Preparation of 5-iodo-2′-deoxycytidine*

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RECENT studies on the biological activities of 5-iodo-2′-deoxyuridine[1-4] and 5-bromo-2′-deoxycytidine[5, 6] have indicated that it should be advantageous to have 5-iodo-2′-deoxycytidine available for comparable studies; however, iodination of 2′-deoxycytidine does not occur as readily as in the case of bromination. Iodination in dilute nitric acid, under similar conditions to those used in the preparation of 5-iodo-2′-deoxyuridine,[1] produced only 5-iodo-cytosine. The standard basic iodination procedure (in the presence of potassium hydroxide and potassium iodide), similar to that used by Johnson and Johns[7] for the iodination of cytosine, led to the production from 2′-deoxycytidine of deaminated materials.

It has been found that 2′-deoxycytidine can be conveniently iodinated in the presence of iodic acid using a method resembling that of Wirth *et al.*[8] for the iodination of aromatic compounds.† The preparation of several batches of the new compound has indicated that solvent, temperature, and the concentration of reactants influence the yield of 5-iodo-2′-deoxycytidine; as a solvent, glacial acetic acid gives a better yield than does water. A considerable amount of 2′-deoxycytidine is converted to a by-product that contains iodine, but does not absorb in the ultraviolet region; the elementary analysis of this substance suggests the formula, $C_9H_{12}O_5N_2I_2$ (possibly 5:6-di-iodo-5:6-di-hydro-2′-deoxyuridine); in addition, a small amount of 5-iodo-cytosine is formed.

In the preparation, on a micromole scale, of 5-iodo-2′-deoxycytidine-^{3}H from tritiated 2′-deoxycytidine, the radioactive compound was purified by the use of a Dowex-1 formate column. Prompt elution of the column was necessary in order to minimize cleavage of the deoxyribonucleoside.

EXPERIMENTAL‡

5-Iodo-2′-deoxycytidine. A mixture of 2′-deoxycytidine hydrochloride§ (2·6 g, 0·01 mole), glacial acetic acid (8 ml), iodic acid (0·9 g), iodine (1·5 g), carbon tetrachloride (2 ml) and water (3 ml) was stirred at 40 °C for 90 min; at this time the iodination was essentially complete (on the basis of ultraviolet absorption, iodination continued to about 80 per cent of the theoretical value). To the now clear solution, water (30 ml) was added and the aqueous solution (after filtering off any solids (0·48 g; by-product) present) was extracted several times with carbon tetrachloride (400 ml). The aqueous phase, after concentration *in vacuo* at 40 °C to dryness, was dissolved in methanol (40 ml) and again concentrated *in vacuo* at 40 °C to dryness. This operation was repeated 5 times in order to remove most of the acetic acid. The yellow crystalline residue obtained was taken up in water (30 ml) and the solution was adjusted to pH 10 with sodium hydroxide (10 N); 5-iodo-2′-deoxycytidine began to precipitate out. Refrigeration of the mixture produced the crude product (1·94 g). After one recrystallization from water, the compound (1·69 g) melted with decomposition at 175–178 °C; λ_{max}^{pH2} 308–309 mμ (ξ 8,150); $\lambda_{max}^{H_2O}$ 293 mμ (ξ 6,020); λ_{max}^{pH11} 294 mμ (ξ 5,930); $\lambda_{max}^{95\%\ EtOH}$ 294 mμ (ξ 5,750). *Analysis*—calcd. for $C_9H_{12}O_4N_3I$: C, 30·61; H, 3·43; N, 11·90; I, 35·94; found: C, 30·68; H, 3·52; N, 11·95; I, 35·82. The mother liquor and all the recrystallization filtrates were combined and the solution was adjusted to pH 10 with sodium hydroxide (10 N). The solution was then put on a column of charcoal||, 2·5 × 12 cm; this was washed quickly with distilled water until free of halides (4–6 l.). The product was eluted from the column with 95% ethanol (5–7 l.) and the solvent was removed *in vacuo* at 40 °C. The residue crystallized to give additional 5-iodo-2′-deoxycytidine (0·5 g), bringing the over-all yield to about 62 per cent.

The solid by-product was dissolved in tetrahydrofuran and reprecipitated by addition of water, m.p. 128–132 °C (decomposition); analysis–calcd. for $C_9H_{12}O_5N_2I_2$: C, 22·42; H, 2·50; N, 5·81; I, 52·65; found: C, 22·69; H, 2·37; N, 5·37; I, 52·21.

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‡ The melting points are uncorrected. Analyses by Schwartzkopf Microanalytical Labs, Woodside, N.Y., and by Midwest Microlab, Inc., Indianapolis, Ind.

§ Purchased from California Corp. for Biochemical Research, Los Angeles, Cal.

|| Composed of activated carbon, grade 20 × 40, Atlas Powder Co., Wilmington, Delaware.

5-Iodo-2′-deoxycytidine-^{3}H. A solution of tritiated 2′-deoxycytidine* in 50 per cent ethanol (2 ml, 2 mc, 1·82 μmole) was reduced to dryness at 40° and vacuum-dried at room temperature. To the dried residue were added non-radioactive 2′-deoxycytidine hydrochloride (2·23 mg, 8·46 μmoles) in sufficient amount to bring the specific activity of the final mixture to 2 mc/10.28 μmoles (194 μc/μmole), dilute hydrochloric acid (0·1 ml, 0·02 N), a solution of iodic acid in water (0·06 ml, 45 mg/ml), and a solution of iodine in carbon tetrachloride (0·1 ml, 20 mg/ml). The mixture was agitated at 40° for 3 hr and taken up in water (1 ml). The carbon tetrachloride layer was separated and washed with a little water (0·2 ml). The aqueous layers were combined and adjusted to about pH 11 with sodium hydroxide (1 N), divided into two equal portions, and put on two 30 × 1-cm Dowex-1 formate columns. These were eluted with water (1 l.) which was agitated magnetically and into which was introduced dropwise, in a continuous manner, 0·025 N formic acid. The eluate was collected in 1-ml fractions; yield, 1366 μg, specific activity, 76 μc/μmole.

* Purchased from Schwarz Bio-Research, Inc., Mt. Vernon, New York.

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Studies on the GABA pathway–II. The lack of effect of pyridoxal phosphate on GABA-KGA transaminase inhibition induced by amino-oxyacetic acid

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IT WAS reported previously[1] that amino-oxyacetic acid (AOAA) is an effective inhibitor of γ-aminobutyric acid (GABA, α-ketoglutaric acid (KGA) transaminase in the brain in five species of animals, inducing elevated levels of γ-aminobutyric acid. Further, it was reported that with a GABA–KGA transaminase preparation of *E. coli*, the kinetics of inhibition indicated that AOAA is a strictly competitive inhibitor for both substrates of the enzyme. Since the brain enzyme is also inhibited by AOAA, it was assumed that this agent was also competitively inhibiting this enzyme in a similar manner.

In animals it was found that toxic doses of AOAA cause convulsions which terminate in a respiratory death.[1] These convulsions can be prevented by the administration of a variety of different compounds which have in common an aldehydic or ketonic group.[2] One such compound of the series studied was pyridoxal phosphate. In common with other transaminases, GABA–KGA transaminase requires pyridoxal phosphate,[3] so that the question arose whether high concentrations of pyridoxal