REGIOSELECTIVE SYNTHESIS OF UNSYMMETRICAL DEUTERIUM LABELLED HYDRAZINES AND AZOXY COMPOUNDS

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SUMMARY

A method for the preparation of deuterium labelled unsymmetrical hydrazines was developed. This method was used to prepare 1-benzyl-2-methyl-$^2H_3$-hydrazine hydrobromide and 1-benzyl-$^2H_7$-2-methylhydrazine hydrobromide, analogs of the cancer chemotheraputic agent procarbazine. These compounds were then used to prepare the corresponding labelled benzyl-(ONN) and (NNO)-azoxymethane isomers. A method was developed whereby the deuterium labelled azoxy isomers were separated by reversed phase semi-preparative HPLC.

Key words: Alkylhydrazine, Alkylazoxy, Procarbazine, Deuterium

INTRODUCTION

Procarbazine is a hydrazine widely used as a chemotherapeutic agent in the treatment of Hodgkins disease, tumors of the brain and other neoplasms[1-3], which requires metabolic activation.[4] Among the characterized metabolites of procarbazine are an azo compound and two azoxy isomers[5]. It has been proposed that these azoxy isomers are then metabolically hydroxylated and that the resulting azoxy-carbinols give rise to benzylating and/or methylating intermediates as the ultimate active species[5].

It is not known which of the azoxy isomers is most important (active) or which site, if any, in the azoxy isomers is hydroxylated preferentially, Fig. 1., R=H, $CONHCH(CH_3)_2$. These hydroxylated intermediates appear to be too

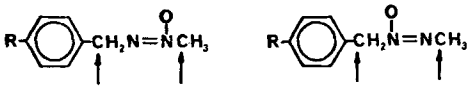

Fig. 1. Possible hydroxylation sites of the azoxy isomers.

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unstable to isolate or observe directly. In order to investigate the importance of the azoxy isomers and to obtain information about the sites of metabolic hydroxylation the unsubstituted procarbazine anologs, methyl-$^2H_3$-benzylhydrazine hydrobromide 7, methylbenzyl-$^2H_7$-hydrazine hydrobromide 8 and their corresponding deuterium labelled azoxy isomers 13-16 were prepared. The results of in vitro metabolic studies with these compounds will be reported elsewhere.

RESULTS AND DISCUSSION

In order to synthesize the labelled hydrazines 7 and 8 a method for the preparation of specifically labelled unsymmetrical hydrazines was needed. This method had to allow for the introduction of a different deutrium labelled group at either end of the hydrazine moiety and to allow for removal of the deactivating or protecting group without loss or scrambling of the incorporated label. The direct alkylation of monsubstituted hydrazines results in 1,1-disubstituted hydrazines rather than the desired 1,2-unsymmetrical compounds. However, Zeller and coworkers have shown that masking of the active hydrazine hydrogens as urethanes allows anions to be generated which can be alkylated to prepare unsymmetrical hydrazines[6].

We found that a modification of this strategy was well suited for the preparation of the deuterium labelled hydrazines 7 and 8. By using different hydrazine starting materials either of the desired labelled groups could be introduced in the alkylation step. No loss or scrambling of the incorporated label occurred on removal of the protecting groups. The methodology and reaction conditions were established with non-labelled substrates before the labelled compounds were prepared. The labelled hydrazines 7 and 8 then served as precursors to the labelled azoxy compounds.

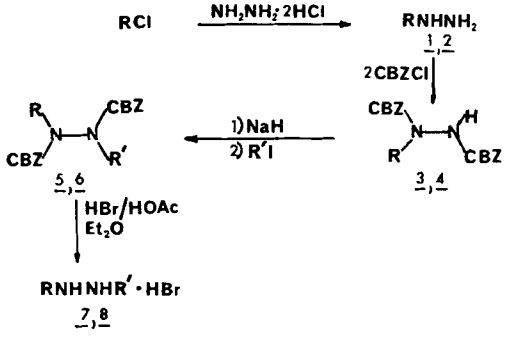
The 1-methyl-$^2H_3$-2-benzylhydrazine hydrobromide 2 was prepared by the reaction sequence shown in Scheme 1. The benzylhydrazine[7] 1 hydrogens were protected by formation of the benzyloxycarbonyl derivative 3[8]. The

protected compound 3 was then converted to its anion by treatment with NaH in THF followed by alkylation with iodomethane-$^2H_3$ for 21 hr at 25°C giving 5. The benzyloxycarbonyl protecting groups were then removed by treatment with HBr and CH_3CO_2H (4 equiv.) in ether, giving the labelled hydrobromide salt 7 in 92% yield. Treatment of 5 with HBr or HCl in CH_3CO_2H as solvent followed by dilution with ether gave much lower yields[9].

A sequence using a ethoxycarbonyl protecting group was also investigated. The alkylations using this method were much more rapid, but the removal of the ethoxycarbonyl protecting occurred in only 10% yield so the method was abandoned.

The ethoxycarbonyl strategy was also used for the preparation of methylbenzyl-$^2H_7$-hydrazine hydrobromide 8. The reaction sequence is summarized in Scheme 1. After protection of the methylhydrazine 2 as its bis-carbobenzoxy derivative 4, the anion was formed by reaction with NaH in THF. The anion was alkylated with benzyl-$^2H_7$ iodide at 25°C for 3 hr giving 6 in 79% yield. The benzyl-$^2H_7$ iodide was prepared from benzyl-$^2H_7$ chloride by the Finkelstein reaction[10]. Attempted

SCHEME 1



1,3 R = $C_6H_5CH_2$; 2,4 R' = CH_3;

5,7 R = $C_6H_5CH_2$ R' = CD_3

6,8 R = CH_3 R' = $C_6D_5CD_2$

CBZ = $C_6H_5CH_2OCO$

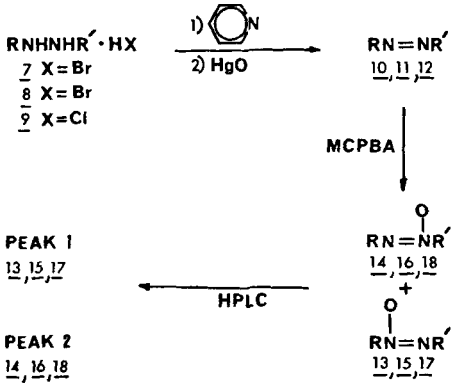
alkylations with benzyl chloride required 2 days and gave only 40-50% yield. The protecting groups were removed by treatment with HBr and CH_3CO_2H (4 equiv.) in ether giving 8 in 93% yield.

The labelled compounds 7 and 8 were characterized by ^1H NMR and chemical ionization mass spectrometry. The ^1H NMR spectra of 7 and 8 were compared to those of the unlabelled 1-methyl-2-benzylhydrazine hydrochloride 9 prepared by the same method[11]. The spectral data of the $^2H_3$-methyl compound 7 and the unlabelled compound 9 were identical except that 7 had no peak for the methyl group at 2.66ppm. The spectral data of the $^2H_7$-benzyl compound 8 was identical to that of 9 except that 8 had no peak for the methylene group at 4.30ppm and no peak for the aromatic protons at 7.38ppm. The ^1H NMR spectra of 7 and 8 showed no loss or scrambling of either deuterium label when the spectra were compared to those of the unlabelled hydrazine 9 or with the labelled precursors 5 and 6. The chemical ionization mass spectrum of 7 gave a base peak at m/z 140 corresponding to loss of Br ion from the parent structure. No significant peaks at m/z 137-139 due to loss of the deuterium label were observed. The chemical ionization mass spectrum of 8 gave a base peak at m/z 144 due to loss of Br ion from the parent structure. A fragment at m/z 113 due to loss of CH_3NH_2 and a fragment for the perdeuteriotropylium ion at m/z 98 were also observed. No peaks due to the loss of the deuterium label were observed.

The azoxy isomers of 7, 8 and 9 were then prepared synthetically by the reactions shown in Scheme 2. The goal of the metabolic studies of these compounds was to determine which sites of the azoxy isomers are hydroxylated during activation by analysis of isotope effects and product identification. To do this it was necessary to separate the methylazoxy isomers 14, 16 and 18 from the benzylazoxy isomers 13, 15 and 17 by semi-preparative HPLC so that methabolisms of the indvidual azoxy isomers could be observed. This was best accomplished by using mixtures of known amounts of 7, 8 and 9.

The benzylmethyl azo isomers 10, 11, 12, were prepared as a mixture on a 0.46mmol scale in a Reacti-Vial. The salts 7, 8 and 9 were suspended in CH_2Cl_2 and were neutralized with pyridine and oxidized with HgO[12]. Use of stronger bases such as Et_3N caused significant isomerization of the azo isomers to hydrazones. The products were obtained by removal of the metallic Hg by filtration. The azo compounds 10, 11, and 12 were then converted directly to the azoxy isomers (see Scheme 2) by oxidation with m-chloroperbenzoic acid (MCPBA)[13]. After aqueous work-up, the benzyl azoxy isomers 13, 14 and 17 were separated from the methylazoxy isomers 14, 16 and 18 by semi-preparative HPLC using a reversed phase 5μ C_{18} column with 43% MeOH/H_2O as the eluting solvent. The products were isolated by collecting and pooling like fractions, the MeOH was removed and the aqueous layer was extracted with CH_2Cl_2. There were two major peaks observed. Peak 1 contained the benzylazoxy isomers and peak 2 contained the methyl azoxy isomers. There was a small amount of overlap between peak 1 and peak 2 fractions that contained both isomers were discarded. The purity of

SCHEME 2



9,10,13,14 R = $C_6H_5CH_2$ R' = CH_3

7,11,15,16 R = $C_6H_5CH_2$ R' = CD_3

8,12,17,18 R = $C_6D_5CD_2$ R' = CH_3

the isomers was established by examining peak 1 and peak 2 on an analytical HPLC column containing the same packing as the semi-preparative column. Both isomers were found to be >97% pure.

The metabolic studies of the indvidual methylazoxy, 14, 16, 18 and benzylazoxy, 13, 15, 17, isomers provides a unique opportunity to determine which is the most important isomer. Since the hydroxylated metabolities are too unstable to isolate, primary kinetic isotope data will show which sites in the azoxy isomers are hydroxylated and to what extent.

EXPERIMENTAL SECTION

The precursors for the deuterium labeled compounds methyl-$^2H_3$ iodide, 99.5 atom %D and benzyl-$^2H_7$ chloride, 98 atom %D were obtained from MSD Isotopes; St. Louis, MO. Hexane was dried over $LiAlH_4$ and was distilled under N_2. THF was predried over CaH_2 and then distilled from $LiAlH_4$ under N_2. Acetone was dried over K_2CO_3 and distilled. All deuterium labeled products were stored desiccated at -30°C. Melting points are uncorrected and were obtained on a Fisher-Johns melting point apparatus. Mass spectra were obtained on a Finnigan 4023 GC-MS-DS in the chemical-ionization mode with isobutane reagent gas. NMR spectra were obtained on a Varian FT-80 spectrometer. Chemical shifts are reported relative to Me_4Si, δ=0. Gas chromatography was done on a Varian Aerograph 2100 gas chromatograph, equipped with a flame ionization detector. Columns were 6' x 1/4" packed with prorapak QS or OV-225 packing. Preparative layer chromatography was performed on 2mm thick 20cmX 20cm silica gel 60 F_{254} plates, HPLC separations were done with an Altex 110A pump and Altex 153 UV detector. Analytical separations were done with an ultrasphere ODS 5μ column using 10 mm 8 ul analytical flow cell and a 20μl injection loop. Preparative separations were done with an ultrasphere ODS 5μ column 10 mm ID x 25 cm with an ultrasphere ODS 5μ pre-column using a 0.5 mm, 2 μl preparative flow cell and a 200 μl injection loop.

Benzylhydrazine (1): A flask was charged with hydrazine dihydrochloride 53 g (0.51 mol) which was slowly neutralized with 255 ml of 4N KOH. To this was added 12 ml (0.08 mol) of benzyl chloride in 35 ml of EtOH dropwise with stirring. After the addition was complete, the reaction was heated under reflux with stirring for 1 hr. The reaction mixture was cooled to room temperature and was extracted with CH_2Cl_2 (4 x 200 ml). The combined organic layers were dried over K_2CO_3. The solvent was removed under reduced pressure and the product 1 was distilled as a clear colorless oil in 54% yield: bp 50-54°C/0.5 torr; ^1H NMR ($CDCl_3$) δ 3.14 (s,3H), 3.78 (s,2H), 7.26 (s,5H); mass spectrum m/z (rel abund) 123 (100) MH^+, 106 (10) $MH^+-NH_3$91 (7) $C_7H_7.^+$

1,2-Bis(benzyloxycarbonyl) benzylhydrazine (2): A flask was charged with 3.0 g (24.6 mmol) of benzylhydrazine, 10 ml of H_2O and 10 ml of ether. The reaction mixture was cooled in an ice bath and 3.6 ml (25.0 mmol) of benzyl chloroformate in 12 ml of ether was added dropwise with stirring. A second portion of benzyl chloroformate 3.6 ml (25.0 mmol) in 12 ml of ether and a solution of 2.8 g of KOH in 5 ml of H_2O were added separately but simultaneously with stirring. After the addition was complete, the reaction mixture was stirred at 0-5°C for an additional 10-15 min. The resulting white precipitate was dissolved in 250 ml of ether. The layers were separated and the ether layer was washed with water (2 x 250 ml). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure. The resulting white solid, dried under vacuum weighed 8.21 g, 85%: mp 80-81°C; ^1H NMR ($CDCl_3$) δ 4.70 (s,2H), 5.11 (s,2H), 5.17 (s,2H), 6.80 (brs, 1H), 7.30 (s,15H) mass spectrum, m/z (rel abund) 391 (37) MH^+, 347 (100) MH^+-CO_2.

1,2-Bis(benzyloxycarbonyl) methylhydrazine (4): A flask was charged with methylhydrazine 4.6 g (0.10 mol), 25 ml of benzene and 10 ml of water. The reaction was cooled to 0°C in an ice bath. To this was added benzyl chloroformate 28 ml (0.20 mol) in 50 ml of benzene. Simultaneously a solution

of 20 ml of 4.5 M KOH was added with the last half of the benzyl cchloroformate solution. The addition was at a rate such that the temperature did not exceed 10°C. After the addiition was complete, the reaction was stirred at room temperature for 12 hr. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 100 ml). The combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure. The resulting white solid was recrystallized from 80% EtOH. The product 4, a white solid weighed 25 g, 80%: mp 71-72°C: ^1H NMR ($CDCl_3$) δ 3.18 (s,3H), 5.13 (s,4H), 7.24 (brs, 1H), 7.31 (s,10H); mass spectrum, m/z (rel abund) 315 (45) MH^+, 271 (100) MH^+-CO_2.

1,2-Bis(benzyloxycarbonyl)-benzyl-2-methyl-$^2H_3$-hydrazine (5): An oven dried round bottom flask with septum inlet, pressure equalized addition funnel and gas stopcock was evacuated and purged with N_2, the reaction was run under a positive N_2 atmosphere. The flask was charged with NaH 0.20 g (4.17 mmol) which was washed with 15 ml of dry hexane. To this was added 7 ml of dry THF. The reaction was cooled in an ice bath and bis-1,2-(carbobenzoxy) benzylhydrazine 1.0 g (2.56 mmol) in 8 ml of dry THF was added dropwise with stirring. After the addition was complete, the reaction was stirred at 0°C for 1 hr. To this was added methyl-$^2H_3$ iodide 0.18 ml (2.90 mmol) in 10 ml of dry THF dropwise with stirring at 0°C. After the addition was complete, the reaction was stirred at room temperature for 21 hr. The reaction mixture was then poured into 40 ml of saturated NaCl solution. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 40 ml). The combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure. The product 5, a clear colorless oil weighed 1.0 g, 96%: ^1H NMR ($CDCl_3$) δ 5.17 (m,2H), 5.25 (m,4H), 7.30 (s,15H); mass spectrum, m/z (rel abund) 408 (100) MH^+, 364 (78) MH^+-CO_2.

Benzyl-$^2H_3$ Iodide: A round bottom flask with septum inlet and gas stopcock was charged with NaI 1.5 g (10.0 mmol) evacuated and purged with

N_2. The reaction was run under a positive N_2 atmosphere. To this was added 30 ml of dried freshly distilled acetone and benzyl-$^2H_7$ chloride 1.0 g (7.5 mmol). A catalytic amount of anhydrous ferric chloride 4 mg was added and the reaction was stirred at room temperature. The reaction was stopped after 20 hr. The NaCl precipitate was removed by suction filtration. The filter cake was washed with benzene (2 x 25 ml). The solvent was removed under reduced pressure. The residue was dissolved in 15 ml of dry THF and was filtered to remove a small amount of NaCl. The solution of THF/benzyl-$^2H_7$ iodide was protected from light and was used immediately in the next reaction.

1,2-Bis(benzyloxycarbonyl)-1-benzyl-$^2H_7$-2-methylhydrazine (6): An oven dried round bottom flask with septum inlet, pressure equalized addition funnel and gas stopcock was evacuated and purged with N_2, the reaction was run under a positive N_2 atmosphere. The flask was charged with NaH 0.26 g (5.5 mmol) which was washed with 15 ml of dry hexane. The NaH was covered with 5 ml of dry THF and the system was cooled in an ice bath. To this was added 1,2-bis(benzyloxycarbonyl)methylhydrazine 1.05 g (3.3 mmol) in 10 ml of dry THF dropwise with stirring. After the addition was complete, the reaction was stirred at 0°C for 1 hr. To this was added benzyl-$^2H_7$-iodide (7.0 mmol) in 15 ml of dry THF, prepared in the previous step. After the addition was complete, the reaction was stirred at 25°C for 3 hr. The reaction mixture was cautiously poured into 30 ml of cold saturated NaCl solution. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 30 ml). The combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure. The resulting orange oil was purified by preparative layer chromatography on silica gel plates which were developed with $CHCl_3$. The band with R_f=0.40 was cut-out and eluted with 1% MeOH/$CHCl_3$. After removal of the

solvent under reduced pressure, the product 6 was obtained as a clear yellow oil 1.07 g, 79%: ^{1}H NMR ($CDCl_3$) δ 2.83 (m,3H); 4.99-5.26 (m,4H), 7.29 (s,10H); mass spectrum, m/z (rel abund) 412 (100) MH^+, 368 (55) $MH-CO_2^+$, 276 (15) $MH-CO_2CH_2Ph^+$.

1-Benzyl-2-methyl-$^2H_3$-hydrazine Hydrobromide (7): A round bottom flask with gas inlet tube and gas stopcock attached to a gas trap was charged with 1,2-bis(benzyloxycarbonyl)-1-benzyl-2-methyl-$^2H_3$-hydrazine 2.1 g (5.1 mmol) dissolved in 25 ml of dry ether. To this was added glacial acetic acid 1.5 ml (23.8 mmol). The solution was then continuously saturated with hydrogen bromide gas for 3 hr. After the addition was complete, the reaction was stirred at room temperature for an additional 30 min. The resulting white precipitate was isolated by suction filtration. The product was washed with ether (4 x 30 ml). The product 7, was a hygroscopic white solid which weighed 1.04 g, 92%: mp 130-131°C; ^{1}H NMR (DMSO-d_6) δ 4.10 (s,2H), 7.38 (s,5H), 8.44 (brs, 3H); mass spectrum, m/z (rel abund), 140 (100) M-Br.

1-Benzyl-$^2H_7$-2-methylhydrazine Hydrobromide (8): A round bottom flask with gas inlet tube and gas stopcock attached to a gas trap was charged with bis-1,2-(carbobenzoxy)-1-benzyl-$^2H_7$-2-methylhydrazine 1.65 g (4.0 mmol) dissolved in 15 ml of dry ether. To this was added glacial acetic acid 1.0 ml (16.0 mmol). The reaction was stirred at room temperature and was continuously saturated with hydrogen bromide gas for 3 hr. After the addition was complete, the reaction was stirred at room temperature for an additional 1 hr. The resulting white precipitate was isolated by suction filtration. The product was washed with ether (3 x 40 ml). The product was dried *in vacuo* at 1.4 torr. The product 8 was a white solid which weighed 836 mg, 93%: mp 127-129°C; ^{1}H NMR (DMSO-d_6) δ 2.69 (s,3H), 8.18 (brs,5H) mass spectrum, m/z (rel abund) 144 (100) M-Br, 113 (58) $M-Br-MeNH_2^+$, 98 (53) $C_7D_7^+$.

Benzylmethylazo isomers (10, 11, 12): A 5 ml Reacti-Vial was charged with 27 mg (0.16 mmol) of methyl-2-benzylhydrazine hydochloride, 35 mg (0.16 mmol) of methyl-$^2H_3$-2-benzylhydrazine hydrobromide and 32 mg (0.14 mmol) of

methyl-2-benzyl-$^2H_7$-hydrazine hydrobromide. The compounds were suspended in 4 ml of CH_2Cl_2. The salts were then neutraliized with 37 µl (0.46 mmol) of pyridine. The reaction mixture was then oxidized by the addition of 100 mg (0.46 mmol) of mercuric oxide. The reaction was stopped after 1 hr. The precipitate was removed by filtration and was washed with 5 ml of CH_2Cl_2. The filtrate was used immediately.

Methylbenzylazoxy isomers (13-18): The CH_2Cl_2 filtrate of 10-12 from the previous reaction was cooled in an ice bath and 238 mg (1.37 mmol) of m-chloroperbenzoic acid in 10 ml of CH_2Cl_2 was added dropwise with stirring at 0°C. After the addition was complete, the reaction was stirred at 0°C for 30 min. and then at 25°C for 19 hr. The reaction miture was treated with 5% sodium sulfite solution and was neutralized to pH 9 with Na_2CO_3. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 ml). The combined organic solutions were evaporated under N_2. The residue was dissolved in 150 µl of MeOH and was purified by semi-preparative HPLC.

Separation of the methylbenzylazoxy isomers: The solution of 13-18 in MeOH was injected on a 10 mm ID x 25 cm ultrasphere ODS 5 µ column with pre-column using 43% MeOH/H_2O as the solvent at a flow rate of 1.9 ml/min monitored at 254 nm. Fractions were collected corresponding to the two major peaks with t_R=60 min (peak 1) and t_R=88 min (peak 2) at a chart speed of 5 min/cm. Fractions that contained overlap of the two peaks were discarded. The peak at t_R=60 min contained the benzyl-(ONN)-azoxymethane compounds 13, 15, 17. The peak at t_R=88 min contained the benzyl-(NNO)-azoxymethane compounds 14, 16, 18. The fractions were concentrated to 5 ml and then extracted with CH_2Cl_2 (3 x 5 ml). The organic solutions were concentrated to dryness under a stream of N_2. The samples were then dissolved in EtOH and were used for metabolic studies.

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