Identification of Products.—C_4F_{10}: This material was recovered from fractionation at between $-1°$ and 1°. It had a molecular weight of between 238 and 240; analysis showed the absence of sulfur.

C_5F_{12}: This material was recovered from fractionation and boiled between 29 and 31°. Its molecular weight was between 286 and 288. It did not show the presence of sulfur. The crude fraction had a melting point range above 10°. Nuclear magnetic resonance spectra were made on aliquots of the sample and from the relative intensity of the CF_3 peaks, it was concluded that the sample contained neo-C_5F_{12}, iso-C_5F_{12} and *n*-C_5F_{12}, crudely in the ratio 3:2:1.

C_6F_{14}: This material boiled between 57 and 59°. It had a molecular weight of between 338 and 340. It contained no sulfur. The refractive index, $n^{25}D$, was 1.2558.

C_7F_{16}: This material boiled between 82 and 83°. It had a molecular weight of 392. It contained no sulfur. The refractive index, $n^{25}D$, was 1.2685.

Six grams of product boiled above 83°, and appeared to be fluorocarbon material.

Although the attack on the nickel packing was not too serious even at 518°, one trial, essentially the same as the third trial in the table, was made with the reactor filled with 0.125″ NaF pellets. From this trial, 5 g. of C_4F_{10}, 2 g. of C_5F_{12} and 5 g. of C_6F_{14} were isolated and characterized. As only 3 g. of material boiled above 60°, no C_7F_{16} was isolated.

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Preparation and Reactions of Methylenedimagnesium Dihalides

BY D. A. FIDLER, J. R. JONES, S. L. CLARK AND HUGO STANGE

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During the course of another investigation it became necessary to prepare methylenedimagnesium dihalides (methylene di-Grignards). Both the dibromide and diiodide had been prepared previously by Emschwiller from the corresponding methylene halide, but the conversion of the magnesium was only of the order of 11%.[1] Chang and Chao-Lun Tseng have confirmed the preparation

$$CH_2X_2 + 2Mg \longrightarrow CH_2(MgX)_2$$

of the diiodide but state that the maximum yield is 10%.[2] We have succeeded in preparing methylenedimagnesium dibromide and methylenedimagnesium diiodide with conversions of the magnesium as high as 54%. The yield of the di-Grignard has been as high as 73%. A small amount of coupling accompanies this reaction, leading to the formation of ethylene.

$$CH_2X_2 + Mg \longrightarrow {}^1/_2CH_2 = CH_2 + MgX_2$$

The reactivity of the methylene di-Grignard reagent was found to be exceptionally low. No reaction was observed when an ethereal solution of benzaldehyde was heated to reflux for three hours with the di-Grignard reagent. The benzaldehyde was recovered quantitatively as the semicarbazone. When an ethereal solution of benzoyl chloride was heated to reflux for one hour with the reagent, there also was no reaction and the benzoyl chloride was recovered quantitatively as benzoic acid. A similar reaction with acetyl chloride, followed by hydrolysis, resulted only in the recovery of solvent from the organic layer. These results are in agreement with Chang and Chao-Lun Tseng who report no reaction with acetone, benzophenone, Michler ketone or carbon dioxide.[2]

Experimental

Methylenedimagnesium Diiodide.—Five grams (0.21 g. atom) of magnesium was covered with anhydrous ethyl ether. While the ether was heated under reflux and the magnesium stirred, 28.0 g. (0.11 mole) of methylene iodide, diluted with ten times its volume of anhydrous ether, was added dropwise over a period of 4.5 hours. After an additional hour of stirring, the flask was cooled and two layers formed. Hydrolysis of the lower layer gave 790 ml. (S.T.P.) of methane as confirmed by its infrared spectrum.[3] The spectrum was recorded by the use of a 10-cm. cell at a gas pressure of approximately 760 mm. Gas which was collected in a cold trap ($-196°$) during the reaction was identified as ethylene by its vapor pressure.

The weight of unreacted magnesium indicated a 54% conversion of which 73% formed methylenedimagnesium diiodide while 27% went to the formation of ethylene. By decreasing the addition time of the methylene iodide to 1.5 hours, both the conversion and yield were reduced to 50%.

Methylenedimagnesium Dibromide.—The dibromide was prepared in the same manner as the diiodide. The addition of 19.0 g. (0.11 mole) of the methylene bromide solution required 15 minutes. Heating under reflux for three hours and the addition of a few crystals of iodine were necessary to initiate the reaction. In this case a 50% conversion of the magnesium was achieved, 58% of which formed methylenedimagnesium dibromide.

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[1] G. Emschwiller, *Compt. rend.*, **183**, 665 (1926).

[2] D. Y. Chang and Chao-Lun Tseng, *Trans. Sci. Soc. China*, **7**, 243 (1932); *C. A.*, **26**, 5544 (1932).

[3] The lower-membered di-Grignard reagents are insoluble in ether, forming a lower layer. A. Kreuchunas, THIS JOURNAL, **75**, 3339 (1953), reports that the solubility increases with chain length and that the C_{10}-compound does not form a separate layer.

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The Effect of Liquid Ammonia on Proteins.
I. Insulin

BY ERIC ELLENBOGEN

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In order to evaluate the use of non-aqueous solvents for tissue fractionation, preliminary experiments were carried out with the insulin–liquid ammonia system[1] employing samples of high potency insulin with known physical chemical properties. These experiments indicated that long exposures of insulin to liquid ammonia did not alter any physical chemical properties as judged by solubility, sedi-

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[1] E. Ellenbogen, Abstr. 124th National Meeting, American Chemical Society, page 20 C, 1953.

mentation constant, crystallizability, biological activity, ratio of maximum/minimum absorptivities in the ultraviolet, infrared absorption spectra and total nitrogen content. Of great interest, also, was the observation that some sixty molecules of water per molecule of insulin (mol. wt. 12,000) seem so tightly bound that they do not react with the solvent.[2] At the low temperatures at which the experiments were carried out, these water molecules (amounting to approximately 9 g. per 100 g. of insulin) might be "frozen" into or onto the protein hydrogen bond network, or might be arranged in a high state of order around CH_2 or CH_3 groups, as observed in some cases.[3] This observation would lead to the conclusion that water molecules play an important part in the stabilization of protein structure whether in solution or in the crystalline state, since a similar role on the part of water molecules is also inferred from studies on small peptides composed of amino acid residues having like configuration.[4]

Experimental

Insulin.—Three different samples of insulin were studied. Two samples were crystalline beef zinc insulin, kindly furnished through the courtesy of the Eli Lilly Co., lots 296,000 and T-2344 (5 times recrystallized). The third sample was amorphous, zinc free, isoelectrically precipitated insulin prepared from crystalline zinc insulin, kindly furnished us by the Armour Co. The biological activities were 25, 27 and 27 I.U./mg., respectively. Extensive physical chemical studies were carried out on lot 296,000[5a,b] and on lot T-2344.[6] Each preparation was thoroughly equilibrated at room temperature until the moisture content, as determined by drying *in vacuo* at 56° over phosphorus pentoxide, became constant. Samples equilibrated in this manner contained 9.3 ± 0.5% moisture.

Liquid Ammonia Studies.—A three-neck flask was equipped with a vacuum tight stirrer in the center, a soda lime–calcium chloride drying tube at one end, and a gas inlet tube at the other. The gas inlet tube was connected to a three-way stopcock, one end of which was connected to a tank of purified, oil pumped nitrogen, and the other through a spiral glass tube to a tank of ammonia. The apparatus was flushed with dry nitrogen at room temperature for one-half hour, and then placed into a Dewar flask kept at −70°. Flushing with dry nitrogen was continued for another 20 minutes. The flask was removed from the cold bath, wiped dry on the outside, and examined for evidence of condensation. Should moisture have condensed on the inside of the apparatus, it was disconnected and another assembly set up. Absence of moisture is critical, since it was observed that slight traces of water will form ammonium hydroxide which will subsequently produce an insoluble precipitate of denatured insulin.

If no condensation was observed, the assembly was returned to the cold bath, and ammonia introduced. This gas will condense faster if it is precooled by placing the spiral glass tube into a bath kept at −33°. When 50 ml. of liquid ammonia was collected, nitrogen was admitted, and the flask transferred to a bath kept at −36°. With nitrogen constantly passing over the surface of the solution, the drying tube was quickly lifted and some 200 mg. of insulin poured into the flask from an inverted test-tube. The drying tube was replaced, the stirrer turned on for a few minutes, and the solution kept at −36° for 18 hours. Under these conditions all of the insulin goes into solution almost as soon as it comes in contact with the liquid ammonia, resulting in a pale yellow solution free of any precipitate.

At the end of the experiment the flask was removed from the cold bath and ammonia removed by means of a stream of nitrogen. The last traces of ammonia were removed by placing the flask in an oil-bath at 60° for two hours and then connecting the flask at room temperature to a vacuum pump without traps for one-half hour, breaking the vacuum by means of dry nitrogen admitted through a drying tube. A small sample of insulin was quickly removed from the flask to a small container previously dried to constant weight, a weight was quickly taken and the sample dried over phosphorus pentoxide at 56° *in vacuo*. Under these conditions, the moisture content of the liquid ammonia-treated insulin samples was found to be 9.0 ± 0.7%. Total nitrogen, based on dry insulin, was identical with values obtained on the starting material,[5b,6] indicating that ammonia was not adsorbed on the molecule, nor that it had reacted with any functional group. The ultraviolet absorption spectra at *p*H 2.2 and 7.4 were identical with those of the starting material,[5b] as were solubility and behavior in the analytical ultracentrifuge as function of charge and ionic strength. Insulin treated with liquid ammonia could be recrystallized either as the zinc salt or as the acid sulfate. The biological activities of the liquid ammonia-treated samples without further purification were 24 ± 2 I.U./mg.[7]

Infrared Spectra.—Infrared spectra were determined as previously described.[8] Fluorolube mulls were used above 1380 cm.$^{-1}$, and nujol mulls between 1380 and 625 cm.$^{-1}$. Since the infrared spectra of the three starting materials were identical with those of the three liquid ammonia treated samples, one spectrum only is shown in Fig. 1 as representative for the whole group. Bands are located at 3230 and 3030 (NH stretch), 2940 (CH stretch), 1700 (hydrogen bonded CO stretch), 1636, 1538 (CO, CN stretch due to resonating peptide linkage), 1500, 1268 (CH stretch and/or NH deformation from peptide linkage), 1463 (symmetrical COO^-), 1444 (CH deformation), 1003 (CNC stretch), 976, 918 (side chain rocking, CH rocking), and 1392, 1237, 1174, 1120, 836 cm.$^{-1}$ (unassigned).[4]

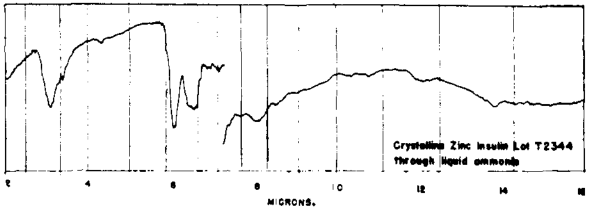

Fig. 1.—Infrared absorption spectrum of liquid ammonia-treated crystalline zinc insulin, Lot T2344.

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[2] It is planned to explore this observation further by studies employing deuterium oxide aud water containing O-18.

[3] H. Frank, personal communication.

[4] E. Ellenbogen, THIS JOURNAL, **78**, in press.

[5] (a) J. L. Oncley and E. Ellenbogen, *J. Phys. Chem.*, **56**, 85 (1952); (b) E. Ellenbogen, Ph.D. Thesis, Harvard University, 1949.

[6] E. Fredericq and H. Neurath, THIS JOURNAL, **72**, 2684 (1950).

[7] We are indebted to the Eli Lilly Co. for some of these assays.

[8] The aid of Dr. Foil A. Miller, Mellon Institute, Pittsburgh, who determined these spectra, is gratefully acknowledged.

The Preparation of Amides of Arylphosphonic Acids. III. Amides of Secondary Amines[1]

BY LEON D. FREEDMAN AND G. O. DOAK

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It long has been known that arylphosphonic dichlorides react with a wide variety of primary

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[1] Presented, in part, before the Division of Organic Chemistry at the 126th Meeting of the American Chemical Society, New York, N. Y., September, 1954.