

¹³C-LABELLED BENZO[a]PYRENE AND DERIVATIVES.
AN EFFICIENT PATHWAY OF LABELLING THE 1-, 2-, AND 3-POSITIONS

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SUMMARY

A ten step synthesis of benzo[a]pyrene-1-¹³C, -2-¹³C, or 3-¹³C from 2,3,7,11b-tetrahydrobenz[d,e]anthracen-3(1H)-one is described in which the initial step involves the condensation of this ketone with the lithium enolate of ethyl acetate. This completes our synthesis of the twelve ¹³C labelled benzo[a]pyrenes.

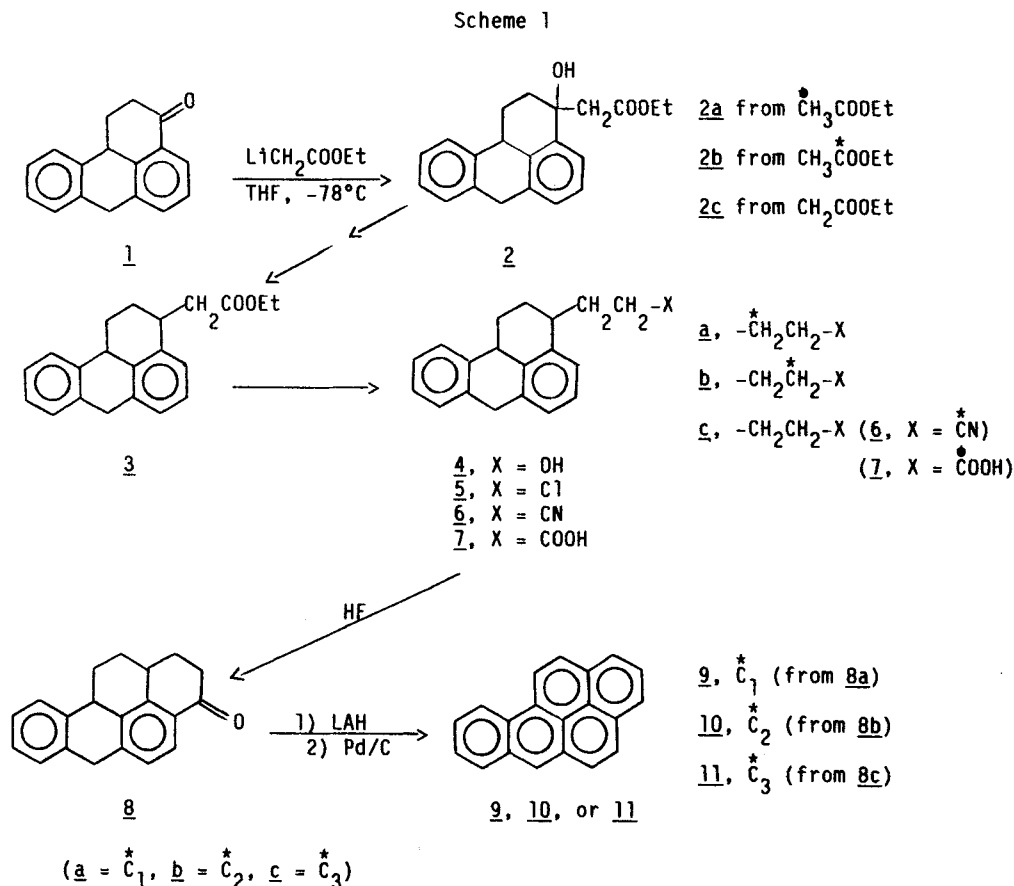
Key word: benzo[a]pyrene, carbon-13, ¹³C-NMR, enolates, PAH

INTRODUCTION

This is the final paper in the series of papers describing the syntheses of all twelve of the possible benzo[a]pyrenes labelled at the peripheral carbon atoms (1-3).

The readily accessible 1,2,7,11b-tetrahydrobenz[d,e]anthracen-3(1H)-one (1) was a suitable starting material (4) for the synthesis of the 1-, 2-, and 3-labelled benzo[a]pyrenes as outlined in Scheme 1. Condensation of 1 with the lithium enolate of ethyl acetate (unlabelled, labelled at C₂, or labelled at C₁) in THF at -78°C afforded the expected hydroxy ester 2 in good yield. Dehydration of the crude hydroxy ester with anhydrous formic acid (5) followed by hydrogenation over Pd/C gave the reduced ester 3 which was directly reduced with lithium aluminum hydride to the alcohol 4. Conversion of the alcohol 4 to the chloride 5 with thionyl chloride followed by treatment with potassium cyanide (labelled or unlabelled as needed) in acetonitrile afforded the nitrile 6 which was hydrolyzed to the corresponding acid 7 by refluxing with a mixture of hydrochloric and formic acids. Cyclization of the acid 7 with anhydrous hydrogen fluoride gave the ketone 8 (appropriately labelled), and

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this was converted to the benzo[a]pyrenes 9, 10, and 11 by reduction with lithium aluminum hydride followed by dehydration and dehydrogenation over Pd/C at 250°C.

The overall yield of BaP-1- ^{13}C (9) and BaP-2- ^{13}C (10) from the ketone 1 was 12% for ten steps. The BaP-3- ^{13}C labelled hydrocarbon 11 was obtained in 21% overall yield from the chloride 5.

The ^{13}C NMR chemical shifts at C_1 , C_2 , and C_3 were 125.38, 125.91, and 124.69 respectively for the labelled compounds. The values obtained were compared with those assigned by Buchanan and Ozubko (6) based on model compounds, selective proton decoupling, empirical correlations, and deuterium substitution. The chemical shift assigned by Buchanan and Ozubko for C_3 was shown to be

correct as 124.69; however, the values of 124.61 and 125.78 assigned by Buchanan and Ozbuko to C₁ and C₂, respectively, were shown to be incorrect by the values reported here. For a complete ¹³C NMR and ¹H NMR analysis of benzo[a]pyrene using these labelled compounds and others reported (1-3), see Unkefer, et al. (7).

EXPERIMENTAL

Melting points were determined using a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected. Elemental analyses were performed by Mrs. Ruby Ju of the Department of Chemistry, University of New Mexico.

Both ¹H and ¹³C NMR spectra were determined on a Varian FT-80A spectrometer. The ¹H and ¹³C chemical shifts are reported as parts per million (ppm) downfield from tetramethylsilane (TMS); and the ¹³C chemical shifts were referenced to the solvent peaks: CDCl₃ (76.9 ppm) or DMSO-d₆ (39.6 ppm). CDCl₃ was passed through basic alumina before use with alkenes or benzo[a]pyrene. ¹³C NMR spectra of labelled compounds were obtained by adding approximately three mg of labelled compound to a solution of the unlabelled compound in an appropriate solvent, unless otherwise noted. Infrared (IR) spectra were taken on a Perkin-Elmer 337 Grating Infrared spectrophotometer. The spectra were referenced with the 1601 and 1030 cm⁻¹ bands of polystyrene.

Ethyl (3-Hydroxy-1,2,7,11b-tetrahydrobenz[de]anthracen-3-(1H)-yl)-acetate (2c). 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 N₂ atmosphere, was added 10.3 mL of 1.6 M butyllithium (16.5 mmol) in hexane. Then 1.32 g (15.0 mmol) of ethyl acetate in 15 mL of anhydrous THF was added dropwise at a rate which maintained the temperature of the reaction mixture below -75°C. After addition was complete, stirring was continued for 15 min after which time 3.51 g (15.0 mmol) of 1,2,7,11b-tetrahydrobenz[de]anthracen-3(1H)-one (**1**), mp 130-131°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, stirring at -78°C

was continued for 2 h, during which time the solution turned from blue-green to pink in color. The complex was hydrolyzed by the dropwise addition of 2 mL of concd HCl in 8 mL of THF at a rate which maintained the temperature below -70°C . The mixture was allowed to warm to room temperature, and 50 mL of water and 50 mL of ether were added. The layers were separated, and the ether layer was washed with two portions of 5% HCl. The aqueous layer was extracted with 25 mL of ether, and the combined ether extracts were dried over MgSO_4 . Removal of the ether gave a pink oil which solidified upon standing. A sample crystallized twice from 95% ethanol afforded 2c as pale yellow irregular prisms, mp $103.5\text{--}105^{\circ}\text{C}$. The crude hydroxyester was used in the next step.

TLC: $R_f = 0.56$ (2:1 benzene/ethyl acetate).

IR (KBr): 3470 (OH), 2940, 1700 (C=O), 1335, 1195, 1085, 1055, 1025, 790, 740 cm^{-1} .

Anal. Calcd. for $\text{C}_{21}\text{H}_{22}\text{O}_3$: C, 78.23; H, 6.88. Found: C, 78.16; H, 6.86.

Ethyl (3-Hydroxy-1,2,7,11b-tetrahydrobenz[de]anthracen-3-(1H)-yl)acetate-2- ^{13}C (2b). In like manner to that described for the synthesis of 2c, 5.86 g (25.0 mmol) of 1, mp $130\text{--}131^{\circ}\text{C}$, was allowed to react with the lithioenolate prepared from 2.22 g (25.0 mmol) of ethyl acetate-2- ^{13}C , 90 mol % ^{13}C . Workup provided 2b as an orange oil which was used directly in the next step.

Ethyl (3-Hydroxy-1,2,7,11b-tetrahydrobenz[de]anthracen-3-(1H)-yl)acetate-1- ^{13}C (2a). In like manner to that described for the synthesis of 2c, 7.03 g (30.0 mmol) of 1 mp $130\text{--}131^{\circ}\text{C}$, was allowed to react with lithioenolate prepared from 2.67 g (30.0 mmol) of ethyl acetate-1- ^{13}C , 90 mol % ^{13}C . Workup provided 2a as an orange oil which was used directly in the next step.

Ethyl (1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)acetate (3c). The crude hydroxyester (2c) dissolved in 10 mL of 97-100% formic acid was warmed on a steam-bath for 10 min. The formic acid was removed under reduced pressure, the residue dissolved in benzene and washed three times with water and once with saturated salt. After drying over MgSO_4 , removal of the benzene afforded

the dehydrated ester as a yellow oil which was dissolved in 100 mL of absolute ethanol and reduced in the presence of 0.75 g 5% Pd/C at 60.0 psi. After 15 h, at room temperature, when the theoretical amount of hydrogen had been taken up, the solution was filtered and the ethanol removed under reduced pressure. The last traces of ethanol were removed by drying at 50°C and 25 torr for 18 h, affording 3.70 g (81% overall yield from 1) of 3c as a highly viscous pale yellow oil consisting of a mixture of isomers which could not be crystallized.

TLC: $R_f = 0.35$ (benzene); $R_f = 0.65$ (2:1 benzene/ethyl acetate).

IR (neat): 2935, 1735 (C=O), 1278 (C-O), 1170, 1033, 778, 740 cm^{-1} .

Ethyl (1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)acetate-2-¹³C (3a). In like manner to that described for the synthesis of 3c, the crude hydroxyester 2a was converted to the unsaturated ester which was dissolved in 125 mL of absolute ethanol and reduced in the presence of 1.25 g of 5% Pd/C at 70.0 psi. Workup afforded 6.06 g (79% overall yield from 1) of 3a as a highly viscous pale yellow oil.

Ethyl (1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)acetate-1-¹³C (3b).

In like manner to that described for the synthesis of 3c, the crude hydroxyester (2b) was converted to the unsaturated ester which was dissolved in 150 mL of absolute ethanol and reduced in the presence of 1.50 g of 5% Pd/C at 70.0 psi. Workup afforded 7.42 g (81% overall yield from 1) of 3b as a highly viscous pale yellow oil.

2-(1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)ethanol (4c). To a stirred slurry of 3.80 g (100 mmol) of lithium aluminum hydride in 90 mL of anhydrous ether was added over 2 h a solution of 10.95 g (35.7 mmol) of 3c in 200 mL of anhydrous ether. After 2.5 h of reflux, TLC indicated completion of the reaction, and 20 mL of ethyl acetate was added slowly to the reaction mixture followed by 20 mL of water to hydrolyze the excess LAH; 5% HCl was added to dissolve the inorganic salts. The layers were separated and the ether solution was washed twice with 5% HCl, and once each with water and saturated salt, dried over MgSO_4 , filtered and the ether removed under reduced

pressure. Drying at 50°C and 25 torr for 15 h afforded 9.45 g (quant. yield) of 4c as a highly viscous pale yellow fluorescent oil as a mixture of isomers.

TLC: $R_f = 0.31$ (2:1 benzene/ethyl acetate).

IR (neat): 3350 (broad, OH), 2935, 1450, 1047 (C-O), 780, 752 cm^{-1} .

The 3,5-dinitrobenzoate ester of a sample of the alcohol was prepared in anhydrous pyridine with 3,5-dinitrobenzoyl chloride. Crystallization twice from benzene/cyclohexane yielded orange powdery crystals, mp 166-174°C (evac).

Anal. Calcd. for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_6$: C, 68.11; H, 4.84. Found: C, 68.31; H, 4.94.

2-(1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)ethanol-2- ^{13}C (4a).

In like manner to that described for the synthesis of 4c, 6.06 g (19.7 mmol) of 3a was reduced to 4a. Workup provided 5.23 g (quant. yield) of 4a as a highly viscous pale yellow oil.

2-(1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)ethanol-1- ^{13}C (4b). In like manner to that described for the synthesis of 4c, 7.42 g (24.1 mmol) of 3b was reduced to 4b. Workup provided 6.41 g (quant. yield) of 4b as a highly viscous pale yellow oil.

2-(1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)chloroethane (5c). To a stirred solution of 9.45 g (35.7 mmol) of 4c in 30 mL of anhydrous chloroform and containing 0.26 g (3.6 mmol) of DMF was added, over 15 min, a solution of 2.7 mL (37.5 mmol) of thionyl chloride in 10 mL of anhydrous chloroform. After 45 min of reflux, TLC indicated completion of the reaction. The reaction mixture was cooled in an ice-bath and 5% NaHCO_3 was added slowly to neutralize any acidic material. The layers were separated and the organic phase was diluted with ether and washed with 5% NaHCO_3 , water and saturated salt, dried over K_2CO_3 , filtered, and the solvents removed under reduced pressure. The brown oil was chromatographed on neutral alumina, eluting with benzene. Removal of the benzene under reduced pressure and drying at 50°C and 25 torr for 15 h afforded 7.17 g (71% yield) of 5c as a highly viscous red oil as a mixture of isomers.

TLC: $R_f = 0.73$ (2:1 benzene/ethyl acetate).

IR (neat): 2930, 1445, 1208, 875, 750 (broad, C-C1) cm^{-1} .

2-(1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)chloroethane-2-¹³C

(5a). In like manner to that described for the synthesis of 5c, 5.23 g (19.7 mmol) of 4a was converted to 5a. Workup afforded 4.12 g (74% yield) of 5a as a highly viscous red oil.

2-(1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)chloroethane-1-¹³C

(5b). In like manner to that described for the synthesis of 5c, 6.41 g (24.2 mmol) of 4b was converted to 5b. Workup afforded 5.46 g (80% yield) of 5b as a highly viscous red-orange oil.

3-(1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)propanenitrile-1-¹³C

(6c). A mixture of 2.98 g (10.5 mmol) of 5c, 0.55 g (2.1 mmol) of 18-Crown-6, 0.70 g (10.5 mmol) of K^{13}CN (99.3% KCN, 90.79 mol % ¹³C) 8 mL of acetonitrile and 4 mL of benzene was refluxed for 48 h. The dark brown solution was diluted with benzene and filtered to remove insoluble components. The solvents were removed under reduced pressure and the dark residue was dissolved in benzene and chromatographed on neutral alumina. The nitrile was eluted with 1:1 benzene/ethyl acetate. After removal of solvents, the residue was dried 18 h at 50°C and 25 torr providing 2.08 g (72% yield) of 6c as a highly viscous yellow fluorescent oil as a mixture of isomers which could not be crystallized.

TLC: $R_f = 0.24$ (benzene); $R_f = 0.64$ (2:1 benzene/ethyl acetate).

IR (neat): 2935, 2240 (CN), 1455, 1035, 878, 737, 680 cm^{-1} .

3-(1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)propanenitrile-3-¹³C

(6a). In like manner to that previously described for the synthesis of 6c, 4.12 g (14.5 mmol) of 5a was allowed to react with 1.89 g (29.0 mmol) of KCN. Workup afforded 2.89 g (73% yield) of 6a.

3-(1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)propanenitrile-2-¹³C

(6b). In like manner to that previously described for the synthesis of 6c,

5.46 g (19.3 mmol) of 5b was allowed to react with 2.51 g (38.5 mmol) of KCN. Workup afforded 3.60 g (68% yield) of 6b.

3-(1,2,7,11b-Tetrahydrobenz[de]anthracen-3-(1H)-yl)propanoic-1-¹³C Acid (7c). A mixture of 2.08 g (7.6 mmol) of 6c, 20 mL of concd HCl, and 40 mL of 97-100% formic acid was refluxed for 6 days. After the first 4 days, 5 mL of additional concd HCl was added. The acid was precipitated by pouring the yellow solution into ice water. The solid collected was dissolved in 5% NH₄OH, extracted with ether and reprecipitated with concd HCl, collected and dried at reduced pressure, affording 1.72 g (78% yield) of 7c as pale yellow crystals, mp 148.5-159°C (reported (9), 150-160°C; isomeric mixture).

IR (KBr): 3200-2700 (broad, C=O-H), 2960 (sharp), 1710 (C=O), 1290, 1270, 1220, 1187, 910 (broad, C=O-H), 790, 755, 730, 700, 635 cm⁻¹.

3-(1,2,7,11b-Tetrahydro-3H-benz[de]anthracen-3-(1H)-yl)propanoic-3-¹³C Acid (7a). A mixture of 2.89 g (10.5 mmol) of 6a, 30 mL of concd HCl, and 75 mL of 97-100% formic acid was refluxed 6 days. After the first two days, 5 mL of concd HCl was added; after two more days, 5 mL of concd HCl and 10 mL of formic acid were added. Workup as previously described for 7c afforded 2.05 g (66% yield) of 7a as yellow granular crystals, mp 133-160°C (isomeric mixture).

3-(1,2,7-11b-Tetrahydro-3H-benz[de]anthracen-3-(1H)-yl)propanoic-2-¹³C Acid (7b). In like manner to that previously described for the synthesis of 7c, the hydrolysis of 3.60 g (13.1 mmol) of 7b afforded 2.31 g (60% yield) of 7b as yellow granular crystals, mp 133.5-158°C (isomeric mixture).

1,6,10b,11,12,12a-Hexahydrobenzo[a]pyren-3(2H)-one-3-¹³C (8c). A solution of 1.71 g (5.8 mmol) of 7c, mp 155-165°C, in 30 mL of anhydrous HF, was stirred for 5 min and then allowed to stand for 6 h. The viscous residue was dissolved in benzene and extracted with 5% NaHCO₃, 5% NH₄OH, water and saturated salt, dried over MgSO₄, filtered and the dark yellow benzene solution was concentrated and chromatographed on neutral alumina. The ketone was eluted with 1:1 benzene/ethyl acetate. Removal of solvents under reduced pressure and drying 4.5 h at 50°C and 25 torr afforded 1.80 g (83% yield) of 8c as pale yellow crystals, mp 127-138°C (reported (9), 135-165°C; mixture of

isomers).

TLC: $R_f = 0.58$ (2:1 benzene/ethyl acetate).

IR (KBr): 2945, 1670 (C=O), 1279, 1205, 827, 764, 743, 717, 659 cm^{-1} .

1,6,10b,11,12,12a-Hexahydrobenzo[a]pyren-3(2H)-one-1-¹³C (8a). In like manner to that described for the synthesis of 8c, 2.05 g (7.0 mmol) of 7a, mp 133–160°C, was cyclized to 8a. Workup provided 1.57 g (82% yield) of 8a as yellow granular crystals, mp 172–178.5°C (isomeric mixture).

1,6,10b,11,12,12a-Hexahydrobenzo[a]pyren-3(2H)-one-2-¹³C (8b). In like manner to that described for the synthesis of 8c, 2.31 g (7.9 mmol) of 7b, mp 133.5–158°C, was cyclized to 8b. Workup provided 1.80 g (83% yield) of 8b as yellow granular crystals, mp 134.5–165°C (isomeric mixture).

Benzo[a]pyrene-3-¹³C (11). To a stirred slurry of 0.48 g (1.3 mmol) of lithium aluminum hydride in 15 mL of anhydrous THF was added over 20 min a solution of 1.30 g (4.7 mmol) of 8c, mp 127–138°C, in 45 mL of anhydrous THF. After 5.5 h of reflux, TLC indicated completion of the reaction, and 10 mL of ethyl acetate was added slowly to the reaction mixture followed by 10 mL of water to hydrolyze the excess LAH. To dissolve the inorganic salts, 5% HCl was added and the organic material was extracted into ether. The layers were separated and the ether solution was washed with 5% HCl, water and saturated salt, dried over MgSO_4 , filtered and the ether removed under reduced pressure. The yellow oily residue was placed in a dehydrogenation tube fitted with a ground-glass cold-finger condenser and gas inlet and outlet tubes and containing 0.13 g of 10% Pd/C, 2.53 g (14.0 mmol) of 1,1-diphenylethene and 10 mL of 1-methylnaphthalene. The reaction mixture was placed in a preheated Woods metal bath and the temperature maintained at 250–260°C for 8 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 and filtered from the catalyst which was washed with benzene. After removal of the benzene on a rotary evaporator, the 1-methylnaphthalene, 1,1-diphenylethane and unreacted 1,1-diphenylethene were removed under reduced pressure (0.025 torr, 50–60°C)

on a Kügelrohr apparatus. The resultant brown solid was chromatographed on neutral alumina, eluting with benzene. The yellow benzene eluate was concentrated and added to a benzene solution of 1.25 g (5.5 mmol) of picric acid. The picrate thus prepared afforded 1.43 g of purple-brown needles, mp 197-198°C (reported (10), 197-198°C). An additional 0.18 g of picrate, mp 194-196°C, was obtained from the mother liquor. The picrate was decomposed on a neutral alumina column, eluting with benzene. The benzene eluate was concentrated and methanol was added to afford 0.55 g (46% yield) of 11 as lemon yellow shiny platelets, mp 175-176°C (reported (11), 176-177°C).

^{13}C NMR (CDCl_3): δ 124.69 (C_3).

Benzo[a]pyrene-1- ^{13}C (9). In like manner to that described for the synthesis of 11, 1.57 g (5.7 mmol) of 8a, mp 172-178.5°C, was converted to benzo[a]pyrene-1- ^{13}C (9). Workup afforded 0.65 g of 9 as lemon yellow fibrous needles, mp 175.5-177°C. An additional 0.07 g (50% overall yield) of product, mp 169-172.5°C, was obtained from the mother liquor.

^{13}C NMR (CDCl_3): δ 125.38 (C_1).

Benzo[a]pyrene-2- ^{13}C (10). In like manner to that described for the synthesis of 11, 1.52 g (5.5 mmol) of 8b, mp 134.5-165°C, was converted to benzo[a]pyrene-2- ^{13}C (10). Workup afforded 0.72 g (51% yield) of 10 as lemon yellow shiny platelets, mp 175-176°C.

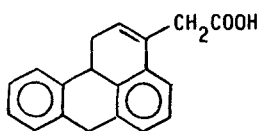
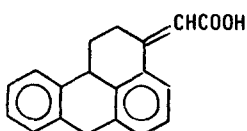
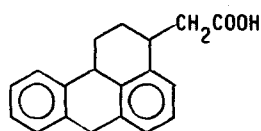
^{13}C NMR (CDCl_3): δ 125.91 (C_2).

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