, **32**, 135 (1943).

(2) F. Sanger, *Adv. Prot. Chem.*, **7**, 1 (1952).

(3) The abbreviations and representation of amino acid sequences follow E. Brand (*Ann. N. Y. Acad. Sci.*, **47**, 187 (1946)) and F. Sanger (ref. 2).

(4) M. Levy and E. Slobodian, *J. Biol. Chem.*, **199**, 563 (1952).

(5) E. Slobodian and M. Levy, *ibid.*, **201**, 371 (1953).

(6) W. A. Schroeder, L. Honnen and F. C. Green, *Proc. Nat. Acad. Sci.*, **39**, 23 (1953).

(7) W. A. Schroeder, L. M. Kay, J. LeGette, L. Honnen and F. C. Green, THIS JOURNAL, **76**, 3556 (1954).

(8) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951).

(9) M. S. Dunn, M. N. Camien, L. B. Rockland, S. Shankman and S. C. Goldberg, *ibid.*, **155**, 591 (1944).

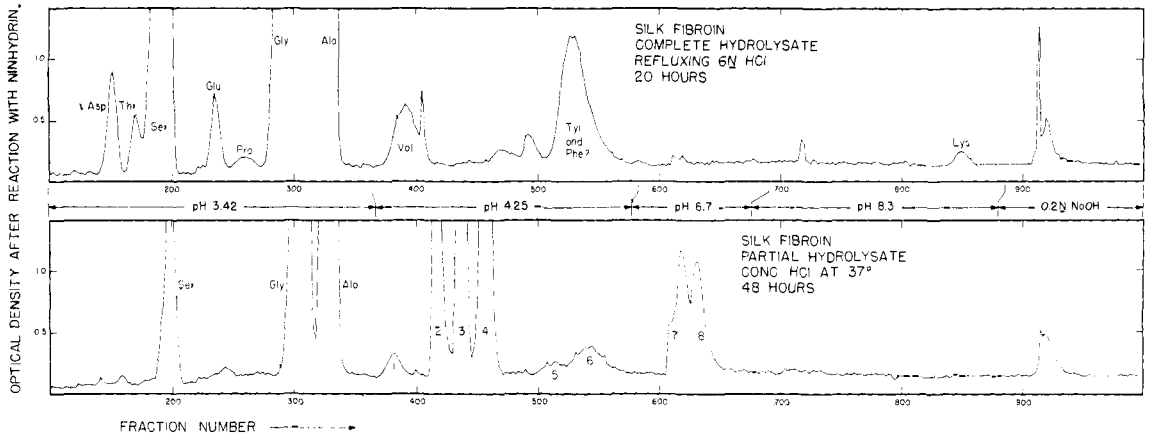

Fig. 1.—Comparison of chromatograms of complete and partial hydrolysates of silk fibroin on Dowex-50. Column dimensions, 1 × 100 cm.; temperature of chromatograms, 37°, except room temperature during 0.2 *N* NaOH; developers, buffers of *p*H shown; fraction size, 0.5-ml. to fraction 220 and 1-ml. thereafter; sample equivalent to 12 mg. of silk fibroin.

The procedures for partial hydrolysis, for ion exchange chromatography of the hydrolysate on Dowex-50, for dinitrophenylation of the peptides so isolated, for the chromatography of the DNP-peptides on silicic acid–Celite, and for the identification and estimation of the peptides have been described previously[7] and need not be repeated here except for certain experimental conditions (such as the hydrolysis) peculiar to this investigation of silk fibroin.

A 1.00-g. sample of the above silk fibroin (moisture, 6.6%; ash 0.3%; nitrogen, 18.1% corrected for moisture and ash[10]) was placed in a 10-ml. volumetric flask and 5 ml. of J. T. Baker analyzed concd. hydrochloric acid was added. The hydrolytic mixture was maintained at 37° for 48 hours. The fibroin dissolved almost completely in 5 min. to give a somewhat viscous, orange-colored solution which took on a purple tinge after about an hour and was intensely purple in color after 24 hours. At the end of 48 hr. the hydrolytic mixture was cooled and diluted to 10 ml. with water. A 3-ml. portion of this solution was diluted to 120 ml. with *p*H 4.25 buffer, adjusted to *p*H 2.0 with 0.3 ml. of 6 *N* hydrochloric acid; 100 ml. was placed on the Dowex-50 column (3.5 × 100 cm.) and chromatographed as previously described. The sample which was chromatographed was equivalent to 232 mg. of silk fibroin (corrected for moisture and ash).

Pooled fractions which contained a given zone from the ion exchange chromatogram were evaporated to dryness over concd. sulfuric acid in a desiccator without neutralizing (except zones S-17 and S-18 which were neutralized) and were stored at 0° until studied further.

Results

The separations which were achieved by the large-scale ion exchange chromatogram are presented in Fig. 2. There is no difficulty in identifying these zones with the corresponding zones of the partial hydrolysate in Fig. 1. Development with *p*H 8.3 buffer was omitted on the large-scale chromatogram because no zones were evident on the exploratory chromatogram when this buffer was used. As was also noticed in the work on gelatin,[6,7] the separation of zones was more satisfactory on the large-scale than on the small-scale chromatograms. Comparison of zones S-12 through S-16 of Fig. 2 with zones 5 through 8 of Fig. 1 points out the improved separation.

Zones S-4 and S-8 to S-18 inclusive have been investigated. Qualitative and quantitative data on the peptides which were isolated are recorded in Table I. Zones S-8 to S-12, S-15 and S-16 contain the peptides enumerated and, in addition, each zone had peptides in smaller amount which were not investigated or could not be characterized with certainty. In general, no attempt has been made in this preliminary work to determine the complete sequence of the amino acids in the tripeptides; the identification of the N-terminal amino acid is a natural result of the procedure. The tripeptide in zone S-12 is gly-val-gly; the ready identification of sequence in this peptide arises from the unusual resistance of the val-gly bond to hydrolysis[11] so that val-gly is still present after hydrolysis under conditions which completely hydrolyzed the other DNP-tripeptides.

One peptide in zone S-10 is listed as ala-(gly, ?) and two peptides in zone S-11 are similarly designated with a question mark. When ala-(gly, ?) was analyzed, the N-terminal alanine and the glycine were found to be present in equimolar amount and equivalent to the amount of peptide which was hydrolyzed; in addition, alanine other than N-terminal was present to the extent of about 10% of the amount of peptide. One might conclude that a mixture of about $^{9}/_{10}$ ala-gly and $^{1}/_{10}$ ala-(gly, ala) was present. The chromatographic properties of the DNP-peptides are such that DNP-ala-(gly, ala) might well be present but DNP-ala-gly has different chromatographic properties and, furthermore, DNP-ala-gly is the main peptide of zone S-10. When, at a later date, the attempt was made to isolate more of the ala-(gly, ?) from another portion of zone S-10, none was detectable but the amount of ala-gly had increased from 229 μmoles/250 mg. of silk fibroin in the first experiment to 264 μmoles. At present, the nature of ala-(gly, ?) in zone S-10 and of gly-(ala, gly, ?) and gly-(ala, ?) in zone S-11 is undetermined.

Zone S-12 emerges in the approximate position of free leucine and isoleucine but neither of these is present. Zone S-13 emerges in the approximate position of free phenylalanine and tyrosine. Free tyrosine was identified as part of this zone but free

(10) Microdeterminations by Dr. Adalbert Elek. Results of individual analyses for N on moisture-free material as follows: 18.10% and 18.05% by Kjeldahl; 17.92% and 17.99% by Dumas. Control analyses on a sample of U.S.P. Reference Standard lysine hydrochloride gave 15.48% N by Kjeldahl and 15.54% by Dumas; theory, 15.34% N.

(11) R. L. M. Synge, *Biochem. J.*, **39**, 351 (1945).

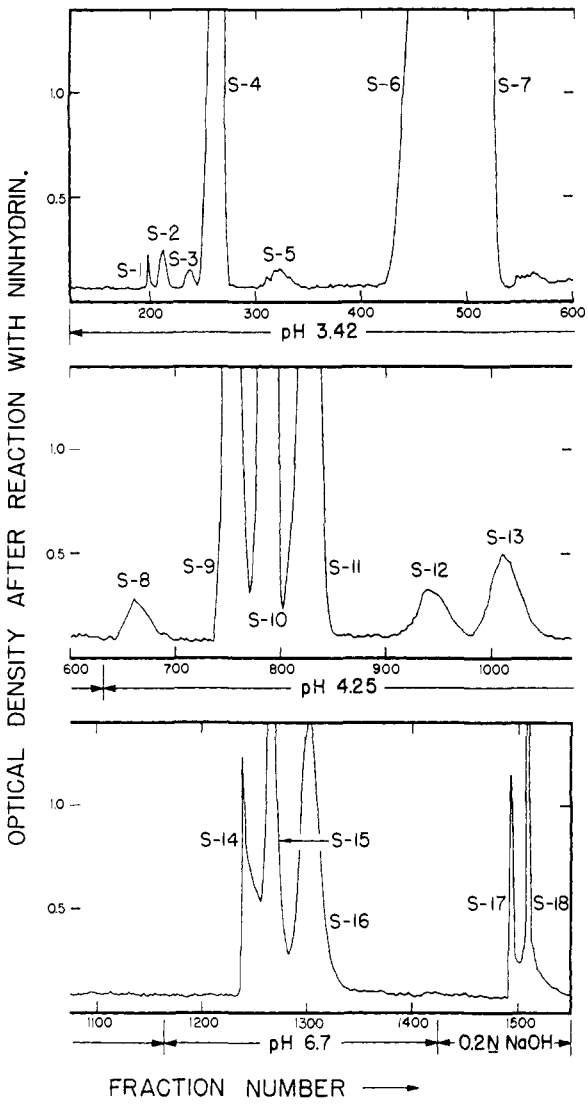

Fig. 2.—Separation of amino acids and peptides in a partial acidic hydrolysate of silk fibroin by chromatography on Dowex-50. Column dimensions, 3.5 × 100 cm.; temperature of chromatogram, 37°, except room temperature during 0.2 *N* NaOH; developers, buffers of *p*H shown; fraction size, about 5 ml.; sample, equivalent to 232 mg. of silk fibroin.

aspartic acid was also present. Because free aspartic acid normally emerges in zone S-2, it may be concluded that some aspartyl peptide in zone S-13 has decomposed. The quantity of aspartic acid as isolated from zone S-13 is about twice that of the tyrosine so that it is improbable that the peptide was (asp, tyr). Zone S-14 contained the colored material from the hydrolysate; a complex mixture is present in this zone and no definite identifications were made. Much the same is true of zones S-17 and S-18. Zone S-4 contained only serine although the quantity isolated was about one-fourth of the anticipated amount. Zones S-2, S-3, S-5, S-6 and S-7 were not investigated but probably contain aspartic acid, threonine, glutamic acid, glycine and alanine, respectively. Zone S-1 contains so small an amount that it was not studied.

Discussion

The amino acid composition of silk fibroin is such that almost half of the residues are glycine, about one-fourth are alanine and much of the remainder of the molecule is composed of serine and tyrosine. It is, therefore, not surprising to find that partial hydrolysates of silk fibroin contain peptides such as are listed in Table I. With one exception, namely, ala-ala, all of the peptides contain glycine and about 99% contain only the four amino acids enumerated. If we exclude the peptides of uncertain identity in zones S-10 and S-11, then 90% of the remainder is composed of dipeptides and 10% of tripeptides. Such a proportion is to be expected from the work of Stein, Moore and Bergmann[12] who showed on the basis of nitrogen distribution that essentially only free amino acids and dipeptides should be present in a partial hydrolysate of silk fibroin which had been prepared essentially under the conditions of the present study.

Quantitative Aspects of the Investigation.—Throughout this work, emphasis has been laid on the acquisition of quantitative data. These data as presented in Table I are minimal amounts. They show that the mixture of peptides in this partial hydrolysate was a relatively simple one and that in many instances very appreciable amounts of some of the peptides were present. According to Tristram,[13] one would expect 250 mg. of silk fibroin to contain about 3200 μmoles of amino acid residues or at a maximum about 1600 μmoles of dipeptides could be present in any hydrolysate. We have isolated about 700 μmoles of peptides of which about 90% is in the form of dipeptides.

On the basis of the colorimetric data from which Fig. 1 is plotted, one can calculate the amount of material in each zone.[14] This has been done on the assumption that the color yield of all peptides is equivalent to that of leucine (an assumption subject to an error of at least ±10%[15,16]). A comparison of these calculated quantities with the quantities actually isolated (Table I) shows a very gratifying recovery in zones S-9 to S-11 and S-16, a somewhat less satisfactory one in zones S-8, S-12 and S-15, and a poor one in zone S-4. The low percentage recovery of thr-gly from zone S-8 is almost the same as that from zone 13 of gelatin, although in both instances only minor amounts of other peptides are present. The poor recovery of serine from zone S-4 is difficult to understand in view of the fact that the recovery of serine was 103% from zone 40 of gelatin. It may be that the method of storing the zone

(12) W. H. Stein, S. Moore and M. Bergmann, *J. Biol. Chem.*, **154**, 191 (1944).

(13) G. Tristram, *Adv. Prot. Chem.*, **5**, 143 (1949). As the basis of calculation, the amino acid composition of silk fibroin as reported by Tristram has been used. His compilation is based on unpublished data. A search of the literature shows that Tristram's values are generally in good agreement with other analyses except for the contents of alanine, phenylalanine, serine and tyrosine. Tristram's value for phenylalanine is two to three times that of other investigators. One cannot with confidence choose any reported or average value as the correct one for the contents of alanine, serine and tyrosine because of the many discordant results which have been obtained.

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

(15) M. Ottesen and C. Villee, *Compt. rend. Lab. Carlsberg Ser. chim.*, **27**, 421 (1951).

(16) Y. P. Dowmont and J. S. Fruton, *J. Biol. Chem.*, **197**, 271 (1952).

TABLE I

QUALITATIVE AND QUANTITATIVE DATA ON PEPTIDES FROM A PARTIAL ACID HYDROLYSATE OF SILK FIBROIN

Zone	Compound	μmoles compound per 250 mg. silk fibroin[a]	Total μmoles per zone: Isol. as DNP der.	Total μmoles per zone: Calcd.[b] from ninhyd.	Isol. as % of calcd.	Percentage[c] of the total amount of amino acid in silk fibroin accounted for by amount of peptide or amino acid: Ala	Gly	Phe	Ser	Thr	Tyr	Val
S-8	Thr-gly	5	5	9.5	53		0.5			14.5		
S-9	Ser-(gly, ala)	27	184	210	87	3	2		7			
	Ala-(gly, ala)	8				2	0.5					
	Ser-gly	149					10.5		40			
S-10	Ala-(gly, ?)	46.5	275.5	291	95	5.5	3					
	Ala-gly	229				27.5	16					
S-11	Gly-(ala, gly, ?)	7	168	208	81	1	1					
	Gly-(ala, gly)	22.5				2.5	3					
	Gly-(ala, ?)	16				2	1					
	Gly-ala	109				13	7.5					
	Ala-ala	13.5				3						
S-12	Gly-val-gly	5.5	5.5	16	34		1					7
S-15	Gly-tyr	18	18	51.5	35		1				10	
S-16	Tyr-gly	44	46.5	65.5	71		3				25	
	Phe-gly	2.5					>0	5				
	Total as peptides	702.5				59.5	50	5	47	14.5	35	7
S-3	Threonine	...		1.5						4.5		
S-4	Serine	19	19	76	25				19.5[d]			
S-6	Glycine	...		410			28					
S-7	Alanine	...		284		34						
S-13	Tyrosine	7	7[e]	28	25						4[f]	
	Total as peptides and amino acids					93.5	78	5	66.5	19	39	7

[a] All values have been recalculated to 250 mg. of moisture- and ash-free silk fibroin. Quantities are listed to the nearest 0.5 μmole and 0.5%. [b] Calculated from the data of the ninhydrin procedure on the assumption that the color yield of all components is equivalent to that of leucine. [c] The amino acid composition of silk fibroin as listed by G. Tristram[13] has been used to calculate these percentages. [d] Based on the ninhydrin data. [e] Zone S-13 contains also 13.5 μmoles of free aspartic acid. [f] Based on amount isolated.

between its isolation from the ion exchanger and its analytical investigation is important: in the present investigation, the pooled fractions were evaporated to dryness over sulfuric acid in a desiccator and stored at 0°, whereas in the case of gelatin they were neutralized to *p*H 6.5 to 7.5 before evaporation and then stored at room temperature. This difference in procedure has not appreciably influenced the recovery of thr-gly.

That some of the peptides account for very significant percentages of the various amino acids may be seen, for example, in the cases of ala-gly, gly-ala, ser-gly and tyr-gly. In all, 60% of the alanine, 50% of the glycine, 47% of the serine and 35% of the tyrosine have been isolated in peptide form: this is equivalent to 45% of the protein molecule. Leucine, isoleucine, proline, the acidic amino acids and the basic amino acids have not been isolated in peptide form; they, however, account for only about 5% of the residues of silk fibroin and may have been present in some of the minor zones which were not investigated.

Conclusions About the Structure of Silk Fibroin. —The literature records evidence for the presence of a number of peptides in partial hydrolysates of silk fibroin, but the older methods which were available when the work was done permitted unequivocal identification of only ala-gly, gly-ala and gly-tyr. Stein, Moore and Bergmann[12] were able to isolate considerable quantities of the first two and Levy and Slobodian[4] have made quantitative determinations of them with the carrier modification of the isotope derivative technique. By the further application of this method, Slobodian and Levy[5] have obtained evidence for the peptide, gly-ala-gly. Levy and Slobodian have proposed the sequence -gly-X-ala-gly-ala-gly-X- as a minimum repeating unit for silk fibroin. They base this suggested sequence on their analyses which show twice as much ala-gly as gly-ala in the hydrolysate and which also indicate the presence of large amounts of gly-ala-gly. They could find no gly-gly but have made no effort to detect ala-ala or ala-gly-ala.

It is of interest to compare the quantitative data of the present investigation with those of Levy and Slobodian and with the suggested minimum repeating unit. The quantitative data are compared in Table II.

TABLE II

QUANTITIES OF SEVERAL COMPOUNDS IN PARTIAL HYDROLYSATES OF SILK FIBROIN AS FOUND IN THREE INVESTIGATIONS

Compound	Calcd. to μmoles per 250 mg. of silk fibroin: Stein, Moore and Bergmann[12]	Levy and Slobodian[4]	This investigation
Ala-gly	94	280	229
Gly-ala	103	81	109
Glycine		420	410
Alanine		310	284

The data of Levy and Slobodian have been chosen from an experiment in which the hydrolytic conditions were almost exactly the same as ours: those

of Stein, Moore and Bergmann were very similar. The agreements generally are surprisingly good when one considers that the three methods of investigation were very different. In no instance have any corrections been applied. Under these conditions of hydrolysis one may then expect to find about twice as much ala-gly as gly-ala. The significance of this result is difficult to assess. Levy and Slobodian point out that one would expect such a ratio from the sequence -gly-X_1-ala-gly-ala-gly-X_2-[17] because Synge[11] found identical rates of hydrolysis for ala-gly and gly-ala. Although one would, indeed, expect twice as much ala-gly as gly-ala from the peptide ala-gly-ala-gly, on the other hand, this reasoning ignores the influence of the bonds X_1-ala and gly-X_2 on the amounts of ala-gly and gly-ala in a partial hydrolysate of silk fibroin.

If we assume that gly-(ala, gly) of zone S-11 actually is gly-ala-gly, the 22.5 μmoles/250 mg. is not unreasonable as compared with the 62 μmoles/250 mg. of Slobodian and Levy[5] because their time of hydrolysis was only 24 hours instead of 48. If we assume that the ala-(gly, ala) of zone S-9 is ala-gly-ala, then the 8 μmoles is considerably less than the 22.5 μmoles of gly-ala-gly whereas we would predict equal amounts from their sequence. (We assume that any losses are equal.)

The sequence in ala-ala definitely is at variance with the repeating unit and it accounts for about 2% of the peptides which were isolated. Because the sequence in most of the tripeptides is unknown, all can be made to fit the sequence -gly-X_1-ala-gly-ala-gly-X_2- but certain data must be ignored if this is done. Gly-tyr and tyr-gly have been isolated but no tyr-ala. If tyrosine were in X_1 we might expect to find tyr-ala but the isolation of gly-tyr and tyr-gly would appear to limit tyrosine to X_2. Ser-gly may be fitted into the position X_2-gly, but ser-(gly, ala) would have to be ser-ala-gly in order to conform to the sequence in the position X_1-ala-gly because X_2-gly-X_1 is not satisfactory. At least 40% of the serine is in the sequence ser-gly but no ser-ala was observed although, if present, it should have been in zone S-10. Hence, it is more reasonable to conclude that ser-(gly, ala) is ser-gly-ala, which would not fit the above sequence.

It would appear that at the present state of our knowledge one can draw few definite conclusions about the structure of silk fibroin. The preliminary chromatographic experiments have shown that the peptide mixture in this partial hydrolysate of silk fibroin is relatively simple and it is to be hoped that future experiments with less completely hydrolyzed material will yield longer peptides from which it may be possible to deduce more about the structure of silk fibroin.

Acknowledgment.—Certain of the experiments which have been described were carried out by Mr. Setty Porto and Miss Nancy Munger. The investigation was supported in part by a contract with the Quartermaster Corps, U. S. Army.

(17) Subscripts have been added in order to distinguish between the "X"s.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER & CO., INC., AND THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

The Structure of Aureomycin[1]

BY C. R. STEPHENS, L. H. CONOVER, R. PASTERNACK, F. A. HOCHSTEIN, W. T. MORELAND, P. P. REGNA F. J. PILGRIM, K. J. BRUNINGS AND R. B. WOODWARD

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The antibiotic Aureomycin has been shown to have the structure II.

Aureomycin is a broad spectrum antibiotic which is produced by *Streptomyces aureofaciens*.[2] Comparison of chemical, biological and physical data for Aureomycin and Terramycin[3] suggested at an early date a close structural similarity between the two substances. When the investigations which led to the establishment of the structure I for Terramycin had been completed,[4] it was possible to propose directly the corollary hypothesis that Aureomycin had the structure II. The correctness of this expression was then confirmed by a series of experiments which are described in this communication.[1,5]

(1) Terramycin XII. The investigations described in this paper were first outlined in part in two preliminary communications: (a) THIS JOURNAL, **74**, 4976 (1952); (b) **75**, 4622 (1953). Terramycin is the registered trade mark of Chas. Pfizer & Co., Inc., for the antibiotic whose generic name is oxytetracycline. Aureomycin is a registered trade mark of Lederle Laboratories Division, American Cyanamid Co., for the antibiotic chlortetracycline.

(2) (a) R. W. Broschard, A. C. Dornbush, S. Gordon, B. L. Hutchings, A. R. Kohler, G. Krupka, S. Kushner, D. V. Lefemine and C. Pidacks, *Science*, **109**, 199 (1949); (b) B. M. Duggar, U. S. Patent 2,482,055 (1949).

(3) (a) A. C. Finlay, G. L. Hobby, S. Y. P'an, P. P. Regna, J. B. Routien, D. B. Seeley, G. M. Shull, B. A. Sobin, I. A. Solomons, J. W. Vinson and J. H. Kane, *Science*, **111**, 85 (1950); (b) P. P. Regna, I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings and W. A. Lazier, THIS JOURNAL, **73**, 4211 (1951).

(4) (a) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, K. J. Brunings and R. B. Woodward, *ibid.*, **74**, 3708 (1952); (b) F. A. Hochstein, C. R. Stephens, L. H. Conover P. P. Regna, R. Pasternack, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **75**, 5455 (1953).

(5) In a series of preliminary communications another group has also suggested the structure II as one of two possibilities for Aureomycin. (a) C. W. Waller, B. L. Hutchings, C. F. Wolf, R. W. Broschard, A. A. Goldman and J. H. Williams, *ibid.*, **74**, 4978 (1952); (b) C. W. Waller, B. L. Hutchings, A. A. Goldman, C. F. Wolf, R. W. Broschard and J. H. Williams, *ibid.*, **74**, 4979 (1952); (c) B. L. Hutchings, C. W. Waller, R. W. Broschard, C. F. Wolf, P. W. Fryth and J. H. Williams, *ibid.*, **74**, 4980 (1952); (d) C. W. Waller, B. L. Hutchings, C. F. Wolf, A. A. Goldman, R. W. Broschard and J. H. Williams, *ibid.*, **74**, 4981 (1952); (e) C. W. Waller, B. L. Hutchings, R. W. Broschard, A. A. Goldman, W. J. Stein, C. F. Wolf and J. H. Williams, *ibid.*, **74**, 4981 (1952).