THE SYNTHESIS OF 2-C^{14} LABELED DDT

by

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I. INTRODUCTION

The compound 2,2-bis-(p-chlorophenyl)1,1,1-trichloroethane, commonly known as DDT, was first synthesized in Germany in 1874 (17, pp.1180-1181), however the insecticidal properties of this compound were not discovered until 1939 (16, p.1). Since then DDT has had unprecedented development as a synthetic insecticide due to its unusual properties of a wide range of insecticidal action (both contact and residual), a simple structure promoting ready synthesis, stability to light and air resulting in enduring residual toxicity and low mammalian toxicity. American domestic consumption had reached 102,000,000 pounds in 1951.

Much work has been attempted in order to elucidate the mode of the insecticidal action of DDT and its metabolism in both insects and mammals (10, pp.47-64). These problems became more pressing when workers recently found that various insects were able to build up an immunity to an extent of several hundred times the normal lethal dose of DDT (11, pp.21-25). Nevertheless, very little was learned concerning these problems mainly due to the difficulties involved in following the translocation and the metabolism of a minute amount of this insecticide in living bodies by conventional methods. It was not until radioactive isotopes became available that a tool was provided which might be used to obtain this information. Not only could the

distribution of the labeled DDT to be followed in the organism's body but many of the degradation products could also be located, isolated and identified.

Hansen et al. in 1944 (7, pp.853-855) and Winteringham et al. in 1951 (16, pp.106-107) synthesized the radioactive bromine analog of DDT in which the two para chlorine atoms had been replaced by radioactive bromine-82 atoms. Various metabolic studies were made using this compound, however, the extent of this work was limited by the relatively short half life of bromine-82. Furthermore, the para bromophenyl analog of DDT did not behave exactly in the same manner as did DDT in respect to the toxicity, solubility and other properties. The iodine-131 analog of DDT, recently synthesized by Jensen and Pearce (8, p.2436), is similarly limited in its application for DDT metabolism studies.

In 1950 Fields and co-workers succeeded in synthesizing carbon-14 benzene ring labeled DDT (3, pp.591-592). Their synthesis consisted of the conversion of C^{14} labeled aniline to chlorobenzene by Sandmeyer reaction with subsequent condensation of the labeled chlorobenzene and chloral to give DDT in 40-50% over-all yield with a specific activity of 54 μc per millimole. Butts and Lindquist and their group at Oregon State using low activity material made in this manner, were able to carry out studies on the distribution of topically applied DDT in common houseflies (9, pp.167-172). Very recently Fukuto and March in their

investigation of the metabolic fate of DDT in susceptible and DDT-resistant strains of houseflies have made use of C^{14}-labeled DDT prepared in a similar manner (6). The extent of the studies using DDT of low specific activity prepared by the above mentioned workers was limited in a different manner mainly because of the necessity of administering many times the normal lethal dose of the low activity DDT in order to furnish enough radioactivity for the tracer studies, and consequently DDT-resistant insect strains were used in most of these experiments.

This laboratory has recently become interested in the study of the metabolism of a sub-lethal dose of DDT in non-resistant insects, which called for the synthesis of DDT having a much higher specific activity. Since at that time a good method of incorporating carbon-14 into a benzene ring was not available and as a result of the considerations rendered on the possible metabolic fate of this compound, it appeared to be most advantageous to label specifically the tertiary carbon atom of the DDT molecule. Using radioactive barium carbonate as a starting material, attempts were first made to develop a synthesis on the 5 millimole scale in which the final step involved the condensation of labeled chloral and chlorobenzene similar to that used in the commercial production of DDT (12, pp.916-923). However, this scheme was later abandoned in view of the low inherent over-all yield and the

difficulties involved in micro scale operations; although very recently a low level synthesis has been reported using this scheme on a relatively large (50 millimole) scale with a yield of 17% of pure p,p' DDT based on carbon-14 labeled ethanol (13). Since ethanol may be synthesized from barium carbonate in approximately 80% yield (2, pp.3176-3180), the total over-all yield of p,p' DDT based on barium carbonate was about 11% and the specific activity of the DDT was reported to be 180 μc per millimole.

Two other suggestive schemes have appeared in the literature. Gunther (5, pp.654-658) and Fry (4, pp.3238-3239) have each proposed a synthesis of DDT starting from carbon-14 carboxyl labeled p-chlorobenzoic acid designed to place isotopic carbon at the point of attachment of the two p-chlorophenyl rings.

Gunther's proposal consisted of the following steps:

(1) Carbonation of p-chlorophenyl magnesium bromide with $C^{14}O_2$ to produce carboxyl labeled p-chlorobenzoic acid.

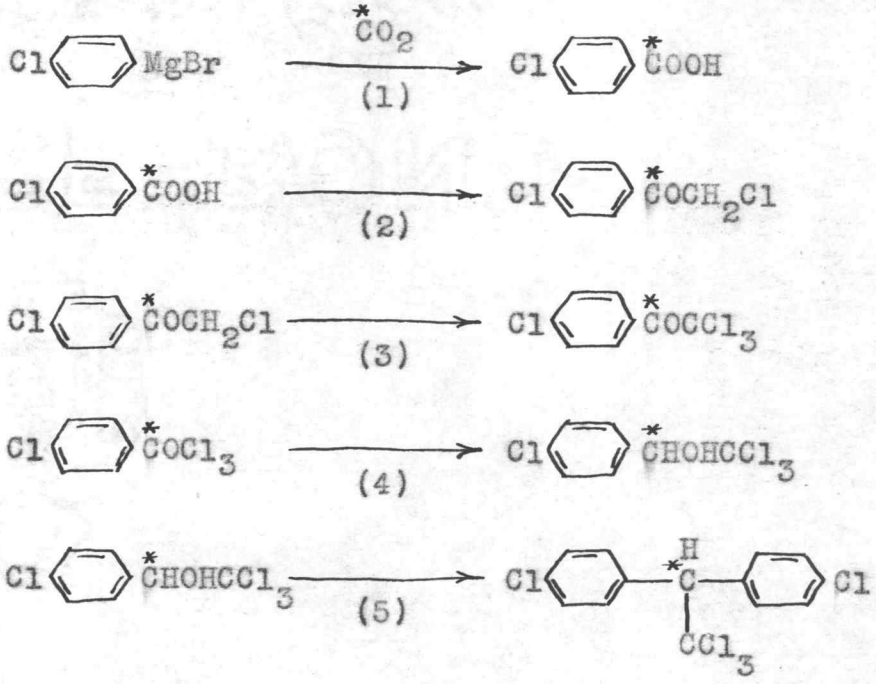
(2) Conversion of p-chlorobenzoic acid to p-chlorobenzaldehyde by methylation and transformation to the hydrazide with subsequent conversion to the desired aldehyde.

(3) Condensation of the aldehyde with chloroform to produce p-chloro-α-(trichloromethyl)-benzyl alcohol.

(4) Condensation of the carbinol with chlorobenzene to produce DDT.

However, the highest possible over-all yield of Gunther's reaction scheme would appear to only 14%, mainly due to the low yields in the chloroform condensation. Because of the potentially low over-all yield, this scheme was evidently not suitable for the present purpose.

The second method for incorporating carbon-14 into the aliphatic portion of the DDT molecule involves five steps:



(1) Carbonation of p-chlorophenyl magnesium bromide to give carboxyl labeled p-chlorobenzoic acid.

(2) Conversion to p-chlorobenzoyl chloride by the action of thionyl chloride followed by reacting the acid chloride in ether solution with cold ethereal diazomethane and then in the cold with dry HCl gas to give p,α dichloro-acetophenone.

(3) The ketone is chlorinated to produce p,$\alpha\alpha\alpha$

tetrachloroacetophenone.

(4) Reduction of the above ketone with aluminum isopropoxide gives p-chloro-α-(trichloromethyl)-benzyl alcohol.

(5) Condensation of the carbinol with chlorobenzene to give DDT.

The over-all yield of p-chloro-α-(trichloromethyl)-benzyl alcohol was calculated by Fry to be about 70% based on barium carbonate. The yield of crude DDT obtained by condensation of chloral with chlorobenzene on a large scale may run as high as 90% (12, pp.916-923) and therefore, the total over-all yield of crude DDT in this series of reactions based on barium carbonate was calculated to be 63%, which appeared to be suitable for a multiple-step radioactive synthesis of this nature. Unfortunately, no experimental details concerning scale, reaction conditions and isolation technique were given in Fry's paper and, although general macro scale reactions of this type and the intermediate products involved were well known, several of the specific reactions involved had never been carried out particularly on the micro scale basis which is essential for the synthesis of isotopically labeled compounds. However, it was felt that this scheme could be redesigned for a radioactive synthesis on a 5 millimole scale because of the good prospect of reasonable yields and the ease of handling the solid intermediate compounds.

A considerable amount of work has been carried out investigating optimum reaction conditions and isolation techniques in order to develop straight forward experimental procedures aimed to give highest possible yield and least contamination hazard. The over-all yield based on numerous trial runs averaged 12% of purified p,p' DDT from barium carbonate, as compared to the calculated 63% described above; the difference presumably derived from the greater losses in a series of micro scale operations and the fact that the previous calculation was based on yield of crude product. However, in view of the simplicity of the isolation techniques involved and the high purity of the product obtained, it can be concluded that the method described here is suitable for the synthesis of carbon-14 labeled DDT with a high specific activity.

II. EXPERIMENTAL

C^{14} carbonyl labeled p,α-dichloroacetophenone

The C^{14} carboxyl labeled p-chlorobenzoic acid (0.176 g.) was synthesized according to the method of Van Bruggen et al. (14, pp.45-48) using 0.2667 gm (1.34 millimole; 10 mc; sp. act. 8.48×10^8 counts per minute per millimole) of $BaCO_3$ in 83% (chemical) or 81% (radioactivity) yield; m.p. 250°C. (sealed tube) and having a sp. act. 8.16×10^8 counts per minute per millimole.

The acid was diluted with 0.7741 g. (4.94 millimole) of ordinary p-chlorobenzoic acid making a total of 6.06 millimoles and converted to the corresponding acid chloride by the use of thionyl chloride. The excess thionyl chloride was removed by distillation and the residual liquid was dissolved in 20 ml of dry benzene. This solution was treated with an ethereal solution of 34 mM of diazomethane in small portions at ice bath temperature with constant stirring, then allowed to warm up to room temperature. After standing overnight the solution was treated with dry HCl gas for 1 hour at ice bath temperature. The solvents were removed by distillation under reduced pressure and the residual crude dichloroacetophenone was purified by recrystallization from aqueous dioxane. Yield: 0.5089 g. (44.5%); m.p. 99.0-99.5° C.

p chloro-α-(trichloromethyl)-benzyl alcohol

The p,α dichloroacetophenone dissolved in an equal

weight of trichloroacetic acid was chlorinated with a stream of chlorine gas at a temperature of 120-125° C. for 48 hours using a modified methoxyl apparatus as the reactor (1, p.276). At the end of this period, the warm liquid was poured immediately into 25 ml of cold water, the trichloracetic acid neutralized with 4% Na_2CO_3 solution and the p,ααα tetrachloroacetophenone then extracted with benzene for 16 hours using a liquid-liquid extractor; the benzene was removed under reduced pressure.

The reduction of the ketone was carried out in an automatic Meerwein-Ponndorf reduction apparatus[1]. The crude p,ααα tetrachloroacetophenone was dissolved in 100 ml of dry isopropyl alcohol, 14.8 millimoles of freshly prepared aluminum isopropoxide was added and acetone removed by slow distillation. Acetone was no longer detected after two hours, but the distillation was continued for eight hours to ensure complete reduction. The remaining isopropyl alcohol was then removed under reduced pressure and the residual viscous mass upon cooling was treated with 2N HCl solution and extracted with benzene in a liquid-liquid extractor for 12 hours. The benzene was removed by distillation first at normal pressure and finally under reduced pressure. The crude p-chloro-α-(trichloromethyl)-benzyl alcohol was used in the next step without further purification. However, in dry runs the

[1] From a design kindly furnished by Dr. Edward M. Fry.

yield was approximately 70% for the chlorination and reduction. The product in this instance was isolated as the acetate m.p. 123-124° C.

2,2 bis-(p-chlorophenyl)1,1,1 trichloroethane

To the crude carbinol was added 4 ml (39 millimoles) dry chlorobenzene and 4 ml (76 millimoles) 98.5% sulfuric acid; the mixture was stirred by means of a glass magnetic stirring bar at room temperature. During the first two hours, 4 ml of 101% sulfuric acid was added in order to maintain the optimum sulfuric acid concentration. Stirring was continued for 40 hours.

The mixture was then poured into water and extracted for 10 hours with petroleum ether using a liquid-liquid extractor. The petroleum ether was removed under reduced pressure and the residue redissolved in 100 ml of n-propyl alcohol. This solution was then concentrated to a volume of 20 ml, any remaining chlorobenzene contaminate being removed as the azeotrope in the process; 20 ml of water was then added. Upon cooling the crude DDT (0.7004 g.) crystallized and was collected in a sintered glass crucible. This was purified by successive recrystallization from 95% ethanol, petroleum ether and 95% ethanol. The yield was 241.2 mg of p,p' DDT m.p. 104-104.5° C. (lit. 108-108.5°) with a specific activity of 1.49×10^8 counts per minute per millimole. This was equivalent to an activity of approximately 1340 μc per millimole. The mother liquor

upon evaporation gave an oily liquid which presumably consisted of a mixture of the isomers of DDT. The over-all chemical and radioactivity yield of purified p,p' DDT based on the original barium carbonate were approximately 10%.

The radioactivity of the various compounds was determined as barium carbonate in the conventional manner; counting data were corrected for background and self-adsorption.

III. SUMMARY

2,2-bis-(p-chlorophenyl)1,1,1 trichloroethane labeled with C^{14} in the tertiary position has been synthesized in 10% chemical and radioactivity yield over-all from barium carbonate. The specific activity of the purified DDT was 1.49×10^{8} cpm/mM.

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