RADIOACTIVELY LABELLED EPOXIDES. PART IV.*
TRITIUM LABELLED α- AND ß-METHYL STYRENE OXIDES

Franz Oesch, Alan J. Sparrow, and Karl L. Platt
Institute of Pharmacology, Section on Biochemical Pharmacology,
University of Mainz, D-6500 Mainz, FRG.

SUMMARY

Tritium labelled α-methyl styrene oxide (2-methyl-2-phenyloxirane) and cis- and trans-ß-methyl styrene oxides (Z- and E-2-methyl-3-phenyl oxirane) have been prepared using tritiated water as the inexpensive source of tritium. The two geometrical isomers of ß-methyl styrene oxide were synthesized by a sequence of reactions which led to stereochemically pure products, and obviated any need to separate the isomers.

Key Words: Alkene oxides, Tritiated water, Stereospecific reactions

INTRODUCTION

Alkene and arene oxides can be enzymatically transformed to dihydrodiols by epoxide hydrolase (1). Epoxide hydrolase is localized in the endoplasmic reticulum where it seems to exist in multiple forms (2-4) as well as in the cytosolic and mitochondrial fraction of the cell (5-7). For the biochemical characterization of the various forms of epoxide hydrolase its specificity towards chemically closely related substrates, e.g. isomeric alkene oxides, is of special interest.

We, therefore, synthesized the three isomeric monomethyl styrene oxides radioactively labelled with tritium in the alkyl side chain using tritiated water as the inexpensive source of tritium.

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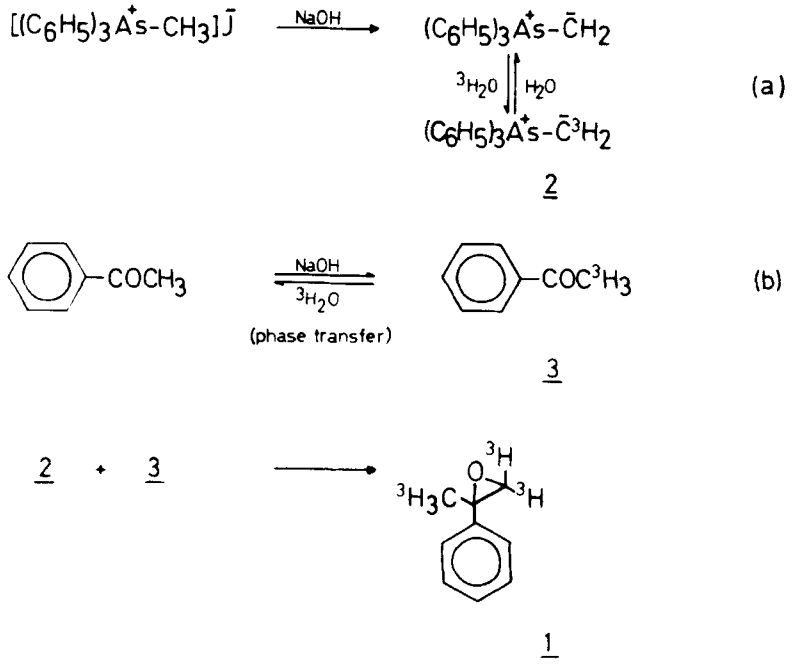
RESULTS AND DISCUSSION

Tritiated α-methyl styrene oxide (1) was prepared in one step from acetophenone and methyltriphenylarsonium iodide by the two-phase aqueous base-catalyzed formation of the ylide (2) and its reaction with the ketone (Scheme I).

SCHEME I



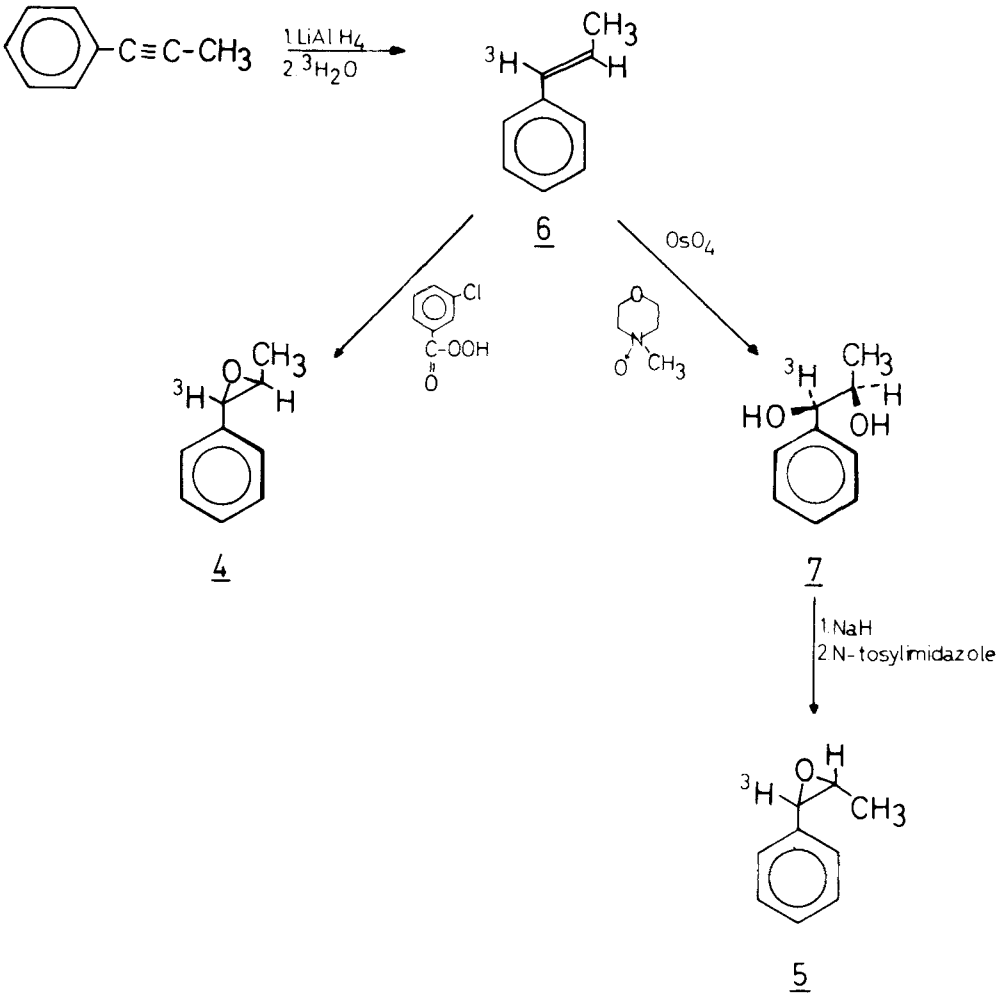
This procedure takes advantage of the liquid-liquid phase-transfer properties of the arsonium salt firstly to produce the ylide (Scheme I, a) (8) and secondly to bring about hydrogen exchange on the methyl group of acetophenone (Scheme I, b) (9). In addition, exchange of the hydrogens on the methylene group of the arsonium ylide also occurs (Scheme I, a), and the α-methyl styrene oxide (1) produced is labelled both on the oxirane ring and on the methyl group.

Utilizing the exchange of hydrogen atoms on a carbon atom in α-position to the hetero atom in different ylide species (phosphorus, sulfur, arsenic) in aqueous base, various labelled olefins or epoxides can be prepared by a one-step reaction, e.g. trans-stilbene oxide from benzyltriphenylarsonium bromide and benzaldehyde (10).

Unfortunately, this useful method of introducing tritium could not be applied to the stereospecific synthesis of the tritiated ß-methyl styrene oxides. The re-

action between benzyltriphenylarsonium bromide and acetaldehyde under conditions similar to those used for the preparation of 1 yielded a mixture of cis- and trans-ß-methyl styrene oxide (85 %) together with cis- and trans-ß-methyl styrene (15 %). For the synthesis of the two isomers of ß-methyl styrene oxide

SCHEME II



(4 and 5, Scheme II) the stereospecific hydrolysis (11) of the reaction product of methylphenyl acetylene and lithium aluminum hydride with tritiated water was used, which yielded [α-^{3}H] -trans-ß-methylstyrene (E-1-methyl-2-phenyl [2-^{3}H]-ethylene) 6 (Scheme II) as the common precursor of 4 and 5.

Epoxidation of 6 with m-chloroperbenzoic acid led to [α-^{3}H]-trans-ß-methyl

styrene oxide (E-3-methyl-2-phenyl [2-^{3}H] oxirane) 4. For the synthesis of the isomeric methyl styrene oxide 6 was cis-hydroxylated to 1,2-dihydroxy-1-phenyl-propane 7 using osmium(VIII)-oxide in catalytic amount and N-methylmorpholine N-oxide as the oxidant (12). Finally, 7 was stereospecifically cyclized to [α-^{3}H]-cis-ß-methyl styrene oxide (Z-3-methyl-2-phenyl [2-^{3}H] oxirane) 5 by treatment with excess sodium hydride and N-tosylimidazole in dry dimethyl-formamide (13,14). The present study demonstrates that tritiated water can be successfully used as the relatively inexpensive source of tritium for the synthesis of the three possible α- and ß-methyl styrene oxides in very good chemical yield and radiochemical purity. The radiosynthesis of the remaining three dimethyl and the trimethyl styrene oxides is under way in our laboratory.

EXPERIMENTAL

The ^{1}H-NMR spectra were recorded on a Varian EM 360 at 60 MHz in carbon tetrachloride using tetramethyl silane as the internal standard. The radiochemical purity of the methyl styrene oxides was determined by high-performance liquid chromatography with UV-detection using LiChrosorb RP18 (7 µm) as stationary and acetonitrile-water (80/20 v/v) as mobile phase at a flow rate of 0.8 ml/min; the total eluate was collected in 240 µl fractions, which were mixed with Unisolve I (Zinsser, Frankfurt,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.

[2- ^{3}H]methyl-2-phenyl [3-^{3}H]oxirane 1

580 mg (1.28 mmol) methyl triphenylarsonium iodide (15) and 130 µl (1.04 mmol) acetophenone in 6 ml dichloromethane are vigorously stirred with 400 mg sodium hydroxide in 400 µl water containing 100 mCi (3.7 GBq) [^{3}H]-water (spec. act. 90 mCi/mmol (3.3 GBq/mmol)). Progress of the reaction can be followed by ^{1}H-NMR spectroscopy on the supernatant organic phase. The reaction ceases after 10-14 days with about 20 % of the acetophenone still present. The aqueous phase is extracted twice with dichloromethane (2 x 4 ml), the combined organic phase is washed twice with water, dried ($MgSO_4$) and the solvent distilled off. The

unreacted acetophenone is transformed to 1 by treatment with trimethylsulfonium iodide (16) in dimethyl sulfoxide in the presence of sodium hydride. Distillation of the crude product yields 1 (118 mg, 85 %) as a colorless oil, b.p. 86 °C/18 torr; ^{1}H-NMR: δ 1.63 (s,3,methyl-H), 2.53 (d,1,ß-H), 2.77 (d,1,ß-H), 7.17 (s,5, arom.-H); radiochemical purity: 98 %; spec. act. 5.44 mCi/ mmol(0.20 GBq/-mmol).

E-2-Methyl-1-phenyl[1-^{3}H]ethylene 6

1.2 ml (10 mmol) methyl phenyl acetylene (17) and 800 mg (21 mmol) lithium aluminum hydride are heated in 40 ml dry tetrahydrofuran at 70 °C for 16 h. A 2 ml-sample is taken and added dropwise to 100 µl water in 1 ml tetrahydrofuran. The resulting mixture is diluted with 10 % aqueous potassium hydrogen sulfate and extracted with petroleum ether. The organic phase is washed with water, dried ($MgSO_4$) and evaporated to dryness. The remaining oil is checked by ^{1}H-NMR spectroscopy for completion of the reaction. If starting material is still present, further lithium aluminum hydride is added and heating resumed. When all the acetylene has been reduced a fraction of the solution is taken corresponding to 2 mmol trans-ß-methyl styrene and added dropwise to an ice- cold solution of 400 µl water containing 100 mCi (3.7 GBq) [^{3}H]-water (spec. act. 90 mCi/ mmol (3.3 GBq/mmol)) in 10 ml tetrahydrofuran. The resulting mixture is treated in the same way as were the samples which were taken during the reaction. After removal of the solvent 6 (217 mg, 92 %) is obtained as a yellowish oil; ^{1}H-NMR: δ 1.87 (d,3,methyl-H), 5.70-6.53 (m,2,α- and ß-H), 7.17 (br.s,5,arom.-H); spec. act. 0.81 mCi/mmol (30.0 MBq/mmol).

E-3-Methyl-2-phenyl [2-^{3}H]oxirane 4

160 µl (1.29 mmol) of 6 is added to an ice-cold solution of 223 mg (1.29 mmol) m-chloroperbenzoic acid in 10 ml dichloromethane. The mixture is then stirred allowing to warm to room-temperature overnight, then filtered into 2 % aqueous sodium hydroxide. After separation the organic phase is washed twice with water, dried ($MgSO_4$) and distilled. 4 (154 mg, 89 %) is obtained as a colorless oil, b.p. 97 °C/23 torr; ^{1}H-NMR: δ 1.38 (d,3,methyl-H), 2.65-2.98 (m,1,ß-H), 3.38 (d,1,α-H), 7.18 (s,5,arom.-H); radiochemical purity: 97 %; spec. act. 0.76 mCi/-mmol (28.1 MBq/mmol).

1,2-Dihydroxy-1-phenyl[1-^{3}H]propane 7

To a solution of 160 mg (1.37 mmol) N-methylmorpholine-N-oxide (12) in 1.2 ml of 50 vol.-% aqueous acetone is added 1.2 mg (0.005 mmol) osmium(VIII)-oxide in 120 µl tert.-butanol and 160 µl (1.29 mmol) 6 under nitrogen at 0 °C. The resulting mixture is stirred at room-temperature for 16 h by which time it is homogeneous. Then 160 mg magnesol and 16 mg sodium dithionite in 1 ml water are added. After stirring for 2 h, the suspension is filtered and the residue washed with methanol and ether. Then the filtrate is evaporated to dryness leaving an oil which is distributed between ether and 0.1 N HCl saturated with sodium chloride. The aqueous phase is extracted twice with ether, the combined organic phase dried ($MgSO_4$) and evaporated to an oil. The oil is chromatographed on silica gel (75 x 1 cm), eluting with dichloromethane-methanol (19 : 1). The fractions containing 7 are combined, freed from methanol by coevaporation with benzene and used directly for the transformation to 5.

Z-3-Methyl-2-phenyl[2-^{3}H]oxirane 5

120 mg (4 mmol) 80 % sodium hydride in mineral oil is washed twice with pentane, then suspended in 10 ml dry dimethylformamide under nitrogen. The total yield of 7 is added and the mixture is stirred for 1 h. Then 260 mg (1.17 mmol) N-tosyl-imidazole (13) is added and the mixture is stirred overnight. The suspension is diluted with ether and treated cautiously with water to destroy unreacted sodium hydride. The aqueous phase is extracted with ether, then the combined organic phase is washed four times with water, dried ($MgSO_4$) and distilled yielding 5 (107 mg, 62 % from 6) as a colorless oil, b.p. 89 °C/21 torr; ^{1}H-NMR: δ 1.01 (d,3,methyl-H), 2.95-3.34 (m,1,ß-H), 3.86 (d,1,α-H), 7.20 (s,5,arom.-H); radiochemical purity: 98 %; spec. act. 0.80 mCi/mmol (29.6 MBq/mmol).

ACKNOWLEDGEMENT

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