THE SYNTHESIS AND CHEMISTRY OF [11-^{14}C]-DIBENZ[b,f][1,4]OXAZEPINE

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SUMMARY

The synthesis of [11-^{14}C]-dibenz[b,f][1,4]oxazepine 1 is described. Chromium trioxide - pyridine oxidation of 1 gives [11-^{14}C]-10,11-dihydrodibenz[b,f][1,4]oxazepin-11-one 3 which on hydrolysis gives 2-amino-2'-[^{14}C]-carboxydiphenylether 4. Hydrogenation of 1 affords [11-^{14}C]-10,11-dihydrodibenz[b,f][1,4]oxazepine 5. The enzymic conversion of 1 into 2-amino-2'-[^{14}C]-hydroxymethyldiphenyl ether 6 is described.

Key Words: Carbon-14, Synthesis, Dibenz[b,f][1,4]oxazepine, Oxidation, Reduction, Hydrolysis

INTRODUCTION

Dibenz[b,f][1,4]oxazepine 1 is a potent sensory irritant whose biological evaluation has been the subject of several recent reports (1 - 5). For use in metabolic studies, the synthesis of 1 radiolabelled with carbon-14 was undertaken. This paper describes the synthesis of [^{14}C] - 1 and its use as a precursor in the preparation of several related radiolabelled compounds (3 - 6; Scheme 1).

For practical and economic reasons, the label was introduced into 1 at C-11 (the azomethine) using with only slight modification, a procedure published elsewhere (6). 2-Aminodiphenylether was treated with [^{14}C]-formic acid for 2 h at 80° to give after column chromatography, [^{14}C]-2-formamidodiphenylether 2, which on ring-closure with polyphosphoric acid (Bischler-Napieralski reaction) gave in good yield, [11-^{14}C]-dibenz[b,f][1,4]oxazepine 1.

Results (7) from both *in vivo* and *in vitro* metabolic studies of 1 indicate that compounds 3 - 6 are probable primary metabolites leading to the observed urinary metabolites. To allow the evaluation of these compounds *in vivo*, their synthesis from [^{14}C] - 1 was undertaken. Oxidation of

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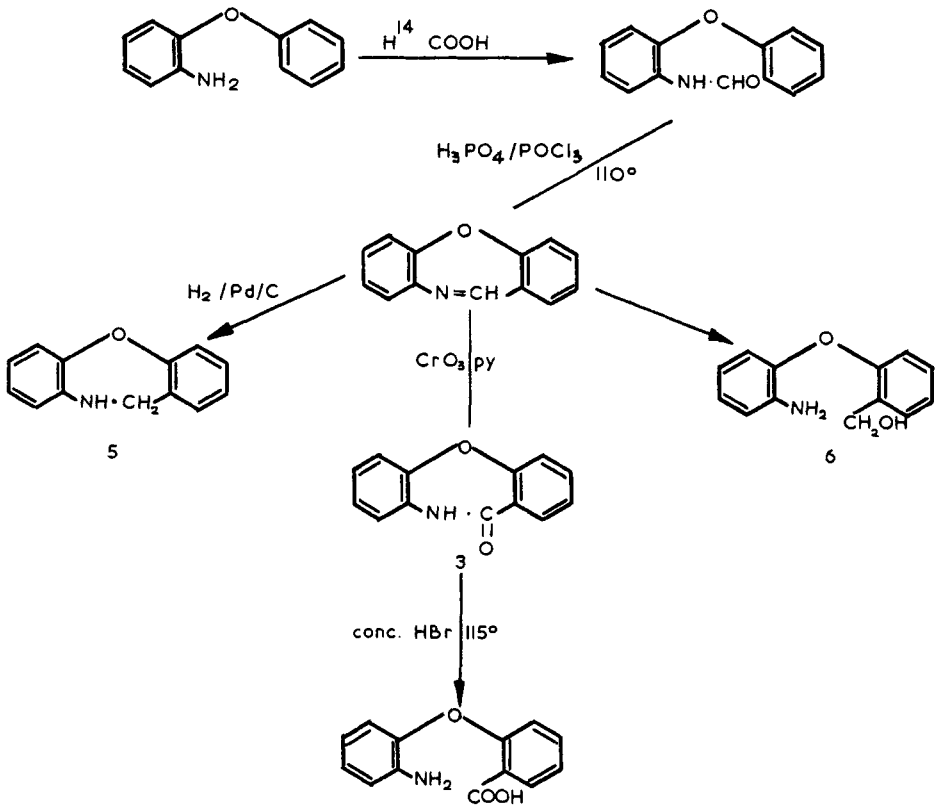
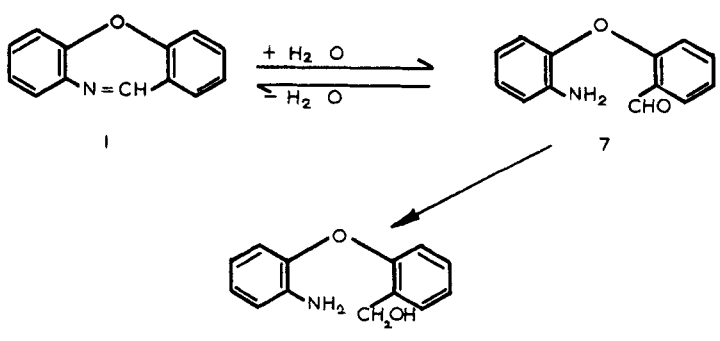


5 6 3 4

Scheme 1



1 7 6

[^{14}C] - 1 using a large excess of chromium trioxide in pyridine gave the lactam, [11-^{14}C]-10,11-dihydrodibenz[b,f][1,4]oxazepin-11-one 3. Conversion of [^{14}C] - 3 into the aminoacid 4 was achieved using concentrated hydrobromic acid at 110 - 120°. Attempts to assay the purity of 4 by autoradiography were unsatisfactory. However, an inverse isotope dilution analysis using pure (by full spectroscopic analysis) unlabelled 4 showed the purity of [^{14}C] - 4 to be in excess of 95%. It is of note that the usual methods of effecting the hydrolysis of amides were unsuccessful. The lactam 3 was stable to both concentrated acids and alkalis when heated under reflux for prolonged periods. Catalytic hydrogenation of [^{14}C] - 1 gave the dihydro-derivative [^{14}C] - 5 in good yield.

Although the chemical conversion of 1 into the amino alcohol 6 by chemical means via the reduction of 4 is clearly possible, it was found to be most convenient to carry out this transformation enzymically using the 10,000 x g supernatant fraction prepared from rat liver homogenate. The enzyme(s) requires NAD or NADP for its activity and is not inhibited by iodoacetate. The conversion of 1 to 6 occurs in good yield and is complete in 30 to 45 minutes. This is an interesting transformation and may result from reduction of the aldehyde moiety of the intermediate amino aldehyde 7 (Scheme 2). Attempts to prepare 7 by chemical methods (6) result in the isolation of 1 only due to spontaneous dehydration and ring closure. Under the aqueous conditions of the liver preparation, a small equilibrium concentration of 7 resulting from enzymic or spontaneous hydrolysis of 1 may allow the transformation to proceed. No stable intermediates have been detected.

EXPERIMENTAL

Except where specified, conventional experimental procedures were used throughout. T.l.c. was performed by upward irrigation on microscope slides coated with Merck silica gel G and column chromatography with Merck silica gel of particle size 0.05 - 0.2 mm in the same solvent as used for t.l.c. Autoradiograms were obtained from thin-layer chromatograms using Kodirex (Kodak) X-ray film.

The identity and homogeneity of labelled compounds was established by full spectroscopic analysis (i.r., n.m.r., m.s. and u.v.) of products obtained under comparable experimental conditions using unlabelled reactants and were homogeneous by t.l.c. in several solvent systems. Radiochemical homogeneity was established by autoradiography.

Scintillation counting was carried out on a Packard Model 3375 Liquid Scintillation Counter fitted with automatic external standardisation.

[2-^{14}C]-2-formamidodiphenylether 2 - 2-Aminodiphenylether (6) (1.0 g, 5.3 mM) and [^{14}C]-formic acid (10 mCi) diluted with cold formic acid (0.126 g, 2.6 mM) were heated at 80 - 90° for 2 h. Chromatography (petrol/ether, 1:1) to remove excess amine (R_f 0.6) gave the formamido compound 2 (R_f 0.3) as a brown crystalline solid (0.50 g, 90% based on formic acid).

[11-^{14}C]-dibenz[b,f][1,4]oxazepine 1 - The formamido compound 2 (0.35 g, 1.64 mM) was treated with a mixture of polyphosphoric acid (s.g. 1.85, 7.2 g) and phosphoryl chloride (2.3 g) for 2.5 h at 110°. The reaction flask was swirled occasionally during the first hour to ensure mixing of the reactants. After neutralisation of the reaction mixture with aqueous ammonia (s.g. 0.88), conventional processing and chromatography (petrol/ether, 2;1) gave [11-^{14}C] - 1 (R_f 0.35) as a pale yellow solid (0.40 g, 87%). The specific activity was 2.85 mCi/mM. The overall yield from [^{14}C]-H·COOH was 79%.

[11-^{14}C]-10,11-dihydrodibenz[b,f][1,4]oxazepine 5 - [^{14}C] - 1 (4.6 mg) diluted with unlabelled 1 (25 mg) was hydrogenated over palladium/charcoal in ethanol (10 ml) at room temperature and pressure for 3.5 h. Chromatography (benzene) gave [11-^{14}C] - 5 (R_f 0.45) as an off-white crystalline solid (26 mg, 86%). The specific activity was 0.41 mCi/mM.

[11-^{14}C]-10,11-dihydrodibenz[b,f][1,4]oxazepin-11-one 3 - [11-^{14}C] - 1 (4.6 mg) with chromium trioxide (1 g) in pyridine solution (13 ml) was stirred overnight at room temperature and then heated at 80°

for 25 min. The reaction mixture was passed directly down a short silica column (to remove chromium salts) eluting with ethyl acetate. The eluate was concentrated to dryness and the residue chromatographed (benzene/ethyl acetate, 9:1) to give [11-^{14}C] - 3 (R_f 0.33) as a white solid (80% by scintillation counting).

2-Amino-2 -[^{14}C]-carboxydiphenylether 4 - [11-^{14}C] - 3 (5 mg), diluted with unlabelled 3 (5 mg) was heated with stirring with conc. hydrobromic acid (2 ml) at 115° for 1.5 h and stripped to dryness in vacuo to give the crude hydrobromide of 4. Chromatography eluting initially with chloroform/methanol, 9:1 to remove non-polar impurities and finally with the same eluant containing 0.25% formic acid gave the free aminoacid 4. Further purification was achieved by rechromatography (chloroform/methanol, 8:2) to give 4 (R_f 0.1 - 0.4) as a white solid (8 mg, 75%). On t.l.c., 4 did not run discretely but gave an elongated spot of variable R_f dependent on plate loading and consequently was not a good criterion of purity. An inverse isotope dilution analysis with unlabelled 4, pure by spectroscopic assay, indicated that the radiochemical purity of [^{14}C] - 4 was in excess of 95%. The specific activity was 1.32 mCi/mM.

2-Amino[2-^{14}C]-hydroxymethyldiphenylether 6 - To each of ten flasks containing NADP (10 μmole), glucose-6-phosphate (20 μmole), magnesium chloride (100 μmole), D-glucose-6-phosphate: NADP oxidoreductase (EC 1.1.1.49, 4 I.U.) in 0.1M phosphate buffer at pH 7.4 (10 ml) and 1 ml of a freshly prepared 10,000 x g supernatant fraction from a Porton strain, rat liver 20% homogenate in 0.25M sucrose was added [^{14}C] - 1 (10 μmole, sp.act. 0.29 mCi/mmole: total added, 19.5 mg). The mixture was incubated at 37°C for 45 min under an oxygen atmosphere. The incubates were combined and extracted with chloroform (x 3). Evaporation and chromatography (chloroform) of the extract gave [^{14}C] - 6 as a yellow solid (14.1 mg, 72%).

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