[CONTRIBUTION FROM THE RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Synthesis of C^{14}-Labeled Guanine, Adenine, 8-Azaguanine and 8-Azaadenine[1]

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Methods are described for the syntheses of adenine-4,6-C^{14}, 8-azaadenine-4,6-C^{14}, guanine-4-C^{14} and 8-azaguanine-4-C^{14} in yields of 20% and 45–50%, respectively, from sodium cyanide. The procedures use known synthetic methods with modifications to produce satisfactory yields for isotopic syntheses.

Interest in 8-azaguanine (5-amino-1-*v*-triazolo(d)-pyrimidine) as a possible anti-cancer agent[3] made the synthesis of this compound as well as guanine, adenine and 8-azaadenine (7-amino-1-*v*-triazolo-(d)pyrimidine) labeled with isotopic carbon desirable for biological studies.

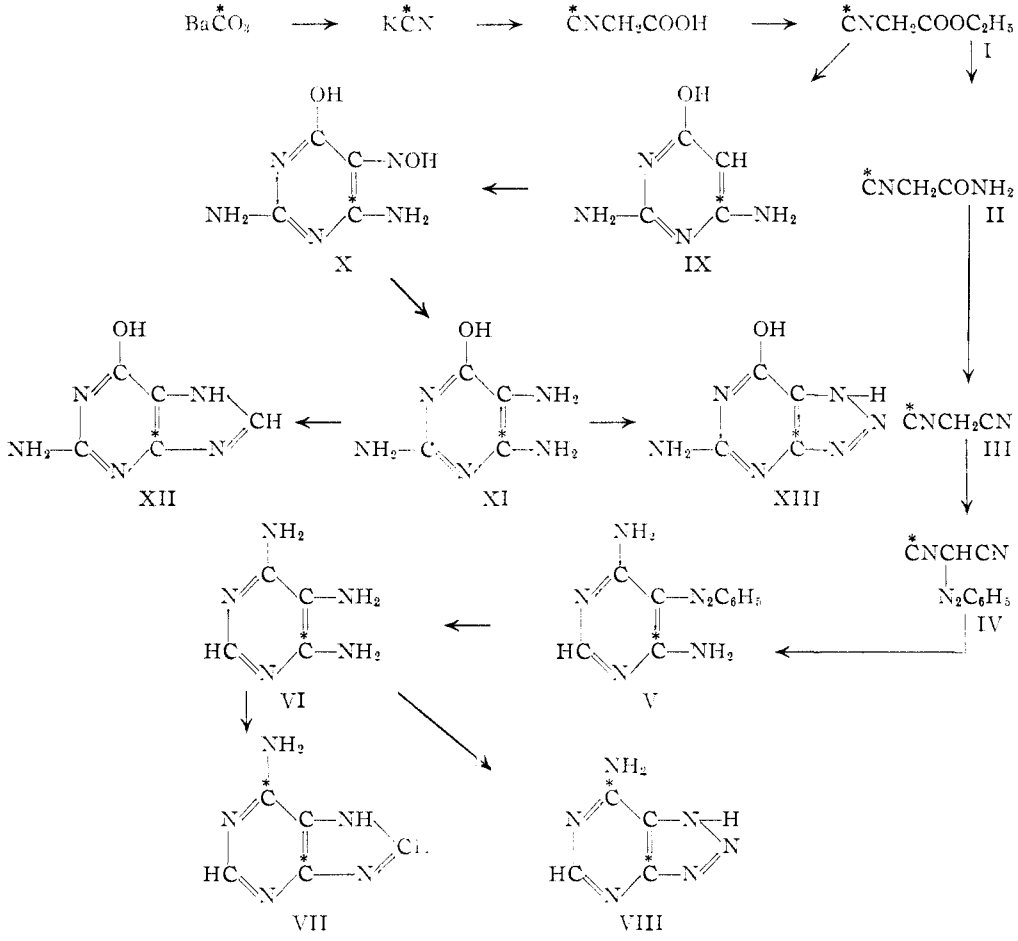

Fig. 1.

The syntheses of several labeled purine compounds have been described[4] including adenine-2-C^{14},[5] adenine-4,6-C^{14},[5] adenine-8-C^{14},[6,7] guanine-2-C^{14},[8] guanine-8-C^{14},[7,9] and 8-azaguanine-2-C^{14}.[8]

The methods indicated in Fig. 1 enabled a common intermediate, ethyl cyanoacetate, to be used in the syntheses of the four desired compounds. The method indicated for adenine-4,6-C^{14} is essentially that described in ref. 4 but modifications in the procedure increased the yield from sodium cyanide from 3.8 to 20%. 8-Azaadenine was prepared in a similar yield. The syntheses of guanine-4-C^{14} and 8-azaguanine-4-C^{14} followed known procedures with modifications that allowed over-all yields from sodium cyanide of 45–50% to be obtained. By suitable modification in the labeling of the ethyl cyanoacetate, the procedures described can be readily adapted to prepare guanine or 8-azaguanine

(1) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

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(4) For a survey of methods of synthesis of these and other heterocyclic compounds see C. E. Crompton and N. H. Woodruff, *Nucleonics*, **7**, No. 4, 44 (1950).

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labeled in the 5- or 6-position, and adenine or 8-azadenine labeled in the 5-position.

Experimental

Ethyl Cyanoacetate-3-C^{14} (I).[5,10]—Forty mc. of barium carbonate (6 mmoles) was converted to potassium cyanide.[11,12] Hydrogen cyanide was generated and collected in 0.3 *M* sodium hydroxide (7.2 mmoles) in 85% yield from barium carbonate. An inactive 2 *M* sodium cyanide solution (34 mmoles) and 2 *M* sodium chloroacetate (44 mmoles) were added and the solution was heated in a water-bath at 70–95° over a 20-minute period. The cooled solution was acidified with 10 ml. of 70–72% perchloric acid (or sulfuric acid) and extracted for 48 hours in a continuous liquid-liquid extractor with ether.

The cyanoacetic-3-C^{14} acid in ether was converted to the ethyl ester by the careful addition of excess diazoethane in ether (prepared from N-nitroso-N-ethyl urea[13]) at 0°. Water was frozen out at −70°, the ether solution of I was decanted and divided into two equal portions each estimated to contain 15 mc. (18 mmoles) of I. The ether solutions were distilled at atmospheric pressure and finally at reduced pressure through a small Vigreux type stillhead until the residual volumes were about 5 ml. In order to dry completely, 50 ml. of benzene was added and the aqueous phase was separated. The solution was again concentrated to 5 ml. (60° bath temperature (40 mm.)) followed by the addition of 30 ml. of benzene and reconcentration.

Cyanoacetamide-3-C^{14} (II).[5,14]—To I was added 3 ml. of concentrated ammonium hydroxide, the solution was allowed to stand at room temperature for one-half hour, then stored at 0°. II was removed, washed with a small amount of cold ethanol and air dried; yield 800 mg. The filtrate and washings were concentrated under reduced pressure to 1 ml., 4 ml. of ethanol was added and the solution was again stored at 0°. II was obtained in a yield of 475 mg.; total yield 1.27 g. (76% from sodium cyanide).

Malononitrile-1-C^{14} (III) and Phenylazomalononitrile-1-C^{14} (IV).—The sublimation reaction apparatus shown in Fig. 2 was used for the preparation of III. By the following procedure, yields of 60–70% in the conversion of II to IV were obtained.[15] II (1.27 g.) was placed in the reaction-sublimation apparatus, 1.3 g. of phosphorus pentachloride was added (operations carried out in a dry-box), and mixed by gentle shaking. The reaction was carried out by heating for 4 minutes at 95–100° (water aspirator), and after the initial reaction had subsided the cold finger was cooled to −70° and the product was sublimed at 30 mm. as rapidly as possible by using an oil-bath previously heated to 160–170°. The sublimation required about 5 minutes and yielded a light yellow product. III was rinsed from the cold finger with 5 ml. of methanol and 22 ml. of 2 *M* sodium acetate and a solution of benzenediazonium chloride (prepared by dissolving 1.9 g. of aniline in 6.75 ml. of concd. hydrochloric acid and 35 g. of ice and adding a solution of 1.75 g. of sodium nitrite) was added. After storage at 0°, the yellow product was filtered and washed with cold water to yield 1.70 g. (66%) of IV.

4,6-Diamino-5-phenylazopyrimidine-4-C^{14} (V).[5]—To IV (10 mmoles, 8.5 mc.) were added 17 ml. of 1.0 *M* sodium butoxide in absolute butanol and 1.24 g. of formamidine hydrochloride.[16] The contents were mixed by swirling at room temperature for 10 minutes, then refluxed for four hours under anhydrous conditions. After storage at 0°, the product was removed and washed with small portions of cold ethanol and water and air-dried. Chromatography of a small portion of this compound on silicic acid–celite (developed with 95% benzene–5% ethanol) indicated that it was 85% pure. The corrected yield was 1.4 g. (70%). Condensations carried out in methanol yielded no product, while one carried out in ethanol gave 55% of V.

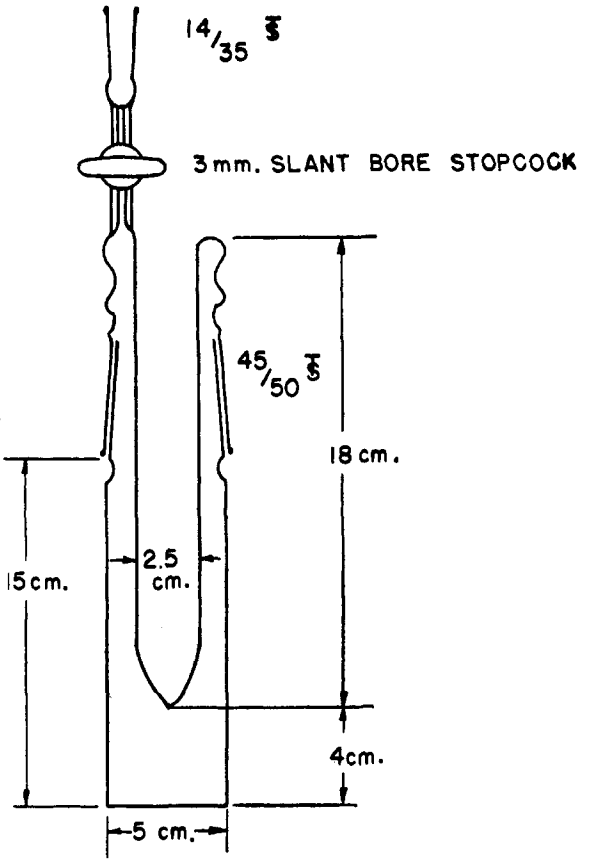

Fig. 2.—Reaction-sublimation apparatus.

4,5,6-Triaminopyrimidine Sulfate-4-C^{14} (VI).—V (1.6 g., 85% pure) was dissolved in 10 ml. of glacial acetic acid and 2.5 g. of powdered zinc was added in small portions over a 15-minute period. The zinc was removed and washed with three 4-ml. portions of warm water containing 1 ml. of glacial acetic acid. The filtrate and washings were cooled and 5 ml. of 9 *M* sulfuric acid was added. After storage at 5°, VI was filtered and washed with three 2-ml. portions of cold water; yield 1.23 g. (78%).

Adenine Sulfate-hydrate-4,6-C^{14} (VII).[5]—Several attempts to repeat the procedure described in ref. (5) resulted in explosions of varying severity either when heating in the Carius tube at 160–170° or especially when opening the cooled tubes with a hot rod at the end of the reaction. Therefore, the following modification was used.

VI (612 mg.) was refluxed in an oil-bath at 160° with 11 ml. of formamide and 0.3 ml. of 98–100% formic acid for 2.5 hours. After 1.2 hours, an additional 0.25 ml. of formic acid was added. The formamide was removed *in vacuo* at a bath temperature of 140–145°. The residue was dissolved by warming in 9 ml. of 0.5 *M* sulfuric acid and the solution was decolorized with Norite and allowed to crystallize at 5°. The light yellow product was filtered, washed with a small amount of water and ethanol and air-dried; yield 341 mg. (67%, specific activity 0.85 mc./mmole).

In order to recover additional VII from the filtrate, it was passed through an ion exchange column (Amberlite IRA-400 in the chloride form), the eluent fractions contain-

(10) J. K. H. Inglis, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 254.

(11) R. E. Selff and B. M. Tolbert, UCRL Report No. 1299.

(12) The author wishes to acknowledge the preparation of the hydrogen cyanide by Mr. R. E. Selff.

(13) F. Arndt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 165.

(14) B. B. Corson, R. W. Scott and C. E. Vose, *ibid.*, Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 179.

(15) E. M. Gal and A. T. Shulgin, THIS JOURNAL, **73**, 2938 (1951), have obtained yields of 85% in the preparation of III by the addition of lithium chloride.

(16) Difficulty was experienced in the preparation of ethyl formimino ether hydrochloride on a 4-mole scale by the procedure described in ref. 5 using a large excess of anhydrous hydrogen chloride. The product appeared to be mainly ammonium chloride. Subsequent preparations using 1.1 moles of hydrogen chloride/mole of hydrogen cyanide[17] yielded a product containing about 65 mole per cent. of the desired product and 35 mole per cent. ammonium chloride. The analysis given for the imino ether in ref. 5 (theory 32.4% chloride) indicates that it also may have been impure.

(17) S. M. McElvain and J. W. Nelson, THIS JOURNAL, **64**, 1825 1942).

ing the adenine were concentrated to 2.5 ml., 60 mg. of inactive adenine sulfate and 0.1 ml. of 9 *M* sulfuric acid were added and, after treatment with Norite, 84 mg. of VII (specific activity 0.55 mc./mmole) was obtained. The total yield of VII from sodium cyanide was 20%.

The purity and identity of VII were shown by ultraviolet absorption spectrum, ϵ_{max} = 12,800 at 263 mμ, *p*H 2.0[6,18] and filter paper chromatography. Only one radioactive spot (also visible under ultraviolet light) was obtained when VII was chromatographed on Whatman No. 1 filter paper using 40 wt. % *n*-butanol–25 wt. % propionic acid–35 wt. % water as the solvent system (R_f 0.42 when applied from hydrochloric acid solution).

8-Azaadenine-4,6-C^{14} (7-Amino-1-*v*-triazolo(d)pyrimidine-3a,7-C^{14}) (VIII).[19]—VI (608 mg.) was dissolved by warming with 10 ml. of 2 *M* sodium acetate. After cooling to room temperature, 1.5 ml. of glacial acetic acid and 213 mg. of sodium nitrite in 5 ml. of water was added, and the solution was treated as described in ref. (19). After allowing VIII to crystallize at 5°, it was filtered, washed with a small amount of water and ethanol and dried; yield 295 mg. (86%). The above product was dissolved by warming in 1 *M* ammonium hydroxide and decolorized with 50 mg. of Norite. Glacial acetic acid was added dropwise to the warm filtrate until a precipitate began to form in the hot solution. The product was removed from the cooled solution and washed with water and ethanol; yield 270 mg. (79%, specific activity 0.85 mc./mmole).

The combined filtrates were concentrated and 51 mg. of inactive VIII added. After one recrystallization, 52 mg. of VIII was obtained, specific activity 0.19 mc./mmole. Thus, the total radioactive yield was 82% from VI, and 20% from sodium cyanide.

The identity and purity of VIII were shown by ultraviolet absorption spectra, ϵ_{max} 10,100 at 264 mμ, *p*H 2.0; ϵ_{max} 10,850 at 275 mμ, *p*H 6.5; and ϵ_{max} 10,900 at 275 mμ, *p*H 9.0. The position of ϵ_{max} at *p*H 2.0 agrees with that reported in ref. (18), but the other values are not in agreement. However, the position of ϵ_{max} for *p*H 6.5 and 9.0 are in substantial agreement with those reported in ref. (19).

Paper chromatography (butanol–propionic acid–water solvent) gave a single spot, R_f = 0.70, visible under ultraviolet light which corresponded with the only radioactive spot obtained.

Anal. Calcd. for $C_4N_6H_4$: C, 35.3; H, 3.0; N, 61.7. Found: C, 35.3; H, 3.0; N, 61.7.

2,4-Diamino-6-hydroxypyrimidine-4-C^{14} (IX) and 2,4-Diamino-5-isonitroso-6-hydroxypyrimidine-4-C^{14} (X).[20,21]—A solution prepared from 4.0 g. of guanidine hydrochloride and 1.04 g. of sodium in 20 ml. of absolute methanol was added to I (18 mmoles) and the mixture was refluxed for 8 hours (protected by a drying tube from moisture). The methanol was removed under reduced pressure to yield a thick brown sirup (IX).[22] This was dissolved in 40 ml. of water; 30 ml. of 2 *M* sodium acetate, 8 ml. of 6 *M* hydrochloric acid and excess sodium nitrite were added. After several minutes, additional 6 *M* hydrochloric acid (24 mmoles) was added and the solution was stored at 0° for several hours; the rose red precipitate of X was filtered and washed with water. It was used to prepare XI without drying since it can be more readily suspended while still moist.

2,4,5-Triamino-6-hydroxypyrimidine Sulfate (XI).[20,21]—Moist X was suspended and partially dissolved in 46 ml. of 0.5 *M* sodium hydroxide. After several minutes 2.5 ml. of 4.5 *M* sulfuric acid was added with vigorous stirring, the solution was warmed to 50° and 10 g. of sodium hydrosulfite was added in small portions over a 15-minute period. The warm solution was filtered and the clear yellow filtrate was acidified with sulfuric acid. After storage at 0°, the crystalline, light yellow precipitate was filtered and washed with water; yield 2.65 g. (52% from sodium cyanide, specific activity 0.85 mc./mmole). By the addition of 215 mg. of carrier XI and additional sulfuric acid to the filtrate from the above crystallization, 338 mg. additional XI was obtained, specific activity 0.44 mc./mmole (equivalent to 3% yield from sodium cyanide).

Guanine Hydrochloride Dihydrate-4-C^{14} (XII).[20]—XI (1.29 g.) was refluxed for 15 hours with 15 ml. of 98–100% formic acid and 357 mg. of sodium formate. The formic acid was removed under reduced pressure and the light yellow solid was dissolved in 25 ml. of 0.5 *M* hydrochloric acid and, after treatment with Norite, the filtered solution was stored for several days at 5°. XII was removed, washed with cold water and air-dried to yield 924 mg. (83%, 45% from sodium cyanide, specific activity 0.85 mc./mmole).

The purity and identity of the compound were shown by paper chromatography (butanol–propionic acid–water solvent); only one spot (R_f 0.40) visible under ultraviolet light or on making a radioautograph was present. The ultraviolet absorption spectrum, ϵ_{max} 11,000 at 248 mμ, 7200 at 270 mμ, *p*H 2.0 agreed with an authentic sample.[18]

8-Azaguanine-4-C^{14} (5-Amino-7-hydroxy-1-*v*-triazolo(d)-pyrimidine-3a-C^{14}) (XIII).[19]—XI (1.36 g.) was dissolved by warming to 80° with 30 ml. of 2 *F* sodium acetate; 1.4 g. of sodium nitrite dissolved in 8 ml. of water was added to the warm solution and it was heated for one-half hour on a steam-bath. Glacial acetic acid (4 ml.) was added and after storage at 5°, the product was filtered, washed with water, and dissolved by warming in 1 *F* ammonium hydroxide and decolorized by treatment with Norite. On storage at 5°, 556 mg. of XIII was obtained (69%). By addition of glacial acetic acid to the warmed filtrate until a precipitate started to form, an additional 194 mg. (24%) of XIII was obtained. Total yield 93% from XI, 51% from sodium cyanide.

XIII exhibited only one radioactive spot (R_f 0.30) after chromatography on paper (butanol–propionic acid–water solvent). This spot fluoresced strongly under ultraviolet light. The ultraviolet absorption spectrum at *p*H 2.0 indicated a single max. at 247 mμ, ϵ_{max} 10,500, and a broad shoulder from 262–272 mμ, and agrees with that reported in ref. (18).

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(21) C. K. Cain, M. F. Mallette and E. C. Taylor, Jr., THIS JOURNAL, **68**, 1996 (1946).

(22) Experiments indicated that the yields using absolute ethanol or butanol were similar although inclined to be more variable. The *p*H for the nitrosation should be more acid than 5.0 at the beginning of the reaction.