Synthesis of Methylglyoxal-^{14}C

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Methylglyoxal-1,3-^{14}C and methylglyoxal-2-^{14}C of relatively high specific activity were prepared by the oxidation of acetone with selenium dioxide followed by distillation under a nitrogen atmosphere and concentration under reduced pressure. Analysis of the synthesized compounds by melting point determination and also by paper and gas-liquid chromatography indicated the samples were of high purity.

THE OCCURRENCE of methylglyoxal in the form of an aberrant metabolite in vitamin B_1 deficiency has held the interest of many investigators (1–8) in the field of nutrition and carbohydrate metabolism. Its existence in the aminoacetone cycle (9), its accumulation in dystrophic muscle (10), and its possible role as a carcinostatic agent (11) have resulted in a revived interest in this compound.

In order to study the metabolic pathway and fate of this compound in animals, radioactive methylglyoxal-1,3-^{14}C and methylglyoxal-2-^{14}C were prepared.

Three methods were considered for the preparation of the labeled compound. (*a*) The conversion of acetone to isonitroacetone and warming the latter with dilute sulfuric acid (12). (*b*) The distillation of dihydroxyacetone in a dilute aqueous solution from calcium carbonate or by *in vacuo* distillation from phosphorus pentoxide (13). (*c*) The oxidation of acetone with selenium dioxide followed by distillation under a nitrogen atmosphere and concentration under reduced pressure (14, 15).

Both methylglyoxal-2-^{14}C ($CH_3{}^{14}COCHO$) and methylglyoxal-1,3-^{14}C ($^{14}CH_3CO{}^{14}CHO$) may be obtained by method (*a*) utilizing ^{14}C-labeled acetone as a precursor. The preparation by method (*b*) requires ^{14}C-labeled dihydroxyacetone which can be prepared enzymatically utilizing ^{14}C-labeled glycerol as a precursor. Both methods (*a*) and (*b*) result in the formation of the following contaminants which are difficult to separate from methylglyoxal: glycols, formaldehyde, and formic acid.

These disadvantages do not exist for method (*c*) which was, therefore, selected for the synthesis of methylglyoxal-^{14}C.

Methylglyoxal-1,3-^{14}C was prepared from 0.5 mc. of acetone-1,3-^{14}C with a specific activity of 9.1 mc./mmole.[1] Methylglyoxal-2-^{14}C was prepared from 0.5 mc. of acetone-2-^{14}C with a specific activity of 5.4 mc./mmole.[1]

METHOD

Procedure for Refluxing.—To a 125-ml. conical flask containing 16.0 ml. (0.19 mole) of *p*-dioxane and 1.1 ml. of water was added 3.04 Gm. (0.027 mole) of purified selenium dioxide. The mixture was slowly brought up to 50° and maintained at this temperature while being constantly stirred by a hot plate magnetic stirrer. The selenium dioxide was completely dissolved in 20 min. The solution was cooled to 25° and transferred to a 50-ml. pear-shaped distillation flask. To this solution was added 2.0 ml. of the radioactive acetone and carrier. A water-cooled upright Leibig condenser was attached to the flask and the mixture was allowed to reflux gently for 4 hr. in an oil bath maintained at 100°. At the completion of the reflux period the solution was dark and tarry in appearance.

Procedure for Distillation.—On completion of the refluxing the distillation flask was removed and attached to a distillation assembly. A capillary was introduced into the distillation flask and served as an inlet tube for the admission of nitrogen gas at a pressure slightly above atmospheric. During the entire distillation process nitrogen gas was allowed to slowly bubble in to prevent the oxidation of methylglyoxal. The mixture was distilled over an oil bath by slowly raising the temperature to 130°. Three separate distillate fractions were collected in an ice bath.

Fraction I consisted of 5 ml. of a clear distillate collected at a vapor temperature of 80 to 90°. It was found to contain less than 5% methylglyoxal as determined by the *m*-nitrobenzhydrazide reaction.

Fraction II consisted of 8 ml. of a pale yellow distillate collected at a vapor temperature of 90 to 100° and contained a 15% solution of methylglyoxal in water and dioxane.

Fraction III consisted of 5 ml. of a distillate collected at a vapor temperature of 100 to 105° and contained a mixture of dioxane, water, oily droplets, and an unstable selenium methylglyoxal complex.

By collecting three fractions it was found that the highest yield came over in fraction II at a vapor temperature of 90 to 100°. Fractions I and III which distilled over at lower and higher temperatures, respectively, produced lower yields and indicated that the ideal temperature range for maximum yield was that of fraction II.

Procedure for Concentration.—The distillate fractions were transferred to a 50-ml. pear-shaped flask and attached to a vacuum system containing two 29 × 200 mm. culture tubes connected in series and immersed in Dewar flasks containing liquid nitrogen. These tubes served as traps for the lower boiling solvents.

At the end of 6 hr. of vacuum distillation at 50 mm. of mercury, water and dioxane were removed and 2 ml. of a solution pale yellow in color and syrupy in consistency remained.

Quantitative Determination of Methylglyoxal-^{14}C as the m-Nitrobenzoylosazone Derivative.—The methylglyoxal-1,3-^{14}C and methylglyoxal-2-^{14}C yields and specific activities were determined by diluting 25.0 μl. of the concentrate in 100 ml. of water and then adding 10 ml. of *m*-nitrobenzhydrazide reagent (16). The precipitate formed was quantitatively transferred to preweighed stainless steel planchets, dried to constant weight, and the radioactive samples counted using a proportional flow counter with a laboratory scaler. A 45.6% yield

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[1] Both ^{14}C labeled acetone precursors were obtained from Nuclear-Chicago Corp., Des Plaines, Ill.

of methylglyoxal-1,3-^{14}C with a specific activity of 9.0 mc./mmole and a 16.6% yield of methylglyoxal-2-^{14}C with a specific activity of 5.0 mc./mmole was obtained.

The purity of the radioactive compounds was assayed by melting point determinations, paper, and gas-liquid chromatography.

The melting point of the 2,4-dinitrophenylhydrazine derivatives of the ^{14}C-labeled methylglyoxal determined on a Fisher-Johns apparatus was found to be 297–298°, uncorrected, in agreement with the theoretical value (17).

Analysis by paper chromatography (18, 19) was carried out with the following modifications. To a solution containing 50.0 mcg. of methylglyoxal-^{14}C was added 0.2 ml. of a 2,4-dinitrophenylhydrazine reagent (0.1% in 2 *N* hydrochloride). After the mixture was allowed to stand for 1 hr., 1 ml. of chloroform was added. The hydrazone formed was extracted by vigorous shaking followed by a 5-min. period of centrifugation at 2000 r.p.m. to separate the layers.

A 100.0 μl. aliquot of the chloroform layer was spotted on Whatman No. 1 paper. The ascending technique was employed and the development solvent used was *n*-butanol–ethanol–aqueous 2 *N* ammonium hydroxide (7:1:2, by volume). After 16 hr., the paper was dried at room temperature in a fume hood and sprayed with alcoholic sodium hydroxide solution (2% sodium hydroxide in 90% ethanol).

The colored spots were identified by comparison with the color and R_f values of a known standard run simultaneously.

Two isomers of methylglyoxal were observed: a blue-violet spot with an R_f value of 0.00 and a pinkish-brown spot with an R_f value of 0.90. Values were in agreement with those reported in the literature (19).

The colored spots identified as methylglyoxal were cut from the paper, macerated 3 times with 0.5-ml. aliquots of 1 *N* ammonium hydroxide, and each quantity was centrifuged. The supernatants of the washings were pooled, transferred to a planchet, evaporated to dryness, and counted. All samples prepared in this manner and identified as methylglyoxal-^{14}C were found to be highly radioactive.

The samples of ^{14}C-labeled methylglyoxal and standards were successfully analyzed by gas-liquid chromatography. The purpose of analyzing the radiolabeled methylglyoxal-^{14}C by gas chromatography was to determine its purity and not its radioactivity since the radioactivity of the compound had previously been established by both paper chromatography and as the *m*-nitrobenzoylosazone of methylglyoxal-^{14}C. The instrument used was a Perkin-Elmer gas chromatograph model 801 with a 6 ft. × 0.085 in. i. d.

The stationary phase used was SE 30/EPON 1001 with a gas-chrom P as the support (ratio of 4/0.2%). Helium gas was used as the carrier gas and introduced at a flow rate of 40 ml./min. The instrument utilized a flame detector and the analysis was carried out using hydrogen gas at 18 psig and air at 46 psig. The temperature of the injection block was 280°, detector 220°, and the initial temperature of the column 200°. A Leeds and Northrop model G (5 MV range) was used and the data recorded at a chart speed of 0.5 in./min.

One major peak was recorded with another following 2.0 cm. behind which represented the other isomer. There was no decomposition, little tailing, and the purity of the methylglyoxal-^{14}C appeared satisfactory from a quantitative as well as qualitative point of view.

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