SYNTHESIS OF 2-FLUOROBENZO[RST]PENTAPHENE AND 2-FLUOROBENZO[RST]PENTAPHENE-5-^{13}C

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SUMMARY

The syntheses of 2-fluorobenzo[rst]pentaphene and 2-fluorobenzo[rst]pentaphene-5-^{13}C (70% ^{13}C) and their precursors are described, and relevant directly-bonded ^{13}C-^{1}H couplings reported. The labelled material was used to differentiate the NMR resonances of the 5- and 8-protons, in connection with our studies of the remote effect exercised by fluorine on their reactivities toward electrophilic aromatic substitution, and on the carcinogenic activity of benzo[rst]pentaphene.

Key Words: carcinogens, 2-fluorobenzo[rst]pentaphene, ^{13}C labelling, ^{13}C-^{1}H coupling constants.

INTRODUCTION

One of the general and widely-used approaches to identifying molecular sites involved in metabolism is the fluorine substitution methodology. This technique, involving replacement of hydrogen by the essentially isosteric fluorine, has been applied by the Millers and others to the study of carcinogens since the nineteen-fifties (1,2). It assumes that, if a molecule retains its biological activity upon fluorination, the position occupied by the fluorine

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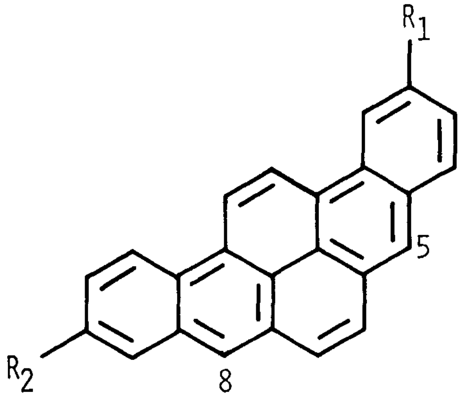
atom is not involved directly in the activity under test. If, on the other hand, a fluorinated derivative is inactive, the position blocked by fluorine may be assumed to participate in the activity of the unfluorinated parent molecule. Thus, our observation (3) that the potent carcinogen dibenzo[a,i]pyrene (benzo[rst]pentaphene, 1) becomes totally noncarcinogenic upon difluorination at positions 2 and 10 (3) provided the first evidence that metabolic activation of 1 occurs in the angular ("bay-region") benzo rings, consistent with the recently enunciated "bay-region" hypothesis (4,5).



	R_1	R_2
1	H	H
2	F	H
3	F	F

However, substitution of fluorine for hydrogen only at the 2-position (2) also leads to complete loss of carcinogenicity (6), suggesting fluorine to be affecting metabolism at the remote, unsubstituted, benzo ring. In search of a chemical precedent for this differential and long-range effect of fluorine, we studied the relative reactivities of the two "mesoanthracenic" 5- and 8-positions in 2 toward electrophilic attack, using deuteriodeprotonation in D_2SO_4. The 5- and 8-positions are peri to the angular (bay-region) benzo rings and may be involved in some way in the activation of those rings leading toward epoxide formation (4,7). Since both protons H-5 and H-8 give rise to singlets in the NMR spectrum, and could not be distinguished spectrally, we chose to effect this differentiation using 2-fluorodibenzo[a,i]pyrene-5-^{13}C (2-5-^{13}C), whose synthesis and spectral characteristics we report here.

RESULTS

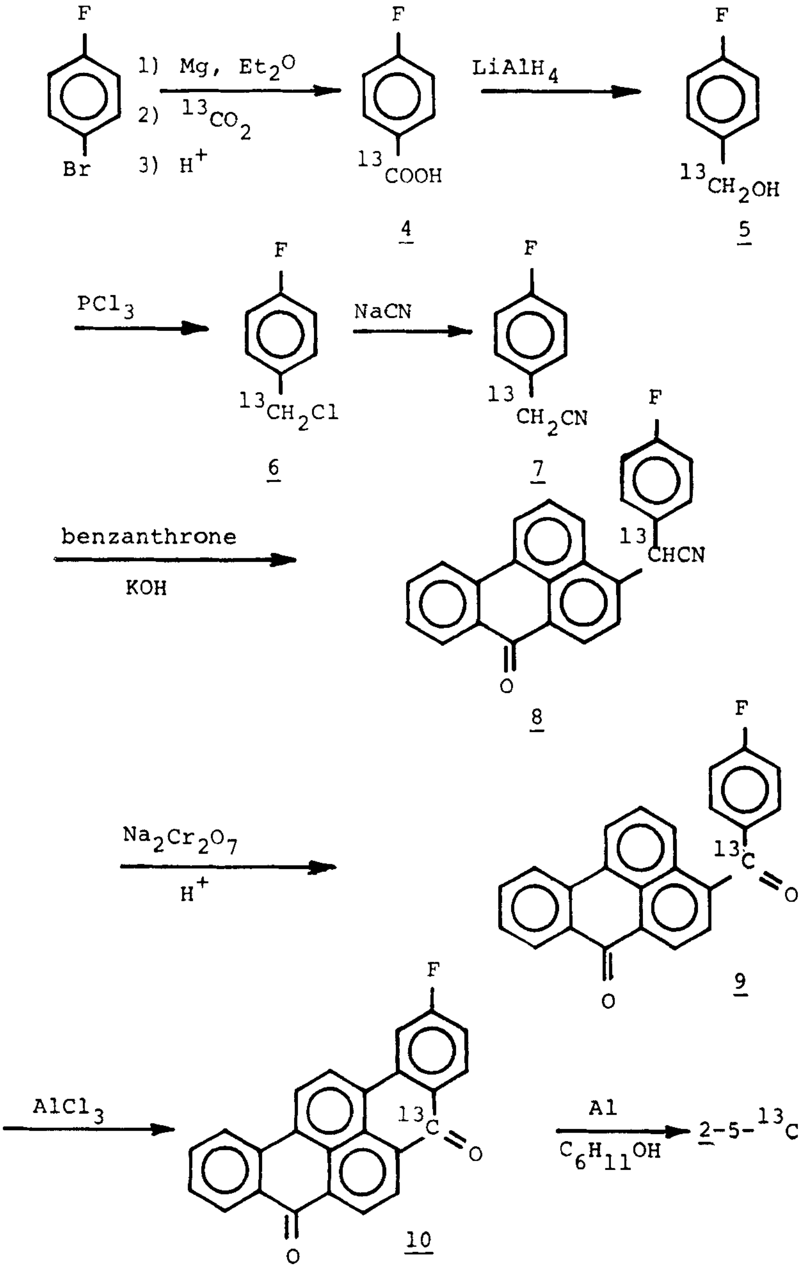
The synthetic scheme employed (Chart I), is a modification of that used for synthesis of the unlabelled material, involving the condensation of p-fluorophenylacetonitrile-α-^{13}C with benzanthrone. The labelled p-fluorophenylacetonitrile was synthesized beginning with p-fluorobromobenzene and labelled CO_2 (70% ^{13}C). None of the labelled compounds has been previously reported. Intermediates were identified by comparison of their physical constants and infrared spectra with those of authentic, unlabelled, samples. In addition, proton and carbon NMR spectra allowed both identification and confirmation of the extent and site of ^{13}C-incorporation, owing to the presence of one-bond ^{13}C-^{1}H couplings, whose magnitudes are reported in the Table. As expected for couplings involving sp^3-hybridized carbon, compounds 5-8 exhibited couplings in the range 130-150 Hz, while 2-5-^{13}C with its sp^2-hybridized carbon, has a coupling constant of 160 Hz, allowing definitive assignment of the resonances of the 5- and 8-protons.

Table 1. ^{13}C-^{1}H couplings

compound	$^{1}J_{CH}$ (Hz)
5	142 Hz
6	148
7	135
8	133
2-5-^{13}C	160

The 270 MHz NMR spectrum of unlabelled 2 is easily assignable, except for the resonances of the "peri" protons, 5 and 8, which exhibit singlets at τ 1.71 and 1.75. Upon incorporation of ^{13}C specifically at position 5, the NMR spectrum was unaffected except for the singlet at τ 1.75, which disappeared (except for a residual peak due to the 30% unlabelled material) and was replaced by a doublet (J = 160 Hz) symmetrically disposed about τ 1.75. Thus, the assignments are: H-5 (τ 1.75) and H-8 (τ 1.71). (Figure 1).

CHART I


F
Br
1) Mg, Et$_2$O
2) ^{13}CO$_2$
3) H$^+$
F
^{13}COOH
4
LiAlH$_4$
F
^{13}CH$_2$OH
5
PCl$_3$
F
^{13}CH$_2$Cl
6
NaCN
F
^{13}CH$_2$CN
7
benzanthrone
KOH
F
^{13}CHCN
O
8
Na$_2$Cr$_2$O$_7$
H$^+$
F
^{13}C
O
O
9
AlCl$_3$
F
^{13}C
O
O
10
Al
C$_6$H$_{11}$OH
2-5-^{13}C

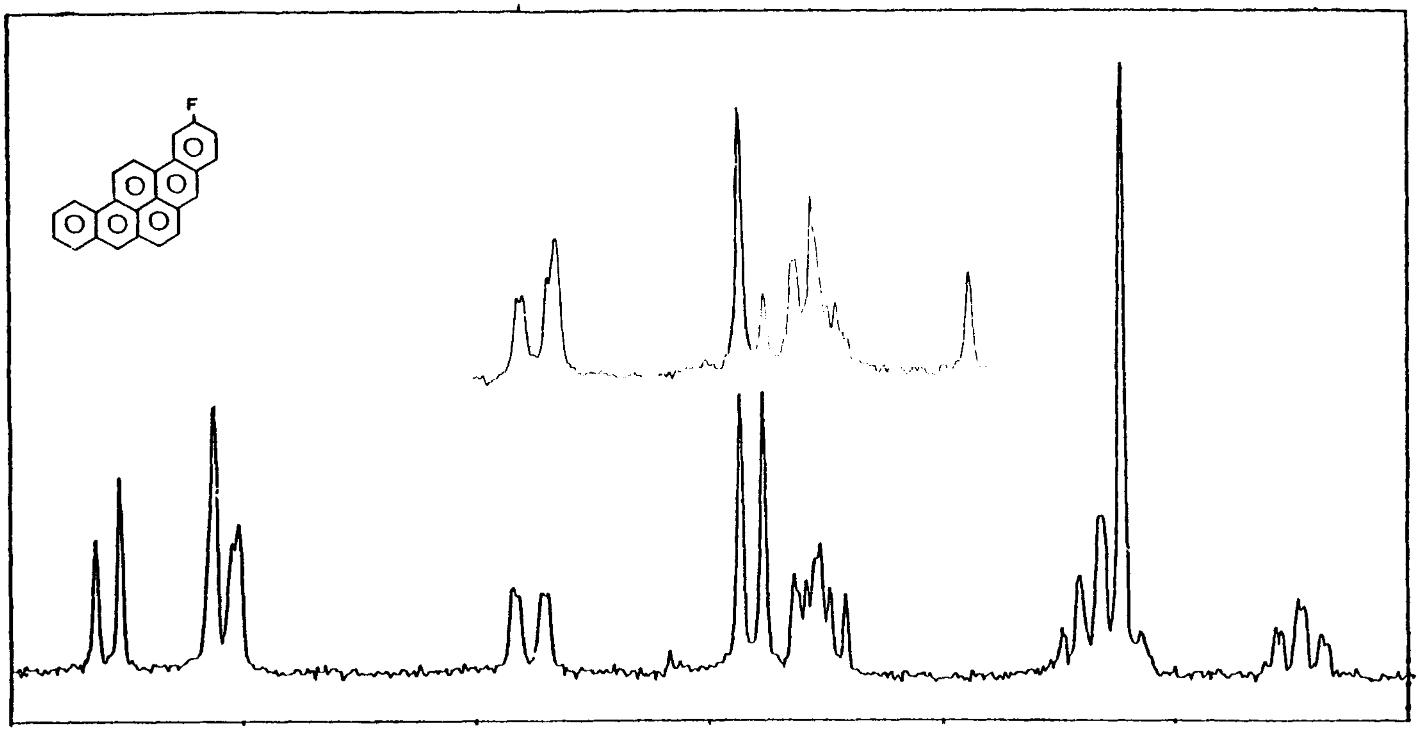


Figure 1. 270 MHz proton spectrum of 2-fluorobenzo[rst]pentaphene (2, in $CDCl_3$). The two singlets at mid-spectrum correspond to H-5 and H-8. Upper trace: 2-5-^{13}C (partial spectrum), showing intensity reduction of H-5 resonance on 70% ^{13}C ennchment, and appearance of ^{13}C-satellites (J = 160 Hz).

The results of our deuteriodeprotonation experiments (to be reported elsewhere) show that, while the 5-position in 2 is less reactive than the 8-position, as anticipated, both positions are deactivated relative to benzo[rst]pentaphene.

EXPERIMENTAL

Melting points and boiling points are uncorrected.

Infrared spectra were obtained on a Perkin-Elmer 421-C spectrophotometer.

Mass spectra were measured on a Hitachi Perkin-Elmer model RMS-4 medium resolution mass spectrometer.

Proton NMR spectra were obtained at 60 MHz on one of three continuous wave instruments: a Varian Associates A-60A NMR spectrometer; a Varian Associates Model T-60 NMR spectrometer; or a Hitachi Perkin-Elmer Model R-24 NMR spectrometer. Spectra of 2-5-^{13}C at 270 MHz were obtained on a Bruker HX-270 Fourier Transform spectrometer. In all cases, the solvent was chloroform-d, with tetramethylsilane serving as an internal reference.

Carbon NMR spectra were obtained on a JEOL Model FX-600 Fourier Transform spectrometer with, and without, proton noise decoupling.

p-Fluorobenzoic acid carbonyl-^{13}C (4) was prepared by carbonation of p-fluorophenylmagnesium bromide, using a modification of a literature procedure (8).

A 500 ml round bottom flask containing 0.094 moles of the Grignard reagent in 200 mls of anhydrous ether was fitted with a bubbler containing mineral oil, and connected to a carbon dioxide generator to form a closed system. The gas generator (consisting of a 100 ml 2-neck round bottom flask, a dropping funnel containing 50 ml of concentrated sulfuric acid, and a gas outlet terminating in a fritted glass aerator extending below the surface of the Grignard reagent) was charged with 20 g of barium carbonate - ^{13}C; Stohler Isotope Chemicals).

The reaction mixture was precooled to -30°C and stirred while $^{13}CO_2$, generated by slow dropwise addition of sulfuric acid to the barium carbonate, was bubbled slowly into the mixture. Addition of

$^{13}CO_2$ required approximately 2 h, during which time the temperature was maintained between -15°C and -30°C. The reaction flask was then warmed to 0°C in an ice bath and a mixture of ice and 6 N sulfuric acid added to decompose the adduct. The aqueous layer was separated and extracted twice with ether. Extraction of the combined, pale yellow, ether layer three times with 5% sodium hydroxide gave an aqueous solution of the sodium salt, which upon acidification (6 N HCl) to pH 1-2, afforded the acid (4) as a white solid which was filtered and dried in a vacuum oven. Yield: 7.33 g (54.6°, based on p-fluorobromobenzene). The ^{1}H NMR spectrum of 4 ($CDCl_3$) was identical with that of the unlabelled acid.

p-Fluorobenzyl alcohol-α-^{13}C (5). A solution containing 7.5 g of 4 in 100 ml of anhydrous ether was added slowly (ca. 1 h) to a solution of 2.5 g of lithium aluminum hydride in 60 ml of ether, after which the mixture was heated under reflux (4 h) and cooled to room temperature. Cautious addition of water (50 ml), followed by acidification with 40% H_2SO_4 (ca. 120 ml) gave two layers. The aqueous layer was extracted with ether (2X) and the combined ether layers dried (Na_2SO_4). Removal of ether in vacuo afforded crude alcohol 5 (5.6 g) which was distilled under vacuum (b.p. 96-99°/ 15 mm) to give pure 5. Yield: 4.5 g (68%).

The ^{1}H NMR spectrum of 5 was identical to that of unlabelled 5, except for the benzylic proton resonance at τ 5.5, which was split into a doublet (J = 142 Hz) by coupling to carbon-13. The coupled ^{13}C spectrum showed only a triplet due to coupling with the benzylic protons.

p-Fluorobenzyl chloride-α-^{13}C (6). To a solution of 7.3 g of 5 in 30 ml of dichloromethane at 0°C was added 2.3 ml of phosphorus trichloride, with stirring, at a rate slow enough to keep the temperature from rising. After addition was complete, the mixture was kept at 0°C for half an hour, then heated at 50°C for 1 h. Upon cooling, the solution separated into two layers. The (upper) halide layer was taken and solvent and excess PCl_3 removed in vacuo to give 6 (Yield: 6 g; 72%).

The ^{1}H NMR spectrum of 6 was identical with that of unlabelled material, except for the benzylic resonance at τ 5.5 which appeared as a doublet (J = 148 Hz). Its ^{13}C spectrum exhibited only a triplet, as expected.

p-Fluorobenzyl cyanide-α-^{13}C (7). Solutions of 6 (7.5 g in 8 ml of 95% ethanol) and potassium cyanide (4 g in 20 ml of H_2O) were combined and the mixture refluxed 4 h. Upon cooling, the liquid was decanted and the solid washed with 50 ml of dichloromethane. The salt, dissolved in water was extracted with dichloromethane (2X 50 ml). The combined organic layers were dried (Na_2SO_4), and the solvent removed in vacuo. The crude product (6.8 g) was distilled (b.p. 119-120°C/18 mm), giving pure 7. Yield: 5.3 g (75%).

The ^{1}H spectra of labelled and unlabelled 7 were identical except for the splitting of the benzylic resonance (d, J = 135 Hz). The carbon spectrum was a triplet.

Compounds 8, 9, 10 and 2 have not been previously reported. Thus procedures for synthesis of unlabelled compounds are described below. Synthesis of labelled compounds involved identical conditions.

α-[p-Fluorophenyl]-7-oxo-7H-benz(de)anthracene-4-acetonitrile; (4-cyano[p-fluorobenzyl]-7H-benz(de)anthracene-7-one) 8. Isopropanol (30 ml) and 7.6 g of powdered potassium hydroxide were mixed with good agitation and cooled to room temperature. Into this mixture, 2.5 g of benzanthrone was added, followed by 10 ml of p-fluorophenylacetonitrile. The reaction mixture was heated to 45°C and air was bubbled through for 5 hours, while the temperature was kept between 40 and 45°C. After the reaction mixture was cooled to room temperature, 7.2 ml of glacial acetic acid was added with stirring and the residue was dissolved to give an orange solution. The reaction mixture was stirred at 30-35°C for five hours. The yellow sticky precipitate was filtered by suction, boiled with methanol and filtered. It was then extracted with hot benzene, and the benzene solution evaporated to give yellowish-brown precipitate. Recrystallization from dimethylformamidebenzene gave 8, m.p. 208°C.

Anal. Calcd: C, 82.63; H, 3.88; F, 5.22; N, 3.86.

Found: C, 82.31; H, 4.02; F, 5.34; N, 4.09.

α-[p-Fluorophenyl]-7-oxo-7H-benz(de)anthracene-4-acetonitrile-α-^{13}C (8-^{13}C) was synthesized as described above using p-fluorobenzyl cyanide-α-^{13}C (7).

Its ^{1}H NMR spectrum was identical to that of unlabelled 8 except for the methine resonance (τ 3.9, 1H) which was split into a doublet (J = 133 Hz) due to coupling with ^{13}C. The carbon spectrum likewise showed only a doublet.

4-[p-fluorobenzoyl]-7H-benz(de)anthracene-7-one (9). A mixture of 3.1 g sodium acetate, 2.75 g sodium dichromate, 1.85 g 4-[p-fluorocyanobenzyl]benzanthrone and 14 ml glacial acetic acid was heated on a boiling water bath for three hours. After cooling, 7 ml of water were added slowly (to avoid precipitation of large particles). The precipitate formed was filtered and washed with hot water until a colorless filtrate was obtained. Recrystallization (benzene-petroleum ether) yielded 1.5 g (84%) of 9. M.p. 212-214°C.

Anal. Calcd. for $C_{24}H_{13}FO_2$: C, 81.81; H, 3.72;

Found: C, 82.20; H, 3.89.

4-[p-Fluorobenzoyl]-7H-benz(de)anthracene-7-one-α-^{13}C (9-^{13}C) synthesized from 8-^{13}C as described above, was obtained in 81% yield. M.p. 215-217°C.

2-Fluorobenzo[rst]pentaphene-5,8-dione (10). A mixture of potassium chloride (0.9 g), sodium chloride (0.9 g) and technical grade aluminum chloride (10.2 g; if pure aluminum chloride is used, no reaction occurs) was heated to 125°C in an Erlenmeyer flask. When the mixture had melted 1.5 g of 9 was added, followed by 0.6 g m-nitrobenzoic acid. Heating was continued for four hours, after which the mixture was boiled with a solution of 5 ml conc. HCl and 50 ml water and filtered. 1.4 g (93% yield) of the dione were obtained. Recrystallization from xylene gave red crystals, m.p. 380°C.

Anal. Calcd. for $C_{24}H_{11}FO_2$: C, 82.28; H, 3.16;

Found: C, 81.45; H, 3.29.

2-Fluorobenzo[rst]pentaphene-5,8-dione-5-^{13}C (10-^{13}C) was synthesized as described above, using 9-^{13}C.

2-Fluorobenzo[rst]pentaphene (2). Aluminum turnings (2 g) were heated under reflux with cyclohexanol (40 ml) after addition of a few milligrams of mercuric chloride as catalyst. 2-Fluorobenzo[rst]pentaphene-5,8-dione (10) (1 g) was added to the opalescent solution and the mixture heated under reflux for 48 h. Cyclohexanol was removed by vacuum distillation and the remaining pasty mass was stirred into 5% sodium hydroxide solution (400 ml). The last traces of cyclohexanol were removed by steam distillation, and the solution was then treated with sodium hydrosulfite (2 g) to dissolve any unconverted quinone. The mixture was filtered and the residual dark brown powder was dissolved in warm chloroform and chromatographed on alumina with 50% hexane-anhydrous ethyl ether as eluent. The yellow fluorescent material was collected. After solvent removal, pale yellow plates of the hydrocarbon were obtained (0.44 g, 46%). After recrystallization from xylene, fluorescent yellow needles, m.p. 241-2°C were obtained.

Anal. Calcd: C, 89.97; H, 4.10; F, 5.93;

Found: C, 89.47; H, 4.06; F, 5.91.

The mass spectrum of 2 exhibited a parent ion peak at 320. Its UV spectrum is very similar to that of benzo[rst]pentaphene.

The ^1H NMR spectrum of 2 (270 MHz, $CDCl_3$) exhibited a qualitative similarity to that of benzo[rst]pentaphene, the major differences being a) shifting of the resonances of protons ortho to fluorine (H-1 and H-3) upfield by ca. 0.3-0.4 ppm to τ 1.43 (d) and τ 2.47 (m), respectively, and b) the nonequivalence of protons H-13 and H-14 (AB, J = 9.6 Hz) and H-5 and H-8 (s).

2-Fluorobenzo[rst]pentaphene-5-^{13}C (2-^{13}C), synthesized as described above from 10-^{13}C, was identical in all respects with unlabelled material. Its ^1H NMR spectrum was also identical with that of 2, except for the resonance of H-5 (τ 1.75), which was split into a doublet (J = 160 Hz) by coupling to ^{13}C.

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Carbon-13 NMR were obtained at Northeastern University.

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