TABLE II

ANALYSES

	Calcd. Cl, %	Found Cl, %	
o-(2,3-Dimethylbenzoyl)-tetrachlorobenzoic acid	36.18	36.24	36.37
o-(2,4-Dimethylbenzoyl)-tetrachlorobenzoic acid	36.18	36.08	36.23
o-(2,5-Dimethylbenzoyl)-tetrachlorobenzoic acid	36.18	36.25	36.13
o-(4-Ethylbenzoyl)-tetrachlorobenzoic acid	36.18	36.03	36.14
2,3-Dimethyl-5,6,7,8-tetrachloroanthraquinone	37.94	37.86	37.83

	C, %	H, %	C, %		H, %	
o-(4-*n*-Butylbenzoyl)-benzoic acid	76.61	6.38	76.57	76.67	6.45	6.49
o-(2,4-Diethylbenzoyl)-benzoic acid	76.61	6.38	76.54	76.58	6.31	6.51
o-(2,4,6-Triethylbenzoyl)-benzoic acid	77.41	7.10	77.52	77.48	7.04	7.16

by suction; the filtrate is acidified with 6 *N* hydrochloric acid. After cooling in an ice-bath the precipitate is brought upon a filter, washed free from acid with small portions of cold water and dried at 105°. It is recrystallized as specified in Table I.

In the case of *o*-xylene and ethylbenzene the melting points of their respective derivatives with tetrachlorophthalic anhydride lie within a range that might conceivably make identification difficult. The following procedure therefore serves as a further means of differentiation by converting the *o*-(2,3-dimethylbenzoyl)-tetrachlorobenzoic acid to the corresponding anthraquinone derivative under conditions in which the ethylbenzoyl derivative gives no quinone.

One-tenth g. of acid in a 15-cm. test-tube is boiled for one minute with 4 cc. of 50% sulfuric acid. The test-tube is cooled under the tap and its contents are poured into 20 cc. of cold water. The yellow solid which separates is collected on a filter, washed with three 2-cc. portions of 6 *N* ammonium hydroxide and then with small portions of cold water until free from alkali. The product is dried in air and recrystallized from 10 cc. of 80% aqueous alcohol in the form of bright yellow needles.

The above procedure might profitably be applied to the dehydration of other acids, *e. g.*, *o*-benzoyl, toluyl and xyloyl benzoic acids. Its general application, however, is impossible due to sulfonation which results in the case of acids with long chain substituents.[3]

In the course of this investigation several new compounds have been obtained, the analyses of which are given in Table II.

In a future paper the applicability of the present method to aromatic chloro compounds will be demonstrated.

Summary

1. A general method for the identification of aromatic hydrocarbons is described.

2. The *o*-aroyl benzoic acid derivatives of nineteen of the more common aromatic hydrocarbons have been obtained.

3. Tetrachlorophthalic anhydride has been substituted for phthalic anhydride with decided advantage, in some cases.

4. The practicability of dehydrating some of the derivatives to the corresponding quinones has been demonstrated.

(3) Scholl, Potschiwauscheg and Lenko, *Monatsh.*, **32**, 687 (1911).

CAMBRIDGE, MASS. RECEIVED MARCH 29, 1935

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

The Synthesis of Methylcholanthrene

BY LOUIS F. FIESER AND ARNOLD M. SELIGMAN

The purpose and the main results of this work have been discussed in a preliminary communication,[1] and the present paper contains the experimental details.

The essential feature of the synthesis (I ⟶ II) is a modification of the Elbs condensation in which one of the meso carbon atoms of the reaction product is originally a part of an alicyclic ring in the diaryl ketone.

The ordinary Elbs reaction often is complicated by the elimination or degradation of alkyl groups[2] and by the migration of an aroyl group in the naphthalene nucleus,[3] and even in a favorable case, as in the synthesis of 1,2,5,6-dibenzanthracene[4] the yields seldom exceed 20–30%. The ketones I and III, however, lose water rapidly at a temperature of 400–410° and, although some low-boiling hydrocarbons result from the hydrolytic

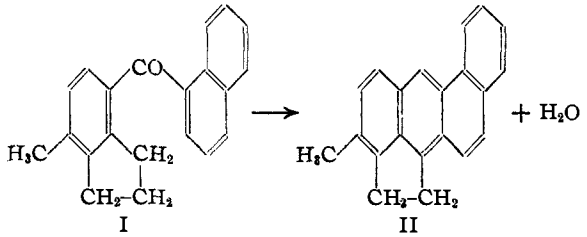
(1) Fieser and Seligman, THIS JOURNAL, **57**, 228 (1935).

(2) Cook, *J. Chem. Soc.*, 456 (1932).

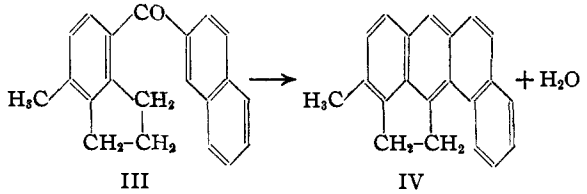
(3) Cook, *ibid.*, 487 (1931).

(4) Clar, *Ber.*, **62**, 350 (1929); Fieser and Dietz, *ibid.*, **62**, 1827 (1929).

fission of the ketones, there is no evidence of any appreciable rearrangement or degradation in the course of the reactions. The hydrocarbons II and IV were obtained in a pure condition in yields as high as 50% of the theoretical amount.



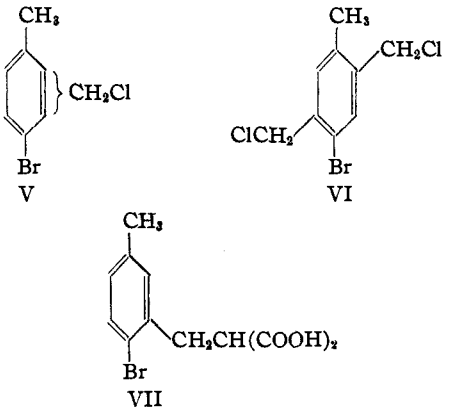
I II

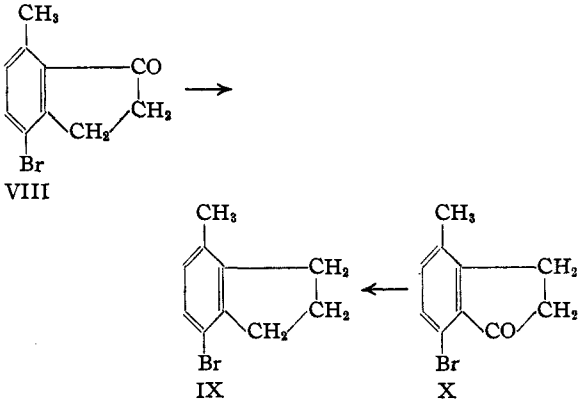


III IV

Our identification of the hydrocarbon II has been confirmed in studies conducted by the Office of Cancer Investigations, U. S. Public Health Service. Dr. Egon Lorenz has found the absorption spectrum of the synthetic material to be identical with that of a sample of methylcholanthrene prepared by the dehydrogenation of dehydronorcholene.[5] Dr. M. J. Shear has observed proliferative changes of a sarcomatous nature forty-five days after the subcutaneous implantation in mice of crystals of the synthetic methylcholanthrene. Definite tumors developed within the next three weeks (65th day). Transplants of the neoplastic tissue on the fifty-third day gave rise to tumors in all of ten cases. The transplants grew slowly and were about 3 cm. in diameter forty-eight days after implantation. The isomeric hydrocarbon IV has given no indication of the production of tumor eighty-four days after implantation. The spectroscopic and biological experiments will be reported in detail later.

The ketones I and III were obtained in 40–45% yield by the reaction of the appropriate naphthoyl chloride with the Grignard reagent from 4-bromo-7-methylhydrindene (IX), which was obtained as follows. From the reaction of *p*-bromotoluene with formaldehyde and hydrogen chloride by the Blanc method a fraction containing the two chloromethyl derivatives V was obtained in 74% yield. Although the mixture could not be separated, it was found from other data that substitution ortho to the bromine atom predominated in the ratio of 1.8 to 1. The disubstituted material (8%) appeared to consist entirely of the crystalline, symmetrical derivative VI, the structure being established by reduction to bromopseudocumene. The product of the reaction of V with sodium malonic ester was a liquid mixture boiling over a narrow range, but a solid product resulted on hydrolysis with barium hydroxide. The less soluble isomer, VII, was isolated in a pure condition and the structure was established by oxidation to 4-bromoisophthalic acid. By decarboxylation of VII and ring closure through the acid chloride the hydrindone VIII was obtained, while the mixture of acids gave a mixture of VIII and X. These hydrindones, m. p. 95 and 154°, differ greatly in properties and





are easily separated, but for the present synthesis this is unnecessary as they both yield the same hydrindene, IX, on reduction by the Clemmensen method. The over-all yield in the eight-step synthesis of methylcholanthrene was 11% of the theoretical amount, or 17 g. of the carcinogenic hydrocarbon from 100 g. of *p*-bromotoluene.

The modified Elbs reaction is being extended to a number of additional cases and this work will be reported separately. We regret to state that

(5) Wieland and Dane, *Z. physiol. Chem.*, **219**, 240 (1933).

an error was made in the preliminary description[1] of ar-α-tetralyl-β-naphthyl ketone and its pyrolysis product.

We are indebted to the Eli Lilly Company for generous supplies of materials, and to the Milton Fund of Harvard University for a grant in support of the work.

Experimental Part[6]

The Blanc Reaction.—In order to avoid polysubstitution the conditions were adjusted to permit only about two-thirds of the *p*-bromotoluene to enter into reaction. The yield was increased about three-fold by the use of a special catalyst.

Anhydrous zinc chloride (280 g.) was fused in a casserole and allowed to cool to 300°, when 2.8 g. of anhydrous aluminum chloride was added with vigorous stirring. As the stirred material began to solidify it was quickly pulverized and added to a mixture of 350 g. of *p*-bromotoluene and 45 g. of trioxymethylene. The mixture was stirred vigorously at 40–50° for a total of eighteen hours (intermittent), while passing in a slow stream of hydrogen chloride. The pasty mass of zinc chloride occasionally was dislodged from the walls and it eventually assumed a semi-solid consistency. The oil was decanted into water, and the residual paste was decomposed carefully with ice. The reaction product was taken up in ether, washed repeatedly with water, dried over sodium sulfate, and fractionated. The recovered *p*-bromotoluene amounted to 112 g., 30 g. (8%) of the disubstitution product was obtained from the after-run as a solid, and, after redistillation of the desired fraction through a Widmer column, there was obtained 225 g. (74%) of a mixture of the two chloromethyl derivatives (V), b. p. 106.5–108.5° at 4 mm., as a lachrymatory liquid.

Anal. Calcd. for C_8H_8BrCl: C, 43.74; H, 3.67. Found: C, 43.68; H, 3.44.

4-Bromo-2,5-di-(chloromethyl)-toluene (VI) was obtained in a practically pure condition by one crystallization from benzene. It forms large, colorless needles melting at 125°.

Anal. Calcd. for $C_9H_9BrCl_2$: C, 40.31; H, 3.39. Found: C, 40.43; H, 3.46.

Reduction with zinc dust and alkali according to v. Braun[7] gave 5-bromopseudocumene, m. p. 71°, in 30% yield. This was identical with a sample (m. p. 72.5°) prepared from pseudocumidine.

Condensation with Malonic Ester.—With but 1 molecular equivalent of sodium there was a considerable amount of dialkylation, and the reagent was best taken in about three-fold excess. Sodium (17.5 g.) was dissolved in 580 g. of ethyl alcohol (freshly distilled from sodium), 245 g. of ethyl malonate was added, and the solution was cooled and treated with 100 g. of the benzyl halide mixture. After heating for two and one-half hours on the steam-bath the mixture was cooled and carefully neutralized with concentrated hydrochloric acid. The precipitated sodium chloride was dissolved in water and extracted with ether, while the bulk of the solvent was removed from the alcoholic solution by distillation *in vacuo*. After washing the product in ether with dilute hydrochloric acid and drying, it was fractionated through a Widmer column. The mixture of methylbromobenzylmalonic esters was obtained as a viscous, odorless liquid, b. p. 166–170° at 2 mm.; yield, 137.5 g. (88%).

Anal. Calcd. for $C_{15}H_{19}O_4Br$: C, 52.46; H, 5.58. Found: C, 52.22; H, 5.50.

The Malonic Acids.—To a hot solution of 152 g. of barium hydroxide octahydrate in 600 cc. of water and 250 cc. of alcohol was added 137.5 g. of the above ester in 90 cc. of alcohol. After shaking for thirty minutes and allowing the mixture to stand until cold, the barium salt which separated was collected and washed with alcohol and ether. The salt was suspended in 500 cc. of ether and shaken with 500 cc. of water containing 75 cc. of concentrated hydrochloric acid until dissolved, and the ethereal solution was washed with a little water, dried and concentrated. The residue was a colorless solid suitable for use in the next step; m. p. about 147–150°; yield, 104 g. (90%).

The material is very readily soluble in ether, readily soluble in hot water and slightly soluble in benzene. By fractional crystallization from ether–benzene the more abundant and less soluble isomer, 2-bromo-5-methylbenzylmalonic acid (a), was obtained in a completely pure condition, as clusters of long needles melting at 159–161° with loss of carbon dioxide. The isomeric 5-bromo-2-methylbenzylmalonic acid (b) (short needles, m. p. 160–162°) was obtained in smaller quantity and the purity is less certain.

Anal. Calcd. for $C_{11}H_{11}O_4Br$: C, 45.99; H, 3.86. Found: (a) C, 46.16; H, 3.79; (b) C, 46.30; H, 3.96.

The less soluble isomer (1.5 g.) was oxidized with alkaline (6 g.) permanganate (5 g.) solution (150 cc.) for one hour at the boiling point, and after treatment with sulfur dioxide the solution was extracted with ether. The purified product had the properties of 4-bromo-isophthalic acid; m. p. 296° (without anhydride formation); analysis: C, 39.07; H, 2.23 (Calcd.: C, 39.20; H, 2.06).

Decarboxylation of the Acids.—The most satisfactory results were obtained by boiling for four hours a solution of the malonic acid mixture (100 g.) in water (400 cc.). The methylbromophenylpropionic acid mixture soon separated as an oil from the hot solution and, after cooling, the product was recovered by ether extraction. No alkali-insoluble material was present, and the acid mixture boiled at 168–172° at 2 mm.; yield, 78 g. (92%). This material, as well as that obtained from either of the pure malonic acids, is a very viscous oil at room temperature.

Anal. Calcd. for $C_{10}H_{11}O_2Br$: C, 49.36; H, 4.56. Found: C, 49.87; H, 4.57.

In an early experiment the decarboxylation was accomplished by heating the malonic acid mixture at 190°, but the desired acid was obtained in only 70% yield and it was accompanied by a neutral substance, b. p. 129° at 1.5 mm. This proved to be a mixture of the two methylbromophenylpropionic acid ethyl esters, $C_6H_3Br(CH_3)CH_2CH_2COOC_2H_5$, for on hydrolysis and ring closure it gave a mixture of the two hydrindones (in the usual ratio).

(6) Microanalyses by Mrs. G. M. Wellwood.

(7) V. Braun and Nelles, *Ber.*, **67**, 1094 (1934).

Anal. Calcd. for $C_{12}H_{15}O_2Br$: C, 53.14; H, 5.58; Br, 29.48. Found: C, 53.43; H, 5.60; Br, 29.24.

The hydrolysis of the malonic ester mixture in this case evidently was incomplete and the material submitted to decarboxylation contained some of the acid ester: $C_6H_3Br(CH_3)CH_2CH(COOH)COOC_2H_5$. In the later runs in which the decarboxylation was conducted in a water solution the acid ester may have become hydrolyzed during the heating or the hydrolysis with barium hydroxide may have been more thorough. At all events no neutral ester was observed and this procedure is regarded as the more reliable of the two.

Ring Closure.—The methylbromophenylpropionic acid mixture (78 g.) was heated on the steam-bath with thionyl chloride (125 g.) for one hour, the excess reagent was largely removed at the pump, and the acid chloride was added in small portions to a stirred suspension of 51 g. of finely powdered aluminum chloride in 1800 cc. of carbon bisulfide at 0°. The metal halide did not darken perceptibly under these conditions and a colorless complex crystallized on the walls of the flask. After twenty minutes at 0° 8 g. of aluminum chloride was added, the mixture was brought to the boiling point in the course of thirty minutes and refluxed for ten minutes. After cautiously adding ice and removing the solvent with steam, the reaction product was obtained as an oil which soon solidified. When this was dried in ether and distilled in vacuum there was obtained 68 g. (94%) of the hydrindone mixture suitable for the next step.

The hydrindones may be separated effectively by steam distillation, although the process is slow, or by fractional crystallization from ether. It is more convenient, however, to use for the separation the crude material obtained after removal of the carbon bisulfide with steam, for the higher melting hydrindone is present in this material in a nicely crystalline condition and it dissolves in ether only with great difficulty, while the isomer easily passes into solution. The hard cake is broken up, dried superficially, and treated with cold ether. The higher melting isomer (a) is left in a nearly pure condition. From the ether solution, after drying and concentrating, a part of the other isomer (b) crystallizes in a nearly pure condition and a further crop is obtained on adding petroleum ether. The final fraction is obtained by removing the solvent and distilling *in vacuo*. From a run of the scale indicated above there was obtained 23 g. of (a) and 42 g. of (b), both samples melting within 1–2° of the correct temperatures. Each hydrindone was also prepared from the corresponding crystalline malonic acid in order to relate the isomers in the different series.

4-Methyl-7-bromohydrindone-1 (X, a) crystallizes from ether as large, colorless prisms melting at 154°. **7-Methyl-4-bromohydrindone-1** (VIII, b) crystallizes from ether–petroleum ether in long, fine needles melting at 95°. It is very soluble in ether or alcohol, and considerably more soluble in solvents and more volatile with steam than the isomer.

Anal. Calcd. for $C_{10}H_9OBr$: C, 53.34; H, 4.03. Found: (a) C, 53.56; H, 3.98; (b) C, 53.64; H, 3.84.

4-Bromo-7-methylhydrindene.—A solution of 25 g. of either of the hydrindones or of the mixture in 150–200 cc. of hot alcohol was added in portions over a period of three hours to a refluxing mixture of 170 cc. of concentrated hydrochloric acid, 80 cc. of water and 250 g. of amalgamated zinc. During this period 150 cc. of concentrated hydrochloric acid was added. After refluxing for two hours more the liquid was decanted and steam distilled to obtain a small crop of the product. The bulk of the material adhered to the zinc, from which it was removed by prolonged steam distillation. For further purification the oil was steam distilled from dilute alkali and fractionated, when a refractive, mobile liquid was obtained; b. p. 100° (2 mm.), 265° (757 mm.); yield, 20.5 g. (87%).

Anal. Calcd. for $C_{10}H_{11}Br$: C, 56.87; H, 5.26. Found: C, 56.68; H, 4.87.

Grignard Reaction.—4-Bromo-7-methylhydrindene reacts only slowly with magnesium and full attention must be given to the quality of all the reagents. The flask containing the magnesium (2.2 g.) was swept with pure, dry nitrogen with stirring and it was then baked thoroughly (free flame) with continued sweeping. Ether (3 cc.) was introduced by distillation from a solution of ethylmagnesium bromide, followed by 10 drops of 4-bromo-7-methylhydrindene and 2 drops of ethyl bromide. The reaction started with very little delay. A solution of 4-bromo-7-methylhydrindene (total of 10 g.) in 50 cc. of the dry ether was added over a period of three to four hours, the solution being maintained in gentle boiling, stirred, and protected with a very slow stream of nitrogen. After twenty to twenty-four hours (estimated conversion, 90%) the solution was cooled to 0°, diluted with 100 cc. of dry ether, and slowly forced under nitrogen pressure into a stirred and protected solution of 15.5 g. of α-naphthoyl chloride in 200 cc. of dry ether at −5°. After thirty minutes in the cold, the solution was refluxed for twelve hours (or for three to four days).

After hydrolysis and removal of the solvent, the crude ketone was subjected to steam distillation both to hydrolyze the greater part of the acid chloride and to remove a liquid by-product having the boiling point of 4-methylhydrindene. The residue was taken up in ether and extracted very thoroughly with dilute alkali. It was found advisable to dry the product in ether, distil in vacuum, and to extract an ether solution of the distillate with several further portions of alkali in order to remove completely all traces of the acid and the acid chloride. After a further vacuum distillation the ketone was ready for pyrolysis, if not completely pure. This ketone (a) was not obtained as a solid and the yield of pure material is not known with accuracy. A sample of **4-(α-naphthoyl)-7-methylhydrindene** (I, a) for analysis was purified by repeated distillation in vacuum, forming a very viscous, faintly yellow oil.

4-(β-Naphthoyl)-7-methylhydrindene (III, b) was prepared by the identical procedure, using β-naphthoy chloride; yield, 6.1 g. (45%). This isomer crystallizes from ether as stout prisms melting at 114°.

Anal. Calcd. for $C_{21}H_{18}O$: C, 88.07; H, 6.34. Found: (a) C, 87.75; H, 6.08; (b) C, 88.39: H, 6.68.

Methylcholanthrene and the Isomer IV.—The crude 4-(α-naphthoyl)-7-methylhydrindene from 10 g. of halide was pyrolyzed in a two-bulb flask in an atmosphere of nitrogen at 405–410° (bath) for twenty-five minutes,

when the formation of water appeared to be at an end. Two distillations at 2 mm. gave a clean orange solid, which was dissolved with an equal weight of picric acid in benzene. After two crystallizations the picrate was obtained as purplish-black needles, m. p. 182.0–182.5°, corr. (5.9 g.). This was converted with ammonia into methylcholanthrene (a), which formed pale lemon yellow needles, m. p. 178.5–179.0°, corr.; yield, 3.2 g. (25%, based on the 4-bromo-7-methylhydrindene used). Samples of the hydrocarbon and its picrate prepared from desoxycholic acid by the known methods melted 0.5–1° lower than the synthetic samples and there was no melting point depression on admixture.

7-Methyl-8,9-dimethylene-1,2-benzanthracene (IV, b) was obtained by the similar pyrolysis of 4-(β-naphthoyl)-7-methylhydrindene (6.1 g.) at 400–405°, the hydrocarbon being crystallized directly from benzene–ether, as purification through the picrate was not found to be of advantage. Fine, very faintly yellow needles melting at 187.5°, corr. were obtained; yield, 2.9 g. (50%). The solution in concentrated sulfuric acid has an orange-green fluorescence. The picrate forms dark crimson needles from benzene, m. p. 164.5–165.5°, corr.

Anal. Calcd. for $C_{21}H_{16}$: C, 93.98; H, 6.02. Found: (a) C, 94.11; H, 6.26; (b) C, 94.23; H, 6.13. Calcd. for $C_{27}H_{19}O_7N_3$: N, 8.45. Found: (b) N, 8.75.

Summary

The cancer-producing hydrocarbon methylcholanthrene has been synthesized in 11% yield from *p*-bromotoluene in an eight-step process involving a modified Elbs condensation.

CONVERSE MEMORIAL LABORATORY
CAMBRIDGE, MASS. RECEIVED APRIL 3, 1935

[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF UPSALA]

The Ultracentrifugal Study of Gliadin[1]

BY LAURA KREJCI AND THE SVEDBERG

Due chiefly to the work of Osborne,[2] gliadin, the alcohol-soluble constituent of wheat gluten, has generally been considered a simple homogeneous protein. His careful investigations threw doubt on the conclusions of Ritthausen[3] that it consisted of three proteins, gliadin, mucedin and glutenfibrin, which differed in their solubility in alcohol of varying concentration; and of Kutscher[4] that gliadin and mucedin were evidently the same protein, but that glutenfibrin contained a lower content of glutamic acid. Recently, however, evidence has been accumulated, chiefly by Haugaard and Johnson,[5] that gliadin does not behave as a simple homogeneous protein. Measurements of sedimentation in high centrifugal fields provide a sensitive test for homogeneity, and the present investigation is an attempt to give a conclusive answer to this question.

Experimental Procedure

In most cases alcohol of medium concentration was used as solvent because of the low solubility of gliadin in neutral aqueous solvents. This necessitated some slight modifications in the ultracentrifuge cells. The plate from which the sectorial aperture is cut was made of elastolite[6] rather than ebonite because of the greater impermeability to alcohol. Thin sheets of vulcanized rubber pressed tightly between this plate and the quartz windows prevented leakage of the solution. Ordinarily the whole was encased in a duralumin collar but for strongly alkaline solutions which attack duralumin a collar of the magnesium alloy electron was used instead.

In order to prevent convection currents, arising from diffusion into the alcohol of the paraffin oil used to cover the solution during centrifuging, each solution was previously agitated with a measured quantity of oil to ensure mutual saturation.

Specially milled Manitoba flour was used as source of the material. The extract resulting from treatment of the well-washed gluten with 64 volume per cent. alcohol was stored in a refrigerator for several days to precipitate the starch, glutenin, and gluten, and the solution decanted; this was used for the study of the stability range. For the remainder of the investigation a quantity of purified gliadin was prepared according to the method of Dill and Alsberg.[7]

Partial Specific Volume

The partial specific volume of gliadin was determined pycnometrically in aqueous solutions of low *p*H and low salt content. The results are collected in Table I. There is a definite downward drift with decreasing concentration; the value for the most concentrated solution is lower than that for practically any of the proteins so far studied. However, it is possible that at higher concentrations the partial specific volume approaches 0.745, the

(1) Original manuscript received February 13, 1934.

(2) T. B. Osborne, "Proteins of the Wheat Kernel," Carnegie Institute of Washington, Publication No. 84, 1907.

(3) H. Ritthausen, "Die Eiweisskörper der Getreidearten, Hülsenfrüchte und Ölsamen," Max Cohen & Son, Bonn, 1872.

(4) F. Kutscher, *Z. physiol. Chem.*, **38**, 111 (1903).

(5) Haugaard and Johnson, *Compt. rend. trav. lab. Carlsberg*, **18**, 2 (1931).

(6) Dr. Rudolf Signer had previously found elastolite cells satisfactory for use with other organic solvents, Signer and Gross, *Helv. Chim. Acta*, **17**, 59 (1934).

(7) Dill and Alsberg, *J. Biol. Chem.*, **65**, 279 (1925).