Final atomic co-ordinates and temperature factors

Atom	x/a	y/b	z/c	b	Atom	x/a	y/b	z/c	b
Br (1)	0.2763	0.1782	0.8309	4.97	C (20)	0.2908	0.1176	0.0950	4.37
Br (2)	0.4980	0.0209	0.3311	8.94	C (21)	0.2868	0.1365	0.2240	1.53
Br (3)	0.4407	0.3539	0.3013	12.31	C (22)	0.2219	0.1185	0.2898	1.43
O (1)	0.5009	0.2357	0.6390	4.43	C (23)	0.2240	0.0575	0.3251	3.66
O (2)	0.3945	0.2495	0.4919	4.02	C (24)	0.1618	0.0345	0.3529	3.99
O (3)	0.2371	0.3610	0.6408	5.42	C (25)	0.1049	0.0415	0.2597	2.45
O (4)	0.2830	0.3778	0.9430	8.13	C (26)	0.0408	0.0170	0.3141	3.77
O (5)	0.2430	0.4448	0.7326	5.92	C (27)	0.4765	0.4707	0.7495	4.72
O (6)	0.4239	0.4623	0.5702	4.46	C (28)	0.4151	0.4926	0.6498	4.53
O (7)	0.4600	0.0450	0.7948	5.31	C (29)	0.3529	0.4728	0.7394	3.96
O (8)	0.4454	0.0543	0.9798	6.18	C (30)	0.3075	0.4511	0.6870	6.63
O (9)	0.1359	0.0621	0.4548	2.11	C (31)	0.1723	0.3564	0.1424	7.41
O (10)	0.2167	0.1459	0.3914	2.53	C (32)	0.2887	0.0432	0.3910	4.02
O (11)	0.1712	0.1827	0.0825	2.88	C (33)	0.1305	0.0249	0.1576	5.99
O (12)	0.0837	0.2790	0.9959	4.60	C (34)	0.4736	0.4077	0.7703	5.38
O (13)	0.3658	0.3005	0.9902	5.83	C (35)	0.4513	0.0736	0.8944	5.39
O (14)	0.3844	0.1733	0.5816	3.37	C (36)	0.4449	0.1386	0.8652	5.72
O (15)	0.1730	0.0075	0.5743	6.84	C (37)	0.4045	0.4936	0.4725	6.53
O (16)	0.1264	0.1903	0.3453	3.27	C (38)	0.3973	0.1975	0.5066	1.52
N (1)	0.0699	0.2572	0.1745	6.70	C (39)	0.4370	0.1663	0.4107	3.02
C (1)	0.4707	0.2555	0.7122	3.54	C (40)	0.4550	0.1950	0.3117	4.68
C (2)	0.5028	0.2614	0.8286	6.23	C (41)	0.4706	0.1683	0.2134	4.87
C (3)	0.4622	0.2749	0.9156	5.18	C (42)	0.4937	0.1150	0.2329	4.39
C (4)	0.4020	0.2895	0.9144	4.61	C (43)	0.4758	0.0907	0.3176	4.15
C (5)	0.3166	0.3233	0.7661	6.06	C (44)	0.4502	0.1143	0.4267	4.26
C (6)	0.2874	0.3294	0.6586	6.44	C (45)	0.1651	0.1788	0.4119	3.91
C (7)	0.3136	0.3029	0.5601	5.66	C (46)	0.1635	0.2012	0.5417	3.88
C (8)	0.3752	0.2735	0.5894	4.14	C (47)	0.1156	0.2327	0.5646	3.99
C (9)	0.3985	0.2742	0.6919	3.44	C (48)	0.1181	0.2469	0.6829	3.27
C (10)	0.3724	0.2981	0.7848	3.65	C (49)	0.1637	0.2292	0.7555	4.73
C (11)	0.2732	0.3696	0.8555	6.16	C (50)	0.2091	0.1980	0.7340	4.28
C (12)	0.2268	0.3925	0.7580	5.19	C (51)	0.2098	0.1810	0.6128	2.89
C (13)	0.1564	0.3895	0.8074	5.46	C (52)	0.1459	0.0437	0.5587	3.82
C (14)	0.2738	0.3039	0.4561	7.56	C (53)	0.1165	0.0728	0.6522	5.55
C (15)	0.1027	0.2757	0.0892	1.80	C (54)	0.1502	0.0703	0.7653	7.52
C (16)	0.1659	0.2953	0.1130	5.27	C (55)	0.1295	0.0992	0.8658	8.10
C (17)	0.2167	0.2651	0.1303	2.59	C (56)	0.0790	0.1157	0.8352	5.94
C (18)	0.2114	0.2016	0.1094	3.14	C (57)	0.0398	0.1158	0.7411	7.69
C (19)	0.2881	0.1744	0.1428	3.64	C (58)	0.0613	0.0902	0.6232	6.37

These facts indicate that the molecules are held together in the crystalline state mainly by van der Waals forces.

Zusammenfassung. Bestimmung der Molekularstruktur des Tolypomycinones durch Röntgenstrukturanalyse seiner *m*-Bromobenzoat-Kristalle. Die auf chemischem Wege aufgeklärte Konstitution wird bestätigt und darüber hinaus Klärung über Konfiguration und Konformation des Tolypomycin Y erreicht.

K. KAMIYA, T. SUGINO, Y. WADA, M. NISHIKAWA and T. KISHI

Research and Development Division, Takeda Chemical Industries, Ltd., Osaka (Japan), 14 April 1969.

Catalytic Properties of Synthetic Pentapeptides

The amino-acid sequences around the active sites of some hydrolytic enzymes have been determined and the functional side chain groups of amino-acids such as serine and histidine have been shown to be essential. Information about the role of these groups has usually been obtained by modifying them and testing the enzymic activity of the modified enzyme[1]. On the other hand many peptides containing in different sequences the amino-acids known to be involved at the active site of hydrolytic enzymes have been synthetized and tested as catalysts in model hydrolytic reactions. The peptide L-threonyl-L-alanyl-L-seryl-L-histidyl-L-aspartic acid (A)[2], which is known to occur at the active site of the enzyme phosphoglucomutase[3] has been shown to exhibit a catalytic activity towards the hydrolysis of *p*-nitrophenyl

[1] Cf. for example the modification of the active L-serines of α-chymotrypsin and subtilisin to dehydroalanine (H. WEINER, W. N. WHITE, D. G. HOARE, and D. E. KOSHLAND JR., J. Am. chem. Soc. *88*, 3851 (1966)) and to L-cysteine (L. POLGAR and M. L. BENDER, J. Am. chem. Soc. *88*, 3153 (1966)) respectively.

[2] Abbreviations follow the tentative rules of IUPAC-IUB Commission on Biochemical Nomenclature, J. biol. Chem. *241*, 2491 (1966).

[3] C. MILSTEIN and F. SANGER, Biochem. J. *79*, 456 (1961).

acetate[4]. The catalytic coefficient k_2[5] expressed in l. mole^{-1} min^{-1} was 92 for A[4] compared with 10^4 for α-chymotrypsin and 20 for imidazol.

Thr-Ala-Ser-His-Asp (A)
Thr-Ala-Cys-His-Asp (B)
DPM
|
Thr-Ala-Cys-His-Asp (C)
Thr-Ala-Abu-His-Asp (D)

DPM = $CH(C_6H_5)_2$

Substitution of the L-serine residue by L-cysteine as in peptide B ($k_2 = 30$)[6] reduced the catalytic activity to about $^1/_3$ of that of Peptide A. Furthermore the S-diphenylmethylpeptide C[6a] showed only $^1/_5$ of the catalytic activity of the SH-peptide B. However this change in the catalytic properties is due rather to steric hindrance than to the lack of a free SH group since oxidation of the SH to –S–S– did not change the catalytic activity of the SH-peptide B[6b].

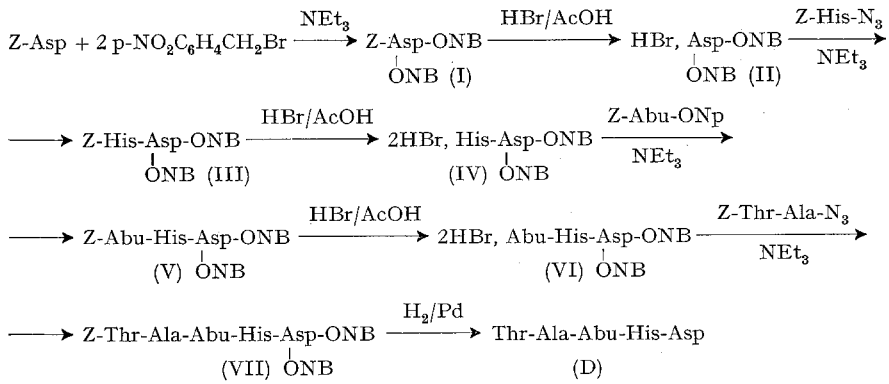
This last finding led us to synthetize another analogue of the pentapeptides A and B with the amino-acid sequence L-Thr-L-Ala-L-Abu-L-His-L-Asp (D) and test it for catalytic activity. In peptide D the sulphur present at the side chain of the third amino-acid residue of peptide B is replaced by the isosteric methylene group.

The synthesis of the α-aminobutyryl pentapeptide D was accomplished as outlined in the scheme.

N-Benzyloxycarbonyl-L-aspartic acid di-*p*-nitrobenzyl ester (I) was obtained (85%, mp 82–84°, $[\alpha]_D^{21}$ −7.9° (*c* 2, ethyl acetate). Anal. calcd. for $C_{26}H_{23}N_3O_{10}$ (537.5): C, 58.1; H, 4.31; N, 7.81. Found: C, 58.2; H, 4.41; N, 7.93) according to SCHWYZER[7] by the interaction of the triethylammonium salt of N-benzyloxycarbonyl-L-aspartic acid[8] with *p*-nitrobenzylbromide. Removal of the benzyloxycarbonyl group with 2*N* hydrogen bromide in acetic acid[9] and coupling of the resulting L-aspartic acid di-*p*-nitrobenzyl ester hydrobromide (II) (82%, mp 160–161°, $[\alpha]_D^{22}$ 0° (*c* 5, dimethylformamide). Anal. calcd. for $C_{18}H_{18}N_3O_8Br$ (484.3): C, 44.6; H, 3.74; N, 8.67; Br, 16.5. Found: C, 44.85; H, 3.83; N, 8.61; Br, 16.4) with N-benzyloxycarbonyl-L-histidine by the azide method[10] in the presence of triethylamine gave N-benzyloxycarbonyl-L-histidyl-L-aspartic acid di-*p*-nitrobenzyl ester (III) (64%, mp 173–174°, $[\alpha]_D^{24}$ −17.1 (*c* 2, dimethylformamide). Anal. calcd. for $C_{32}H_{30}N_6O_{11}$ (674.6): C, 57.0; H, 4.48; N, 12.5. Found: C, 56.85; H, 4.67; N, 12.2). Treatment of III with 3*N* hydrogen bromide in acetic acid removed the benzyloxycarbonyl group giving L-histidyl-L-aspartic acid di-*p*-nitrobenzyl ester dihydrobromide (IV) (93%, mp dec. above 108°, $[\alpha]_D^{25}$ +9.8° (*c* 2, ethyl alcohol). Anal. calcd. for $C_{24}H_{26}N_6O_9Br_2$ (702.35): C, 41.0; H, 3.73; N, 12.0; Br, 22.8. Found: C, 40.7; H, 4.25; N, 11.8; Br, 22.4).

From a reaction of IV with N-benzyloxycarbonyl-L-α-aminobutyric acid *p*-nitrophenyl ester[11] in the presence of triethylamine the protected tripeptide N-benzyloxycarbonyl-L-α-amino-butyryl-L-histidyl-L-aspartic acid di-*p*-nitrobenzyl ester (V) was obtained (76%, mp 114° dec., $[\alpha]_D^{26}$ −15° (*c* 2, dimethylformamide). Anal. calcd. for $C_{30}H_{37}N_7O_{12}$ (759.75): C, 56.9; H, 4.91; N, 12.9. Found: C, 56.7; H, 5.12; N, 12.7) Decarbobenzoxylation of V with 3*N* hydrogen bromide in acetic acid afforded L-α-amino-butyryl-L-histidyl-L-aspartic acid di-*p*-nitrobenzyl ester dihydrobromide (VI) (91%, mp dec. above 208°, $[\alpha]_D^{27}$ +4.5° (*c* 2, dimethylformamide). Anal. calcd. for $C_{28}H_{33}N_7O_{10}Br_2$ (787.5): C, 42.7; H, 4.22; N, 12.45; Br, 20.3. Found: C, 42.3; H, 4.58; N, 12.2; Br, 19.9). Compound V was reacted with N-benzyloxycarbonyl-L-threonyl-L-alanine azide[6] in the presence of triethylamine to give N-benzyloxycarbonyl-L-threonyl-L-alanyl-L-α-aminobutyryl-L-histidyl-L-aspartic acid di-*p*-nitrobenzyl ester (VII) (50%, mp 182–184°, $[\alpha]_D^{26}$ −21.5° (*c* 1, dimethylformamide). Anal. calcd. for $C_{43}H_{49}N_9O_{15}$ (931.9): C, 55.4; H, 5.29; N, 13.5. Found: C, 55.4; H, 5.66; N, 13.5). Catalytic hydrogenation of VII in the presence of palladium charcoal removed the amino and carboxyl protecting groups. The free pentapeptide L-threonyl-L-alanyl-L-α-aminobutyryl-L-histidyl-L-aspartic acid (D) was obtained by evaporating the filtrate to dryness, adding water repeatedly and evaporating, and finally adding acetone (73%, mp 248–252° dec., $[\alpha]_D^{29}$ −51.8° (*c* 1, water). Anal. calcd. for $C_{21}H_{35}N_7O_9$, H_2O (545.6): C, 46.2; H, 6.46; N, 18.0. Found: C, 46.3; H, 6.86; N, 17.9). All the intermediate compounds I–VII and the pentapeptide D were crystalline and homogeneous by thin-layer chromatography on Silica Gel G. The homogeneity of compound D was also proved by paper electro-

[4] P. CRUICKSHANK and J. C. SHEEHAN, J. Am. chem. Soc. *86*, 2070 (1964); J. C. SHEEHAN, G. B. BENNETT and J. A. SCHNEIDER, J. Am. chem. Soc. *88*, 3455 (1966).
[5] E. KATCHALSKI, G. D. FASMAN, E. SIMONS, E. R. BLOUT, F. R. N. GURD and W. L. KOLTUN, Arch. Biochem. Biophys. *88*, 361 (1960).
[6] (a) I. PHOTAKI and V. BARDAKOS, Chem. Comm. *275* (1967); (b) I. PHOTAKI, V. BARDAKOS, A. W. LAKE and G. LOWE, J. chem. Soc. (C) *1860* (1968).
[7] R. SCHWYZER and P. SIEBER, Helv. chim. Acta *42*, 972 (1959).
[8] M. BERGMANN and L. ZERVAS, Ber. dt. chem. Ges. *65*, 1192 (1932); German Patent 556,798 (1932).
[9] D. BEN ISHAI and A. BERGER, J. org. Chem. *17*, 1564 (1952).
[10] R. W. HOLLEY and E. SONDHEIMER, J. Am. chem. Soc. *76*, 1326 (1954). – R. F. FISCHER and R. R. WHETSTONE, J. Am. chem. Soc. *76*, 5076 (1954).
[11] V. DU VIGNEAUD, G. S. DENNING JR., S. DRABAREK and W. Y. CHAN, J. biol. Chem. *239*, 472 (1964).



Synthetic scheme (NB, nitrobenzyl; AcOH, acetic acid; NEt_3, triethylamine).

phoresis (in 0.5 N acetic acid or in pyridine-acetic acid buffer pH 4.9, 3 h, 350 V, it moved as a single band towards the cathode). A semi-quantitative thin-layer two-dimensional chromatography[12] of an acid hydrolysate of either peptides VII or D revealed the presence of the expected amino-acids in equimolar ratios.

The α-aminobutyryl peptide D, when tested as a catalyst for the hydrolysis of nitrophenyl acetate, showed a catalytic activity of about $^1/_3$ ($k_2 = 11$) of that of the cysteine (or cystine) peptide B and half of that of imidazole. First order kinetics were observed from about 10% to about 70% of completion of the reaction. The concentration of *p*-nitrophenyl acetate was $3.58 \times 10^{-5} M$ and the peptide D concentration ranged from $1.48 \times 10^{-4} M$ to $4.43 \times 10^{-4} M$ (in phosphate buffer 0.2 M, pH 7.7 containing 5% dioxan (V/V) at 22–24°). The formation and decomposition of an N^{im}-acetyl peptide intermediate (measured by the optical density at 245 nm) is probable as was also shown for the cysteine peptide B[6b].

Although the peptide analogues A–D are poor enzyme models, the role of the neighbouring amino-acid side chain to the catalytic imidazole group of histidine is obvious. A comparison of the catalytic coefficients of peptide B in the SH- or –S–S-form, and of peptides B and D suggests that the polarity rather than the reactivity or the geometry of the side chain group next to the histidine residue plays a more important role in the exhibition of the catalytic activity. A better peptide model showing a much higher catalytic activity is sought to study the effect of changing the functional side chain groups on the catalytic properties[13].

Zusammenfassung. Es wurde das Pentapeptid L-Thr-L-Ala-L-Abu-L-His-L-Asp synthetisiert, seine katalytische-hydrolytische Wirkung auf Essigsäure-*p*-nitrophenylester geprüft und die katalytische Aktivität ca. $^1/_3$ derjenigen seines isosteren L-Thr-L-Ala-L-Cys-L-His-L-Asp festgestellt.

I. Photaki and S. Moschopedis[14]

Laboratory of Organic Chemistry, University of Athens, Athens 144 (Greece), 28 April 1969.

[12] M. Brenner and A. Niederwieser, Experientia *16*, 378 (1960).

[13] This investigation was supported by Grant No. 814 to I.P. from the Royal Hellenic Research Foundation. Microanalyses were performed by Dr. H. Mantzos, Analytical Laboratory of the Royal Hellenic Research Foundation.

[14] Work done on a leave of absence from the Research Council of Alberta, Canada.

Isolation and Characterization of a Cystine-Containing Octapeptide from Silk

The controversy as to whether silk fibroin (*Bombyx mori*) contains a small amount of cystine or whether this amino acid is entirely absent was finally resolved by the isolation of the 2:4-dinitrophenyl derivative of cysteic acid from a silk hydrolysate which had been treated with 2:4-dinitrofluorobenzene and then oxidized[1]. It is now generally accepted that the cystine content of silk is approximately 0.2%[2].

The isolation and characterization of a cystine-containing octapeptide from *Bombyx mori* silk fibroin is now reported and it is estimated that approximately $^1/_3$ of the cystine residues present in silk form part of the structure given. The experimental procedures used were as follows: Acid-degummed[3] *Bombyx mori* fibroin (1.0 g) was dissolved in 60% (w/v) lithium thiocyanate solution (5 ml) and after diluting to 50 ml with distilled water the solution was dialysed against dilute ammonia solution of pH 8. The solution of fibroin was digested with chymotrypsin (2.5 mg) for 24 h at 40 °C, the solution being maintained at pH 8 by the intermittent addition of 0.05 N ammonia. The precipitate formed was removed by centrifuging and the supernatant solution evaporated down to 20 ml and freeze dried.

The residue (0.35 g) was dissolved in 5% acetic acid (1.5 ml) and applied to a 175×2.0 cm column of Sephadex G 15 and eluted with 5% acetic acid and 5 ml fractions were collected. An aliquot of each fraction was treated with ninhydrin-hydrazine sulphate reagent (pH 5.5) and another, after acid hydrolysis, with acid ninhydrin reagent[4] to determine the distribution of the cystine residues. The cystine peptides, which were eluted before the bulk of the other peptides, gave rise to 2 main peaks, the first of which corresponded to high molecular weight material and was rejected, the second corresponding to peptides with a mean chain-length of 10 residues being retained for further examination.

The latter was subjected to diagonal electrophoresis and by this means a homogeneous cystine (oxidized)-containing peptide was isolated which contained $(CySO_3H)_2$, Asp, Pro, Ala, Val, Leu, Arg. From a partial acid hydrolysate (6 N-HCl for 45 min at 100 °C) of this material 11 peptides were isolated. The amino acid composition and N-terminal amino acid residues of these was determined, the latter by the DNS method. In addition, complete sequences of 2 of these peptides were established by the DNS-Edman method and they were shown to be Arg. Ala, and $CySO_3H$. Asp. Val. $CySO_3H$.

It was possible for these peptides to arise only from the unique structure, Arg. Ala. Leu. Pro. $CySO_3H$. Asp. Val. $CySO_3H$. Since the unoxidized peptide is basic it is most probable that the aspartic acid is present as asparagine residue and further, since it is unlikely that the half-cystine residue are in the reduced (SH) state, they must form a four-membered ring to give the structure: Arg. Ala. Leu. Pro. CyS. Asp(NH_2), Val. CyS.

Although this structure is the smallest known cystine-containing ring found in proteins, the octapeptide may be constructed without difficulty from space-filling atomic (Courtauld's) Models, the half-cystine residues being in suitable positions for linking.

Zusammenfassung. Isolierung und Strukturaufklärung eines Oktapeptides aus Seidenfibroin aus *Bombyx mori*.

C. Earland and S. P. Robins

Postgraduate School of Fibre Science, The University, Bradford 7 (England), 22 May 1969.

[1] C. Earland and D. J. Raven, Nature *192*, 1185 (1961).
[2] F. Lucas, Nature *210*, 952 (1966).
[3] A. F. Burgess, British Patent 563,745 (1944).
[4] M. K. Gaitonde, Biochem. J. *104*, 627 (1967).

[5] We wish to thank the Wool Textile Research Council for financial assistance towards this investigation, a fuller account of which will be given in a later publication.