mole (50 g.) of antimony pentachloride added. There was attached to the reaction flask an eight-inch air reflux condenser which in turn was connected to an air condenser for distillation. On the application of heat the reaction proceeded smoothly and approximately one-half the product was distilled. Thereupon the reaction mixture was kept at reflux for thirty minutes and then the remainder of the product distilled after which it was washed with warm hydrochloric acid, then with water and dried over calcium chloride. Fractionation indicated 64 g. (VI), b. p. 92–92.5°, $n^{20}\text{D}$ 1.3787, d^{20}_4 1.645 and 58 g. (V), b. p. 134.6°, m. p. 65.1° (from freezing curve). *Anal.* Calcd. for (VI), $C_3HF_4Cl_3$: Cl, 48.48. Found: Cl, 47.61. Calcd. for (V), $C_3HF_3Cl_4$: Cl, 60.13. Found: Cl, 60.44.

Dehydrochlorination of $CHCl_2$-CCl_2-CHF_2 (II).—In a two-liter three-neck flask, 2.35 moles (512 g.) of (II) was treated with 2.6 moles (104 g.) of sodium hydroxide dissolved in 780 ml. of methyl alcohol. The sodium hydroxide was dropped into the organic material with constant stirring, the temperature being kept at 25–30° by cooling with water as necessary. At the conclusion of the reaction the mixture was poured into water, the product was separated, dried and fractionated to give 345 g. of (VII), boiling from 113–114°. Passage of chlorine into this olefin in ultraviolet light produced (III).

Acknowledgment.—The authors are indebted to the Research Fund of the University of South Carolina for some financial assistance.

Summary

The hydrogen in a $-CHF_2$ has been found to be more resistant to displacement by chlorine and attack by alkali than that in a $-CHCl_2$ group.

Four new compounds, $CHCl_2$–CCl_2–CHF_2, CCl_3–CCl_2–CHF_2, $CFCl_2$–CCl_2–CHF_2 and CF_2Cl–CCl_2–CHF_2 were prepared in the course of this investigation.

COLUMBIA, S. C. RECEIVED MARCH 13, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MARYLAND]

Reaction Rates by Distillation. III. The Effect of Changes in Structure on the Rate of the Knoevenagel Reaction[1,2]

BY ERNEST F. PRATT AND ELEANOR WERBLE

An improved method of carrying out the Knoevenagel type of condensation, developed several years ago, involves continuous removal of the byproduct water by distilling it into a phase separator.[3,4] Under the conditions used by previous investigators, however, the reaction mixture ordinarily was not homogeneous and the aqueous phase which appeared in the separator was not pure water so that no attempt was made to use the rate of appearance of the aqueous phase as anything more than an indication of the time necessary for complete reaction. The results described in previous papers of this series suggested that if certain modifications were introduced reactions of the type

$$p\text{-}RC_6H_4CHO + R'CH_2R'' \longrightarrow p\text{-}RC_6H_4CH{=}CR'R'' + H_2O$$

in which R′ and R″ are electron attracting groups, could be accurately followed by noting the rate of evolution of water. It seemed desirable to investigate the effects on the rate of variations in R, R′ and R″ since in spite of the wide application of condensations of this type little quantitative information on these fundamental points has heretofore been available. To our knowledge none of the condensations considered here have previously been carried out by the distillation method.

Experimental[5,6]

Purification of Materials.—All starting materials were purified by the usual methods until their physical constants agreed closely with the values in the literature. These constants were as follows: benzene, $n^{25.5}\text{D}$ 1.4978; toluene, $n^{25.5}\text{D}$ 1.4935; caproic acid, $n^{20}\text{D}$ 1.4147; piperidine, $n^{20}\text{D}$ 1.4530; benzaldehyde, $n^{20}\text{D}$ 1.5450; anisaldehyde, $n^{28.5}\text{D}$ 1.5700; *p*-chlorobenzaldehyde, m. p. 46.5–47.5°; *p*-nitrobenzaldehyde,[7] m. p. 105–106°; 2-ethylhexanal, $n^{22.5}\text{D}$ 1.4154; diethyl malonate, $n^{20}\text{D}$ 1.4136; ethyl cyanoacetate, $n^{22.5}\text{D}$ 1.4165; ethyl acetoacetate, $n^{22.5}\text{D}$ 1.4188; phenylnitromethane,[8] $n^{21.8}\text{D}$ 1.5318: phenylacetone, $n^{21.7}\text{D}$ 1.5161; phenyl benzyl ketone,[9] m. p. 58.5–59.5°; malononitrile,[10] m. p. 30–32°; acetylacetone, $n^{24.2}\text{D}$ 1.4485; dibenzoylmethane, m. p. 78–79°; phenylacetonitrile, $n^{28.5}\text{D}$ 1.5189; ethyl phenylacetate, $n^{20}\text{D}$ 1.4985; phenyl benzyl sulfone,[11] m. p. 146–147°.

Standard Procedure.—The precautions previously described[12] were taken and the same apparatus and procedure were used except for the following modifications. One-eighth mole of active methylene compound and the desired amounts of piperidine and caproic acid were dissolved in sufficient benzene to fill a 250-ml. volumetric flask to the mark. A second 250-ml. volumetric flask was filled to the mark with benzene, a volume equal to the volume of aldehyde to be added was removed with a pipet and the remainder was mixed in the reaction flask with the solution of active methylene compound and catalyst. Because of its decreased volatility and water solubility the

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[1] From a portion of the Ph.D. thesis of Eleanor Werble, September, 1948.

[2] For the preceding article in this series, see Pratt, Preston and Draper, THIS JOURNAL, **72**, 1367 (1950).

[3] Cope, Hofmann, Wyckoff and Hardenbergh, *ibid.*, **63**, 3452 (1941), and earlier papers.

[4] Mowry, *ibid.*, **67**, 1050 (1945).

[5] Microanalyses by Mrs. Mary Aldridge. Values given are averages of duplicates.

[6] All melting points are corrected.

[7] Prepared by procedure of Leiberman and Connor, "Org. Syntheses," Coll. Vol. 2, p. 441 (1943).

[8] Prepared by procedure of Black and Babers, *ibid.*, p. 512.

[9] Prepared by procedure of Ballard and Dehn, THIS JOURNAL, **54**, 3970 (1932).

[10] Prepared by procedure of Corson, Scott and Vose, "Org. Syntheses," Coll. Vol. 2, p. 379 (1943).

[11] Prepared by procedure of Pummerer, *Ber.*, **43**, 1407 (1910).

[12] Pratt and Draper, THIS JOURNAL, **71**, 2846 (1949).

readily available caproic acid was used in place of acetic acid. An excess of the acid prevented volatilization of the piperidine. The phase separator was filled with benzene, the solution was brought to reflux and a vial containing the proper amount of aldehyde was dropped in. One-eighth mole of aldehyde was used unless otherwise noted. The maximum voltage which would not cause flooding of the condenser was applied to the heating mantle; a number of check runs at lower voltage gave the same rates. The total yield of water was 98 to 102% unless otherwise noted. After evolution of water ceased the reaction mixture was washed four times with 10% sodium bicarbonate solution, then with water and finally with 5% acetic acid solution. The solvents were distilled off and the products isolated by crystallization or distillation. The tabulated yields are for once-distilled or once-crystallized products. This standard procedure was followed in all cases except where obvious modifications were required as indicated by the data in the tables.

Over the 20 to 70% portion of a given experiment the maximum temperature variation was 0.3° from the median value. Among all the experiments the temperature was almost invariably 83.8 ± 1.0° and for most of the experiments the temperature was 83.8 ± 0.5°. The probable variations were only about half these values for the total variations.

The rate constants tabulated were calculated as previously described.[12] For some unknown reason the rate constants for about half the experiments tended to drift downward slightly during the latter half of the reaction. By not extending the calculations beyond the point for 70% reaction the total drift was kept to less than 5% except that with acetoacetic ester (Table I) the drift was 8%. Most of the experiments were repeated and in all cases the rate constants were readily checked to within 3 to 6%.

For three of the compounds in Table I the first order rate "constants" steadily decreased as the reaction progressed. With malononitrile the value was 0.023 min.$^{-1}$ at 30% reaction and 0.0071 min.$^{-1}$ at 80% reaction; the corresponding values for acetylacetone were 0.017 and 0.0093 min.$^{-1}$; for dibenzoylmethane the values were 0.0070 and 0.0040 min.$^{-1}$. Calculations of rate constants based on the assumption that the reactions were first order with respect to both the active methylene compound and the benzaldehyde were then made. With malononitrile the "constants" fell steadily from 0.13 at 30% reaction to 0.076 at 80% reaction; with acetylacetone the values rose only slightly from 0.089 to 0.10; with dibenzoylmethane the values rose gradually from 0.037 to 0.045. In all these cases the dimensions were liters × moles^{-1} × min.$^{-1}$.

Reaction Products.—Physical constants and analytical results for the new compounds follow: *p*-Chlorobenzalmalonic ester, b. p. 156–158° (1.5 mm.), $n^{27.5}\text{D}$ 1.5470. *Anal.* Calcd. for $C_{14}H_{15}O_4Cl$: C, 59.47; H, 5.37. Found: C, 59.63; H, 5.68. 2-Ethylhexylidenemalonic ester, b. p. 108–110° (0.6 mm.), $n^{27.5}\text{D}$ 1.4471. *Anal.* Calcd. for $C_{15}H_{26}O_4$: C, 66.63; H, 9.72. Found: C, 66.69; H, 9.96. Benzaldibenzoylmethane, recrystallized from alcohol, m. p. 87–88°. *Anal.* Calcd. for $C_{22}H_{16}O_2$: C, 84.59; H, 5.16. Found: C, 84.38; H, 5.60.

The refractive index or melting point of all known products was determined and found to agree closely with the recorded values except in the cases of anisalmalonic ester and benzalacetoacetic ester. Although anisalmalonic ester is reported in the literature as a low-melting solid (m. p. 38–40°) our attempts to crystallize it were unsuccessful. The analytical results on the liquid, b. p. 166–168° (1.2 mm.), $n^{30.0}\text{D}$ 1.5563, were satisfactory. *Anal.* Calcd. for $C_{15}H_{18}O_5$: C, 64.74; H, 6.57. Found: C, 64.84; H, 6.63. Five of the $R'CH_2R''$ compounds in Table I are unsymmetrical and hence might give both *cis* and *trans* products. Sharply melting solids were obtained, however, from cyanoacetic ester, phenylnitromethane and phenylacetone with no indication of a second isomer. Ethyl acetoacetate gave a liquid product, b. p. 125–126° (0.9 mm.), $n^{26.3}\text{D}$ 1.5545, which resisted attempts at crystallization although a solid compound, m. p. 60–61°, has been reported.[13] This suggests that our product contained at least some of the second isomer. *Anal.* Calcd. for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.88; H, 7.04. With phenyl benzyl ketone recrystallization of the total solid product from 60–80° ligroin gave a first crop of material, in 55% yield, which melted at 100–100.5° (lit. m. p. for one isomer 101–102°[14]). A mixture of two types of crystals deposited from the mother liquor. Mechanical separation followed by further recrystallization gave a large proportion of crystals which melted at 99–100° and only a small amount of material which melted at 87–89° (lit. m. p. for other isomer 88–89°[15]).

Study of Side Reaction of Benzaldehyde.—When expt. 2 of Table III was repeated using 0.250 mole of benzaldehyde (100% excess) 182% of the theoretical amount of water was evolved in about one hundred hours. Benzalmalonic ester was isolated from the reaction mixture in 60% yield together with 22.6 g. of a tarry residue. In a similar experiment except that no malonic ester was present 0.062 mole of water (50%) was evolved in twenty-eight hours and 0.11 mole (88%) was obtained in ninety-one hours. No sharply boiling product could be isolated from the reaction mixture; a resinous distillation residue which weighed 13 g. was obtained.

This side reaction of the benzaldehyde was apparently the only reaction which occurred to an appreciable extent when an attempt was made to condense the aldehyde with ethyl phenylacetate. The standard procedure was followed using 0.250 mole of piperidine and 0.500 mole of caproic acid. The theoretical amount of water (0.125 mole) was evolved, half of it being evolved in about nine hours. Upon working up the reaction mixture the ethyl phenylacetate was recovered almost quantitatively and about 11 g. of the resinous by-product was obtained. Essentially the same results were obtained with phenylacetonitrile and with phenyl benzyl sulfone.

Calculations based on the assumption that the reactions were first order with respect to the active methylene compound gave satisfactory rate constants in most cases as shown in the tables. The proportionality between rate and catalyst concentration found for benzaldehyde and malonic ester (Table III) was assumed to hold for the other condensations for which relative reactivities are given in Table I.[16] Unit reactivity was arbitrarily assigned to malonic ester.

Table I

Condensation of Benzaldehyde with $R'CH_2R''$

$R'CH_2R''$	Cat. concn.[a]	$t^1/_2$, min.	Yield, %	k, min.$^{-1}$ × 10^{-3}	Rel. rate
$CNCH_2CN$	1	53	74	.........[c]	...
$CNCH_2COOC_2H_5$	2	36	72	20.40 ± 0.31	66
$CH_3COCH_2COOC_2H_5$	8	25	83	26.74 ± 0.81	22
$CH_3COCH_2COCH_3$	4	48	67	.........[c]	...
$C_6H_5COCH_2COC_6H_5$	8	111	61	.........[c]	...
$C_6H_5CH_2NO_2$	64	49	68	11.75 ± 0.22	1.2
$C_2H_5OCOCH_2COOC_2H_5$	64	75	89	9.96 ± 0.07	1
$C_6H_5CH_2COCH_3$ ·	128	63	59	11.42 ± 0.32	0.58
$C_6H_5CH_2COC_6H_5$	128	61	88[b]	11.01 ± 0.20	0.56

[a] Unit catalyst concentration equals 9.76 × 10^{-4} mole of piperidine and 2(9.76 × 10^{-4}) mole of caproic acid per 500 ml. [b] Both *cis* and *trans* isomers obtained; see Experimental. [c] Rate "constants" rapidly decreased as the per cent. reaction increased; see Experimental. In these three experiments the yield of water was only 88 to 92%.

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(13) Claisen and Matthews, *Ann.*, **281**, 177 (1883).

(14) Knoevenagel and Weisgerber, *Ber.*, **26**, 442 (1893).

(15) Thiele and Ruggli, *Ann.*, **393**, 71 (1914).

(16) An experiment with cyanoacetic ester at twice the catalyst concentration listed in Table I gave a rate constant of 34.7 × 10^{-3} min.$^{-1}$. It should be noted that the effect on the reaction medium of the rather large changes in catalyst concentration required for the experiments of Table I may not have been insignificant.

TABLE II

CONDENSATION OF ALDEHYDES WITH MALONIC ESTER[a]

Aldehyde	$t_{1/2}$, min.	Yield, %	k, min.$^{-1}$ × 10^{-3}
p-$CH_3OC_6H_4CHO$	62	86	11.77 ± 0.15
C_6H_5CHO	75	89	9.96 ± 0.07
p-ClC_6H_4CHO	91	85	7.78 ± 0.11
p-$O_2NC_6H_4CHO$	98	56	6.85 ± 0.11
$CH_3(CH_2)_3CH(C_2H_5)CHO$	120	87	5.17 ± 0.11

[a] The catalyst concentration was 0.0625 mole of piperidine and 0.125 mole of caproic acid per 500 ml. This corresponds to a value of 64 in Table I.

TABLE III

CONDENSATION OF BENZALDEHYDE WITH MALONIC ESTER[a]

Expt.	Mole of piperidine	Mole of caproic acid	$t_{1/2}$, min.	k, min.$^{-1}$ × 10^{-3}
1	0.0625	0.0625	63	11.07 ± 0.10
2	.0625	.125	75	9.96 ± 0.07
3	.0625	.250	114	6.09 ± 0.05
4[b]	.0312	.0625	117	5.96 ± 0.08
5[b]	.0625	.125	71	10.18 ± 0.05
6[b]	.125	.250	34	21.53 ± 0.16
7[c]	.0625	.125	60	9.00 ± 0.25
8[d]	.0625	.125	38	7.38 ± 0.13
9[b,e]	.0312	.0625	33	22.51 ± 0.15

[a] In expts. 2, 3, 4 and 5 the product was isolated in 83 to 89% yields; in the other cases the product was not isolated. [b] A 10% excess of benzaldehyde was used in these expts. [c] A 20% excess of malonic ester was used. Calculated on the basis of the benzaldehyde the value of k was 11.9 ± 0.21 × 10^{-3} min.$^{-1}$. [d] A 100% excess of malonic ester was used. Calculated on the basis of the benzaldehyde the rate "constants" steadily increased from 17.2 × 10^{-3} min.$^{-1}$ at 30% reaction to 22.6 × 10^{-3} min.$^{-1}$ at 80% reaction. [e] Toluene was used as a solvent in place of benzene; reaction temperature 114.5 ± 0.1°.

Although, as noted in Table I, three of the compounds followed kinetics which were higher than first order[17] a comparison of plots of per cent. reaction *versus* time allowed them to be tabulated according to decreasing reactivity.[18] Phenylacetonitrile, ethyl phenylacetate and phenyl benzyl sulfone were all found to be less reactive than any of the compounds of the table, but with none of these three was an appreciable yield of the expected product obtained.

The approximate order of decreasing activating ability of the electron attracting groups is: $-NO_2$, $-CN$, $-COCH_3$, $-COC_6H_5$, $-COOC_2H_5$ and $-C_6H_5$.[19] This series can be only approximate since in a given methylene compound the activating effect of one electron attracting group alters the effect of the second electron attracting group because of some type of interaction such as chelation, resonance or steric hindrance. A comparison of cyanoacetic ester and acetoacetic ester (or of malononitrile and acetylacetone), for example, indicates that the $-CN$ group is more strongly activating than the $-COCH_3$ group while a comparison of phenylacetone and phenylacetonitrile, which reacted little, if at all, indicates that the reverse is true. However, if all compounds containing a $-COOC_2H_5$ group, for example, are compared the relative activating abilities of the $-CN$, $-COCH_3$ and $-COOC_2H_5$ groups are shown by the values 66, 22 and 1.

The rate of condensation of malonic ester with para-substituted benzaldehydes decreased only gradually as the electron releasing ability of the substituent decreased (Table II).[20] There is an apparent conflict between the fact that the overall kinetics were found to be first order and the fact that the rate changes as the structure of either the aldehyde or the active methylene compound is changed. When, as in most cases, equivalent amounts of the two reactants were used, the calculations do not determine which reactant is involved in the rate controlling step. The large changes in rate obtained upon varying the structure of the active methylene compound, however, as well as the effects observed upon carrying out the reaction in the presence of excess malonic ester (expts. 2, 7 and 8, Table III) suggest that it is the active methylene compound rather than the benzaldehyde which enters into the rate expression. The fact that the effect of decreasing the electron releasing ability of the para-substituent in the benzaldehydes is the reverse of that ordinarily observed for addition to the carbonyl group[21] is also consistent with this view. Conceivably the changes in rate shown in Table II are the result of medium effects which are often large in benzene type solvents.[12,22] 2-Ethylhexanal (Table II) reacted only slightly more slowly than the benzaldehydes. The products from the reaction with heptaldehyde distilled over a wide range and neither diethyl ketone nor cyclohexanone reacted with malonic ester to an appreciable extent even at high catalyst concentrations.

The reaction of benzaldehyde with malonic ester was studied in further detail as shown in Table III. As mentioned above, the rate is approximately proportional to the catalyst concentration

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[17] The different kinetic results obtained with acetylacetone and dibenzoylmethane may be ascribable to their exceptionally high degree of enolization (Zuffanti, *J. Chem. Ed.*, **22**, 230 (1945)).

[18] It is apparent that the small difference in reactivity found for benzyl methyl ketone and benzyl phenyl ketone is not significant; ethyl acetoacetate also appears to be only very slightly more reactive than acetylacetone.

[19] Related series arrived at from other standpoints are given in: (a) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 188; (b) Fieser and Fieser, "Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1940, p. 558; (c) McElvain, "The Characterization of Organic Compounds," The Macmillan Co., New York, N. Y., 1945, p. 58; (d) Burnett and Levitt, THIS JOURNAL, **70**, 2778 (1948).

[20] For the condensation of para-substituted acetophenones with malononitrile, Mowry (ref. 4) found that both nitro and ethoxyl groups had an activating effect.

[21] Johnson, "Organic Reactions," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1942, p. 218.

[22] The following is proposed as a possible explanation: As the electron releasing ability of the para substituent of the aldehyde decreases, the availability of the unshared electrons of the carbonyl oxygen would be expected to decrease. The extent to which these oxygens form hydrogen bonds with, and thereby "deactivate," the caproic acid would then be diminished and the reaction rate would decrease according to the results of expts. 1, 2 and 3, Table III

(expts. 4, 5 and 6) and the rate decreased as the concentration of caproic acid increased (expts. 1, 2 and 3).[23,24] A comparison of the results of expts. 4 and 9 shows the effect of carrying out the reaction in refluxing toluene instead of benzene. When excess malonic ester was used, the rate constants decreased appreciably (*cf.* expts. 2, 7 and 8); again this may be a medium effect. Attempts to determine the effect on the kinetics of a large excess of benzaldehyde were unsuccessful because under these conditions a normally slow side reaction seriously interfered (see Experimental).

Because of this side reaction and the somewhat anomalous effects encountered upon using excess malonic ester and upon varying the para substituent in the benzaldehydes a detailed interpretation of the results in terms of the mechanism is not given here. It should be noted, however, that the weight of the evidence is consistent with the favored mechanism[25] if the over-all rate is chiefly controlled by the rate at which the piperidine removes a proton from the active methylene group. The question of mechanism is, of course, not necessarily involved in using the results for predicting the optimum conditions for condensations of this type.

Acknowledgment.—It is a pleasure to acknowledge generous financial support by the Research Corporation which greatly aided the progress of this work.

Summary

It has been found that the rates of condensation of certain active methylene compounds with benzaldehyde decreased in the order: $CNCH_2COOC_2H_5$ (66), $CH_3COCH_2COOC_2H_5$ (22), $C_6H_5CH_2NO_2$ (1.2), $H_2C(COOC_2H_5)_2$ (1), $C_6H_5CH_2COCH_3$ (0.58) and $C_6H_5COCH_2C_6H_5$ (0.56). The numbers in parentheses are a quantitative measure of the relative reactivities. The rates of condensation of malonic ester with benzaldehydes decreased only gradually in the order: *p*-$CH_3OC_6H_4CHO$, C_6H_5CHO, *p*-ClC_6H_4CHO and *p*-O_2N C_6H_4CHO. The over-all decrease was about 40%.

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[23] This negative effect of increasing acid concentration and the relatively small effect of varying the para substituent in the benzaldehyde indicate that the dehydration step of the process is not the rate controlling one.

[24] The decrease in catalytic activity of piperidine with increasing acidity has been previously noted for the reaction of formaldehyde with cyanacetamide. See Enkvist, *J. prakt. Chem.*, **149**, 65 (1937) and Enkvist and Andersson, *ibid.*, **153**, 116 (1939).

[25] See Hammett, ref. 19a, p. 343, for a general discussion of the mechanism of these reactions. Also see Coombs and Evans, *J. Chem. Soc.*, 1295 (1940).

COLLEGE PARK, MD. RECEIVED AUGUST 2, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Light Scattering in Solutions of Serum Albumin: Effects of Charge and Ionic Strength[1,2]

BY JOHN T. EDSALL, HAROLD EDELHOCH,[3] RENÉ LONTIE AND PETER R. MORRISON

The determination of molecular weights of proteins, from measurement of light scattered by their solutions, was apparently first carried out by Putzeys and Brosteaux[4] who studied egg albumin, serum albumin and certain hemocyanins. They employed amandin as a standard protein, assuming its molecular weight to be 330,000; the relative molecular weights of the other proteins they studied agreed well with those obtained by other methods. More recently, Bücher[5] has used light scattering to measure the molecular weight of enolase, employing edestin as a standard of reference. Heller[6a] has reported measurements on egg albumin; and Halwer, Nutting and Brice[6b] have determined on an absolute scale the molecular weights of β-lactoglobulin, ovalbumin and bovine serum albumin with results in good agreement with the values obtained by other methods. Campbell, Blaker and Pardee[7] have determined the molecular weight of a rabbit antibody. Oster, Doty and Zimm[8] studied tobacco mosaic virus, which, unlike the other molecules previously mentioned, is in its longest dimension comparable with the wave length of visible light; thus they were able to determine not only the molecular

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[1] This paper is Number 88 in the series "Studies on the Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross, and Number XXVII in the series "Preparation and Properties of Serum and Plasma Proteins" from the same Department. A preliminary account of some of this work was presented at the 114th Meeting of the American Chemical Society in Washington, D. C., August 30 to September 3, 1948; see the abstracts of this meeting, page 25C.

[2] This work was originally supported by grants from the Rockefeller Foundation and from funds of Harvard University. It was aided early in 1941 by grants from the Committee on Medicine of the National Research Council, which included a grant from the American College of Physicians. From August, 1941, to July, 1946, it was carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University. Since then it has been aided by a grant recommended by the Panel on Hematology of the National Institutes of Health.

[3] National Cancer Institute Postdoctorate Research Fellow, 1947–1949.

[4] P. Putzeys and J. Brosteaux, *Trans. Faraday Soc.*, **31**, 1314 (1935); *Med. Kon. Vl. Acad. Wetensch.*, **III**, No. 1 (1941).

[5] T. Bücher, *Biochim. et Biophys. Acta*, **1**, 467 (1947).

[6] (a) W. Heller and H. B. Klevens, *Phys. Rev.*, **67**, 61 (1945). (b) M. Halwer, G. C. Nutting and B. A. Brice, Abstracts of Papers, 116th Meeting, American Chemical Society, page 3G, 1949.

[7] D. H. Campbell, R. H. Blaker and A. B. Pardee, THIS JOURNAL, **70**, 2496 (1948).

[8] G. Oster, P. Doty and B. H. Zimm, *ibid.*, **69**, 1193 (1947)