THE SYNTHESIS OF [ring-U-^{14}C] DDD FROM [ring-U-^{14}C] DDT

using rat liver homogenate

S.C. FAWCETT, L.J. KING, P.I. STANLEY* and P.J. BUNYAN*
Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH and *Ministry of Agriculture, Fisheries and Food, Tolworth Laboratory, Tolworth, Surrey KT6 7NF

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SUMMARY

1,1-Di[ring-U-^{14}C] (4-chlorophenyl)-2,2-dichloroethane ([^{14}C] DDD) was prepared by the anaerobic incubation of 1,1-di[ring-U-^{14}C] (4-chlorophenyl)-2,2,2-trichloroethane ([^{14}C] DDT) with homogenised rat liver in buffer. This gave a good yield of DDD which was purified chromatographically. The overall recovery of 99.5% pure DDD was 68%.

Introduction

1,1-Di(4-chlorophenyl)-2,2,2-trichloroethane (DDT) is still used as an insecticide in many countries and its metabolic fate in animals is currently under investigation in this laboratory. 1,1-Di(4-chlorophenyl)-2,2-dichloroethane(DDD) is an important metabolite of DDT as it appears to be the first one produced in the metabolic pathway to 1,1-di(4-chlorophenyl) acetic acid (DDA) in rats (Peterson and Robison, 1964). A radiolabelled sample of DDD which was required for metabolic studies, was synthesised from commercially available [^{14}C] DDT. Pure [^{14}C] DDD with a high specific activity was required. It was particularly important to ensure that there was minimal contamination of the DDD with other DDT metabolites as these could give rise to misleading results. The presence of 1,1-di(4-chlorophenyl)-2,2-dichloroethylene (DDE), in particular, has invalidated previous studies as it is slowly eliminated by most animals and even a low level in the original dose of DDD would accumulate in the tissues. A chemical method for the synthesis of DDD has been described by Zimmer and Klein (1972) and this was evaluated but it was difficult to obtain consistently good yields and the DDD required extensive purification prior to use. Several authors have reported that animal liver preparations readily dechlorinate DDT under anaerobic conditions. The method used by Bunyan *et al.* (1966) for pigeon liver was modified and used with a rat liver preparation. The method described in this paper is suitable for the synthesis of DDD [ring-U-^{14}C] of high purity and with a high specific activity.

Materials

DDT [ring-U-^{14}C] was obtained from The Radiochemical Centre, Amersham, Bucks. DDT was prepared from technical DDT (Rohm Haas) by the method of West and Campbell (1950) with final recrystallisation from ethanol to constant melting point 108-109°C. Streptopen (500 mg procaine penicillin BP, 500mg dihydrostreptomycin sulphate, 2, procaine hydrochloride and 0.25% cetrimide BP/ml) was obtained from Glaxovet, Greenford, England. Neutral alumina (Woelm, Superactive) was obtained from Koch-Light Laboratories, Colnbrook, Bucks. Silica gel G (Type 60) was obtained from Anderman & Co., East Molesey, Surrey. Scintillant was prepared using 4g 2,5-diphenyloxazole (PPO) from Hopkins and Willaims, Romford, Essex and 0.2g l,4-di[2-(5-phenyloxazolyl)] benzene (POPOP) from Koch-Light Laboratories Ltd. dissolved in 1L toluene. All other chemicals were of the highest grade commercially available.

The male Wistar rats (TAS) strain weighing at least 200g were supplied by the Ministry of Agriculture, Fisheries and Food, Tolworth, Surrey.

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Method

A rat was killed by cervical dislocation and the liver was removed, washed with sucrose solution (42.8g l^{-1} sucrose in 0.1% Triton solution) and weighed. The liver was homogenised in sucrose solution (2ml g^{-1}) and poured onto a 250ml conical flask containing 60ml phosphate buffer, pH 7.4 (17.8g Na_2HPO_4 and 20ml 1N HC1 L^{-1} in 0.1% Triton solution) and 60mg ascorbic acid. Approximately 1 ml of *Streptopen* was added and the flask was closed with a rubber seal. A continuous stream of oxygen free nitrogen was supplied through needles inserted into the seal and the flask was incubated for 4 hours at 37–38°C in a shaking water bath. DDT (4.9mg, 90.677μCi) in 1ml methanol was added using a syringe and the incubation was allowed to proceed for a further 42 hours. Approximately 40ml of 10% trichloroacetic acid solution was added. The solution was centrifuged (2,000 r.p.m. for 15 minutes), the supernatant removed and extracted with n-hexane for 16 hours in a liquid-liquid extractor and the pellet was mixed with anhydrous sodium sulphate then soxhlet extracted with hexane for 16 hours. The glassware was also thoroughly rinsed with hexane. The hexane extracts and washings were combined and dried over anhydrous sodium sulphate and reduced in volume to 3ml. Aliquots (500μl) were applied to alumina columns (2.5g neutral alumina activity IV) and eluted in 30ml n-hexane. The eluates were pooled, reduced in volume to 2ml and applied as a continuous band to the bottom of preparative layer chromatography plates. These were prepared from a slurry of silica gel G (type 60) spread to a thickness of 1mm. The plates were developed in hexane and air dried a total of 3 times. The active bands were located using a 4π Tracerlab Scanner with the following settings; ratemeter 10K cpm, time constant 0.25s, slit width 0.25 ins, scanning speed 12 in hr^{-1} and gas flow of 500ml $min.^{-1}$. The bands were scraped off and eluted in hexane as described by Odam *et al.*, 1975. The band corresponding to DDD on electron capture gas chromatography was reapplied to plates and the procedure repeated. Samples (50μl) were taken for scintillation counting from each hexane extract and the glassware washings, from the column eluate and the final hexane extract. The instrument used was a Nuclear Enterprise NE8310 and counting efficiencies using a PPO/POPOP/toluene scintillant were greater than 85%.

Results

The recoveries of radioactivity in hexane from the various fractions and after each clean-up stage are shown in the table below:-

	μCi	% recovered
Liver pellet	56.1	61.9
Liver supernatant	0.1	0.1
Flask washes	7.6	8.4
Total	63.8	70.4
After alumina columns	63.6	70.1
After preparative layer chromatography	62.1	68.4

The DDD obtained had a specific activity of 20.4μCi/mg and was greater than 99.5% pure by electron capture gas chromatography.

Discussion

The method of synthesis described gave a good yield of [ring-U-^{14}C] DDD of high purity. The dechlorination was probably carried out largely by porphyrins in the liver homogenate although enzymic conversion may also have occurred (Hassall, 1972). The extraction procedures recovered most of the radioactivity from the precipitate and very little was recovered from the aqueous fraction of the homogenate. There was also a considerable recovery of radioactivity in the glassware washings. The hexane extracts contained traces of 1,1-di(4-chlorophenyl)-2-chloroethylene (DDMU) and DDE but no DDT before purification was undertaken indicating that the liver preparation had metabolised all the available DDT. The chromatographic purification efficiently removed traces of DDE and DDMU. The DDD was then used in metabolism studies or converted to DDMU, which is another important metabolite of DDT.

This latter conversion involves a simple chemical dehydrohalogenation reaction (Bunyan *et al.* 1977). DDE and DDMU are difficult to separate completely using chromatography and so it is of considerable advantage to have removed traces of DDE before synthesising DDMU.

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