RADIOACTIVELY LABELLED EPOXIDES PART II. (1) TRITIUM LABELLED CYCLOHEXENE OXIDE, TRANS-STILBENE OXIDE AND PHENANTHRENE 9,10-OXIDE

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SUMMARY

Tritium labelled cyclohexene oxide, trans-stilbene oxide and phenanthrene 9,10-oxide were prepared with specific activities of 0.7 - 1.1 mCi per mmole starting with mono- or diketo compounds.

Tritium was introduced by reducing the ketone precursors with tritiated complex metal hydrides.

The resulting alcohols were transformed to the epoxides by methods described for the unlabelled compounds.

The syntheses require only two or three steps and yield cyclohexene oxide, trans-stilbene oxide and phenanthrene 9,10-oxide, important substrates for the study of epoxide hydratase and glutathione S-transferases in high radiochemical purity.

Key Words: Alkene oxides, Arene oxides, Complex metal hydrides, Tritium

INTRODUCTION

Epoxides play a fundamental role in the metabolism of olefinic and aromatic compounds (2-10).

Due to their electrophilicity epoxides can bind covalently to biopolymers,

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e. g. DNA and proteins causing cytotoxic, mutagenic and cancerogenic effects (2-10).

It is therefore of vital interest to study the metabolic behaviour of epoxides towards the enzymes epoxide hydratase (3,4) and the glutathione S-transferases (11). Studies concerning this objective required the synthesis of tritium labelled cyclohexene oxide, trans-stilbene oxide and phenanthrene 9,10-oxide.

RESULTS AND DISCUSSION

A recently described synthesis of tritiated arene oxides (12) requires four steps in the case of phenanthrene 9,10-oxide-^{3}H and uses tritium gas to introduce the label.

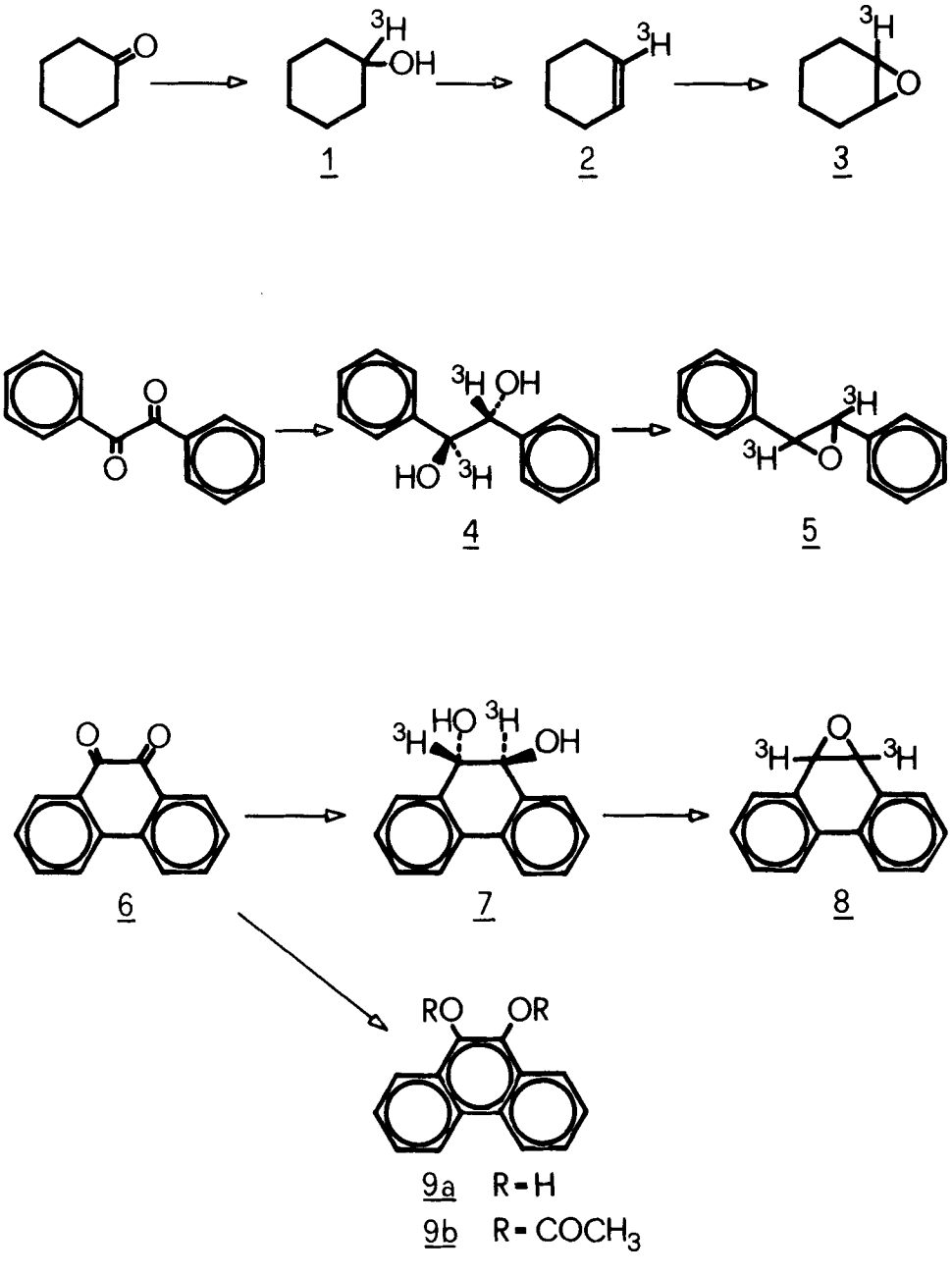
We used a more simple approach which required fewer steps. The key step involved the reduction of a keto function -CO- to a secondary alcohol -C^{3}HOH- by tritium labelled complex metal hydrides (KB^{3}H$_4$, LiAl^{3}H$_4$).

In the case of a monoketone reduction led to the labelled alcohol which was dehydrated to the olefin followed by epoxidation with m-chloroperbenzoic acid (Scheme).

In the case of 1,2-diketo compounds or K-region quinones the resulting trans-glycols were cyclized with N-tosylimidazole (13) to yield epoxides (Scheme).

Borohydride reductions are usually performed in methanol solution. It is well known (14) that borohydride reacts rapidly with this solvent, and it is generally assumed that a species such as $[BH(OCH_3)_3]^-$ is the active reducing agent. Clearly, methanol is an unsuitable solvent when maximum use of the tritium content of labelled borohydride is desired. For our purposes, experimentation showed that isopropanol was a suitable reaction medium. In this medium, reaction, of BH_4^- with solvent was negligible under the conditions required for reduction of the carbonyl compounds of interest, so that efficient and predictable incorporation of label was possible.

Some difficulty was experienced in isolating 2 in the actual preparation of the labelled compound, although no such problem was encountered in a pilot (unla-



Scheme

belled) experiment. The difference is attributable to a rather greater degree of contamination of 1 with isopropanol in the labelled preparation. In the end, 2 was obtained as a dilute solution in dichloromethane (with no other impurity), perfectly suitable for the epoxidation step 2 to 3.

In the reduction of benzil to 4, small amounts (< 5 %) of the threo isomer were detectable by n.m.r. (15), but this, together with similar amounts of benzoin, was easily removed by recrystallisation.

Since reports in the literature describe the use of potassium borohydride for the reduction of K-region quinones of polycyclic aromatic hydrocarbons to the corresponding trans-dihydrodiols (16,17,18) it was planned to introduce the label into K-region arene oxides in the same manner as described above for the alkene oxides.

But all attempts to reduce 6 to 7 with potassium borohydride in methanol or ethanol at 0-5° resulted in the formation of an extremely air-sensitive product that turned yellow upon recrystallization or chromatography, thereby forming almost quantitatively the starting quinone.

Acetylation of the air-sensitive compound gave 9b in very good yield, thus proving that the reduction of the K-region quinone 6 with potassium borohydride in methanol or ethanol does not lead to the dihydrodiol 7, but almost exclusively to the catechol 9a, thus providing a synthetic approach to these important derivatives of polycyclic aromatic hydrocarbons.

The same reduction type was observed recently (19) when sodium borohydride was used in polar aprotic solvents, e.g. N,N-dimethylformamide.

When lithium aluminum hydride in ether was used 6 could be reduced to the trans-dihydrodiol 7 as described in the literature (20).

7 is quite stable in the solid state at room temperature whereas slow oxidation to the quinone takes place in solution.

Therefore tritium was introduced into 7 with lithium aluminum hydride-^{3}H which possesses the slight disadvantage of being more expensive than potassium borohydride-^{3}H.

The method described should be generally useful for the radiosynthesis of both alkene and K-region arene oxides in few steps and high radiochemical yield.

EXPERIMENTAL

The ^{1}H-NMR spectra were recorded on a Varian EM 360 spectrometer. Melting points are uncorrected.

Cyclohexanol-1-^{3}H 1

1.0 g Potassium borohydride (finely crushed) and 54.4 mCi potassium borohydride-^{3}H (Amersham Buchler, Braunschweig, FRG) together with 7.5 g distilled cyclohexanone and 10 ml isopropanol was stirred in the dark for 65 hr and then poured into 50 ml water. The water was extracted with 4 x 50 ml dichloromethane. The combined organic phases were distilled. The fraction boiling at 85°/37 torr weighed 7.2 g and, from n.m.r. contained 85 % 1, the rest being almost exclusively dichloromethane and isopropanol. This was used for the next step.
Yield of 1 5.8 g (77 %).

Cyclohexene-1-^{3}H 2

7.2 g impure (85 wt-%) 1 was mixed with 2.9 g 85 % phosphoric acid and distilled using coldfinger condenser and cooling receiver in ice. Bath initially was at 120°, rising over 2 hr to 170°. The distillate was almost homogeneous so was diluted with dichloromethane and distilled from solid sodium sulphate using a short Vigreux column. Everything 40° and above was collected in several fractions. All contained cyclohexene in concentrations ranging from 1 to 3 %. Total yield of 2 was 2.2 g (46 %).

Cyclohexene oxide-1-^{3}H 3

The dilute solution of 2 (2.2 g) was cooled to 0° and 5.1 g 85 % m-chloroperbenzoic acid was added. The mixture was stirred 5 min at 0°, then allowed to warm to room temperature and stirred 1 hr. The suspension was filtered directly into 10 ml 2 % sodium hydroxide washing with dichloromethane. The phases were separated and the organic phase washed 2 x 80 ml water then dried over sodium carbonate and distilled. After redistillation (b.p. 48°/40 torr) the yield of 3 was 2.1 g (75 %) Specific activity: 0.65 mCi/mmole
n.m.r. (CCl_4): δ 1.0-2.1 (m, 8H), 3.0 (m, 2H).

erythro-1,2-Dihydroxy-1,2-diphenylethane-1,2-^{3}H 4

5.0 g Benzil (crystallised from tetrahydrofuran) and 0.65 g potassium borohydride (finely crushed) with 25.3 mCi potassium borohydride-^{3}H in 60 ml isopropanol was refluxed in dark for 15 hr. The suspension was then evaporated to dryness and the solid transferred to a separator with 100 ml water and 100 ml dichloromethane. The water was extracted twice more with dichloromethane. Evaporation of the combined organic phase produced crystallisation. Further product was obtained from the mother liquor by evaporation with methanol. On filtration, the crystals were washed with carbon tetrachloride until colourless.

Yield 3.2 g (63 %) m.p. 133° Lit. (15) 134°

n.m.r. ($CDCl_3$/$(CD_3)_2CO$): δ 4.8 (s, 2H), 7.2 (s, 10H)

trans-2,3-Diphenyloxirane-2,3-^{3}H 5

1.8 g 80 % Sodium hydride was washed three times with 50 ml petroleum benzene then suspended in 150 ml dry N,N-dimethylformamide. 3.2 g of 4 was then added and the mixture stirred for 2 hr. Then 3.55 g N-tosylimidazole (13) added and stirring continued for 1 hr. The mixture was then poured into 1.5 l ice water. After stirring over 2 days (to coagulate the precipitate) it was filtered, washed well with water and dried in air. Recrystallisation from benzene/methanol gave 1.43 g (48 %) 5. m.p. 69-71°. Lit. (15) 69°. A further 0.73 g (24 %) slightly impure material (65-70°) was also isolated. Specific activity: 1.02 mCi/mmole

n.m.r. (CCl_4): δ 3.7 (s, 2H), 7.3 (s,10H)

Phenanthrene 9,10-quinone 6

is commercially available. It was prepared for this study according to (21) by oxidation of phenanthrene (Schuchardt, Hohenbrunn, FRG) with iodic acid which gives 6 in 67 % yield.

trans-9,10-Dihydroxy-9,10-dihydrophenanthrene-9,10-^{3}H 7

2.08 g of 6 was placed in the thimble of a Soxhlet apparatus and continuously extracted into a round bottom flask with 200 ml of dry ether and 355 mg lithium aluminum hydride containing 25 mCi of lithium aluminum hydride -^{3}H (New England Nuclear, Boston, Mass., USA). The extraction was complete after 24 hr.

Water (30 ml) and 15 % sodium hydroxide (10 ml) was added dropwise with stirring to the cooled grey-greenish solution. The resulting slightly yellow suspension was filtered through a Büchner funnel and the residue washed with 3 x 100ml warm ether. The combined filtrates were evaporated to dryness and recrystallized from chloroform/methanol yielding 1.29 g (61 %) of 7 in fine white needles with a specific activity of 1.3 mCi/mmole. m.p. 186° Lit. (20) 185°.

Phenanthrene 9,10-oxide-9,10-^{3}H 8

To 288 mg of pentane-washed sodium hydride was added 50 ml of dry N,N-dimethylformamide and the grey suspension stirred under argon for 20 min at room temperature. 954 mg of 7 was added and stirring was continued for an additional 45 min. Then 1.10 g of N-tosylimidazol (13) was added to the red solution. After one more hour of stirring at room temperature the deep red solution was poured into 250 ml of ice water and the off-white precipitate isolated by filtration. Yield: 742 mg (85 %) of 8 with a specific activity of 1.1 mCi/mmole. m.p. 147°. Lit. (21) 148°.

n.m.r. ($CDCl_3$): δ 4,5 (s, 2H), 7.2-7.7 (m, 6H), 7.9-8.2 (m, 2H). 8 was pure according to TLC (silica gel, benzene + 10 % triethylamine) and n.m.r. but can be further purified by chromatography according to (22).

9,10-Dihydroxyphenanthrene 9a

4.2 g of 6 was suspended in 300 ml of dry ethanol and 4.3 g potassium borohydride was added in small portions under argon with stirring over 3 hr at room temperature. Stirring was continued for an additional 16 hr when an ice-cold mixture of 37 wt-% hydrochloric acid (30 ml) and water (70 ml) was added slowly to the yellowish suspension. The resulting solution was concentrated to 60 ml at 40° in vacuo and 300 ml ice-water were added. The precipitate was isolated by filtration yielding 3.8 g (90 %) of 9a as yellowish crystals.
m.p. 145° Lit. (23) 147-148°.

9,10-Diacetoxyphenanthrene 9b

Acetylation of 9a (3.2 g) with acetic anhydride/pyridine under argon at 5° yields after recrystallization from ethanol/chloroform 3.9 g (87 %) of 9b as white

powder.

m.p. 203° Lit. (19) 203°

n.m.r. ($CDCl_3$): δ 2.4 (s, 6H), 7.4-7.9 (m, 6H), 8.4-8.7 (m, 2H)

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REFERENCES

1. Part I: Platt K. L. and Oesch F. - J. Lab. Comp. Radiopharm. 13: 471 (1977).

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

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

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

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

6. Wiebel F. J., Selkirk J. K., Gelboin H. V., Haugen D. A., Van der Hoeven T. A. and Coon M. J. - Proc. Nat. Acad. Sci. USA 72: 3917 (1975).

7. Heidelberger C. - Ann. Rev. Biochem. 44: 79 (1975).

8. Nebert D. W., Robinson J. R., Niwa A., Kumaki K. and Poland A. P. - J. Cell Physiol. 85: 393 (1975).

9. Baird W. M., Harvey R. G. and Brookes P. - Cancer Res. 35: 54 (1975).

10. Oesch F., Bentley P. and Glatt H. R. - In: Biological Reactive Intermediates (Eds. Jollow D. J., Kocsis J. J., Snyder R. and Vainio H.) Plenum Press, New York, pp. 181-206, 1977.

11. Arias I. M. and Jakoby W. B. - Glutathione, Metabolism and Function. Kroc Foundation Series 6. Raven Press, New York, 1976.

12. Yagi H., Dansette P. and Jerina D. M. - J. Lab. Comp. Radiopharm. 7: 127 (1976).

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

14. Gaylord N. G. - Reduction with Complex Metal Hydrides. Interscience, New York, 1956.

15. Dansette P. M., Ziffer H. and Jerina D. M. - Tetrahedron 32: 2071 (1976).

16. Dey A. S. and Neumeyer J. L. - J. Med. Chem. 17: 1095 (1974).

17. Moriarty R. M., Dansette P. and Jerina D. M. - Tetrahedron Lett. 2557 (1975).

18. Lehr R., Taylor C. W., Kumar S., Mah H. D. and Jerina D. M. - J. Org. Chem. 43: 3462 (1978).

19. Cho H. and Harvey R. G. - J. C. S. Perkin 1 836 (1976).

20. Booth J., Boyland E. and Turner E. E. - J. Chem. Soc. 1188 (1950).

21. Williams A. G. - J. Am. Chem. Soc. 43: 1911 (1921).

22. Harvey R. G., Goh S. H. and Cortez C. - J. Am. Chem. Soc. 97: 3468 (1975).

23. Schmidt J. and Kämpf A. - Ber. 35: 3117 (1902).