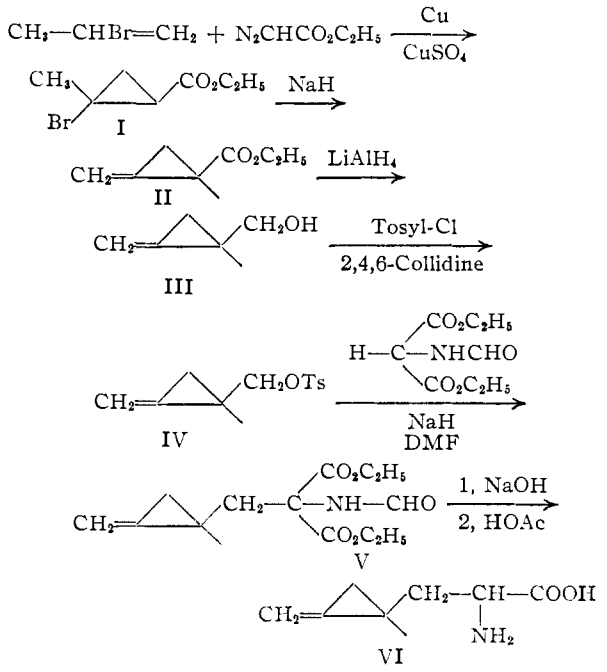
SYNTHESIS OF α-AMINO-METHYLENECYCLOPROPANEPROPIONIC ACID (HYPOGLYCIN A)

Sir:

We wish to report on the synthesis of Hypoglycin A, a natural product obtained from the unripe fruit of *Blighia sapida*.[1] This interesting material, which exhibits marked hypoglycemic properties, was first assumed to possess a polypeptide structure.[1,2] However, recent work has shown the compound to be a new amino acid, α-amino-methylenecyclopropanepropionic acid (VI).[3] We have now succeeded in synthesizing racemic VI and have shown it to be identical with the natural material.

The synthetic route which was finally successful is shown below



Treatment of 2-bromopropene with ethyl diazoacetate in the presence of a copper–bronze catalyst resulted in a 17–20% yield of ethyl 2-bromo-2-methylcyclopropanecarboxylate (I), obtained as a mixture of the *cis* and *trans* forms, b.p. 71–86° (11 mm.); $n^{25}D$ 1.4653–1.4666. *Anal.* Calcd. for $C_7H_{11}BrO_2$: C, 40.59; H, 5.36; Br, 38.59. Found: C, 40.88; H, 5.14; Br, 38.42. Although the bromo-ester I was inert to boiling 2,4,6-collidine, it reacted with sodium hydride in refluxing ether containing a few drops of ethanol[4] to form a 60% yield of ethyl methylenecyclopropanecarboxylate (II), b.p. 152–154°; $n^{25}D$ 1.4447. *Anal.* Calcd. for $C_7H_{10}O_2$: C, 66.64; H, 7.99; O, 25.37. Found: C, 66.68; H, 8.11; O, 25.45. Compound II was also obtained by treatment of allene with ethyl diazoacetate. This unsaturated ester II could not be prepared by the treatment of I with sodium ethoxide in ethanol, due to the predominant formation of the ether, ethyl 2-ethoxy-2-methylcyclopropanecarboxylate.[5] The ester II was reduced readily in 75% yield to methylenecyclopropanemethanol (III), b.p. 138–139°; $n^{25}D$ 1.4644. *Anal.* Calcd. for C_5H_8O: C, 71.41; H, 9.59; O, 19.00. Found: C, 71.40; H, 9.86; O, 18.95. The infrared spectrum of this alcohol exhibited bands at 5.73 and 11.26 μ, which are considered typical of methylenecyclopropane.[6] Conversion of compound III in 2,4,6-collidine[7] to the tosylate IV proceeded in 63% yield (crude). This tosylate IV was used to alkylate sodio diethyl formamidomalonate in N,N-dimethylformamide and the crude reaction product was hydrolyzed and decarboxylated. The crude amino acid was purified by chromatography over powdered cellulose, using *n*-butanol saturated with water as the eluent. α-Amino-methylenecyclopropanepropionic acid (VI) was thus obtained as colorless leaflets from water–acetone; darkens above 200° and does not melt to 300°. *Anal.* Calcd. for $C_7H_{11}NO_2$: C, 59.54; H, 7.85; N, 9.92. Found: C, 59.73; H, 7.78; N, 9.92. Although VI is capable of existing as two diastereoisomers, only one form could be isolated from the reaction. This material was shown to be identical with natural Hypoglycin A by paper chromatography, electrophoresis and infrared spectra.

(1) C. H. Hassall, K. Reyle and P. Feng, *Nature*, **173**, 356 (1954); C. H. Hassall and K. Reyle, *Biochem. J.*, **60**, 334 (1955).

(2) C. v. Holt and W. Leppla, *Bull. soc. chim. Belges*, **65**, 113 (1956); W. Leppla and C. v. Holt, *Arch. exp. Pathol. Pharmakol.*, **228**, 166 (1956).

(3) C. v. Holt, W. Leppla, B. Kroner and L. v. Holt, *Naturwissenschaften*, **43**, 279 (1956); C. v. Holt and W. Leppla, *Angew. Chem.*, in press.

(4) M. S. Newman and S. Merrill, THIS JOURNAL, **77**, 5549 (1955).

(5) A similar result has been reported recently by K. B. Wiberg, R. K. Barnes and J. Albin, *ibid.*, **79**, 4994 (1957), who obtained only ethyl 2-*t*-butoxycyclopropanecarboxylate from the treatment of ethyl 2-bromocyclopropanecarboxylate with potassium *t*-butoxide.

(6) J. T. Gragson, K. W. Greenlee, J. M. Derfer and C. E. Boord, *ibid.*, **75**, 3344 (1953).

(7) C. G. Bergstrom and S. Siegel, *ibid.*, **74**, 145 (1952).

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THE SIZE AND SHAPE OF BOVINE SERUM ALBUMIN AS A FUNCTION OF *p*H, DETERMINED BY SMALL-ANGLE SCATTERING OF X-RAYS

Sir:

The size and shape of bovine serum albumin molecule at different *p*H has been studied recently in several laboratories by viscosity, diffusion, sedimentation and light scattering experiments.[1] Since the interpretation of the observed data is still incomplete,[2] we have tried to collect some new information by small-angle X-ray scattering techniques.

The protein was supplied by Armour Laboratories. Its molecular weight is 66,400[1]; the sample we used contains a small amount (8% by weight) of a heavier impurity.[1]

The X-ray diffraction experiments were carried out with strictly monochromatic radiation (Cu $K\alpha_1$, as obtained with a bent quartz monochromator), *in vacuo*. The protein solution was kept in a small, vacuum tight, Plexiglas cell, provided with

(1) M. Champagne, *J. chim. phys.*, **378**, 410 (1957).

(2) Yang and Foster, THIS JOURNAL, **76**, 1588 (1954); Tanford, *et al.*, *ibid.*, **77**, 6421 (1955); Harrington, *et al.*, *Biochem. J.*, **62**, 569 (1956); Aoki and Foster, THIS JOURNAL, **79**, 3385 (1957); J. F. Foster, *J. Phys. Chem.*, **61**, 704 (1957).

thin mica windows. The optics of the camera was of the "infinite slit" type:[3] in interpreting the experimental results we performed the necessary corrections. Experimental and mathematical details will be given elsewhere.

Granted some assumptions (that are likely to hold true for serum albumin),[3] small-angle X-ray diffraction experiments can provide the values of the volume (V) and of the ratio external surface/volume (S/V), of one molecule, besides the length of the radius of gyration (R_0).[3] To eliminate interparticle scattering effects V and R_0 values have to be extrapolated to zero concentration; S/V is fairly independent of concentration.

The protein was dissolved in saline water (0.15 M NaCl). The pH was adjusted by adding some diluted HCl to the solution, and was checked before and after the X-ray diffraction experiment (the maximum shift was 0.15 pH unit). The experiments were carried out at pH values ranging from 5.6 to 3.5, where all the physico-chemical properties of the protein undergo reversible changes, and the molecular weight is constant.[1]

In a preliminary stage it was observed that the experimental parameters undergo continuous changes as a function of pH. A complete determination of R_0, V and S/V was then undertaken at pH 5.1 and 3.6. The experimental values of R_0, V and S/V, extrapolated to zero concentration, are reported in Table I. The major effect of decreasing pH from 5.1 to 3.6 seems to be to lower the volume by as much as 30%.

TABLE I

pH	R_0 (Å.)	V (Å.^{3})	$\frac{S}{V}$ (Å.$^{-1}$)
5.1	31.5	130,000	0.160
3.6	30.5	90,000	0.200

This result should be compared to previous work. In the same conditions the "hydrodynamic volume" of the molecule increases by lowering the pH: one of us[1] obtained V = 171,000 Å.^{3} at pH 5.3 and V = 262,000 Å.^{3} at pH 3.48, by viscosity, diffusion and sedimentation experiments.

These two sets of results are not necessarily incompatible, since X-ray and hydrodynamic techniques are not concerned exactly with the same "particle." The "X-ray particle" is the region of the solution where the electron density is higher than in the solvent; the "hydrodynamic particle" is likely to be bulkier since it includes the shell of solvent that the molecule drags along in its movement. So, if for instance some segments of the peptide chains unfolded at acidic pH, and took a random configuration (reversible denaturation), the "hydrodynamic volume" would become larger, while the "X-ray volume" would decrease, since the electron density of the solvent around one molecule would be hardly raised by the presence of a few random polypeptide chains.

This explanation is only tentative. We intend to compare X-ray and hydrodynamic results in more detail elsewhere.

(3) A. Guinier and G. Fournet, "Small-angle Scattering of X-Rays," John Wiley and Sons, New York, N. Y., 1955.

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THE FORMATION OF A HELICAL COMPLEX BETWEEN POLYINOSINIC ACID AND POLYCYTIDYLIC ACID

Sir:

The polyribonucleotides are a series of enzymatically produced polymers which have the same ribose-phosphate backbone as ribonucleic acid.[1] Previous work has shown that polyadenylic acid and polyuridylic acid can combine to form a two-stranded helical complex with a structure similar to that of deoxyribonucleic acid.[2] Evidence now has been obtained which demonstrates that polyinosinic acid (poly I) and polycytidylic acid (poly C) can also react together to form a helical complex.

The ultraviolet absorption spectra of poly I and poly C and a 1:1 mixture of the polymers (Fig. 1) clearly indicate that some interaction takes place. To determine the stoichiometry of the reaction, the optical density has been measured at 235 mμ for a series of mixtures containing varying proportions of poly I and poly C, while keeping the total nucleotide concentration constant. The resulting curve is composed of two straight lines which intersect sharply at a 1:1 mole ratio, thereby demonstrating the formation of a strong 1:1 complex. Ultracentrifuge studies at low concentrations using ultraviolet absorption measurements show that the sedimentation coefficient of the complex (S_{20} = 13.9) is considerably greater than that of the separate polymers (poly I, S_{20} = 7.4; poly C, S_{20} = 5.5). These results all apply to solutions containing 0.1 M NaCl and 0.01 M sodium cacodylate buffer at pH 6.7.

The reaction is controlled by the amount of electrolyte that is present. No reaction takes place at very low concentrations (*e.g.* $< 10^{-4}$ M NaCl, without buffer at pH 6.5). In the presence of 0.01 M sodium cacodylate at pH 6.7, the reaction proceeds very slowly, taking about two hours to reach completion, while in 0.1 M NaCl, equilibrium is reached within a few minutes. At high salt concentrations (*e.g.*, 1 M NaCl, 0.01 M sodium cacodylate at pH 6.7) no reaction takes place.

Fibers can be drawn from concentrated solutions of the 1:1 mixture and these yield a moderately well oriented X-ray diffraction pattern which is characteristic of a helix. This diffraction pattern is unlike that of the complex formed between polyadenylic acid and polyuridylic acid and in many respects resembles that of natural ribonucleic acid.[3] The layer line spacing is near 28 Å. There is a strong second layer line and two strong reflections on or near the meridion at 3.3 and 3.9 Å.

There are a number of possible two (or four) stranded configurations for this helical complex in

(1) M. Grunberg-Manago, P. J. Ortiz and S. Ochoa, *Science*, **122**, 907 (1955).

(2) A. Rich and D. R. Davies, THIS JOURNAL, **78**, 3548 (1956).

(3) A. Rich and J. D. Watson, *Proc. Nat. Acad. Sci.*, **40**, 759 (1954).