A SIMPLE METHOD FOR THE SYNTHESIS OF SPECIFIC [^{2}H] AND [^{3}H]LABELLED METHYL-HYDROXYLATED DERIVATIVES OF 7,12-DIMETHYLBENZ[a]ANTHRACENE

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SUMMARY

Specific [^{2}H] and [^{3}H]labelled methyl-hydroxylated derivatives of 7,12-dimethylbenz[a]anthracene (DMBA), 7-hydroxymethyl-12-methylbenz[a]anthracene, 7-methyl-12-hydroxymethylbenz[a]anthracene, and 7,12-dihydroxymethylbenz[a]anthracene, were synthesized with good yield and high specific activity. The method involved two simple steps; oxidation of the unlabelled methyl-hydroxylated DMBA to the corresponding formyl derivatives followed by reduction with either sodium borodeuteride or sodium borotriteride to yield the [^{2}H] or [^{3}H]labelled methyl-hydroxylated derivatives with the isotope specifically attached to benzylic carbons.

Key Words: 7-hydroxymethyl-12-methylbenz[a]anthracene, 7-methyl-12-hydroxymethylbenz[a]anthracene, 7,12-dihydroxymethylbenz[a]anthracene, deuterium, tritium

INTRODUCTION

Several studies have shown that the potent carcinogen 7,12-dimethylbenz-[a]anthracene (DMBA) is metabolized by the microsomal enzyme systems to hydroxymethyl derivatives 1 - 3 (1,2). Hydroxylation at the methyl groups is one of several types of metabolic reactions which may be the initial step in the biotransformation *in vivo* to the ultimate carcinogenic metabolite (1,3-7). Evidence indicated that 1 was more adrenocorticolytic and had similar or reduced carcinogenicity in rats than its parent hydrocarbon (3,4). Recent findings indicated

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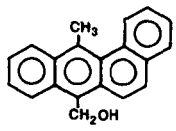
that further metabolism of 2 and 3 may also contribute to the biological activities of DMBA (6, 7). For the quantitative determinations of metabolism and the biochemical and biological studies both in vitro and in vivo, availability of radiolabelled compounds of 1 - 3 with high specific activity are obviously desirable.

Randomly [^{3}H]labelled compounds have been prepared by a catalytic exchange reaction which involved passing gaseous tritium through a solution of organic compounds in the presence of platinum oxide or palladium black (8). This method, however, has been found in our laboratories to be not applicable to the preparation of randomly [^{3}H]labelled compounds of 1 - 3 due to the removal of hydroxyl groups by hydrogenolysis which occurred in the catalytic exchange reaction. This report describes a convenient two-step method for the synthesis of specifically deuterium (D) and tritium (T) labelled compounds 4 - 9 from compounds 1 - 3.



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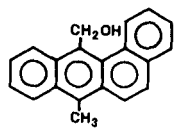

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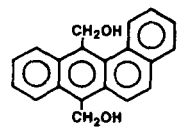

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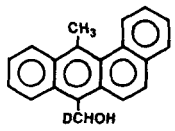

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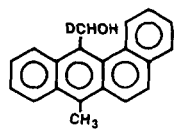

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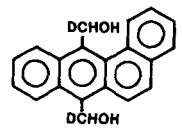

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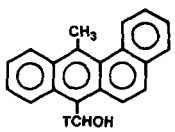

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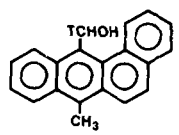

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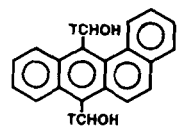

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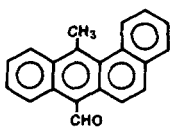

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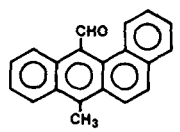

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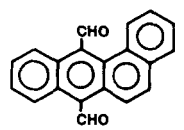

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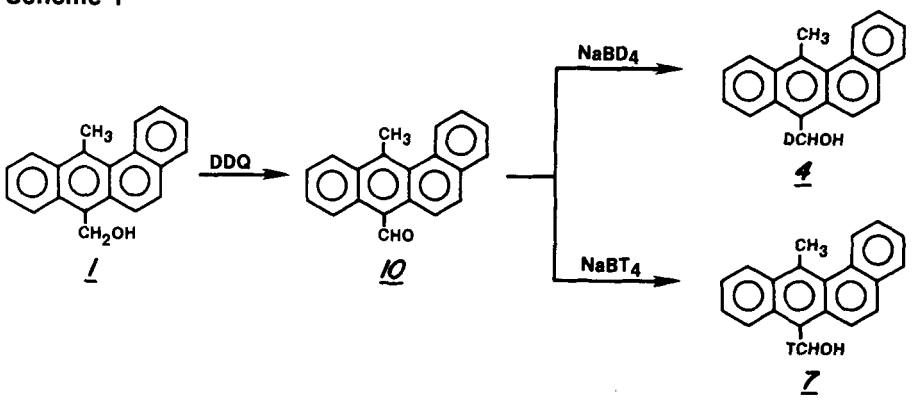
RESULTS AND DISCUSSION

Our simple approach to the synthesis of 4 - 9 is the oxidation of 1 - 3 to the formyl derivatives 10 - 12 which are subsequently reduced to the specifically deuterium- and tritium- labelled compounds 4 - 9 by reduction reaction with $NaBD_4$ and $NaBT_4$, respectively. A representative synthetic route is illustrated in Scheme 1 for the synthesis of 4 and 7.

Scheme 1



Formyl derivatives 10 - 12 were prepared according to the method of Pataki, et al (9). The purity of 10 - 12 was confirmed by high pressure liquid chromatography (HPLC, Fig. 1A). Thus, oxidation of 1 - 3 by 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in refluxing benzene yielded 10 - 12 (9). Reduction of 10 - 12 with $NaBD_4$ was conducted in anhydrous methanol-tetrahydrofuran solution at ambient temperature in a nitrogen atmosphere for 1 to 3 hr, which yielded the corresponding compounds 4 - 6 in 80, 85 and 82% yield, respectively. Compounds 4 - 6 were recrystallized from acetone-hexane and their purities were confirmed by HPLC (Fig. 1B). The specificity of labelling was confirmed by mass and nmr spectral analysis (see experimental section). A reaction time as short as 1 hr is sufficient for >98% conversion of 10 - 12 to 4 - 6, respectively. Lengthening the reaction time up to 3 hr did not alter the specificity of labelling.

Synthesis of the tritium-labelled compounds 7 - 9 was similarly conducted by reaction of 10 - 12 (84, 88, and 84% yield, respectively) with $NaBT_4$ for 1 hr in a screw-capped test tube flushed with nitrogen. There was greater than 98% conversion of the formyl derivatives 10 - 12 to the corresponding labelled compounds 7 - 9 as determined by HPLC analysis (Fig. 1B). The specific activity of

7 - 9 (1.67, 1.68 and 2.91 Ci/mmol, respectively) was each determined after HPLC purification.

The method of specific deuterium and tritium labelling described in the foregoing is very simple and it may be a general method for the synthesis of other tritium-labelled hydroxymethyl-substituted polycyclic aromatic hydrocarbons

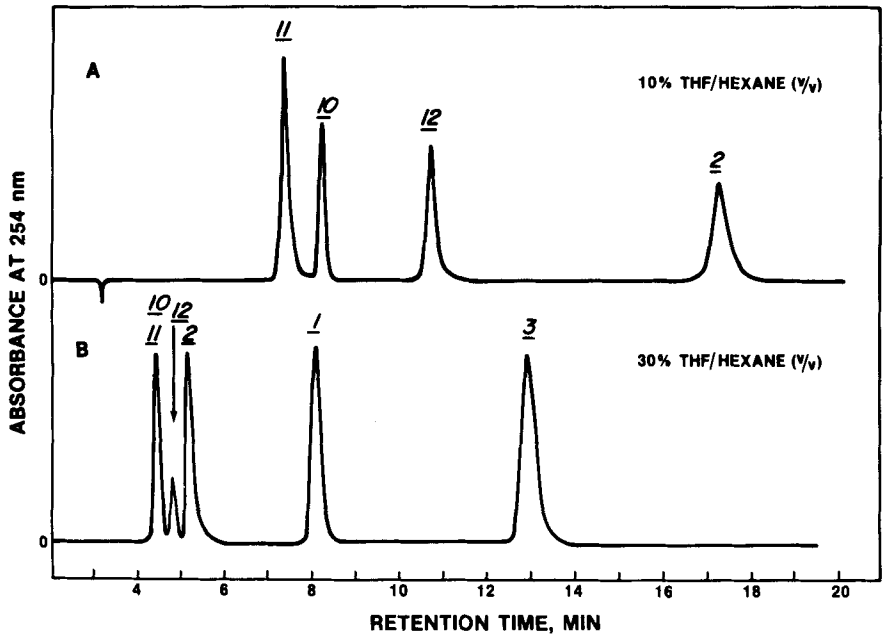


Fig. 1. HPLC separation of formyl derivatives (A) and methyl-hydroxylated derivatives (B) of DMBA on a DuPont 6.2 mm x 25 cm Zorbax SIL column. Solvents are indicated in the figure and flow rate was 2 ml/min. Deuterium- and tritium- labelled compounds cochromatographed with the respective unlabelled compounds in this HPLC system.

(PAH). The method involves only one step in handling the radioactive material, thus minimizing the potential hazards. The specific radioactivity of the final products 7 - 9 can be increased depending on the ratio of reactants and the specific activity of $NaBT_4$. Recent advances in the field of chemical carcinogenesis has prompted the investigation of the carcinogenic alkyl-substituted PAH. Thus our convenient method for the preparation of specific deuterium- and tritium-labelled hydroxyalkyl-substituted PAH would facilitate research efforts which employ radiolabelled compounds of this type.

EXPERIMENTAL

Materials and Methods: $NaBT_4$ (specific activity, 5 Ci/mmol) was purchased from Amersham Corp., Arlington Heights, Illinois. $NaBD_4$ (98 atom %) was purchased from ICN Corp., Irvine, California and DDQ was purchased from Aldrich Chemical Co. The hydroxy derivative 7-hydroxymethyl-12-methylbenz[a]anthracene (1), 7-methyl-12-hydroxymethylbenz[a]anthracene (2), and 7,12-dihydroxymethylbenz-[a]anthracene (3) were prepared essentially according to the method of Boyland and Sims (10). The acetoxylated DMBA derivatives, obtained by reaction of DMBA with lead tetraacetate (10), were not separated by column chromatography as described previously (10), but were hydrolyzed directly to the hydroxy derivatives 1 - 3 which were separated by HPLC (Fig. 1B). The HPLC system can isolate up to 5 mg mixture of 1 - 3 with baseline separation in 15 min. The formyl derivatives 7-formyl-12-methylbenz[a]anthracene (10), 7-methyl-12-formylbenz[a]anthracene (11), and 7,12-diformylbenz[a]anthracene (12) were prepared by oxidation of the corresponding hydroxy precursors (1 - 3) with DDQ according to the published procedure (9). The purity of 10 - 12 was confirmed by HPLC analysis (Fig. 1A) and when necessary, the formyl derivatives were purified by HPLC before use.

NMR spectra were obtained on a Varian T-60 spectrometer. HPLC was performed with DuPont Zorbax SIL 6.2 mm x 25 cm column. The column was pre-equilibrated with the eluting solvent and the formyl derivatives were eluted with 10% tetrahydrofuran (THF) in hexane (v/v) and the hydroxy derivatives with 30% THF in hexane (v/v) at a flow rate of 2 ml/min. The solvent was delivered by an Altex model 110 metering pump and the eluents were monitored at 254 nm. Mass spectral analysis was performed on a Finnigan 4000 GC/MS/Data System at 70 eV with a solid probe. The radioactivity was determined on a Packard Tricarb model B2450 liquid scintillation spectrometer. Ultraviolet spectra were recorded on a Cary 118C spectrophotometer. The deuterium content (mole %) was calculated from the mass spectra of labelled compounds and corresponding unlabelled compounds according to the method of Biemann (11). The extinction coefficients (M^{-1} cm^{-1} at λ_{max}) determined in methanol are: DMBA, 79,200 (at 295.5 nm); 1, 83,500 (at 294 nm); 2, 79,900 (at 295 nm); 3, 82,100 (at 293.6 nm). These values and data obtained from

liquid scintillation counting were used to determine the specific activity of compounds 7 - 9.

7-Hydroxydeuteromethyl-12-methylbenz[a]anthracene (4)

$NaBD_4$ (28 mg, 0.67 mmole) was added to a solution of 7-formyl-12-methyl-BA 10 (27 mg, 0.1 mmol) in freshly distilled absolute methanol (2 ml) and THF (1 ml) in a flame dried round bottom flask containing a small magnetic stirring bar. The resulting solution was then stirred under nitrogen atmosphere at ambient temperature for 3 hr. The excess deuteride was quenched by addition of water, and the product was partitioned between water and ethyl ether. The ethereal layer was dried over $MgSO_4$ and solvent removed, yielding 4 as white solid (~99% pure as determined by HPLC analysis). Upon recrystallization of this material from acetone-hexane gave the pure 4 as light yellow plates (22 mg, 80% yield); mp 158-160° (lit. (10) 162°); nmr ($CDCl_3$) δ 3.33 (s, 3, 12-CH_3), 5.54 (s,1,7-methine), and 7.40-8.58 ppm (m,10, aromatic). The above nmr spectra and mass spectral analysis (m/e of M^+, 273; 4.7 mole % d_0, 95.1 mole % d_1, and 0.2 mole % d_2) indicated that this compound was specifically labelled.

7-Methyl-12-hydroxydeuteromethylbenz[a]anthracene (5)

This compound was prepared in 85% yield upon reaction of 11 with $NaBD_4$ similarly as described above, except the reaction time was 2 hr; mp 163-164° (lit. (10) 164°); nmr ($CDCl_3$) δ 3.05 (s,3,7-CH_3), 5.57 (s,1,12-methine), and 7.3-9.3 ppm (m, 10, aromatic). The above nmr spectra and mass spectral analysis (m/e of M^+, 273; 6.4 mole % d_0, 93.2 mole % d_1 and 0.4 mole % d_2) indicated that compound 5 was specifically labelled.

7,12-Dihydroxydeuterobenz[a]anthracene (6)

This compound was prepared in 82% yield by allowing 12 to react with $NaBD_4$ exactly as described for the preparation of 4; mp 228-229° (lit. (10) 221-223°); nmr (DMSO-d_6) δ 5.42 (s,1,7-methine), 5.48 (s,1,12-methine), and 7.4-9.3 ppm (m,10,aromatic). The above nmr spectra and mass spectral analysis (m/e of M^+, 290; 1.8 mole % d_0, 4.7 mole % d_1, 93.5 mole % d_2, and 0 mole % d_3) indicated that this compound was specifically labelled.

7-Hydroxytriteromethyl-12-methylbenz[a]anthracene (7)

Compound 10 (2.5 mg, 9.3 μmol) was allowed to react with $NaBT_4$ (100 mCi, 20

μmol) in 3 ml methanol-THF at ambient temperature for 1 hr in a screw capped test tube flushed with nitrogen. Water (2 ml) was added to stop the reaction. Organic solvent was removed by a gentle stream of nitrogen and the aqueous phase was then twice extracted with ethyl ether. Upon removal of ether by nitrogen, compound 7 was purified by HPLC (Fig. 1B) and yielded 2.1 mg (84% yield) with specific activity of 1.67 Ci/mmol.

7-Methyl-12-hydroxytriteromethylbenz[a]anthracene (8)

This compound was prepared in 88% yield by the reaction of compound 11 (2.5 mg, 9.3 μmol) with $NaBT_4$ (100 mCi, 20 μmol) by the same procedure as described for 7. HPLC analysis (Fig. 1B) indicated that this compound was about 98% pure and the specific activity of HPLC purified 8 was found to be 1.68 Ci/mmol.

7,12-Dihydroxytriteromethylbenz[a]anthracene (9)

This compound was prepared in 84% yield by the same procedure as described above by the reaction of compound 12 (2.5 mg, 8.7 μmole) with $NaBT_4$ (100 mCi, 20 μmole). HPLC analysis (Fig. 1B) indicated that compound 9 was about 98% pure and the specific activity of HPLC purified 9 was found to be 2.91 Ci/mmol.

The procedures for the synthesis of 7 - 9 were carried out in a laboratory equipped for the synthesis of radiolabelled compounds.

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