barbs and down may differ. However, both instances are borderline and because duplicate hydrolyses of goose barbs and down were not made, one cannot conclude with confidence that differences exist: probably they do not. Thus, in all likelihood, appreciable dissimilarities in the amino acid content of the two types of goose feathers are negligible.

On the other hand, it would appear that turkey barbs differ from goose barbs and down in content of cystine, glycine, isoleucine, phenylalanine, proline, tyrosine and valine. However, the differences in glycine, isoleucine, proline and valine are borderline and probably are not significant. It seems quite definite, however, that turkey feather barbs contain less cystine and tyrosine and more phenylalanine than goose feather barbs or goose down. The sulfur contents (Table I) substantiate the conclusion about differences in cystine content. Thus, species variations are indicated.

Conclusions

The results of the present investigation show that the morphologically distinct parts of a white turkey feather differ in amino acid composition. The barbs are most distinctly different from the other parts and the contents of alanine, glycine, isoleucine and tyrosine are most manifestly dissimilar. Although one cannot assume *a priori* that feathers of other birds will show similar variations from part to part, it is apparent that in studies of so-called "feather keratin" definite portions rather than the whole feather should be investigated if the results are to be meaningful.

Although the data are less extensive, it may tentatively be concluded that different types of feathers from a given species of bird probably do not vary greatly in amino acid content but, on the other hand, some variation in amino acid composition of feathers from different species seems to be probable. This conclusion is also suggested by the work of Wilson and Lewis[19] on the cystine content of feathers from several types of birds.

The general amino acid composition of the feather proteins is such that about 10% of the residues are proline and another 40% are composed of amino acids with small side chains, that is, glycine, alanine and serine. Serine and threonine with their polar side chains make up 20% of the residues. If any attempt is to be made to interpret the complex X-ray diffraction pattern of the rachis in terms of the structure of the protein, the general amino acid composition must be kept in mind. For example, it is of interest to note that a proline residue will profoundly influence the configuration of a polypeptide chain and that the feather proteins contain one residue of proline in ten. Therefore, if the proline residues are at all randomly distributed in the feather keratin, it is highly unlikely that the polypeptide chains could assume any of the pleated sheet or simple helical configurations,[20,21] which have thus far been suggested.

Acknowledgments.—The assistance and interest of Mr. Holmes of the Colby Ranch, Covina, California, in supplying us with turkey feathers is greatly appreciated. This investigation was supported in part by a contract between the Quartermaster Corps, U. S. Army, and the California Institute of Technology.

(19) R. H. Wilson and H. B. Lewis, *J. Biol. Chem.*, **73**, 543 (1927).
(20) L. Pauling, R. B. Corey and H. R. Branson, *Proc. Nat. Acad. Sci.*, **37**, 205 (1951).
(21) L. Pauling and R. B. Corey, *ibid.*, **37**, 251 (1951).

PASADENA 4, CALIFORNIA

[CONTRIBUTION NO. 1974 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

The Amino Acid Composition of *Bombyx mori* Silk Fibroin and of Tussah Silk Fibroin

BY W. A. SCHROEDER AND LOIS M. KAY WITH THE TECHNICAL ASSISTANCE OF BARRY LEWIS AND NANCY MUNGER

RECEIVED FEBRUARY 28, 1955

Chromatographic determinations of the amino acid content of *Bombyx mori* silk fibroin and of Tussah silk fibroin (*Antheraea pernyi*) have revealed marked differences in composition. Although glycine, alanine, serine and tyrosine account for about 90% of the residues in both fibroins, the major difference lies in the almost exact reversal of the amounts of glycine and alanine in one fibroin as compared to the other. Some of the minor constituents also are present in very different amounts.

The composition and structure of the silk fibroin of *Bombyx mori* has been the subject of study and speculation over a period of many years. One evidence of this interest lies in the many fragmentary determinations of amino acid composition which have been described in the literature. It is, therefore, the more surprising to find that apparently the only complete analysis of a single sample of silk fibroin in one laboratory was made by Tristram who cites his own unpublished results in two review articles.[1] Bergmann and collaborators[2-4] determined certain amino acids in a sample of silk fibroin and portions of this sample were apparently used by many other workers who report that they obtained silk fibroin from Bergmann and co-workers: thus, a complete analysis of this sample seems to have been made.

The common cultivated silkworm, *Bombyx mori*, feeds on mulberry leaves and produces a silk which after degumming is almost white. Tussah silk, however, is produced by wild silk-

(1) G. Tristram, *Adv. Prot. Chem.*, **5**, 143 (1949), and "The Proteins," Vol I, Part A, edited by H. Neurath and K. Bailey, Academic Press, Inc., New York, N. Y., 1953, p. 220.
(2) M. Bergmann and C. Niemann, *J. Biol. Chem.*, **122**, 577 (1937–1938).
(3) S. Moore and W. H. Stein, *ibid.*, **150**, 113 (1943).
(4) W. H. Stein, S. Moore and M. Bergmann, *ibid.*, **154**, 191 (1944).

worms of several species which feed on oak leaves; after degumming, the fibroin of Tussah silk retains a green or tan color. It has long been known that the X-ray diffraction patterns of *Bombyx mori* fibroin and of Tussah fibroin differ[5] and it may be assumed that the amino acid compositions also are dissimilar. However, determinations of the amino acid composition of Tussah silk fibroin are few and fragmentary.

In the course of investigations of the structure of silk fibroin, we have determined the amino acid compositions of the fibroins from *Bombyx mori* silk and from Tussah silk; the results are reported in this paper.

Experimental

Materials.—The *Bombyx mori* silk fibroin (BSF) was a portion of a sample whose preparation has been described.[6] Redetermination of the nitrogen content[7] showed 18.25% (moisture- and ash-free basis) by the Kjeldahl method and 18.31% by the Dumas method, in excellent agreement with the average of 18.1% previously reported.[6]

The Tussah silk was given to us by Professor S. Mizushima of Tokyo University and had been obtained by him from Dr. F. Nakagawa. The silkworms, *Antherea pernyi*, had been fed on the leaves of the oak, *Quercus acutissima carruther*. The degumming procedure of Dunn, *et al.*,[8] was followed in detail. The decantates of the fourth and fifth autoclavings were not colorless but the amount of color which they removed was small compared to that removed by the preceding autoclavings. After the fifth autoclaving, and after drying at 50°, the Tussah silk fibroin (TSF) was light tan in color; 13% of the original weight had been removed in the degumming. The dried material was further extracted with the azeotrope of benzene and methanol in the manner described by Corfield, Howitt and Robson.[9] It was passed through approximately 50 cyclings of a Soxhlet extractor, washed thoroughly with water, and air-dried. The loss in weight was 0.4% and the fibroin had less luster and was more matted. The nitrogen content of the moisture- and ash-free protein was 18.81% by the Kjeldahl method and 18.95% by the Dumas method; duplicate determinations of the moisture content gave 7.35 and 7.13% and of the ash content 0.18 and 0.21%.

Acidic Hydrolyses.—Samples of 60 or 100 mg. of fibroin were hydrolyzed in 10 ml. of refluxing double distilled 6 *N* hydrochloric acid. Samples of BSF were hydrolyzed for 24 and 72 hr. and of TSF for 24 hr. Hydrolysates of BSF were light yellow-brown in color and those of TSF were dark muddy brown. Further preparation for chromatography followed the procedure previously described.[10]

Oxidation of the Fibroins and Subsequent Acidic Hydrolysis.—In order to determine cystine as cysteic acid according to the method of Schram, Moore and Bigwood,[11] about 170 mg. of each fibroin were oxidized with performic acid and subsequently hydrolyzed with 6 *N* hydrochloric acid. Exact details have been described.[10]

Chromatographic Methods.—The amino acid compositions of the silk fibroins were determined chromatographically by the starch[12] and ion-exchange[13] methods of Moore and Stein with only minor modifications in technique which have been discussed.[10]

When 100-cm. columns of Dowex 50 were used for the determination of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine and valine, a quantity of sample which produced reasonably sized zones of serine, glycine and alanine contained so little of the other amino acids that their zones were very small and the quantitative accuracy was low. Consequently, chromatograms on the 100-cm. columns were run at two levels of sample size; a quantity of hydrolysate equivalent to about 12 mg. of fibroin was used to obtain satisfactory zones of the minor constituents and one-tenth this amount for the major constituents. For the estimation of amino acids on 15-cm. columns of Dowex 50, the sample was equivalent to 12 to 13 mg. of fibroin and on the starch columns equivalent to 3 to 4 mg. On Dowex 2 for the determination of cysteic acid, the sample size was 16 to 17 mg. and the chromatogram was developed first with 100 ml. of 0.01 *N* monochloroacetic acid and then changed to 0.1 *N* acid.[11]

Tyrosine was also estimated by a recently published method of Hirs, Moore and Stein.[14] Substitution of Dowex 2-X 10 for the prescribed Dowex 1-X 8 was without effect on the separation. Half-ml. fractions were collected at the rate of 8–10 ml. per cm. of cross-sectional area per hr. These fractions were neutralized with 0.05 ml. of 2.5 *N* sodium hydroxide before analysis by means of the original photometric ninhydrin method.[15] Under these conditions the color yield of tyrosine was 0.97. Recovery of known amounts of tyrosine was satisfactory.

Determination of Tryptophan.—The method of Spies and Chambers[16] was used to determine tryptophan in the intact protein by Procedures N and P. The rate of solution of BSF in 19 *N* sulfuric acid is not influenced by the presence or absence of the *p*-dimethylaminobenzaldehyde and the sample usually dissolved within 15 min. However, TSF dissolves much more readily in its absence (15 min. compared to 90 min.) and as a consequence Procedure P was preferred. However, the results from Procedures N and P did not agree; relative to Procedure P, Procedure N gave values which were 15% higher for TSF and 7% higher for BSF. The results determined by Procedure P have been accepted because the spectrum of the color coincided more nearly with the color which tryptophan standards develop. The results of the determination were not different even if the time of reaction I was varied from 4 to 16 hr. The suggested optimum conditions for reaction II (0.1 ml. of 0.045% sodium nitrite per 10 ml. and 30 min.) were used.

Results

The data are recorded in Table I. Both fibroins were submitted to one 24-hr. hydrolysis and the BSF to one 72-hr. hydrolysis. In most instances, duplicate determinations of each amino acid in each hydrolysate were made. The final values are the averages of the individual results with the exception of those of serine, tyrosine and ammonia. To obtain the final values for serine and ammonia, the results of the 24- and 72-hr. hydrolyses were extrapolated to zero time; because no 72-hr. hydrolysis of TSF was made, the quantities of serine and ammonia in TSF were corrected to zero time by assuming that the percentage decrease or increase with time was the same in TSF as in BSF. In order not to overemphasize the greater number of determinations of tyrosine in BSF by means of Dowex 2, the final value is the average of the individual averages on starch and Dowex 2.

No corrections have been applied to histidine[10] or to glutamic acid.[13]

The nature of the chromatograms which resulted when hydrolysates of the two fibroins were chromatographed on 100-cm. and 15-cm. columns of Dowex 50 is shown in Fig. 1.

(5) C. Trogus and K. Hess, *Biochem. Z.*, **260** 376 (1933). See W. T. Astbury and F. O. Bell, *Tabulae Biologicae*, **17**, 90 (1939), for a resume.

(6) L. M. Kay and W. A. Schroeder, THIS JOURNAL, **76**, 3564 (1954).

(7) All microanalyses by Dr. Adalbert Elek.

(8) M. S. Dunn, M. N. Camien, L. B. Rockland, S. Shankman and S. C. Goldberg, *J. Biol. Chem.*, **155**, 591 (1944).

(9) M. C. Corfield, F. O. Howitt and A. Robson, *Nature*, **174**, 603 (1954).

(10) W. A. Schroeder, L. M. Kay, B. Lewis and N. Munger, THIS JOURNAL, **77**, 3901 (1955).

(11) E. Schram, S. Moore and E. J. Bigwood, *Biochem. J.*, **57**, 33 (1954).

(12) W. H. Stein and S. Moore, *J. Biol. Chem.*, **176**, 337 (1948).

(13) S. Moore and W. H. Stein, *ibid.*, **192**, 663 (1951).

(14) C. H. W. Hirs, S. Moore and W. H. Stein, THIS JOURNAL, **76**, 6063 (1954).

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

(16) J. R. Spies and D. C. Chambers, *Anal. Chem.*, **21**, 1249 (1949).

TABLE I: AMINO ACID COMPOSITIONS OF *Bombyx mori* SILK FIBROIN (BSF) AND OF TUSSAH SILK FIBROIN (TSF)

(Results are expressed in terms of g. of amino acid per 100 g. of moisture- and ash-free protein except as shown.)

Amino acid	*Bombyx mori* silk fibroin Individual values 24-hr. hydrolysis	*Bombyx mori* silk fibroin Individual values 72-hr. hydrolysis	Final values[a]	Tussah silk fibroin Individual values 24-hr. hydrolysis	Final values[a]	Residues per 10^5 g. protein BSF	Residues per 10^5 g. protein TSF	Tristram[c]	Literature on *Bombyx mori* silk fibroin Bergmann's sample	Literature on *Bombyx mori* silk fibroin Dunn, *et al.*	Literature on *Bombyx mori* silk fibroin Others	Lit. on Tussah silk fibroin
Alanine	34.71, 34.49	34.10, 34.17	34.37	(51.83),[b] 47.13, 47.22	47.18	385.7	529.5	29.7	26.4[d]	34.9[t]	32.4,[ab] 25.5,[ah] 37.6 and 34.0,[aj] 17.9–23.2,[al] 25.4[ao]	19.0,[al] 34.6–35.8[ao]
Arginine	0.92, 0.94	0.91, 1.15	0.98	5.45, 5.36	5.41	5.6	31.1	1.1	0.95,[d] 0.98[e] 0.97,[h] 1.11[k]		1.000,[z] 1.1,[ad] 2.4 and 0.8,[aj] 0.76,[ap] 0.74[aq]	
Aspartic acid	2.27, 2.39	2.26, 2.45	2.34	7.62, 7.42	7.52	17.6	56.5	2.76	2.8[f]		2.4,[am] 2.65[ab]	
Cystine (?)	0.18, 0.17	....	0.18	....	....	0.8	...	0				
Glutamic acid	1.83, 1.79	1.79, 1.52	1.73	1.48, 1.44	1.46	11.8	9.9	2.16	2.2[g]	2.16[l]	2.0,[ab] 2.1 and 3.5,[ac] 0.8[ao]	5.6[ao]
Glycine	42.59, 43.02	42.73, 42.05	42.60	24.01, 24.10, 23.52	23.88	567.2	318.0	43.6	43.8,[d] 43.8[i]	43.6,[s] 43.4[t], 43.6[u]	36.0,[w] 40.3,[aa] 41.0,[ah] 39.9 and 42.4,[aj] 34.3–41.4,[al] 35.5[ao]	22.1,[al] 18.9–19.2[ao]
Histidine	0.31, 0.33	0.33, 0.27	0.30	1.55, 1.55	1.55	1.9	10.0	0.36	0.36,[e] 0.41[k]	0.34,[o] 0.34[q]	0.47,[y] 0.37,[z] 0.15,[aa] 0.37,[ad] 0.2,[ao] 0.07[aq]	0.8–1.9[ao]
Isoleucine	0.84	0.95, 0.92	0.90	0.52, 0.79	0.66	6.9	5.0	1.1	1.15[k]		Trace and 2.5[aj]	
Leucine	0.77	0.78, 0.88	0.81	0.62, 0.59	0.61	6.2	4.6	0.91	0.8,[i] 0.93[k]		0.8,[v] 1.2[am]	
Lysine	0.56, 0.55	0.62, 0.52	0.56	0.15, 0.18	0.17	3.8	1.2	0.68	0.72[k]	0.6[r]	0.537,[z] 0.7,[aa] 0.7,[ad] 0.3,[ao] 0.25[aq]	7.6–7.9[ao]
Methionine	....	....	....	....	....	.....	.....	0	0.15[k]	0.136[m]	Absent[aj]	
Phenylalanine	1.24	1.25, 1.47	1.32	1.15, 0.97	1.06	8.0	6.4	3.36	1.49[k]	1.28[p]	Absent,[aj] 1.3[ao]	0.6–0.7[ao]
Proline	0.58	0.60	0.59	0.32, 0.54	0.43	5.1	3.7	0.74			1.5,[y] 0.5,[aa] trace[aj]	
Serine	14.63, 14.73	12.13, 12.02	15.98	13.95, 13.24	14.80	152.0	140.8	16.2	14.5[j]		12.6,[y] 14.7,[aa] 12.7,[ah] 9.45,[ag] 13.57,[ai] 12.7 and 11.9,[aj] 16.24,[ak] 12.3–15.7,[al] 11.2[ao]	15.6[al]
Threonine	1.32	1.65	1.49	0.1–0.3	0.2	12.5	1.7	1.6	1.3,[e] 1.36[k]	1.24[n]	1.5,[y] 1.2,[aa] 1.07,[ab] 0.6,[ae] 1.36,[ai] 1.37,[ak] 1.9[ap]	
Tryptophan			0.50		2.74	2.5	13.4	0	0.34,[e] 0.44[k]		0,[an] 0.6[ap]	1.4–2.1[ao]
Tyrosine { Starch	10.73	10.56	11.29	10.12, 10.14	10.60	62.3	58.5	12.8	13.2[d]			
Tyrosine { Dowex 2	12.09, 12.66 11.59, 11.39			10.91, 11.24							13.2,[r] 10.6[y] 10.8 and 10.6,[af] 5.9 and 8.3,[aj] 9.7–12.3,[al] 12.2[ao]	9.7,[al] 9.2–9.6,[ao] 10.42–12.89[ar]
Valine { Starch	2.98	2.95	3.12	0.77, 0.91	0.87	26.7	7.4	3.6	3.45,[e] 3.2[h]			
Valine { Dowex 50	3.13	3.42		0.94					3.5[k]		4.4 and 5.7,[aj] 4.3[am]	
Ammonia	0.44, 0.44	0.83, 0.84	0.24	0.30, 0.31	0.17	14.1[b]	10.0[b]	?			0.46[r]	
Total			119.3		119.3	1277	1198					
% Wt. accounted for			96.3		97.8							
% N accounted for			101.0		99.1							

[a] The considerations which lead to these final values are discussed under "Results." [b] Not included in average or total. [c] G. Tristram, *Adv. Prot. Chem.*, **5**, 143 (1949). [d] M. Bergmann and C. Niemann, *J. Biol. Chem.*, **122**, 577 (1937–1938). [e] M. J. Boyd, M. A. Logan and A. A. Tytell, *ibid.*, **174**, 1027 (1948). [f] L. R. Hac and E. E. Snell, *ibid.*, **159**, 291 (1945). [g] L. R. Hac, E. E. Snell and R. J. Williams, *ibid.*, **159**, 273 (1945). [h] J. R. McMahan and E. E. Snell, *ibid.*, **152**, 83 (1944). [i] S. Moore and W. H. Stein, *ibid.*, **150**, 113 (1943). [j] J. L. Stokes and M. Gunness, *ibid.*, **157**, 651 (1945). [k] J. L. Stokes, M. Gunness, I. M. Dwyer and M. C. Caswell, *ibid.*, **160**, 30 (1945). [l] M. S. Dunn, *et al.*, *ibid.*, **155**, 591 (1944). [m] M. S. Dunn, *et al.*, *ibid.*, **163**, 577 (1946). [n] M. S. Dunn, *et al.*, *ibid.*, **163**, 589 (1946). [o] M. S. Dunn, *et al.*, *ibid.*, **159**, 653 (1945). [p] M. S. Dunn, *et al.*, *ibid.*, **161**, 643 (1945). [q] M. S. Dunn, *et al.*, *ibid.*, **161**, 669 (1945). [r] M. S. Dunn, *et al.*, *ibid.*, **156**, 715 (1944). [s] T. A. McCoy, M. K. Patterson, Jr., and S. H. Wender, *Arch. Biochem. Biophys.*, **43**, 485 (1953). [t] L. B. Rockland and M. S. Dunn, THIS JOURNAL, **71**, 4121 (1949). [u] S. Shankman, M. N. Camien and M. S. Dunn, *J. Biol. Chem.*, **168**, 51 (1947). [v] E. Brand, F. J. Ryan and E. M. Diskant, THIS JOURNAL, **67**, 1532 (1945). [w] E. Cherbuliez, P. Plattner and S. Ariel, *Helv. Chim. Acta*, **13**, 1390 (1930). [x] A. C. Chibnall, *Proc. Roy. Soc.* (*London*), **131B**, 136 (1942). [y] D. Coleman and F. O. Howitt, *ibid.*, **190A**, 145 (1947). [z] M. C. Corfield, F. O. Howitt and A. Robson, *Natvre*, **174**, 603 (1954). [aa] R. Glanzman and R. Signer, *Makromol. Chem.*, **8**, 134 (1952). [ab] M. Levy and E. Slobodian, *J. Biol. Chem.*, **199**, 563 (1952). [ac] J. C. Lewis and H. S. Olcott, *ibid.*, **157**, 265 (1945). [ad] H. T. Macpherson, *Biochem. J.*, **40**, 470 (1946). [ae] A. J. P. Martin and R. L. M. Synge, *ibid.*, **35**, 294 (1941). [af] K. H. Meyer, M. Fuld and O. Klemm, *Helv. Chim. Acta*, **23**, 1441 (1940). [ag] R. Michel and M. Bozzi, *Bull. soc. chem. biol.*, **29**, 884 (1947). [ah] R. Michel and O. Michel, *ibid.*, **29**, 886 (1947). [ai] B. H. Nicolet and L. J. Saidel, *J. Biol. Chem.*, **139**, 477 (1941). [aj] A. Polson, V. M. Moseley and R. W. G. Wyckoff, *Science*, **105**, 603 (1947). [ak] M. W. Rees, *Biochem. J.*, **40**, 632 (1946). [al] J. Roche, R. Michel and M. Bozzi-Tichadou, *Compt. rend. soc. biol.*, **144**, 100 (1950). [am] J. Roche and M. Mourgue, *ibid.*, **137**, 766 (1943). [an] A. Rossi and A. Vescia, *Boll. soc. ital. biol. sper.*, **17**, 129 (1942). [ao] E. P. Tsintsevich and M. M. Botvinik, *J. Applied Chem. USSR*, **23**, 793 (1950); *C. A.*, **46**, 5623g (1952). [ap] H. B. Vickery, *J. Biol. Chem.*, **132**, 325 (1940). [aq] H. B. Vickery and R. J. Block, *ibid.*, **93**, 105 (1931). [ar] R. Murase and S. Sakaguchi, *J. Soc. Textile Cellulose Ind.* (*Japan*), **7**, 97 (1951); *C. A.*, **46**, 3764i (1952).

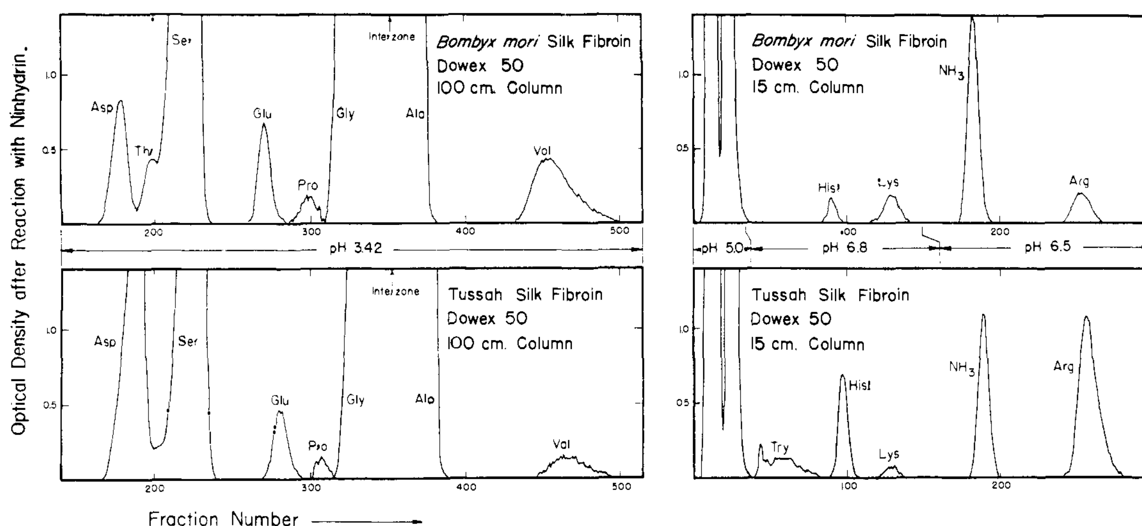


Fig. 1.—Comparison of chromatograms of approximately equal quantities of hydrolysates of BSF and TSF. On the 100-cm. chromatograms, the fraction size was 0.5 ml. to about fraction no. 235 and 1 ml. thereafter. On the 15-cm. chromatograms, the fraction size was 1 ml.

Discussion

Chromatographic Aspects of the Results.—The zones of alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, valine and ammonia were well-formed and well-separated on these chromatograms and the determinations proceeded without difficulty.

As has been mentioned above, the 100-cm. columns were run with different amounts of sample because of the unusual variation in the quantities of amino acids within a given fibroin. It was anticipated that the determinations of serine, glycine and alanine would be less satisfactory where the large sample was used because the amount of each of these amino acids is much greater than is commonly placed on columns of this size. The quantity of glycine itself, for example, was about 5 mg. The anticipated poor results have not been realized: even when the quantities are unusually large, the quantitative values have been excellent and the agreement of results from the two levels has been very good. Note the very satisfactory agreement of duplicate determinations of serine, glycine and alanine in a given hydrolysate despite the fact that the quantities in the duplicates differed by a factor of 10. It is apparent from this outcome that the ninhydrin method is applicable over an exceedingly wide range of concentration.

When BSF was oxidized with performic acid, hydrolyzed, and then chromatographed on Dowex 2, a minor zone was present in the position of cysteic acid. One cannot say unequivocally that cystine therefore is present in BSF although it may be there in very small amount. On the other hand, no zone in the position of cysteic acid appeared when the method was applied to TSF.

No methionine was detected on the chromatograms. If it occurs to the minor extent reported in the literature (Table I), it would not have been noticed on the chromatograms.

Proline is found in the hydrolysates in detectable amounts but the quantitative estimation is rather rough because of the low color yield of this amino acid in the ninhydrin procedure. The zones are rather ill-formed.

The large amount of serine makes the serine–threonine separation even less satisfactory than usual[10] as may be seen by inspection of Fig. 1. The method of calculation for badly overlapping zones[12] has been used to calculate the amount of threonine in BSF but the result may be much in error. The chromatogram of TSF (Fig. 1) reveals no discrete zone of threonine. In order to determine whether or not any threonine was present, the fractions from the aspartic acid–threonine–serine region of a duplicate chromatogram were combined, and dinitrophenylated. The DNP-amino acids were chromatographed according to the method of Green and Kay.[17] In this way, the presence of threonine was confirmed and the quantity was estimated at 0.1–0.3 g. per 100 g. of protein.

The occurrence of tryptophan in appreciable amount in TSF was immediately suspected on the basis of the 15-cm. chromatograms on Dowex 50 (see Fig. 1). The qualitative identification of this zone as tryptophan was made by comparing its chromatographic behavior with that of known tryptophan on Dowex 50 and also by converting to the DNP-amino acid and chromatographing on silicic acid–Celite.[17] Its quantitative determination in the intact protein by the method of Spies and Chambers[16] has been discussed above.

On starch chromatograms, the zone of tyrosine from BSF was very poorly formed. Instead of a symmetrical peak, the zone took the form of a peak superimposed on the trailing edge of a plateau. This malformation of the zone probably was caused by overloading of the column (4 to 4.5 mg. of hydrolysate) because more symmetrical zones were obtained from TSF by reducing the load to about 3 mg. As a further check on the determina-

(17) F. C. Green and L. M. Kay, *Anal. Chem.*, **24**, 726 (1952).

tion of tyrosine, the new method of Hirs, Moore and Stein[14] was applied. The results from both fibroins were definitely higher than when starch was used (Table I). One 24-hr. hydrolysate of BSF showed 10.73 g. of tyrosine per 100 g. of protein from the starch method and 12.09 and 12.66 from the Dowex 2 method; a second 24-hr. hydrolysate yielded 11.59 and 11.39 g. per 100 g. of protein from Dowex 2 columns. Various experiments have failed to ascertain the cause of the discrepancy. Chromatography of a synthetic mixture of all amino acids except tyrosine showed complete lack of interference in the region of tyrosine. The recovery of known amounts of tyrosine from Dowex 2 columns was excellent and it was generally satisfactory from starch although on occasion the recovery was only about 90% for reasons unknown. The possibility exists that a contaminant from the hydrolysis, which, of course, would not be present in a synthetic mixture, coincides with the tyrosine on Dowex 2. Since tyrosine emerges rapidly from Dowex 2 and slowly from starch, there is greater likelihood that a contaminant would interfere on Dowex 2. Since one set of results cannot with justification be chosen over the other, both have been used in arriving at the final value.

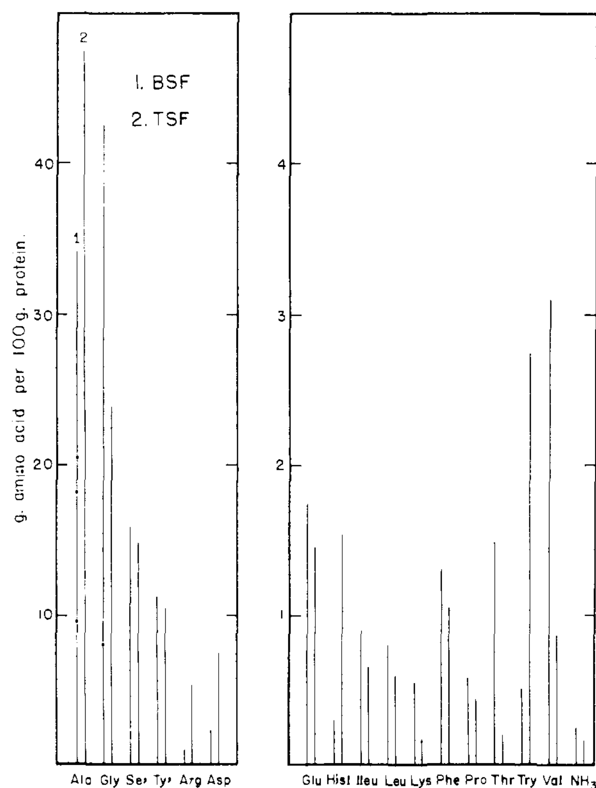


Fig. 2.—Comparison of the amino acid composition of BSF and TSF.

Effect of Time of Hydrolysis.—That more than one time of hydrolysis is required for satisfactory determination of amino acid composition has been pointed out by Harfenist and by Smith and coworkers. A discussion of this subject and its relation to the analysis of feather proteins is given in the preceding paper.[10] In the analysis of the silk fibroins, increased time of hydrolysis seems to alter only the content of serine and ammonia. Although it may actually influence the content of the other amino acids, the effect is difficult to detect because the quantity of most amino acids is small and the determinations have greater error. The destruction of 8% of the serine during 24 hr. of hydrolysis is not abnormal.[10,18]

Comparison of the Amino Acid Composition of the Silk Fibroins.—That the two fibroins differ very appreciably in composition is evident from mere inspection of Fig. 1 in which the zones of aspartic acid, threonine, valine, tryptophan, histidine, lysine and arginine show marked dissimilarity in size. Graphical representation in Fig. 2 demonstrates the difference in an even more striking way. In summary, relative to BSF, TSF contains about 40% more alanine, three times as much aspartic acid, and five times as much arginine, histidine and tryptophan; about 55% as much glycine, 35% as much lysine, 25% as much valine, 10% as much threonine, and no cystine; about equal amounts of serine, tyrosine, glutamic acid, isoleucine, leucine, phenylalanine, proline and ammonia. Both contain little or no methionine.

Despite the very marked differences in amino acid composition, the four amino acids, glycine, alanine, serine and tyrosine, make up almost exactly 90% of the residues in both proteins. The residue percentage of serine and tyrosine is the same in both but the residue percentage of glycine and alanine is almost completely reversed in one as compared to the other; this is the major variation. In the remaining 10% of the residues, the TSF is enriched in histidine, arginine and aspartic acid at the expense especially of such neutral amino acids as threonine and valine in BSF. Because the increase in acidic and basic amino acids is approximately equal in amount, the over-all acidity of the molecule is not greatly altered.

The difference in the amino acid composition of the two fibroins is so far reaching that it is not surprising that the X-ray diffraction patterns do not coincide.

Comparison with Results in the Literature.—The literature references in Table I take note of no work reported prior to 1940. The references on BSF are many and may be divided as shown in Table I into four categories. It is interesting to compare the results of Tristram, the analyses of Bergmann's sample, and the data of Dunn. There is rather good agreement, but a few exceptions occur. Tristram reports no methionine or tryptophan, his value for phenylalanine is vastly different from any other, and his value for serine is higher than for Bergmann's sample. The three do not agree on alanine. Our data on glycine and serine agree well with Tristram and on alanine with Dunn but all others are about 80% of Tristram's. A great variety of values is to be found in the other references. Many of these used a presumably specific chemical procedure with all of its attendant difficulties when applied to a complex mixture and,

(18) E. L. Smith and A. Stockell, *J. Biol. Chem.*, **207**, 501 (1954).

consequently, the results must be treated with some reserve. It may be noted that the analysis for the basic amino acids by Corfield, Howitt and Robson (Table I, footnote *z*) used the chromatographic technique on 15-cm. columns which we have employed; the results are in good agreement. The work of Roche, Michel and Bozzi-Tichadou (Table I, footnote *al*) suggests that the composition of the silk fibroin of *Bombyx mori* may depend upon the exact geographical source of the silk; perhaps some variations in the results of the literature may be attributed to this cause.

Literature references to amino acid analyses of TSF are fragmentary but would indicate a difference between BSF and TSF.

NOTE ADDED IN PROOF.—Since this manuscript was submitted, a translation of the paper by Tsintsevich and Botvinik (Table I, footnote *ao*) has been obtained. They comment also upon the reversal in the ratio of glycine to alanine in the two fibroins. Attention should be called to a recent paper of F. Lucas, J. T. B. Shaw and S. G. Smith (*Shirley Institute Memoirs*, **28**, 77 (1955)), which lists partial analyses of fibroins from *Bombyx mori* silk, from several Tussah silks, and from three *Anaphe* silks.

Acknowledgments.—We wish to express our thanks to Professor S. Mizushima for supplying the sample of Tussah silk and to Dr. C. H. W. Hirs for the details of the separation of tyrosine on Dowex 2 prior to publication. This investigation was supported in part by a contract between the Quartermaster Corps, U. S. Army and the California Institute of Technology.

PASADENA 4, CALIFORNIA

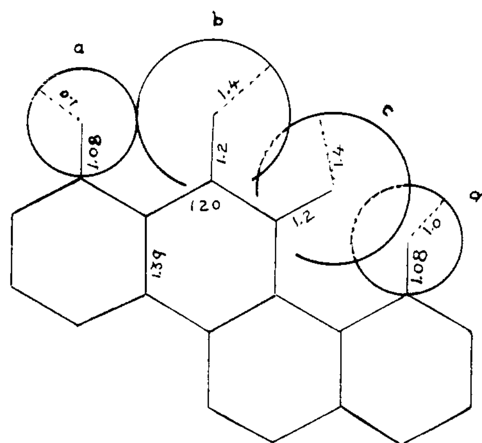
NOTES

Studies on Chrysenoxazoles

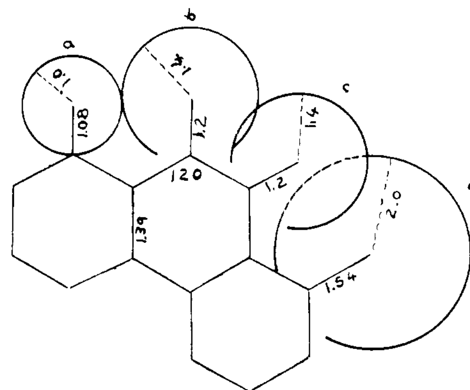
BY WILLIAM IBRAHIM AWAD AND ABDEL REHIM ABDEL RAOUF

RECEIVED MARCH 1, 1955

It was found recently[1] that chrysenequinone (I) reacts with aldehydes and ammonium acetate in acetic acid to give chrysenoxazoles and not imidazoles as expected from the general procedure for the preparation of phenanthrimidazoles[2] and have concluded that, in contrast to phenanthraquinone, one carbonyl group of chrysenequinone is more active than the other.



I, a and d = H
b and c = carbonyl group



II, a = H
b, c = carbonyl group
d = methyl group

—, bond distances; ······ van der Waals radii.

In continuing this investigation, we have allowed some amines ($R-CH_2-NH_2$) to react with chrysenequinone under pressure. The products obtained were even under these conditions the corresponding oxazoles (II) and not the imidazoles.[3]

2-Methylchrysenoxazole also has been prepared by the action of diazoethane on chrysenequinonimine in a similar manner to the action of diazomethane on chrysenequinonimine.[1]

Another attempt to prepare chrysenimidazoles through the interaction of the diimine acetate and aldehydes was unsuccessful since chrysenequinone when allowed to react with ammonium acetate in acetic acid did not yield the diimine acetate[4] as expected. A pale yellow compound was obtained which we believe to be chrysenequinonimine anhydride (III), since it proved to be identical with the

(1) W. I. Awad and A. R. A. Raouf, THIS JOURNAL, **77**, 1013 (1955).

(2) E. A. Steck and A. R. Day, *ibid.*, **65**, 452 (1943).

(3) Compare the reactions of phenanthraquinone and retenequinone with amines under pressure by G. M. Jaffe and A. R. Day, *J. Org. Chem.*, **8**, 43 (1943).

(4) For the preparation of phenanthraquinone diimine acetate and its interaction with aldehydes to give phenanthrimidazoles, compare ref. 2.