SYNTHESIS OF 4-AMINO-3,2'-DIMETHYLBIPHENYL-3-METHYL-^{14}C AND 4-AMINO-2'-METHYLBIPHENYL-2'-METHYL-^{14}C

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SUMMARY

The syntheses of 4-amino-3,2'-dimethylbiphenyl-3-methyl-^{14}C, a potent carcinogen and mutagen, and 4-amino-2'-methylbiphenyl-2'-methyl-^{14}C, an analogue having weaker activity, are described. In both cases, label was introduced with Cu^{14}CN.

Key words: 4-Amino-3,2'-dimethylbiphenyl, 4-Amino-2'-methylbiphenyl, carcinogens, carbon-14.

INTRODUCTION

4-Amino-3,2'-dimethylbiphenyl (1) is a potent carcinogen, inducing colon and breast tumors in rats and bladder cancer in Syrian golden hamsters (1-3). In contrast, 2 , as the corresponding acetamide, failed to induce tumors in rats (4). The

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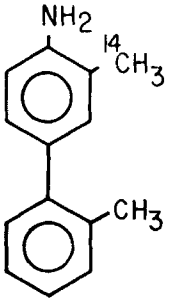
mutagenic activity of 1 toward *S. typhimurium* TA 1538 also exceeded that of 2 (5). These results are typical of several carcinogenic aromatic amines having a methyl group *ortho* to the amino functionality. The *ortho* substituted compounds are frequently more carcinogenic or mutagenic than the unsubstituted analogues (6). To investigate the origin of this effect, we synthesized 1-3-methyl-^{14}C and 2-2'- methyl-^{14}C.



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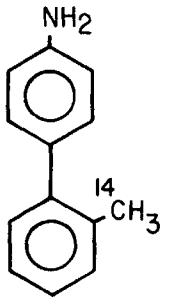

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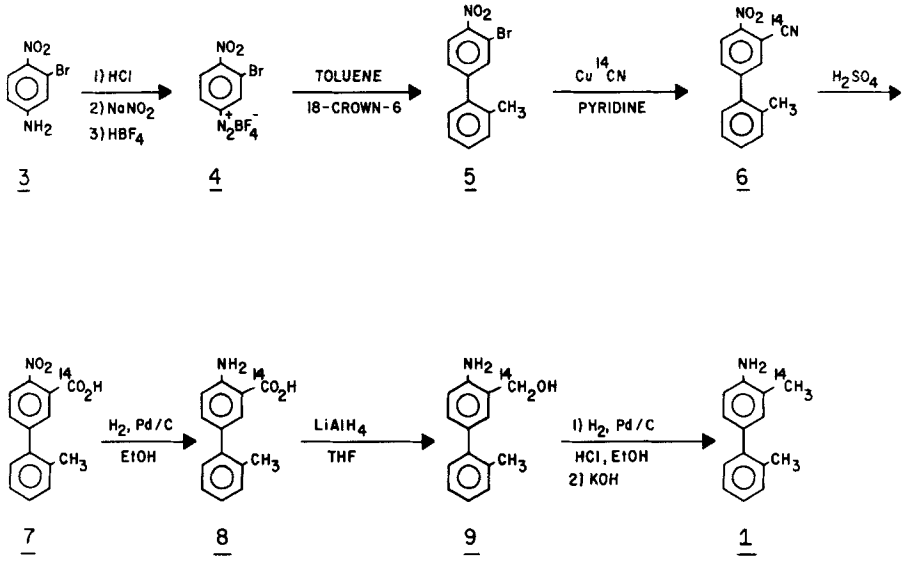
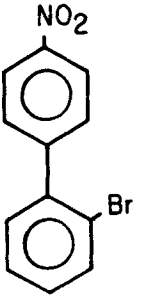
The synthesis of 1-3-methyl-^{14}C is outlined in Scheme 1. The coupling product 5 was separated from the two other isomers by column chromatography, and characterized by its NMR spectrum. The purity of 5 was established by GLC, which separated all 3 isomers. We preferred to introduce the label by reaction of the carbanion derived from 5 with $^{14}CO_2$, by analogy to our previous syntheses of *o*-toluidine-methyl-^{14}C and 3-methyl-2-naphthylamine-3-methyl-^{14}C (7). However, this reaction was unsuccessful in the case of 5, probably because of intramolecular quenching of the carbanion. Therefore, we reacted 5 with Cu^{14}CN generated from K^{14}CN and obtained 6 in 68% yield (34% based on K^{14}CN). The remaining steps in Scheme 1 all proceeded in good yields.

A similar scheme was used for the synthesis of 2-2'-methyl-^{14}C. 2'-Bromo-4-nitrobiphenyl (10) was prepared by coupling of p-nitro-

SCHEME 1: SYNTHESIS OF 4-AMINO-3,2'-DIMETHYLBIPHENYL-3-METHYL-^{14}C



benzenediazonium tetrafluoroborate with bromobenzene; 10 was purified and characterized prior to introduction of label with $Cu^{14}CN$. Hydrolysis and successive reductions, as in Scheme 1, yielded 2-2'-methyl-^{14}C. The yields of 1-3-methyl-^{14}C and 2-2'-methyl-^{14}C from $Cu^{14}CN$ were 13% and 16%, respectively.



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EXPERIMENTAL

Melting points were determined with a Thomas-Hoover Capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Hitachi Perkin-Elmer R-24 high-resolution NMR Spectrometer. Chemical shifts are reported in parts per million relative to Me_4Si as an internal standard. IR spectra were recorded on a Perkin-Elmer Model 267 infrared spectrometer. Mass spectrometry was performed with a Hewlett-Packard Model 5982A instrument. For GC analysis, we used a Hewlett-Packard Model 5710A gas chromatograph equipped with a flame-ionization detector and a 6 ft x 0.12 in. column packed with 10% UCW-98 on WHP-7620. The oven temperature was programmed from 150 to 240°C at 8°/min; helium was used as a carrier gas at a flow rate of 50 ml/min. To check the radiochemical purity, the GLC was interfaced with a Packard Model 894 gas proportional counter or TLC plates were scanned with a Packard Model 7201 radiochromatogram scanner. Scintillation counting was performed with a Nuclear Chicago Isocap 300 scintillation system. TLC was carried out with 0.25 mm silica gel $60F_{254}$ (E. Merck) glass plates. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. and were within $\pm$ 0.1% of the calculated values.

3-Bromo-4-nitrobenzenediazonium tetrafluoroborate (4)

3-Bromo-4-nitroaniline (3)(8) (4.3 g, 0.019 mole) was suspended in CH_3OH (10 ml) and conc. HCl (25 ml) was added. The reaction mixture was heated on a steam bath for 15 min., cooled and the hydrochloride filtered. The salt was washed with benzene, giving the HCl salt (3g,0.012 mole, 63%). This hydrochloride was suspended in 48% HBF_4 (15 ml, Columbia Chem.) and cooled to 0°C. A

saturated aq. solution of $NaNO_2$ (2.07 g, 0.03 mole) was added dropwise with vigorous stirring while the temperature was maintained below 5°C. After addition was complete, the reaction mixture was stirred for 90 min. as the temperature rose to ambient. The diazonium salt 4 was filtered and washed with benzene yielding 3.2 g, 0.01 mole, 84%. IR (Nujol) 2300 cm^{-1}.

3-Bromo-2'-methyl-4-nitrobiphenyl (5) The reaction was performed under N_2 and protected from light. To a stirred mixture of the diazonium tetrafluoroborate 4 (3.1 g, 0.01 mole) and 18-Crown-6 (0.08 g, 0.3 mmole, Aldrich Chem. Co.) in dry toluene (30 ml) was added anhydrous potassium acetate (0.6 g, 0.6 mmole). The color of the reaction mixture changed from yellow to red within a few min. Stirring was continued for 2 h. followed by filtration. The solid was washed with benzene (25 ml) and the combined filtrates were washed with saturated aq. NaCl and H_2O, dried ($MgSO_4$) and concentrated. The resulting oil was chromatographed on Silicar CC-7 (Mallinckrodt) with elution by hexane. A mixture of 3 isomers was obtained (0.73 g, 2.5 mmole, 25%) having relative retention times on GLC of 1.00, 1.05, and 1.07. The predominant isomer (relative retention time, 1.00) was 5 which was purified by another column chromatography on Silicar CC-7 with elution by hexane. This gave 0.43 g (1.5 mmole, 15%) 5, mp 55-56°C and pure by GLC analysis. Spectral properties of 5: NMR ($CDCl_3$) δ 7.91 (d, 1H, J=9 Hz, H *ortho* to NO_2), 7.69 (d, 1H, J=2-3 Hz, H *ortho* to Br), 7.29 (m, 5H, aromatic H), MS m/e (rel intensity) 293 (49.4), 291 (47.9), 166 (57), 165(100). Anal. Calcd for $C_{13}H_{10}BrNO_2$: C, 53.44; H, 3.45; Br, 27.36; N, 4.79 Found C, 53.45; H, 3.49; Br, 27.49; N, 4.81.

3-Cyano-2'-methyl-4-nitrobiphenyl-3-cyano-^{14}C (6) In a dry 15 ml round bottom flask fitted with a reflux condenser and protected from moisture by a $CaCl_2$ tube were placed 5 (150 mg, 0.52 mmole), anhydrous $Cu^{14}CN$ (9) (from 5 mCi $K^{14}CN$, 5 mCi/mmole, New England Nuclear) and dry pyridine (10 ml). The mixture was heated under reflux for 5 h. The resulting dark brown solution was poured while still hot into a flask containing aq. NH_3 (Sp. gr. 0.9, 20 ml) and H_2O (20 ml). The mixture was extracted with ethyl acetate (3 x 50 ml) and the organic layer washed with H_2O (50 ml), 6 N HCl (2 x 50 ml), H_2O (50 ml), dried (Na_2SO_4) and concentrated to give a pale yellow solid. This material (80 mg, 0.34 mmole, 1.7 mCi, 34% based on $K^{14}CN$) showed mainly one radioactive band of R_f= 0.64 (4/1: EtOAc/MeOH) identical to unlabeled 6. Another minor, unlabeled component with R_f=0.65 (same solvent system for TLC) corresponded to 5. The cyano compound 6 was used in the next step without further purification. Spectral properties of unlabeled 6, mp 125-127°C; IR (Nujol) 2215 cm^{-1} (CN); MS m/e (rel. intensity) 238 (M^+, 100), 191 (33), 190 (43.7), 165 (31.7).

3-Carboxy-2'-methyl-4-nitrobiphenyl-carboxyl-^{14}C (7) A mixture of 6 (80 mg, 0.34 mmole, 1.7 mCi) and 40% H_2SO_4 (20 ml) was heated under reflux for 7 h. The mixture was added to cold H_2O (40 ml) and extracted with EtOAc (3 x 50 ml). The organic layer was concentrated to about 50 ml and extracted with 10% aq. $NaHCO_3$ (50 ml). The aq. layer was separated, acidified with conc. HCl and extracted with EtOAc (3 x 50 ml). The organic extract was dried (Na_2SO_4) and concentrated to yield a colorless solid (56 mg, 0.22 mmol, 1.09 mCi, 64%) which showed a single radioactive band of R_f=0.36 (4/1:EtOAc/

MeOH), identical to unlabeled 7; mp 149°C. Spectral properties: IR (Nujol) 1695 cm^{-1} (br), MS m/e (rel intensity) 257 (M^+, 100), 165 (66.3). Anal. (unlabeled 7) Calcd for $C_{14}H_{11}NO_4$: C,65.36; H, 4.31; N, 5.45. Found: C, 65.52; H, 4.45; N, 5.45.

4-Amino-3-carboxy-2'-methylbiphenyl-carboxyl-^{14}C (8) The nitro compound 7 (56 mg, 0.22 mmole, 1.09 mCi) was dissolved in absolute ethanol (25 ml) containing 10% Pd/C (20 mg) and hydrogenated in a Parr Shaker at 15 psi. After 30 min., the catalyst was filtered, washed with 20 ml absolute ethanol and the solvent removed to give a solid (42 mg, 0.19 mmole, 0.8 mCi, 73%) which showed a single radioactive band of R_f=0.50 (4/1: EtOAc/MeOH) identical to unlabeled 8 (mp 201-203°C). MS m/e (rel. intensity) 227 (M^+, 100), 209 (82), 180 (33.4).

4-Amino-3-hydroxymethyl-2'-methylbiphenyl-methylene-^{14}C (9). The reduction was done under an N_2 atmosphere in a 25 ml three neck round bottom flask fitted with a gas inlet tube and a pressure equalizing dropping funnel. To a suspension of lithium aluminum hydride (100 mg, 2.5 mmole) in dry THF (10 ml) was added dropwise a solution of 8 (42 mg, 0.19 mmole, 0.8 mCi) in dry THF (15 ml). After addition was complete, stirring was continued for 2 h at room temperature. The reaction was quenched by cautious addition of 20 ml H_2O, filtered, and the inorganic solid washed with EtOAc (20 ml). The filtrate was further extracted with EtOAc and the organic layers were combined, washed with 25 ml 10% aq. $NaHCO_3$ and dried (Na_2SO_4) to give 9 (0.68 mCi, 85%) with R_f=0.55 (4/1: EtOAc/MeOH). This was used without further purification.

4-Amino-3,2'-dimethylbiphenyl-3-methyl-^{14}C (1) A mixture of 9 (32 mg, 0.15 mmole, 0.68 mCi) and 10% Pd/C in 30 ml absolute ethanol containing 1 ml conc. HCl was shaken for 45 min in a Parr apparatus at room temperature and a hydrogen pressure of 13-15 psi. The catalyst was filtered, washed with 20 ml absolute ethanol and the combined ethanol solutions concentrated to give the crude hydrochloride of 1 (0.41 mCi) as a colorless solid. The free amine was obtained by treating the salt with 30 ml 10% aq. KOH. Chromatography on 10 g Silicar CC-7 with elution by hexane gave pure 1 (12 mg, 0.061 mmole, 0.32 mCi), R_f=0.3 (9/1: EtOAc/benzene) identical to unlabeled 1. GLC analysis of 1 showed no radioactive or nonradioactive impurities. The amine was stored as the hydrochloride salt; MS of the labeled hydrochloride m/e (rel. intensity) 197 (M^+-HCl, 100), 180 (29.2), 165 (29.7), identical to unlabeled 1 (5).

2'-Bromo-4-nitrobiphenyl (10) The compound was obtained as described above for 5 using p-nitrobenzenediazonium tetrafluoroborate (Eastman) and dry bromobenzene. Three isomers were obtained in 30% yield with relative retention times on GLC 1.00:1.09:1.11. Compound 10 (rel. retention time, 1.00) was obtained in 16% yield after column chromatography, mp 81-83°C, lit[10] 82.5°C. MS m/e (rel. intensity) 277 (72.2), 279 (69.3), and 152 (100).

2'-Cyano-4-nitrobiphenyl-cyano-^{14}C (11) A mixture of 10 (140 mg, 0.5 mmole) and Cu^{14}CN (9) (from 5 mCi K^{14}CN, 5 mCi/mmole, New England Nuclear) was heated under reflux overnight in dry quinoline (15 ml). The reaction mixture was worked up as described

above for 6. The product (95 mg, 0.42 mmole, 1.9 mCi, 38% based on starting $K^{14}CN$) had R_f= 0.45 (9/1:EtOAc/benzene), identical to that of unlabeled 11; mp 114-116°C. IR (Nujol, unlabeled 11) 2210 cm^{-1}. MS (unlabeled) m/e (rel intensity) 224 (M^+, 100), 178 (44), 177 (51.5), 166 (40.6).

2'-Carboxy-4-nitrobiphenyl-carboxyl-^{14}C (12) The cyano compound 11 (95 mg, 1.9 mCi, 0.42 mmole) was heated under reflux for 24 h in 40% H_2SO_4 (25 ml). The reaction was worked up as above for compound 7. Colorless crystals were obtained (1.5 mCi, 78% based on 11) which had R_f=0.4 (4/1:EtOAc/MeOH) identical to an unlabeled sample, mp 224-226°C. IR (Nujol, unlabeled 12) 1690 cm^{-1}. MS m/e (rel intensity) 243 (M^+,100), 226 (33.1) and 151 (28.2). The radioactive purity was greater than 99% as shown by radiochromatography.

4-Amino-2'-carboxybiphenyl-carboxyl-^{14}C (13) The nitro compound 12 (70 mg, 0.29 mmole, 1.5 mCi) was hydrogenated in a Parr Shaker (10 psi) in absolute ethanol (50 ml) using 10% Pd/C (20 mg). After 30 min, the product 13 (45 mg, 0.21 mmole, 1 mCi, 67% based on 12) was obtained as described above for compound 8. The product had R_f=0.5 (4/1: EtOAc/MeOH) identical to unlabeled 13,mp 208-210°C. MS (unlabeled 13) m/e (rel intensity) 213 (M^+,100), 196 (17.6) 167 (26.4), 168 (19.9).

4-Amino-2'-hydroxymethylbiphenyl-methylene-^{14}C (14) Compound 13 (45 mg, 0.21 mmole, 1 mCi) was dissolved in dry THF (20 ml) and lithium aluminum hydride (100 mg, 2.5 mmole) in dry THF (10 ml) was used for reduction. The procedure was identical to that described above for 9; after 2.5 h reaction time, 14 (0.71 mCi, 71% based on 13) was obtained. Compound 14 had R_f=0.54 (4/1:EtOAc/MeOH) and

was used without further purification in the following step.

4-Amino-2'-methylbiphenyl-methyl-^{14}C (2) The hydrogenation of compound 14 (0.71 mCi) was performed as described above for 9. The amine 2 (16 mg, 0.073 mmole, 0.4 mCi, 16% based on Cu^{14}CN) was obtained by treating the hydrochloride with 10% aq. KOH. The radiochemical purity of 2 was greater than 99% as shown by radiochromatography. The chromatographic and spectral properties of 2-2'-methyl-^{14}C were identical to those of unlabeled 2, prepared by a different route (11). MS of 2-HCl salt,m/e (rel intensity) 183 (M^+-HCl, 100), 182 (52), 165 (32). The amine was stored as the hydrochloride.

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