adenylate. This formulation is supported by the recent O^{18} exchange studies of Boyer[12] and by Jencks[13] who obtained a coA-independent activation of octanoic and similar fatty acids by pyrophosphate split of ATP. Definitive proof of this general mechanism must await experiments demonstrating the enzymatic synthesis of adenyl acetate from ATP and acetate (reaction 2), and the formation of adenyl acetate from acetyl-coA and A5P (reaction 3).

During the course of the above work a methionine requiring PP–ATP exchange system was also purified from yeast.[14] It is possible that this and the previously reported pantoic[15] and amino acid activated PP-ATP exchanges[5] may occur by the formation of the corresponding adenyl-acyl group derivatives. Thus, the pantothenate peptide bond formation may represent a variation of the acetate activation with the amino group of β-alanine serving as the acyl group acceptor in place of the sulfhydryl group of coA.

(12) P. D. Boyer, O. J. Koeppe, W. W. Luchsinger, and A. B. Falcone, *Fed. Proc.*, **14**, 185 (1955).
(13) W. P. Jencks, *ibid.*, **12**, 703 (1953).
(14) P. Berg, unpublished.
(15) W. K. Maas, quoted by F. Lipmann, *Science*, **120**, 855 (1954).

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RECEIVED APRIL 21, 1955

REDUCTION OF ESTER AND OTHER DIFFICULTLY REDUCIBLE GROUPS BY SODIUM BOROHYDRIDE

Sir:

Since its original discovery[1] sodium borohydride has proven a very useful reagent for the selective reduction of aldehyde and ketone groups.[2] It has not been applicable to the reduction of ester and similar functional groups which are relatively difficult to reduce. Recently Kollonitsch, Fuchs and Gabor[3] reported that they had succeeded in reducing esters by the use of calcium borohydride[3a] and magnesium borohydride.[3b] We wish to report the rapid reduction at room temperatures of esters, carboxylic acids and nitriles by sodium borohydride in the presence of aluminum chloride. 1-Olefins are reduced at 75°; unconjugated 2-olefins are not affected by these conditions. Nitro and amide groups are not reduced by the reagent. Consequently the method offers a convenient procedure for the selective reduction of nitro esters.

The addition of aluminum chloride to a solution of sodium borohydride in diethylene glycol dimethyl ether ("diglyme") results in a clear solution of the reagent. Sodium chloride does not precipitate. Therefore it is improbable that aluminum borohydride is present in other than minor amounts. (We plan to investigate this point.) The reagent has been exposed to dry air with only minor loses in activity. It can be poured in air without difficulty.

Other polyvalent metal halides, such as gallium trichloride and titanium tetrachloride also bring about the reduction of esters. However, aluminum chloride possesses obvious advantages for general laboratory use so that our studies have been concentrated on the applicability of this reagent.

In a typical preparative procedure ethyl *p*-chlorobenzoate was reduced to *p*-chlorobenzyl-alcohol in 89% yield as follows. A stirred solution of 0.25 mole of sodium borohydride (99% purity) in 250 ml. of diglyme and 0.4 mole of ethyl *p*-chlorobenzoate was treated slowly with a total of 0.084 mole of anhydrous aluminum chloride (42.0 ml. of a freshly prepared 2 *M* solution of $AlCl_3$ in the same solvent). The reaction was vigorous in the initial stages and the rate of addition was controlled to maintain the temperature below 50°. The flask was then heated for a few minutes on the steam-bath to complete the reaction. The reaction mixture was poured onto crushed ice and dilute acid. The solid product (50.9 g., 89%) was recrystallized from hot water to give pure *p*-chlorobenzyl alcohol, m.p. 75°, in a yield of 84%.

We have examined the utilization of hydride from the reagent by various compounds usually at 25°. The first figure gives the time of reaction in hours and the second gives the moles of hydride per mole of compound (one mole of hydride utilized to form hydrogen in the case of carboxylic acids and amides is not included in the figure): ethyl acetate (0.5, 2.0); ethyl stearate (0.5, 2.0); ethyl *p*-chlorobenzoate (0.5, 2.0); ethyl oleate (0.5, 2.2); ethyl cinnamate (0.5, 3.0); ethyl *p*-nitrobenzoate (0.5, 2.0); benzoic acid (0.5, 2.0); benzamide (3.0, 0.0); benzoyl chloride (3.0, 2.0); benzonitrile (3.0, 2.0); acetonitrile (3.0, 2.0); nitrobenzene (3.0, 0.0); 1-nitropropane (3.0, 0.0); pyridine-N-oxide (3.0, 1.0); benzaldehyde (0.5, 1.0); benzophenone (0.5, 1.0); styrene (1.0, 1.0); 1-hexene (25°, 3.0, 0.8; 75°, 1.0, 1.0); cyclohexene (75°, 1.0, 0.0).

These results suggest that the reducing properties of sodium borohydride can be profoundly modified by addition of various polyvalent metal halides. We are continuing to explore the potentialities of these reducing systems.

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(1) H. I. Schlesinger, H. C. Brown, H. R. Hoekstra and L. R. Rapp, THIS JOURNAL, **75**, 199 (1953).
(2) S. W. Chaikin and W. G. Brown, *ibid.*, **71**, 122 (1949).
(3) (a) J. Kollonitsch, O. Fuchs, V. Gabor, *Nature*, **175**, 346 (1955); (b) **173**, 125 (1954).

NON-SOLVATED ALUMINUM HYDRIDE[1]

Sir:

Previous preparations of aluminum hydride have resulted either in a very low yield of impure product[2] or in a solid solvated polymer from which it has been impossible to remove all the solvent without decomposition.[3]

We have now succeeded in preparing non-

(1) The work reported herein was carried out under the auspices of the Office of Naval Research under Contract ONR-494(04).
(2) O. Stecher and E. Wiberg, *Ber.*, **75**, 2003 (1942).
(3) A. E. Finholt, A. C. Bond, Jr., and H. I. Schlesinger, THIS JOURNAL, **69**, 1199 (1947).

solvated aluminum hydride as follows: A solution of aluminum hydride in diethyl ether is prepared in the usual fashion[3] and filtered promptly (before polymerization can occur) through sintered glass under nitrogen into an inert liquid, which is not a solvent. Pentane and ligroin have been found to be suitable. It is essential that the hydride solution be rapidly mixed with a relatively large volume of this inert liquid (at least 100 ml. for each gram of aluminum hydride); a satisfactory method is to run the solution in a thin film down a wire while the precipitant is vigorously stirred by a magnetic stirrer. Precipitation is instantaneous and a very fluffy product results.

The bulk of the ether–precipitant mixture is removed by vacuum, and the apparently dry residue is subjected to high vacuum at room temperature. At least twelve hours are ordinarily required to remove all volatile material from the product. During evacuation, it is beneficial to grind and stir the product by means of the magnetic stirrer.

Aluminum hydride samples prepared in the above manner were analyzed for aluminum by precipitation as the 8-hydroxyquinolinate and for hydrogen by measuring the gas evolution produced by a water-dioxane mixture.

Anal. Calcd. for AlH_3: Al, 89.93; H, 10.07. Found, for a sample precipitated by ligroin: Al, 89.28; H, 9.96.

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RECEIVED MAY 5, 1955

ENZYMATIC SYNTHESIS AND BREAKDOWN OF POLYNUCLEOTIDES; POLYNUCLEOTIDE PHOSPHORYLASE[1]

Sir:

In the course of experiments on biological phosphorylation mechanisms[2] it was found that extracts of *Azotobacter vinelandii* catalyze a rapid exchange of P^{32}-labelled orthophosphate with the terminal phosphate of ADP,[3] IDP, UDP, CDP and (less rapidly) GDP. There is no reaction with the corresponding nucleoside triphosphates or monophosphates (tried ATP, ITP, AMP, IMP). The exchange is accompanied by the liberation of P_i and requires Mg^{++}. Employing the rate of the ADP-P_i exchange as an assay, the enzyme activity has been purified about 40-fold through ammonium sulfate fractionation and $Ca_3(PO_4)_2$ adsorption steps. The ratio of the rates of ADP-P_i exchange to P_i liberation remained constant.

On incubation of the purified enzyme with IDP, in the presence of Mg^{++}, 50–60% of the nucleoside diphosphate disappears with liberation of a stoichiometric amount of P_i. The missing nucleotide is accounted for by a water-soluble, non-dialyzable product which is precipitated by TCA or alcohol. Its solutions are rather viscous and exhibits a typical nucleotide ultraviolet absorption spectrum. Judging from its chromatographic behavior on Dowex anion exchange columns[4] the material is strongly acidic. It yields IMP (Fig. 1) on mild alkaline hydrolysis[6] and thus appears to be an

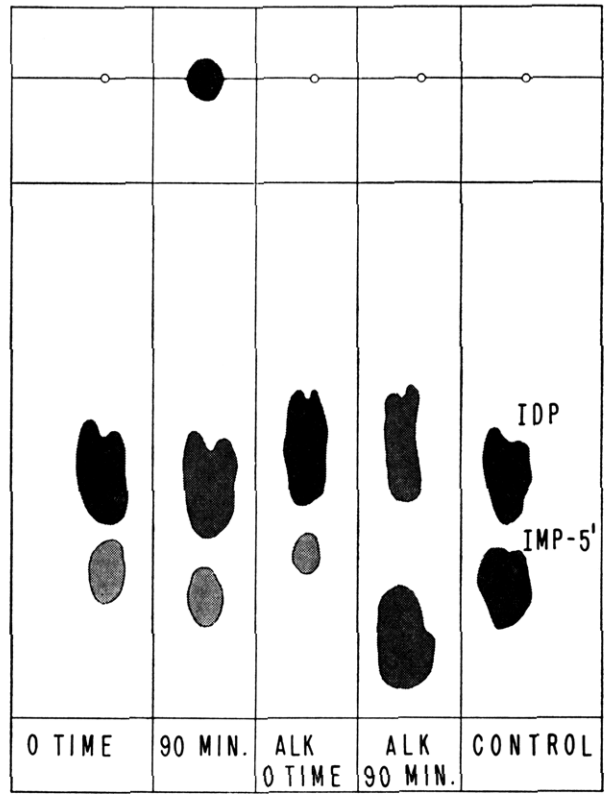

Fig. 1.—Identification of product of reaction of IDP with *Azotobacter* enzyme by paper chromatography. Solvent system of Krebs and Hems[5]; spots located by UV absorption. The three degrees of shading indicate strong, medium, and weak absorption respectively; 0.97 mg. of enzyme (SA, 12) incubated 90 minutes at 30° with 25 μM. IDP, in the presence of 12 μM. $MgCl_2$ and 90 μM. Tris buffer, *p*H 8.1; final volume, 2.5 ml. Mixture deproteinized by heating 1 minute at 100° and equal aliquots (without and with hydrolysis with 0.4 *N* KOH for 22 hours at 37°) used for chromatography. The IDP is contaminated with small amounts of 5′-IMP. Incubation results in decrease of IDP and appearance of an ultraviolet absorbing material which remains at the origin of the chromatogram. After alkaline hydrolysis, this material disappears and is replaced by a product migrating somewhat faster than 5′-IMP. This has been identified as a mixture of 2′- and 3′-IMP. The SA of the enzyme is defined as units/mg. protein. One enzyme unit catalyzes the exchange of 1.0 μM. of P_i^{32} with ADP in fifteen minutes at 30° under standard assay conditions. SA of initial enzyme extract was 0.3.

(1) Supported by grants from the U. S. Public Health Service, the American Cancer Society (recommended by the Committee on Growth, National Research Council), the Rockefeller Foundation, and by a contract (N6onr279, T.O. 6) between the Office of Naval Research and New York University College of Medicine. Presented at the April, 1955, meeting of the Federation of American Societies for Experimental Biology in San Francisco.

(2) M. Grunberg-Manago and S. Ochoa, *Fed. Proc.*, **14**, 221 (1955).

(3) Abbreviations: diphosphates of adenosine, inosine, guanosine, uridine, and cytidine, ADP, IDP, GDP, UDP, and CDP; orthophosphate, P_i; adenosine and inosine monophosphates, AMP, and IMP; inosine-2′- and 3′-monophosphates, 2′-IMP and 3′-IMP; inosine diphosphatase, IDPase; trichloroacetic acid, TCA; tris(hydroxymethyl)aminomethane, Tris; specific activity, SA; micromoles μM.

(4) Based on W. E. Cohn, THIS JOURNAL, **72**, 1471 (1950).

(5) H. A. Krebs and R. Hems, *Biochem. et Biophys. Acta*, **12**, 172 (1953).

(6) E. Vischer and E. Chargaff, *J. Biol. Chem.*, **176**, 715 (1948); C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).