SYNTHESIS OF IODINE-125 LABELLED ANALOGUES OF METYRAPONE AND METYRAPOL

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SUMMARY

Metyrapone (1) and metyrapol (2) are potent and reversible inhibitors of the 11β-hydroxylase enzyme system of the adrenal cortex. Iodine-125-labelled derivatives of 1 and 2 were required for biodistribution studies. Utilizing a new exchange procedure these radioiodinated analogues were synthesized with radiochemical yields ranging from 24-100% and specific activities as high as 1.72 Ci/mmol.

Key Words: Iodine-125; metyrapone; metyrapol; 11β-hydroxylase

INTRODUCTION

Both metyrapone, 2-methyl-1,2-di-3-pyridyl-1-propanone (1), and its respective alcohol, metyrapol (2) have been shown to be potent and reversible inhibitors of the adrenal cortical 11β-hydroxylase enzyme system[1-3]. Structure-activity-relationship studies of metyrapone derivatives have indicated that inhibition of 11β-hydroxylase activity is enhanced by replacement of the A-ring pyridyl group

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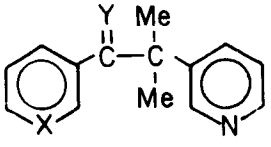
by a phenyl ring exemplified by compounds 3 and 4[4-5]. We required the iodine-125 labelled analogues of compounds 3 and 4 for initial biodistribution studies aimed at development of a clinically useful adrenocortical imaging agent. The syntheses of these iodinated compounds and their subsequent radioiodination are described herein.



1 X=N, Y=O

2 X=N, Y=H,OH

3 X=C, Y=O

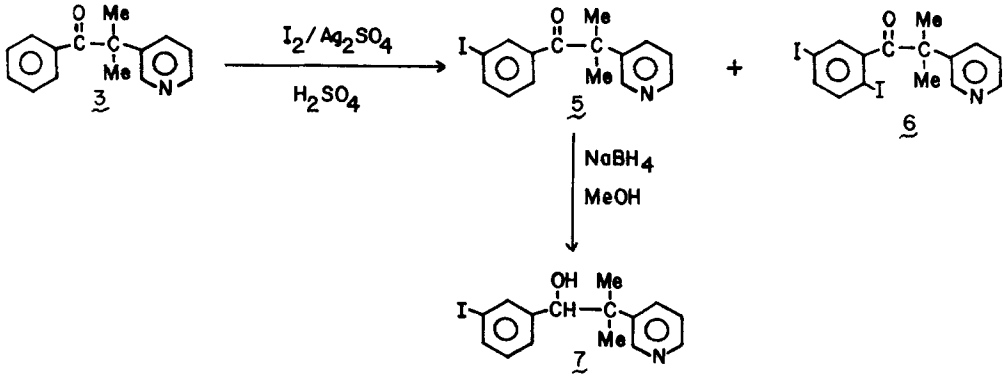
4 X=C, Y=H,OH

SYNTHESIS

Iodination of the previously synthesized ketone 3 [5] was accomplished with silver sulfate and iodide under Derbyshire conditions[6,7] to produce the mono 5 and diiodo 6 products shown in Scheme 1. Compounds 5 and 6 could be readily separated by silica gel chromatography. The iodometyrapone derivative 5 underwent sodium borohydride reduction to yield the iodinated metyrapol derivative 7.

Confirmation of the position of the iodine atom on the phenyl ring was achieved by taking advantage of the nonenolizable nature of the ketones 5 and 6. Treatment of either ketone with potassium hydroxide yielded 3-isopropylpyridine

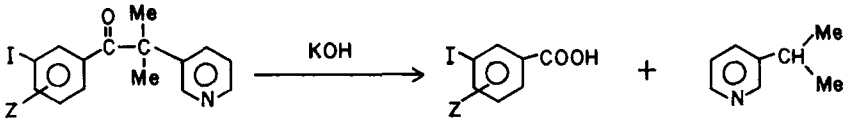
Scheme 1



and the corresponding iodobenzoic acids (see Scheme 2). Utilizing authentic samples of o-, m- and p-iodobenzoic acids for comparison by HPLC analysis, the base cleavage of ketone 5 yielded m-iodobenzoic acid in greater than 98.5% purity. The para isomer (<2%) was the only other component present.

Alkali treatment of ketone 6 yielded an acid whose ^{1}H NMR spectrum revealed the presence of both 2,5-diiodo- and m-iodobenzoic acid upon comparison to published spectra for these compounds[8,9]. Since a 200 MHz ^{1}H NMR spectrum of ketone 6 confirmed the presence of only the 2,5-diiodo-isomer, the m-iodobenzoic acid derived from its alkali treatment is probably the result of ortho deiodination either before or after cleavage.

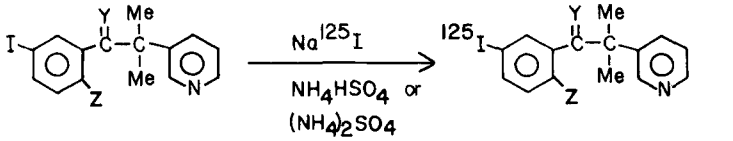
Scheme 2



5 Z=H
6 Z=I

Compounds 5-7 were radioiodinated by a new iodide exchange technique developed in our laboratory[10]. Treatment of the appropriate nonradioactive iodinated compound with Na^{125}I and ammonium sulfate or ammonium bisulfate afforded the iodine-125-labelled derivatives in radiochemical yields of 24-100%. Specific activities ranging from 0.45 to 1.72 Ci/mmol (see Table 1) were obtained.

Scheme 3



5 Y=O Z=H
6 Y=O Z=I
7 Y=H,OH Z=H

TABLE 1

SOLID-PHASE EXCHANGE REACTION OF IODINATED METYRAPONE DERIVATIVES 5-7.

Compound Number	Number of Exchange Reactions	Isolated Radiochemical Yield (average yield)	Highest Specific Activity (Ci/mmol)	R_f of Compound Silica Gel Plate EtOAc/hexane (1:1)	R_f of Compound C-18 Plate MeCN/H_2O (4:1)
5	8	24-100% (66%)	1.72[a]	.32	.33
6	1	88%	1.05[b]	.44	.22
7	2	53,64%	1.17[c]	.18	.47

[a]Specific activity was determined by UV quantitation of the ^{125}I-labelled ketone 5 by HPLC using nonradioactive alcohol 7 as the internal standard and measuring the radioactivity.

[b]Specific activity was determined based on the starting quantity of ketone 6 and final isolated radioactivity.

[c]Specific activity was determined by UV quantitation of the ^{125}I-labelled alcohol 7 by HPLC using nonradioactive ketone 5 as the internal standard and measuring the radioactivity.

EXPERIMENTAL

Chemicals and Equipment

No-carrier-added $Na^{125}I$ (ca. 500 mCi/mL) in reductant-free 0.1N NaOH was obtained from New England Nuclear. m-Iodobenzoic acid was purchased from Aldrich Chemical Co. o-Iodo- and p-iodobenzoic acids were obtained from Pfaltz and Bauer.

Radioactivity measurements were made using either a Capintec Model CRC-2 radioisotope calibrator or a Packard Model 5260 autogamma counter. Analytical HPLC was performed on a Waters Model 272 equipped with a Radiomatic Flo-one radioactive flow detector (200 μL solid scintillator cell). Simultaneous ultraviolet (254 nm) and radioactive detection were utilized. Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, Michigan. Infrared spectra were recorded on a Beckman IR Acculab 8 spectrophotometer. Nuclear magnetic resonance spectra were recorded on either a Varian EM-360A (60 MHz) or a Varian XL-200 (200 MHz) spectrophotometer. All melting points were taken on a Laboratory Devices Mel-temp capillary melting point apparatus and are uncorrected.

3'-Iodo-2-methyl-2-(3-pyridyl)propiophenone (5) and 2',5'-Diiodo-2-methyl-2-(3-pyridyl)propiophenone (6)

Ketone 3 (0.75 g, 3.3 mmol) was dissolved in 10 mL of 80% H_2SO_4. Silver sulfate (1.16 g, 3.7 mmol) was added and the reaction flask was heated to 85°C. Finely crushed iodine (1.80 g, 7.5 mmol) was then added in small batches over the course of 1 h. The reaction solution was heated an additional 1.5 h, cooled to 25°C and poured over ice. The insoluble silver salts were removed by vacuum filtration and the filtrate was made alkaline with solid Na_2CO_3 to precipitate additional silver salts. The aqueous layer and solid salts were washed liberally with CH_2Cl_2. The CH_2Cl_2 layer was washed with 10% $Na_2S_2O_3$, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue (1.09 g) was chromatographed on a silica gel column (28 x 4.2 cm) (E. Merck #9385, silica gel 60

40-63 μm) and eluted with EtOAc/hexane (1:1) according to the procedure of Still et al.[11]. The diiodo compound 6 eluted first (elution volume 500-700 mL) as a yellow oil (0.23 g, 14.6%). IR (neat) 1698 (C=O); 1385, 1360 cm^{-1}(geminal methyls). ^1H NMR (200 MH_z)(d_6-acetone) δ 1.67 (s, 6H, CH_3), 6.70 (d, 1H, phenyl C_6-H, $J_{4,6}$= 2.0 Hz), 7.45 (m, 2H, phenyl C^4-H, pyridine C_5-H), 7.67 (d, 1H, phenyl C_3-H, $J_{3,4}$=8.4 Hz), 7.83 (d/d, 1H, pyridine C_4-H, $J_{4,6}$=1.5 Hz, $J_{4,5}$=7.1 Hz) 8.55 (d/d, 1H, pyridine C_6-H, $J_{4,6}$=1.5 Hz, $J_{5,6}$=4.7 Hz) 8.67 (d, 1H, pyridine C_2-H, $J_{2,5}$= 2.0 Hz). Mass spectrum, EI m/e 477 (M^+, 22), 356 (100).

Analysis: Calculated for the picrate salt (mp 160-161°C) $C_{15}H_{13}NOI_2 \cdot C_6H_3N_3O_7$; C, 35.71; H, 2.28; N, 7.93; Found: C, 35.79; H, 2.25; N, 7.82.

The iodoketone 5 was eluted (elution volume 800-950 mL) from the column as a white solid (0.61 g, 52.6%). The solid was recrystallized from hexane, mp 99-100°C. IR (KBr) 1673 (C=O), 1388, 1365 cm^{-1} (geminal methyls). ^1H NMR (60 MH_z) ($CDCl_3$) δ 1.66 (s, 6H, CH_3), 7.45 (m, 2H, arom.), 8.62 (m, 6H, arom.).

Analysis: Calculated for $C_{15}H_{14}NOI$: C, 51.30; H, 4.02; N, 3.99; Found: C, 51.42; H, 4.21; N, 3.86.

α-(m-Iodophenyl)-β,β-dimethyl-3-pyridineethanol hydrochloride (7).

The monoiodoketone 5 (0.21 g, 0.6 mmol) was dissolved in 8 mL of absolute ethanol. Sodium borohydride (0.045 g, 1.2 mmol) was added in one batch and the reaction was stirred at 25°C for 3 h. H_2O (0.2 mL) was then added and the reaction was stirred another 0.5 h. The solvent was removed under reduced pressure and the residue partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude alcohol (0.21 g, 97.2%) was converted to the HCl salt with ethanolic HCl and the solvent was removed. The salt was recrystallized from absolute EtOH/Et_2O to yield 7 as a white solid (0.18 g, 77.8%), mp 190-192°C. IR (KBr) 3330 cm^{-1} (OH). ^1H NMR (60 MH_z) (d_6-DMSO) δ 1.28 (d, 6H, CH_3), 4.64 (s, 1H, CH), 7.90 (m, 8H, arom.).

Analysis: Calculated for $C_{15}H_{16}NOI \cdot HCl$: C, 46.23; H, 4.40; N, 3.59; Found: C, 46.08; H, 4.33; N, 3.50.

Determination of iodide position on the phenyl ring.

A. Cleavage of iodoketone 5. A sample of the iodoketone 5 (3.0 mg) was added to 1.0 mL of Claisen's alkali[12] (KOH, H_2O/ MeOH) and stirred for 18 h at 25°C. The reaction was extracted with CH_2Cl_2 (3 x 1 mL). The aqueous solution was injected into the HPLC employing ultraviolet (254 nm) detection. A Waters μBondapak C-18 column (4.6 x 250 mm) was used for the analysis with the following solvent system: 3% Et_3N (pH 7.5 with H_3PO_4)/ CH_3CN (83/17). The k' values for the authentic samples of o-iodobenzoic acid, m-iodobenzoic acid and p-iodobenzoic acid were, respectively, 1.7, 6.9 and 7.6. Based on the difference in extinction coefficient between the authentic samples of m- and p-iodobenzoic acid (p-iodobenzoic acid has an extinction coefficient 13.3 times larger than m-iodobenzoic acid), the ratio of the meta/para product in the cleavage reaction was calculated to be 98.8/1.2.

B. Cleavage of ketone 6. The diiodoketone 6 (10.0 mg) was dissolved in 2.0 mL of Claisen's alkali and stirred for 18 h at 25°C. The reaction was extracted with CH_2Cl_2 (3 x 1 mL). The aqueous solution was cooled to 5°C and acidified to pH 2 with concentrated HCl. The aqueous solution was then re-extracted with CH_2Cl_2 (3 x 1 mL). The CH_2Cl_2 layer from the second extraction was dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield 4.2 mg of the benzoic acid. The material was analyzed by ^1H NMR and a comparison with literature spectra[8,9] showed that the reaction product was a mixture of the 2,5-diiodo- and m-iodobenzoic acids.

Exchange Radioiodinations of Compounds 5-7.

Preparative exchange radioiodinations of compounds 5-7 were carried out in 5 mL multidose vials closed with a Teflon-lined septum. These were fitted with a 5 mL disposable glass syringe connected to a 1 1/2" 18 ga needle bent at 90° and inserted through the septum, as the distillate condenser and receptacle. The condenser outlet was vented through a sodium thiosulfate trap to sequester any volatile radioiodine produced. A mixture of 1-2 mg of substrate, 5-15 mCi of

$Na^{125}I$, and 5-7 mg of $(NH_4)HSO_4$ (for exchange of ketone 5 or 6) or $(NH_4)_2SO_4$ (for exchange of alcohol 7) in 0.5-1.0 mL of H_2O was heated to dryness in an oil bath and the dry reaction mixture maintained at 140°C for 2-5 h. The course of the exchange was monitored, at appropriate intervals, by TLC analysis after redissolving the reaction medium in 0.5 mL of H_2O. Heating was stopped when unbound radioiodide could no longer be detected or after a reaction time of 5 h if the exchange was incomplete. The dry reaction mixture was then cooled, dissolved in 1 mL of absolute EtOH and passed through a Cellex-D (Biorad) anion exchange column (1 x 4 cm) eluted with EtOH to remove unbound radioiodide from the preparation. Radiochemical yields of 23-88% were obtained. Following TLC and HPLC analyses, the ethanol was removed from the radioiodinated product and the product was formulated to a specific concentration of approximately 100 μCi/mL using ethanol/Tween 80/ water (1.0/ 0.25/8.75).

Radio-TLC analyses were performed on 2.5 x 20 cm silica gel-coated glass plates (Whatman K6F) or reverse phase C-18-coated glass plates (Whatman $KC_{18}F$). The silica gel plates were developed with EtOAc/hexane (1:1) and the C-18 plates with MeCN/H_2O (4:1). The plates were examined on a Packard Model 720 radiochromatogram scanner. Free ^{125}I remained at the origin on the developed silica gel plates and travelled with the solvent front on the reverse phase C-18 plates. The R_f values for the iodinated compounds 5-7 are shown in Table 1.

Specific activity was determined by using HPLC with a nonradioactive internal standard (see Table 1). Radiochemical purity was confirmed by HPLC analysis and was always > 98%. A Waters μBondapak C-18 column (4.6 x 250 mm) was used for all analyses with the following solvent system: 0.03 M phosphate (pH 7.4)/CH_3CN (60:40). The k' values for the iodinated compounds are 10.8 (5), 16.8 (6), 6.8 (7).

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