mol. wt. calcd., 108). The freezing curve had a good plateau at −122°, but some solid began to separate at −100°. It was concluded that fractions 4 and 5 were inseparable constant boiling mixtures, not necessarily azeotropic, presumably consisting largely of $SF_4$ (mol. wt., 108), but probably containing considerable amounts of $CSF_2$ (mol. wt., 82), together with some material of high molecular weight, and quite possibly $SiF_4$ (mol. wt., 104) as well.

**Fractions 6 and 7.**—The first of these portions (18 cc.) was heterogeneous with a top layer (1 cc.) which was shown to be $CS_2$. Most of the lower layer was combined with the second portion (3 cc.), and fractionated yielding a central cut (3.5 cc.) believed to be pure $CF_3SF_3$, b.p. −7°, f.p. −110°, mol. wt., 160 (calcd., 158). The analysis, although roughly approximate, was unacceptable (S, 22.6%; calcd., 20.3%), but it should be stated that the technical difficulties involved in the microanalysis of compounds of this nature are very great.

**Fraction 8.**—This portion was partially crystallized at −80° and filtered by means of a filter stick yielding a residue (8 cc.) and a filtrate (12 cc.). The residue was redistilled, discarding a small forerun, and the remainder (about 8 cc.) had b.p. −10 to −9° at 168 mm., f.p. −52°, mol. wt., 242-263. This was refractionated and yielded a portion believed to be pure $SF_5CF_2SF_5$ (3 cc.), b.p. −20° at 90 mm., 26° (Siwoloboff), f.p. −51°, mol. wt., 265 (calcd., 266). The analysis, although closely approximate, was not entirely satisfactory (S, 23.4%, calcd., 24.1%). The filtrate on rectification yielded a portion (3 cc.), b.p. 17° at 550 mm., f.p. −72°, mol. wt., 236; and this on further fractionation gave a central cut of pure $S_2F_{10}$ (1 cc.), b.p. −19° at 100 mm., 25° (Siwoloboff), (known[5] b.p. 29°, f.p. −92°). This very good sample liberated iodine from KI paper as expected, but froze twenty degrees higher than previously reported.

*Anal.* Calcd. for $S_2F_{10}$: S, 25.2; F, 74.8; mol. wt., 254. Found: S, 25.3; F, 75.1; mol. wt., 251.

**Fraction 9.**—This final portion was redistilled and yielded pure $SF_5CF_2SF_5$ (4 cc.), b.p. 23° at 170 mm., f.p. −70°. This portion was refractionated yielding a cut, b.p. 24° at 170 mm., mol. wt., 307, while the remainder was again redistilled in a small precision column yielding a central cut, b.p. 62° at 1 atm., from which the analytical sample was taken.

*Anal.* Calcd. for $CS_2F_{12}$: S, 21.1; mol. wt., 304. Found: S, 20.7; mol. wt., 307.

The analyses were by the Huffman Micro-analytical Laboratories, Wheatridge, Colorado.

DURHAM, N. C.

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[CONTRIBUTION FROM THE SOUTHERN REGIONAL RESEARCH LABORATORY[1]]

# Elevation of the Intrinsic Viscosity of Peanut Protein by Treatment with Terephthalyl Dichloride[2]

BY GODFREY E. MANN

RECEIVED FEBRUARY 16, 1953

It has been determined that the treatment of aqueous alkaline dispersions of peanut protein with the bifunctional acid chloride, terephthalyl dichloride ($p$-$C_6H_4(COCl)_2$), results in modified proteins of elevated intrinsic viscosity as measured at 25.0°, using 10 $M$ urea as the solvent. The treatment also results in depression of the solubility of the protein in this solvent. The viscosity elevating effect appears to vary inversely with the temperature of the treatment; a greater rise occurring in the vicinity of 0° than at room temperature. At the low temperature the intrinsic viscosity of a protein preparation could be elevated to a maximum of about 6 times that of the original protein (from 0.24 to 1.5 deciliters/g.) while the solubility was maintained at 80% of the original. Under the same conditions, treatment with equivalent amounts of monofunctional benzoyl chloride did not lead to significant elevations of intrinsic viscosity.

Many organic and inorganic reagents have been used to harden or insolubilize proteins for industrial applications. In general, the mechanism of action for such reagents has been visualized as the formation of cross-links between neighboring protein molecules with the development of a tridimensionally stabilized structure, although denaturation of the protein might play an important role in some cases.[3,4]

Usually, the existence of cross-links in the treated protein has been inferred from analytical data and from the alteration in mechanical properties undergone by the protein upon treatment. Fraenkel-Conrat and Mecham,[4] however, demonstrated that treating a variety of proteins with formaldehyde resulted in elevations of the apparent molecular weights of the proteins as measured by osmotic pressure. Determination of sedimentation constants and light-scattering enabled Hughes[5] to demonstrate that a protein from human serum albumin forms a dimer when treated with mercuric chloride. There appear to be no viscosity data recorded on such protein derivatives of relatively unequivocal cross-linked structures.

The intrinsic viscosities of synthetic polymers have been related to such of their physico-chemical properties as molecular weights, molecular shapes and fiber-forming abilities.[6,7] Relationships between intrinsic viscosities and molecular weights and molecular shapes which have been established for proteins[8] make it appear that elevation of the intrinsic viscosity of a protein may markedly alter other of its properties, possibly resulting in enhanced fiber-forming capacity. This report describes the attainment of an increase in the intrinsic viscosity of peanut protein by treatment with the reagent terephthalyl dichloride (1,4-benzenedicarbonyl chloride), $p$-$C_6H_4(COCl)_2$. The bifunctional nature of the dichloride and the recognized reactivity of proteins toward aryl acyl chlorides[9] suggested that this reagent should be capable of

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(2) Presented at the National Meeting of the American Chemical Society, Atlantic City, N. J., September 14-19, 1952.

(3) J. Bjorksten, *Advances in Protein Chem.*, **6**, 343 (1951).

(4) H. Fraenkel-Conrat and D. K. Mecham, *J. Biol. Chem.*, **177**, 477 (1949).

(5) W. L. Hughes, Jr., *Cold Spring Harbor Symposia Quant. Biol.*, **14**, 79 (1950).

(6) B. L. Johnson and R. D. Wolfangel, *Ind. Eng. Chem.*, **41**, 1580 (1949).

(7) H. Mark, *ibid.*, **34**, 1343 (1942).

(8) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Pub. Corp., New York, N. Y., 1943, p. 512.

(9) R. M. Herriott, *Advances in Protein Chem.*, **3**, 169 (1947).

linking the protein molecules in a manner similar to that of formaldehyde and of mercuric chloride.

For comparison, reactions were carried out using equivalent amounts of benzoyl chloride, $C_6H_5COCl$, in place of the terephthalyl dichloride. The monofunctional acyl chloride failed to effect marked increases of intrinsic viscosity, indicating that the aryl acid chloride group is not inherently capable of markedly elevating the viscosity of peanut protein under the conditions employed.

A fresh aqueous solution of 10 *M* urea was selected as a solvent for the viscosity determinations to minimize aggregation and to ensure "unfolding" of the peptide chains.[8] In general, the intrinsic viscosity is as much a function of molecular asymmetry as it is of molecular weight[10]; hence the values obtained should be interpreted accordingly.

## Experimental

**Materials.**—Terephthalic acid (1,4-benzenedicarboxylic acid) was prepared by the oxidation of *p*-cymene (4-isopropyl-1-methylbenzene) with chromic acid according to the method of Bogert and Harris[11]; neutral equivalent 82.5, theoretical value 83.0. Terephthalyl dichloride (m.p. 83.5–84.0°) was prepared from the acid by treatment with phosphorus pentachloride according to the procedure of Cohen and de Pennington.[12] The benzoyl chloride was a commercial product which had been redistilled once and preserved in a well-stoppered container; neutral equivalent 73.4, theoretical value 70.3.

Peanut protein A was prepared on a large scale in a manner similar to that previously described.[13] A 200-pound portion of hexane-extracted peanut meal was slurried at room temperature with about 3000 pounds of water, and the *p*H was elevated to 7.45 by the slow addition of sodium hydroxide. The extract was clarified by use of an automatic valve-type centrifuge operated at 5000 times gravity, and the protein in the extract precipitated by adding sulfur dioxide until the *p*H was lowered to 4.4. The curd was separated from the mother liquor by use of the valve-type centrifuge, and drying was carried out by spreading the curd on trays in a 50° circulating oven for 3 days 18 hours. A yield of 43 pounds of protein was obtained.

Protein B was isolated in essentially the same manner as was preparation A; a 281-pound portion of the hexane-extracted peanut meal was extracted with about 15 times its weight of dilute sodium hydroxide, *p*H 7.50. After clarification by centrifugation, the curd was precipitated with sulfur dioxide (*p*H 4.5), centrifuged down, and dried as previously described. A yield of 83 pounds was obtained.

Peanut protein C was prepared by use of ion-exchange fabrics.[14] A 2.5-kg. portion of skin-free, hexane-extracted peanut meal was mixed with 14 l. of water at 4°. Pieces of freshly regenerated aminized cotton fabric, about 0.5 sq. yard in area, were added slowly (two each 15 min.) with occasional stirring until the apparent *p*H rose from 6.85 to 7.82. A total of 17 pieces of fabric were required. The extract was clarified by filtration and centrifugation for 10 minutes at 700 times gravity, and the apparent *p*H was lowered to 4.26 by the slow addition of 18 pieces of freshly regenerated phosphorylated cotton fabric, each about 0.5 sq. yard in area, one piece being added every 10 min. The cold acidified mixture was allowed to stand overnight, and 5 pieces of aminized fabric and 3 pieces of phosphorylated fabric were added alternately until the apparent *p*H became stable at 4.22–4.24. The precipitated protein was centrifuged down in an 8-inch basket head, washed with 5 times its volume of 4° water, slurried with an equal volume of water, and vacuum-dried from the frozen state. A yield of 460 g. was obtained.

Peanut protein C was prepared by extracting 1.00 kg. of skin- and oil-free peanut meal with a total of 15 l. of water (in 3 successive portions) at about 25°. The extract, *p*H 6.78, was clarified by filtration and centrifugation, and then dialyzed for 7 days against 74-l. portions of distilled water at about 6°; cellophane sausage casing was used and the outer liquid was changed once a day. The protein in the dialyzed extract was isolated by vacuum drying from the frozen state; the yield was 287 g.

Analytical data on the various protein preparations are given in Table I. Electrophoretic examination (*p*H 9.4, glycine buffer, ionic strength 0.1) indicated that the soluble portions of the four substances were qualitatively and quantitatively similar; furthermore, the patterns were virtually identical with those previously determined[15] for buffer extracts of peanut meal.

TABLE I

ANALYTICAL DATA ON PEANUT PROTEIN PREPARATIONS

| Protein | Moisture, % | Nitrogen content,[a] % | Ash content,[a] % | Phosphorus content,[a] % | Solubility,[b] % |
|---|---|---|---|---|---|
| A, large-scale | 6.76 | 16.00 | 0.80 | 0.42 | 83.0 |
| B, large-scale | 6.54 | 16.22 | 1.10 | .40 | 87.6 |
| C, ion-exchange fabric | 8.37 | 17.12 | 0.27 | .46 | 100 |
| D, water-extracted | 9.65 | 16.92 | 1.99 | .34 | 94.9 |

[a] Moisture-free basis. [b] Solubility the percentage of protein nitrogen which dissolved in *p*H 9.4 glycine buffer, ionic strength 0.1, on agitating 24 hours at about 6°; 200 ml. of buffer per 2.00 g. of protein.

**Preparation of the Modified Proteins.**—A 20.0-g. portion of the protein was stirred vigorously with 100 ml. of water, and the *p*H value at room temperature (25–26°) was raised to 9.50 by dropwise addition of 1 *N* sodium hydroxide. Two drops of 2-ethyl-1-hexanol were added to minimize foaming, and the turbid alkaline mixture was made to a total volume of 200 ml. by the addition of water. About one hour was required for the addition of the alkali and the peptization of the protein. The reaction vessel (a 600-ml. beaker), containing the 10% protein solution, was immersed in a bath of the specified temperature, and the solution stirred with a "slotted disc"-type of propeller, 1.75 inch in diameter, operated at 630 r.p.m. After thermal equilibrium had been reached and maintained for 12 to 15 min., the "apparent" *p*H—that is, the glass electrode reading without temperature correction—was noted, and the given weight of reagent in 60 ml. of ether was added in six 10-ml. portions, at 2-min. intervals. A transient temperature rise of the mixture was observed when the ether solution of the reagent was first added; in the treatments at 0° bath temperature the rise amounted to about 4° during the first 8 min., dropping to the equilibrium value thereafter. An additional 20 ml. of ether (four 5-ml. portions) was used to ensure quantitative transfer of the reagent. Acid liberation started at once, and the "apparent" *p*H value was maintained by the careful addition of 1 *N* sodium hydroxide as the reaction proceeded. After 4 to 5 hours, acid liberation ceased, and and the "apparent" *p*H was raised to about 10.8 with 1 *N* sodium hydroxide. The mixture was transferred to a cellulose sausage casing and dialyzed for 6 days against running distilled water at about 6°; after this operation the *p*H values at 25–26° of all the reaction mixtures fell within the range 6.8–8.2. These dialyzed solutions were then vacuum-dried from the frozen state, and the fluffy solids obtained were allowed to equilibrate with atmospheric moisture for at least 24 hours, ground to coarse powders with a small Wiley-type mill (20-mesh screen), and stored at room temperature in sealed containers.

**Determination of Intrinsic Viscosities.**—Solutions of the derivatives for viscosity determinations were prepared by mixing weighed amounts of the substances with quantities of 10 *M* urea sufficient to yield final volumes of 200 ml. The 10 *M* urea was prepared each day it was used by dissolving 300.3 g. of reagent-grade urea crystals in about 200

(10) H. Neurath, J. P. Greenstein, F. W. Putnam and J. O. Erickson, *Chem. Revs.*, **34**, 157 (1944).

(11) M. T. Bogert and C. P. Harris, THIS JOURNAL, **41**, 1676 (1919).

(12) J. B. Cohen and H. S. de Pennington, *J. Chem. Soc.*, **113**, 57 (1918).

(13) J. C. Arthur, Jr., A. J. Crovetto, L. J. Molaison, W. F. Guilbeau and A. M. Altschul, *J. Am. Oil Chemists' Soc.*, **25**, 389 (1948).

(14) C. L. Hoffpauir and J. D. Guthrie, *J. Biol. Chem.*, **178**, 207 (1949).

(15) M. L. Karon, M. E. Adams and A. M. Altschul, *J. Phys. Colloid Chem.*, **54**, 56 (1950).

ml. of water with vigorous agitation and careful warming; adjustment to a final volume of 500 ml. was made at 25.0°. The solutions were allowed to stand for about 24 hours at room temperature (25–26°) with occasional agitation, and then clarified by centrifuging successively for 30 min. at 1160 times gravity and for 15 min. at 9160 times gravity, followed by pressure filtration through a sintered glass disc of medium porosity. Two or three such solutions of nominal concentrations in the range of 0.250 to 0.750 g./100 ml. were prepared for each derivative, and the intrinsic viscosity (expressed as deciliters per g., dl./g.) at 25.0° was estimated by extrapolation of the plot of reduced viscosity against corrected concentration to zero concentration as described elsewhere.[16] The nominal concentrations were corrected for the moisture contents of the derivatives and for the solubilities in 10 *M* urea, the latter correction being obtained by Kjeldahl nitrogen determinations on aliquots of the urea solutions after dialyzing for 3 days against running tap water to remove the urea. The *p*H values at 25–26° of the clarified 24 hour-old urea solutions of all the derivatives fell in the range 7.3–8.0, but the solutions of protein A had *p*H values in the range 6.5–7.0. It was noted that the urea-insoluble portions of the derivatives of high viscosity had loose, jelly-like structures.

**Determination of Terephthalic Acid.**—These estimations were carried out by refluxing 2.000-g. samples with 150 ml. of 6 *N* hydrochloric acid for 24 hours in the presence of 5 g. of mossy tin. After cooling overnight the mixtures were filtered and the residues washed successively with four 10-ml. portions of 6 *N* hydrochloric acid and with six portions of water. Each washed residue was extracted with nine 10-ml. portions of 1 *N* sodium hydroxide and with several portions of water, and the pooled extracts were filtered to remove paper fibers. The terephthalic acid in each extract was precipitated by acidification with 20 ml. of concentrated hydrochloric acid, the precipitates were centrifuged down, and the supernatants carefully drawn off with an aspirator and discarded. Using the centrifuge–aspirator technique each precipitate was washed twice with 30-ml. portions of 6 *N* hydrochloric acid and four times with 30-ml. portions of water. The moist precipitates were dissolved in accurately known volumes of 0.1 *N* sodium hydroxide (about 1.5 hours was required for complete solution), and the amount of alkali equivalent to the acid in the precipitates was determined by back-titration with 0.1 *N* hydrochloric acid. These data and the known equivalent weight of terephthalic acid were used to calculate the apparent acid contents of the protein derivatives. The protein obtained from the "zero reagent" control reaction was found to contain an apparent terephthalic acid content of 3.5 mg./g.; this correction has been applied to the values reported in Tables III and IV. To check the validity of this procedure several recovery experiments were carried out, known amounts of terephthalic acid being added to 2.00-g. samples of peanut protein A and the analyses performed as described. The results of these experiments are given in Table II. Fair recoveries (104–105%) were achieved providing the total amount of acid titrated was about 50 mg. or greater, a criterion fulfilled by all of the derivatives reported in Tables III and IV.

TABLE II

TEREPHTHALIC ACID RECOVERY IN PRESENCE OF 2.00 G. OF PEANUT PROTEIN A

| Acid added, mg. | Acid found (uncor.), mg. | Acid found (cor.),[a] mg. | Recovery, % |
|---|---|---|---|
| 0 | 7 | 0 | ... |
| 27 | 27 | 20 | 74 |
| 53 | 62 | 55 | 104 |
| 106 | 118 | 111 | 105 |

[a] Seven mg. of apparent acid in 2.00 g. of protein subtracted from uncorrected figures.

The hydrolysis technique was used to determine the efficiency with which the 6-day dialysis procedure separated terephthalate ions from peanut protein, since such ions undoubtedly occur owing to partial hydrolysis of terephthalyl dichloride in the reactions described above. Twenty grams of protein A was peptized at *p*H 9.5, 2.001 g. of terephthalic acid (as a solution of the disodium salt) was added, and the mixture brought to a final volume of 200 ml. The *p*H was then elevated to 10.8, and the 6-day dialysis performed as usual. The dialyzed solution was hydrolyzed by refluxing for 24 hours with 6 *N* hydrochloric acid in the presence of tin, and the residue was washed and examined for terephthalic acid in a manner similar to that employed for the analysis of the derivatives. A considerable quantity of oily brown material was found; this, unlike terephthalic acid, dissolved in ether, leaving a small amount of brown solid which gave a titration equivalent to 6 mg. of terephthalic acid. This experiment demonstrates the efficiency of the dialysis procedure, and shows that the derivatives should be free of disodium terephthalate and terephthalic acid.

(16) J. C. Arthur, Jr., and B. H. Saik, *J. Colloid Sci.*, **5**, 326 (1950).

TABLE III

CHARACTERIZATION OF DERIVATIVES OF PEANUT PROTEIN A

| Reaction conditions: Reagent used | Amount of reagent,[a] g. | Bath temp., °C. | Data on derivatives[b]: Nitrogen content, % | *p*-$C_6H_4(COOH)_2$ content, mg./g. |
|---|---|---|---|---|
| None | None | 0 | 16.02 | 0 |
| *p*-$C_6H_4(COCl)_2$ | 0.800 | 0 | 15.93 | 28 |
| *p*-$C_6H_4(COCl)_2$ | 1.200 | 0 | 15.53 | 39 |
| *p*-$C_6H_4(COCl)_2$ | 1.600 | 26 | 15.71 | 47 |
| *p*-$C_6H_4(COCl)_2$ | 1.600 | 6 | 15.68 | 51 |
| *p*-$C_6H_4(COCl)_2$ | 1.600 | 0 | 15.38 | 52 |
| *p*-$C_6H_4(COCl)_2$ | 2.000 | 0 | 15.24 | 57 |
| *p*-$C_6H_4(COCl)_2$ | 2.400 | 0 | 15.23 | 63 |
| $C_6H_5COCl$ | 2.216 | 0 | 15.32 | .. |
| $C_6H_5COCl$ | 3.326 | 0 | 14.94 | .. |

[a] Quantity of reagent used per 200 ml. of 10% protein solution of *p*H 9.5 at 25–26°. [b] Moisture-free basis.

TABLE IV

CHARACTERIZATION OF DERIVATIVES[a] OF VARIOUS PEANUT PROTEIN PREPARATIONS

| Protein preparation used | Data on derivatives[b]: Intrinsic[c] viscosity, dl./g. | Solubility,[c] % | Nitrogen content, % | *p*-$C_6H_4(CO\text{-}OH)_2$ content, mg./g. |
|---|---|---|---|---|
| A, large-scale | 1.64 | 87 | 15.38 | 52 |
| B, large-scale | 1.94 | 69 | 15.32 | 51 |
| C, ion-exchange fabric | 2.45 | 84 | 15.79 | 49 |
| D, water-extracted | 0.56 | 92 | 15.89 | 35 |

[a] 1.600 g. of terephthalyl dichloride per 200 ml. of 10% protein solution at 0° bath temperature. *p*H 9.5 at 25–26°. [b] Moisture-free basis. [c] In freshly prepared 10 *M* urea.

## Results and Discussion

Shown in Fig. 1 is the effect of varying the temperature of the treating bath on the intrinsic viscosities and solubilities of the derivatives which resulted when peanut protein A was treated with 1.600 g. of terephthalyl dichloride. It is evident that attainment of a derivative of high intrinsic viscosity is favored by a low bath temperature.

The results obtained when protein A was treated with varying amounts of terephthalyl dichloride at a bath temperature of 0° are given in Fig. 2. The reagent appears to exert its maximum effect of viscosity elevation when 1.200 to 1.600 g. is employed per 200 ml. of 10% protein solution; further increases in reagent result in considerable depression of the solubility and intrinsic viscosity. The "zero reagent" derivative constituted a control showing that treatment with alkali and ether under

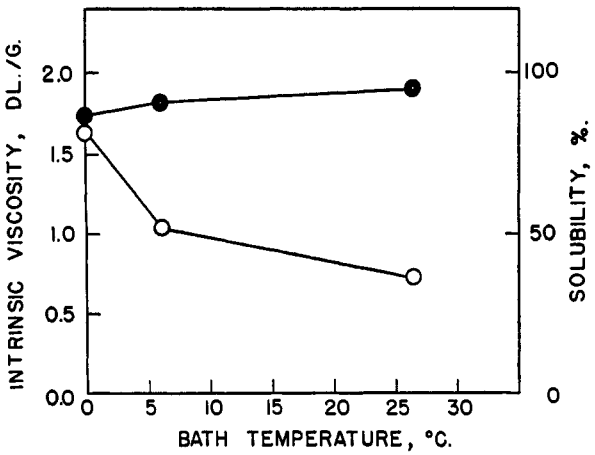


Fig. 1.—Variation of intrinsic viscosity, O, and solubility ● (freshly prepared 10 *M* urea solvent, 25°) of modified proteins with temperature of treating bath; 1.600 g. of terephthalyl dichloride used to treat 200 ml. of 10% peanut protein A, *p*H 9.5 at 25–26°; intrinsic viscosity of untreated protein A, 0.24 dl./g.; solubility 97%.

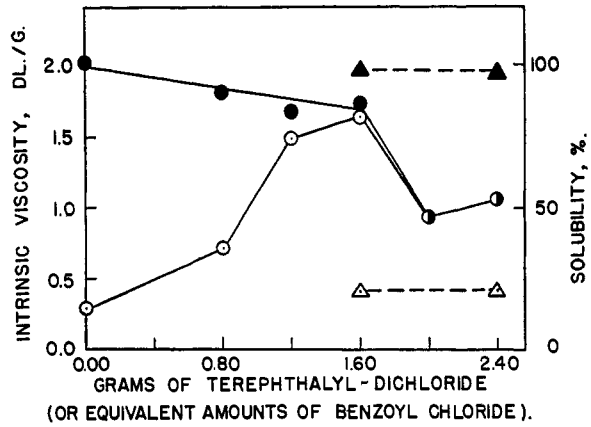


Fig. 2.—Variation of intrinsic viscosity, O, and solubility ● (freshly prepared 10 *M* urea solvent, 25.0°) of modified proteins with amount of terephthalyl dichloride used to treat 200 ml. of 10% peanut protein A at 0° bath temperature; intrinsic viscosity, Δ, and solubility, ▲, of benzoyl chloride derivatives; intrinsic viscosity of untreated protein A, 0.24 dl./g., solubility 97%.

the given conditions did not result in a significant rise in intrinsic viscosity. Included in Fig. 2 are the data on two products resulting from treating the protein with 2.216 and 3.326 g. of benzoyl chloride (equivalent to 1.600 and 2.400 g. of terephthalyl dichloride, respectively). The monofunctional acyl chloride did not yield derivatives of viscosities markedly higher than that of the "zero reagent" control, nor was there any depression of the solubility.

Table III gives additional data on the derivatives of protein A. For the reactions at 0° with terephthalyl dichloride, the amount of terephthalic acid combined is almost directly proportional to the amount of reagent employed, providing that the latter is 1.600 g. or less. When more reagent is used the combined acid is less than the amount calculated on the basis of a direct proportionality. The data on the effect of lowering the bath temperature from 26 to 0° tend to indicate that more reagent reacts with the protein in the 6 to 0° range than at 26°, although the maximum difference of 5 mg./g. in terephthalic acid contents is considered barely significant for the analytical procedure employed. The products prepared at bath temperatures of 0 and 6° displayed a considerable difference in intrinsic viscosities, about 1.6 dl./g. and 1.0 dl/g., respectively, although their terephthalic acid contents were identical. This result indicates that some factor other than combination of the protein with terephthalyl dichloride appears to play a role in the viscosity elevation. This factor seems to be associated with low reaction temperatures, for it is to be noted that the use of 0.800 g. of reagent at 0°, yielding a derivative with a terephthalic acid content of 28 mg./g., was just about as effective in elevating the intrinsic viscosity as was the use of 1.600 g. of reagent at 26°, even though the latter derivative had the much greater acid content of 47 mg./g.

The data in Table IV show that products of high intrinsic viscosity can be obtained from peanut proteins other than protein A by treatment with 1.600 g. of terephthalyl dichloride in a bath at 0° under the described conditions. The second large-scale preparation, protein B, yielded a derivative similar to the derivative from protein A, while protein C, made using the ion-exchange fabric technique, yielded a product of very high intrinsic viscosity, but with a terephthalic acid content practically identical with the acid contents of the derivatives from A and B. Protein D, a water-extracted preparation of relatively high ash content, gave a product which was low in both intrinsic viscosity and acid content.

The interactions in a system composed of an ether solution of terephthalyl dichloride and an alkaline aqueous dispersion of peanut protein appear to be quite complex. The failure of benzoyl chloride to yield products of elevated viscosity seems to preclude the possibility that the viscosity-elevating effect is owing to the well-known denaturation observed when a protein is subjected to the action of various reagents such as acids, alcohol, detergents, etc. The bifunctional nature of the terephthalyl dichloride, the reduced solubilities of the derivatives, and the jelly-like nature[17] of the urea-insoluble portions of the products suggest that the increase of intrinsic viscosity is due to a cross-linking phenomenon, although the possibility exists that charged centers of the type protein—$COC_6H_4COO^-$ have been introduced and that the increase in molecular asymmetry is owing to mutual repulsion of these groups.

**Acknowledgment.**—The author is indebted to J. Pominski for protein preparations A and B; to W. B. Carney for preparation C; and to M. L. Karon for the electrophoretic analyses of the various proteins.

NEW ORLEANS, LA.

(17) J. D. Ferry, *Advances in Protein Chem.*, **4**, 1 (1948).