SYNTHESIS AND SPECTROSCOPIC CHARACTERISATION OF 3-CHLOROPERBENZOIC ACID-^{17}O,^{18}O, NITROSOBENZENE-^{17}O,^{18}O AND NITROSOBENZENE-^{15}N

Christine Bleasdale,[a*] Martin K. Ellis,[b] Peter B. Farmer,[c] Bernard T. Golding,[a*] Kevin F. Handley,[a] Peter Jones,[a] and William McFarlane[a]

a *Department of Chemistry, Bedson Building, The University, Newcastle upon Tyne NE1 7RU.*

b *ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ.*

c *MRC Toxicology Unit, Hodgkin Building, University of Leicester, P.O. Box 138, Lancaster Road, Leicester LE1 9HN*

* Address correspondence to this author.

Key words: Nitrosobenzene, 3-chloroperbenzoic acid, ^{17}O-labelling, ^{15}N-labelling.

Summary: 3-Chloroperbenzoic acid-^{17}O,^{18}O was prepared from 3-chlorobenzoyl chloride and hydrogen peroxide-^{17}O,^{18}O (obtained by subjecting water-^{17}O,^{18}O to an electric discharge) and used to oxidise aniline to nitrosobenzene-^{17}O,^{18}O. Nitrobenzene-^{15}N was reduced to aniline-^{15}N, which was oxidised by 3-chloroperbenzoic acid to nitrosobenzene-^{15}N. ^{15}N and ^{17}O NMR data are given for the corresponding labelled nitrosobenzene.

1 INTRODUCTION

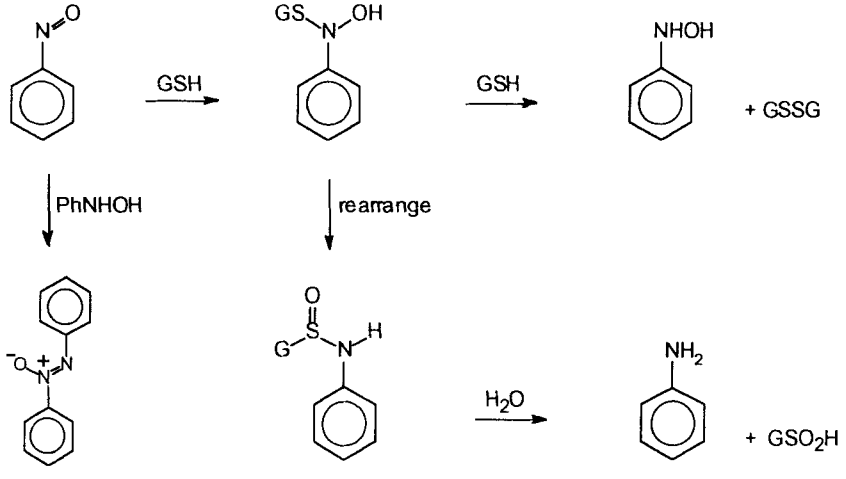
Nitroso compounds are important metabolites of nitro compounds and are implicated in the toxicity of arylnitro compounds (1). Arylnitroso compounds react with thiol nucleophiles and this has been demonstrated to occur intracellularly for nitrosobenzene, which is reactive towards cysteine residues of hemoglobin and towards glutathione (2). The initial step in the reaction of an arylnitroso

0362–4803/93/080739–08$09.00

Received 9 March, 1993
Revised 28 March, 1993

compound with a thiol is the formation of a 'semimercaptal', which either rearranges to a sulphinanilide or is attacked by a second molecule of thiol to yield a disulphide and arylhydroxylamine (see Scheme 1); the sulphinanilide is hydrolysed to a sulphinic acid and arylamine (3).

Scheme 1: Alternative pathways for the reaction of nitrosobenzene with glutathione.



The definition of reaction pathways and mechanisms of key steps for arylnitroso/thiol systems is hampered by the difficulty of using NMR for monitoring reactions of functional groups that obviously lack ^{1}H and ^{13}C (*ie* in non-exchangeable form for ^{1}H in *eg* an arylamine), whilst possessing nitrogen and oxygen nuclei at natural abundance. We therefore sought to devise efficient syntheses of arylnitroso compounds enriched with ^{15}N and/or ^{17}O because this should enable reactions with substrate concentrations in the millimolar range to be monitored by NMR (for a recent study showing the power of this approach with ^{15}N see ref. 4; for reviews of ^{15}N and ^{17}O NMR see ref. 5).

In this paper we describe syntheses of nitrosobenzene-^{15}N and nitrosobenzene-$^{17}O,^{18}O$ by methodology that, in principle, can be applied to a wide range of arylnitroso compounds. Furthermore, the precursor of nitrosobenzene-$^{17}O,^{18}O$ is 3-chloroperbenzoic acid-$^{17}O,^{18}O$, which could also be used to prepare ^{17}O-labelled oxiranes for mechanistic studies. The application of ^{17}O NMR to monitoring oxygen chemistry is well-known (6), but requires efficient methods for

incorporating ^{17}O into organic molecules (7). The results reported in this paper should significantly extend the range of possible investigations.

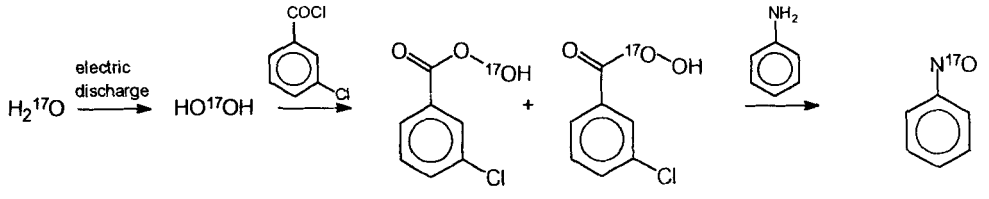
2 RESULTS AND DISCUSSION

2.1 Preparation of 3-chloroperbenzoic acid-$^{17}O,^{18}O$ and nitrosobenzene-$^{17}O,^{18}O$

An exchange reaction between nitronium tetrafluoroborate and water-^{18}O, and its application to the preparation of nitrobenzene-^{18}O has been reported (8). However, we considered this method unsuitable for adaptation to the preparation of nitrosobenzene-^{17}O, because of the inevitable inefficient utilisation of valuable ^{17}O in an exchange process, and the need for a two-step conversion of the initially obtained nitrobenzene-^{17}O into nitrosobenzene-^{17}O. We have therefore revived the little known procedure of D'Ans and his coworkers (9) for preparing arylnitroso compounds by direct oxidation of the corresponding arylamine with a peracid (NB D'Ans used peracetic acid). Using HPLC to monitor the reaction enables over-oxidation of the nitrosobenzene to nitrobenzene to be minimised. In this way the incorporation of ^{17}O can be achieved by oxidising aniline with 3-chloroperbenzoic acid-$^{17}O,^{18}O$, which we have prepared from 3-chlorobenzoyl chloride and hydrogen peroxide-$^{17}O,^{18}O$. The latter is readily available by subjecting water-$^{17}O,^{18}O$ (containing 20 atom % ^{17}O, and 30 atom % ^{18}O) to an electric discharge, in the manner described for preparing hydrogen peroxide-^{18}O from water-^{18}O (10-12).

Although half the ^{17}O in the peracid is lost to 3-chlorobenzoic acid during the oxidation of aniline, the percentage of ^{17}O in the nitrosobenzene-$^{17}O,^{18}O$ obtained is necessarily the same as that in the water-$^{17}O,^{18}O$, and so the procedure described (summarised in Scheme 2) is superior to any exchange process. Hydrogen peroxide-^{18}O has also been prepared by hydrolysis of potassium hydroperoxide-^{18}O obtained by base-catalysed oxidation of diphenylmethanol with dioxygen-^{18}O, and was used for the synthesis of peroxyformic acid-^{18}O (13).

Scheme 2: Synthetic route to nitrosobenzene-$^{17}O,^{18}O$ (NB the labelled water used, shown as $H_2{}^{17}O$ below, contained 20% ^{17}O and 30% ^{18}O).



2.2 Preparation of nitrosobenzene-^{15}N

We have also used the method of D'Ans (9) to advantage in the preparation of nitrosobenzene-^{15}N. In this case the efficiency problem to solve is to maximise utilisation of nitric acid-^{15}N. This was achieved by nitration (14) of an excess of benzene using nitric acid-^{15}N (97 atom % ^{15}N), to give nitrobenzene-^{15}N in 98% yield. Reduction (15) of the nitrobenzene-^{15}N gave aniline-^{15}N which was oxidised by unlabelled 3-chlorobenzoic acid to give nitrosobenzene-^{15}N in 32% overall yield from nitric acid-^{15}N.

2.3 Spectroscopic characterisation

The isotopic contents of the 3-chloroperbenzoic acid-^{17}O,^{18}O and nitrosobenzene-^{17}O,^{18}O prepared were found to be *ca* 50% ^{16}O, 20% ^{17}O and 30% ^{18}O by electron impact mass spectrometry, which reflects exactly the isotopic content of the water used. The ^{17}O NMR resonance for nitrosobenzene has been previously reported (16) as δ 680 ppm. We found the resonance for nitrosobenzene-^{17}O at the remarkable high frequency position of δ 1534 ppm (solvent deuteriochloroform, external reference water), and confirmed this value by a measurement on a concentrated solution of unlabelled nitrosobenzene in deuteriochloroform. On addition of a solution of the nitrosobenzene-^{17}O,^{18}O in CD_3CN to glutathione in buffered D_2O (pH 7.1) the resonance at δ 1534 ppm disappeared immediately, and was replaced by a resonance at δ 85 ppm, which is assigned to phenylhydroxylamine-^{17}O. It is known that under these conditions the principal reaction pathway from reacting nitrosobenzene with glutathione is the formation of oxidised glutathione and phenylhydroxylamine *via* a semimercaptal intermediate (see Scheme 1) (2,3).

The nitrosobenzene-^{15}N showed a ^{15}N NMR resonance at δ 915 ppm (in deuteriochloroform, relative to external nitromethane) in agreement with that reported for unlabelled nitrosobenzene (17). Both nitrobenzene-^{15}N and nitrosobenzene-^{15}N showed ^1J and longer range couplings to the phenyl carbons and protons (for details see the Experimental Section).

The labelled nitrosobenzenes described are being used to study the detailed mechanisms of reactions of nitrosobenzenes with amine and thiol nucleophiles.

3 EXPERIMENTAL

NB All of the following procedures were tested with unlabelled compounds. Yields at least as good as those given below were obtained and all organic compounds were authenticated by

spectroscopic and combustion analysis. NMR spectra were measured with a Bruker AMX500 instrument operating at frequencies of 125.76 (^{13}C), 50.70 (^{15}N) and 67.80 MHz (^{17}O). Mass spectra were acquired by direct insertion of samples into a VG 70-SEQ instrument, using electron impact ionisation (70 eV).

3.1 Preparation of hydrogen peroxide-$^{17}O,^{18}O$

Water containing 20 atom % ^{17}O (Cambridge Isotope Laboratories) was passed through an electrical discharge and the resulting hydrogen peroxide was collected in two cooled traps (liquid nitrogen). The discharge apparatus was a modification of that described (12) and consisted of an inverted Pyrex U-tube (i.d. 28 mm), with two aluminium electrodes (2 mm thick) supported by tungsten rods. The electrodes were positioned at opposite ends of the tube (2 m apart). Water vapour was drawn into the tube from a thermostatted reservoir (15 ^{o}C), through a narrow capillary at one end of the tube, by applying a vacuum at the other end (approximately 0.1 mmHg). The trapping points were positioned 60 cm and 170 cm downstream from the feed. The apparatus was composed of a number of cone and socket jointed pieces, for ease of cleaning and storage. The tube was comprised of three pieces with detachable electrodes, each with B24 joints, the traps with B19 joints and the inlet system a pair of B10 joints. Apiezon L grease was used to lubricate all joints except those at the top of the inverted U-tube, which become very hot within the discharge, and were greased with Apiezon T. The discharge tube was connected in series with a transformer rated as 1850 V/350 mA, a 5 kΩ, 200 W load limiting resistor and an ammeter. The secondary current was approximately 100 mA. The feed rate was 500 mg of water in 140 min.

Yields of hydrogen peroxide were optimised by conditioning the tube by discharge experiments with unlabelled water. The hydrogen peroxide collected was diluted to 100 cm^3 with water and to a 30 cm^3 aliquot was added 6 cm^3 of 20% v/v aqueous sulphuric acid. Hydrogen peroxide in the resulting solution was titrated against 0.05 M cerium(IV) sulphate with 1,10-phenanthroline-ferrous sulphate as indicator (18). Under optimum conditions hydrogen peroxide was obtained in 39 % yield.

3.2 Preparation of 3-chloroperbenzoic acid-$^{17}O,^{18}O$

NB This preparation is based on that described for unlabelled 3-chloroperbenzoic acid (19).

A beaker was charged with anhydrous magnesium sulphate (8.5 mg), sodium hydroxide (0.45 g), water (4.5 ml), hydrogen peroxide-$^{17}O,^{18}O$ (1 ml, *ca* 10 mmol H_2O_2) and 1,4-dioxane (5.4 ml). The

stirred mixture was cooled to 15 °C and 3-chlorobenzoyl chloride (0.63 g, 3.6 mmol, freshly prepared from 3-chlorobenzoic acid using thionyl chloride: b.p. 103-104 °C at 14 mm Hg) was added in one portion, maintaining the temperature below 25 °C. After 15 minutes 20% v/v H_2SO_4 (10.8 ml) was added and the product was extracted into dichloromethane (3 x 10 ml). The combined extracts were dried ($MgSO_4$) and the organic solvents were removed *in vacuo*, leaving crude 3-chloroperbenzoic acid-^{17}O,^{18}O (620 mg, 88% pure by iodometric titration): *m/z* (positive ion electron impact, ions above *m/z* 110 and with intensity > 10% of base peak given) 160 (11), 159 (10), 158 (42), 157 (21), 156 (42), 141 (51), 140 (20), 139 (100%), 113 (21), 111 (68).

3.3 Preparation of nitrosobenzene-^{17}O,^{18}O

Crude 3-chloroperbenzoic acid-^{17}O,^{18}O (120.5 mg, 0.61 mmol) in dichloromethane (2 ml) was added to redistilled aniline (27.8 mg, 0.3 mmol), with vigorous stirring, at 0 °C. After 1 h, sodium carbonate (7.5 mg, 0.71 mmol) in water (10 ml) was added, the mixture was allowed to warm to ambient temperature and stirred for a further 5 min. The organic layer was separated, washed with saturated brine (10 ml), dried ($MgSO_4$), and concentrated. The residual solid was recrystallised thrice from ethanol (degassed by nitrogen sparging) to give sand-coloured crystals of nitrosobenzene-^{17}O,^{18}O (13.8 mg, 43%), m.p. 67 °C: δ_O ($CDCl_3$, external reference H_2O) 1534 ppm; *m/z* (positive ion electron impact, ions with intensity > 10% of base peak given) 109 (26), 108 (18), 107 (38), 77 (100%), 51 (60), 50 (15).

NB To monitor the above reaction and to establish product purity HPLC was performed (using a Gilson gradient system with UV detector operating at 254 nm) with a Hichrom C_{18} 10μ bonded reverse phase column (25 cm x 4.6 mm i.d.). A gradient was run from 80% A/20% B to 100% B over 20 min (where A is water and B is 80% aq methanol) with a total flow rate of 2 ml min^{-1}.

3.4 Preparation of nitrobenzene-^{15}N

Nitric acid-^{15}N (42.9% w/w, 1.53 g, 10.3 mmol HNO_3, 98 atom % ^{15}N) was added to a mixture of benzene (10.5 ml), sulphuric acid (98% w/v, 7.4 ml) and water (2.7 ml). After 3 h, the mixture was diluted with water and neutralised by the gradual addition of a solution of sodium hydroxide (5.94 g, 0.149 mol) in water (20 ml) with cooling. The product was extracted into ether, dried ($MgSO_4$) and the solvent removed to give nitrobenzene-^{15}N (1.26 g, 98%), pure by HPLC: δ_N ($CDCl_3$, relative to external $MeNO_2$) - 9.7 ppm [^1J (^{15}N-^{13}C) 14.6, ^2J (^{15}N-^{13}C) 2.5, ^3J (^{15}N-^{13}C) 1.8, ^3J (^{15}N-H) 1.9 Hz, ^4J (^{15}N-H) 0.8 Hz].

3.5 Preparation of aniline-^{15}N

Conc hydrochloric acid (2.5 ml) was added to a mixture of nitrobenzene-^{15}N (0.599 g, 0.483 mmol) and granulated tin (1.12 g, 9.4 mmol). After the initial reaction had subsided the reaction mixture was heated (100 ^{o}C) for 90 min, (at which time the smell of nitrobenzene was no longer perceptible). The mixture was allowed to cool, neutralised by the addition of sodium hydroxide (1.65 g, 41.3 mmol) in water (2.8 ml) and steam distilled. The distillate was saturated with sodium chloride and extracted with ether. The combined extracts were dried ($MgSO_4$) and the solvent was reomoved to give a crude product that was distilled (Kugelrohr) to give aniline-^{15}N (0.453 g, 84%) b.p. 74 ^{o}C at 14 mmHg: δ_N (CH_2Cl_2, relative to external $MeNO_2$) - 324.2 ppm [^1J (^{15}N-^{13}C) 11.3, ^2J (^{15}N-^{13}C) 2.6, ^3J (^{15}N-^{13}C) 1.3 Hz].

3.6 Preparation of nitrosobenzene-^{15}N

This was obtained from aniline-^{15}N in a similar manner and in comparable yield as for -nitrosobenzene-$^{17}O,^{18}O$, except that the 3-chloroperbenzoic acid (unlabelled, prepared according to ref. 19) was purified before use. Thus, crude 3-chloroperbenzoic acid was washed with 0.1 M phosphate buffer (pH 7.5) to give 3-chloroperbenzoic acid (97% pure by iodometric titration), m.p. 82-83 ^{o}C. The nitrosobenzene-^{15}N obtained showed δ_N ($CDCl_3$, relative to external $MeNO_2$) + 915 ppm [^1J (^{15}N-^{13}C) 12.2, ^2J (^{15}N-^{13}C) 4.9, ^3J (^{15}N-^{13}C) 1.5, ^3J (^{15}N-H) 1.2 Hz].

ACKNOWLEDGEMENTS

We thank SERC and ZENECA for a CASE award to KH, and the Leverhulme Trust for support (W McF).

REFERENCES

1 P M D Foster, S C Lloyd, and M S Prout, *Toxicology in Vitro*, 1987, **1**, 31.

2 P Eyer, *Xenobiotica*, 1988, **18**, 1327.

3 P Eyer, *Chem-Biol Interactions*, 1979, **24**, 227; for recent studies see S Kazanis and R A McClelland, *J Am Chem Soc*, 1992, **114**, 3052 and M K Ellis, S Hill, and P M D Foster, *Chem-Biol Interactions*, 1992, **82**, 151.

4 C Bleasdale, B T Golding, J McGinnis, S Müller, and W P Watson, *J Chem Soc, Chem Commun*, 1991, 1726.

5 ^{15}N: J Mason in *Multinuclear NMR*, ed J Mason, Plenum Press, New York, 1987, p 355; ^{17}O: W McFarlane and H C E McFarlane, *ibid*, p 403.

6 *eg* E Curzon, B T Golding, and A K Wong, *J Chem Soc, Chem Commun*, 1982, 63; E Curzon, B T Golding, C Pierpoint, and B W Waters, *J Organomet Chem*, 1984, **262**, 263; B Gordillo and E L Eliel, *J Am Chem Soc*, 1991, **113**, 2172.

7 B T Golding and A K Wong, *Angew Chem Int Ed Engl*, 1981, **20**, 89.

8 J H Hoeg, *J Labelled Compd Radiopharm*, 1971, **7**, 179.

9 J D'Ans and W Frey, *Chem Ber*, 1912, **45**, 1845; J D'Ans and A Kniep, *ibid*, 1915, **48**, 1136.

10 R C Jarnagin and J H Wang, *J Am Chem Soc*, 1958, **80**, 786.

11 R E Ball, J O Edwards, and P Jones, *J Inorg Nucl Chem*, 1966, **28**, 2458.

12 V Conte, G Miozzo, and R Salmaso, *Chim Oggi*, 1987, **7**, 31.

13 G Grassi, M Oldani, and A Bauder, *Helv Chim Acta*, 1983, **66**, 400.

14 C Hanson, M W T Prat, and M Sohrabi, 'Some Aspects of Aromatic Nitration in Aqueous Systems' in *Industrial and Laboratory Nitrations*, ed L F Albright and C Hanson, ACS Symposium Series, 22, American Chemical Society, Washington DC, 1976, p 225 (especially p 237); for a previous preparation of nitrobenzene-^{15}N see R J Konior, L Yang, and R I Walter, *J Labelled Compd Radiopharm*, 1990, **28**, 1243.

15 Vogel's Textbook of Practical Organic Chemistry, ed B S Furniss, A J Hannaford, V Rogers, P W G Smith, and A R Tatchell, Longman, London, 1978, p 659.

16 A I Rezvukhin, G G Furin, and G G Yakobson, *Izv Akad Nauk SSSR, Ser Khim*, 1981, 2512.

17 G C Levy and R L Lichter (eds), *Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy*, Wiley-Interscience, New York, 1979.

18 G Charlot and D Bézier, *Quantitative Inorganic Analysis*, Methuen and Co Ltd, London, 1957, p 522.

19 R N McDonald, R N Steppel, and J E Dorsey, *Org Synth*, 1963, Coll Vol IV, 276.