Anal. Calcd. for $C_{12}H_{12}O_2Fe$: C, 59.0; H, 4.92. Found: C, 59.0; H, 4.94.

Preparation of Triphenylsilylferrocene and Bis-triphenylsilylferrocene.—A solution of 10 g. (0.054 mole) of ferrocene in 225 ml. of anhydrous ether was placed in a dried 3-neck flask, fitted with a dropping funnel and a Friedrichs condenser. To this was added 0.108 mole of butyllithium,[2] and the mixture was stirred for 37 hours in an atmosphere of nitrogen.

After the addition of 200 ml. of anhydrous ether, 35.3 g. (0.108 mole) of triphenylchlorosilane (*ca.* 90% pure) was added as a solid. The mixture was refluxed for three hours, stirred for an additional nine hours and hydrolyzed with 300 ml. of water.

A precipitate was filtered from the two-phase system and recrystallized from benzene to give 2.5 g. (7% conversion or 12% yield) of bis-triphenylsilylferrocene, m.p. 253–254° (cor.).

Anal. Calcd. for $C_{46}H_{38}Si_2Fe$: C, 78.62; H, 5.45. Found: C, 78.91, 79.10; H, 5.68, 5.81.

The ether layer of the filtrate was separated and evaporated to give an oil. The oil was steam distilled to remove unreacted ferrocene. The pot residue was taken up in benzene and dried with Drierite. A solid was obtained upon evaporation of the benzene. It was recrystallized from petroleum ether (b.p. 90–100°) to yield 6.50 g. (27% conversion or 49% yield) of triphenylsilylferrocene, m.p. 142–143°.

Anal. Calcd. for $C_{28}H_{24}SiFe$: C, 75.68; H, 5.44. Found: C, 75.55, 75.62; H, 5.70, 5.72.

Determination of the Dissociation Constant of Ferrocenemonocarboxylic Acid.—The *p*H of a dilute solution containing known amounts of the acid and its sodium salt was measured at 24° with a Beckman *p*H meter equipped with a shielded glass and a calomel electrode. The *p*H meter was standardized with a potassium dihydrogen phosphate–disodium hydrogen phosphate buffer[3] having a *p*H of 6.86 at 24°. The pK_a of the acid was calculated by means of the Henderson equation.[4]

Each sample of acid permitted three determinations, one after exactly half neutralization and the others at approximately three-eighths and five-eighths neutralization. The six pK_a values determined for each acid differed by 0.02 or less from the average value. The pK_a of ferrocenecarboxylic acid was found to be 6.78 while that of benzoic acid under identical conditions was 6.32.

A portion of a stock solvent was used for dissolving each acid and for the preparation of a standardized sodium hydroxide solution. This solvent was prepared by mixing at 23.5° one volume of water (distilled from a sodium hydroxide–potassium permanganate solution) with two volumes of ethyl alcohol (dried with diethyl phthalate[5] and distilled); d^{25}_4 0.8863.

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Effect of Pressure on a Plant Agglutinin[1]

BY WILLIAM C. BOYD

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The discovery of plant proteins having blood group specific agglutinating activity[2,3] for human erythrocytes suggested a determination of the inactivating effect of hydrostatic pressures. In a previous experiment[4] it had been found that hemagglutinins of human origin were inactivated by pressures, and that the so-called "blocking" type of antibody resisted inactivation up to pressures 1000 atmospheres higher than did the ordinary "saline" agglutinins for Rh. It seemed to be of some interest to determine the magnitude of the pressures required to inactivate the plant agglutinins.

Twenty-four hour treatment of a plant anti-A agglutinin with various pressures gave the following results

Pressure, atm.	3000	6000	4250
Agglutinating activity remaining, %	100	0	30

It is apparent that these plant agglutinins are inactivated by hydrostatic pressures of the same order as those which inactivate hemagglutinins of human origin (it was found previously[4] that the saline type of human agglutinins were nearly but not quite completely inactivated by being subjected to a pressure of 4000 atmospheres for 24 hours).

After exposure of another sample to a pressure of 4250 atmospheres for 48 hours, 20% of the activity was found to remain. Considering the crudeness of the method of estimating activity, this is consistent with the notion that the inactivation reaction follows a first-order course, which has been reported by other workers for pressure denaturation of various proteins.[5]

The rate of inactivation by pressure seems to increase rapidly with increasing pressure within a certain critical region, somewhat similarly to the increase in rate of heat inactivation with rising temperature, for it was found that a sample exposed to a pressure of 3000 atmospheres for 170 hours was only slightly affected compared with a control kept in contact with the outside of the apparatus.

Experimental

The plant agglutinin used was extracted from Lima beans and partially purified and concentrated[6] by precipitation in the cold at *p*H 4.5 by adding ethyl alcohol up to 20% by volume, having first removed an inactive precipitate at 10% alcohol. The 20% precipitate was dissolved in 0.15 *M* NaCl to a concentration of about 1.5% protein. About 8% of this protein was specifically precipitable with blood group A substance prepared from hog gastric mucin.

Prof. P. W. Bridgman kindly consented to apply the pressures to this material in the apparatus described by him.[7] The pressure was applied through mercury, as previously described.[4] Contact with mercury at one atmosphere had no detectable effect on the material. The material was allowed to stand under pressure at room temperature (about 25°) for 24 hours, unless otherwise specified. After the pressure treatment, the material was tested by mixing successively doubled dilutions in 0.15 *M* NaCl with human erythrocytes blood group A, centrifuging, and examining for agglutinated erythrocytes after shaking. From the degree of agglutination observed at various dilutions, an adjusted "titer" was arrived at by interpolation formulas which will be presented elsewhere. These were compared with the adjusted titers obtained with the untreated material.

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I am indebted to Prof. P. W. Bridgman for subjecting the samples to pressure, and to Mrs. Elizabeth Shapleigh for technical assistance.

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An Improved Synthesis of Perfluoroaldehydes[1]

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Published methods for the preparation of perfluoroaldehydes by reduction of the corresponding acid chloride,[2] or nitrile[3] and the oxidative nitration of 1,1,1-trifluoropropane[4] are inconvenient and give low yields of the desired aldehydes. The reduction of the corresponding perfluoro acid with lithium aluminum hydride,[2b] while more direct and convenient since it does not require the preparation of intermediates or catalysts, does not give a good yield of the perfluoroaldehyde. A substantial amount of the corresponding 1,1-dihydroperfluoro alcohol is also obtained by this reduction.

Greatly improved yields of perfluoroaldehydes have been obtained in this laboratory by the reduction of the appropriate perfluoro acids with lithium aluminum hydride by employing an inverse addition of the hydride to the acids.[5] The reductions were carried out at low temperature (−5 to 0°) using a 1:2 *M* ratio of hydride to acid and relatively concentrated ethereal solutions of the reactants. Only small quantities of the corresponding 1,1-dihydroperfluoro alcohols are obtained by this method. The liberation of the free aldehyde from its hydrate, the product of the reduction reaction, was most advantageously accomplished by adding it to a preheated mixture of phosphoric anhydride and concentrated sulfuric acid rather than either reagent alone. The dehydration medium did not thicken and the aldehyde was isolated without prolonged refluxing which leads to polymerization. Trifluoroacetic, pentafluoropropionic and heptafluorobutyric acids have been reduced by this method to give the corresponding perfluoroaldehydes in 77.5, 60 and 64% yield, respectively.

If the lithium aluminum–acid complex II is less easily reduced than the acid I, it will undergo little further attack in the absence of excess reducing agent (present in the normal order of addition) while any unreacted acid is present. When the transformation of the acid to complex II has been completed, the addition of more reducing agent will yield the aldehyde precursor III. The presence of an excess of hydride would favor further attack on III to give the alcohol precursor IV at the expense of the aldehyde. Thus the inverse order of addition favors the formation of the aldehyde.

$$\underset{\text{I}}{CF_3(CF_2)_nCOOH} \xrightarrow{LiAlH_4} \underset{\text{II}}{CF_3(CF_2)_nC(=O)OM} \xrightarrow{LiAlH_4} \underset{\text{III}}{CF_3(CF_2)_nCH(OM)_2} \xrightarrow{LiAlH_4} \underset{\text{IV}}{CF_3(CF_2)_nCH_2OM} \quad M = \frac{LiAl}{4}$$

A similar explanation has been reported[6] for the reduction of lactones to hydroxyaldehydes by lithium aluminum hydride using the inverse addition procedure.

Experimental

A solution of one mole of the perfluoro acid in 1 l. of anhydrous ether was cooled to −5° (brine-bath) in a 3-l. flask fitted with addition funnel, stirrer and condenser. The system was flushed with nitrogen while cooling. A slurry of 21.5 g. of lithium aluminum hydride in 750 ml. of anhydrous ether was added slowly with continuous stirring at −5° to 0° during 1.5 hours. Stirring was continued at −5° for one hour.

The reaction mixture was hydrolyzed with 40 ml. of water followed by 80 ml. of concentrated sulfuric acid in 200 ml. of water. The ether was decanted, and the solids remaining in the flask were dissolved in 300 ml. of water. The aqueous solution was extracted with ether, and the extracts were combined with the main ether portion and fractionally distilled to remove the solvent and alcohol leaving as a residue the crude aldehyde hydrate.

The crude aldehyde hydrate was dropped slowly into a vigorously stirred mixture of phosphorus pentoxide and concentrated sulfuric acid heated to 85–90°. The free aldehyde was collected in a suitably cooled receiver.

One mole of trifluoroacetic acid, after being subjected to the described reduction procedure, gave 21 g. of trifluoroethanol, crude, b.p. 68–85° and 110 g. of the crude aldehydrol. A 50-g. portion of the crude aldehydrol was dehydrated in a mixture of 21.6 g. of phosphorus pentoxide and 83 ml. of 96.7% sulfuric acid. There was obtained 34.5 g. of trifluoroacetaldehyde, representing a 77.5% overall yield. The aldehyde gave a 2,4-dinitrophenylhydrazone, m.p. 149°, and a hydrate, m.p. 69–70°.[2b]

Similarly treated, perfluoropropionic acid gave a 60% yield of perfluoropropionaldehyde, b.p. 1–2°, and perfluorobutyric acid gave a 64% yield of perfluorobutyraldehyde, b.p. 29°.

The physical constants observed for the aldehydes or their derivatives are in agreement with previous literature values.[2b]

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Synthesis of 8-C^{14} and of S^{35}-6-Mercaptopurine

BY GERTRUDE B. ELION AND GEORGE H. HITCHINGS

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The marked biological activities of 6-mercaptopurine[1–5] made the study of its metabolism in various species of considerable interest. For this reason

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