temperature overnight. A 1-cc. aliquot was diluted with 4 cc. of water and 0.03 cc. of 2 *N* sulfuric acid and then distilled. The distillate was found to contain 0.87 mg. of formaldehyde (83% of theory) by chromotropic acid titration.

The bulk of the reaction mixture was diluted with water and the cyano ketone separated by filtration. The crude ketone melted at 121–123° and exhibited bands in the infrared at 3.1 μ (OH), 4.58 μ (CN) and 5.80 μ (CO). It was found later that the m.p. depended on the rate of heating. Thus a sample melting ordinarily at about 125° was found to melt at 221–235° when heated very slowly to 130°. An analytical sample was prepared by recrystallization from methanol–water, m.p. 237–238°.

Anal. Calcd. for $C_{27}H_{39}O_4N$: C, 73.43; H, 8.90. Found: C, 73.82; H, 9.17.

Saponification Rate Determinations of the Diacetates of Rockogenin and 12-Epi-rockogenin.—Solutions containing 0.0960-millimole samples of the diacetates in 55 cc. of ethanol were treated with a solution containing 0.4140 meq. of lithium hydroxide and the total volume of the solutions was brought to 80 cc. with water. The temperature was maintained at 25.0 ± 0.2°. Aliquots were removed at intervals, treated with an excess of hydrochloric acid and back-titrated potentiometrically with alkali.

Rockogenin diacetate consumed one equivalent of base in about eight hours and two equivalents in four days. The diacetate of 12-epi-rockogenin, however, took up only one equivalent of alkali in 24 hours. In one experiment 0.0099 millimole of 12-epi-rockogenin diacetate dissolved in ethanol was treated with 0.0135 meq. of lithium hydroxide and diluted to 10 cc. with ethanol. After heating at 74° for six hours, 1.93 equivalents (96.5% of the theory) had been consumed.

Determination[47] of the Rates of Acetolysis of the 3-Methyl Succinate-12-mesylate Derivatives of Rockogenin and 12-Epi-rockogenin.—An 0.0041 *N* solution of IIc in acetic acid (m.p. 16.3°) was divided into aliquots which were kept frozen in sealed ampules until the beginning of the experiment. Acetolysis was carried out at 64.4 ± 0.2°. The rate of reaction was determined by potentiometric titration of 5-cc. aliquots with a solution of sodium acetate.

The first order rate constant for IIIc was similarly determined on a 0.0039 *N* solution.

Deuteration of 22a,5α-C-Nor/D-homo-18-nor-spirostane-3β-ol-17a-one (XV).—Butanol (46 cc.) was washed successively with two 24-cc. and three 10-cc. portions of deuterium oxide. A 0.054-g. sample of XIIa (m.p. 179–183°) was refluxed with 12 cc. of the above deuterized butanol in the presence of 0.240 g. of potassium hydroxide for 48 hours. The solution was concentrated *in vacuo* and cooled. A large excess of methanol was added, the solution was filtered and the product isolated by the addition of water. The infrared spectrum of the product, m.p. about 179–183°, was very similar to the starting material but differed in detail. Analysis by infrared spectroscopy of the water formed on combustion revealed the presence of 4.2 ± 0.1% D (85% of the theory for two gram atoms). XV was recovered unchanged (m.p., m.m.p., infrared spectrum) on treatment with potassium hydroxide in butanol under these conditions.

(47) See reference 27 for method.

RAHWAY, N. J.

NOTES

A Route to Monosubstituted Ferrocene Compounds

BY ROBERT A. BENKESER, DONALD GOGGIN AND GENE SCHROLL

RECEIVED APRIL 15, 1954

Thus far relatively few monosubstituted ferrocene (bis-cyclopentadienyliron) compounds have been reported.[1] We have found that ferrocene can be metalated conveniently with *n*-butyllithium to yield a mixture of the mono- and dimetalated products, with the former predominating.

The metalation mixture can be carbonated in the usual way to yield the mono- and the disubstituted acids, separable by fractional crystallization. The methyl ester of the mono-acid can be prepared in conventional fashion.

The pK_a of the mono-acid, determined in ethanol–water solution (2:1 by volume), is 6.78, while that of benzoic acid under identical conditions is 6.32. This indicates that the ferrocene acid is weaker by approximately a factor of 3 than benzoic acid.

If the metalation product is treated with triphenylchlorosilane, a mixture of mono- and bis-triphenylsilylferrocene results, which again can be separated by fractional crystallization. It would seem that this metalation procedure may thus be a key in preparing other monosubstituted ferrocene types.

It is of interest that ferrocene does undergo the metalation reaction with *n*-butyllithium. Benzene itself does not react appreciably with this reagent, indicating that the hydrogen atoms in ferrocene are more acidic.

Experimental

Preparation of Acids.—A solution of 5.0 g. (0.027 mole) of ferrocene[2] in 75 ml. of anhydrous ether was placed in an oven-dried 3-neck flask fitted with a stirrer, a dropping funnel and a water-cooled condenser. To this 0.08 mole of *n*-butyllithium was added dropwise with stirring. The stirring was stopped after one hour and the mixture was allowed to stand for 24 hours under an atmosphere of nitrogen.

The reaction mixture was poured jetwise with stirring into a Dry Ice ethereal slush. Cold water was added cautiously after the Dry Ice had disappeared and the two layers were separated. The water layer was acidified with 6 *N* hydrochloric acid whereupon 0.4 g. of a mixture of crude acids precipitated out. An elemental analysis of the mixture indicated it to be approximately 70% ferrocenemonocarboxylic acid and 30% dicarboxylic acid. On this basis, the reaction gave a 65% total yield of acids, or a 35% conversion.

The crude acids were separated by fractional crystallization from glacial acetic acid. The reddish brown crystals of the mono-acid did not melt up to 200° and then slowly decomposed.

Anal. (monocarboxylic acid) Calcd. for $C_{11}H_{10}O_2Fe$: C, 57.4; H, 4.29. Found: C, 57.0; H, 4.36.

Preparation of Methyl Esters.—Each acid was esterified, using methanol and a trace of mineral acid. The esters were recrystallized from a methanol–water mixture. The methyl ester of the dicarboxylic acid melted at 114–115°.[1] The methyl ester of ferrocenemonocarboxylic acid melted at 70–71°.

(1) R. B. Woodward, M. Rosenblum and M. C. Whiting, THIS JOURNAL, **74**, 3458 (1952); P. L. Pauson, *ibid.*, **76**, 2187 (1954).

(2) T. J. Kealy and P. L. Pauson, *Nature*, **168**, 1039 (1951). See also G. Wilkinson, *et al.*, THIS JOURNAL, **74**, 2125 (1952).

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.

(3) G. G. Manov, "Symposium on *p*H Measurement," Tech. Bull. No. 73, Am. Soc. Testing Materials, Philadelphia, Pa., p. 31.

(4) S. Glasstone, "Textbook of Physical Chemistry," D. Van Nostrand Co., Inc., New York, N. Y., 1940, p. 982.

(5) R. H. Manske, THIS JOURNAL, **53**, 1106 (1931).

DEPARTMENT OF CHEMISTRY
PURDUE UNIVERSITY
LAFAYETTE, INDIANA

Effect of Pressure on a Plant Agglutinin[1]

BY WILLIAM C. BOYD

RECEIVED APRIL 1, 1954

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.

(1) The work reported in this paper was made possible by support extended to Boston University by the United States Atomic Energy Commission, under contract no. AT(30-1)-1395, the Navy Department (Office of Naval Research) under contract no. Nonr-492(01) and by a research grant (H-1076(2)) from the National Heart Institute, of the National Institutes of Health, Public Health Service.

(2) K. O. Renkonen, *Ann. med. exp. Biol. Fenn.*, **26**, 66 (1948).

(3) W. C. Boyd and R. M. Reguera, *J. Imm.*, **62**, 333 (1949).

(4) W. C. Boyd, *J. Exp. Med.*, **83**, 401 (1946).

(5) F. W. Putnam, Protein denaturation, in "The Proteins," Ed. H. Neurath and K. Bailey, Vol. IB, Academic Press, New York, N. Y., 1953, p. 807–892.

(6) W. C. Boyd and E. Shapleigh, *J. Immunol.*, in press (1954).

(7) P. W. Bridgman, "The Physics of High Pressure," G. Bell and Sons, London, 1931.