We are also grateful to Dr. G. J. M. van der Kerk (Organisch Chemisch Laboratorium, Rijksuniversiteit, Utrecht) who very kindly prepared the samples of inactive iodoxybenzene used in this investigation.

AMSTERDAM, NETHERLANDS RECEIVED JULY 18, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, BOWMAN GRAY SCHOOL OF MEDICINE, WAKE FOREST COLLEGE]

Preparation of C^{14}-Methyl Labeled Dimethylaminoethanol

BY CAMILLO ARTOM AND MARIETTA CROWDER

2-Methylaminoethanol (1 mM./ml.) is refluxed for 6 hours with C^{14}-formaldehyde (1 mM./ml.). Unreacted 2-methylaminoethanol and formaldehyde are destroyed with nitrous acid and alkaline iodine, respectively. C^{14}-Dimethylaminoethanol is recovered by two successive steam distillations (yield 40% based on the isotopic formaldehyde). The solution is of isotopic purity sufficient for biological experiments. No dimethylaminoethanol is formed at pH < 7, unless formic acid is also added.

In the course of our studies on the role of dimethylaminoethanol (DMA) as a likely intermediate in choline metabolism,[1-3] C^{14}-methyl-DMA was prepared by refluxing 2-methylaminoethanol (MMA) with C^{14}-formaldehyde (FA) in alkaline solution.

$$CH_3NHCH_2CH_2OH + 2HC^{14}HO \longrightarrow C^{14}H_3CH_3NCH_2CH_2OH + HC^{14}OOH$$

The highest yield, based on the FA, was obtained with about 1 mM. each of FA and MMA per ml. of solution, and was but very slightly increased by addition of formate. No DMA was formed at pH < 7, unless formic acid was also added. A comparison between these conditions and those of other methods for the reductive methylation of amines[4-7] may be of some theoretical interest. From the practical viewpoint, the present procedure, in spite of its lower yield, is extremely simple and the product is of a sufficient isotopic purity to be used directly for most biological experiments.

Experimental

For the radioactive measurements (thin mica window or Q-gas flow counter), both standard and unknown samples were brought to a uniform weight by adding the proper amounts of inactive materials (egg lipides), and were spread as thin films onto aluminum dishes covered by lens paper.

2-Dimethylaminoethanol.—Into a small flask fitted with a ground glass condenser, 2 ml. of a 2.5 *M* solution of 2-methylaminoethanol, b.p. 158–160° (5 mM.), 1.8 ml. of a solution of C^{14}-labeled formaldehyde[8] (300 microcuries, 4.6 mM.) and 0.7 ml. of alkaline borate, pH 10.0 (6.2 g. of boric acid in 100 ml. of *N* NaOH) are added. The mixture is refluxed for 6 hours, cooled and neutralized with 2 *N* HCl. The unreacted 2-methylaminoethanol is destroyed by adding 10 ml. of 30% sodium nitrite and 5 ml. of glacial acetic acid. After 10 minutes in ice, the mixture is made alkaline with saturated NaOH, aerated vigorously for 0.75 hour (to remove the oxides of nitrogen), then steam distilled (Parnas–Wagner apparatus[9]) for 0.5 hour into 4 ml. of *N* HCl. To eliminate traces of C^{14}-formaldehyde which may be carried over in the distillate, 1 ml. of 1% inactive FA, 1 ml. of *N* iodine and a slight excess of *N* NaOH are added. After acidification, the excess iodine is reduced with 5% $NaHSO_3$. The solution is concentrated to a few ml., made alkaline and steam distilled again into 4 ml. of *N* HCl. The distillate contained 1.87 mM. of DMA (by titration: a 40.6% yield, based on the added FA) and 41.0% of the counts introduced as C^{14}-FA.

The extent to which other isotopic products, introduced or formed during the reaction, had been eliminated was determined. Inactive formaldehyde, formic acid, or methanol was added to separate aliquots of the solution of isotopic DMA. The mixtures were acidified and distilled. In the distillates the per cent. recoveries of C^{12} and C^{14} (measured as $BaCO_3$ after oxidation with cold alkaline $KMnO_4$) were, respectively, formaldehyde, 97 and 0.08; formic acid, 90 and 0; methanol, 99 and 0.09.

From both active and inactive materials the picrolonic acid derivative was prepared[10] with variable yields (60–95%, depending on the amounts of DMA present or added as a carrier). The m.p. was 196° (unchanged by admixture with an authentic sample of DMA picrolonate; reported,[11] 197°). Within the limits of error of our measurements, the specific activity of the picrolonate was the same as that calculated for the DMA in solution and was not changed after several recrystallizations from water and alcohol.

Anal. Calcd. for $C_{14}H_{19}N_5O_6$: C, 47.59; H, 5.42; N, 19.82. Found: C, 47.48, 47.69; H, 5.35, 5.58; N, 19.45, 19.71.

Acknowledgment.—This work was done under contract No. AT-(40-1)-288, Title II, between the Bowman Gray School of Medicine and the U. S. Atomic Energy Commission. The authors wish to express their appreciation to Mr. H. Wainer for technical help and to Dr. C. Tiedcke for the microanalyses on the DMA picrolonate.

WINSTON-SALEM, N. C. RECEIVED AUGUST 22, 1951

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