PREPARATION OF RING-TRITIATED AND OF ALDEHYDE-^{14}C-LABELLED 1-PYRENECARBOXALDEHYDE

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SUMMARY

A short, direct, and efficient synthesis of very high specific-activity ring-tritiated 1-pyrenecarboxaldehyde is described. A novel aspect of this synthesis involves direct bromination of an aromatic pyrene system carrying an electron-withdrawing aldehyde group. Synthesis of ^{14}C-labelled 1-pyrenecarboxaldehyde is also described. Each of these compounds was converted in one step into the correspondingly radiolabelled trans-1-(2'-methoxyvinyl)pyrene.

Key Words: tritium, ^{14}C, 1-pyrenecarboxaldehyde, 1-(2'-methoxyvinyl)pyrene, radiolabelled PAH

INTRODUCTION

We have designed and synthesized trans-1-(2'-methoxyvinyl)pyrene (trans-MVP) to serve as a model for the mechanism of the observed microsomal chemiluminescence (CL) of benzo[a]pyrene 7,8-dihydrodiol, the proximate carcinogenic metabolite of benzo[a]pyrene [1]. This model compound (trans-MVP) has been found to be a highly efficient and specific chemiluminescent probe for detection of picomole quantities of singlet oxygen and singlet oxygen equivalents, and it produces significant CL when allowed to react with cytochrome P-450 enzymes [2]. We wanted to have tritiated and ^{14}C-labelled trans-MVP (^{3}H-2 and ^{14}C-2) in order to study the details of its metabolism and the intricacies of the reaction(s) of its metabolite(s) with various biomolecules. We describe here preparation of ring-tritiated and of aldehyde-^{14}C-labelled 1-pyrenecarboxaldehydes (^{3}H-1 and ^{14}C-1) and conversion of each into the correspondingly labelled trans-MVP (^{3}H-2 and ^{14}C-2).

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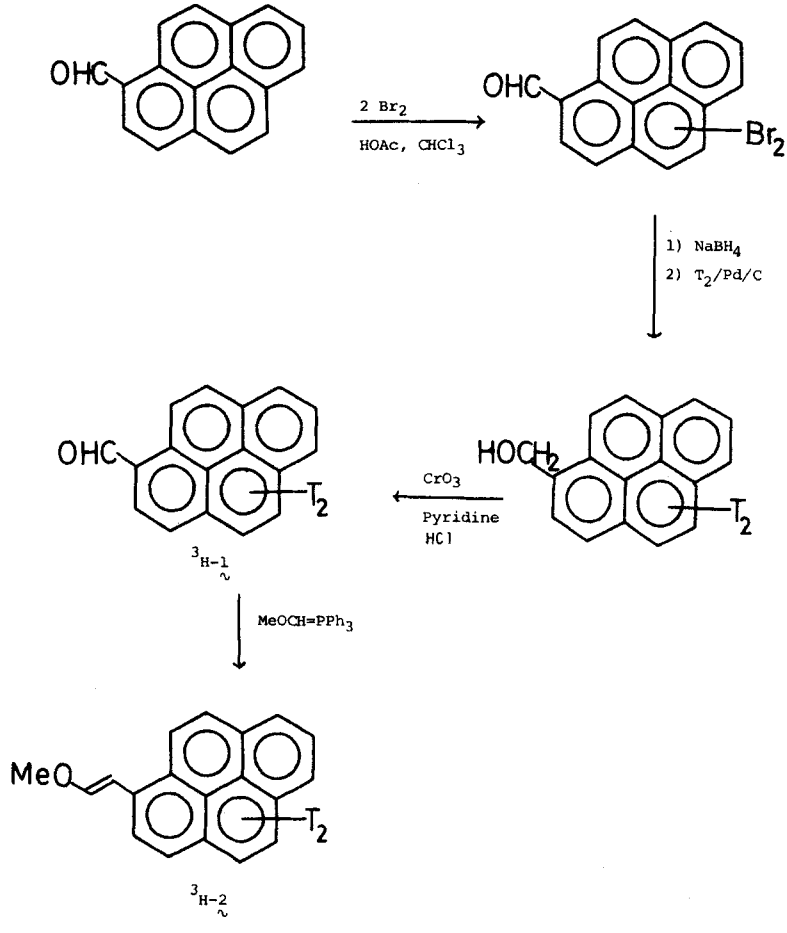
DISCUSSION

Our synthetic route for preparation of ring-tritiated trans-MVP (^{3}H-2) is shown in Scheme I. Highlights of this synthetic scheme include the following three points:

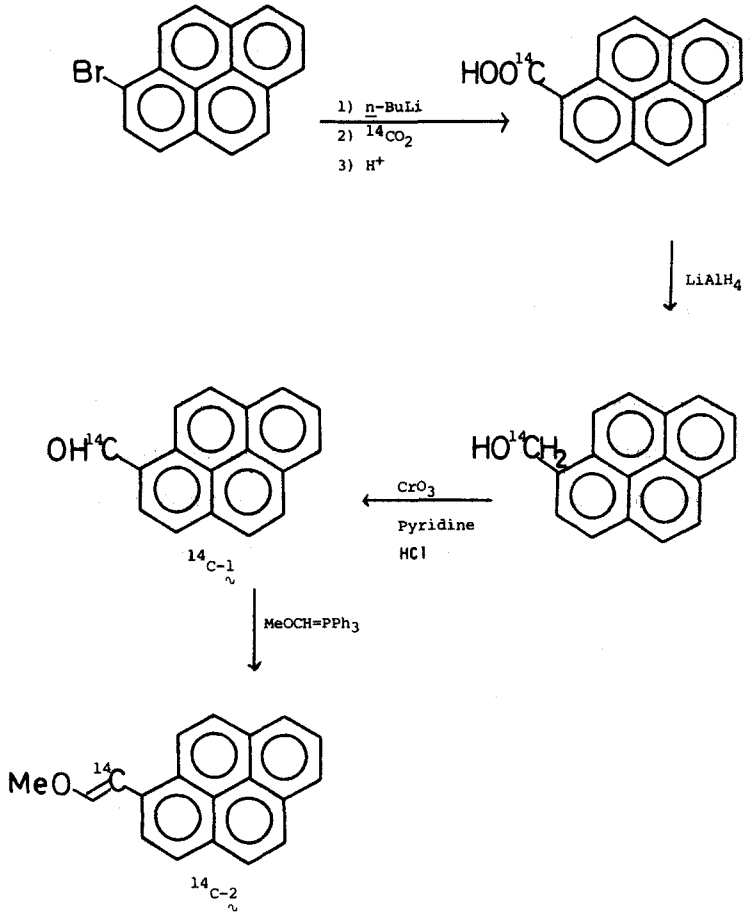
(1) Normally, electrophilic aromatic bromination of electron-deficient (e.g. aldehyde-substituted) aromatics is very difficult. For example, 1-pyrenecarboxaldehyde is unreactive toward cupric bromide, whereas 1-ethylpyrene is easily brominated by cupric bromide [3]. Nevertheless, exposure of 1-pyrenecarboxaldehyde to two equivalents of molecular bromine in acetic acid/chloroform produces mainly dibrominated 1-pyrenecarboxaldehyde;



SCHEME I

(2) effective catalytic replacement of the two bromine atoms by tritium atoms using tritium gas ($^3H_2$) produces the ring-tritiated pyrene alcohol shown in Scheme I in high specific activity (38.5 Ci/mmol);

(3) finally, after some decomposition upon storage for one month at 0°C, this tritiated pyrene alcohol was oxidized into aldehyde ^{3}H-1 (specific activity of 14.8 Ci/mmol). The aldehyde was diluted with some cold 1-pyrenecarboxaldehyde and then was converted in one step into the desired ring-tritiated trans-MVP ^{3}H-2 which, after meticulous purification by column chromatography, had a specific activity of 1.6 Ci/mmol and which exhibited a single peak on high performance liquid chromatography (HPLC). The



SCHEME II

radiochemical purity of this freshly prepared material was estimated to be 98% as assayed (see Experimental) by liquid scintillation counting (LSC) after correction for efficiency of tetrahydrofuran (THF) extraction of a single thin layer chromatography (TLC) spot of ^{3}H-2. After storage in cyclohexane at -85°C for six months, 12% loss of labelled ^{3}H-2 occurred due to radiation-induced decomposition of this high specific activity material.

1-Pyrene-^{14}CHO (^{14}C-1) was prepared as shown in scheme II. 1-Bromopyrene underwent bromine → lithium exchange and then carboxylation with $^{14}CO_2$ generated from $Ba^{14}CO_3$ [4]. Reduction of the carboxylic acid followed by oxidation produced 1-pyrene-^{14}CHO (^{14}C-1) which was directly converted into the corresponding ^{14}C-labelled trans-MVP (^{14}C-2, specific activity 50 mCi/mmol). This freshly prepared material was estimated to be 98% radiochemically pure; storage in cyclohexane at -85°C for six months resulted in only a 3% loss of labelled ^{14}C-2 due to decomposition.

EXPERIMENTAL

High Performance Liquid Chromatography

Chemical purity and quantification measurements were made by high performance liquid chromatography (HPLC). The HPLC system consisted of a Milton Roy mini Pump with a Bodine NSI 33R motor; a Valco sample injection valve model CV-6-UHPa-N60; a Whatman Partisil PXS 5/25 ODS03 reverse phase column; a Kratos Spectroflow 575 variable wavelength UV absorbance detector and a Kratos BD-40 recorder. The mobile phase was 90% methanol:10% water for t-MVP, pyrene-1-carboxaldehyde and 65% methanol:35% water for pyrene-1-carboxylic acid (methanol by Burdick and Jackson, HPLC grade). The flow rate was 1.0 ml/min. Samples were dissolved in the respective mobile phases and 10 µl of this solution (0.1-1.0 nmole) was injected.

Radioassay

Radioactivity measurements were made using a Packard Tri Carb Model 3385 liquid scintillation spectrometer. Radiochemical purity was estimated using THF extraction from TLC on Macherey-Nagel Polygram 4x8 cm. silica gel sheets with fluorescent indicator. Three solvent systems were used for TLC: anhydrous ether (to isolate pyrene-1-methanol and pyrene-1-carboxylic acid), dichloromethane:hexane 1:1 (to isolate pyrene-1-carboxaldehyde) and hexane:ether:triethylamine 90:8:2 (to isolate MVP). Generally, 2 µl containing 10^5 dpm was spotted on TLC plates side by side with standard compounds for R_F comparison. All visible fluorescent spots were cut out and extracted with 1 ml of THF by shaking overnight. Extraction into THF, as estimated from controls, indicated recovery efficiencies of 86.5% (C.V. = 5.3%; n = 4) (t-MVP), 86.6% (C.V. = 1.5%; n = 4) (pyrene-1-aldehyde) and 94.6% (C.V. = 2.4%; n = 2) (pyrene-1-COOH). 1 ml of THF containing the extracted material was dissolved in 20 ml of Bio-Fluor liquid scintillation cocktail (New England Nuclear Corp.), and the count rate was converted into dpm using commercial radioactive standards and was corrected for quench by solvent which in this case (1 ml THF) was minimal.

Dibromopyrene-1-carboxaldehyde.

1-Pyrenecarboxaldehyde (Aldrich, 460 mg, 2.0 mmol) was dissolved in 30 ml of acetic acid (glacial), and a solution of bromine (0.72 g, 4.5 mmol) in 24 ml of chloroform was added dropwise with stirring over 5 hr. The mixture was stirred for 72 hr., and the precipitate was collected by filtration, washed with acetic acid, water and dried to yield 633 mg of a solid. The product was crystallized from 140 mL benzene to provide 426 mg (54% yield) of dibromopyrene-1-carboxaldehyde, mp 258°C. IR (KBr) 1720 cm^{-1} (-CHO); MS: base peak at m/e 388 is consistent with a dibromo product.

Dibromopyrene-1-methanol

Dibromopyrene-1-carboxaldehyde (200 mg, ~0.5 mmol) was suspended in a solution of 150 ml of THF and 30 ml of isopropyl alcohol, and $NaBH_4$ (200 mg,

5.3 mmol) was added. The mixture was stirred at room temperature under nitrogen for 24 hr. During this time the mixture became colorless and clear. The solution was then cooled in an ice bath, 10 ml of water and 10 ml of acetone were added, and the mixture was stirred for 1/2 hr. After neutralization with 1 N HCl, the mixture was concentrated to a 10 ml volume and 50 ml of water was added. The solid was collected by filtration, washed with water, and dried to yield 193 mg (99% yield) of the desired alcohol, mp 220°C. IR (KBr): 3300 cm^{-1} (broad OH); MS: m/e 390 (100%), 310 (48%), 202 (82%).

Ring Tritiated Pyrenecarboxaldehyde ^{3}H-1

Dibromopyrene-1-methanol (40 mg, 0.1 mmol) was dissolved in 13 ml of tetrahydrofuran and 0.5 ml dry triethylamine; to this was added 50 mg of 5% Pd/C catalyst and 85 curies of tritium gas (this procedure was performed by the New England Nuclear Chemical Co.). The reaction mixture was stirred for 1.5 hours at room temperature and uptake of 6.1 c.c. was noted. Labile tritium was removed in vacuo using THF/MeOH 1:1 as solvent. After filtration from the catalyst the product was again taken to dryness to yield crude ring-tritiated pyrenecarboxaldehyde ^{3}H-1 with total activity of 3.85 Ci and was stored in 10 ml THF at 0°C. After one month the solution was evaporated under reduced pressure and worked up as above to yield 20 mg of ^{3}H-1 with a radiopurity of 35% (due to decomposition during storage). To this, 11 mg of cold 1-pyrenecarboxaldehyde in 3 ml of dry CH_2Cl_2 was added. Then 50 mg (0.23 mmol) pyridinium chlorochromate was added, stirred for 15 minutes and 1 g of cold (0°C) silica gel (70-230 mesh) was added. The mixture was evaporated until the silica gel flowed freely. The absorbed compound was loaded on a silica gel column (10 mm, containing 12 ml dry silica gel, 70-230 mesh) and eluted with hexane:dichloromethane 1:1 to yield 15 mg of ^{3}H-1 with a specific activity of 14.8 Ci/mmol: HPLC (λ = 287nm) retention time (t_R) = 6.08

minutes, coefficient of variation (C.V. = 0.95%; n = 10); TLC R_f = 0.19, C.V. = 3.3%; n = 4. Initially 98% of the radioactive label could be immediately recovered from a single TLC spot; after six months storage as a dry compound at -85°C, only 65.1% (C.V. = 1.1%; n = 3) of the radioactive label was located in a single TLC spot which was eluted and determined by HPLC to be essentially a single peak identical to that of authentic ^{1}H-1.

Ring-Tritiated trans-Methoxyvinylpyrene ^{3}H-2.

Methoxymethyltriphenylphosphonium chloride (355 mg, 1.04 mmol) was suspended with stirring in dry THF (10 ml) at -25°C under argon. To this 0.8 ml (1.3 mmol) of n-butyllithium in hexane was added. The red solution which resulted was stirred for 20 minutes and 3 ml of this solution was taken and injected into a solution of ^{3}H-1 (23 mg, 0.1 mmol, specific activity 1.6 Ci/mmol) in 2 ml dry THF at 25°C under argon. After 20 minutes the reaction mixture was placed in a refrigerator overnight. Then 0.7 g of silica gel (70-230 mesh) was added and carefully was evaporated until the siica gel flowed freely. The adsorbent compound was loaded on a silica gel column (230-240 mesh, 40 g, 220 mm diameter) and eluted with hexanes:diethyl ether:trimethylamine 90:8:2 to provide 6.4 mg of trans-MVP ^{3}H-2 and 4.4 mg of the corresponding cis isomer. trans-MVP ^{3}H-2: HPLC (λ = 352nm) t_R = 7.79 minutes, c.v. = 0.8%, n = 11. TLC R_f = 0.22, (C.V. = 3.2%; n = 8). After six months storage in cyclohexane at -85°C, the initial activity (98% of the TLC spotted radioactive material was recovered as a single spot) was reduced to 86.5% recovery of the spotted radioactive label at R_f = 0.22; 13% of the radioactivity was recovered as a green fluorescing spot, R_f = 0.11 (C.V. = 10.1%; n = 9), and 0.5% remained at the origin.

1-Pyrene-^{14}COOH

1-Bromopyrene (Cambridge Chemical Co., 282 mg, 1.0 mmol) was dissolved

under argon in 10 ml of THF. The mixture was cooled to -78°C and 0.85 ml of 1.3 M (1.1 mmol) of n-butyllithium in hexane was added, and stirred for 20 minutes. The yellow precipitate was carboxylated at -78°C with 25 mCi (~0.5 mmol) of $^{14}CO_2$ released from 97 mg of $Ba^{14}CO_3$ with 1.2 ml of H_2SO_4. The mixture was stirred for 20 minutes at -78°C and then for one hour at -40°C and then raised to room temperature. The carboxylation apparatus was purged with argon, and the reaction mixture was acidified with 5 ml of 2N HCl. The mixture was concentrated under reduced pressure and the excess of $^{14}CO_2$ was trapped in NaOH. Ether (30 ml) was added to the residue and the mixture was extracted with 5% $NaHCO_3$ until no precipitate occurred upon acidification. The aqueous solution was acidified at 0°C with conc. HCl. The precipitate was collected and washed with water and dried under vacuum to yield 1-pyrene-^{14}COOH, 61 mg, (50% yield) as a light yellow solid; specific activity 49.3 mCi/mmol. 1-Pyrene-^{14}COOH eluted (HPLC, λ = 278nm) as a single peak, t_R = 3.05 minutes, n = 2. TLC R_f = 0.29, (C.V. = 4.2%, n = 4). After six months storage as dry material at -85°C, 92.3% (C.V. = 1.7%; n = 4) of the spotted activity was recovered from this single peak.

Pyrenecarboxaldehyde ^{14}C-1

1-Pyrene-^{14}COOH (52 mg, 0.21 mmol, specific activity 49-50 mCi/mmol) was dissolved under argon in 3 ml of THF and 20 ml of ether. Then 40 mg of lithium aluminum hydride was added, the mixture stirred for four hours at room temperature and then kept refrigerated for two days. The mixture was hydrolyzed with 10 ml of water and then with 10 ml of HCl (1N). Then 60 ml of ether was added and the organic layer was extracted with H_2O and 5% NaOH, then dried ($MgSO_4$) and evaporated under reduced pressure to yield a yellow crystalline residue. To this residue CH_2Cl_2 (6 ml) and 100 mg of pyridinium chlorochromate were added and stirred for 15 minutes. The resultant ^{14}C-1 was

purified as before to yield 34 mg of ^{14}C-1. Specific activity: 49-50 mCi/mmol; HPLC (λ = 287nm): t_R = 6.08 minutes, n = 3. TLC R_f = 0.19; n = 2. The total activity recovered was 98%; n = 2 after six months storage as dry material at -85°C.

trans-MVP ^{14}C-2.

As above for conversion of aldehyde ^{3}H-1 into trans-MVP ^{3}H-2, aldehyde ^{14}C-1 (28 mg, 0.11 mmol, specific activity 49-50 mCi/mmol) was converted into trans-MVP ^{14}C-2 [8.2 mg; specific activity 49-50 mCi/mmol] HPLC (λ = 352nm): t_R = 7.79 minutes. TLC R_f = 0.22. After six months storage in cyclohexane at -85°C, 95.2% (C.V. = 0.47%; n = 4) of the spotted radioactivity was recovered as a single spot and was identified by HPLC as ^{14}C-1.

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