^{13}C LABELED BENZO[a]PYRENES AND DERIVATIVES

3. BENZO[a]PYRENE-4,5-OXIDE-4-^{13}C and BENZO[a]PYRENE-4,5-OXIDE-5-^{13}C.

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SUMMARY

The synthesis of benzo[a]pyrene-4,5-oxide-4-^{13}C and benzo[a]-pyrene-4,5-oxide-5-^{13}C from the corresponding ^{13}C labeled 4,5-dihydrobenzo[a]-pyrene-4,5-diones is described. Reduction of the quinone with potassium borohydride in freshly distilled peroxide-free 2-propanol gave the trans-diol which was directly cyclized to the oxide by refluxing with DMF-DMA in chloroform/DMF. The overall yield of the oxide was 48-53% for the two steps. The requisite quinones were available in five steps from 1,2-dihydrochrysen-4(3H)-one as described in an earlier paper. Overall yield of the oxide from the chrysenone was 27%. ^{13}C NMR of the labeled oxides in $CDCl_3$ gave chemical shifts of 56.80 for C_5 and 56.20 for C_4 (δ_C from TMS) of benzo[a]pyrene-4,5-oxide.

Key Words: arene oxides, benzo[a]pyrene-4,5-oxide, carbon-13, carcinogenic hydrocarbons.

INTRODUCTION

As part of a program designed to synthesize benzo[a]pyrene and its derivatives labeled with ^{13}C (90%) at each of the peripheral carbon atoms of the ring system, we have prepared benzo[a]pyrene-4,5-oxide-4-^{13}C and benzo[a]-pyrene-4,5-oxide-5-^{13}C for use in studies of nucleophilic additions to the oxide and of its acid catalyzed rearrangement (NIH shift) using ^{13}C NMR as a structural probe (1).

0362-4803/83/091121-09$1.00 Received April 29, 1983

DISCUSSION

The reaction of 1,2-dihydrochrysen-4(3H)-one (1) with the lithioenolate of ethyl acetate-1-^{13}C (or 2-^{13}C) at -78°C in anhydrous THF afforded the hydroxyester 2a (or 2b) in 82% yield (2). Dehydration and dehydrogenation of 2a (or 2b) was accomplished by heating with 10% Pd/C in 1-methylnaphthalene containing 1,1-diphenylethene at 250-260°C for three hours. Isolation of the ester 3a (or 3b) followed by hydrolysis gave 4-chryseneacetic acid-1-^{13}C (4a) (or -2-^{13}C, 4b) in 69% overall yield from the hydroxyester 2a (or 2b). The arylacetic acid 4a (or 4b) was readily cyclized by warming (50°C) with methanesulfonic acid to give the phenol 5a (or 5b) which was directly oxidized with dipotassium nitrosodisulfonate (Fremy's salt) in buffered aqueous acetone to the labeled quinone 6a (or 6b). Overall yield of the quinone 6a (or 6b) from the acid 4a (or 4b) was 71-74%.

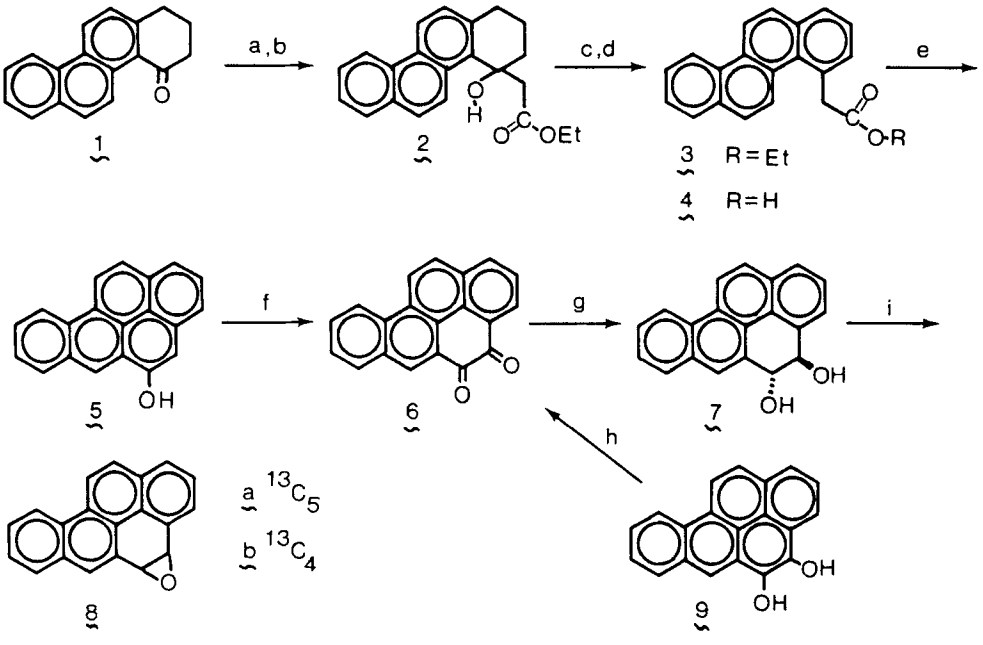
Initial studies on the reduction of the quinone 6 to the trans-dihydrodiol 7 were carried out on unlabeled 6. The use of lithium aluminum hydride in ethyl ether or a mixture of ether/THF (3,4) proved unsatisfactory, because, in addition to incomplete reaction even after long reflux periods, the desired trans-dihydrodiol 7 was obtained in poor yield and was contaminated with unreacted quinone and benzo[a]pyrene-4,5-diol (9). Upon workup, the diol 9 readily formed the quinone 6 in the presence of air. The reduction of the quinone 6 with potassium borohydride was attempted in absolute ethanol (5), methanol (6), and THF. In each case, unsatisfactory results were obtained; however, the use of freshly distilled 2-propanol as solvent at room temperature afforded excellent yields of the trans-dihydrodiol 7. As with the LAH reduction in ether, a significant amount of the diol 9 could be isolated when the reaction and workup procedures were performed in an inert atmosphere (N_2). When conducted in the presence of air, the reduction went to completion, and no diol 9 was detected. Apparently, any benzo[a]pyrene-4,5-diol (9) formed during the reduction is oxidized by air back to the quinone 6 which, in turn, is reduced to the trans-dihydrodiol 7. In this manner, the quinone 6a (or 6b) was converted to the trans-dihydrodiol 7a (or 7b) in 86-91% yield.

This behavior is not without precedence, since previous studies with phenanthrene-9,10-diol have demonstrated that a rapid oxidation to 9,10-dihydrophenanthrene-9,10-dione takes place in the presence of air (7). The formation of such catechol type intermediates in the LAH reduction of 4,5-dihydrobenzo-[a]pyrene-4,5-dione has been observed previously (4).

SCHEME

Series a from ethyl acetate-1-^{13}C ($CH_3\overset{*}{C}OOEt$)

Series b from ethyl acetate-2-^{13}C ($\overset{*}{C}H_3COOEt$)



(a) $LiCH_2COOEt$, THF, -78°C; (b) HCl, THF, -78°C; (c) Pd/C, 1-Me-naphthalene, $\Phi_2C{=}CH_2$, 250°C → 3; (d) KOH, EtOH → 4; (e) $MeSO_3H$, 50°C; (f) Fremy's salt, acetone/H_2O, buffer; (g) KBH_4, iPrOH, air; (h) O_2 (air); (i) DMF-DMA, $CHCl_3$/DMF, reflux.

The diol 7a (or 7b) was directly cyclized to the labeled benzo[a]pyrene-4,5-oxide-5-^{13}C (8a) (or -4-^{13}C, 8b) by refluxing with DMF-DMA in chloroform/DMF solution for seven hours. The oxide 8a (or 8b) was isolated in 48-53% yield after purification by treatment of a benzene solution of the crude product with activated charcoal, filtration, removal of the benzene, and crystallization of the residue from hexane/benzene.

EXPERIMENTAL

Materials and Methods--Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{13}C NMR spectra were determined on a pulse Fourier transform Varian FT-80 or Varian CFT-20 spectrometer using $CDCl_3$, acetone-d_6, or CD_2Cl_2 solutions of the labeled compounds. Peaks were referenced to solvent $CDCl_3$ (76.9 ppm), acetone-d_6 (29.2 ppm), or CD_2Cl_2 (53.6 ppm), and are reported relative to TMS. Product purity and reaction progress were detected with analytical thin-layer chromatography using 5.0 x 10 cm Analtech plates coated with silica gel GF. Sodium acetate-1-^{13}C and -2-^{13}C were supplied by the Stable Isotope Resource (LASL/NIH/ERDA) and were converted into the ethyl esters as described in an earlier paper (2).

Ethyl 4-Hydroxy-1,2,3,4-tetrahydro-4-chryseneacetate-1-^{13}C (2a)--To a mixture of 2.33 g (16.5 mmol) of N-isopropylcyclohexylamine and 10 ml of anhydrous THF, cooled to -78°C and under a N_2 atmosphere, was added 10.62 ml of 1.6 M n-butyllithium (16.5 mmol) in hexane. This mixture was cooled again to -78°C, and 1.34 g (15.0 mmol) of ethyl acetate-1-^{13}C in 10 ml of anhydrous THF was added dropwise at such a rate to maintain the temperature of the reaction mixture below -75°C. After the addition was complete, stirring was continued for 15 min after which time 3.69 g (15.0 mmol) of 1,2-dihydrochrysen-4(3H)-one (1), mp 124-125°C, dissolved in 40 ml of anhydrous THF was added at a rate which maintained the temperature below -75°C. After the addition was complete (1 h), stirring at -78°C was continued for 1 h. The yellow reaction mixture was hydrolyzed by the dropwise addition of 10 ml of 2.4 M HCl in THF at such a rate as to maintain the temperature of the reaction mixture below -70°C. The mixture was allowed to warm to room temperature, and water and ether were added. The layers were separated, and the ether layer was extracted with two 25 ml portions of 5% HCl, then with two 25 ml portions of water. The aqueous layer was extracted with an additional 25 ml of ether, and the combined ether extracts were dried over anhydrous $MgSO_4$. Removal of the ether afforded a pale yellow oil (8) which solidified on trituration with 95% ethanol. Crystallization of this solid from 95% ethanol gave 3.67 g (73% yield) of colorless ethyl 4-hydroxy-1,2,3,4-tetrahydrochrysene-

acetate-1-^{13}C, mp 77-81°C, which was shown by TLC to contain trace amounts of starting ketone. The mother liquor which contained additional amounts of the hydroxy ester 2a and traces of starting ketone was later dehydrated and dehydrogenated to give additional acid 4a (8).
^{13}C NMR ($CDCl_3$) of 2a: 176.06 (-$\overset{*}{C}$OOEt).

Ethyl 4-Hydroxy-1,2,3,4-tetrahydro-4-chryseneacetate-2-^{13}C (2b)--Same procedure as for 2a gave 3.80 g (77% yield) of 2b as colorless crystalline material, mp 74-78°C. Hydroxy ester 2b remaining in mother liquor was also converted to 4b (8).
^{13}C NMR ($CDCl_3$) of 2b: 43.06 (-$\overset{*}{C}H_2$COOEt).

Ethyl 4-Chryseneacetate-1-^{13}C (3a) and 4-Chryseneacetic Acid-1-^{13}C (4a)--In a dehydrogenation system fitted with a ground glass cold finger condenser and gas inlet and outlet tubes was placed 2.5 g (7.5 mmol) of ethyl 4-hydroxy-1,2,3,4-tetrahydro-4-chryseneacetate-1-^{13}C (2a), mp. 77-81°C, 0.25 g of 10% Pd/C, 1.5 g (8.2 mmol) of 1,1-diphenylethene, and 25 mL of 1-methylnaphthalene (9). The reaction mixture was heated to 250-260°C (Woods metal bath) for 3 h while steam was passed through the condenser and with maintenance of a slow flow of N_2. The cooled reaction mixture was diluted with benzene, filtered, and the catalyst was washed with benzene. After removal of the benzene (rotary evaporator), the 1-methylnaphthalene, 1,1-diphenylethane, and unreacted 1,1-diphenylethene were removed under reduced pressure (55°C, 0.05 torr) on a Kugel-Rohr. The resulting brown oily residue (8) was crystallized from 95% EtOH affording 1.47 g (62% yield) of ethyl 4-chryseneacetate-1-^{13}C (3a) as colorless prisms, mp 60.5-62.0°C.

The residue in the mother liquor from the crystallization of 3a was heated with 0.4 g of KOH in 25 mL of 95% ethanol for 3h. The ethanol was removed, the residue was dissolved in water, filtered through filter-cel, and acidified to give crude 4-chryseneacetic acid-1-^{13}C. Recrystallization from toluene afforded 0.44 g (20% yield) of colorless needles of 4-chryseneacetic acid-1-^{13}C (4a), mp

204-206°C. The total yield of dehydrated and dehydrogenated product from 2a thus amounted to 82% (8).

^{13}C NMR (acetone-d_6) of 3a: 171.67 ppm (-$\overset{*}{C}$OOEt).

^{13}C NMR (acetone-d_6) of 4a: 172.64 ppm (-$\overset{*}{C}$OOH).

Ethyl 4-Chryseneacetate-2-^{13}C (3b) and 4-Chryseneacetic Acid-2-^{13}C (4b)--In like manner to that described above for 3a and 4a the ester 3b was obtained in 73% yield (mp 64-65.5°C) and the acid 4b was obtained in 8% yield (mp 205-206.5°C) for a total yield of dehydrated and dehydrogenated material of 81% (8).

^{13}C NMR (acetone-d_6) of 3b: 43.85 ppm (-$\overset{*}{C}H_2$-COOEt).

^{13}C NMR (acetone-d_6) of 4b: 43.46 ppm (-$\overset{\bullet}{C}H_2$COOH).

4,5-Dihydrobenzo[a]pyrene-4,5-dione-5-^{13}C (6a)--A solution of 0.58 g (2.0 mmol) of 4-chryseneacetic acid-1-^{13}C, mp 205-207°C, in 35 mL of methanesulfonic acid under a N_2 atmosphere was stirred for 30 min at 50°C. The deep red complex was hydrolyzed by pouring into 200 g of water and ice, and the yellow-green precipitate of benzo[a]pyren-5-ol-5-^{13}C (5a) which was collected was directly dissolved in 100 mL of acetone and added to a solution of 1.30 g (4.8 mmol) of dipotassium nitrosodisulfonate (Fremy's salt) in 80 mL of water buffered with 20 mL of 0.167M KH_2PO_4. The purple solution was shaken in a stoppered Pyrex hydrogenation bottle on a Parr shaker for 1 h after which time the reaction mixture was red in color. The red-brown precipitate which formed during the reaction was collected and heated with 5% aqueous Na_2CO_3 releasing the bright orange-red quinone 6a. This crude product was collected, dried, and dissolved in $CHCl_3$ and applied to a silica gel column; the quinone was eluted with $CHCl_3$. After removal of the solvent, the dark red solid was dissolved in hot $CHCl_3$, the solution reduced in volume and EtOAc was added to facilitate crystallization, affording 400 mg (71% yield) of 4,5-dihydrobenzo[a]pyrene-4,5-dione-5-^{13}C (6a) as red-orange crystals, mp 257.5-259°C, reported (4) mp 255-256°C.

^{13}C NMR (CD_2Cl_2): 180.94 ppm ($\overset{\bullet}{C}_5$).

4,5-Dihydrobenzo[a]pyrene-4,5-dione-5-^{13}C (6b)--In like manner to that described for 6a, 0.58 g of 4b yielded 420 mg (74% yield) of 6b, mp 257-258°C.

^{13}C NMR (CD_2Cl_2): 180.65 ppm ($\overset{*}{C}_4$).

Benzo[a]pyrene-4,5-oxide-5-^{13}C (8a)--Freshly distilled peroxide-free 2-propanol (35 mL) was added to 100 mg (0.35 mmol) of 4,5-dihydrobenzo[a]pyrene-4,5-dione-5-^{13}C (6a), mp 254-255.5°C, in a 125 mL Erlenmeyer flask equipped with a Claisen head and drying tube. The orange suspension was placed in a vibrasonic bath for 2 min. The resulting fine suspension of the quinone 6a was stirred rapidly at which time 1.00 g of potassium borohydride was added. The reaction mixture was stirred for 17 h at RT in the dark under air. The bright yellow solution was then cooled in an ice bath and hydrolyzed by the dropwise addition of 20 mL of cold 2M HCl. The liberated trans-diol 7a was taken up in 150 mL of cold peroxide-free ether. The ether phase was washed several times with 5% HCl and water and then dried over $MgSO_4$. Removal of the drying agent followed by removal of the ether (roto-evaporator) yielded 92 mg of pale yellow trans-diol 7a, mp 211-213°C, reported mp 211.5-213°C (4), (Rf = 0.23 benzene/EtOAc, 1:1). The trans-diol 7a was not further purified but was cyclized directly to the oxide 8a by the following procedure.

Crude trans-4,5-dihydro-4,5-dihydroxybenzo[a]pyrene-5-^{13}C (7a) (92 mg, 0.32 mmol) was suspended in 4.6 mL of freshly distilled $CHCl_3$ and 1.55 mL of DMF was injected into the stirred suspension. Complete solution occurred upon final addition of 0.09 mL (0.64 mmol) of dimethylformamide-dimethylacetal (DMF-DMA) to give a pale orange solution. The solution mixture was allowed to reflux with stirring in the dark for 7 h. The reaction mixture was worked up by adding 25 mL of cold water followed by 75 mL of cold peroxide-free ether. The pale red ether layer was washed with water (3 x 50 mL) and was dried over $MgSO_4$. After removal of the drying agent, the ether was removed (roto-evaporator) under reduced pressure in the cold. The pale red solid residue was dissolved in 12 mL of dry benzene, activated charcoal (Norit) was added, and the mixture was stirred for 10 min. The charcoal was removed by filtration yielding a benzene solution with a pale green tint. The benzene solution was concentrated (cold) to 1.5 mL and 15 mL of hexanes was added to induce crystallization of 8a. The

crystalline arene oxide 8a was separated from the mother liquor by centrifugation, was washed with 3 mL of hexanes, and dried to afford 50 mg (53% yield) of 8a as pale yellow needles, mp 150-152°C (ps 130°C) reported (4), mp 150°C.
^{13}C NMR (acetone-d_6) of diol 7a: 74.33 ppm (C_5).
^{13}C NMR ($CDCl_3$) of oxide 8a (10): 56.80 ppm (C_5).

Benzo[a]pyrene-4,5-oxide-4-^{13}C (8b)--In like manner to that described above for 8a, 100 mg (0.35 mmol of the dione 6b, mp 257-258°C, was reduced to the diol 7b which was further cyclized to the arene oxide 8b (45 mg, 48% yield), mp 150-151°C (ps 130°C).
^{13}C NMR (acetone-d_6) of diol 7b: 73.77 ppm (C_4).
^{13}C NMR ($CDCl_3$) of oxide 8b (10): 56.20 ppm (C_4).

ACKNOWLEDGEMENT

The authors wish to acknowledge the technical assistance of Robin D. Walker in running some of the ^{13}C NMR spectra. We also wish to thank Dr. R. M. Blazer and Dr. T. W. Whaley of the Los Alamos National Laboratory for helpful discussions. Discussions with Dr. D. M. Jerina were invaluable regarding the KBH_4 reductions and we thank him for his help and interest.

This investigation was supported in part by Grant Number CA-16871 awarded by the National Cancer Institute, DHEW. We are also indebted to the Stable Isotope Resource (LANL/NIH/ERDA) for supplying the labeled sodium acetates used.

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8. Both the crystalline esters 3a and 3b are readily hydrolyzed in excellent yields to the respective acids 4a and 4b with ethanolic KOH. The acid 4a (or 4b) is most conveniently prepared by subjecting the crude (uncrystallized) ester 2a (or 2b) to the dehydration and dehydrogenation step described followed by directly saponifying the crude ester 3a (or 3b) as described to give the acid 4a (or 4b) in 69-72% overall yield from the ketone 1.

9. Commercial 1-methylnaphthalene was dissolved in hexane, chromatographed over basic Woelm alumina, and distilled at reduced pressure. Failure to do so resulted in low yields of 3a (or 3b) due to elimination of ethylene from the ester group (acid catalyzed) and loss of CO_2 from the resulting acid at 250-260°C with the formation of considerable amounts of 4-methylchrysene.

10. The $CDCl_3$ used in the ^{13}C NMR studies should be passed over basic alumina (Woelm) just prior to use. Failure to do this may result in obtaining the spectra of the C_4 and C_5 phenols resulting from an NIH shift transformation of the oxides 8a or 8b due to traces of acid in the $CDCl_3$.