

STABILITY UPON STORAGE, ANALYSIS AND PURIFICATION OF ^{14}C - AND ^3H -LABELED
POLYCYCLIC AROMATIC HYDROCARBONS (PAH) AND THEIR METABOLITES

Alexander B. Susàn, Timothy P. Rohrig, and James C. Wiley, Jr.

Organic and Radiochemical Synthesis Section
Midwest Research Institute, 425 Volker Boulevard
Kansas City, Missouri 64110, USA

SUMMARY

The studies on the stability, analysis and purification of several ^{14}C - and ^3H -labeled polycyclic aromatic hydrocarbons such as benzo[a]-pyrene, benzo[e]pyrene, and dibenz[a,c]anthracene and their metabolites such as dihydrodiols, phenols, and tetrahydrodiol epoxides are reported.

Key Words: Tritium, Carbon-14, Labeled polycyclic aromatic hydrocarbons, Thin-layer chromatography, Column chromatography

INTRODUCTION

Carcinogenesis studies of polycyclic aromatic hydrocarbons (PAH) necessitate the availability of a variety of radiolabeled (^3H and ^{14}C) cancer suspect or established metabolites of PAH such as arene oxides, phenols, dihydrodiols, quinones, etc.

During the past years, a number of such radiolabeled compounds have been prepared [1-7] in our laboratory by known or modified literature methods or by novel reactions. The establishment of a NCI Repository at Midwest Research Institute for a limited distribution of such radiolabeled PAH derivatives prompted the investigation of the stability upon storage as well as the analysis and repurification procedures of these compounds. Fairly comprehensive reviews concerning the decomposition of tritium-labeled organic compounds have been published in the literature [8-10], but very little information has appeared dealing specifically with the stability and purification of the title radiolabeled PAH's and their derivatives.

RESULTS AND DISCUSSION

A summary of the studies of stability, analysis and purification of a number of ^{14}C - and ^3H -labeled polycyclic aromatic hydrocarbons and their metabolites is

outlined in the Table. The details concerning the purification procedures of these compounds are given in the experimental section.

The stability upon storage of the title compounds was monitored by reanalysis of the stock samples. The frequency of the analyses depended upon the known or suspected rates of decomposition of the individual compounds. No attempts have been made to identify the impurities generated upon storage. The labeled PAH's were stored at -5° and -20°C in solutions of concentrations ranging from 0.5 to 1.5 mg/ml, protected from light and packaged in one of the following solvents as indicated in the Table: (I) THF:Et₃N (19:1); (II) benzene; (III) benzene:ethyl acetate (4:1); (IV) benzene:ethanol (9:1). The beneficial effects of storing tritiated compounds of relatively high specific activities in purified organic solvents such as those above listed are well-known.

Addition of Et₃N to THF (solvent I) was found to minimize unwanted acid-catalyzed reactions such as epoxy ring opening in the corresponding BaP derivatives; further, ethyl acetate and ethanol were added to benzene for enhanced solubility purposes and, in the case of ethanol, also for scavenging of free radicals due to self-radiolysis.

The results presented in the Table are in accordance with previous general observations concerning the stability of ¹⁴C- and ³H-labeled compounds [1-9]. Thus, parent hydrocarbons such as benz[a]anthracene-G-³H, dibenz[a]anthracene-G-³H, benzo[a]pyrene-G-³H and benzo[e]pyrene-G-³H, of specific activities ranging between 300 mCi/mole to 1,200 mCi/mole, exhibited a chemical and radiochemical purity of $\geq 98\%$ for a year or more. Further, oxygenated derivatives of these hydrocarbons such as 2- and 3-hydroxybenzo[a]pyrene-G-³H were found to exhibit the same degree of purity for a shorter time, i.e., approximately 6 months. The least stable compound among those studied was 9-hydroxybenzo[a]pyrene-G-³H, which kept the initial purity of $\geq 98\%$ only for approximately 3 months. It is interesting to note also that, in the case of the latter compound, the rate of decomposition was apparently not influenced by the amount of isotope contained; two different batches of 9-hydroxybenzo[a]pyrene-G-³H of 324 and 3,681 mCi/mole, respectively, exhibited similar stabilities for approximately 3 months. These

TABLE

Compound Name	Specific Activity mci/nmole	TLC System	Purification Method	% Radiochemical Purity Storage Time (months)			Storage Conditions		UV Spectral Data		
				0	3	6	12	Solvent	Temp. °C	λ _{max}	log ε
(±)-t-7,8-Dihydrobenzo[a]pyrene-7,8-diol-7,14C	30.1	a	1	98	-	98	90	I	-20	365 nm	4.67
(±)-t-7,8-Dihydrobenzo[a]pyrene-7,8-diol-7,14C	58.4	a	1	98	-	98	98	I	-20	365 nm	4.67
(±)-t-7,8-Dihydrobenzo[a]pyrene-7,8-diol-G-3H	438	a	1	98	-	98	98	I	-20	365 nm	4.67
(±)-t-7,8,9,10-Tetrahydrobenzo[a]pyrene-7,8-dibenzoate-G-3H	14,500	b	N/A	97	-	-	97	II	-5	-	-
r-7,t-8-Dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-G-3H (anti)	406	c	2	98	97	97	97	I	-20	344 nm	4.68
r-7,t-8-Dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-7,14C (anti)	54.8	c	2	98	-	98	98	I	-20	344 nm	4.68
r-7,t-8-Dihydroxy-c-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene-7,14C (syn)	17.9	c	2	98	-	98	98	I	-20	344 nm	4.68
4,5-Dihydrobenzo[a]pyrene-4,5-epoxide-G-3H	283	d	3	98	-	-	98	I	-20	274 nm	5.06
Benzo[a]pyrene-G-3H	310	e	4	98	-	-	98	-	-5	383 nm	4.35
Benzo[e]pyrene-G-3H	414	g	4	99	-	-	99	IV	-5	330 nm	4.53
2-Hydroxybenzo[a]pyrene-G-3H	155	d	5	99	-	98	-	II	-5	380 nm	4.28
3-Hydroxybenzo[a]pyrene-G-3H	466	a	5	98	-	98	-	III	-5	378 nm	4.42
9-Hydroxybenzo[a]pyrene-G-3H	3,681	a	5	98	97	90	-	III	-5	377 nm	4.35
9-Hydroxybenzo[a]pyrene-G-3H	324	a	5	98	97	90	-	III	-5	377 nm	4.35
Benzo[a]anthracene-G-3H	1,240	b	6	98	-	-	98	II	-5	285 nm	4.97
Dibenz[a,c]anthracene-G-3H	292	f	7	98	-	98	98	II	-5	349 nm	3.45
7,8-Dihydrobenzo[a]pyrene-7,8-diol-G-3H	3,760	a	1	98	-	80	-	II	-20	365 nm	4.67
	298	a	1	99	-	99	98	II	-20	365 nm	4.67

results indicated that the decomposition on storage of the hydroxybenzo[a]pyrene- $G-^3H$ derivatives is due most likely to chemical reactions rather than to self-radiolysis.

EXPERIMENTAL

Radioactivity was determined in Packard liquid scintillation counters (Models 3003 and 2425) using Liquifluor[™] or Aquasol[™] (New England Nuclear) as the counting medium. The radiochemical purity was determined by autoradiography (on Eastman Kodak SB-5 X-ray film) and radiochromatogram scanning (Packard Model 7201 Radiochromatogram Scanner) of the TLC plates (precoated fluorescent silica gel, 5 x 20 cm, Brinkman 60F-254). Known standards were used on TLC for visual comparison with the radiolabeled samples. Several developing solvent systems were used for the analysis of individual samples by TLC. The following systems were found to give the best resolution of the samples analyzed, as listed in the Table: (a) benzene:isopropanol (4:1); (b) benzene; (c) THF:Et₃N (19:1); (d) benzene:ethyl acetate (4:1); (e) cyclohexane:benzene (9:1); (f) hexanes:benzene (4:1); and (g) hexanes:ethyl acetate (9:1).

Direct spotting onto the silica gel plate of the labeled and the "cold standard" of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene showed an almost complete decomposition of both samples. This artifact was avoided by applying the above samples immediately over a drop of Et₃N.

The specific activity was determined by assaying the radioactivity of the storage solution of a known concentration determined directly by UV. If the storage solvent interfered in the UV determinations, both radioactivity and UV assays were done in suitable solvents, after removal *in vacuo* of the original solvent. The sample concentrations were determined from the UV spectra (Varian Superscan 3), and the standard extinction coefficient was determined from quantitative UV spectra using authentic standards of $\geq 98\%$ purity. The λ_{\max} and ϵ standard values used are given in the Table.

The repurification of the compounds was carried out, when possible or practical, by column chromatography. The average amounts purified ranged from 25 mg

to 350 mg. Silica Gel Woehlm (70-250 mesh) was used as the solid phase, and solvents degassed via nitrogen bubbling were utilized.

(±)-trans-7,8-Dihydrobenzo[a]pyrene-7,8-diol, 1

The impure diol 1 (25 to 50 mg) was dissolved in 10 ml benzene and placed on a silica gel column (250 x 20 mm) packed with the same solvent. The impurities were removed first by elution with 10% THF in benzene followed by an equal volume of 5% ethanol in benzene. The desired compound was then eluted with 15% ethanol in benzene followed by a double amount of 10% ethanol in benzene. The eluates were concentrated in vacuo followed by recrystallization of the solid residue from benzene:ethanol. Yield 60 to 70% of 1 with a chemical and radiochemical purity of $\geq 98\%$ as established by TLC (solvent system I) and by UV.

(±)-trans-7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, 2

The crude epoxide 2 (155 mg) was dissolved in 25 ml of THF:triethylamine (19:1) solution and placed on a silica gel column (100 x 15 mm) prepared with the same solvent. The column was eluted with additional THF:triethylamine (19:1) and the first 10-ml fraction was discarded. The desired product was then eluted in the following 30 ml of solution to which 10 ml of dioxane (freshly dried by passing through a short alumina column) was added. The eluate was concentrated to a volume of 5 ml by heating at 40 to 50°C in a stream of nitrogen and the crystalline product was removed by filtration followed by recrystallization from 5 ml dry dioxane. Yield 60 mg (39%) of 2 as off-white crystals with a chemical and radiochemical purity of $\geq 98\%$ as established by TLC (solvent system I) and by UV.

4,5-Dihydrobenzo[a]pyrene-4,5-epoxide, 3

The impure epoxide (100 mg) was dissolved in 20 ml of benzene and placed on a 200 x 20 mm column consisting of alumina (neutral, grade IV) packed in benzene. The column was eluted with benzene and the first 200 ml containing the desired product were collected and evaporated to dryness in vacuo. The resulting yellow-orange solid was recrystallized from a 1:1 mixture of benzene and hexane, yielding 70 mg (70%) of 3 (yellow-golden solid) with a chemical and radiochemical purity of $\geq 98\%$ as established by TLC (solvent system I) and by UV.

Benzo[a]pyrene, 4

The impure hydrocarbon 4 (350 mg) was dissolved in 50 ml benzene to which 2 g of silica gel was added followed by evaporation to dryness in vacuo. The resulting residue was then slurried with 10 ml cyclohexane and placed on a silica gel column (300 x 20 mm) packed with the same solvent. The main impurities which eluted in the first 300 ml of cyclohexane were discarded and the pure product was collected in an equal volume of cyclohexane:benzene (9:1) eluates. The combined eluates containing the desired product were concentrated in vacuo until crystallization started to occur. Yield 259 mg (74%) of 4 with a chemical and radiochemical purity of $\geq 98\%$ as established by TLC (solvent system IV) and by UV.

Hydroxybenzo[a]pyrenes, 5

All the hydroxybenzo[a]pyrene derivatives could be purified by column chromatography as illustrated below for the impure 3-hydroxybenzo[a]pyrene.

The crude phenol (25 mg) was dissolved in 25 ml of benzene and placed on a silica gel column (500 x 20 mm) packed with a 3:1 mixture of benzene:chloroform. The desired product was eluted in the first 75 ml of the above solvent mixture, which was then concentrated to a volume of 2 ml by heating at 40°C in a stream of nitrogen. The crystalline material was removed by filtration, yielding 15 mg (75%) of 5 with a radiochemical and chemical purity of $\geq 98\%$ as established by TLC (solvent system III) and by UV.

Benz[a]anthracene, 6

A concentrated solution of impure 6 (321 mg) in 5 ml benzene was placed on a silica gel column (120 x 25 mm) packed with benzene. The desired product was eluted in 60 ml of solvent after approximately 200 ml benzene were discarded. The eluate was then concentrated in vacuo to 3 ml and the crystalline material was removed by filtration, yielding 308 mg (96%) of 6 with a radiochemical and chemical purity of $\geq 98\%$ as established by TLC (solvent system II) and by UV.

Dibenz[a,c]anthracene, 7

To a solution of impure 7 (177 mg) in 25 ml benzene, 2 g of silica gel was added, and the resulting suspension was evaporated to dryness in vacuo. The residue was then slurried with 10 ml cyclohexane and placed on a silica gel column (200 x 20 mm) packed with the same solvent. The column was first eluted with 300 ml cyclohexane followed by 100 ml benzene. The desired product was collected in the benzene fractions, which were combined and evaporated to dryness. Yield 154 mg (87%) of 7 with a chemical and radiochemical purity $\geq 98\%$ as established by TLC (solvent system II) and by UV.

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