A STUDY OF CHEMICAL CARCINOGENESIS 4. SYNTHESIS OF THE CARBON-14 LABELLED CARCINOGENS 5-METHYLCHRYSENE, 2-METHYL-ANILINE AND 3-METHYL-2-NAPHTHYLAMINE

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SUMMARY

Procedures were developed for synthesis of the carcinogenic compounds 5-methylchrysene-5-^{14}C, 2-methylaniline-methyl-^{14}C and 3-methyl-2-naphthylamine-methyl-^{14}C. In each case, label was introduced with $^{14}CO_2$ and yields were acceptable for preparation of these compounds for metabolic studies.

Key Words: 5-Methylchrysene, 2-Methylaniline, o-Toluidine, 3-Methyl-2-naphthylamine, Carbon-14.

INTRODUCTION

Substitution of methyl groups in an aromatic system can have important effects on the carcinogenic activity of that system. For example, 5-methylchrysene (Scheme 1,5) is a potent tumor initiator and complete carcinogen on mouse skin, with activity comparable to benzo(a)pyrene, whereas chrysene shows only marginal

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activity (1-4). In fact, the tumor initiating strength of 5-methylchrysene is approximately 100 times that of chrysene. The position of methyl substitution is also critical; the other methylchrysene isomers are much less active than 5.

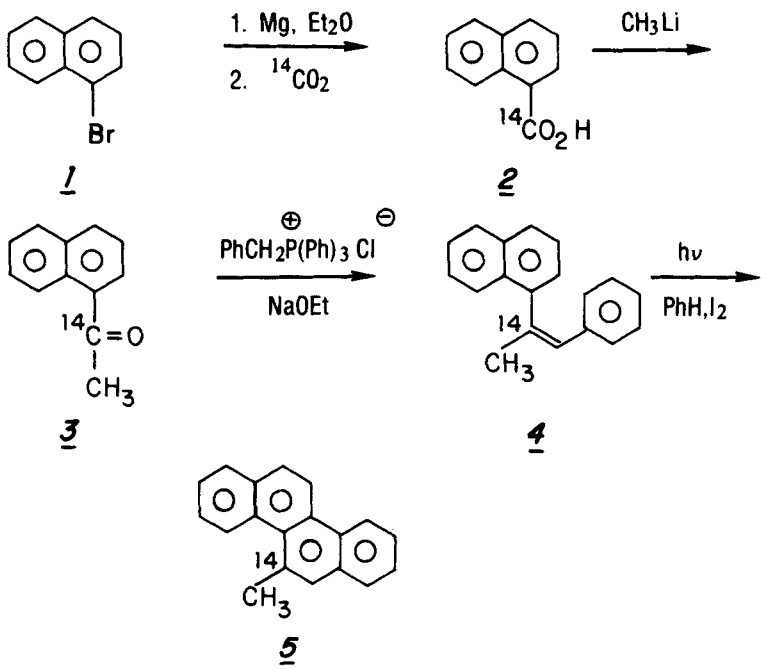
Similarly, both 3-methyl-2-naphthylamine (11) and 2-methylaniline (o-toluidine) (10) are more active as carcinogens in the rat than the corresponding unmethylated compounds, 2-naphthylamine and aniline (5,6). 3-Methyl-2-naphthylamine induces colon tumors in male rats and mammary tumors in female rats while o-toluidine, when administered to rats in high doses, induces subcutaneous and bladder tumors. For o-toluidine (and several other aromatic amines), o-methyl substitution is particularly effective in increasing carcinogenicity (7).

To study the metabolism of these compounds, the ^{14}C labelled compounds were required. These investigations are not merely of academic interest because both 5-methylchrysene and o-toluidine occur in cigarette smoke (3,8) and o-toluidine is used in the chemical manufacturing industry. 3-Methyl-2-naphthylamine is closely related to the human bladder carcinogen, 2-naphthylamine (9).

The synthesis of 5-methylchrysene-5-^{14}C (5) is summarized in Scheme 1. 1-Bromonaphthalene (1) was allowed to react with Mg and the resulting 1-naphthylmagmesium bromide was carboxylated with $^{14}CO_2$, generated from $Ba^{14}CO_3$, to give 1-naphthoic acid-carboxyl-^{14}C (2) in 79% yield. Reaction of 2 with methyllithium afforded 1-acetonaphthone-carbonyl-^{14}C (3) (71%). Wittig condensation of 3 with benzyltriphenylphosphonium chloride gave alkene 4 (36%) which was cyclized photochemically (30%) to 5-methylchrysene-5-^{14}C (5).

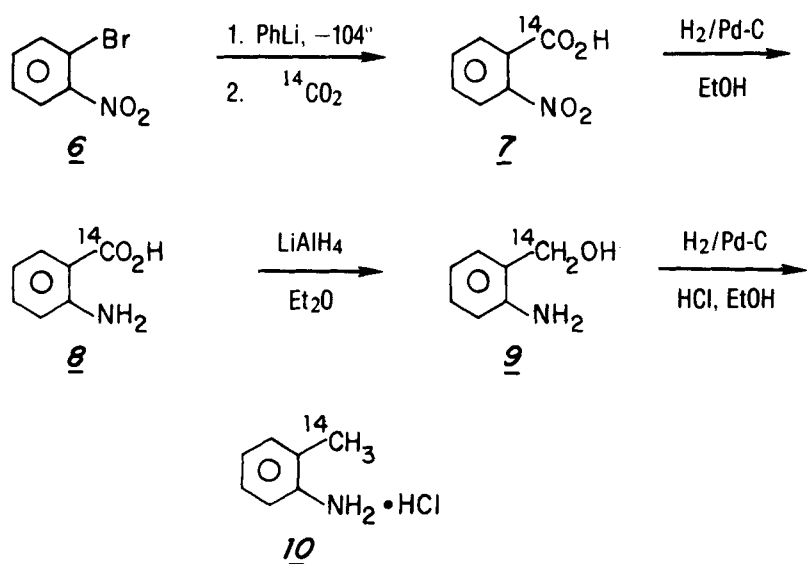
For the synthesis of o-toluidine (10, Scheme 2), o-nitrobromobenzene (6) was allowed to exchange at -104° with phenyllithium. Carboxylation with $^{14}CO_2$ then gave o-nitrobenzoic acid carboxyl-

SCHEME 1: SYNTHESIS OF 5-METHYLCHRYSENE-5-^{14}C



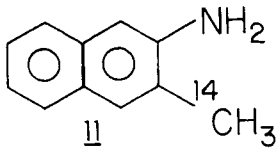
1 2 3 4 5

SCHEME 2: SYNTHESIS OF 2-METHYLANILINE-METHYL-^{14}C



6 7 8 9 10

^{14}C (7), as described previously for unlabelled material (10). The acid 7 was catalytically reduced to anthranilic acid-carboxyl-^{14}C (8), which was further reduced with $LiAlH_4$ to o-aminobenzyl alcohol-methylene-^{14}C (9). Hydrogenolysis of 9 gave o-toluidine-methyl-^{14}C (10) in 53% overall yield, based on starting $Ba^{14}CO_3$. For 3-methyl-2-naphthylamine-methyl-^{14}C (11), the same sequence was employed, but starting with 2-bromo-3-nitronaphthalene.



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The overall yield in this case was 40%.

The syntheses of all three ^{14}C-labelled compounds, none of which has been previously described to our knowledge, were designed to use $Ba^{14}CO_3$ for introduction of label. Thus they can all be prepared inexpensively despite a low overall yield in the case of 5. The photochemical cyclization procedure (11) was chosen for 5 because previously published synthetic methods (12-16) would not allow convenient introduction of label using $Ba^{14}CO_3$ and at the same time provide material of unambiguous isomeric purity. The synthesis of 10 avoids costly separation of isomers which would have been necessary if it had been prepared by nitration of toluene and reduction. Unlabelled 3-methyl-2-naphthylamine (11) can also be prepared in good yield from 2-hydroxy-3-naphthoic acid (17) or by nitration of the Diels-Alder adduct of 2-methylnaphthalene and hexachlorocyclopentadiene followed by retro Diels-Alder cleavage (18) and reduction. However, neither of these methods appeared attractive for the synthesis of ^{14}C-labelled material.

EXPERIMENTAL

1-Naphthoic acid-carboxyl-^{14}C (2) Naphthylmagnesium bromide was prepared from 4.0 g (0.02 mole) 1-bromonaphthalene (Aldrich,

Milwaukee, Wisc.) and 0.5 g (0.02 mole) Mg in 100 ml anhydrous ether. After the reaction was complete (refluxing, 2 hrs), $^{14}CO_2$, generated from $Ba^{14}CO_3$ (2.0 g, 10 mmol, 51 mCi), was added, and the mixture was heated under reflux for 0.5 hr. Workup, as previously described (19,20) gave 1-naphthoic acid-carboxyl-^{14}C (1.35 g, 7.8 mmol, 78%), mp 157-159°, lit. (19,20), 159-161.

1-Acetonaphthone-carbonyl-^{14}C (3) To an ice cooled solution of 1-naphthoic acid-carboxyl-^{14}C (2, 1.35 g, 7.8 mmol, 39.8 mCi) in 50 ml anhydrous ether, under N_2, was added with stirring a solution of 10 ml 1.7 M methyllithium in hexane-ether. After the addition was complete, the ice bath was removed and the mixture was stirred at room temperature for 3 hrs. It was then poured slowly into stirred ice-cold 1N HCl. The layers were separated and the aqueous layer extracted 3 times with etner. The combined ether solutions were washed (sat'd $NaHCO_3$ and H_2O), dried ($MgSO_4$) and concentrated to give a residue, 0.94 g crude 3, which by ir and tlc was slightly contaminated with dimethyl-(1-naphthyl)carbinol. This material was used without further purification in the synthesis of 4.

1-Phenyl-2-(1-naphthyl)propene-2-^{14}C (4)

To a stirred solution of crude 3 (0.94 g, 5.5 mmol, 28.2 mCi) and benzyltriphenylphosphonium chloride (3.1 g, 8.0 mmol) in 50 ml absolute ethanol, was added a solution of sodium ethoxide (8.0 mmol) in 15 ml ethanol. The mixture was refluxed overnight, after which some starting ketone remained. Another 8.0 mmol phosphonium salt and sodium ethoxide were added and the mixture was refluxed for 48 hrs more until the reaction was complete. The residue was chromatographed on silica gel with elution by hexane to give 4 as a mixture of *trans*-(21%) and *cis*-(79%) isomers (0.5 g, 2.0 mmol, 10.4 mCi) identical to unlabelled 4 synthesized by the same route and pure according to glc analysis [8 ft, 10%

OV-17 on 60/80 Gas Chrom Q, 200°, 40 ml/min He; retention times, 16 min (*trans*-) and 28 min (*cis*-)]. Spectral properties of 4: ir(film) 3060, 3020, 1598, 1590, 1490, 1440, 1390, 1373, 795, 772, 750, 690 cm $^{-1}$; nmr ($CDCl_3$) 8.2-6.5 ppm (ArH and =C-H) m, 13H; 2.35 ppm (*cis*-CH_3) s, 2.33 ppm (*trans*-CH_3) s, 3H; ms, m/e (rel. int.); 244(80), 230(22), 229(100), 228(25), 227(20). Analysis: Calcd for $C_{19}H_{16}$: C,93.40; H,6.60. Found: C,93.45; H,6.60.

5-Methylchrysene-5-^{14}C (5) Alkene 4 (0.5 g, 2.0 mmol, 10.4 mCi) was dissolved in 1 ℓ dry benzene and 100 mg I_2 was added. The solution was simultaneously aerated and irradiated with a Hanovia 250 watt medium pressure mercury arc, using a corex filter. The reaction mixture was filtered and the filtrate concentrated to give a residue which was chromatographed on silica gel with elution by 4/1 chloroform/hexane. The fractions containing 5 were combined and recrystallized twice from ethanol to give pure 5-methylchrysene-5-^{14}C (150 mg, 0.62 mmol, 3.2 mCi) mp 116.5-117, lit. (12) 117-118. The purity of this material was greater than 99.9% according to analysis by glc (conditions as above) and hplc (6 mm x 30 cm microbondapak/C_{18} 50% CH_3OH/H_2O-80% CH_3OH/H_2O, 2.0 ml/min, 2500-3000 psi). No other radioactive material eluted either before or after 5 under these hplc conditions. Additional 5 could be obtained from the earlier silica gel chromatographic fractions which were contaminated with traces of starting alkene.

o-Nitrobenzoic acid carboxyl-^{14}C (7) A 50 ml wide mouth round bottom flask, equipped with a four neck head, was fitted with an overhead mechanical vacuum stirrer, a gas inlet tube, and a hose connecting adaptor which in turn was connected to an oil bubbler and a vacuum pump *via* a three-way stopcock. The gas inlet tube was connected to a N_2 source and a CO_2 generator *via* a three-way stopcock. To the reaction flask was added

o-bromonitrobenzene (2.02 g, 10 mmole, Eastman, Rochester, N.Y., purified by recrystallization from hexane and sublimation, mp 42-43°). The system was dried by alternately evacuating and filling it with dry N_2 several times. Under a slow flow of N_2 the reaction flask was charged with THF (40 ml) which had been dried by distillation from $LiAlH_4$. The fourth neck of the reaction flask was then fitted with a dropping funnel capped with a septum. Stirring was begun as the flask was cooled to -104° to -109° in a liquid N_2-isooctane bath.

Phenyllithium (5.5 mmole, 3.7 ml, 1.5 M in benzene-ether) was transferred by syringe to the dropping funnel and added dropwise over a 15 min. period to the o-bromonitrobenzene solution. The mixture was allowed to continue stirring for an additional 45 min. after which $^{14}CO_2$ generated from $Ba^{14}CO_3$ (0.99 g, 5.0 mmol, 25.0 mCi) was introduced into the reaction mixture.

Stirring was continued for 2 hrs while the temperature was maintained below -104°. The system was then filled with N_2 and 50 ml of 10% aq. NaOH was slowly added. After stirring for a few minutes, the mixture was allowed to come to room temperature and the layers were partitioned. The aq. layer was washed with ether (2 x 50 ml) and acidified with conc. HCl.

Cooling of the acidic solution resulted in precipitation of a white solid which was filtered and dried. Additional product was recovered by extracting the aq. acid with ether to give a total of 0.80 g crude o-nitrobenzoic acid-carboxyl-^{14}C. Analysis by glc-ms of a previous, identically prepared reaction product from non-labelled $BaCO_3$ indicated 88% o-nitrobenzoic acid and 12% of an unidentified component. Benzoic acid was not present. A radiochromatogram (silica gel) showed a single isotopically pure product of R_f=0.65 (EtOH:MeOH-1:1) identical to unlabelled material (10). Assuming 88%

purity, this represented 84% yield of o-nitrobenzoic acid-carboxyl-^{14}C, which was used without further purification.

Anthranilic acid-carboxyl-^{14}C (8) The crude o-nitrobenzoic acid-carboxyl-^{14}C (0.80 g) from the previous step was hydrogenated in a Parr shaker in 95% EtOH (50 ml) at 20° and 4 atm using 10% Pd-C (0.12 g) as catalyst. After 5 min. filtration of catalyst and evaporation of the solvent left 0.64 g crude anthranilic acid-carboxyl-^{14}C as a yellow powder: TLC (silica gel, MeOH) R_f=0.65; identical to a reference sample; a radiochromatogram showed the presence of a single isotopically pure product. Anthranilic acid was previously prepared from o-bromoacetanilide (21).

o-Aminobenzyl alcohol-methylene-^{14}C (9) A three neck, 50 ml round bottom flask was fitted with an N_2 gas inlet and a pressure equalizing dropping funnel. The funnel was fitted with a cotton plug over which the crude anthranilic acid carboxyl-^{14}C (0.64 g) from the previous step was added. The dropping funnel was connected *via* a condenser to an oil bubbler. The flask was charged, under N_2, with anhydrous ether (15 ml) and $LiAlH_4$ (10 mmole, 10 ml, 1 M in ether). The solution was heated with stirring to reflux, allowing the ether to condense back into the dropping funnel onto the anthranilic acid. As the anthranilic acid dissolved, it was slowly added to the $LiAlH_4$ solution so as to maintain a gentle reflux. After all the acid had been added, the reaction was quenched by addition of just enough H_2O to decompose the excess $LiAlH_4$. The resulting mixture was separated from the solid inorganic salts by centrifugation. The supernatant was removed and the inorganic solid washed with additional ether. The ether layers were combined and dried ($MgSO_4$), and the solvent was evaporated to leave 0.42 g (3.4 mmole, 16.8 mCi) of crude o-aminobenzyl alcohol-methylene-^{14}C. TLC (silica gel,

ether, R_f=0.40, identical to reference material) showed only minor impurities: a radiochromatogram indicated the presence of two minor ^{14}C containing impurities, R_f=0.63 and 0.73. This material was used without further purification.

2-Methylaniline hydrochloride-methyl-^{14}C (10) The crude o-aminobenzyl alcohol-methylene-^{14}C (0.42 g, 3.4 mmole, 16.8 mCi) from the previous step was hydrogenolized in a Parr shaker in abs. EtOH (50 ml) which was acidified with 0.5 ml conc. HCl. The reaction was carried out at room temperature and 6 atm pressure with 10% Pd-C (0.12 g) for 30 min. The mixture was filtered of catalyst and most of the solvent was evaporated from the filtrate. The product was precipitated from the residue by addition of anhydrous ether. The precipitate was filtered and dried to yield 2-methylaniline hydrochloride-methyl-^{14}C (0.48 g, 3.3 mmole), which was recrystallized from ethanolic HCl to give material [mp 213-215°, lit. (22) 215°, 0.38 g, 2.65 mmole, 13.3 mCi] of greater than 99.9% purity according to analysis of the salt by TLC (silica gel, ether, R_f=0.55) and radiochromatography and of the free base by GLC (6 ft, 10% SP 2250 on 100/120 Supelcoport, 100-200°, 8°/min, 40 ml/min He, retention time = 7 min.), combined GLC-mass spectrometry and UV (hexane) λmax (ε)232 nm (8400), 284 (2160).

3-Nitro-2-naphthoic acid carboxyl-^{14}C A 100 ml wide mouth round bottom flask with a four neck head was assembled as described for the preparation of 7. To the reaction flask was added 2-bromo-3-nitronaphthalene (2.52 g, 10 mmole, Eastman, purified by recrystallization from abs. ethanol, mp 83-84°). The system was dried by alternately evacuating and filling it with dry N_2 several times.

Under a slow flow of N_2 the reaction flask was charged with THF (980 ml) which had been dried by distillation from $LiAlH_4$. The fourth neck of the reaction flask was then fitted

with a dropping funnel capped with a septum. Stirring was begun as the flask was cooled to -115° to -120° in a liquid N_2-methylcyclohexane bath.

Phenyllithium (5.5 mmole, 3.7 ml, 1.5 M in benzene-ether) was then added as in the preparation of 7; after the addition was complete, stirring was continued for 30 min. $^{14}CO_2$, generated from $Ba^{14}CO_3$ (0.99 g, 5.0 mmol, 25.0 mCi), was then introduced, after which stirring was continued for 1.5 hrs while the temperature was maintained below -115°. Workup as for 7 yielded 0.49 g (45%) of crude 3-nitro-2-naphthoic acid-carboxyl-^{14}C, which was identical by tlc (silica gel; EtOAc: MeOH;3:1;R_f=0.24) to an identically prepared sample of unlabelled 3-nitro-2-naphthoic acid: mp 217-219° (lit (23) 220.5) and was isotopically pure according to thin layer radiochromatography.

3-Amino-2-naphthoic acid-carboxyl-^{14}C The crude nitronaphthoic acid-carboxyl-^{14}C (0.49 g) from the previous step was hydrogenated in a Parr shaker in 95% EtOH (50 ml) at 20° and 5 atm using 10% Pd-C (0.15 g) as catalyst. After 15 min. filtration of catalyst and evaporation of the solvent left 0.41 g (2.2 mmol) crude 3-amino-2-naphthoic acid-carboxyl-^{14}C as a light brown powder identical on tlc (silica gel; EtOAc: MeOH; 3:1; R_f 0.42) to an unlabelled sample which had been prepared in the same way: mp 210-212° (lit (24) 214°). This was used immediately in the next step.

3-Amino-2-hydroxymethylnaphthalene-methylene-^{14}C To a suspension of the 3-amino-2-naphthoic acid (0.41 g, 2.2 mmole) in dry ether (20 ml) was added dropwise with stirring $LiAlH_4$ (5 mmole, 5 ml, 1M in ether). Stirring was continued for 1 hour at room temp. and then at reflux for 15 min. The excess $LiAlH_4$ was decomposed with a minimum of H_2O. THF (15 ml) was added to the mixture and the inorganic salts filtered off. The

filter cake was washed several times with THF. The filtrates were combined, dried ($MgSO_4$) and the solvent evaporated to leave 0.35 g (2.1 mmole) of crude 3-amino-2-hydroxymethyl-naphthalene-methylene-^{14}C which coeluted on tlc (silica gel; EtOAc; R_f 0.55) with an identically prepared sample of unlabelled compound: mp 177-180° (lit (25) 183°). It showed only minor impurities by thin layer radiochromatography and was used without further purification.

2-Methyl-3-aminonaphthalene-methyl-^{14}C (11) The crude 3-amino-2-hydroxymethylnaphthalene (0.35 g, 2.1 mmole) from the previous step was hydrogenolized on a Parr shaker in abs EtOH (50 ml) which was acidified with 5 drops conc. HCl. The reaction was carried out at room temp. and 7 atm with 10% Pd-C (0.10 g) for 30 min. The mixture was filtered of catalyst. Tlc (silica gel, ether) showed the presence of substantial amounts of starting material. Another portion of catalyst was added and the mixture was returned to the Parr shaker for an additional 30 min. Filtration and evaporation of solvent yielded the crude product. This was dissolved in degassed H_2O (50 ml) and stirred with decolorizing charcoal. The mixture was filtered and the free base precipitated by basification with sat. Na_2CO_3. The precipitate was filtered off and dried to yield 2-methyl-3-aminonaphthalene-methyl-^{14}C mp 123-125°, lit (17) 120-120.5° (0.312 g, 1.99 mmol, 9.9 mCi) which was identical (tlc, ir, glc, glc-mass spec., UV) both to an independently synthesized sample of unlabelled 2-methyl-3-aminonaphthalene (26) and to an unlabelled sample prepared by the same route. The spectral and chromatographic properties of 2-methyl-3-aminonaphthalene-methyl-^{14}C indicated greater than 99% purity: tlc (silica gel, EtOAc) R_f=0.76; glc (10% SP 2250 on Supelcoport, 100-250°, 32°/min; retention time, 8 min); ms, m/e (rel intensity):

157(100), 156(47), 140(16), 139(23), 129(29), 128(35), 127(18); UV (hexane) $\lambda_{max}(\varepsilon)$260 nm (3,480), 270.5(4,660), 281.5(5,270), 293(3,510). The labelled compound was stored most effectively as its HCl salt in ethanol.

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