LABELED METABOLITES OF POLYCYCLIC AROMATIC HYDROCARBONS I. DETERMINATION OF TRITIUM AT THE 6-POSITION IN BENZO [a] PYRENE-G-^{3}H

Received on October 2, 1974.

INTRODUCTION

For studies of metabolic reactions, specific labeling is often essential for conclusive results. Although many investigations relating to the metabolic fate of tritium labeled benzo[a]pyrene have appeared in recent years [1], very little information has been reported concerning the tritium distribution in tritiated benzo[a]pyrene prepared via various techniques. However, Lijinsky and Garcia have reported the distribution of tritium at certain positions in tritiated benzo[a]pyrene prepared by Wilzbach's method [2].

In this note we report a simple convenient procedure for determination of the amount of tritium at the 6-position of benzo[a]pyrene-G-^{3}H. This commercially available material was prepared by catalytic exchange in solution [3] as described by Evans, et al. [4].

EXPERIMENTAL

Liquid scintillation counting was done with a Packard Model 3375 liquid scintillation spectrometer, using ECONOFLUOR^{TM} premixed scintillation solution (New England Nuclear Corporation, Boston, Massachusetts). Benzo[a]pyrene-G-^{3}H was obtained from Amersham/Searle Corporation, Arlington Heights, Illinois.

GENERAL PROCEDURE FOR PREPARATION OF 6-ACETOXYBENZO[a]PYRENE-G-^{3}H

Benzo[a]pyrene-G-^{3}H was reacted with lead tetraacetate using the procedure of Fieser and Hershberg [5]. Thus, 493 mg (1.95 mmol) of benzo[a]pyrene-G-^{3}H specific activity 19.44 mCi/mmol dissolved in 20 ml of benzene was mixed with 910 mg (2.02 mmol) of lead tetraacetate in 25 ml of glacial acetic acid at room temperature. The reaction mixture was homogeneous at this point and there was no exotherm on mixing. A copious flocculent yellow precipitate formed after 25 min. The precipitate was filtered and recrystallized from acetic acid. The yellow-green product was then recrystallized from benzene, leaving 356 mg (60%) of 6-acetoxybenzo[a]pyrene-G-^{3}H, mp 208 - 210° (uncorrected), specific activity 18.02 mCi/mmol. Three additional runs were made, the results of which are presented below.

DISCUSSION

The data in the Table indicate an average of 8.55% of the tritium in benzo[a]pyrene-G-^{3}H is located at the 6-position. This value agrees nicely with the expected loss of radioactivity if the tritium were distributed uniformly in the molecule. In addition, we have ruled out exchange since both benzo[a]pyrene-G-^{3}H and 6-acetoxybenzo[a]pyrene-G-^{3}H failed to show any loss of radioactivity upon recrystallization from acetic acid and benzene. Furthermore, this value is compatible with the value of 11% recently communicated to us by Evans and coworkers [3], who determined the tritium distribution in a similarly prepared sample of benzo[a]pyrene-G-^{3}H using triton nmr.

TABLE

	SPECIFIC ACTIVITY (mCi/mmol)		
Reaction	Benzo[a]pyrene-G-^{3}H	6-Acetoxybenzo[a]pyrene-G-^{3}H	^{3}H at 6-Position (%)
I	19.44	18.02	7.31
II	15.77	14.28	9.44
III	16.06	14.40	8.92
IV	17.21	15.74	8.54
		Average	8.55

ACKNOWLEDGEMENT

We gratefully acknowledge the support of this work by the National Cancer Institute, Contract No. N01-CP-33387.

W. P. Duncan and James F. Engel
Midwest Research Institute, 425 Volker Boulevard
Kansas City, Missouri 64110

REFERENCES

1. For leading references see:
    - (a) Borgen, A., H. Darvey, N. Castagnoli, T. T. Crocker, R. E. Rasmussen, and I. Y. Wang, J. Med. Chem., **16**, 502 (1973).
    - (b) Selkirk, James K., Robert G. Croy, and Harry V. Gelboin, Science, **184**, 169 (1973).
    - (c) Kinoshita, Nadao, Barbara Shears, and Harry V. Gelboin, Cancer Res., **33**, 1937 (1973).
2. Lijinsky, William and Humberto Garcia, Biologica, **35**, 135 (1963); Nature, **197**, 688 (1963).
3. Evans, E. A., The Radiochemical Centre, Amersham, Buckinghamshire, England, personal communications, 1974.
4. Crowter, D. G., E. A. Evans, and R. Rasdell, Chem. and Ind. (London), 1622 (1962).
5. Fieser, L. F. and E. B. Hershberg, J. Amer. Chem. Soc., **60**, 2542 (1938).