Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 11

RADIOACTIVELY LABELLED EPOXIDES. PART VI.* TRITIUM-LABELLED MONO- AND DIMETHYL SUBSTITUTED PHENYL OXIRANES (STYRENE OXIDES)

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 (E)- and (Z)-2,3-dimethyl-2-phenyl oxirane 4, (E)- and (Z)-2-methyl-3-phenyl oxirane 7 and 2,2-dimethyl-3-phenyl oxirane 11 have been prepared by reduction of the corresponding bromoketones with sodium borotritide to the corresponding bromohydrins followed by cyclization to the oxiranes. These oxiranes were successfully used as diagnostic substrates to distinguish between different forms of epoxide hydrolase and glutathione transferase.

Key Words: Phenyl Oxiranes, Bromoketone, Bromohydrin, Sodium Borotritide

INTRODUCTION

Epoxides can be formed *in vivo* by the metabolic conversion of olefinic or aromatic precursors by mammalian monooxygenases (1-3). Due to their electrophilic reactivity, epoxides can exert cytotoxic, mutagenic and/or carcinogenic effects and thus be deleterious to living organisms. Therefore, enzymes that convert the electrophilic epoxides to less reactive compounds are of interest. Epoxides are hydrolyzed by epoxide hydrolases to vicinal dihydrodiols (3,4) or metabolized to glutathione conjugates as catalyzed by glutathione transferase

---

* Part V: Setiabudi, F., Oesch F. and Platt K.L. - J. Label. Compound. Radiopharm. 24: 979 (1987).

§ To whom correspondence should be addressed.

0362-4803/88/111209-09$05.00

Received November 6, 1987
Revised March 28, 1988

(5,6). These enzymes exist in different organs and in multiple forms. In order to distinguish between different forms of epoxide hydrolase and glutathione transferase, epoxides that are selectively converted by these isozymes are needed as diagnostic substrates.

Phenyl oxirane (styrene oxide) is converted rather unspecifically by various epoxide hydrolases (7) and glutathione transferases (8). A small steric alteration of phenyl oxirane by introduction of methyl groups in the 2- and/or 3-position of the oxirane ring changed the conversion by microsomal epoxide hydrolase drastically (9) and thus should provide the desired substrate specificity for different isozymes of epoxide hydrolase and of glutathione transferase. We, therefore, prepared five tritium-labelled mono- and dimethyl substituted phenyl oxiranes.

RESULTS AND DISCUSSION

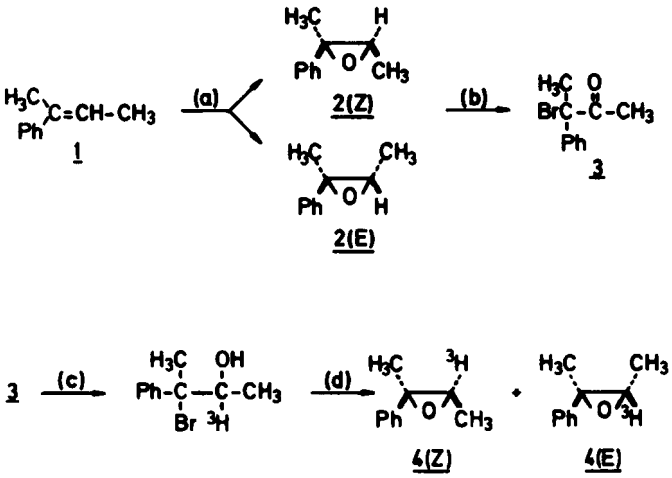
In an earlier report (10), we described the synthesis of tritium-labelled (Z)- and (E)-2-methyl-3-phenyl oxiranes using tritiated water as the source of tritium. It required a high amount of radioactivity (3.7 GBq) of tritiated water yet resulted in only low specific radioactivity (30 MBq/mmol).

In view of these disadvantages we have developed a versatile and convenient synthetic pathway preparing five tritium-labelled methyl substituted styrene oxides (cf. Scheme I, II, III).

Introduction of tritium with sodium borotritide appeared to be the method of choice considering its price and its convenience of handling.

Bromoketones generally react readily with sodium borohydride to yield bromohydrins which are in turn easily converted to the oxirane on treatment with base. There are a number of methods to prepare bromoketones. The photo-catalytic bromination (11) of 2,3-dimethyl-2-phenyl oxirane 2 nevertheless did not yield the desired product; however, the treatment of 2 (Scheme I) with bromodimethylsulfoniumbromide (12) gave 2-bromo-2-phenyl-butan-3-one 3, the key compound for the synthesis of (Z)-2,3-dimethyl-2-phenyl oxirane 4(Z) and (E)-2,3-dimethyl-2-phenyloxirane 4(E). Reduction of the bromoketone 3 with sodium borotritide

SCHEME I[a]



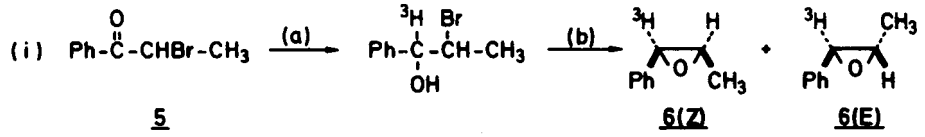
[a](a) m-CPBA , (b) bromodimethylsulfoniumbromide , (c) NaB^3H_4 , (d) NaOH

followed by treatment with base furnished a mixture of (Z)- and (E)-2,3-dimethyl-2-phenyl [3-^{3}H]oxiranes 4(Z) and 4(E) in 61 % overall yield and these could be separated by HPLC. The ratio of the diastereomers 4(Z)/4(E) was 55/45.

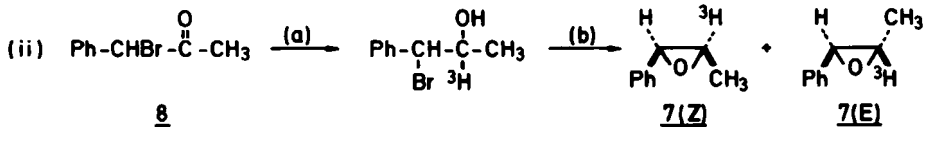
For the preparation of 2, 2-phenyl-2-butene 1 (13) was epoxidized with 3-chloroperoxybenzoic acid (mCPBA) (14) yielding the isomeric mixture of (Z)- and (E)-2,3-dimethyl-2-phenyl oxirane 2(Z) and 2(E), the precursor of the bromoketone 3. The epoxidation of (Z)-2-phenyl-2-butene 1(Z) (13) with m-CPBA (14) gave specifically (Z)-2,3-dimethyl-2-phenyl oxirane 2(Z) which was confirmed by ^{1}H-NMR. 2(Z) was required for assigning the structure of the tritium-labelled isomers 4(Z) and 4(E) by HPLC.

There are two different pathways for the preparation of tritium-labelled (Z)- and (E)-2-methyl-3-phenyl oxiranes 6 or 7 (Scheme II), (i); reduction of commercially available α-bromopropiophenone 5 with sodium borotritide to the bromohydrin and treatment of this with base afforded a mixture of (Z)- and (E)-2-methyl-3-phenyl [3-^{3}H]oxiranes 6(Z) and 6(E) in 65 % overall yield with the isomers 6(Z)/6(E) in a ratio of 90/10 and (ii); starting with the bromoketone

SCHEME II[a]



5



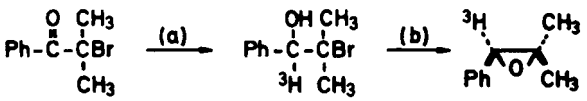
8

[a](a) NaB^3H_4 ,(b) NaOH

8 (15) and proceeding in essentially the same way as in (i), resulted in an isomeric mixture of (Z)- and (E)-2-methyl-3-phenyl [2-^{3}H]oxiranes 7(Z) and 7(E) in 93 % overall yield with the diastereomers 7(Z)/7(E) in a ratio of 60/40. Both diastereomers of 7 were separated by HPLC. Epoxidation of the commercially available (Z)- or (E)-1-phenyl-1-propene with mCPBA (14) yielded the corresponding unlabelled (Z)- or (E)-2-methyl-3-phenyl oxiranes which were required for assigning the structure of the tritium-labelled isomers isolated from the mixture of 6 and 7 by comparing their chromatographic properties on HPLC.

Reduction of α-bromoisobutyrophenone 10 with sodium borotritide followed by

SCHEME III[a]



10

[a](a) NaB^3H_4 ,(b) NaOH

treatment of the bromohydrin with base gave the tritium-labelled 2,2-dimethyl-3-phenyl [3-^{3}H]oxirane 11 in 81 % overall yield (Scheme III).

These five phenyl oxirane derivatives indeed showed distinct specificity toward different epoxide hydrolases and glutathione transferases. They were successfully used (i) to distinguish rat liver cytosolic form from the microsomal epoxide hydrolase (16) and (ii) as diagnostic substrates to differentiate glutathione transferases of the B- and C-group (17).

EXPERIMENTAL

3-Chloroperoxybenzoic acid (85 %) (mCPBA), α-bromopropiophenone, α-bromoisobutyrophenone and unlabelled sodium borohydride were supplied by Aldrich (Steinheim, FRG), poly-(4-vinylpyridine-hydrobromide-perbromide) resin by Fluka (Neu-Ulm, FRG).

Sodium boro[^{3}H]hydride was obtained in screw-cap bottles by New England Nuclear (Dreieich, FRG). The shipping container was used for performing the reaction.

Neutral alumina (type 90, activity II-III) for column chromatography was from Merck (Darmstadt, FRG).

Separation of the diastereomeric mixture 4 and 7 as well as determinations of the chemical and radiochemical purity of 4(Z) and 4(E), 7(Z) and 7(E) and 11 were carried out on a Spectra Physics SP 8700/ 8750 LC system, an LKB 2140 diode array detection unit and a silica gel column (Macherey & Nagel, Polygosil 60, 5 µm, 8 x 250 mm or 4 x 250 mm). The flow rate for the preparative separation was 3.2 mL/min and for the analytical purpose 0.8 mL/min.

For determination of radiochemical purities the total eluate of one chromatographic run was collected in 0.5 mL fractions, and these 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 CCl_4 on a Varian EM60 spectrometer at 60 MHz using tetramethylsilane as internal standard.

General method for the preparation of tritium labelled methyl substituted phenyl oxiranes:

To a solution of the corresponding bromoketone (i.e.: 2-bromo-2-phenyl-butan-3-one 3, α-bromopropiophenone 5, α-bromo-α-phenylacetone 8, α-bromoisobutyrophenone 10) in 2 mL absolute ethanol was added 0.5 mg sodium borohydride at 0 °C. After stirring for 8 min, the entire reaction mixture was transferred to a bottle containing sodium borohydride and sodium borotritide in required amounts. The mixture was stirred for 30 min at 0 °C, then 2 h at room temperature.

The resulting reaction mixture containing the bromohydrin was treated with 0.5 mL 1 N aqueous sodium hydroxide for 20 min at room temperature and a further 20 min at 50 °C, diluted with 10 mL water and extracted three times each with 10 mL n-hexane. The organic phase was washed with brine and dried over anhydrous magnesium sulfate. After evaporation of the solvent under reduced pressure the crude product was purified by column chromatography on neutral alumina eluting with n-hexane/ triethylamine (99.5:0.5, v/v). The (Z) and (E) diastereomers were separated by HPLC on silica gel; the details of the individual cases are described below.

2,3-Dimethyl-2-phenyl oxirane, 2:

To a stirred solution of 5.0 g (37.8 mmol) of 1-phenyl-1-butene 1 (13) in 125 mL dichloromethane and 400 mL sodium phosphate buffer of pH 8 was added 6.5 g (32 mmol) m-CPBA (85 %) in small portions over a 20 min-period at 0 °C. After stirring for 5 h at room temperature, 6.5 g (32 mmol) m-CPBA (85 %) was added in small portions at 0 °C over a second 20 min-period. The mixture was stirred at room temperature for 1.5 h and the organic layer was then separated, washed with saturated sodium thiosulfate then water and then dried over anhydrous magnesium sulfate. 2 was obtained after evaporation of the solvent at reduced pressure as a colourless oil (5.5 g, 98 %). The diastereomer 2(Z) was prepared in 89 % yield by the above method, starting with (Z)-1-phenyl-1-butene 1 (13). Purification by column chromatography on neutral alumina eluting with n-hexane/triethylamine (99.5:0.5, v/v) gave 2(Z) as a colourless oil: ^{1}H-NMR δ 0.85 (d, 3, 3-CH_3, J = 6 Hz), 1.50 (s, 3, 2-CH_3), 3.00 (q, 1, 3-H, J = 6 Hz), 7.10-7.25 (m, 5, arom. H).

2-Bromo-2-phenylbutan-3-one, 3:

To a stirred solution of 16.8 g (75.7 mmol) bromodimethylsulfoniumbromide (12) in 150 mL of dry dichloromethane at 0 °C was added 11.5 g (77.6 mmol) 2 in 75 mL dry dichloromethane. The reaction mixture was stirred at room temperature for 2 h and then cooled to 0 °C. A solution of 10.7 mL dry triethylamine in 37 mL dry dichloromethane was then added to the reaction mixture, the ice bath was removed and stirring continued for 20 min. The reaction mixture was diluted with 150 mL water, the organic layer was separated and the aqueous layer was extracted with dichloromethane (2 x 100 mL). The organic phase were combined and washed with water (2 x 200 mL) followed by 50 ml brine and dried over anhydrous magnesium sulfate. After removal of the solvent the crude product was purified by distillation, yielding 11.0 g (62 %) of a light yellow oil, b.p. of 80-83 °C/ 0.025 Torr. Column chromatography on neutral alumina eluting with n-hexane/dichloromethane (5:1, v/v) furnished a pure sample of 3: ^{1}H-NMR δ 2.05 (s, 3, CH_3), 2.20 (s, 3, CH_3), 7.2-7.5 (m, 5, arom. H).

(Z)-2,3-dimethyl-2-phenyl [3-^{3}H]oxirane, 4(Z):
(E)-2,3-dimethyl-2-phenyl [3-^{3}H]oxirane, 4(E):

These were prepared as an isomeric mixture in 61 % yield (63.8 mg) by the above general method, starting with 160 mg (0.71 mmol) 2-bromo-2-phenylbutan-3-one 3, 2.64 mg (0.07 mmol) sodium borotritide (specific activity 13.32 GBq/mmol) and 17.4 mg (0.46 mmol) sodium borohydride. Separation of the diastereomers by HPLC eluting with n-hexane/diethylether (99.5:0.5, v/v) showed the crude product as a 55/45 mixture of 4(Z) and 4(E). The first diastereomer to be eluted from the column was 4(Z) whose structure was assigned by co-chromatography with the authentic compound prepared by epoxidation of (Z)-2-phenyl-2-butene 1 (13) with m-CPBA (14). 4(Z) and 4(E) are colourless liquids with specific activities of 133 MBq/mmol and proved to be identical with the authentic unlabelled compound 2 as judged by HPLC and UV-spectroscopy. Radiochemical purity of 4(Z) was 99 % and of 4(E) 97 %.

(Z)-2-Methyl-3-phenyl [2-^{3}H]oxirane, 7(Z):
(E)-2-Methyl-3-phenyl [2-^{3}H]oxirane, 7(E):

These were prepared as an isomeric mixture in 93 % yield (100 mg) by the above general method, starting with 170 mg (0.8 mmol) α-bromo-α-phenylacetone 8 obtained from the bromination of phenylacetone with poly-(4-vinylpyridine-hydrobromide-perbromide) resin (15), 1.7 mg (0.045 mmol) sodium borotritide (specific activity 18.5 GBq/ mmol) and 14 mg (0.37 mmol) sodium borohydride. Separation of both diastereomers by HPLC eluting with n-hexane/dichloromethane (85:15, v/v) showed the crude product being a 60/40 mixture of 7(Z) and 7(E). The first diastereomer to be eluted from the column was 7(Z) whose structure was assigned by co-chromatography with the authentic compound prepared by epoxidation of the commercially available (Z)-1-phenyl-1-propene with m-CPBA (14). 7(Z) and 7(E) are colourless liquids with specific activities of 148 MBq/mmol and proved to be identical with the authentic unlabelled compound (14) as judged by HPLC and UV-spectroscopy. Radiochemical purity of 7(Z) was 99 % and of 7(E) 97 %. ^{1}H-NMR of unlabelled (Z)-2-methyl-3-phenyl oxirane: δ 1.00 (d, 3, 2-CH_3, J = 8 Hz), 3.15 (m, 1, 2-H), 3.90 (d, 1, 3-H, J = 4 Hz), 7.15-7.35 (m, 5, arom. H) and of (E)-2-methyl-3-phenyl oxirane: δ 1.35 (d, 3, 2-CH_3, J = 5 Hz), 2.85 (m, 1, 2-H), 3.40 (d, 1, 3-H, J = 1.8 Hz), 7.10-7.25 (m, 5, arom. H).

2,2-Dimethyl-3-phenyl [3-^{3}H]oxirane 11:

This was prepared in 81 % yield (52 mg) by the above general method, starting with 99 mg (0.436 mmol) α-bromoisobutyrophenone 10, 3.12 mg (0.082 mmol) sodium borotritide (specific activity 11.25 GBq/mmol) and 6.1 mg (0.16 mmol) sodium borohydride. Purification by column chromatography gave a colourless liquid with specific activity of 2.04 GBq/mmol and proved to be identical with the authentic unlabelled compound as judged by HPLC eluting with n-hexane/diethylether (99.5: 0.5, v/v) and UV-spectroscopy. Radiochemical purity of 11 was 99 %. ^{1}H-NMR of the unlabelled 2,2-dimethyl-3-phenyl oxirane: δ 1.00 (s, 3, 2-CH_3), 1.40 (s, 3, 2-CH_3), 3.73 (s, 1, 3-H), 7.2-7.3 (m, 5, arom. H).

ACKNOWLEDGEMENT

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 302). We wish to thank Mrs. I. Böhm for skillful help in the preparation of this manuscript.

REFERENCES

1. Sims P. and Grover P.L. - Adv. Cancer Res. 20: 165 (1974)
2. Jerina D.M. and Daly J.W. - Science 185: 573 (1974)
3. Oesch F. - Xenobiotica 3: 305 (1973)
4. Guengerich F.P. - Rev. Biochem. Toxicol. 4: 5 (1982)
5. Habig W.H., Pabst M.J. and Jakoby W.B. - J. Biol. Chem. 249: 7130 (1974)
6. Jerina D.M. and Bend J.R. - In: Biological Reactive Intermediates; Jollow D.J., Koscis J.J., Snyder R. and Vainio H. (Eds.) Plenum Press, New York p.p. 207-236 (1977)
7. Oesch F., Jerina D.M. and Daly J. - Biochim. Biophys. Acta 227: 685 (1971)
8. DePierre J.W. and Moron M.S. - Pharmacol. Res. Commun. 11: 421 (1979)
9. Oesch F., Kaubisch N., Jerina D.M. and Daly J.W. - Biochemistry 10: 4858 (1971)
10. Oesch F., Sparrow A.J. and Platt K.L. - J. Label. Compound. Radiopharm. 20: 1297 (1983)
11. Calo V., Lopez L. and Valentino D.S. - Synthesis 139 (1978)
12. Olah G.A., Vankar Y.D. and Arvanaghi M. - Tetrahedron Lett. 3653 (1979)
13. Adam W., Baeza J. and Liu J.C. - J. Am. Chem. Soc. 94: 2000 (1972)
14. Imuta M. and Ziffer H. - J. Org. Chem. 44: 1351 (1979)
15. Frechet J.M.J., Farrall M.J. and Nuyens L.J. - J. Macromol. Sci.-Chem. A11: 507 (1977)
16. Schladt L., Thomas H., Hartmann R. and Oesch F., Eur. J. Biochem., in press
17. Milbert U., Thomas H., Setiabudi F. and Oesch F., submitted to Biochem. J.