Synthesis of 6-(1-Methyl-4-nitro-5-[4,5-^{14}C]imidazolyl)thiopurine (Azathioprine).

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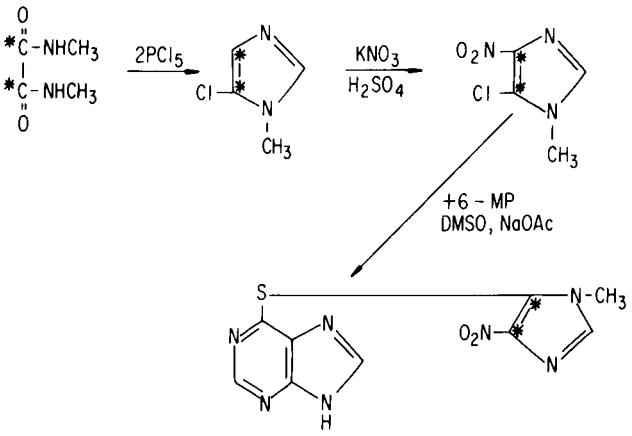
Azathioprine (6-(1-methyl-4-nitro-5-imidazolyl)thiopurine, "Imuran") is widely used for the suppression of the immune response in renal transplantation. It was originally synthesized and studied as a "masked" form of the antimetabolite 6-mercaptopurine (6-MP), which is released from azathioprine by various nucleophiles both *in vitro* and *in vivo* (1-3). Metabolic studies of azathioprine in the past have used ^{35}S-azathioprine, prepared by the reaction of ^{35}S-6-mercaptopurine with 5-chloro-1-methyl-4-nitroimidazole. This made it possible to investigate the fate of the purine moiety of azathioprine, which was found to be metabolized, qualitatively but not quantitatively, like 6-MP itself (4,5). In addition, one ^{35}S-labeled imidazole, 1-methyl-4-nitro-5-[^{35}S]thioimidazole, was identified among the urinary metabolites. This product accounted for approximately 10% of the methyl-nitroimidazole moiety of the azathioprine administered. In order to follow the metabolic fate of the major portion of the methyl-nitroimidazole moiety, it was necessary to synthesize azathioprine labeled in that portion of the molecule. Since demethylation is often encountered in metabolic processes, it seemed advisable to have the radioactivity present in the ring carbons of the methyl-nitroimidazole.

The steps for the radioactive synthesis were similar to those used for the synthesis of non-radioactive azathioprine (1) (Scheme I). However, unexpected difficulties were encountered in the first step when attempts were made to conduct the reaction on a 2.5 millimolar scale. The reaction of *N,N'*-dimethyloxamide with 2 molecular equivalents of phosphorus pentachloride to give 5-chloro-1-methylimidazole was originally described by Wallach (6). It is a complex reaction, the exact mechanism of which is still not understood. On a large scale, when *N,N*-dimethyloxamide is mixed with dry phosphorus pentachloride, the reaction is exothermic and proceeds spontaneously or with slight warming (7) in 60-70% yield. In addition to the formation of the principal product, 5-chloro-1-methylimidazole, a few percent of the 4-chloro-1-methyl isomer is also formed. When model experiments were conducted with 3 g. (25.8 mmoles) of *N,N'*-dimethyloxamide, yields for this ring



closure varied from 33 to 50%. However, when the size of the batch was reduced to the 2.5 mmole scale, the yields became very erratic and sometimes fell to zero. It was apparent that not enough heat was being generated by this usually highly exothermic reaction to proceed normally.

A variety of conditions was tried in an attempt to provide the degree of heat necessary to accomplish the ring closure on a 280 mg. scale. These investigations revealed that overheating was deleterious, especially at the beginning of the reaction. The procedure which was finally found to give the best and most reproducible yields consisted of preheating a mixture of phosphorus pentachloride and phosphorus oxychloride to 60° before adding the *N,N'*-dimethyloxamide, and then raising the temperature slowly and keeping it elevated for about 2 hours.

The 5-chloro-1-methylimidazole was isolated by chloroform extraction, after removal of the phosphorus oxychloride. In order to obtain the maximum recovery of product, non-radioactive product was added after the initial extractions and the solutions were re-extracted with chloroform. The yield for this first step was estimated to be about 70%. No attempt was made to remove any 4-chloro-1-methylimidazole which may have formed, since this was known to be only a minor contaminant and would be diluted out still further in subsequent steps.

The nitration step was carried out with a 20% excess of potassium nitrate in concentrated sulfuric acid overnight on a steam bath. This is a modification of the method of Sarasin and Wegmann (8) who used the nitrate salt of 5-chloro-1-methylimidazole. The nitro derivative, unlike 5-chloro-1-methylimidazole itself, has a distinctive ultraviolet absorption maximum at 295 nm at *p*H 1, which enables one to follow the progress of the nitration and to determine the specific activity of the product.

The final step, condensation of 5-chloro-1-methyl-4-nitroimidazole with 6-MP, proceeded in over 85% yield in dimethylsulfoxide in the presence of anhydrous sodium acetate. The total yield of radioactive azathioprine was 0.57 mCi, 23.7% of the radioactivity of the starting material, *N,N'*-dimethyloxamide.

The ^{14}C-azathioprine prepared in this manner has been used for metabolic studies in animals and in man, and several 5-substituted-1-methyl-4-nitroimidazoles have been identified as metabolic products (9,10). These investigations are continuing.

EXPERIMENTAL

5-Chloro-1-methyl[4,5-^{14}C]imidazole.

A mixture of 1.055 g. (0.51 mmole) of phosphorus pentachloride and 3 ml. of phosphorus oxychloride was heated to 60° in a 50 ml. round-bottomed flask, in an oil bath, with magnetic stirring. To this hot mixture was added 280 mg. (2.4 mmoles) of *N,N'*-dimethyloxamide [1,2-^{14}C] (1 mCi/mmole) (11). A considerable amount of gas evolution ensued and the internal temperature rose to 63°. The temperature was raised slowly during 1 hour to 100° and maintained at that temperature for 45 minutes, followed by an additional 75 minutes at 92-94°. The reaction mixture was then allowed to remain in the oil bath until it cooled to 40° (1 hour). Approximately one-half of the phosphorus oxychloride was removed by distillation under reduced pressure. The residue was diluted with 20 ml. of ice water and make alkaline (*p*H 9) with 15 *N* sodium hydroxide. The solution was extracted with 6 x 40 ml. of chloroform. The chloroform extracts were dried over anhydrous sodium sulfate. At this time, the aqueous solution still contained 2.5 x 10^8 dpm (0.12 mCi) of radioactivity. On the assumption that this may have represented unextracted 5-chloro-1-methylimidazole, 32 mg. of the non-radioactive compound was added to the aqueous solution and re-extracted with 4 x 40 ml. of chloroform. This process of dilution with 32 mg. of non-radioactive product was then repeated. Following this, the aqueous solution still contained 0.1 mCi of radioactivity, indicating that the residual radioactivity was not due to 5-chloro-1-methylimidazole.

All of the chloroform extracts were combined and dried over sodium sulfate. The chloroform was removed by evaporation under reduced pressure. The residual yellow oil was dissolved in 20 ml. of dry ether and filtered to remove 5 mg. of a white solid, which proved to be unchanged dimethyloxamide. The ethereal solution was evaporated to give 260 mg. of yellow oil. Since 64 mg. of non-radioactive product had been added, the yield of radioactive material was calculated to be 196 mg. (70%). This material was used for the next step, nitration, without any further characterization or purification.

5-Chloro-1-methyl-4-nitro[4,5-^{14}C]imidazole.

To 260 mg. (2.23 mmoles) of crude 5-chloro-1-methylimidazole were added 300 mg. (2.7 mmoles) of potassium nitrate and 3 ml. of concentrated sulfuric acid. The mixture was protected from moisture with a drying tube and heated on a steam bath overnight. The pale brown mixture was cooled, diluted with 20 g. of ice, and extracted with 5 x 50 ml. of chloroform. The aqueous layer retained 1.4 x 10^8 dpm, but did not show a significant absorption at 285 nm. Consequently, these counts were judged not to be due to the nitroimidazole.

The chloroform extracts were combined, dried over sodium sulfate and evaporated to dryness. The residue, 220 mg., of 5-chloro-1-methyl-4-nitroimidazole showed a λ max = 295 nm at *p*H 1. The specific activity was determined on the basis of the concentration as determined by the ultraviolet absorption spectrum. At *p*H 1, λ max = 295 nm, E_m = 7,500. The specific activity of the product was 8.5 x 10^3 dpm/μg or 0.61 mCi/mmole. If the 220 mg. of 5-chloro-1-methyl-4-nitroimidazole isolated were pure, this would represent 0.844 mCi, a yield of 34.7% based on the radioactivity of the starting *N,N'*-dimethyloxamide. This material was diluted with 280 mg. of non-radioactive 5-chloro-1-methyl-4-nitroimidazole for use in the next step.

6-(1-Methyl-4-nitro-5-[4,5-^{14}C]imidazolyl)thiopurine.

A mixture of 471 mg. (3 mmoles) of anhydrous 6-mercaptopurine, 500 mg. of 5-chloro-1-methyl-4-nitro[4,5-^{14}C]imidazole and 250 mg. of anhydrous sodium acetate in 7.5 ml. of dry dimethylsulfoxide was heated in a round-bottomed flask, with magnetic stirring, in an oil bath at 97-100° for 4 hours. At that time, a drop was removed from the reaction mixture and diluted with 0.1 *N* hydrochloric acid to an appropriate concentration for measuring the ultraviolet absorption spectrum. The ratio of O.D. at 280 nm to O.D. at 325 nm was 2.9 (theory = 3.4). The mixture was allowed to cool slowly in the oil bath for 1 hour and was then poured into 40 ml. of water and cooled in an ice bath. The yellow precipitate was collected, washed with 10 ml. of ice water, and dried at 100° *in vacuo* (720 mg., 84%). The ultraviolet absorption spectrum at *p*H 1, showed a ratio of $\frac{OD_{280}}{OD_{325}} = 3.2$, indicating less than 1% contamination with 6-mercaptopurine. Based on a λ max = 280 nm, E_m = 16,900 at *p*H 1, the product had a specific activity of 1560 dpm/μg (0.195 mCi/mmole). It was radiochemically homogeneous, as judged by ascending paper chromatography in the biphasic system of 5% disodium hydrogen phosphate-isoamyl alcohol (R_f = 0.53). When the material was eluted from the paper chromatogram, its specific activity was unchanged. The R_f of 5-chloro-1-methyl-4-nitroimidazole in this solvent was 0.65.

An additional 100 mg. of 6-(1-methyl-4-nitro-5-[4,5-^{14}C]-imidazolyl]thiopurine, of specific activity 700 dpm/μg, was obtained by adding 75 mg. of non-radioactive material in 2 ml. dimethylsulfoxide to the aqueous filtrate from the main batch and collecting the precipitate after three hours. The total yield of radioactive product was 0.57 mCi (23.7%).

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