148–155°. Further cooling and volume reduction gave 2.4 g. of material melting over the range 145–155°. Fractional crystallization of the above materials from petroleum ether (b.p. 77–115°) gave 3.6 g. (37%) of triphenylgermyldiphenylcarbinol melting at 153–155° and 1.0 g. (16%) of hexaphenyldigermoxane (mixed melting point) melting at 184–186°.

A repetition of the above reaction gave 1.1 g. (16.3%) of hexaphenyldigermoxane[10] melting at 185–187° and 1.0 g. (10.3%) of triphenylgermyldiphenylcarbinol melting at 153–155°.

Anal. Calcd. for $C_{31}H_{26}OGe$: Ge, 14.91. Found: Ge, 14.93, 15.21.

Preparation of Triphenylgermyldiphenylcarbinol.—To 22 ml. of an ether solution of phenylmagnesium bromide prepared from 0.34 g. (0.014 g. atom) of magnesium and 2.17 g. (0.014 mole) of bromobenzene was added dropwise 2.0 g. (0.0055 mole) of methyl triphenylgermanecarboxylate dissolved in 50 ml. of ether. After refluxing for 4 hours the mixture was hydrolyzed by pouring it into a mixture of ammonium chloride and cracked ice. The ether layer was separated and the aqueous portion washed three times with ether. The combined ether portions were dried over anhydrous sodium sulfate and the solvent was distilled to leave a residue which was crystallized from petroleum ether (b.p. 60–70°) to give 1.54 g. (64%) of impure triphenylgermyldiphenylcarbinol melting over the range 143–153°. Purification was extremely difficult due to the instability of the material. After numerous crystallizations from ethanol and petroleum ether (b.p. 60–70°) the melting point was raised to 153–155°. A mixed melting point with the product from the addition of triphenylgermyllithium to benzophenone showed no depression and the infrared spectra were identical, showing a typical hydroxyl absorption. On standing in air the melting point of this compound was lowered, indicating that some change was taking place.

A repetition of the above experiment using phenyllithium in place of the phenylmagnesium bromide gave 1.7 g. (71%) of impure triphenylgermyldiphenylcarbinol melting over the range 142–147°. Repeated recrystallization from petroleum ether (b.p. 60–70°) gave 0.6 g. (25%) of product melting at 154–155°. Again a mixed melting point with the product from the reaction of triphenylgermyllithium with benzophenone showed no depression and the infrared spectra were identical.

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(10) If any benzohydryloxytriphenylgermane were formed in this reaction, it is probable on the basis of some other unpublished studies that it would be hydrolyzed under these experimental conditions of working up the mixture to give hexaphenyldigermoxane.

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Benzhydrylmagnesium Bromide from Potassium Diphenylmethide and Magnesium Bromide

BY CHARLES R. HAUSER AND DAVID S. HOFFENBERG[1]

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Because of its tendency to couple to form tetraphenylethane, benzhydryl bromide or chloride has been difficult to convert satisfactorily to the Grignard reagent.[2] Even when the reagent was prepared from the bromide in a high dilution cyclic reactor and then carbonated, only a 25% yield of diphenylacetic acid was obtained.[3]

We have prepared benzhydrylmagnesium bromide by a new method in which the coupling reaction was avoided. This consisted in metalating diphenylmethane with potassium amide in liquid ammonia, replacing the ammonia with ether and treating the resulting red suspension of potassium diphenylmethide with magnesium bromide in the latter solvent (equation 1). In the last step the red color characteristic of the diphenylmethide ion disappeared rapidly indicating the formation of the more covalent carbon–magnesium bond.

$$(C_6H_5)_2CH_2 \xrightarrow[2,\ \text{ether}]{1,\ KNH_2\ (\text{liq. } NH_3)} (C_6H_5)_2CHK \xrightarrow[\text{ether}]{MgBr_2} (C_6H_5)_2CHMgBr \quad (1)$$

The reagent prepared in this manner was shown to undergo a reaction characteristic of the Grignard reagent but not of potassium diphenylmethide. Thus, it reacted with α-amino ether I to form tertiary amine II in 82% yield, whereas the potassium reagent failed to react under similar conditions. This yield was based on amino ether I since, as usual, an excess of the reagent was employed. Recently[4] a somewhat lower yield (50%) of II was obtained with benzhydrylmagnesium chloride prepared from a large excess of benzhydryl chloride and powdered magnesium.

$$(C_6H_5)_2CHMgBr + \underset{\text{I}}{C_4H_9O{-}CHC_6H_5} \longrightarrow \underset{\text{II}}{(C_6H_5)_2CH{-}CHC_6H_5} + MgBrOC_4H_9$$

(N(CH₃)₂ in I; N(CH₃)₂ in II)

It should be pointed out that for many reactions there is no advantage in converting the potassium diphenylmethide to the Grignard reagent since equally good yields may be obtained with the potassium reagent. For example, potassium diphenylmethide has been carbonated to form diphenylacetic acid in 90% yield.[5]

Experimental

An ether suspension of potassium diphenylmethide was prepared from 0.11 mole of potassium amide and 0.10 mole of diphenylmethane essentially as described previously.[5] The potassium amide was obtained from 4.4 g. (0.11 g. atom) of potassium, a crystal of ferric nitrate and 300 ml. of liquid ammonia. After adding the diphenylmethane, the ammonia was replaced by an equal volume of ether, and the resulting suspension refluxed until practically all of the ammonia had been removed (1 hour).

To the stirred red suspension was added during 15–20 minutes a magnesium bromide–ether mixture which was added through a stopcock attached to the bottom of the flask in which it was prepared, the potassium diphenylmethide reagent being decolorized rapidly. The magnesium bromide–ether mixture was prepared essentially as described by Swain and Boyles[6] by adding dropwise with stirring 17.0 g. (0.105 mole) of bromine to 3.0 g. (0.12 g. atom) of magnesium turnings and 300 ml. of ether, the stirring being continued until the bromine color disappeared (about 15 minutes).

To the resulting gray benzhydrylmagnesium bromide re-

(1) Allied Chemical and Dye Corp. Fellow, 1954–1955.

(2) See H. Gilman and J. E. Kirby, THIS JOURNAL, **48**, 1773 (1926).

(3) D. C. Rowlands, K. W. Greenlee and C. E. Boord, Abstracts of Papers, 117th Natl. Meeting, Am. Chem. Soc., Philadelphia, Pa., April 9–13, 1950, p. 8-L.

(4) A. T. Stewart and C. R. Hauser, THIS JOURNAL, **77**, 1098 (1955).

(5) R. S. Yost and C. R. Hauser, *ibid.*, **69**, 2325 (1947).

(6) C. G. Swain and H. B. Boyles, *ibid.*, **73**, 870 (1951).

agent (containing suspended potassium bromide) was added with stirring 10.0 g. (0.0485 mole) of α-amino ether I in 50 ml. of ether. After stirring for 4 hours the reaction mixture was poured onto iced hydrochloric acid, and the resulting precipitate collected on a funnel (sucked dry). This crude hydrochloride was warmed on a steam-bath with an excess of sodium carbonate solution until carbon dioxide ceased to be evolved. The liberated amine was collected on a funnel, washed with water, dried, and recrystallized from petroleum ether (cooled in Dry Ice) to give 11.0 g. (76%) of tertiary amine II, m.p. 125–126°, reported m.p. 126.5–127.5°.[4] More (0.8 g.) of II, m.p. 125–126°, was isolated from the filtrate; total yield 82%. The melting point was not depressed on admixture with an authentic sample of II.[4]

When an experiment was carried out with potassium diphenylmethide and amino ether I under similar conditions, the red color was not discharged and no precipitate of the amine hydrochloride was obtained on pouring the reaction mixture into iced hydrochloric acid. A similar observation has been reported.[4]

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Chromatography of Chymotrypsin-α

BY C. H. W. HIRS

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This investigation has been carried out to determine the chromatographic behavior of chymotrypsin-α on ion-exchange columns under conditions similar to those which have proved suitable for the chromatography of chymotrypsinogen.[1] The curve shown in Fig. 1 was obtained when a sample of crystalline chymotrypsin-α (Worthington Biochemical Corp., Freehold, New Jersey), prepared by the method of Kunitz,[2] was chromatographed at 25° on a 0.9 × 30 cm. column of the sodium form of Amberlite IRC-50 (XE-64) using as eluent a 0.2 *M* phosphate buffer at *p*H 6.02. The enzyme emerges at about the same effluent volume observed previously for the zymogen under the same conditions.[1] The recovery of chymotrypsin activity in the single large peak at 25 effluent ml. was quantitative within the limits of precision of the spectrophotometric assay method of Kunitz[3] in which casein is used as substrate. The recovery of ninhydrin color varied from 95 to 112%[4] when the more rapidly eluted inactive components were included. These fast moving peaks probably represent some of the split products formed during tryptic activation of chymotrypsinogen, for Rovery, Fabre and Desnuelle,[5] using the DNP technique, have demonstrated the presence in chymotrypsin-α of adsorbed peptides not removable by crystallization, dialysis, or trichloroacetic acid precipitation.

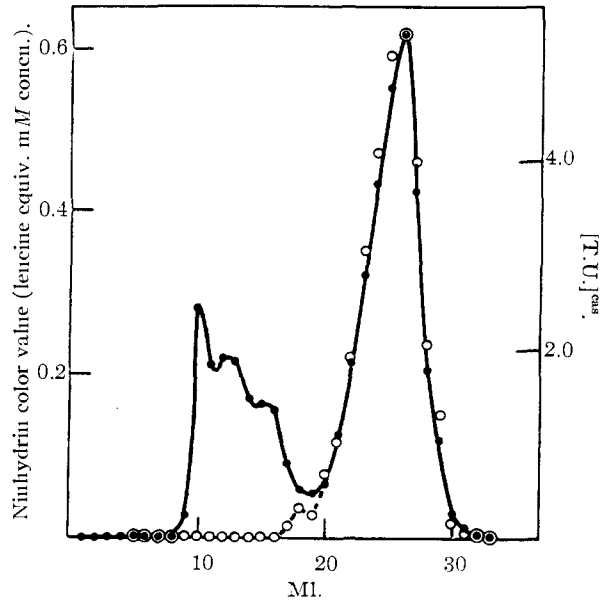

Fig. 1.—Chromatography of crystalline chymotrypsin-α (6.5 mg.) on a 0.9 × 30 cm. column of IRC-50 (XE-64). Elution was performed with a 0.2 *M* phosphate buffer at *p*H 6.02 at a rate of 2 ml. per hour. The effluent was collected in 1-ml. fractions, and aliquots were pipetted for ninhydrin analyses and for determinations of enzymatic activity (T.U. = trypsin units)[2] using Hammarsten's casein as substrate; ●—●, ninhydrin color; O—O, enzymatic activity.

Under the conditions employed for the chromatogram shown in Fig. 1, the enzyme and the zymogen would not be well separated. By the use of a slightly more acidic (*p*H 5.67) citrate buffer as elu-

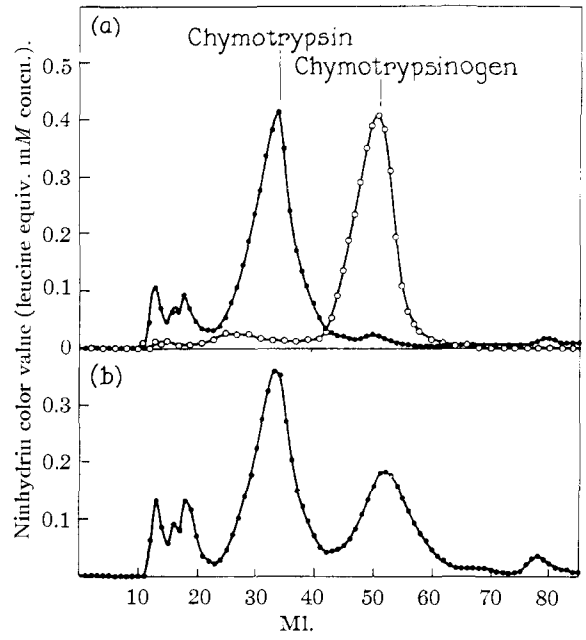

Fig. 2.—Chromatography of chymotrypsin and chymotrypsinogen on a 0.9 × 30 cm. column of IRC-50 (XE-64). Elution was performed with a citrate buffer at *p*H 5.67, 0.1 *M* in respect to citric acid and 0.255 *N* in respect to sodium, at a rate of 2 ml. per hour. The effluent was collected in 1-ml. fractions; ●—●, chymotrypsin; O—O, chymotrypsinogen. Figure 2a, chymotrypsin-α (9.0 mg.) and chymotrypsinogen (10.5 mg.) chromatographed in separate experiments; Figure 2b, chromatography of a mixture of chymotrypsin α (8.0 mg.) and chymotrypsinogen (5.7 mg.).

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(2) M. Kunitz, *J. Gen. Physiol.*, **32**, 265 (1948).

(3) J. H. Northrop, M. Kunitz and R. M. Herriott, "Crystalline Enzymes," Columbia University Press, New York, N. Y., 2nd Edition, 1948.

(4) Some autodigestion of the enzyme may occur during chromatography or while the effluent fractions are on the fraction collector.

(5) M. Rovery, C. Fabre and P. Desnuelle, *Biochim. Biophys. Acta*, **12**, 547 (1953).