amino acids are subsequently liberated in an undetermined sequence.

The lysozyme molecule is believed to consist of only one peptide chain having lysine as its N-terminal group.[12,13] Leucine was found to be the only amino acid formed by digestion of lysozyme with carboxypeptidase and it is concluded from this observation that leucine occurs at the carboxyl end of the lysozyme molecule.[14]

(12) F. C. Green and N. A. Schroeder, THIS JOURNAL, **73**, 1385 (1951).

(13) A. R. Thompson, *Nature*, **168**, 390 (1951).

(14) A similar result was recently reported by A. R. Thompson, *Nature*, **169**, 495 (1952).

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THE BIOLOGICAL ACTIVITY OF ENZYMATIC DIGESTS OF INSULIN[1]

Sir:

It has recently been demonstrated[2] that both alanine and asparagine are liberated simultaneously by the action of carboxypeptidase on insulin, the rate of formation of the alanine being of the order of eight times faster than that of the asparagine. In an experiment designed to correlate biological activity with loss of terminal carboxyl groups, insulin[3] solutions (80 mg. in 16 cc., *p*H 7.8) were treated with carboxypeptidase (160 μg. N); at the appropriate time intervals enzyme action was stopped by adjusting the *p*H to 5.4; the resulting precipitates were collected by centrifugation and subsequently assayed for insulin activity by the mouse convulsion test.[4] Fractions soluble at *p*H 5.4 were analyzed by the ninhydrin reaction[5] and the micro-Kjeldahl procedure. The presence of alanine and asparagine as the main products of digestion was confirmed by chromatography on paper; starch column analysis[6] showed that 1.9 moles of alanine and 0.4 mole of asparagine were present in the *p*H 5.4 soluble fraction after six hours digestion with carboxypeptidase. Results are summarized in Table I.

TABLE I

EFFECT OF CARBOXYPEPTIDASE ON THE HYPOGLYCEMIC ACTIVITY OF INSULIN

Time of digestion, hr.	Soluble N at *p*H 5.4, %	αNH_2-N alanine equiv.,[a] μM	Insulin activity, %
0	0.4	1.0	100
2	1.5	13.4	98
4	1.9	13.8	87
6	2.4	14.3	80
20	2.8	18.8	40

[a] Based on 80 mg. of insulin.

It may be concluded from the assay figures that, contrary to the results reported by Lens,[7] the two alanine C-terminal residues of the constituent phenylalanine chains of the insulin molecule are not essential for its biological activity; it would appear, however, that loss of terminal asparagine leads to a decrease of activity.

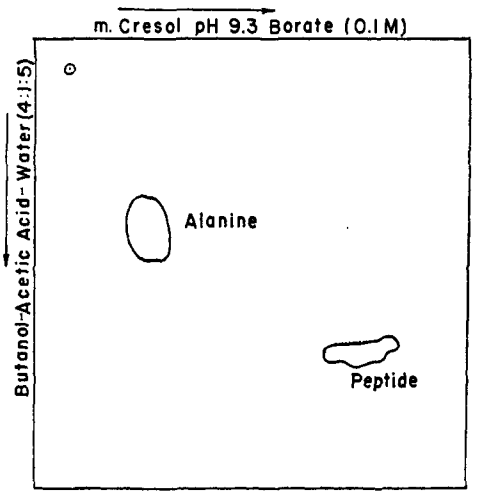

Fig. 1. Two-dimensional chromatogram of the *p*H 5.4 soluble fraction from a tryptic digest of insulin (enzyme/substrate ratio 1:5000 by weight).

Butler, *et al.*,[8,9] studied the action of trypsin on insulin and concluded that the observed diminution of biological potency was due to the splitting of one or two peptide bonds in the insulin molecule. Using lower enzyme concentrations Van Abeele and Campbell[10] demonstrated the formation of a stable trypsin-insulin complex which retained full insulin activity. The finding by Sanger and Tuppy,[11] that alanine and the heptapeptide gly.-phe.phe.tyr.thr.pro.lys are formed by the action of trypsin on Fraction B, led us to reinvestigate the action of this enzyme on insulin. Since no basic amino acids are present in the glycine chains of insulin, it might be predicted in accordance with the postulated specificity of the enzyme that trypsin would split four peptide bonds in the insulin molecule to give the same products as were obtained from Fraction B by Sanger and Tuppy.[11] In a series of preliminary experiments insulin (0.5% solutions) was incubated with crystalline trypsin (enzyme/substrate ratios varied from 1:50–1:5000) at *p*H 8.0 and 25° for 12–18 hours. Precipitates formed by adjusting digestion mixtures to *p*H 5.4 were removed by centrifugation and supernatant fractions were examined for digestion products by chromatography on paper (see Fig. 1). As predicted, the main products of digestion were found to be alanine and a heptapeptide of the same amino acid composition as the one identified by Sanger and Tuppy.[11] Under conditions where Van Abeele and Campbell[10] reported no loss of biological potency we found alanine to be the main

(1) This work has been supported in part by a grant from the Eli Lilly Laboratories.

(2) J. I. Harris, THIS JOURNAL, **74**, 2944 (1952).

(3) We are indebted to Drs. E. D. Campbell and O. K. Behrens of the Eli Lilly Laboratories for the generous supply of the crystalline insulin sample and the assay results.

(4) R. E. Thompson, *Endocrinology*, **39**, 1 (1946).

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

(6) W. H. Stein and S. Moore, *ibid.*, **176**, 337 (1948).

(7) J. Lens, *Biochimica et Biophysica Acta*, **3**, 367 (1949).

(8) J. A. V. Butler, E. C. Dodds, D. M. P. Phillips, and J. M. L. Stephen, *Biochem. J.*, **42**, 116 (1948).

(9) Butler, *et al.*, *ibid.*, **44**, 224 (1949).

(10) F. R. Van Abeele and E. D. Campbell, *Fed. Proc.*, **10**, 263 (1951).

(11) F. Sanger, and H. Tuppy, *Biochem. J.*, **49**, 481 (1951).

product of digestion, although a small amount of the heptapeptide was also present. This finding provides additional evidence for the view that the C-terminal alanine groups are not essential for the biological activity of insulin.

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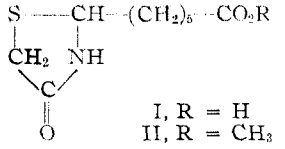
RECEIVED APRIL 30, 1952

THE STRUCTURE AND SYNTHESIS OF A NEW THIAZOLIDONE ANTIBIOTIC[1]

Sir:

We have established the structure (−)2-(5-carboxypentyl)-4-thiazolidone (I) for a new *Streptomyces* antibiotic[2] exhibiting *in vitro* antitubercular activity.



I, R = H
II, R = CH_3

Reaction of I in ether solution with excess diazomethane gave the microbiologically active methyl ester (II), crystallized from ether–hexane as colorless needles, m.p. 53–54°, $[\alpha]^{25}D$ −50.9°, (*c* 1, methanol). (*Anal.* Calcd. for $C_{10}H_{17}O_3NS$: C, 51.92; H, 7.41; N, 6.06; S, 13.86; CH_3O, 13.41. Found: C, 51.92; H, 7.43; N, 6.15; S, 13.62; CH_3O, 12.90.) Ultraviolet absorption studies of I (in methanol) and II (in 2,2,4-trimethylpentane) in the region 210–400 mμ revealed only end absorption. The infrared spectrum of I (Nujol mull) exhibits two characteristic carbonyl bands near 1640 and 1710 cm.$^{-1}$. Comparable bands at 1680 and 1720 cm.$^{-1}$ are apparent in the spectrum of II (chloroform solution). The lower frequency carbonyl band has been attributed to a carboxamide and the higher frequency carbonyl band to an aliphatic carboxyl grouping. Hydrogen bonded N–H absorption has been assigned to bands at 3140 cm.$^{-1}$ in I and 3170 cm.$^{-1}$ in II. A cleanly resolved band near 3450 cm.$^{-1}$ (free N–H) is apparent in the spectrum of II.

I rapidly loses optical activity in dilute alkali to give the racemate, obtained in two crystalline modifications: (1) from water as colorless needles, m.p. 122–123° and (2) from chloroform as colorless needles, m.p. 116–117°. The infrared spectra of (1), (2) and I in dioxane solution are indistinguishable. Mild alkaline hydrolysis of II gave racemic I.

Oxidation of I with alkaline permanganate or dilute nitric acid gave a crystalline dibasic acid, m.p. 104°, unequivocally identified as pimelic acid (III) by comparison with an authentic sample.

Mercuric chloride hydrolysis of I was accompanied by rapid loss of optical activity and liberation of an aldehyde identified as the semi-aldehyde of pimelic acid (IV), characterized as the oxime, m.p. 110–111°[3] and by oxidation to pimelic acid (III).

Desulfurization of I with Raney nickel in ethanol solution gave acetamide as well as a mixture of acidic products. However, when the desulfurization was carried out on the ester (II) in anhydrous dioxane under mild conditions, desthio-II was obtained in good yield. It crystallized from ether–petroleum ether as colorless needles, m.p. 31–32°. (*Anal.* Calcd. for $C_{10}H_{19}O_3N$: C, 59.67; H, 9.52; N, 6.96. Found: C, 59.55; H, 9.58; N, 6.87.) Alkaline hydrolysis of the desthio compound gave ω-aminoheptanoic acid, colorless plates from acetone–methanol–water, m.p. 193–194°.[4] (*Anal.* Calcd. for $C_7H_{15}O_2N$: C, 57.90; H, 10.41; N, 9.65. Found: C, 58.11; H, 10.34; N, 9.57.) Desthio-II was shown to be methyl ω-acetamidoheptanoate (V) by comparison with an authentic sample prepared by hydrogenation of methyl ω-cyanocaproate in acetic anhydride.

$$CH_3CONH—CH_2—(CH_2)_5—CO_2CH_3$$
V

Oxidation of I in acetic acid solution with hydrogen peroxide gave the microbiologically inactive sulfone (VI), crystallized from water as colorless needles, m.p. 143°, $[\alpha]^{25}D$ +43 (*c* 1, methanol). (*Anal.* Calcd. for $C_9H_{15}O_5NS$: C, 43.37; H, 6.07; N, 5.62; S, 12.85. Found: C, 43.65; H, 6.18; N, 5.85; S, 13.10.) The infrared spectrum of VI (Nujol mull) exhibits a strong band in the region 1120 to 1160 cm.$^{-1}$, characteristic of sulfones.[5]

Dissolving VI in *N* sodium hydroxide at 27° resulted in extensive hydrolysis within a few minutes as evidenced by the rapid formation of titratable sulfite.

Mild hydrochloric acid hydrolysis of VI yielded products identified as sulfur dioxide, ammonium chloride and IV. Comparable acid hydrolysis of I also gave ammonium chloride together with a mixture of products identified as IV and a sulfur-containing dicarboxylic acid believed to be VII, which was purified as the dimethyl ester (VIII).

$$RO_2C—CH_2—S—CH=CH—(CH_2)_4—CO_2R$$
VII, R = H
VIII, R = CH_3

Compound VIII is a colorless, odoriferous oil, b.p. 162–168° (0.4 mm.), $n^{25}D$ 1.4850, d^{25}_4 1.128. The light absorption properties, $\lambda^{m\mu}_{max}$ 226, ε 4600 (in 2,2,4-trimethylpentane), are consistent with the above formulation.[6] (*Anal.* Calcd. for $C_{11}H_{18}O_4S$: C, 53.64; H, 7.36; S, 13.02; CH_3O, 25.2; *M.R.*, 63.9. Found: C, 53.60; H, 7.49; S, 13.30; CH_3O, 22.3; *M.R.*, 62.6.)

The structure of I was confirmed by synthesis. Methyl ω-aldehydopimelate (IX)[7] was condensed with mercaptoacetamide in benzene in the presence of *p*-toluenesulfonic acid to give racemic II. Saponification of the synthetic ester yielded racemic I which was resolved by means of fractional crystallization of its brucine salts. Synthetic I, m.p. 139–

(1) Since the completion of this work, we have learned that this antibiotic (actithiazic acid) has been independently isolated and synthesized by a group at the Abbott Laboratories.

(2) B. A. Sobin, THIS JOURNAL, **74**, 0000 (1952).

(3) M. Kershbaum, *Ber.*, **60**, 902 (1927).

(4) Lit. m.p. 186–187°. A. Manasse, *ibid.*, **35**, 1369 (1902); O. Wallach, *Ann.*, **312**, 205 (1900).

(5) (a) K. C. Schreiber, *Anal. Chem.*, **21**, 1168 (1949). (b) D. Barnard, J. M. Fabian and H. P. Koch, *J. Chem. Soc.*, 2442 (1949).

(6) K. Bowden, E. A. Braude and E. R. H. Jones, *ibid.*, 948 (1946).

(7) IX was prepared by a modified Rosemund reduction (5% palladium on carbon catalyst) of methyl pimelyl chloride in refluxing xylene solution. IX distilled at 70° (0.5 mm.), as a colorless liquid, $n^{25}D$ 1.4310.