SYNTHESIS OF LABELLED HISTAMINE H_2-RECEPTOR ANTAGONISTS

I. SYNTHESIS OF DEUTERIUM, SULFUR AND TRITIUM LABELLED CIMETIDINE AND METIAMIDE BY EXCHANGE.

Hesselbo, T.*, and Slade, S. W.,
The Research Institute, Smith Kline & French Laboratories Limited,
Welwyn Garden City, Hertfordshire, England
And
Villani, A. J.+, Petka, L.,
Research & Development Division, Smith Kline & French Laboratories,
Philadelphia, Pennsylvania, U.S.A.

SUMMARY

The H_2-antagonists, metiamide and cimetidine were labelled with deuterium and tritium in the 2-position of the imidazole ring by the uncatalyzed exchange reaction with deuterium and tritium oxide at 100°C. The sulfur-35 labelled metiamide was prepared by an exchange reaction with elemental sulfur-35 in refluxing pyridine and by the reaction of 2[(4-methyl-5-imidazolyl) methylthio]ethylamine with methylisothiocyanate-^{35}S in refluxing ethanol.

Key Words: Tritium, Sulfur-35, Exchange, Imidazole, Deuterium and Methylisothiocyanate-^{35}S.

INTRODUCTION

A number of imidazole compounds have been synthesized as potential H_2-receptor antagonists. Among such compounds metiamide(1) and cimetidine(2) showed excellent biological activity in inhibiting gastric secretions(1-3). For pharmacokinetic(4) and metabolism(5-6) studies with 1 and 2, the isotopically labelled drugs were required. Isotopic exchange

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*Inveresk Research International, Musselburgh-Midlothian, Scotland.
+To whom correspondence should be addressed.

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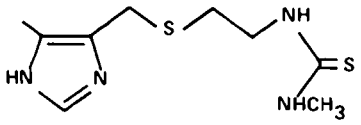
methods were first used because the fewer synthetic steps required would accelerate the start of these studies.



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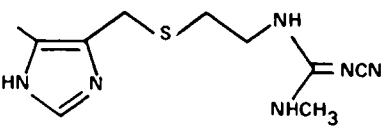
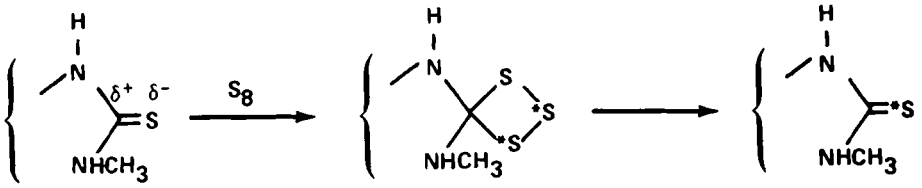


2

Harris(7) had shown that imidazole and N-methylimidazole undergo facile exchange with deuterium oxide at the C-2 position in the absence of base catalysis and at room temperature. It seemed reasonable that metiamide and cimetidine should behave similarly with deuterium and tritium oxide.

Sulfur exchange in thioureas was studied by Vulterin (8) who showed that the rate of exchange was dependent on the degree of polarization of the carbon to sulfur double bond and to a lesser extent on the degree of N-substitution of the thiourea. Mikhlukin(9) explained this facile sulfur exchange in organosulfur compounds having a thione group, as involving a cyclic activated complex formed by the interaction of the S_8 ring of elemental sulfur with the polarized carbon to sulfur double bond.



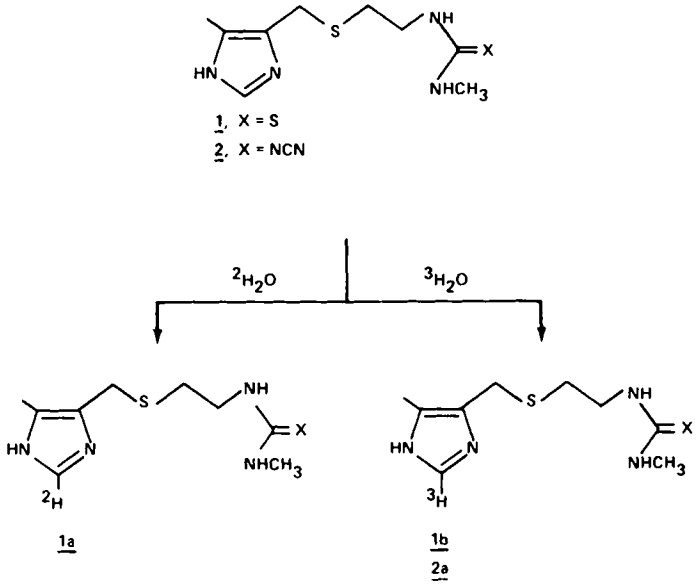
It was reasonable to assume that metiamide, having a thiourea moiety, would undergo a similar sulfur exchange. This was indeed the case, albeit with concomitant by-product formation.

DISCUSSION

The C-2 imidazole proton in 1 was exchanged for deuterium by refluxing in deuterium oxide for one hour. The lack of any measurable peak for this

proton in the NMR, suggests that the deuteration was better than 97%. Integral ratios over the aliphatic region suggest that no deuteration of the three methylenes and the two methyl groups occurred.

The tritium labelling was carried out in a similar fashion to give the 2-tritio derivatives of 1 and 2. The stability of 1b was studied under several conditions (table I) and was found to have a half-life of 2.4 days at 37°C and pH 7.



SCHEME I

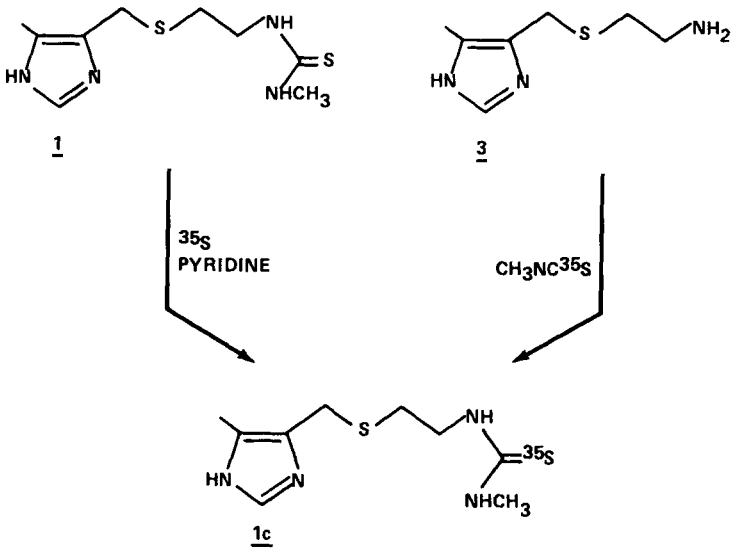
Table I

Effect of Temperature and pH on Stability of 1b[a]

Temperature (°C)	pH	$t_{1/2}$ (days)
24±2	5.4[b]	242
ambient	7.0[c]	16.5
	9.0[d]	8.7
37±0.1	5.4[b]	19.0
	7.0[c]	2.5
	9.0[d]	1.8

a - 10^{-3}M
b - 0.01M phosphate
c - 0.025M phosphate
d - 0.025M borate

The exchange of sulfur was accomplished by treatment of 1 with elemental sulfur-35 in refluxing pyridine for 15 hours. Under these conditions, an 18% incorporation of sulfur-35 in the thiourea moiety of 1 was achieved. The sulfur exchange was attended by the formation of several by-products (Fig. 1) which were not identified and which impeded subsequent purification of 1c. However, by use of dry-column chromatography(10) and phase solubility equilibria techniques(11-12), a radiochemical purity of 96% for 1c was obtained. Ultimate purification, giving a radiopurity of greater than 99%, was achieved by use of preparative thin-layer chromatography. Compound 1c was also prepared by the reaction of methylisothiocyanate-^{35}S with 3. The reaction was complete in 2 hours at 82°C in ethanol.



SCHEME II

EXPERIMENTAL

The elemental sulfur-35, purchased from New England Nuclear Corporation, Boston, Massachusetts, had a radiochemical purity of 99% and a specific activity of 493 mCi/mmol. The methylisothiocyanate-^{35}S, purchased from the Radiochemical Center, Amersham, England, had a specific activity of 54 mCi/mmol. Tritiations were carried out at the Radiochemical Center, Amersham, England. Radiochromatographs were examined by the Berthold radioscanner/integrater (Model 6000-10), exposure against Kodirex X-ray film and by use of spark chamber techniques(13). Specific activities were determined with a Packard Tri-Carb 3003 and a Nuclear Enterprise NE 8212 scintillation spectrometers.

N-Methyl-N'-[2-[[(2-deutero-5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]thiourea(1a)

400 mg (1.6 mmol) of 1 was refluxed with deuterium oxide for 1 hour. After cooling to room temperature, the pH was adjusted to 3 by the addition of 20% deuteriochloric acid. The deuterium oxide was removed by rotary evaporation and the oily residue dissolved in water and the process was repeated to remove labile deuterium. The exchange was approximately 97% by NMR.

N-Methyl-N'-[2-[[(2-tritio-5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]thiourea(1b)

Preparative Procedure:

244 mg (1 mmol) of 1 was dissolved in tritiated water (2 ml, 200 Ci/ml) at 100°C. The reaction was allowed to equilibrate for 1 hour with occasional shaking to ensure complete solution. The tritiated water was distilled off and the residual oil dissolved in methanol (10 ml) and evaporated to dryness to remove labile tritium. The residue was redissolved in methanol (10 ml) and benzene (90 ml) added. The specific activity of the solution was 15.5 mCi/ml for a total of 1,550 mCi. The theoretical specific activity of 1b was calculated to be 1,550 mCi/mmol.

Purification/Dilution Procedure:

35.5 ml (550 mCi, 0.35 mmol) of 10% v/v methanol in benzene solution of 1b was evaporated to dryness under aspirator vacuum. The residual solid was combined with pure carrier 1 (5 g, 20 mmol) and dissolved in water (20 ml)

containing 1N hydrochloric acid. The flask was cooled in ice and 2M sodium carbonate added to pH 8. After crystallization, the solid 1b was filtered and dried under an infra-red lamp. The product weighed 5 g (488 mCi) with a specific activity of 24.4 mCi/mmol. Spark chamber photographs of the radiochromatographs of 1b showed the compound to have a high radiochemical purity.

N''-Cyano-N-methyl-N'-[2-[[(2-tritio-5-methyl-1H-imidazol-4yl)methyl]thio]ethyl] guanidine(2a)

Preparative Procedure:

500 mg (2.0 mmol) of 2 was dissolved in tritiated water of specific activity in excess of 1.8 Ci/mmol and heated at 100°C for 1 hour. The tritiated water was distilled off and the residue dissolved in methanol which was also removed by distillation. This procedure removed labile tritium. The product (2a) contained 2 Ci of radioactivity and was diluted with methanol to a final volume of 25 ml. The specific activity of the solution was 80 mCi/ml and the theoretical specific activity of 2a was 1 Ci/mmol.

Purification/Dilution Procedure:

6.25 ml (500 mCi, 0.5 mmol, 126 mg) of the methanol solution of 2a was evaporated to dryness under vacuum. The residual solid was combined with pure carrier 2 (1.0 g, 4.1 mmol) and dissolved in 1N hydrochloric acid (4 ml). The solution was cooled in ice and the free base of 2a precipitated by the slow addition, with stirring, of 1N sodium hydroxide to pH 9. The product was filtered off and washed well with water to remove traces of sodium chloride. The solid (2a) was dried under an infra-red lamp and then over phosphorous pentoxide under vacuum. The specific activity was 126 mCi/mmol. The radiochemical purity of this material was approximately 99%. This was determined by examination of radiochromatographs in a coil spark chamber. The chromatographs were obtained by TLC on silica gel F of 50, 5, 2.5, and 0.5 µl amounts of solution and developed using a solvent system of ethylacetate/methanol/ammonium hydroxide (5:1:1 v/v).

N-Methyl-N'-[2-[[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]thiourea-^{35}S(1c)

Sulfur-35 Exchange:

To a suspension of 1 (99 mg, 0.41 mmol) in pryidine (1 ml) was added 200 mCi of elemental sulfur-35 (6 mg, 0.41 mmol) having a specific activity of 493 mCi/mmol. The reaction mixture was heated to a temperature of 115°C (bath) for 15 hours, while maintaining a constant flow of nitrogen through the system and good stirring. At the end of this period, an 18% exchange had been obtained (a maximum of 35 mCi 1c). This was determined by TLC radiochromatography (Fig. 1) on a silica layer using a developing solvent system of chloroform/methanol (90:10 v/v).

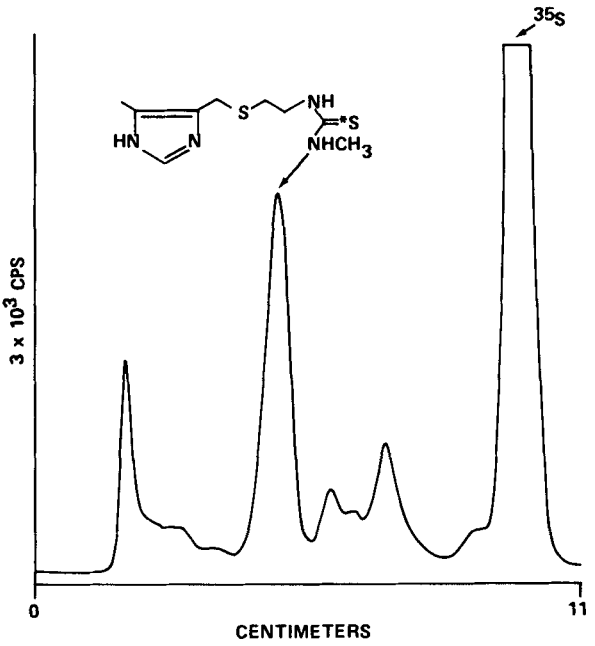


Figure 1 - Radiochromatogram of crude sulfur exchange product(s) using silica gel GF and a solvent system of chloroform/methanol (90:10 v/v).

The unreacted sulfur-35 was removed by treatment of the reaction mixture with benzene (25 ml) followed by decantation of the benzene layer. The residual, crude, 1c was dissolved in methanol (5 ml) containing 100 mg of carrier 1. The solution was dry-column chromatographed on a 2.5 x 25 cm silica column using

chloroform/methanol (90:10 v/v) as the developing solvent system. The band representing 1c was cut out of the column and extracted from the absorbent with methanol. The solvent was removed by rotary evaporation in vacuo and the residual solid magnetically stirred as a suspension in acetone (10 ml) for 24 hours. The solid was filtered and combined with and additional 100 mg of carrier 1 and recrystallized from water (10 ml).

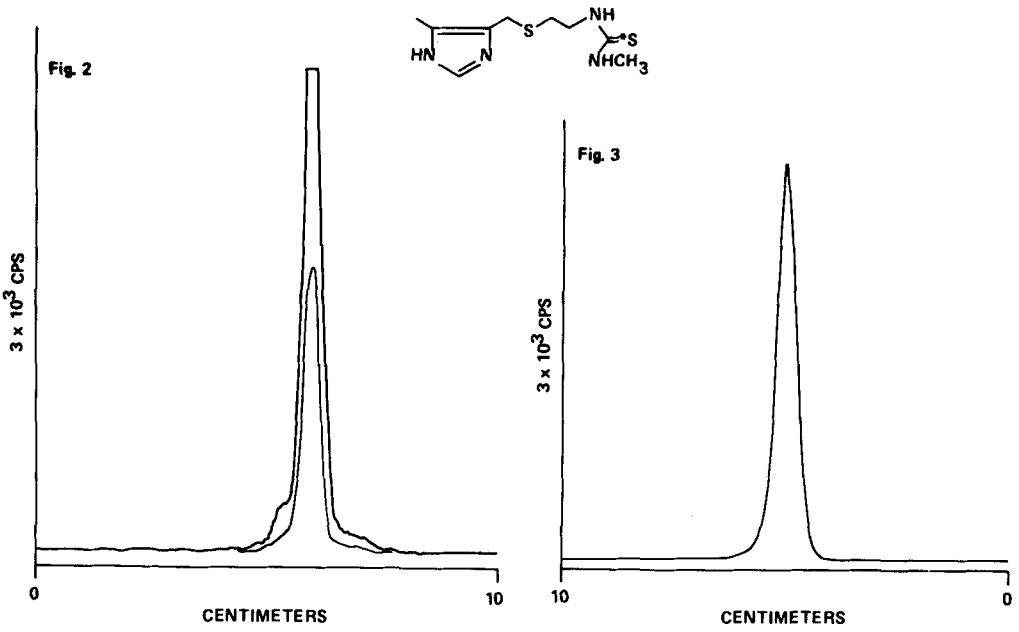
The first crop weighed 158.1 mg (14.2 mCi) and had a specific activity of 22 mCi/mmol (0.09 mCi/mg). The overall radiochemical yield was 7.1% and the product (1c) was 96% pure by TLC radiochromatography (Fig. 2).

Preparative TLC Purification:

Approximately 2 mCi (~ 8 mCi/mmol) of 1c having a radiopurity of 93% was dissolved in chloroform/methanol (1:1 v/v) (4 ml) containing 40 mg of carrier 1. The solution was streaked on four Quantum silica preparative thin-layer plates (500 μ) and developed in a solvent system of chloroform/methanol/ammonia (89:20:5% v/v). The band on the silica layer representing 1c was removed from the plates and slurried in chloroform/methanol (1:1 v/v) (30 ml). The silica was removed by vacuum filtration and the filtrate concentrated to dryness under aspirator vacuum. The residual white solid was suspended in ether and filtered to give 42 mg of product after drying at 50°C for 4 hours under vacuum. The solid amounted to 0.84 mCi with a specific activity of 4.85 mCi/mmol. The radiochromatographic purity (Fig. 3) was 99.3%.

From methylisothiocyanate-^{35}S:

The dihydrobromide salt of 3 (350 mg, 1.1 mmol) was dissolved in a minimum volume of water. The pH was raised to 11 by the addition of 1M potassium carbonate. The solution was evaporated to dryness using a rotary evaporator under aspirator vacuum and the residual base and inorganic salts were extracted with isopropanol. The isopropanol extracts were combined and evaporated to dryness. 180 mg (1.1 mmol) of 3 was dissolved in ethanol to a final volume of 5 ml.



Radiochromatograms of purified 1c obtained via sulfur exchange using silica gel GF and a solvent system of chloroform/methanol/ammonia (4:1:0.5% v/v).

To 3.6 ml of this solution (130 mg, 0.76 mmol) was added methylisothiocyanate (40 mCi, 0.74 mmol) in benzene (4 ml) and the solution heated under reflux at 82°C for 2 hours. The ethanol/benzene solution was evaporated under vacuum in a rotary evaporator and the residual yellow oil acidified with 1N hydrochloric acid (1.1 ml). This solution was then neutralized with 1 M sodium carbonate (1 ml) and the resulting solid filtered. The solid was recrystallized from water (1.5 ml), filtered and dried overnight under vacuum in a dessicator.

The product (1c, 150 mg, 0.6 mmol, 33 mCi) had a radiochromatographic purity of 97.5% as determined by TLC on silica gel F using a solvent system of ethylacetate/methanol/ammonia (10:1:1 v/v). The radiochemical yield was 83% (based on methylisothiocyanate-^{35}S) with a specific activity of 54 mCi/mmol.

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