cinchonine hydrogen salt was stirred and intimately mixed with 90 ml. of cold 2 *N* hydrochloric acid at −5 to −10°. The mixture was filtered (sintered glass funnel) and the residual dicarboxylic acid was macerated with an additional 100 ml. of cold 2 *N* hydrochloric acid at −5° for a period of 15–20 minutes. Filtration was followed by washing the cold residue with an additional 100 ml. of cold 3 *N* hydrochloric acid and then with three successive 100-ml. portions of cold water (0°). The residual cold dicarboxylic acid was then dissolved in 25 ml. of cold (−5 to 0°) 0.5 *N* ammonia in 5% aqueous urea solution and filtered. The resulting solution of the ammonium salt of 2,7-dihydro-3,4-5,6-dibenzothiepin-1-dioxide-2′,3″-dicarboxylic acid when examined in a 2 dm. polarimeter tube, had $\alpha^{29}\text{D}$ +0.14°; this corresponds to $[\alpha]^{29}\text{D}$ +1.81° (*l* 4 cm.; *c* 1.936 g., diammonium salt, 0.5 *N* ammonia in 5% aqueous urea) for the diammonium salt of the acid.

In exactly the same manner as that just described, the more dextrorotatory fraction (0.860 g.) gave a diammonium salt in 25 ml. of 0.5 *N* ammonia and 5% urea having $\alpha^{25}\text{D}$ +0.225° when examined in a 4 dm. macro polarimeter tube; this corresponds to $[\alpha]^{25}\text{D}$ +3.1° (*l* 4 dm.; *c* 1.828 g., 0.5 *N* ammonia in 5% aqueous urea) for the acid, or expressed in respect to the diammonium salt, $[\alpha]^{25}\text{D}$ +2.85° (*l* = 4 dm.; *c* 1.98 g., diammonium salt; 0.5 *N* ammonia in 5% aqueous urea).

Since there is abundant evidence that in most simple cinchonine salts optical activity is relatively additive,[23] it would appear that the material is extensively racemized during the conversion of the cinchonine acid salt to the ammonium salt. This is confirmed by the difficulty in obtaining an active acid from the brucine salt (see above), by the fact that, effectively, only one (the dextrorotary) antipode can be isolated from recrystallization of the acid cinchonine salt.

Anal. Calcd. for $C_{19}H_{22}N_2O \cdot C_{16}H_{12}SO_6 \cdot 2H_2O$: C, 63.42; H, 5.78; N, 4.23. Found: C, 63.70; H, 5.50; N, 4.28; $[\alpha]^{23}\text{D}$ +90°.

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(23) H. Landolt, ref. 21, p. 681; A. C. Oudemans, *Ann.*, **182**, 55 (1876). Anomalous activities are sometimes observed with alkaloid salts, especially salts, of unsaturated acids, *cf.* T. P. Hilditch, *J. Chem. Soc.*, **93**, 1388 (1908); **95**, 335, 1570 (1909); **99**, 224 (1911).

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[CONTRIBUTION NO. 389 FROM THE CHEMICAL DEPARTMENT, EXPERIMENTAL STATION, E. I. DU PONT DE NEMOURS AND CO.]

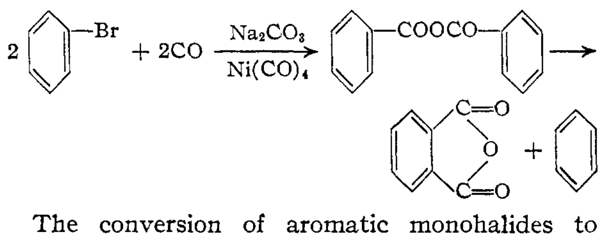
Reactions of Carbon Monoxide at High Temperature. I. A New Synthesis of Phthalic Anhydrides

BY W. W. PRICHARD

RECEIVED JUNE 27, 1956

A mixture of benzoic anhydride, phthalic anhydride and benzene is formed by the reaction of bromo- or chlorobenzene, carbon monoxide and alkali metal carbonates or orthophosphates in the presence of nickel tetracarbonyl. Under optimum conditions, phthalic anhydride is obtained in 75% conversion. Benzoic anhydride may be the immediate precursor of phthalic anhydride, as shown by its conversion to phthalic anhydride and benzene by heating with nickel carbonyl and carbon monoxide at 250–375° and 300–600 atm. Certain substituted phthalic anhydrides have been prepared from substituted aromatic halides and from aromatic acid anhydrides. Under similar conditions N-phenylphthalimide and benzene are formed from N,N-dibenzoylaniline.

During an investigation of high-temperature reactions of carbon monoxide with aromatic halides, it was found that chlorobenzene, carbon monoxide and nickel carbonyl gave traces of benzoyl chloride, a small amount of benzoic anhydride and much tar. When anhydrous sodium fluoride was added in molar amounts, the product contained benzoyl fluoride in up to 27% conversion and tar formation was suppressed.[1] An attempt to extend this reaction to the synthesis of tribenzoyl phosphate, by substituting dry trisodium phosphate for the sodium fluoride, resulted in a mixture of benzoic anhydride, phthalic anhydride and benzene. Since the conversion of a monosubstituted aromatic nucleus to an aromatic dicarboxylic acid derivative was an unexpected result, the reaction was studied in detail.



The conversion of aromatic monohalides to phthalic anhydrides occurred when stoichiometric amounts of the aromatic halide and certain inorganic salts were heated at 250–375°, under carbon monoxide pressure, in the presence of nickel carbonyl.[2] The nature of the inorganic salt was very important, since good yields of phthalic anhydride were obtained only with the alkali metal carbonates and orthophosphates. Traces of phthalic anhydride were formed with calcium phosphate and sodium fluoride.

Nickel tetracarbonyl appeared to be necessary for the synthesis of phthalic anhydride, since no phthalic anhydride was obtained from bromobenzene, carbon monoxide and sodium carbonate with either iron or cobalt carbonyl. Precursors of nickel carbonyl such as Raney nickel and nickel chloride were equivalent to the carbonyl.

The reaction of chloro- or bromobenzene with carbon monoxide and sodium carbonate was sufficiently exothermic to cause the temperature of the reaction mixture to exceed 400°. The products from such overheated reactions were black and difficult to purify. Dilution of the aromatic halide with three times its weight of an inert diluent such as toluene or cyclohexane not only eliminated this flash to extreme temperatures, but also increased the ratio of phthalic to benzoic anhydride in the product from 1:1 in the undiluted system to 15:1. The amount of non-distillable tarry by-products in the diluted system was only 0.3% of the weight of bromobenzene.

Nearly stoichiometric ratios of alkali metal to halide must be present in the reaction mixture to obtain maximum yields. Variation of the carbon

(1) W. W. Prichard, U. S. Patent 2,696,503, Dec. 7, 1954.

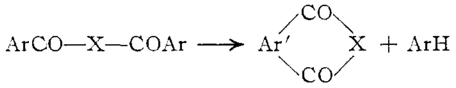
(2) W. W. Prichard, U. S. Patent, 2,680,751, June 8, 1954.

monoxide pressure from 100 to 600 atm. did not affect the reaction. The optimum temperature for reaction of bromobenzene was 275°, while chlorobenzene gave best results at 325°.

Certain substituted phthalic anhydrides were prepared from substituted aromatic halides (Table I). The yields varied greatly with the particular compound employed.

Course of the Reaction.—Since benzoic anhydride and benzene were always present in the reaction product, and since the amount of benzene was roughly equivalent to that of the phthalic anhydride produced, it seemed probable that benzoic anhydride was the immediate precursor of phthalic anhydride. Heating benzoic anhydride with nickel carbonyl under carbon monoxide pressure readily converted it to phthalic anhydride and benzene.[3] When benzoic anhydride was heated with a metallic nickel catalyst in the absence of carbon monoxide, no phthalic anhydride was formed. It was thus possible that carbon monoxide was actually a reactant in the conversion of benzoic anhydride to phthalic anhydride and that one or both of the carbonyl groups in the phthalic anhydride came from the carbon monoxide. In an attempt to demonstrate this, a sample of benzoic anhydride tagged with C^{14} in both carbonyl carbons was prepared and heated with carbon monoxide and nickel carbonyl. The radioactivity of the resulting phthalic anhydride was equal to that of the recovered benzoic anhydride. The activity of each, however, had decreased to the extent required for complete equilibration between the anhydride carbonyl groups and the gaseous carbon monoxide present in the reaction vessel. Since equilibration could have occurred before, after or during the conversion of the benzoic anhydride to phthalic anhydride, the function of the carbon monoxide in the reaction is not explained.

Cyclization of Imides.—It was of interest to determine the scope of this new type of reaction in which Ar represents an aryl group having an unsubstituted *o*-position and Ar′ the corresponding group in which the *o*-hydrogen atom has been displaced.



$$\underset{\text{I}}{\text{ArCO—X—COAr}} \longrightarrow \underset{\text{II}}{\text{Ar}'\begin{matrix}\diagup\text{CO}\diagdown\\ \\ \diagdown\text{CO}\diagup\end{matrix}\text{X}} + \text{ArH}$$

I II

N,N-Dibenzoylaniline was chosen as an analog of benzoic anhydride (I, X = NC_6H_5). Heating of this imide with carbon monoxide and nickel carbonyl at 325° gave N-phenylphthalimide (II, X = NC_6H_5) and benzene. Similarly, an equimolar mixture of benzonitrile and benzoic acid (which are known to react to form dibenzamide at temperatures above 250°)[4] when heated at 325° with carbon monoxide and nickel carbonyl gave phthalimide. Similar treatment of dibenzoylmethane (I, X = CH_2) failed to yield the desired diketohydrindene.

Acknowledgment.—The assistance of Dr. J. H. Peterson of this Laboratory in planning the experiments with radioactive tracers and in measuring the activity of tagged compounds is gratefully acknowledged.

Experimental

All reactions were carried out in silver-lined 400-ml. shaker tubes. The reactants were charged into the tube under nitrogen, the tube sealed, cooled to −40° and evacuated. The vessel was then pressured with carbon monoxide and heated to reaction temperature. After the desired reaction period, the shaker tube was cooled to room temperature, bled to atmospheric pressure, opened, and discharged. A preliminary separation of the products was obtained by distilling the crude reaction mixture from a large-bore tube heated by refluxing xylene vapors, first at atmospheric pressure to remove low-boiling materials (cut 1) and then at 10–20 mm. (cut 2). The pressure was then further reduced to 1 mm. (cut 3), and finally all materials volatile at 255° and 1 mm. pressure were distilled by using refluxing Dowtherm A as a heating bath (cut 4). A non-volatile residue composed of tars and inorganic salts remained in the distillation vessel. This preliminary separation greatly facilitated the purification of the products.

Phthalic Anhydride from Bromobenzene.—A mixture of 39.2 g. (0.25 mole) of bromobenzene, 120 g. of toluene, 13.25 g. (0.125 mole) of sodium carbonate and 2.5 g. of nickel carbonyl, was charged into the shaker tube, which was pressured at room temperature to 100 atm. with carbon monoxide and heated at 275° for 2 hours. The reaction mixture contained 112.4 g. of volatile material and 24 g. of non-volatile solid. All but 0.1 g. of the non-volatile solid was water soluble. The *p*H of the aqueous solution was 4.0. Addition of hydrochloric acid precipitated 0.5 g. (0.003 mole) of phthalic acid. The volatile portion of the product contained recovered toluene, 7 g. (0.089 mole) of benzene, 13.5 g. (0.092 mole) of phthalic anhydride and 0.8 g. (0.003 mole) of benzoic anhydride. The total conversion of bromobenzene to phthalic anhydride and phthalic acid was 76%. The use of chlorobenzene in place of bromobenzene at a reaction temperature of 325° gave a slightly lower yield of phthalic anhydride accompanied by a larger amount of tarry by-products.

TABLE I

CONVERSION OF SUBSTITUTED AROMATIC HALIDES TO DICARBOXYLIC ACID ANHYDRIDES

Halide	Inorg. salt	Temp., °C.	Dicarboxylic acid anhydride isolated	M.p.,[a] °C.	Conv., %
p-Chlorotoluene	Na_2CO_3	325	4-Methylphthalic	88.5–89[b]	20
o-Bromotoluene	Na_3PO_4	325	3-Methylphthalic	115–116[c]	45
p-Bromodiphenyl	Na_2CO_3	275	4-Phenylphthalic	141–142[d]	36
α-Chloronaphthalene	Na_2CO_3	325	Naphthalic	272–275[e]	4

[a] All m.p.'s taken on block between cover-slips and are uncorrected. [b] Reported m.p. 92°, S. Niementowski, *Monatsh.*, **12**, 626 (1891). Hydrolysis gave 4-methylphthalic acid, m.p. 152–153° dec.; reported m.p. 152° dec. [c] Reported m.p. 114–115°, V. Jurgens, *Ber.*, **40**, 4413 (1907); 118–119°, E. D. Parker and L. A. Goldblatt, THIS JOURNAL, **72**, 2155 (1950). [d] Reported m.p. 138–139°, E. C. Butterworth, I. M. Heilbron, D. H. Hey and R. Wilkenson, *J. Chem. Soc.*, 1386 (1938). [e] Reported m.p. 272–274°, H. H. Hodgson and E. R. Wood, *ibid.*, 590 (1954). *Anal.* Calcd. for $C_{12}H_6O_3$: C, 72.73; H, 3.05. Found: C, 72.69; H, 3.10.

Substituted Phthalic Anhydrides.—Utilization of this reaction for the preparation of several substituted phthalic anhydrides is summarized in Table I. The formation of the anhydride of 1,8-naphthalenedicarboxylic acid as the only dicarboxylic acid derivative from α-chloronaphthalene in preference to the corresponding anhydride of 1,2-naphthalenedicarboxylic acid is interesting.

Conversion of Monocarboxylic Acid Anhydrides to Dicarboxylic Acid Anhydrides.—A solution of 25 g. of benzoic anhydride and 5 g. of nickel carbonyl in 100 g. of toluene was pressured to 100 atm. with carbon monoxide and heated at 325° for 2 hours. Distillation of the product gave 3 g. of recovered nickel carbonyl, 3.4 g. of benzene, 91 g. of recovered toluene, 10.1 g. of phthalic anhydride, 1.4 g. of recovered benzoic anhydride and 3.6 g. of non-volatile residue. This represents a 61.8% conversion to phthalic anhydride and benzene.

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[3] W. W. Prichard, U. S. Patent 2,680,750, June 8, 1954.

[4] C. E. Colby and F. D. Dodge, *Am. Chem. J.*, **13**, 1 (1891).

A solution of 78 g. of anisic anhydride and 5 g. of nickel tetracarbonyl in 80 g. of benzene reacted under the same conditions to give 43 g. (88.6% conversion) of 4-methoxyphthalic anhydride, and 20 g. (68%) of anisole. The anhydride was identified by its melting point of 94–95° and by hydrolysis to 4-methoxyphthalic acid, m.p. 171–172°.[5]

Tracer Studies.—A sample of C^{14}-tagged benzoic anhydride with an activity of 2.08×10^6 counts/min./mmole was prepared from carboxyl tagged benzoic acid. A silver-lined shaker tube was charged with 15 g. (0.0664 mole) of the anhydride, 100 g. of benzene and 3 g. of $Ni(CO)_4$. The tube was sealed, pressured with CO to 100 atm. (total CO estimated as 1.26 moles including that bound as $Ni(CO)_4$) and heated at 300° for 2 hours. The product consisted of 4.3 g. of phthalic anhydride and 7.7 g. of recovered benzoic anhydride. The molar activity of the phthalic anhydride was 1.99×10^5 counts/min./mmole or 9.5% of the activity of the original benzoic anhydride. The recovered benzoic anhydride gave a value of 2.02×10^5 counts/min./mmole or 9.7% of the original value. Since a total of 1.39 eq. of carbonyl groups was charged, of which 0.133 was in the original tagged benzoic anhydride, complete equilibration with gaseous CO would reduce the molar activity of the benzoic anhydride to 9.57% of the original activity. In a second experiment, 0.044 mole of benzoic anhydride was treated with 3.09 moles of CO under similar conditions. The recovered benzoic anhydride showed a specific activity which was 2.26% of the original value. The value for complete equilibration should be 2.77%, so that agreement is within the limits of accuracy by which the equivalents of CO charged were measured.

Conversion of N,N-Dibenzoylaniline to N-Phenylphthalimide.—A solution of N,N-dibenzoylaniline (8 g.) in 200 g. of toluene containing 5 g. of $Ni(CO)_4$ was heated at 325° under 200 atm. CO pressure for 2 hours. The product contained 3.6 g. of N-phenylphthalimide (m.p. 203° after recrystallization from chloroform–methanol) in addition to recovered N,N-dibenzoylaniline. A mixed melting point with an authentic sample of N-phenylphthalimide, melting at 203°, was not depressed.

Anal. Calcd. for $C_{14}H_9O_2N$: C, 75.32; H, 4.06; N, 6.28. Found: C, 74.98; H, 4.13; N, 6.32.

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[5] 4-Methoxyphthalic anhydride is reported to melt at 93–96° and 4-methoxyphthalic acid at 168–170°; M. Freund and E. Göbel, *Ber.*, **30**, 1932 (1897).

[CONTRIBUTION FROM THE DAJAC LABORATORIES OF THE CHEMICAL DIVISION OF THE BORDEN COMPANY, AND THE DEPARTMENTS OF SURGERY, SINAI HOSPITAL OF BALTIMORE AND THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE]

Syntheses of Some *p*-Nitrophenyl Substituted Tetrazolium Salts as Electron Acceptors for the Demonstration of Dehydrogenases[1]

BY KWAN-CHUNG TSOU, CHAO-SHING CHENG, MARVIN M. NACHLAS AND ARNOLD M. SELIGMAN

RECEIVED JULY 23, 1956

A number of *p*-nitrophenyl substituted tetrazolium salts have been synthesized for the histochemical demonstration of dehydrogenase in tissues. One of these, 2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride (XA, "nitro-BT") (Table IV) was found to be superior to any tetrazole so far developed for histochemistry and makes possible the demonstration of the dehydrogenases at the cytological level. It was also found that during the coupling of a tetrazotized biphenylenediamine with a phenylhydrazone, one of the diazonium groups could be replaced by hydrogen to form a phenyl group. Thus, in the formation of the above-mentioned ditetrazolium salt, a monotetrazolium salt, 2-*p*-nitrophenyl-5-phenyl-3-(3,3′-dimethoxy-4-biphenylyl)-tetrazolium chloride (IIIA) (Table III), was obtained concomitantly. The isolation of the mono- and diformazans is described and mechanisms for the formation of the monoformazans are discussed. The nature of the monotetrazole that is found as a contaminant in commercial preparations of ditetrazoles is thus ellucidated.

Tetrazolium salts form a unique class of oxidation–reduction indicators in the study of dehydrogenases.[2] The advantages of tetrazolium salts over the classical methylene blue reaction arise from the facts that (1) with the former compounds a color is produced on reduction (Fig. 1), while in the latter a decoloration occurs and (2) the formazans are not readily reoxidized in air, in contrast to methylene blue. The tetrazolium salts should have certain other properties in order to serve as an ideal electron acceptor for histochemical purposes; *i.e.*, (1) ease of reduction, (2) low lipid solubility (3) low light sensitivity, (4) amorphous or fine granular particle size, (5) insolubility in aqueous solution, (6) low solubility in common organic solvents and (7) aerobic as well as anaerobic reduction. In spite of the fact that a large number of tetrazolium salts have been synthesized in recent years,[3] one possessing all the desired features listed above has not yet been described.

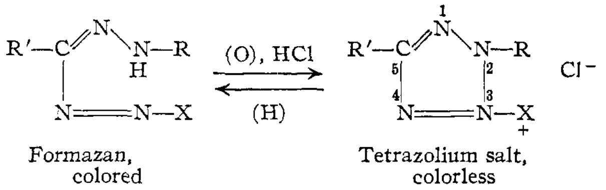

Fig. 1.

Among the commonly used biological tetrazolium salts[4] are: "TTC" (2,3,5-triphenyltetrazolium chloride), "BT" (2,2′,5,5′-tetraphenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-ditetrazolium chloride), "NT" (2,2′,5,5′-tetraphenyl-3,3′-(*p*-biphenylene)-ditetrazolium chloride), and "INT" (3-*p*-iodophenyl-2-*p*-nitrophenyl-5-phenyltetrazolium chloride). The last reagent was found to reduce easily but was lacking in many of the other features essential for histochemical use; in fact, its crystalline nature resulted in the destruction of cellular detail in the tissue section. Since the introduction of the nitro group in "INT" markedly increased its ability to serve as an electron acceptor, and since it was known that the less soluble diformazans yielded blue rather than red pigments, it

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[1] This investigation was supported by research grants (C-2530 and C-2478) from the National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare.

[2] H. A. Hölscher, *Z. Krebborsch*, **56**, 587 (1900); A. M. Seligman and A. M. Rutenberg, *Science*, **113**, 317 (1951); A. M. Rutenburg, R. Gofstein and A. M. Seligman, *Cancer Research*, **10**, 113 (1950).

[3] A. W. Nineham, *Chem. Revs.*, **55**, 355 (1955).

[4] N. D. Cheronis and H. Stein, *J. Chem. Ed.*, **33**, 120 (1956).