THE PREPARATION OF N^{τ}-[^{3}H]METHYLHISTAMINE DIHYDROCHLORIDE AND N^{τ}-METHYL[α,β,2,4,5-$^{14}C_5$]HISTAMINE DIHYDROCHLORIDE

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SUMMARY

In order to study some aspects of the central metabolism of histamine we have prepared N^{τ}-[^{3}H]methylhistamine dihydrochloride from [^{3}H]methyl iodide by chemical synthesis and N^{τ}-methyl[α,β,2,4,5-$^{14}C_5$]histamine dihydrochloride from [α,β,2,4,5-$^{14}C_5$]histamine dihydrochloride by an enzymatic method.
Key words: N^{τ}-[^{3}H]methylhistamine dihydrochloride, N^{τ}-methyl[α,β,2,4,5-$^{14}C_5$]-histamine dihydrochloride, chemical synthesis, enzymatic synthesis.

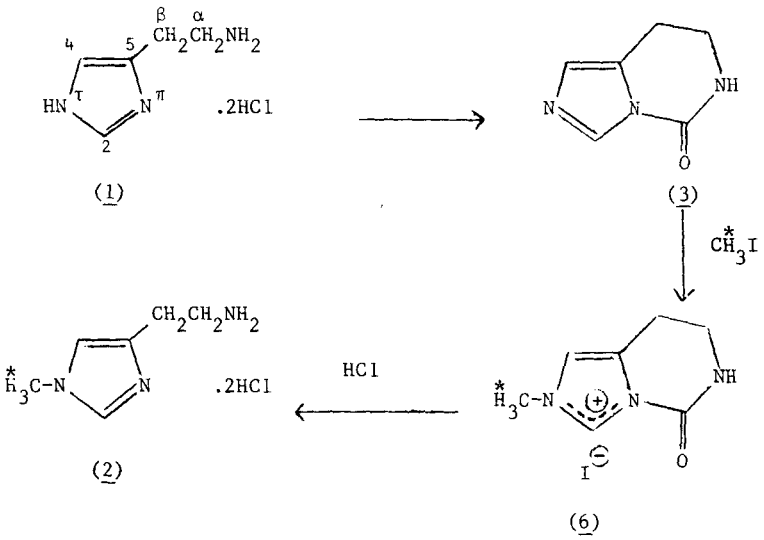
INTRODUCTION

A major route of catabolism of the biologically important substance histamine (1) [1,2] is by methylation to yield N^{τ}-methylhistamine catalyzed by the enzyme histamine N-methyltransferase (EC 2.1.1.8) [3] with the coenzyme S-adenosylmethionine as methyl group donor. Since N^{τ}-methylhistamine appears to be virtually devoid of physiological activity, this pathway represents an inactivation route for histamine. As part of our investigation of the function of histamine in the brain, we wished to study some aspects of the central metabolism of N^{τ}-methylhistamine. [4] During the course of this work we have prepared radiolabelled N^{τ}-methylhistamine by chemical and enzymatic methods.

0362-4803/79/0516-0761$01.00 Received November 7, 1978

Rothschild and Schayer[5] prepared low specific activity (178μCi/mmol) N^{τ}-methyl[^{14}C]histamine dihydrochloride chemically in unquoted yield by methylation of acetyl[^{14}C]histamine, separating the co-product N^{π}-methyl[^{14}C]-histamine by recrystallisation of the dipicrate salts. We wish to describe an unambiguous chemical synthesis of N^{τ}-methylhistamine dihydrochloride (2) labelled specifically in the N^{τ}-methyl position based on the method developed by Durant and co-workers.[6] Thus we have prepared N^{τ}-[^{3}H]methylhistamine (2) from unlabelled histamine dihydrochloride (1) via the cyclic urea (3) using tritiated methyl iodide as shown in Scheme 1.

SCHEME 1



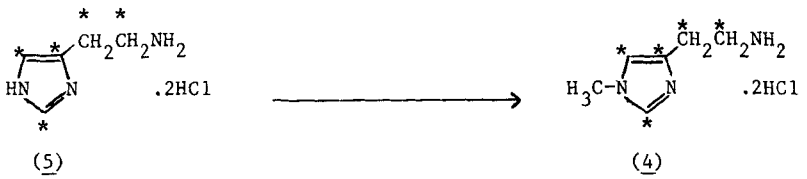
* denotes tritium radiolabel

We considered this synthetic route to be particularly suitable as this method of amine protection would ensure an unambiguous methylation at the N^{τ}-position at a sufficiently late stage in the synthesis to ensure a reasonable radiochemical yield. Using this method the product (2) was obtained at a specific activity of 66.9 mCi/mmol and, after extensive purification, in a 21% overall radiochemical yield. The authenticity of the N^{τ}-[^{3}H]methylhistamine dihydrochloride (2) was confirmed by co-chromatography with authentic material and a radiochemical purity of 98% was established.

We have also prepared by an enzymatic method a high specific activity carbon-14 labelled N^{τ}-methylhistamine dihydrochloride (4), labelled in the histamine moiety rather than the N^{τ}-methyl group. This afforded a higher specific activity and radiochemical yield than could be obtained using a multistage chemical synthesis such as that described above. Several workers [7-10] have described both *in vivo* and *in vitro* enzymatic preparations of radiolabelled N^{τ}-methylhistamine. The method most appropriate to our needs was that described by Beaven and Jacobsen [10] who prepared N^{τ}-methyl[β-^{3}H] histamine dihydrochloride from [β-^{3}H]histamine using a 3 hour incubation with a histamine methyltransferase preparation and excess S-adenosylmethionine. The product was purified by the addition of dilute sodium hydroxide to the reaction medium, extraction into chloroform and back-extraction into dilute hydrochloric acid. Although the product was shown to be of high purity no radiochemical yield was quoted.

We have developed this method using [α,β,2,4,5-$^{14}C_5$]histamine dihydrochloride (5) and established conditions which give virtually quantitative conversion to the required N^{τ}-methyl[α,β,2,4,5-$^{14}C_5$] histamine dihydrochloride (4) as shown in Scheme 2.

SCHEME 2



(5) (4)

* denotes carbon-14 radiolabel

Isolation of the product was achieved by thin layer chromatography on cellulose to yield a product of specific activity 290 mCi/mmol in an overall radiochemical yield of 60%. The authenticity of the product (4) was confirmed by co-chromatography with authentic material and radiochemical purities of over 95% were obtained. [α,β,2,4,5-$^{14}C_5$]Histamine dihydrochloride (5) if present accounted for less than 0.5% of the total radioactivity.

A major departure from the method of Beaven and Jacobsen[10] was in the method of isolation of the radiolabelled N^{τ}-methylhistamine (4). We found that the extraction procedure using chloroform and strongly alkaline solutions on a microscale resulted in substantial conversion of the N^{τ}-methylhistamine (4) to an unidentified product. This product was not formed when the purification was carried out by thin layer chromatography.

EXPERIMENTAL

General

Tritiated methyl iodide and [α,β,2,4,5-$^{14}C_5$]histamine dihydrochloride were supplied by the Radiochemical Centre Ltd. S-Adenosylmethionine iodide (Grade 1) was supplied by Sigma. N,N-Dimethylformamide (Fisons Ltd) was dried over molecular sieves type 3A (BDH Chemicals Ltd) before use. Unlabelled methyl iodide (May and Baker Ltd) and all other reagents were of analytical grade.

The silica gel (type 60F_{254}) and cellulose (Avicel) chromatography plates were supplied by E. Merck and Anachem Ltd. respectively. Eluant systems used for the development of the chromatography plates contained chloroform, methanol and 0.88 ammonia solution in the following ratios by volume.

System A; 10:7:1, System B; 10:5:1, System C; 25:25:2

Liquid scintillation counting was carried out using Nuclear Enterprises Liquid Scintillation Spectrometer models 8310 or 8312 and micellar scintillator NE260. Digestion of silica gel prior to liquid scintillation counting was effected using 40% hydrofluoric acid (BDH Chemicals Ltd). The radioactive scanning of thin-layer chromatograms was performed using a Panax Thin Layer Scanner (Type RTLS-1A). Kodirex photographic paper (Kodak Ltd.) was used for autoradiography.

N^{τ}-[^{3}H]Methylhistamine dihydrochloride (2)

N^{τ}-[^{3}H]Methylhistamine dihydrochloride (2) was prepared by a method based on that described by Durant and co-workers.[6]

Unlabelled methyl iodide (32.4 µl, 74.2 mg, 0.52 mmol) was transferred

via a vacuum manifold into a limb containing [^{3}H]methyl iodide (0.93 µl, 2.1 mg, 0.015 mmol, 25 mCi, 1.68 Ci/mmol).

The resulting isotopically diluted [^{3}H]methyl iodide was transferred under vacuum into dry N,N-dimethylformamide (200 µl). After careful venting of the apparatus under dry nitrogen at -70° the [^{3}H]methyl iodide was transferred with further dry N,N-dimethylformamide (300 µl) into a glass vial containing 5-oxo-5,6,7,8-tetrahydroimidazo-[1,5-c]pyrimidine (3) (33.8 mg, 0.25 mmol) which had been prepared by the method of Mechoulam and Hirshfeld.[11]

The sealed vial was heated at 85°C for 24 hours. At the end of this time the reaction mixture was cooled to 20°C and dry ether (3ml) was added causing 2-[^{3}H]methyl-5-oxo-5,6,7,8-tetrahydroimidazo-[1,5-c]pyrimidinium iodide (6) to precipitate as a buff coloured solid. The supernatant liquid was removed and the residual solid was dried for 1 hour *in vacuo* and then heated at 100°C in the dark for 20 hours in 6M hydrochloric acid (300 µl).

Thin layer chromatography of the freeze-dried product on silica gel using System A as eluant showed the presence of the required N^{τ}-[^{3}H]methylhistamine dihydrochloride (2) together with several radiochemical impurities.

The product was purified by preparative layer chromatography on silica gel using system A as eluant, the product being extracted from the silica gel with 0.1M hydrochloric acid. The acid extract was basified with excess sodium bicarbonate and freeze-dried. The N^{τ}-[^{3}H]methylhistamine (2) was extracted from the freeze-dried solid by repeated washing with chloroform, which was in turn evaporated to dryness under a stream of nitrogen. The resulting oil was dissolved in 1M hydrochloric acid which was then freeze-dried to yield the required N^{τ}-[^{3}H]methylhistamine dihydrochloride (2) in 21% radiochemical yield

(15.6 mg, 0.080 mmol, specific activity [12] 66.9 mCi/mmol).

The radiochemical purity of the product was determined by thin layer chromatography on silica gel using System A as eluant followed by radioactive scanning. This indicated that the product (2) was chromatographically identical with authentic N^{τ}-methylhistamine and had a radiochemical purity greater than 98%.

Histamine N-methyltransferase

The enzyme was prepared from guinea-pig brain using the method of Miller and co-workers [13], except that the final pellet was dissolved in 0.01 M phosphate buffer (60 ml, pH 7.4) for overnight dialysis. The dialysate (6.8 mg protein per ml) was not freeze-dried but was frozen in aliquots using liquid nitrogen and stored at -40°C.

N^{τ}-Methyl [α,β,2,4,5-$^{14}C_5$]histamine dihydrochloride (4)

[α,β,2,4,5-$^{14}C_5$]Histamine dihydrochloride (5) (0.033 mg, 0.17 μmol, 50μCi, 290 mCi/mmol), S-adenosylmethionine iodide(3 mg, 0.006 mmol) and histamine N-methyltransferase dialysate (1 ml) were incubated in 0.05M phosphate buffer (pH 7.4, total volume 20 ml) for 7 hours at 37°C, after which time thin layer chromatography on silica gel using system B as eluant indicated a virtually quantitative conversion to the required N^{τ}-methyl[α,β,2,4,5-$^{14}C_5$]histamine dihydrochloride (4). The reaction mixture was freeze-dried and the residue taken up in a small volume of water for purification by thin layer chromatography on cellulose using system C as eluant. The area on the developed chromatogram containing N^{τ}-methyl[α,β,2,4,5-$^{14}C_5$]histamine (4) was extracted with 0.01 M hydrochloric acid which was then freeze-dried.

The residue was redissolved in 0.01 M hydrochloric acid and re-chromatographed using the same chromatographic system. The 0.01 M hydrochloric acid extract of the N^{τ}-methylhistamine band was freeze-dried and the product (4) was isolated as a solution in 0.01 M hydrochloric acid (7 ml) to give 30 μCi of radioactivity in a 60% radiochemical yield (specific activity assumed to be unchanged at 290 mCi/mmol).

Thin layer chromatography on silica gel using eluant system B and cellulose using eluant system C indicated that the product (4) was chromatographically identical with authentic N^{τ}-methylhistamine dihydrochloride with a radiochemical purity of not less than 95%. [α,β,2,4,5-$^{14}C_5$]Histamine dihydrochloride (5) if present accounted for less than 0.5% of the total radioactivity.

ACKNOWLEDGEMENT

We wish to thank Ms. Carol Dent for technical assistance

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