Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 4

Synthesis of 1-([$^{18}O_2$]-2-Nitro-1-imidazolyl)-3-methoxy-2-propanol ([$^{18}O_2$]-Misonidazole)

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SUMMARY

A short, efficient synthesis for 1-(2-[$^{18}O_2$]-nitro-1-imidazolyl)-3-methoxy-2-propanol ([$^{18}O_2$]-misonidazole) from 2-aminoimidazole is described. The required ^{18}O-labeled sodium nitrite (82.6 atom %) was conveiently synthesized by the treatment of sodium nitrite with DOWEX 50W (H^+) in $H_2{}^{18}O$. When 2-aminoimidazole reacted with excess of ^{18}O-labeled sodium nitrite in the presence of sulfuric acid containing copper sulfate in $H_2{}^{18}O$, the ^{18}O-labeled 2-nitroimidazole was formed with a reasonable yield (43%). [$^{18}O_2$]-Misonidazole (70 atom %) was achieved with good yield by the coupling of sublimed ^{18}O-labeled 2-nitroimidazole with freshly distilled 1,2-epoxy-3-methoxypropane in ethanol in the presence of a catalytic amount of anhydrous potassium carbonate under reflux for one hour. The desired product was purified by recrystalization and TLC, and characterized by ^1H NMR and mass spectra. The ^{18}O content and distribution of synthetic ^{18}O-labeled misonidazole was determined from the FAB-MS spectra.

Key Words: 2-aminoimidazole, 2-nitroimidazole, 1-methoxy-2-hyd- roxy-3-chloropropane, 1,2-epoxy-3-methoxypropane, 1-(2-nitro-imidazolyl)-3-methoxy-2-propanol (misonidazole), fast atom bombardment mass spectroscopy (FAB-MS).

INTRODUCTION

The nitroimidazoles belong to the family of nitroheterocyclic compounds which play an extremely important role as drugs in

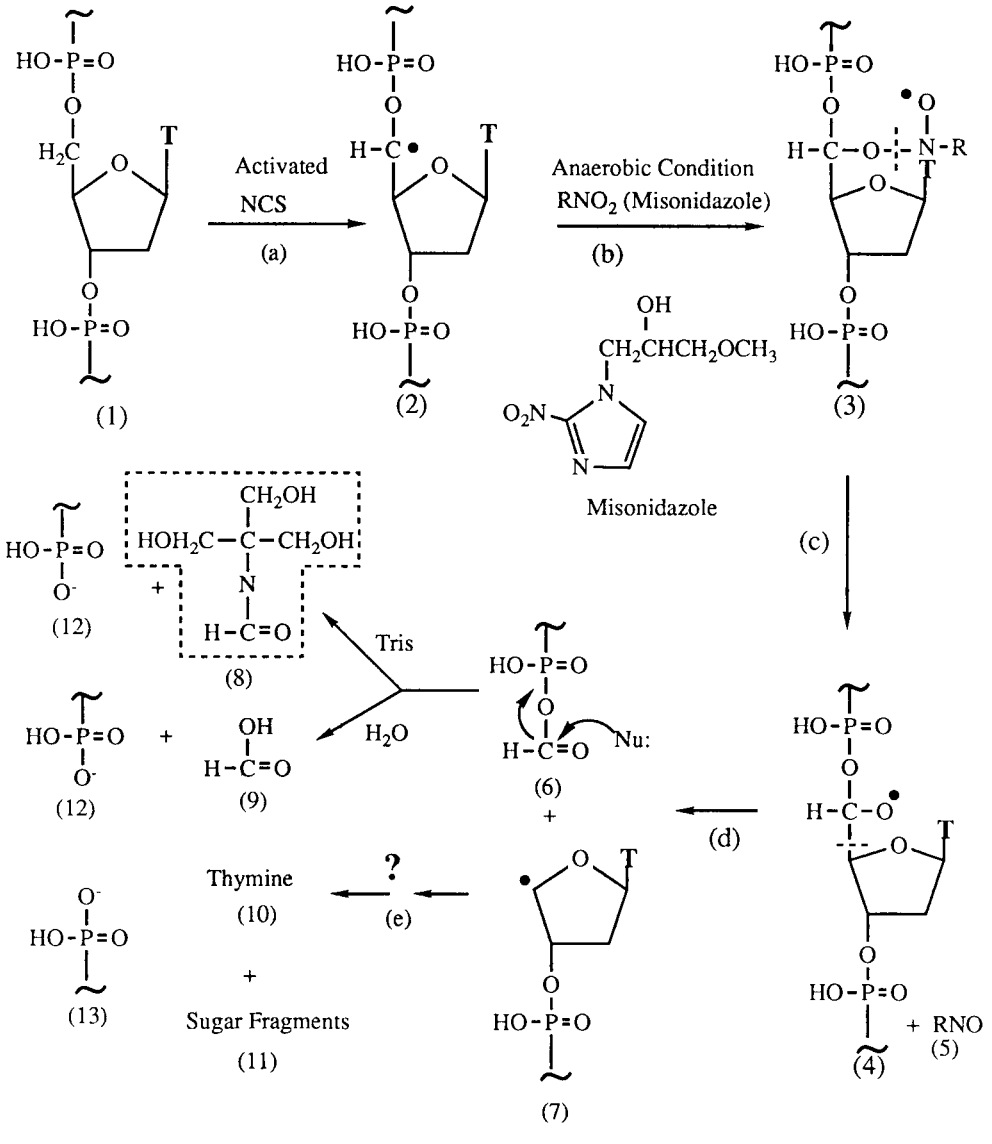
0362–4803/89/040423–12$06.00

Received May 23, 1988
Revised September 2, 1988

chemotherapy. Because of their important antiprotozoal activity, nitroimidazoles have been studied extensively in terms of chemical synthesis and properties, biosynthesis, and biochemical mode of action.[1] For instance, the antibiotic azomycin (2-nitroimidazole), first isolated by Maeda in 1953 from *Streptomyces*,[2] has shown antiparasitic and anticancer activity, and has been widely used as the drug for the treatment of human trichomoniasis and as a hypoxic cell radiosensitizer in radiotherapy. Moreover, a series of investigations at Hoffmann-La Roche resulted in the discovery of very active 1-substituted 2-nitroimidazoles such as misonidazole (**A**), 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol, which exhibited the best activity against *Trichomonas vaginalis* in mice.[3,4]

Misonidazole, a nitroaromatic radiation-sensitizer, has been used to study the mechanism of DNA strand breakage by our laboratory. Under anaerobic conditions where misonidazole substitutes for dioxygen, DNA strand breakage by the nonprotein chromophore of the antitumor antibiotic neocarzinostatin (NCS-Chrom) is associated with the generation of a reactive form of formate (**6**) from the C-5' of deoxyribose of thymidylate residues (scheme 1). Amino-containing nucleophiles such as tris(hydroxymethyl)aminomethane (Tris) acts as acceptors for the activated formate to give formyltris (**8**).[5]

A possible mechanism was proposed to account for the reaction products.[5] The carbon radical at C-5' (**2**), produced by the hydrogen atom abstraction by a radical species of NCS-Chrom complex[6] (reaction a, Scheme 1) forms a labile nitroxyl radical adduct (**3**) with misonidazole (RNO_2) (reaction b, Scheme 1). This nitroxyl radical intermediate can transform to (reaction c, Scheme 1) oxy radicals (**4**) and the nitroso reduction product of misonidazole[7] (**5**) by the fragmentation process. The oxy radicals undergo β-fragmentation reaction, resulting in the cleavage of the C-5',4' bond of the sugar moiety (reaction d, Scheme 1) with the formation of 3'-formylphosphate-ended DNA (**6**) and 5'-sugar-radical-phosphate-ended DNA (**7**). Spontaneous hydrolysis of the labile formyl phosphate-ended DNA or transfer of the activated formyl moiety to a nucleophile (for instance, tris) results in the formation of a gap with phosphates at both ends (**12**).


Activated
NCS
(a)
Anaerobic Condition
RNO2 (Misonidazole)
(b)
(c)
(d)
(e)
Misonidazole
Tris
H2O
Nu:
Thymine
(10)
+
Sugar Fragments
(11)
+ RNO
(5)
(1)
(2)
(3)
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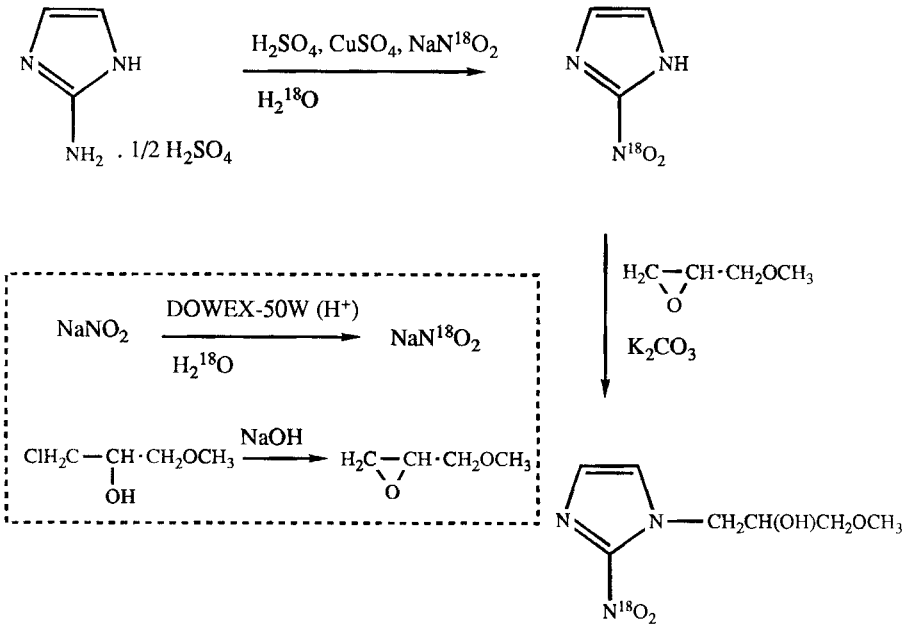
(12)
(13)

SCHEME 1



SCHEME 2

This proposal involves the nucleophilic attack of the oxygen atom of the nitro group of misonidazole on the radical at C-5' of the sugar of the thymidylate residue, resulting in the formation of formate (or formyltris) in which the carbonyl oxygen atom is derived from the oxygen atoms of misonidazole. In order to investigate the mechanism further, we have devised a method to synthesize [$^{18}O_2$]-misonidazole with high ^{18}O content via the nitration of 2-aminoimidazole with [$^{18}O_2$]-sodium nitrite followed by the alkylation at N-1 position of 2-[$^{18}O_2$]-nitroimidazole with 1,2-epoxy-3-methoxypropane, as shown in scheme 2.

RESULTS AND DISCUSSION

The synthesis of misonidazole have been recorded in some papers,[3,8] but unfortunately the detailed procedure has not been published. We report, herein, a convenient and efficient synthesis of 1-(2-[$^{18}O_2$]-nitro-1-imidazolyl)-3-methoxy-2-propanol with a good yield and high ^{18}O-enrichment.

There are two questions which had to be solved in order to achieve this ^{18}O-labeled synthesis. First of all, the required ^{18}O-labeled sodium nitrite, not commercially available, needed to be synthesized with high ^{18}O-enrichment. Secondly, the synthetic protocol for ^{18}O-labeled 2-nitroimidazole needed to be established in the minimum amount of ^{18}O-labeled water as the solvent, due to the high cost of $H_2{}^{18}O$ ($120/g). The use of ^{16}O-water might cause extensive ^{18}O-^{16}O exchange for sodium nitrite and/or 2-nitroimidazole resulting in low ^{18}O-enrichment of 2-nitroimidazole.

^{16}O-labeled sodium nitrite was converted to ^{18}O-labeled sodium nitrite (82 atom %) efficiently according to the literature procedures.[10] Sodium nitrite, dissolved in ice-cold $H_2{}^{18}O$ (98 atom %), was treated with dry DOWEX-50W (H^+) with stirring at 0 °C, and then the mixture was allowed to warm to room temperature. The reaction mixture was allowed to proceed at room temperature for 24 hours and then passed through filter paper. The filtrate was adjusted to pH 5.4 with NaOH powder at 0 °C. This solution was then distilled by short path distillation under reduced pressure and gave ^{18}O-labeled sodium nitrite (85% yield) and $H_2{}^{18}O$ (88% recovery). The ^{18}O-content and distribution of labeled sodium nitrite was determined by FAB-MS. Low resolution FAB-Mass spectrometric analysis displayed a sodiumnated parent ion at 96 ($Na^+[Na^+N^{18}O^{18}O^-]$) with an absolute intensity 70.6%, a sodiumnated parent ion at 94 ($Na^+[Na^+N^{18}O^{16}O^-]$) with an absolute intensity 24.1%, and a sodiumnated parent ion at 92 ($Na^+[Na^+N^{16}O^{16}O^-]$) with an absolute intensity 5.3%. The fragmentation pattern of ^{18}O-labeled sodium nitrite showed a similar pattern to ^{16}O-sodium nitrite and the intensity of each peak was corrected by using unlabeled compound as an internal standard. The choice of DOWEX-50W (H^+) for the synthesis of ^{18}O-labeled sodium nitrite led to a clean desired product with high yield, while mineral acids resulted in low yield and the formation of side products.

Preliminary nitration experiments, varying the amount of H_2O used, were carried out to establish optimum reaction conditions for the synthesis of ^{18}O-labeled 2-nitroimidazole. The protocol for the synthesis of $^{18}O_2$-2-nitroimidazole, a modification of A.G. Beaman's procedures,[3,8] was

accomplished; the amount of the ^{18}O-labeled water used was the key factor for this nitration reaction. When the amount of ^{18}O-labeled water used was too little, the reaction mixture became a suspension solution which resulted in a very low yield. The synthesis of [$^{18}O_2$]-2-nitroimidazole employed unlabeled 2-aminoimidazole as the starting material. When 2-aminoimidazole (1 equiv.) reacted with excess of ^{18}O-labeled sodium nitrite (20 equiv.) in sulfuric acid (80 equiv.) in the presence of copper sulfate (1 equiv.) with the proper amount of ^{18}O-labeled water (11 mL), the ^{18}O-labeled 2-nitroimidazole was formed and gave a characteristic maxminum ultraviolet peak at 374 mu (in 0.1 N NaOH) after 60 hours reaction time.

Sulfuric acid in $H_2{}^{18}O$ was reacted with a $H_2{}^{18}O$ solution of $CuSO_4$ and 2-aminoimidazole sulfate in $H_2{}^{18}O$ at 0 °C with stirring. The temperature of the reaction mixture was maintained below 20 °C during the above additions. The reaction mixture was cooled to -20 °C and stirred vigorously. A $H_2{}^{18}O$ solution of ^{18}O-labeled sodium nitrite was added very slowly and carefully through a long needle several inches below the surface of the liquid while the temperature was kept below -15 °C. The reaction mixture was allowed to stand at room temperature for about 60 hr without stirring. The completion of the reaction was monitored by the UV absorption at 370 m*u*. The solution was worked up by a careful titration of pH to 1 at -10 °C. The titration was done by bubbling NH_3 gas through the vigorously stirring solution, and yellow ^{18}O-labeled 2-nitroimidazole precipitated. The yellow precipitate was filtered and washed to give 40 mg of product. The aqueous solution was extracted by ethyl acetate 6 times to give 28 mg of brown yellow product. The combined crude product was purified by sublimation in three portions to give 50 mg (yield 43%) of ^{18}O-labeled 2-nitroimidazole (mp, 288 °C, decomposition). This sample was suitable for synthetic reaction without further purification. The titration was carried out very carefully, otherwise there was no precipitate formation. An attempt to determine the ^{18}O content and distribution of ^{18}O-labeled 2-nitroimidazole by FAB-MS failed because of its sublimate property. Unreacted ^{18}O-labeled water could not be recovered due to technical difficulties.

The required 1,2-epoxy-3-methoxypropane was freshly prepared by the treatment of 1-methoxyl-2-hydroxy-3-chloropropane with 1.5 equivalent of sodium hydroxide in diethyl ether at room temperature for eight hours.[9] The completion of the reaction was monitored by TLC. The reaction mixture was worked up by the addition of H_2O and the aqueous layer was extracted with organic solvent. The organic portions were combined, washed with H_2O and brine and dried over magnesium sulfate. This diethyl ether solution was distilled by short-path distillation at low temperature to remove diethyl ether followed by the distillation of 1,2-epoxy-3-methoxypropane at reduced pressure. The desire product, a colorless liquid (70% yield), was collected at 85-90 °C at 250 mm. Success in the alkylation of ^{18}O-labeled 2-nitroimidazole to obtain ^{18}O-labeled misonidazole required a pure and dry 1,2-epoxy-3-methoxypropane.

Preparation of [$^{18}O_2$]-Misonidazole was carried out conveniently by the coupling of sublimed ^{18}O-labeled 2-nitroimidazole with 3 equivalents of freshly distilled 1,2-epoxy-3-methoxypropane in ethanol containing the catalytic amount of anhydrous potassium carbonate under reflux for one hour.[3] The hot reaction mixture was filtered, and the insoluble material was washed with boiling ethyl alcohol. The volatile material was removed by the rotary evaporatory and gave a brown solid crude product. This crude product was purified by recrystalization in ethanol at 0 °C and by TLC (62% yield). The purity of this purified ^{18}O-labeled misonidazole was checked by ^1H NMR and FAB MS. The ^{18}O content and distribution of this synthetic ^{18}O-labeled misonidazole was determined from the FAB-MS spectra which showed [$^{18}O^{18}O$]-misonidazole at peak 206 with 52.3% absolute intensity; [$^{18}O^{16}O$]-misonidazole at peak 204 with 35.4% absolute intensity and [$^{16}O^{16}O$]-misonidazole at peak 202 with 12.3% absolute intensity. The fragmentation pattern of ^{18}O-labeled misonidazole displayed a similar pattern to ^{16}O-misonidazole and the intensity of each peak was corrected by using unlabeled compound as an internal standard. The synthesis of ^{18}O-labeled misonidazole was achieved with reasonable yield and high ^{18}O-enrichment (70%). On the basis of the starting ^{18}O-enrichment of sodium nitrite (82.6%) and the final

^{18}O-enrichment of misonidazole (70%), it appeared that 12.6% of ^{18}O-sodium nitrite was exchanged to ^{16}O-sodium nitrite or 12.6% of ^{18}O-labeled 2-nitroimidazole was exchanged to ^{16}O-labeled 2-nitroimidazole during the nitration reaction, containing 450 mmole of $H_2{}^{18}O$ and 80 mmole of H_2SO_4.

EXPERIMENTAL

Instrumentation and General Procedures

^1H NMR spectra were obtained on Bruker WM 250 FT NMR spectrometers at 250.0 MHz. NMR spectra are reported as parts per million downfield of Me_4Si (δ = 0). Multiplicities are as follows: s = singlet, d = doublet, t = triplet, q = quartet. The low resolution fast-atom-bombardment mass spectra (FAB-MS) were determined by the MAT731 mass spectrometer at the Mass Spectrometry Facility at the Massachusetts Institute of Technology and principal molecular fragments are reported.

Preparative and analytical thin-layer chromatography were carried out by using 20 x 20 cm Merk precoated glass-backed, silica-gel 60 F-254 ultraviolet light, 0.25 mm plates. Visualization of spots was effected by heating to 150 °C after spraying with p-anisaldehyde solution, by ultraviolet illumination, or by exposure to iodine vapor. Diethyl ether was distilled from sodium under nitrogen. $H_2{}^{18}O$ (98 atom %) was purchased from Cambridge Isotopes. Merck silica gel 60 (0.05 - 0.2 mm) was used for column chromatography. Anhydrous reactions were performed in flamed-dried glassware under a positive pressure of nitrogen or argon. Reaction mixtures were stirred magnetically unless otherwise indicated. Sensitive liquids and solutions were transferred via syringe and were introduced into reaction vessels through rubber septa. Reaction product solutions were concentrated using a Buchi rotary evaporator at 20-25 mm Hg.

Synthesis:

(1) Preparation of H^+-resin AG 50W-X8

The analytical grade cation exchange resin AG 50W-X8, 10 g, (100-200 Mesh, hydrogen form, 5.1 meq/dry g moisture content: 50-56%, medium pore size) was reacted with 100 ml of 2N HCl for two hours, and then filtrated and

washed with distilled water twice. These resins were stirred with 2N HCl for another 12 hours followed by the filtration and two distilled water washes. The resins were dried by the high vacuum pump overnight, and then stored at room temperature for the future work.

(2) Preparation of $^{18}O_2$-labeled sodium nitrite

Sodium nitrite (1.74 g, 25.3 mmole), dissolved in ice-cold $H_2{}^{18}O$ (98 atom %, 8 ml, 444.5 mmole), was treated with dry DOWEX-50W (H^+) (0.48 g) with stirring at 0 °C, and then the mixture was allowed to warm to room temperature. After standing at room temperature for 24 hours, the reaction mixture was filtered and brought to pH 5.4 with NaOH powder at 0 °C. This solution was then distilled (88% recovery) by short path distillation under reduced pressure (aspirator) and gave 1.57 g of $^{18}O_2$-labeled sodium nitrite (85.1% yield). Low resolution FAB-MS data: $Na^+(Na^+N^{18}O^{18}O^-)_{found}$ = 96, relative intensity 100; $Na^+(Na^+N^{18}O^{16}O^-)_{found}$ = 94, relative intensity 34.1; $Na^+(Na^+N^{16}O^{16}O^-)_{found}$ = 92, relative intensity 7.5.

(3) Preparation of [$^{18}O_2$]-2-nitroimidazole ([$^{18}O_2$]-azomycin)

Sulfuric acid (4.4 ml, 80 mmol) in 2.5 mL of ^{18}O-labeled water was cooled to 0 °C in a 15 mL round bottom flask, equipped with mechanical stirrer. With stirring, a 2.5 mL $H_2{}^{18}O$ solution of $CuSO_4$ (160 mg, 1 mmole) was added slowly, followed by the careful addition of 2-aminoimidazole sulfate (132.2 mg, 1 mmole) in 1.5 ml of $H_2{}^{18}O$ to give a clear green solution. The temperature of the reaction mixture was maintained below 20 °C during the above additions. After the addition the reaction mixture was cooled to -20 °C (CCl_4 in dry ice) and stirred vigorously. A solution of [$^{18}O_2$]-sodium nitrite (1.46 g, 20 mmol) in 4.5 ml of $H_2{}^{18}O$ was added slowly and carefully through a long needle several inches below the surface of the liquid while the temperature was kept below -15 °C. Gas evolved during the 30 minute addition period, and the reaction mixture turned a dark blue. The reaction mixture was allowed to stand at room temperature for about 60 hr without stirring and gave a clear green solution with some white precipitate at the bottom of the flask. The final solution was worked up by a careful titration of to pH 1 at -10 °C. The titration was done by bubbling the NH_3 gas through the

vigorously stirring solution for 2 hrs. Yellow 2-[$^{18}O_2$]-nitroimidazole precipitated, and the solution was allowed to stir for another 2 hr at 0 °C. The yellow precipitate was filtered, washed with distilled water two times, and dried by air; yield, 40 mg. The aqueous solution was extracted by ethyl acetate 6 times to give 28 mg of brown yellow product. The combined crude product (68 mg, 58% yield)) was purified by sublimation (170 °C, 0.1 mm) in three portions to give 50 mg (43% yield) of 2-[$^{18}O_2$]-nitroimidazole (mp, 288 °C, decomposition). This sample was suitable for synthetic reaction without further purification.

(4) Preparation of 1,2-epoxy-3-methoxypropane

Into a flamed-dried, 50-mL, 2-neck, round-bottom flask equipped with a mechanical stirrer and a reflux condenser with a drying tube was added 2 mL of diethyl ether solution and 2.49 g (2.2 mL) of 1-methoxyl-2-hydroxy-3-chloropropane (20 mmol). The vigorously stirred solution was cooled to 0 °C in an ice-water bath for 15 minutes, and then 1.2 g of finely powdered sodium hydroxide (30 mmol) was added in small portions. After stirring at 0 °C for 10 minutes, the reaction mixture was warmed to room temperature and was stirred for 8 hours. The completion of the reaction was monitored by TLC (ethylacetate/petroleum ether, 1/4) until all the starting material was consumed. The reaction mixture was worked up by the addition of 5 mL of H_2O, and the aqueous layer was extracted with three portions of diethyl ether. The organic extracts were combined, washed with two portions of H_2O and one portion of brine and dried over magnesium sulfate. The diethyl ether solution was distilled by short-path distillation at a temperature below 60 °C at one atmosphere to remove diethyl ether, and then the 1,2-epoxy-3-methoxypropane was distilled under vaccum (aspirator). The desired product was collected at 85-90 °C at 250 mm to give 1.23 g (1.3 mL) of colorless liquid (70% yield).

(5) Preparation of 1-(2-[$^{18}O_2$]-nitro-1-imidazolyl)-3-methoxy-2-propanol ([$^{18}O_2$]-misonidazole)

To a dry, 2-neck, 5 mL flask equipped with a magnetic stirrer, a septum inlet and a reflux condenser with a drying tube was introduced 50 mg of

sublimed 2-[$^{18}O_2$]-nitroimidazole (0.5 mmol) in 1.5 mL of ethanol (99%). The reaction solution with stirring was charged with a mixture of 0.14 g (0.14 mL) freshly distilled 1,2-epoxy-3-methoxypropane (1.5 mmol) and 10 mg of anhydrous potassium carbonate (0.084 mmol) and refluxed for one hour. The hot reaction mixture was filtered, and the insoluble material was washed with boiling ethyl alcohol twice. The volatile material was removed by the rotary evaporatory and gave a brown solid crude product. This crude product was purified by recrystalization in ethanol at 0 °C and TLC (ethylacetate/methanol, 9/1; R_f=0.6) and gave 62 mg of pure misonidazole (62% yield). ^{1}H NMR (250 MHz), $CDCl_3$) δ 3.35 (s, -CH_2-O$\underline{CH_3}$, 3H), δ = 3.4 (t, J = 5.0 Hz, -N$\underline{CH_2}$CH(OH)-, 2H), δ = 4.04 (m, -NCH_2C$\underline{H}$(OH)-, 1H), δ = 3.64 (dd, J = 13.8 Hz, J = 8.18 Hz, -CH(OH)C$\underline{H_2}$OCH_3, 1H), δ = 4.68 (dd, J = 13.8 Hz, J = 3.74 Hz, -CH(OH)C$\underline{H_2}$OCH_3,1H), δ = 4.87 (s, NCH_2CH(O$\underline{H}$), 1H), δ = 7.10 (d, J = 1 Hz,-CH_2NC$\underline{H}$CHN-, 1H), δ = 7.41 ((d, J = 1.0 Hz,-CH_2NCHC$\underline{H}$N-, 1H). Low resolution FAB-MS data: MH^+_{found} ($^{18}O_2$-midonidazole) = 206, relative intensity 100; MH^+_{found} ($^{18}O^{16}O$-midonidazole) = 204, relative intensity 68.2; MH^+_{found} ($^{16}O_2$-midonidazole) = 202, relative intensity 23.5.

ACKNOWLEDGEMENT

We acknowledge support for this work from National Institutes of Health Grant GM 12573.

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