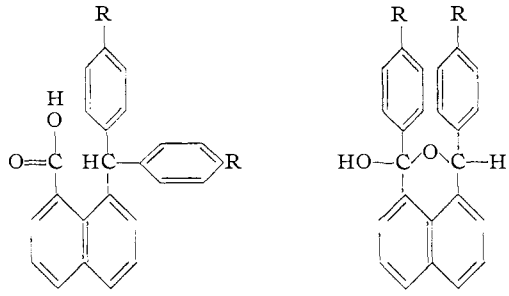


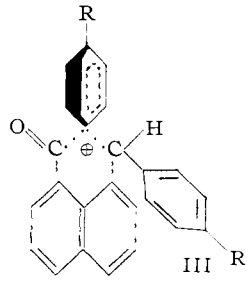
Ia, b IIa, b

a, R = H; b, R = OCH_3

137–137.5 (*Anal.* Calcd. for $C_{24}H_{18}O_2$: C, 85.18; H, 5.36. Found: C, 85.12; H, 5.21). The presence of hydroxyl and absence of carbonyl was indicated by the infrared spectra. IIa was also prepared (0.10 g.) by warming 0.50 g. of Ia with 16 ml. of sulfuric acid and 4 ml. of water for two hours on a steam-bath; 0.30 g. of Ia was recovered from this reaction.

Oxidation of IIa with chromium trioxide in acetic acid yielded (45%) 1,8-dibenzoylnaphthalene, m.p. and mixed m.p. 188.5–189.5°; reduction of IIa with lithium aluminum hydride in ether yielded (60%) a diol, m.p. 199–200 (*Anal.* Calcd. for $C_{24}H_{20}O_2$: C, 84.68; H, 5.92: Found: C, 84.62; H, 5.88.), which was identical with the substance obtained by reducing 1,8-dibenzoylnaphthalene with lithium aluminum hydride. In further agreement with the assigned structure, IIa reacted with ethanol to form a ketal, m.p. 203–204.5, no hydroxyl or carbonyl by infrared (*Anal.* Calcd. for $C_{26}H_{22}O_2$: C, 85.21; H, 6.05. Found: C, 85.43; H, 6.00).

Further insight into the course of the reaction was provided by the rearrangement of the di-*p*-methoxy analog, Ib.[3] On successive treatment with thionyl chloride, stannic chloride and water, it was converted to IIb, m.p. 142–143 (*Anal.* Calcd. for $C_{26}H_{22}O_4$: C, 78.37; H, 5.57. Found: C, 77.79; H, 5.40). The structure of IIb is based on the analysis, infrared spectra, and the fact that oxidation with chromium trioxide yielded (47%) 1,8-di-*p*-anisoylnaphthalene (m.p. and mixed m.p. 217–218) as the only isolable product. This reaction shows that the migrating aryl group undergoes detachment and attachment at the same carbon atom.



III

We believe that the rearrangement is an intramolecular one, proceeding through a transition state of the type represented by III. The alternative intermolecular transfer of aryl groups seems highly improbable on steric grounds. Furthermore, the conditions of the reaction appear to be too mild to effect an intermolecular reaction.[4] This was demonstrated by refluxing a solution of benzoyl chloride, stannic chloride and triphenylmethane in carbon disulfide for fifteen hours. No ketonic material could be detected as a product and 92% of the triphenylmethane was recovered. Under similar conditions, I was isomerized in less than ninety minutes.

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(4) Stannic chloride is a good reagent for closing rings by intramolecular acylation (W. S. Johnson, "Organic Reactions," Vol. II, p. 114), but it is not useful for the intermolecular acylation of benzene (G. Stadnikoff and A. Baryochewa, *Ber.*, **61**, 1996 (1928)).

(5) National Science Foundation Fellow, 1955–1956.

CHROMATOGRAPHY OF PROTEINS ON CELLULOSE ANION-EXCHANGERS USING WATER-CARBON DIOXIDE SYSTEMS

Sirs:

We have found that proteins can be chromatographed on cellulose anion-exchangers in a system of distilled water and carbon dioxide. This observation was unexpected since weak anion-exchangers do not normally remove carbon dioxide from water.[1] The proteins so fractionated were free of small ions. Recent publications have indicated that proteins can be fractionated on cellulose ion-exchangers.[2] However, these workers used a method involving buffer solutions of various ionic strengths making it necessary, in certain circumstances, to introduce additional steps for the removal of salts.

All the proteins tested, except the very basic ones, were sorbed from distilled water by the free-base form of the anion-exchanger. The proteins were not desorbed by simple washing with distilled water. In fact, usually the last traces of salt and some of the other non-protein materials were removed by the washing. The introduction of an atmosphere of carbon dioxide over the distilled water in contact with the protein-containing exchanger caused the release of part or all of the protein. Eluted proteins yielded solutions which, after the gas was permitted to escape, approached the isoionic *p*H's of the proteins. The exchanger can be regenerated with distilled-water washing, which removes carbon dioxide. In cases of accumulation of mineral acids, nucleic acids, and some proteins which are not removed by carbon dioxide, strong bases must be used to regenerate the exchanger.

Several proteins were examined batch-wise to help predict what to expect on a column. A protein, dissolved in distilled water or dialyzed against distilled water, was equilibrated for fifteen minutes with the 2-(diethylamino)-ethyl ether of cellulose (0.25–0.50 m.e./g.).[3] The sorbate was separated by centrifugation, washed, and eluted. Typical results are shown in Table I.

(1) R. Kunin and R. J. Myers, "Ion Exchange Resins," J. Wiley & Sons, New York, N. Y., 1950, p. 41.

(2) E. A. Peterson and H. A. Sober, THIS JOURNAL, **78**, 751, (1956); H. A. Sober, F. J. Gutter, M. M. Wyckoff and E. A. Peterson, *ibid.*, **78**, 756 (1956).

(3) C. L. Hoffpauir and J. D. Guthrie, *Textile Research J.*, **20**, 617 (1950).

TABLE I

ELUTION OF PROTEINS BY CARBON DIOXIDE

100 mg. of exchanger, 10 mg. of protein in 10 ml. of H_2O; washed with 10 ml. of H_2O; and eluted with 10 ml. of H_2O in equilibrium with 1 atm. of CO_2.

	% Sorbed by exchanger	% Desorbed by CO_2[a]
Hemoglobin[b]	60	35
Serum Albumin[c]	25	25
Egg Albumin[d]	60	10
Catalase[e]	30	45
Cathepsin[f]	25	40
Pepsin[g]	30	<5
Casein	75	10
Lysozyme[h]	0	...
Gamma Globulin[i]	60	85
Nucleic Acids[j]	40	0

[a] Calculated from the amount sorbed on the exchanger. [b] Bovine, Armour. [c] Bovine plasma Fraction V, Armour. [d] Crystallized, Armour. [e] Bovine liver, Armour. [f] Bovine kidney, J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **130**, 19 (1939). [g] Porcine crystallized, Armour. [h] Crystallized, Armour. [i] Porcine plasma Fraction II, Armour, [j] Yeast, Schwarz.

Fractionation of kidney cathepsin (Fig. 1) and liver catalase on the exchanger in a column resulted in a two- to ten-fold purification on distilled water–carbon dioxide elution, with a recovery of 90–100% of the total enzymatic activity in each case. The extent of purification of these proteins depended on the purity of the starting material. Porcine plasma Fraction II was also purified by this method.

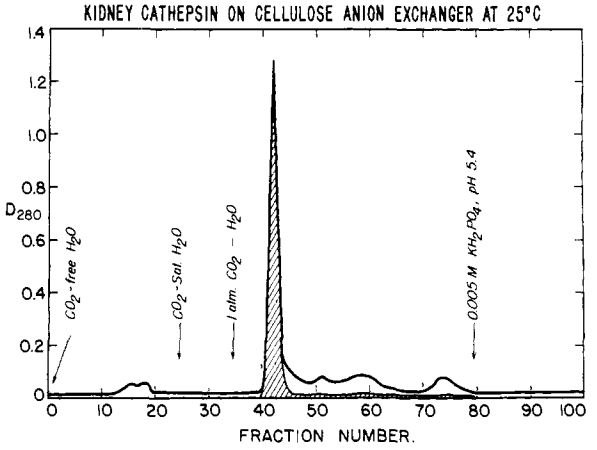

Fig. 1.—Eight grams exchanger (free-base form) in 50 × 1.1 cm. column; load in 5.5 ml.: 250 mg. of $(NH_4)_2SO_4$-fractionated cathepsin dialyzed against CO_2-free water. Fraction volume, 4 ml.; flow rate, 0.5 ml./min. Shaded area represents proportion of catheptic activity in protein fractions. Specific activity of the preparation by Anson's hemoglobin assay, 0.08 ΔD_{280}/mg. protein/10 min. Maximum specific activity of 0.5 was in fraction 42. More than half of the protein remained on the column after all the activity had been eluted.

After carbon dioxide elution, proteins which remain on the column may be subjected to further fractionation by other methods.[2]

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RECEIVED MARCH 16, 1956

THE OPTICAL ROTATORY POWER OF POLYAMINO ACIDS AND PROTEINS

Sir:

In a recent article,[1] we derived an expression for the optical rotatory power of an infinitely long helical molecule. Our result for polyglycine in the conformation of a right-handed alpha helix may be generalized for an arbitrary polyamino acid $(-NH-CRH-CO)_n$ in the form

$$[M]_D = [M_0]_D + 49.4(n^2 + 2)/3$$
$$[\alpha]_D = [\alpha_0]_D + 4940(n^2 + 2)/3M \qquad (1)$$

where $[M_0]_D$ and $[\alpha_0]_D$ are the intrinsic residue and the specific rotations of the monomer for the sodium D line, M the residue molecular weight, and n the refractive index of the solvent. We have neglected the effect of vicinal interactions of the residue side chains on the rotation. Under this approximation, the destruction of the right-handed helical conformation should result in a decrease in specific rotation given by

$$-\Delta[\alpha]_D = +4940(n^2 + 2)/3M \qquad (2)$$

If we assume that the L-amino acid residues of a representative natural protein of average residue molecular weight 120 form a right-handed alpha helix, reversible or irreversible denaturation in water, involving destruction of the helical conformation, should lead to a decrease in specific rotation of 52°. The specific rotations of many proteins decrease by approximately this amount upon denaturation.[2]

From equation (2), we predict that the destruction of a right-handed helical structure in poly-γ-benzyl-L-glutamate (PBG) in 20:80 ethylene dichloride–dichloroacetic acid ($n \sim 1.45$) would result in a decrease in specific rotation of 31°. This value agrees well with the experimental decrease of 28° observed by Doty and Yang.[3] Equation (2) predicts a decrease of 49° for the destruction of the helical configuration of poly-L-glutamic acid (PGA) in water. This change would be expected in passing from solutions of low pH to those of high pH, in which the negative charges of the carboxyl groups would extend the chain. This value is in semi-quantitative agreement with the change of 75° observed by Blout and Idelson.[4] A part of this change is unquestionably due to the direct effect of pH on the intrinsic residue rotations.

Since the observed contributions of the helical conformation to the specific rotations of both PBG and PGA are positive, we conclude that both polypeptides are right-handed helices. Our conclusion is in agreement with Huggins'[5] calculations, which indicate that the right-handed helix is the more stable conformation for polymers of L-amino acids.

On the basis of his equivalent theory of optical rotation, Moffitt[6] has predicted for helical molecules a special type of anomalous rotatory dispersion of the form

$$\Delta[\alpha] = -B\nu^2/(\nu_0^2 - \nu^2)^2 \qquad (3)$$

(1) D. D. Fitts and J. G. Kirkwood, *Proc. Natl. Acad. Sci. (Wash.)*, **42**, 33 (1956).
(2) C. Cohen, *Nature*, **175**, 129 (1955).
(3) P. Doty and J. T. Yang, THIS JOURNAL, **78**, 497 (1956).
(4) E. R. Blout and M. Idelson, *ibid.*, **78**, 498 (1956).
(5) M. L. Huggins, *ibid.*, **74**, 3963 (1952).
(6) W. Moffitt, private communication. The theory is contained in a forthcoming article in the *Journal of Chemical Physics*.