RADIOACTIVELY LABELLED EPOXIDES. PART V.* TRITIUM LABELLED K-REGION OXIDES AND TRANS-DIHYDRODIOLS OF PYRENE, BENZO[a]PYRENE AND DIBENZ[a,h]ANTHRACENE

Frans Setiabudi, Franz Oesch, and Karl L. Platt§
Institute of Toxicology, University of Mainz, Obere Zahlbacher Straße 67, D-6500 Mainz, FRG

SUMMARY

Tritium labelled K-region oxides of pyrene, benzo[a]pyrene and dibenz[a,h]anthracene have been prepared by cyclization of the corresponding trans-dihydrodiols which were obtained by reduction of K-region quinones with sodium borotritide in the presence of oxygen. This synthetic pathway not only yields two metabolically important radioactively labelled derivatives of polycyclic aromatic hydrocarbons in a simple and efficient manner, but also requires a rather inexpensive source of tritium.

Key Words: Arene oxides, Dihydrodiols, Pyrene, Benzo[a]pyrene, Dibenz-[a,h]anthracene, Sodium borotritide

INTRODUCTION

Enzymatic attack of cytochrome P-450 dependent monooxygenases at the K-region of polycyclic aromatic hydrocarbons (PAH) results in the formation of arene oxides (1-3). These could be further transformed by epoxide hydrolases (4) to trans-dihydrodiols (5,6) and/or by glutathione transferases (7) to glutathione conjugates (8-10). K-Region trans-dihydrodiols could serve as substrates for dihydrodiol dehydrogenase (11,12), or be converted to glucuronides (9,13) or sulfates (9,14). In order to study the role of these enzymes in the metabolic processing of K-region derivatives of PAH, a simple and efficient radiosynthesis of tritium

* Part IV: Oesch F., Sparrow A.J. and Platt K.L. - J. Label. Comp. Radiopharm. 20: 1297 (1983).

§ To whom correspondence should be addressed.

0362-4803/87/080979-08$05.00

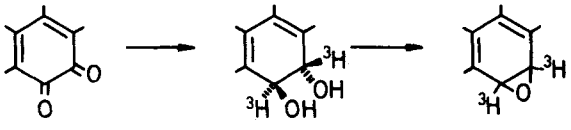
Received January 5, 1987

labelled K-region oxides and trans-dihydrodiols is required.

K-Region arene oxides have been prepared from PAH by (a) direct oxidation (15-19) or (b) cyclization of trans-bromohydrin acetates (20), (c) dialdehydes (21,22), (d) cis-dihydrodiols (23,24) and (e) trans-dihydrodiols (25-27). Application of synthetic routes (a)-(d) for the preparation of tritium labelled arene oxides requires tritium labelled PAH while in case of the route (e) tritium can be introduced via reduction of unlabelled o-quinones of PAH (28,29) by complex metal tritides, e.g. lithium aluminum [^{3}H]hydride (27), sodium or potassium boro-[^{3}H]hydride (30,31) yielding tritium labelled trans-dihydrodiols. This furnishes both radioactively labelled metabolically relevant K-region derivatives of PAH by the same reaction sequence.

Since it has been shown that o-quinones of PAH can efficiently be reduced with sodium borohydride in the presence of oxygen to the corresponding trans-dihydrodiols (28,32,33) synthetic route (e) (cf. SCHEME) was followed to prepare the

SCHEME



1 2 3

a) Pyrene
b) Benzo [a] pyrene
c) Dibenz [a,h] anthracene

K-region trans-dihydrodiols and arene oxides of pyrene, benzo[a]pyrene and dibenz-[a,h]anthracene.

RESULTS AND DISCUSSION

For efficient introduction of tritium into suitable substrates by [^{3}H]borohydride isopropanol has been found to be the solvent of choice (27) as it does not exhibit undesired tritium-hydrogen exchange as in the case of methanol or ethanol. Unfortunately the solubility of 4,5-pyrenequinone, 1a, 4,5-benzo[a]-

pyrenequinone, 1b, and 5,6-dibenz[a,h]anthracenequinone, 1c, in isopropanol is very poor. Since K-region quinones are quite soluble in tetrahydrofurane (THF) a mixture of dry THF and isopropanol (5:3, v/v) was used for the reduction of 1a-c.

In the case of unlabelled 1 sodium borohydride is applied in ten molar excess to achieve complete conversion to 2 (28). We observed that satisfying yields of 2 can also be obtained if borohydride is used in 1.5-3 molar excess as compared to 1. A specific activity of 1.0-1.2 GBq/mmol was reached when the reduction of 1a-c was started by the addition of about 10 % of the final amount of borohydride as boro[^{3}H]hydride followed by the remaining 90 % as unlabelled borohydride. However, when in the case of 1c the addition of boro[^{3}H]hydride was preceded by one fifth of the amount of unlabelled borohydride in order to remove traces of moisture in the reaction mixture the specific activity decreased to 0.3 GBq/mmol. These results indicate that the major part of 2c is formed directly from 1c and not via the corresponding o-diphenol and its reoxidation to 1c, as it has been postulated (28,33).

The cyclization of 2 to 3 with N-tosylimidazole (34) worked well with trans-9,10-dihydroxy-[9,10-^{3}H]dihydrophenanthrene (27) and unlabelled 2c but proved unsuccessful with tritium labelled 2c for reasons not yet determined. 2a-c were finally transformed to 3a-c with N,N-dimethylformamide dimethylacetal (DMF-DMA) (25,26) in high yields and with almost complete retention of the label.

EXPERIMENTAL

Sodium boro[^{3}H]hydride was obtained in sealed glass tubes from Amersham Buchler (Braunschweig, FRG) or in screw-cap bottles by New England Nuclear (Dreieich, FRG). In the latter case the shipping container could directly be used for performing the reaction which proved to be quite convenient.

DMF-DMA was supplied by Aldrich (Steinheim, FRG). Neutral alumina (typ 90, activity II-III) for column chromatography was from Merck (Darmstadt, FRG). Thin-layer chromatography (TLC) was performed on ALF sheets (Riedel-de-Haen, Seelze, FRG) with chloroform-methanol (99:1, v/v) as mobile phase.

The chemical and radiochemical purity of 3a-c was determined by high-performance chromatography with UV-detection (280 nm) using LiChroscrb RP-18 (5µm; 4 x

250 mm; Merck, Darmstadt, FRG) as stationary and methanol- 0.1 % aqueous triethylamine (3a: 65/35; 3b: 75/25; 3c: 90/10, v/v) as mobile phase at a flow rate of 1 ml/min; the total eluate of one chromatographic run was collected in 0.5 ml fractions, which were mixed with Lumagel SB (LKB, Gräfelfing, FRG) and counted in a liquid scintillation counter. The radiochemical purity is given as the percentage of total radioactivity that is eluted within the same elution volume as the pure unlabelled compound.

^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker WH 90 spectrometer at 90 MHz using tetramethylsilane as internal standard.

trans-4,5-Dihydroxy-[4,5-^{3}H]dihydropyrene, 2a

A suspension of 4,5-pyrenequinone (28) (110.4 mg, 0.48 mmol) in a mixture of 3 ml dry isopropanol and 5 ml dry THF was added to 0.925 GBq sodium boro[^{3}H]hydride (spec. act. 12.6 GBq/mmol) and stirred at room temperature under air and exclusion of moisture. After 30 min unlabelled sodium borohydride (24 mg, 0.63 mmol) was added and stirring was continued for 20 h. After removal of the solvent under reduced pressure and addition of water (20 ml) the reaction product was isolated by extraction with chloroform (3 x 75 ml). Washing of the combined organic phases with water (2 x 75 ml), drying over anhydrous $MgSO_4$ and evaporation under reduced pressure yielded crude 2a which was purified by column chromatography on neutral alumina with chloroform-methanol (95:5, v/v). Pure 2a (27.6 mg, 25 %) was obtained as a white solid (spec. act. 1.2 GBq/mmol) and proved to be identical with authentic unlabelled 2a (28) as judged by TLC.

trans-4,5-Dihydroxy-[4,5-^{3}H]dihydrobenzo[a]pyrene, 2b

4,5-Benzo[a]pyrenequinone (28) (134 mg, 0.48 mmol) was transformed to 2b with 1.85 GBq sodium boro[^{3}H]hydride (spec. act. 12.6 GBq/mmol) and unlabelled sodium borohydride (48 mg, 1.27 mmol) in the same manner as described for the synthesis of 2a. Pure 2b (97 mg, 71 %) was obtained as a white solid (spec. act. 0.96 GBq/mmol) and proved to be identical with authentic unlabelled 2b (28) as judged by TLC.

trans-5,6-Dihydroxy-[5,6-^{3}H]dihydrodibenz[a,h]anthracene, 2c

5,6-Dibenz[a,h]anthracenequinone (28) (146.5 mg, 0.48 mmol) was transformed to 2c with 0.925 GBq sodium boro[^{3}H]hydride (spec. act. 16.7 GBq/mmol) and unlabelled sodium borohydride (25 mg, 0.66 mmol) in the same manner as described for the synthesis 2a. Pure 2c (49.3 mg, 33 %) was obtained as a white solid (spec. act. 1.1 GBq/mmol) and proved to be identical with authentic unlabelled 2c (28) as judged by TLC.

When the amount of unlabelled sodium borohydride was split, one portion (5 mg) being added before and the other portion (20 mg) being added after the addition of sodium boro[^{3}H]hydride to the solution of 1c the yield of 2c was somewhat improved (40 %) yet the specific activity decreased to 0.32 GBq/mmol.

[4,5-^{3}H]Pyrene 4,5-oxide, 3a

A solution of 2a (27.6 mg, 0.12 mmol; spec. act. 1.2 GBq/mmol) in a mixture of 6 ml $CHCl_3$ and 2 ml dry N,N-dimethylformamide was treated with DMF-DMA (37 µl, 0.28 mmol) and stirred under reflux for 2.5 h. The solvent was removed under reduced pressure and the residue was washed with water and dried. Purification by column chromatography on neutral alumina with methylene chloride-triethylamine (99:1, v/v) yielded 3a (21.2 mg, 83 %) as an off-white solid; ^{1}H-NMR ($CDCl_3$) δ 4.75 (s,2, $H_{4,5}$), 7.3-8.0 (m, 8, ArH); radiochemical purity: 96 %; spec. act. 0.96 GBq/mmol.

[4,5-^{3}H] Benzo[a]pyrene 4,5-oxide, 3b

2a (93.9 mg, 0.33 mmol; spec. act. 0.96 GBq/mmol) was transformed with DMF-DMA (155 µl, 1.17 mmol) to 3b in the same manner as described for the synthesis of 3a. 3b (78.7 mg, 89 %) was obtained as an off-white solid. ^{1}H-NMR ($CDCl_3$) δ 4.71 (d, 1, H_4 or H_5, $J_{4,5}$ = 4.0 Hz), 4.79 (d, 1, H_4 or H_5, $J_{4,5}$ = 4.0 Hz), 7.4 - 8.8 (m, 10, ArH); radiochemical purity: 96 %; spec. act. 0.87 GBq/mmol.

[5,6-^{3}H] Dibenz[a,h]anthracene 5,6-oxide, 3c

2c (54.1 mg, 0.17 mmol; spec. act. 0.32 GBq/mmol) was transformed with DMF-DMA (47 µl, 0.35 mmol) to 3c in the same manner as described for the synthesis of 3a. 3c (41 mg, 80 %) was obtained as an off-white solid. ^{1}H-NMR ($CDCl_3$) δ 4.63 (d, 1, H_5, $J_{5,6}$ = 4.1 Hz), 4.81 (d, 1, H_6, $J_{5,6}$ = 4.1 Hz), 7.2 - 9.0 (m, 12, ArH); radiochemical purity: 99 %; spec. act. 0.28 GBq/mmol.

ACKNOWLEDGEMENT

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 302).

We thank Ms. S. Pollok for expert help in preparing the manuscript.

REFERENCES

1. Daly J.W., Jerina D.M. and Witkop B. - Experientia 28: 1129 (1972)

2. Sims P. and Grover P.L. - Adv. Cancer Res. 20: 165 (1974)

3. Jerina D.M. and Daly J.W. - Science 185: 573 (1974)

4. Oesch F. - Xenobiotica 3: 305 (1973)

5. Gelboin H.V. - Physiol. Rev. 60: 1107 (1980)

6. Sims P. and Grover P.L. - In: Polycyclic Hydrocarbons and Cancer, Vol. 3, Gelboin H.V. and Ts'o P.O.P. (Eds.), Academic Press, New York, pp. 117-181 (1981)

7. Habig W.H., Pabst M.J., Jakoby W.B. - J. Biol. Chem. 249: 7130 (1974)

8. Bend J.R., Ben-Zvi Z., van Anda J., Dansette P.M. and Jerina D.M. - In: Carcinogenesis, Vol. 1, Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis, Freudenthal R.I. and Jones P.W. (Eds.) Raven Press, New York, pp. 63-75 (1976)

9. Nemoto N. - In: Polycyclic Hydrocarbons and Cancer, Vol. 3, Gelboin H.V. and Ts'o P.O.P. (Eds.) Academic Press, New York, pp. 213-258 (1981)

10. Cobb D., Boehlert C., Lewis D. and Armstrong R.N. - Biochemistry 22: 805 (1983)

11. Vogel K., Bentley P., Platt K.L. and Oesch F. - J. Biol. Chem. 255: 9621 (1980)

12. Smithgall T.E., Harvey R.G. and Penning T.M. - J. Biol. Chem. 261: 6184 (1986)

13. Nemoto N. and Gelboin H.V. - Biochem. Pharmacol. 25: 1221 (1976)

14. Nemoto N., Takayama S. and Gelboin H.V. - Chem.-Biol. Interact. 23: 19 (1978)

15. Krishnan S., Kuhn D.G. and Hamilton G.A. - J. Am. Chem. Soc. 99: 8121 (1977)

16. Ishikawa K., Charles H.C. and Griffin G.W. - Tetrahedron Lett. 427 (1977)

17. Krishan S., Kuhn D.G. and Hamilton G.A. - Tetrahedron Lett. 1369 (1977)

18. Nagano T., Yokoohji K. and Hirobe M. - Tetrahedron Lett. 24: 3481 (1983)

19. Jeyaraman R. and Murray R.W. - J. Am. Chem. Soc. 106: 2462 (1984)

20. van Bladeren P.J. and Jerina D.M. - Tetrahedron Lett. 24: 4903 (1983)

21. Newman M.S. and Blum S. - J. Am. Chem. Soc. 86: 5598 (1964)

22. Fu P.P. and Harvey R.G. - J. Label. Comp. Radiopharm. 13: 619 (1977)

23. Dansette P. and Jerina D.M. - J. Am. Chem. Soc. 96: 1224 (1974)

24. Kolwyck K.C., Duncan W.P., Engel J.F. and Selkirk J.K. - J. Label. Comp. Radiopharm. 12: 153 (1976)

25. Goh S.H. and Harvey R.G. - J. Am. Chem. Soc. 95: 242 (1973)

26. Harvey R.G. and Fu P.P. - J. Label. Comp. Radiopharm. 12: 259 (1976)

27. Oesch F., Sparrow A.J. and Platt K.L. - J. Label. Comp. Radiopharm. 17: 93 (1980)

28. Platt K.L. and Oesch F. - Synthesis 459 (1982)

29. Platt K.L. and Oesch F. - Tetrahedron Lett. 23: 163 (1982)

30. Vyas K.P., van Bladeren P.J., Thakker D.R., Yagi H., Sayer J.M., Levin W. and Jerina D.M. - Mol. Pharmacol. 24: 115 (1983)

31. Thakker D.R., Levin W., Yagi H., Yeh H.J.C., Ryan D.E., Thomas P.E., Conney A.H. and Jerina D.M. - J. Biol. Chem. 261: 5404 (1986)

32. Thakker D.R., Levin W., Yagi H., Tada M., Ryan D.E., Thomas P.E., Conney A.H. and Jerina D.M. - J. Biol. Chem. 257: 5103 (1982)

33. Platt K.L. and Oesch F. - J. Org. Chem. 48: 265 (1983)

34. Hicks D.R. and Fraser-Reid B. - Synthesis 203 (1974)