[CONTRIBUTION FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH AND FROM THE CHEMICAL LABORATORIES OF THE POLYTECHNIC INSTITUTE OF BROOKLYN]

Light Scattering Studies of Tobacco Mosaic Virus

BY GERALD OSTER,[1] P. M. DOTY[2] AND B. H. ZIMM[3]

Introduction

Recent investigations have shown that considerable information about large molecules can be obtained from the turbidity of their solutions and from the angular dependent of the intensity of light scattered from their solutions. If the scattering particles are small compared with the wave length of light, then, according to Einstein,[4] Raman,[5] and Debye[6] the molecular weight of the particles can be determined by measuring the turbidity of the solutions. By this method the molecular weights of polystyrene[7] and of various proteins[8] have been determined and these are in good agreement with values determined by other methods. However, if the particles possess a linear dimension equal to or greater than about one tenth the wave length of the incident light, the particles can no longer be considered as point sources and the scattering from different parts of the same particle must be considered separately.[9] According to Debye,[10] a characteristic dimension of the particles may be determined by measuring the ratio of the intensity of light scattered in the forward direction to that scattered in the backward direction at two angles symmetrically located about the direction transverse to the incident beam. Furthermore the molecular weight of large particles can be determined by combining the data from angular dissymmetry measurements with those from turbidity measurements. The molecular weights of cellulose acetate[11] and of polystyrene[12] have been obtained in this way.

In this paper are reported studies of the particle length and weight of tobacco mosaic virus as determined from dissymmetry and turbidity measurements. The size distribution of the virus particles is determined from electron micrographs and the shape of the particles in solution is determined from viscosity measurements. These latter two measurements serve as independent checks of the results obtained from the light scattering experiments. Tobacco mosaic virus particles are thin rigid rods having a length comparable with the wave length of visible light and therefore serve as excellent molecular models to test the theory of light scattering from thin rod-like particles.[13]

Light Scattering from Thin Rods

Molecular Size.—The solution of the problem of light scattering by a rod-shaped particle shows that the angular dependence of this intensity of the light[10] is given by

$$I = (1 + \cos^2\theta)\,\frac{1}{x}\int_0^{2x} \frac{\sin w}{w}\,\mathrm{d}w - \left(\frac{\sin x}{x}\right)^2 \qquad (1)$$

where θ is the angle of observation measured with respect to the incident beam and $x = 2\pi L/\lambda \sin \theta/2$. L is the length of the rod-like particle and λ is the wave length of light in the medium in which the particle is located. In Fig. 1 are illustrated plots of the angular intensity as a function of $\sin \theta/2$ for various ratios of rod length to wave length. The factor $1 + \cos^2\theta$ has been omitted since it is symmetrical about 90°. The two vertical lines correspond to θ equal to 42.5 and 137.5°, the two angles of observation in air used in this study, when a correction is made for refraction in the liquid.

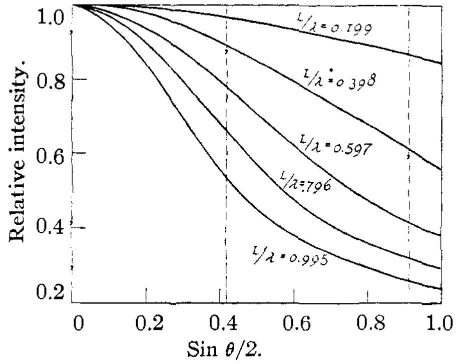

Fig. 1.—Relative intensity of light scattering as a function of $\sin \theta/2$ and L/λ.

At two angles symmetrical about the plane per-

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[4] A. Einstein, *Ann. Physik*, **33**, 1275 (1910).

[5] C. V. Raman, *Ind. J. Phys.*, **2**, 1 (1927).

[6] P. Debye, *J. Applied Phys.*, **15**, 338 (1944).

[7] P. M. Doty, B. H. Zimm and H. Mark, *J. Chem. Phys.*, **13**, 159 (1945).

[8a] P. Putzeys and J. Brosteaux, *Trans. Faraday Soc.*, **31**, 1314 (1935), *Mededeel. Kon. Vlaamsche Akad. Wetensch.*, **3**, 3 (1941) (ovalbumin and various hemocyanins).

[8b] G. Oster, *Science*, **103**, 306 (1946) (influenza and bushy stunt viruses).

[9] G. Mie, *Ann. Physik.*, **25**, 377 (1908).

[10] P. Debye, Lecture given at the Polytechnic Institute of Brooklyn, Nov. 25, 1944; *J. Phys. and Colloid Chem.* **51**, 18 (1947); review paper, B. H. Zimm, R. S. Stein and P.D oty, *Polymer Bull.*, **1**, 90 (1945).

[11] R. S. Stein and P. Doty, THIS JOURNAL, **68**, 159 (1946).

[12] P. M. Doty, W. A. Affens and B. H. Zimm, *Trans. Faraday Soc.*, in press.

[13] For experiments with spherical particles see, for example, V. K. LaMer and M. D. Barnes, *J. Colloid Sci.*, **1**, 71 (1946), and P. P. Debye, *J. Applied Phys.*, **17**, 392 (1946). See also W. Heller, H. B. Klevens and H. Oppenheimer, *J. Chem. Phys.*, **14**, 566 (1946).

pendicular to the incident beam the ratio of the intensities may be written

$$\frac{I_1}{I_2} = \frac{\frac{1}{x_1}\int_0^{2x_1}\frac{\sin w}{w}\,\mathrm{d}w - \left(\frac{\sin x_1}{x_1}\right)^2}{\frac{1}{x_2}\int_0^{2x_2}\frac{\sin w}{w}\,\mathrm{d}w - \left(\frac{\sin x_2}{x_2}\right)^2} \qquad (2)$$

for a given pair of angles of observation the ratio in equation 2 is in reality a function only of L/λ, and consequently a determination of the ratio of the intensity of scattered light at two angles should permit the evaluation of L.

This relation between the ratio of the intensities and L/λ has been calculated from equation 2 and is shown in Fig. 2 where the ratio is plotted against L/λ for the angles of observation of 42.5 and 137.5° (in air) used in this work.

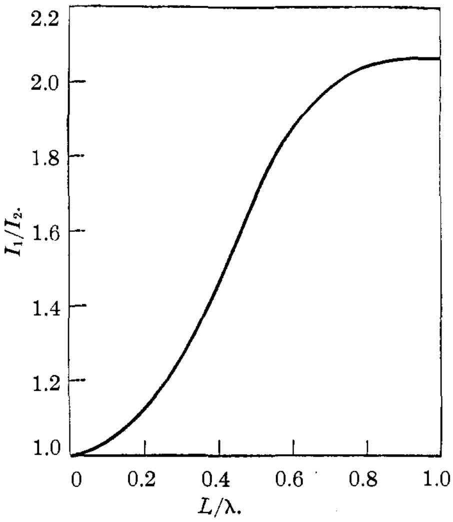

Fig. 2.—Dissymmetry (θ equals 42.5 and 137.5° in air) as a function of L/λ.

The foregoing remarks apply to the scattering from single particles, *i. e.*, at infinite dilutions, and only the effect of *intramolecular* interference on the dissymmetry, I_1/I_2, was considered. At finite concentrations the effect of *intermolecular* interference on the dissymmetry must also be considered. The magnitude of this effect is intimately connected with the interaction between the dissolved particles. If there is little interaction between the solute particles, each particle will, in dilute solution, move about nearly independently of the other dissolved particles. Each particle will behave as an independent source of light with the result that the dissymmetry will be approximately independent of concentration in dilute solution. However, if the forces between the solute particles are such as to tend to arrange them in an orderly array there will be greater intermolecular interference of the scattered light (coherent scattering) with a consequent decrease in the dissymmetry with increasing concentration.

Molecular Weight.—For spherical particles small compared with the wave length of light, the molecular weight of the particles can be obtained (Debye[6]) by determining the turbidity, τ, at several concentrations, c, and then plotting Hc/τ against c. ($H = 32\pi^3n^2(\mathrm{d}n/\mathrm{d}c)^2/3\lambda^4N$ where n is the index of refraction of the medium, $\mathrm{d}n/\mathrm{d}c$ is the refractive index increment, λ is the wave length of incident light, and N is Avogadro's number.) The intercept of such a plot on the ordinate axis is the reciprocal of the molecular weight.

For particles having a dimension comparable with the wave length of light there is a general decrease in the scattered light intensity and a greater reduction in intensity in the backward direction due to destructive interference. In order to determine the molecular weight of the particles responsible for the scattering of light the turbidity must be multiplied by a correction factor to account for the intermolecular interference of scattered light characterized by the dissymmetry.

In Fig. 3 is illustrated this turbidity correction factor as a function of the dissymmetry, I_1/I_2. The turbidity correction factors are the reciprocals of the points of intersection of the plots in Fig. 1 with the ordinate drawn at $\sin \theta/2 = 0.707$ ($\theta = 90°$). Figure 3 is a plot of the turbidity correction factor as a function of the values of I_1/I_2 corresponding to the L/λ values at the intersection of the ordinate at 0.707 in Fig. 1.

The means are now at hand for determining the length and molecular weight of the tobacco mosaic virus from light scattering measurements. The ratio of the intensity of light at 42.5 and 137.5° is determined and the length of the rods is obtained from Fig. 2. The molecular weight of the virus is obtained by applying to the turbidity measurements the correction factor of Fig. 3 for the observed dissymmetry.

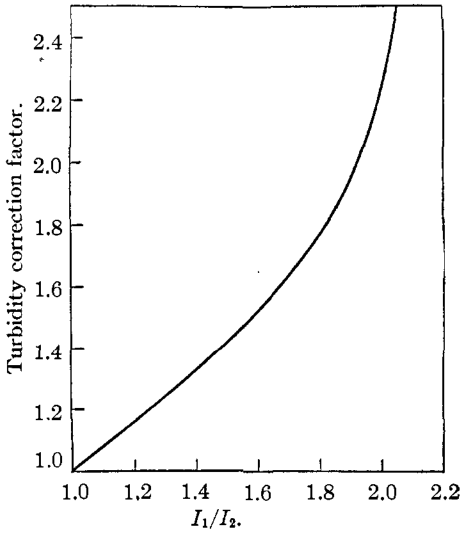

Fig. 3.—Turbidity correction factor as a function of dissymmetry (θ equals, in air, 42.5 and 137.5°).

Preparation of Purified Tobacco Mosaic Virus

Ultracentrifugation, diffusion, electron microscope and viscosity studies, carried out mainly by Lauffer and Stanley,[14] have established that the infectious principle of tobacco mosaic virus is a rod-shaped particle of about 15 by 280 mμ in size. Electron micrographs[15] of the contents of plant cells infected with the virus show that nearly 70% of the rod-shaped particles are of this size. The variation in the reported size of these particles has been shown by Sigurgeirsson and Stanley[16] to be due to variations in the treatment of the material. When solutions of tobacco mosaic virus are allowed to stand for long periods of time they show the presence of a greater percentage of particles aggregated end on end. In the present work a fresh solution of tobacco mosaic virus was used and viscosity and electron microscope studies were made on the same sample used in the light scattering measurements.

The tobacco mosaic virus was purified by centrifugation methods.[17] However in order to ensure freshness of the material, the leaves of Turkish tobacco plants infected for twenty-five days with the virus were frozen, ground in a meat grinder, and the juice extracted during the course of eight hours. The material was purified the next day by differential centrifugation. The final purified material was obtained by sedimentation for fifteen minutes in a Bauer and Pickels air-driven quantity centrifuge at 24,000 r. p. m. The sample was diluted with distilled water to a concentration of 3.19 mg. of virus per cc. The electron microscope, viscosity and light scattering studies were all made within two days after the final purification of the virus.

Electron Microscope Studies

In order to determine the size distribution of the tobacco mosaic virus, electron micrographs of the purified material were made. The electron microscope was an RCA console type having an accelerating voltage of 30,000 volts and a magnification of 5,800 times. The samples were gold shadow cast in order to increase the photographic contrast.[18] Representative pictures were enlarged to a total magnification of 35,000 times and the lengths of all the particles were measured. The size distribution of 500 particles is illustrated in Fig. 4. About 67% of the particles are of the length 280 ± 20 mμ. The particle lengths were not determined with greater accuracy since errors due to spherical aberration of the microscope, unequal shrinkage of the photographic paper on which the pictures were enlarged, and normal errors of measuring lengths negate such an attempt.

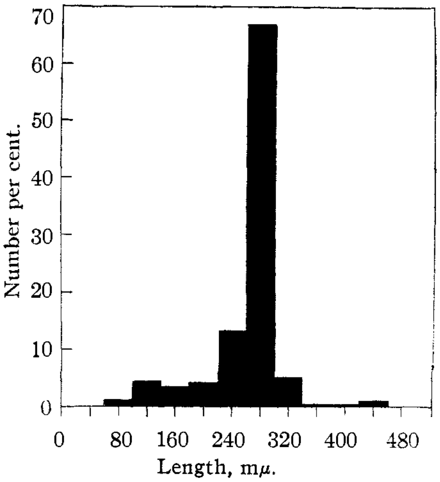

Fig. 4.—Size distribution of tobacco mosaic virus sample from electron micrographs.

From the size distribution the length averages defined as follows

average length $L_n = \Sigma n_i L_i / \Sigma n_i$

root mean square average length $L_{rms} = \sqrt{\Sigma n_i L_i^2 / \Sigma n_i}$

weight average length $L_w = \Sigma n_i L_i^2 / \Sigma n_i L_i$

may be calculated, where n_i is the number of particles of length L_i. These averages, calculated from Fig. 4, are 262, 269 and 271 mμ, respectively.

Viscosity Studies

Lauffer[14b] has applied recent theories of the viscosity of solutions of elongated ellipsoids to viscosity data of solutions of tobacco mosaic virus and has obtained axial ratios which are in agreement with those obtained from electron microscope, ultracentrifugation and diffusion studies.

In the present study the viscosity of solutions of tobacco mosaic virus at various concentrations in water was determined in an Ostwald type viscometer at a temperature of 25°. The flow times for the solutions were greater than 125 seconds for a two-cc. bulb. The strict proportionality between the specific viscosity, $\eta/\eta_0 - 1$ (η and η_0 are the viscosities of the solution and the solvent, respectively), and the concentration is illustrated in Fig. 5. The viscosity observed is in agreement with values obtained by Lauffer when the different size distributions of his samples are taken into account. The high viscosities observed by Frampton[19] for low rates of shear were not obtained—the difference probably being due to the high state of aggregation of his sample brought about by his method of purification of the virus.

Using a value[14b,20] of 0.73 for the partial specific volume of the virus and assuming an unhydrated

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(14) (a) M. A. Lauffer and W. M. Stanley, *Chem. Rev.*, **24**, 303 (1939); (b) M. A. Lauffer, THIS JOURNAL, **66**, 1188 (1944).

(15) G. Oster and W. M. Stanley, *Brit. J. Exp. Path.*, **27**, 261 (1946).

(16) T. Sigurgeirsson and W. M. Stanley, *Phytopathology*, **37**, 26 (1947).

(17) W. M. Stanley, THIS JOURNAL, **64**, 1804 (1941).

(18) R. C. Williams and R. W. G. Wyckoff, *J. Applied Phys.*, **15**, 712 (1944).

(19) V. L. Frampton, *J. Biol. Chem.*, **129**, 233 (1939).

(20) F. C. Bawden and N. W. Pirie, *Proc. Roy. Soc.* (London), **B125**, 274 (1937).

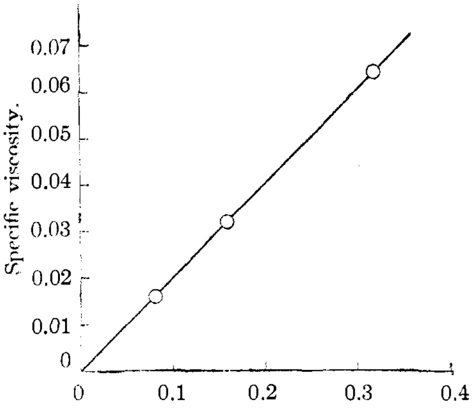

Fig. 5.—Specific viscosity as a function of concentration of tobacco mosaic virus.

particle, a value of 28 for the intrinsic viscosity is obtained. This corresponds in the theory of Simha[21] for the viscosity of very elongated ellipsoids, to an axial ratio of 17.3. Since the principal terms in this theory are proportional to the square of the axial ratio, the intrinsic viscosity is, for a mixture of ellipsoids, proportional to the root mean square axial ratio. If we approximate the virus particles, known from electron micrographs to be cylindrical in shape, as ellipsoids of minor axis of 15 mμ, the diameter of the cylinders as determined by X-ray studies[22] and observed in electron micrographs,[16] then the root mean square length is, according to Simha's theory, 260 mμ. This value agrees with that observed in the electron micrograph studies of this sample.

Light Scattering Studies

Dissymmetry Measurements.—The value of the dissymmetry, I_1/I_2, has been determined for tobacco mosaic virus dissolved in distilled water and in 0.1 *M* sodium phosphate buffer (*p*H 7) at several concentrations in a simple apparatus previously described.[11] At all concentrations measured the background scattering from the solvent was negligible in comparison with that of the solution; hence no correction for solvent scattering was applied. The results of the measurements are summarized in Fig. 6, where I_1/I_2 is plotted against concentration.

Within the probable experimental error the dissymmetry at infinite dilution (called the intrinsic dissymmetry since it is due to intramolecular scattering alone) is the same for both water and buffer solutions, and is equal to 1.94. From the plot in Fig. 2 this value of I_1/I_2 corresponds to a L/λ value of 0.66. The 546 mμ monochromatic light has a wave length of 409 mμ in water and consequently *L*, the length of the scattering particle, is found to be 270 mμ. The estimated probable error is ±15 mμ. These measurements are seen to agree closely with those obtained from electron microscope and viscosity measurements described earlier. *This agreement indicates that the light scattering dissymmetry technique is essentially correct.*

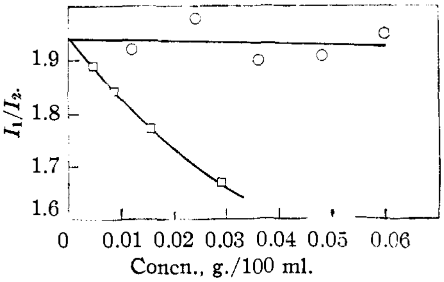

Fig. 6.—Dissymmetry (θ equals 42.5 and 137.5°) as a function of concentration of tobacco mosaic virus (○, in 0.1 *M* sodium phosphate buffer *p*H 7; □, in water).

Bernal and Fankuchen[22] have shown, from their extensive X-ray studies on gels of tobacco mosaic virus, that such gels show considerable regularity in distances perpendicular to the rods. That such an ordering effect may also take place in extremely dilute solutions of the virus in distilled water is illustrated in Fig. 6. The ordering effect, with a consequent decrease in dissymmetry, is enhanced at greater concentrations of the virus. However, phosphate buffer at *p*H 7 apparently lowers the interaction between the particles, since I_1/I_2 for virus in buffer is independent of the concentration of the virus.

Turbidity studies made by one of us[23] indicate that, for higher concentrations of solutions of tobacco mosaic virus, there is real liquid crystal formation with a consequent sharp decrease in intensity of light scattering.

Turbidity Measurements.—The turbidities of solutions of tobacco mosaic virus in water were measured at right angles to the incident monochromatic light in a turbidimeter previously described.[7,11] The measurements are given in Table I together with the steps used in calculating Hc/τ. The turbidimeter was calibrated by use of a butanone solution of a polystyrene fraction of 1% concentration which exhibited negligible dissymmetry. The turbidity of this solution was accurately determined by transmission measurements. The solution was then used to calibrate the turbidimeter. It is not necessary to know the molecular weight of the material used to calibrate the turbidimeter—the only requirement is that it exhibit no dissymmetry of scattering.

In Table I the turbidity correction factor is obtained from Fig. 3. From refractive index measurements made with a Zeiss dipping refractometer $dn/dc = 0.169$ and since $\lambda = 546$ mμ it follows that $H = 3.15 \times 10^{-6}$. The reciprocal of the intercept of Hc/τ *versus* *c* gives a value for the

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[21] R. Simha, *J. Phys. Chem.*, **44**, 25 (1940), J. W. Mehl, J. L. Oncley and R. Simha, *Science*, **92**, 132 (1940); see also L. Onsager, *Phys. Rev.*, **40**, 1028 (1932).

[22] J. D. Bernal and I. Fankuchen, *J. Gen. Physiol.*, **25**, 111 (1941).

[23] G. Oster, data to be published.

TABLE I

Concn. g./100 ml.	$\tau \times 10^2$ (cm.$^{-1}$)	I_1/I_2	Turbidity correction factor	$\tau \times 10^2$ (cor.)	$H\frac{c}{\tau} \times 10^8$
0.0290	2.26	1.67	1.59	3.60	2.54
.0145	1.035	1.78	1.74	1.80	2.54
.0097	0.68	1.83	1.81	1.23	2.49

weight-average molecular weight of $M = 40 \times 10^6$ with a probable error of 5%. In this determination of the molecular weights from light scattering no assumptions need be made about the percentage of hydration of the particles. If the length and molecular weight of the particles as determined by the light scattering measurements are used with a value of 0.73 for the partial specific volume of the virus, the diameter of the cylindrically shaped virus particles can be calculated to be 15.2 mμ. This value is in agreement with that obtained in X-ray[22] and electron microscope[16] studies.

Summary

Light scattering measurements were made on a freshly prepared sample of purified tobacco mosaic virus. The length of the particles determined from dissymmetry of light scattered by the solutions were found to be 270 mμ. Application of the dissymmetry to turbidity measurements gave a weight average molecular weight of 40 millions for the particles. These values are in complete agreement with sizes determined from electron microscope and viscosity studies made on the same sample. This agreement indicates the validity of the light scattering method.

For dilute solutions of tobacco mosaic in water the dissymmetry decreases with increasing virus concentration thus indicating that strong interaction with a consequent increase in order of the scattering particles is occurring. However, the dissymmetry is independent of concentration for dilute solutions of the virus in 0.1 M sodium phosphate buffer at pH 7.

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Reduction of Organic Compounds by Lithium Aluminum Hydride. I. Aldehydes, Ketones, Esters, Acid Chlorides and Acid Anhydrides

BY ROBERT F. NYSTROM[1] AND WELDON G. BROWN

Following the discovery of lithium aluminum hydride by Finholt, Bond and Schlesinger[2] some preliminary experiments were carried out on a vacuum-line scale by Finholt, Schlesinger and Wilzbach[3] to ascertain its behavior toward some common types of organic compounds. These experiments indicated that this hydride is capable of reducing carbonyl, carbalkoxy, acyl chloride and nitro groups, but is without action on the double bond of simple olefins. It was thus evident that the reagent offered some promise as a synthetic tool and, with the consent and coöperation of these authors, we have undertaken a more extensive survey of such applications.

The attractive features of lithium aluminum hydride as a reagent which, combined, place it in a unique position are (1) that it is easily prepared on either a large or a small scale from commercially available lithium hydride, (2) that it is indefinitely stable at room temperature, (3) that it is ether-soluble, (4) that as compared with other reducing agents, excepting hydrogen, it has a favorable ratio of reducing capacity to mass, (5) that the reductions occur at room temperature, and (6) that no unusual equipment is needed. The present high cost of lithium hydride is partially offset by the consideration stated under (4) above. As the technique stands at present, compounds which are insoluble in ether do not react satisfactorily, and there is a further limitation connected with the fact that some substances containing active hydrogen decompose the reagent with the liberation of hydrogen.

This paper summarizes the results achieved in the reduction of representative aldehydes, ketones, acid chlorides and acid anhydrides. These functional groups, in the cases thus far examined, are reduced to the alcohol stage with extreme rapidity and with virtually no side-reactions. The yields appear to be limited principally by the efficiency of the procedures employed subsequently in isolating the products. As determined by the hydride consumed, the reductions proceed quantitatively inac cordance with the following general equations

Aldehydes and ketones

$$4R_2CO + LiAlH_4 \longrightarrow LiAl(OCHR_2)_4$$

Esters

$$2RCOOR' + LiAlH_4 \longrightarrow LiAl(OR')_2(OCH_2R)_2$$

Acid chlorides

$$2RCOCl + LiAlH_4 \longrightarrow LiAlCl_2(OCH_2R)_2$$

Acid anhydrides

$$(RCO)_2O + LiAlH_4 \longrightarrow LiAlO(OCH_2R)_2$$

The unsaturated compounds for which data are included in this summary are crotonaldehyde, methyl oleate and sorbyl chloride, the product in

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(1) Naval Research Laboratory Fellow.

(2) Finholt, Bond and Schlesinger, THIS JOURNAL, **69**, 1199 (1947).

(3) Finholt, Schlesinger and Wilzbach, unpublished work.