SYNTHESIS OF 6-SUBSTITUTED BENZO[a]PYRENE-α-^{14}C DERIVATIVES

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SUMMARY

6-Lithiobenzo[a]pyrene was carbonated to produce benzo[a]pyrene-6-carboxylic-^{14}C acid, which was in turn used in the preparation of several derivatives. Methylation to the ester was followed by $LiAlH_4$ reduction to 6-hydroxymethyl-^{14}C-benzo[a]pyrene. An efficient oxidation using DDQ produced the corresponding aldehyde. Direct reduction of the ester using $LiAlH_4$ and $AlCl_3$ produced 6-methyl-^{14}C-benzo[a]-pyrene.

Key Words: Benzo[a]pyrene-6-carboxylic-^{14}C acid, methyl benzo[a]pyrene-6-carboxylate-^{14}C, 6-hydroxymethyl-^{14}C-benzo[a]pyrene, benzo[a]pyrene-6-carboxaldehyde-^{14}C, 6-methyl-^{14}C-benzo-[a]pyrene

INTRODUCTION

A variety of 6-substituted derivatives of benzo[a]pyrene (BP) have been demonstrated to be carcinogens in various studies [1-4]. These compounds include 6-methyl-BP, 6-hydroxymethyl-BP and some of its organic and inorganic esters, BP-6-carboxaldehyde and 6-halomethyl-BPs (Cl, Br, I). The metabolic interrelationships of these and similar derivatives and how they relate to the mechanism of carcinogenic induction are matters of much current interest. Convenient syntheses of such compounds containing a ^{14}C label would be valuable for facilitating such studies. This paper describes the synthesis of several derivatives of BP labeled with ^{14}C in the 6-substituent. These basic derivatives can be used for simple conversions to other compounds of biochemical interest.

Direct formation of BP-6-carboxaldehyde by a Vilsmeier-Haack formylation on BP has long been known [5], and in a labeled case Royer, *et al.*, [6] have synthesized BP-6-carboxaldehyde-^{13}C in a similar reaction using N-methylformanilide-1-^{13}C. However, these authors required a two-fold excess of labeled formylating agent, which in turn had to be synthesized from simpler materials. More recently,

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Cavalieri, et al., [2] have synthesized BP-6-carboxylic acid by carbonation of 6-lithio-BP derived from 6-bromo-BP. This was an attractive route for the present case because of the ready availability of inexpensive $^{14}CO_2$.

BP-6-carboxylic-^{14}C acid (2) (Figure I) produced in this manner was converted to the more stable and easily purifiable methyl ester. Reduction of this ester was readily accomplished with $LiAlH_4$ to provide 6-hydroxymethyl-^{14}C-BP, which in turn could be oxidized in excellent yield using DDQ in benzene to give BP-6-carboxaldehyde-^{14}C. In addition, the synthesis of 6-methyl-^{14}C-BP was accomplished by direct reduction of methyl BP-6-carboxylate-^{14}C using $LiAlH_4$ and $AlCl_3$.

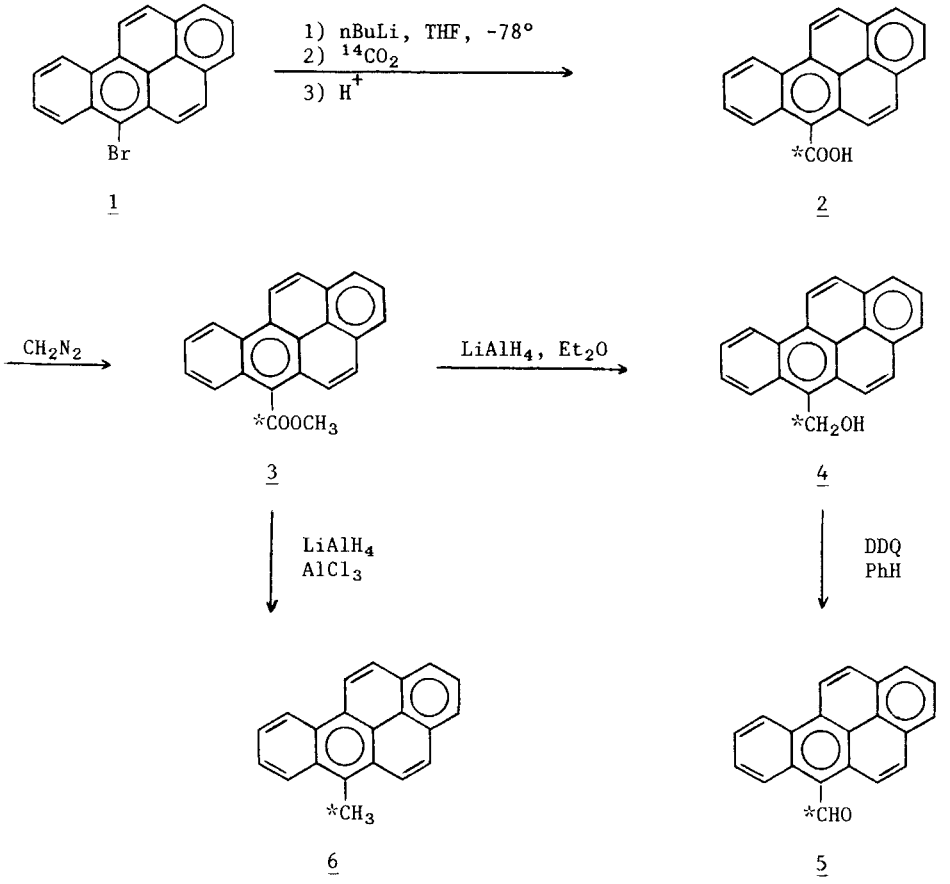

Figure 1 - Synthesis of 6-^{14}C-Substituted Benzo[a]pyrene Derivatives

EXPERIMENTAL

General

Thin-layer chromatograms were carried out on Merck Silica Gel 60 F-254 glass plates and visualized by UV light. Radiochemical purity was determined by radiochromatogram scanning with a Packard Model 7201 radiochromatogram scanner and autoradiography using Kodak SB-5 X-ray film. Radioactivity was determined using a Beckman LS-100C liquid scintillation system. Mass spectra were determined using a Varian-MAT 311-A double focusing mass spectrometer with a Finnigan/Incos 2300 data system or a Varian-MAT CH4 single focusing mass spectrometer at 70 ev. GLC was carried out with a Varian Aerograph Series 2400; argon carrier gas (30 ml/min), 3% Dexsil 400 on 80/100 Supelcoport, 1.8 m x 2 mm ID glass column, isothermal at 290°, FID at 330°, injector at 200°C. Barium carbonate-^{14}C at a specific activity of 54 mCi/mmole was purchased from the Atomic Energy Commission of Canada. Benzo[a]pyrene (99%) was purchased from Aldrich Chemical Company. All reactions were carried out under an argon atmosphere.

Benzo[a]pyrene-6-carboxylic-^{14}C acid (2)

6-Bromobenzo[a]pyrene (1) (1.31 g, 3.96 mmoles), prepared by the method of Rogan, *et al.*, [7] was dissolved in 100 ml THF. The solution was cooled to -78° and treated with 2.6 ml, 3.9 mmoles, of n-butyllithium (1.5 M in hexane). An orange-red color developed. After 1 hr the solution was frozen (liquid N_2) and evacuated on a vacuum line. Barium carbonate-^{14}C (180 mCi, 3.3 mmoles) was reacted with concentrated sulfuric acid, and the $^{14}CO_2$ generated was distilled into the frozen reaction solution. This was isolated from the vacuum line, warmed to -78° and stirred for 3 hr. Water (0.1 ml) was added and the solution was allowed to warm to room temperature, followed by addition of 0.33 ml (4.0 mmoles) of concentrated hydrochloric acid. The THF was evaporated *in vacuo* and the residue was taken up in ethyl acetate, washed with dilute HCl, and water and dried (Na_2SO_4). The product was used without purification in the next step.

Methyl benzo[a]pyrene-6-carboxylate-^{14}C (3)

Diazomethane [8] was added to the ethyl acetate solution of the carboxylic acid and after 1 hr, acetic acid was added to decompose the excess diazomethane. The solution was washed with water and dried (Na_2SO_4). The product was absorbed onto 10 g of silica gel and layered onto a 4 x 30 cm column of silica gel packed in hexane. Elution with hexane removed small amounts of BP and 6-bromo-BP, and further elution with 4:1 benzene:hexane gave, after evaporation of the solvent, 130 mCi (72% based on $^{14}CO_2$) of 3. R_f = 0.29 (silica gel/benzene), R_f = 0.57 (silica gel/ethyl acetate). This compound was chromatographically indistinguishable from an unlabeled sample of ester made from the carboxylic acid by the method of Cavalieri et al. [2].

6-Hydroxymethyl-^{14}C-benzo[a]pyrene (4)

Lithium aluminum hydride (74 mg, 1.9 mmole) was added to a solution of 40.0 mCi (0.74 mmole) of 3 in 35 ml of ether at room temperature. The mixture was stirred for 15 min then quenched by addition of 1 ml of water, 1 ml of 15% NaOH, and 3 ml water. The reaction mixture was filtered. The collected solid was dissolved in 1 M HCl and washed with ethyl acetate. The combined organic fractions were dried (Na_2SO_4) and evaporated to afford 33.1 mCi (83%) 4. TLC: R_f = 0.12 (alumina/benzene:ethyl acetate, 1:1), R_f = 0.48 (alumina/benzene:ethanol, 9:1), m.p. 234-5 (sealed, evacuated tube) (lit. [2] 231-232); mass spectrum m/e (I%): 284 (98, M^+); 285 (24), 282 (36), 268 (95), 267 (100), 253 (77), 252 (59), 133 (22), 132 (35), 127 (22), 162 (42), 125 (26), 44 (72); UV (95% EtOH), λ_{max} (log ε): 254 (4.51), 264 (4.60), 273 (4.36), 285 (4.56), 297 (4.64), 351 (4.04), 369 (4.35).

Benzo[a]pyrene-6-carboxaldehyde-^{14}C (5)

A suspension of 150 ml of benzene, 15.2 mCi (0.28 mmoles) of 4 and 150 mg of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was stirred for 20 hr at room temperature. The reaction mixture was subjected to chromatography on a 2 x 30 cm column of silica gel eluted with benzene and the bright yellow fluorescent band of 5 was collected: 14.0 mCi (92%) as a yellow solid. TLC: R_f = 0.60 (silica gel/benzene:ethyl acetate, 1:1).

6-Methyl-^{14}C-benzo[a]pyrene (6)

Lithium aluminum hydride (318 mg, 8.3 mmoles) was added to a solution of 44 mCi (0.82 mmoles) of 3 in 100 ml of diethyl ether at room temperature. After 30 min, 2.27 g (17 mmoles) of anhydrous aluminum chloride was added. The reaction was nearly complete by TLC analysis after 30 min but was continued for an additional 90 min to assure completion of the reaction. To decompose excess hydride, 10 ml of ethyl acetate was added, followed by 10 ml of ethanol and then 20 ml of 20% KOH. The aqueous layer was washed with benzene and ethyl acetate, and the combined extracts were dried (Na_2SO_4) and evaporated to give 217 mg (43 mCi, 97% yield) of crude 6. The product was purified by passage through a 2 x 35 cm column of silica gel eluted with hexane then 10% benzene in hexane to give 35.5 mCi of light yellow crystals of 6-methyl-^{14}C-benzo[a]pyrene at a chemical purity > 98% as determined by GLC and of > 98% radiochemical purity by autoradiography. TLC: R_f = 0.63 (silica gel/benzene), R_f = 0.38 (silica gel/CCl_4); mass spectrum, m/e (I%): 268 (100, M^+), 267 (74), 266 (30), 265 (59), 263 (23), 133 (16), 132 (27); UV (95% EtOH), λ_{max}: 256, 267, 275, 288, 300, 355, 373, 394, 409.

DISCUSSION

Most previously reported syntheses of 6-substituted BP derivatives depended on the direct formylation of BP as done by Fieser and Hershberg [5] and adapted to labeling by Royer, et al. [6]. The expense and extra steps involved in introducing the label using the Royer procedure induced us to seek a more efficient approach to obtaining 6-substituted BP derivatives containing ^{14}C attached to position 6 of BP. Initial attempts were made to adapt the method of Cavalieri, et al., [2] which involved the carbonation of BP-6-Li, made from BP-6-Br and n-butyllithium in benzene. The low solubility of BP-6-Br, BP-6-Li and BP-6-^{14}COOLi required a long reaction time and gave low yields. It was found that using tetrahydrofuran eliminated the solubility problem. With the change to THF it also became necessary to lower the reaction temperature to -78°, to minimize, among other side reactions, the coupling between BP-6-Br and BP-6-Li. The method

developed here gave 70 to 80% yields of BP-6-carboxylic acid based on barium carbonate, using a relatively short reaction time. In most cases BP-^{14}COOH was not isolated but instead converted quantitatively to its more stable and easily purifiable methyl ester by use of diazomethane, and purified by column chromatography, providing an overall radiochemical yield of 3 of 72% from $Ba^{14}CO_3$. This was then converted to 6-hydroxymethyl-^{14}C-BP by reduction with lithium aluminum hydride in diethyl ether at room temperature, in a yield of 83%. The reduction in THF solvent, in contrast, produced substantial amounts of side products.

The use of high-potential quinones has been described for the oxidation in a few cases of alcohols to ketones and aldehydes [9] and the benzylic alcohol 4 seemed particularly suited electronically for application of DDQ to its oxidation to BP-6-carboxaldehyde-^{14}C. Trial reactions on unlabeled substrate proved the reagent's efficacy, and the reaction of 6-hydroxymethyl-^{14}C-BP with DDQ in benzene at room temperature for 20 hr provided a 92% yield of BP-6-carboxaldehyde-^{14}C on a 0.3 mmolar scale. Shortly after development of this work a report by Becker, et al., [10] appeared describing similar high-yield oxidations of various benzylic alcohols by DDQ in dioxane solution. Our observation of the facile oxidation of the hydroxymethyl group at the electron-rich [11] 6-position of BP correlates well with the observations of Becker, et al., [10] that electron-donating ring substituents, in general, greatly accelerate the DDQ oxidations of benzylic alcohols.

The synthesis of 6-methyl-BP has previously been accomplished by the reduction of BP-6-carboxaldehyde using Wolff-Kishner conditions [12] or from 6-hydroxymethyl-^{14}C-BP by reduction of its tosylate with lithium aluminum hydride [13] or by $LiAlH_4/AlCl_3$ reduction [14]. In fact, several examples exist of the $LiAlH_4/AlCl_3$ reduction not only of benzylic alcohols but of aromatic acids to aryl methyl groups [15]. It was attractive to attempt, in our case, the latter, but the difficulty of obtaining pure samples of BP-6-carboxylic-^{14}C acid would have negated any increase in efficiency over alternative procedures. Surprisingly, we have found no previous reference to the $LiAlH_4/AlCl_3$ reduction of arylcarboxylic esters to methylarenes [16]. In our hands, reductions of methyl BP-6-carboxylate

with $LiAlH_4$ alone usually produced some 6-methyl-BP, and we quickly found that addition of $AlCl_3$ indeed resulted in complete reduction to 6-methyl-BP. The highest yields were obtained when $LiAlH_4$ was added to a suspension of methyl BP-6-carboxylate in ether at room temperature, followed after a short while by the $AlCl_3$, rather than adding the ester to the $LiAlH_4/AlCl_3$ complex. This strongly implies a two-stage reduction first to a hydroxymethyl intermediate followed upon $AlCl_3$ addition by further reduction. Upon reduction of the labeled ester by this procedure, an 80% yield of ≧ 98% pure 6-methyl-^{14}C-BP was obtained.

CONCLUSION

The ^{14}C-carboxylation of 6-lithio-BP derived from 6-bromo-BP followed by methylation of the resulting carboxylic acid results in a high yield of methyl BP-6-carboxylate-^{14}C. Reduction with $LiAlH_4$ affords 6-hydroxymethyl-^{14}C-BP, and its oxidation with DDQ in benzene gives BP-6-carboxaldehyde-^{14}C, both in excellent yield. These compounds are useful for the preparation of a range of ^{14}C-labeled 6-substituted BP derivatives of biological interest such as 6- halomethyl-^{14}C-BPs, various organic and inorganic esters of 6-hydroxymethyl-^{14}C-BP, and higher homologs. We have, for instance, converted BP-6-carboxaldehyde-^{14}C to either 6-ethenyl-(1-^{14}C)-BP or 6-ethyl-(1-^{14}C)-BP in moderate yield. Alternatively, reduction of methyl BP-6-carboxylate-^{14}C with $LiAlH_4$ and $AlCl_3$ provides an efficient synthesis of 6-methyl-^{14}C-BP. This reaction is potentially useful in the conversion of other arylcarboxylic esters to methylarenes.

The basic carboxylation method used here has been extended to the synthesis of similar 7-functionalized derivatives of benz[a]anthracene [14] and in principle may be used for the synthesis of such derivatives from other specifically halogenated polynuclear aromatic hydrocarbons.

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