[From the University of California, Los Alamos Scientific Laboratory]

MICRO-SYNTHESES WITH TRACER ELEMENTS. II. METHYLDI-(BETA-CHLOROETHYL)AMINE HYDROCHLORIDE (NITROGEN MUSTARD) AND METHYL BROMIDE LABELED WITH C^{14} [1]

W. W. FOREMAN, ARTHUR MURRAY, III, and ANTHONY R. RONZIO

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"Nitrogen Mustard", methyldi-(*beta*-chloroethyl)amine hydrochloride, has received a great deal of attention because of its use in the treatment of Hodgkin's disease. It is now recognized that lymphatic tumors which are responsive to x-radiation will also respond to treatment with nitrogen mustard.

In addition, the physiologic effects of excessive doses of the mustard are so strikingly similar to the effects of radiation injury that the possibility of investigating the mechanism of this type of cell damage using labeled nitrogen mustard has been proposed.

The synthesis herein reported leads to nitrogen mustard labeled with C^{14} in the methyl group. Labeled methanol was converted to methyl bromide using concentrated hydrobromic acid. Methyl bromide was then condensed with diethanolamine. Methyl iodide does not work satsifactorily in this reaction, since it is decomposed by the diethanolamine. When equal mole ratios of methyl bromide and amine were used, an appreciable percentage (20–35%) of the amine was converted to the quaternary salt. Upon halogenating with thionyl chloride a mixture of products was obtained which were exceedingly difficult to separate from each other. However, a mole ratio of halide to amine of 1:5 gave no amine higher than the tertiary amine desired.

Attempts at separating the tertiary from the secondary amine using acetylation, benzoylation, and the Hinsberg reaction were unsuccessful. The isocyanates were also useless for this purpose. Fractional recrystallization and fractional sublimation gave only partial separation. Treatment with nitrous acid gave a good separation, doubtlessly accompanied, however, by some decomposition.

EXPERIMENTAL

Methyl bromide labeled with C^{14}. Flask *A* containing 5 ml. of HBr-H_2SO_4 mixture (made by the dropwise addition of 3.6 ml. of conc'd H_2SO_4 to 23.03 g. of chilled 48% HBr) and a glass-enclosed iron slug was attached to the manifold at the point indicated in Figure 1. After a brief initial period of outgassing, the flask was chilled with liquid nitrogen, the system evacuated to 10^{-4} mm, and the stopcock *G* closed. Methanol (0.8–10 *mM*) contained in "breakofsky" tubes *B* sealed to a manifold was attached to the line at *C*. The entire system was evacuated under high vacuum and closed off from the vacuum manifolds. With stopcock *D* closed, the methanol was distilled into *A* by chilling flask *A* with liquid nitrogen. Flask *A* was allowed to warm to room temperature and then stirred continuously by means of a magnetic stirrer *I*, while the temperature was gradually brought to 100° with oil-bath *H* (1.25 hours). The mixture was then cooled to room temperature and the product distilled into *E* through the purifying train *F* consisting of soda-lime and P_2O_5 on glass wool.

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E was a light flask of 100-ml. capacity equipped with a micro stopcock. Quantitative transfer was attained by cooling *E* with liquid nitrogen. Any non-condensible gas present in the system was pumped off. The transfer of known amounts of methyl bromide by this method was quantitative (99.8 %). The yields of methyl bromide obtained were 94–96%; the product was checked for purity by analysis for Br.

The oil-bath heater was made by wrapping resistance wire around a crystallizing dish; voltage was controlled with a variable transformer. An HBr-H_2SO_4 reagent of mole ratio 1:1.55 gave slightly lower yields than the one used which corresponds to a 1:2.06 ratio.

Experiments using PBr_3 for the brominating agent resulted in yields of 60–70% of theory with runs on the 10-millimole level.

Methyldi-(beta-chloroethyl)amine hydrochloride. Methyl bromide, made from 3.86 *mM.* of methanol labeled with C^{14}, was transferred *in vacuo* in the vapor phase into a 25-ml. reaction flask *S*, Figure 2, attached to a vacuum line, by cooling the flask with liquid nitrogen. Inside the flask were a short glass-enclosed magnet and an outgassed solution of 2.1065 g. (20.04 *mM.*) of diethanolamine dissolved in 15 ml. of absolute ethanol. Stopcock *T* was closed and the mixture in flask *S* was melted as quickly as possible with the magnetic stirrer set for maximum stirring. Stirring was maintained continuously for 42 hours. At the end of this period about ¼ of the solvent was distilled into receiver *U*, containing an alcoholic solution of silver nitrate. The receiver was then stoppered and the contents of the flask were digested 48 hours at 45°. There was no trace of silver halide, indicating that the methylation reaction had been quantitative. A slight excess of potassium hydroxide over that required to free the base was dissolved in absolute alcohol and added to the cooled reaction mixture. After allowing the mixture to stir ½ hour in an ice-bath, the precipitated potassium bromide was filtered, washed several times with absolute ethanol, dried, and weighed. Yield, 0.4376 g. (95.5%) of methyl bromide from the labeled methanol. The filtrate which contained a mixture of methylated and unmethylated diethanolamine was saturated with hydrogen chloride and then concentrated to a small volume with a gentle stream of air. If too large an excess of potassium hydroxide was used, the volume of KCl at this stage was excessive and it was filtered off through a layer of diatomaceous earth. A small amount of precipitate did no harm and did not require removal. The combined filtrate and washings were transferred to a 60-ml. flask and concentrated as before. The degree of removal of ethanol was checked by weighing.

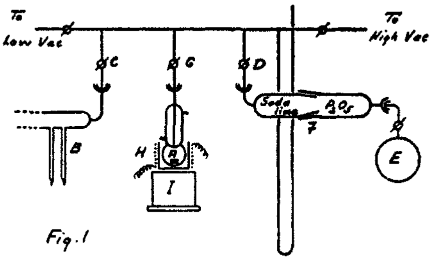

Fig. 1

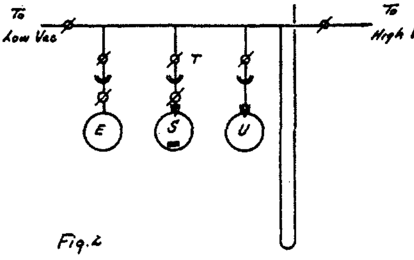

Fig. 2

The flask was equipped with a reflux condenser and mechanical stirrer. Twenty-five ml. of benzene was added to the flask and the mixture was saturated with anhydrous hydrogen chloride. Stirring was begun and the calculated amount of purified thionyl chloride, based upon the methylated and unmethylated diethanolamine and the residual ethanol remaining in the mixture (5 ml. in all in this experiment) was added in small portions through the condenser.

At the end of the reaction, the mixture was warmed to 60° for 3.5 hours. The mixture was opalescent at the beginning of the reaction, then became successively opaque, gummy, and finally crystalline. When evolution of hydrogen chloride ceased, an additional 2 ml. of thionyl chloride was added in 0.5-ml. portions. Stirring was maintained continuously throughout the reaction. When reaction again ceased, the excess thionyl chloride was destroyed by the dropwise addition of the calculated amount (1.4 ml.) of absolute methanol to the cooled mixture. Care was exercised to avoid any excess of methanol since this exerted some solvent action on the product. The mixture was then treated with 20 ml. of benzene, cooled in an ice-bath, filtered through a sintered-glass funnel, and washed with several 5-ml. portions of cold benzene. The crystalline mustards were roughly separated from each other by extraction with boiling chloroform in the following manner. The crystalline mass was transferred to the original flask, stirred briefly with 30 ml. of boiling chloroform, and allowed to cool for 15 minutes, while being stirred, before filtration. This was repeated four times. After the fourth extraction the residue, wt. 2.2149 g., melted at 209–212.5° (m.p. of pure $HN(C_2H_4Cl)_2 \cdot HCl$ is 214–215°) and was discarded. This quantity of unmethylated nitrogen mustard corresponded to 76.9% of the excess diethanolamine originally added to the reaction.

The chloroform was removed by evaporation at room temperature and the crude product was quantitatively transferred to a liquid-liquid extractor, dissolved in 8–10 ml. of water, and acidified with 1 ml. of conc'd. HCl. The solution was cooled in an ice bath, and 0.4 g. of sodium nitrite dissolved in the minimum amount of water was slowly added. After one hour, the nitrosoamine was removed by continuous extraction with ether for seven hours. The almost colorless raffinate was concentrated to a small volume in a gentle stream of air Final drying was accomplished by placing the residue first in a desiccator over $CaCl_2$, then over P_2O_5.

The pale yellow, powdery residue was extracted six times with boiling chloroform in order to separate the mustard from sodium chloride. The chloroform was removed by evaporation in a gentle stream of air. The crude product was dried over P_2O_5, then was dissolved in 10 ml. of pure acetone, decolorized with Norit, and filtered through a small sintered-glass funnel into a 30-ml. centrifuge cone. The cone was stoppered and the product was crystallized by cooling to −25°. After removing the mother liquor using a filter stick, the crystals were washed with several 10-ml. portions of absolute ether. The product was recrystallized by solution in 17 ml. of acetone followed by cooling to −40°. The yield from 2 runs made on this scale, using a total of 17 mc. of methanol (0.2225 g.) was 0.6995 g. (yield 52.5% based on methanol); m.p. 109–110°. None of the acetone mother liquors was reworked for a second crop of crystals.

The nitrogen mustard (cold run) yielded a chloroaurate from water solution, which, recrystallized three times from water, melted at 82–84°.

Anal. Calc'd for $CH_3N(CH_2CH_2Cl)_2 \cdot HAuCl_4$: Au, 39.80.
Found: Au, 39.50.

Methyldi-(*beta*-chloroethyl)amine hydrochloride is volatile with boiling organic solvents. When 0.0148 gram was placed in a distilling flask with dry benzene (250 ml.) and 200 ml. of benzene was distilled, the residue remaining in the flask amounted to 0.0014 g., indicating that over 90% had co-distilled with the benzene.

In neutral water solution the nitrogen mustard will undergo hydrolysis. Attempts to evaporate such solutions resulted in the loss of mustard. This hydrolysis can be totally prevented by acidifying with HCl before evaporation.

Dimethyldi-(beta-chloroethyl)ammonium bromide. When the mole ratio of methyl bromide to diethanolamine was 1:1, a mixture was obtained after treatment with thionyl chloride as described above, which left an insoluble product when extracted with acetone. After several recrystallizations from acetonitrile, colorless crystals were obtained which melted at 217–218°; yield, 20–35%. Analysis indicated the compound was the quaternary amine hydrobromide.

Anal. Calc'd for $(CH_3)_2N(C_2H_4Cl)_2 \cdot HBr$: N, 5.55. Found: N, 5.52, 5.58.

The acetone-soluble fraction was a mixture of methylated and unmethylated nitrogen mustard hydrobromide and hydrochloride.

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SUMMARY

Methyl bromide labeled with C^{14} has been synthesized from labeled methanol. Methyldi-(*beta*-chloroethyl)amine hydrochloride, a nitrogen mustard, has been labeled at the methyl group with C^{14}. The compound obtained by this synthesis possessed an activity corresponding to 22 μc. per milligram.

Los Alamos, New Mexico