

Synthesis of Benzo [RST] Pentaphene-5,15-¹⁴C and of its Oxidation Products *

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SUMMARY

¹⁴C labeled benzo [rst] pentaphene (V) known as 3, 4, 9, 10-dibenzpyrene, ¹⁴C labeled benzo [rst] pentaphene-5,8-diol, diacetate (VI) and ¹⁴C labeled benzo [rst] pentaphene-5,8-dione (IV) have been synthesized for the study of the distribution, the metabolic fate and the mode of action of the carcinogenic hydrocarbon dibenzpyrene, which appears to differ from other known carcinogens with regard to these factors[1, 2].

Benzo [rst] pentaphene (3, 4, 9, 10-dibenzpyrene)² when injected subcutaneously in mice has been found not to be excreted in urine or feces [1, 2]. These results have been obtained by the use of the ultraviolet absorption technique. In order to confirm these data and to show that not even trace amounts of the carcinogen are excreted or metabolized, benzo [rst] pentaphene-5,15-¹⁴C was synthesized. The results of the biological experiments obtained will be reported elsewhere. The labeled carcinogen and its oxidation products (IV and VI) are also being used for the study of the mechanism of carcinogenesis of DP and for the study of the possible metabolism of DP by TPNH-dependent inductive hydroxylases in liver and the gastro-intestinal tract [3].

The synthesis of DP-¹⁴C involved eight steps and started with the commercially available phthalic anhydride-7,¹⁴C as source of radioactivity. Condensation with benzene gave *o*-benzoyl benzoic acid [4] (¹⁴C equally distributed between carbonyl and carboxylic carbons). Ring closure yielded anthraquinone-9,¹⁴C [5]. The latter substance was partially reduced with copper to

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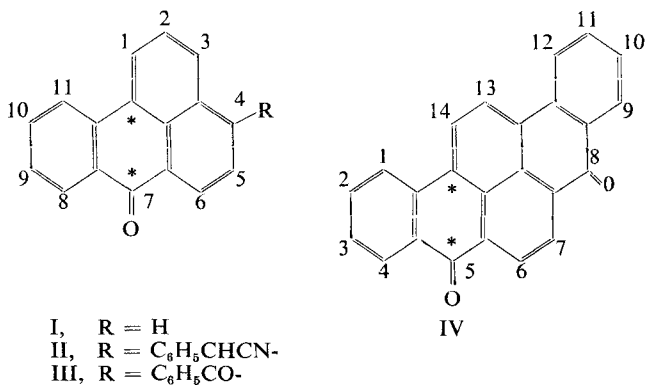
² The abbreviation DP for 3,4,9,10-dibenzpyrene (benzo[rst]pentaphene) will be used in this article.

anthrone-9,10- ^{14}C [6]. Condensation of anthrone with glycerol yielded 7-H-benz [de] anthracene-7-one-7,12- ^{14}C (I, benzanthrone) [6]. Reaction with benzyl cyanide in pyridine resulted in the formation of 4-cyanobenzyl benzanthrone-7,12- ^{14}C (II) which yield 4-benzoyl benzanthrone-7,12- ^{14}C (III) upon oxidative hydrolysis [7].

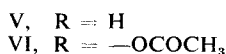
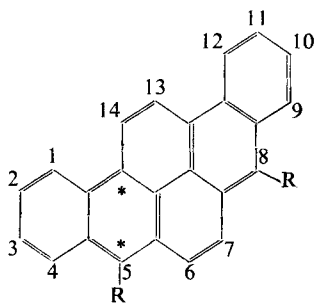
Fusion with a mixture of technical aluminum chloride, anhydrous, (AlCl_3 practical, EASTMAN) and sodium chloride gave benzo [rst] pentaphene-5,8-dione-5,15- ^{14}C (IV) also named 3,4,9,10-dibenzpyrene-5,8-quinone. When pure aluminum chloride (product of MATHESON, COLEMAN and BELL) was used instead of the technical one, no reaction took place. The analysis¹ of these two products showed that the technical AlCl_3 contained between 10^{-3} and $10^{-2}\%$ Ni, which was not detected in the pure material. On the other hand, the pure AlCl_3 contained more than 1% Mg, 10^{-2} to $10^{-1}\%$ Si and Zn and 10^{-3} to $10^{-2}\%$ Cd, while the technical product contained 10^{-4} to $10^{-3}\%$ Mg and Si, 10^{-3} to $10^{-2}\%$ Zn and no Cd. Some other elements (Na, Ca, Mn, Fe, Co, Ga, Sn and Pb) were found in quantities of less than $10^{-3}\%$ in both materials. It is unknown whether Ni is required for the catalytic action, or whether Mg, Si, Zn and Cd might act as inhibitors of the reaction. It is not known whether a possible difference in water content of the two AlCl_3 samples may have had any influence on this reaction.

The quinone (IV) was reduced to benzo [rst] pentaphene-5,15- ^{14}C (V), also named 3,4,9,10-dibenzpyrene-5,12- ^{14}C , by fusion with zinc and zinc chloride [2]. Treatment of the quinone (IV) with zinc in acetic anhydride gave benzo [rst] pentaphene-5,8-diol, diacetate-5,15- ^{14}C (VI), also called 5,8-diacetoxy-3,4,9,10-dibenzpyrene-5,12- ^{14}C .

It is understood that the ^{14}C label is evenly divided between the 5 and 15 positions in the benzopentaphenes, but that each molecule of benzopentaphene and of compounds I through VI contains only a single ^{14}C label.



¹ Carried out by Jarrell-Ash Co., Waltham, Mass.



EXPERIMENTAL

o-Benzoyl benzoic acid-¹⁴C [4].

A mixture of 205 mg of phthalic anhydride-7-¹⁴C (radioactivity 5.0 mC, available from NEW ENGLAND NUCLEAR Corp., Boston) and 1.3 g regular phthalic anhydride was heated in a porcelain dish on a hot plate until a clear melt with no foaming was obtained. It was then dried in a desiccator and ground.

The phthalic anhydride thus prepared was mixed with 5.5 g of dry benzene and 2.7 g anhydrous aluminum chloride (pure, obtained from MATHESON, COLEMAN and BELL. The mixture was heated to 70° C and kept at this temperature until no more HCl gas was evolved. It was then poured into a solution of 4 g sodium carbonate in 25 ml water. The excess benzene was eliminated by steam distillation and the alkaline reaction mixture was filtered off from the precipitated aluminum hydroxide, which was washed with hot water.

The combined filtrates were acidified and cooled. The precipitated *o*-benzoyl benzoic acid was washed with water and dried in vacuum over a water bath. M.p. 126-127° C. Yield, 2.0 g (87%). The ¹⁴C is equally distributed between the carbonyl and the carboxyl carbons.

Anthraquinone-9-¹⁴C [5].

2.0 g of the anhydrous *o*-benzoyl benzoic acid-¹⁴C and 10 ml concentrated sulfuric acid were heated on a steam bath until a solution was formed. This was heated to 150° C for 5 minutes, then cooled to 100° C. 2 ml of water were added dropwise and the mixture was let cool. An additional 28 ml of water were then added, the mixture was cooled in an ice bath, and filtered by suction. The precipitate was washed with water and dried. 1.84 g of anthraquinone-9, 10-¹⁴C were obtained (quantitative yield).

Benzanthrone-¹⁴C [6].

1.84 g of the anthraquinone-¹⁴C were dissolved in 28 ml concentrated sulfuric acid. To the red solution 1.1 ml water were added. The flask was immersed in an oil bath at 40° C, and 1.8 g of precipitated copper (prepared according to Org. Syn., Vol. 14, p. 67) were introduced over a period of 1 1/2 hours, keeping the reaction at 38-42° C until all the copper was dissolved (altogether three hours).

Into the reaction flask a mixture of 1.81 g glycerol and 1.81 g water was introduced during half an hour, while the temperature of the bath was raised to 85-90° C. When all the glycerol was introduced, the temperature was raised gradually to 120° C at a rate of 1° C per three minutes. It was maintained at 118-120° C for an additional three hours.

Finally the mixture was cooled to 70-80° C and carefully poured into 102 ml of boiling water while stirring. Spattering was avoided by pouring the mixture down the walls of the beaker while stirring. The water suspension was boiled for a few minutes and let stand a few hours. It was then filtered by suction and washed with water, then boiled in 30 ml of 1 % NaOH, filtered, and again washed with water, filtered and dried at 120° C. Recrystallization from methyl isobutyl ketone gave 1.38 g (yield 68 %) of benzanthrone-¹⁴C. M. p. 168-170° C.

Phenyl-7-oxo-7-H-benz [de] anthracene-4-acetonitrile-7,12-¹⁴C (II).

24.2 ml isopropanol and 7.6 g of powdered potassium hydroxide were mixed with good agitation and cooled to room temperature. Into this mixture there were added rapidly 1.38 g of the benzanthrone-¹⁴C and 1.12 g recrystallized commercial benzanthrone, followed by 7.7 ml of freshly distilled benzyl cyanide (b.p. 110° C/15 mm). The reaction flask was heated to 45° C and air was bubbled through for five hours, keeping the temperature between 40° C and 45° C. After cooling to room temperature, 7.2 ml of glacial acetic acid were added, and the reaction mixture was stirred at 30-35° C for five hours. The solid material was filtered by suction, boiled with methanol and filtered. It was then extracted with hot benzene, and the benzene solution was concentrated and cooled. Recrystallization from dimethyl formamide-benzene gave 1.85 g (48 % yield) 4-cyanobenzyl benzanthrone-¹⁴C (II). M. p. 209-210° C.

4-benzoyl-7-H-benz [de] anthracene-one-7,12-¹⁴C (III).

A mixture of 3.1 g sodium acetate, 2.75 g sodium dichromate, 1.85 g 4-cyanobenzyl benzanthrone-¹⁴C (II) and 14 ml glacial acetic acid were heated on a boiling water bath for three hours. After cooling, 6.9 ml of water were added gradually (to avoid the precipitation of large particles). The precipitate formed was filtered by suction, then washed with hot water until a colorless filtrate was obtained. Recrystallization from benzene-petroleum ether gave 1.5 g (84 %) 4-benzoyl benzanthrone-¹⁴C (III). M. p. 170-171° C.

Benzo [rst] pentaphene-5,8-dione-5,15-¹⁴C (IV).

A mixture of 0.9 g KCl, 0.9 g NaCl and 10.2 g technical aluminum chloride (EASTMAN) was heated to 125° C in a small erlenmeyer flask. When it melted, 1.5 g of 4-benzoyl benzanthrone-¹⁴C (III) were added, followed by 0.6 g *m*-nitrobenzoic acid. Heating was continued for three to four hours, after which the mixture was boiled with a solution of 5 ml concentrated HCl and 50 ml water. The precipitate formed was filtered then boiled with a solution of 5 ml concentrated HCl, 5 ml ethanol and 25 ml water, and filtered. 1.4 g of the quinone (IV) were obtained (yield 93 %). M. p. 370° C (from xylene).

Benzo [rsi] pentaphene-5,15-¹⁴C (V).

A mixture of 1.05 g sodium chloride, 1.05 g zinc dust, 5.25 g zinc chloride and 1.05 of the quinone (IV) (0.55 g of ¹⁴C labeled and 0.50 g of nonlabeled) was heated for one hour at 290-300° C. After cooling, dilute hydrochloric acid was added to dissolve the inorganic materials. The dibenzpyrene (IV) formed was treated with charcoal in boiling xylene and recrystallized from *o*-dichlorobenzene. M. p. 279° C. Yield, 330 mg (35 %).

Measurement of the radioactivity.

The radioactivity was determined in a NUCLEAR-CHICAGO liquid scintillation counter (efficiency 75 %). A weighed sample was dissolved in toluene containing PPO and POPOP. The DP (V) obtained had a specific activity of 450,000 c.p.m./mg., which is equivalent to 600,000 d.p.m./mg. or 0.3 μC/mg. This represents an overall yield of 5 % based on the radioactivity of the starting material.

Benzo [rst] pentaphene-5,8-diol, diacetate-5,15-¹⁴C (VI).

Into a boiling mixture of 250 mg of the quinone (IV) (100 mg¹⁴C labeled and 150 mg nonlabeled), 283 mg sodium acetate and 24 ml acetic anhydride, 1.3 g zinc dust were added gradually. Refluxing was continued for two hours, after which 25 ml of glacial acetic acid were added. The supernatant solution was decanted and left overnight, then cooled in ice. The diacetoxy derivative (VI) was precipitated out and recrystallized from xylene in yellow needles. M. p. 332° C. 273 mg were obtained (78 %). The specific activity of this material was 110,000 c.p.m. per mg, or 147,000 d.p.m./mg (0.067 μC/mg).

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