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SYNTHESES OF C^{14}-LABELED GUANINE, ADENINE, 8-AZAGUANINE AND 8-AZAADENINE

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ABSTRACT

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Methods are described for the syntheses of adenine-4,6-C^{14}, 8-aza-adenine-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.

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

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SYNTHESES OF C^{14}-LABELED GUANINE, ADENINE, 8-AZAGUANINE AND 8-AZAADENINE

by

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Interest in 8-azaguanine (5-amino-1-y-triazolo(d) pyrimidine) as a possible anti-cancer agent[2] made the synthesis of this compound as well as guanine, adenine and 8-azaadenine (7-amino-1-y-triazolo(d) pyrimidine) labeled with isotopic carbon desirable for biological studies.

The syntheses of several labeled purine compounds have been described[3] including adenine-2-C^{14},[4] adenine-4,6-C^{14},[4] adenine-8-C^{14},[5] guanine-2-C^{14},[6] guanine-8-C^{14},[7] and 8-azaguanine-2-C^{14}.[6]

The methods indicated in Figure 1 enabled a common intermediate, ethyl

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

(2) G. W. Kidder, V. C. Dewey and R. E. Parks, Jr., Science, 109, 511 (1949).

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

(4) L. F. Cavalieri, J. F. Tinker and A. Bendich, J. Am. Chem. Soc. 71, 533 (1949).

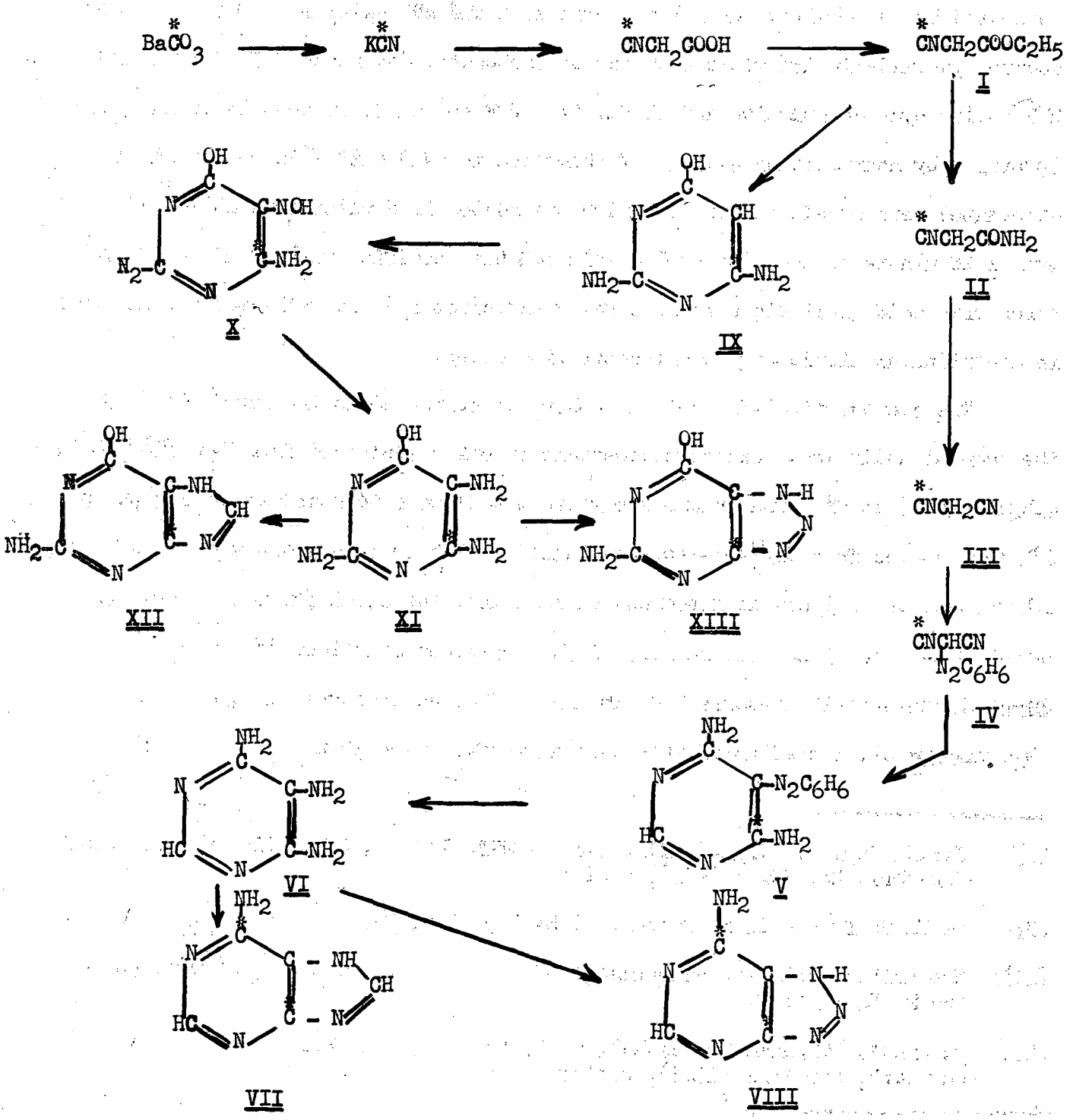
(5) V. M. Clark and H. Kalckar, J. Chem. Soc. 1029 (1950).

(6) "Progress Report on the Chemotherapy of Leukemia and Studies on the Mechanism of Action of Certain Anti-Cancer Agents", Southern Research Institute, Birmingham, Alabama, August 15, 1950.

(7) M. E. Balis, G. B. Brown, G. B. Elion, G. B. Hitchings and H. Wanderververff, J. Biol. Chem. 188, 217 (1951).

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 reference 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 overall yields from sodium cyanide of 45-50% to be obtained. By suitable modification in the labeling of the ethyl cyanoacetate, the procedure described below can be readily adapted to prepare guanine or 8-azaguanine labeled in the 5- or 6-position, and adenine or 8-azaadenine labeled in the 5-position.

Figure 1


$Ba^{*}CO_3$
$K^{*}CN$
$^{*}CNCH_2COOH$
$^{*}CNCH_2COOC_2H_5$
I
$^{*}CNCH_2CONH_2$
II
$^{*}CNCH_2CN$
III
$^{*}CNCHCN$
$N_2C_6H_6$
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII

Experimental

Ethyl cyanoacetate-3-C^{14} (I)[4,8]: - Forty mc. of barium carbonate was converted to potassium cyanide[9] in three 2 mmole preparations[10]. Hydrogen cyanide was generated by the addition of 3 F sulfuric acid and collected in 24 ml. of 0.3 F sodium hydroxide in 85% yield from barium carbonate. Seventeen ml. of an inactive 2 F sodium cyanide solution and 22 ml. of a 2 F sodium chloroacetate solution (prepared by neutralizing 4.15 g. of chloroacetic acid with 2.36 g. of sodium carbonate) 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 (sulfuric acid is equally satisfactory) 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[11]) at 0°. The reaction mixture was allowed to stand several hours at 0°, then cooled in a Dry Ice-isopropyl alcohol bath to freeze out water. 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 first at atmospheric pressure and finally at reduced pressure through a small Vigreaux-type still head until the residual volumes were about 5 ml. In order

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(8) J.K.H. Inglis, "Organic Syntheses", Coll. Vol. I, John Wiley & Sons, Inc., New York, New York (1941), p. 254.

(9) R. E. Selff and B. M. Tolbert, UCRL Report #1299.

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

(11) F. Arndt, "Organic Syntheses", Coll. Vol. II, John Wiley & Sons, Inc., New York, New York (1943), p. 165

to dry completely, 50 ml. of benzene was added, and the small 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. The combined ether-benzene distillates contained about 6% of the C^{14} activity when plated into sodium hydroxide.

Cyanoacetamide-3-C^{14} (II)[4,12]: - 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° overnight. II was removed by filtration, 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. (the distillated contained 1 mc. of a volatile compound), 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, 1.275 g. (76% from sodium cyanide). The filtrate from this fraction contained 2-3 mc. but attempts to isolate additional cyanoacetamide by isotope dilution were not successful.

Malononitrile-1-C^{14} (III) and Phenylazomalononitrile-1-C^{14} (IV)[4]: - The sublimation reaction apparatus shown in Figure 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. II (1.27 g.) was placed in the reaction-sublimation apparatus (Figure 2), 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 isopropyl alcohol and Dry Ice were placed in the cold finger and the product was sublimed

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(12) B. B. Corson, R. W. Scott and C. E. Vose, "Organic Syntheses", Coll. Vol. I, John Wiley & Sons, Inc., New York, New York (1941), p. 179.

at 30 mm. as rapidly as possible onto it 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 into a 125 ml. conical flask with 5 ml. of methanol and 22 ml. of 2 F sodium acetate, and then a solution of benzene diazonium chloride (prepared by dissolving 1.9 g. of aniline in 6.75 ml. of concentrated hydrochloric acid and 35 g. of ice and slowly adding a solution of 1.75 g. of sodium nitrite in 6 ml. of water[13]) were added and after storage at 0° the hellow product was filtered and washed with cold water to yield 1.70g . (66%) of IV.

4,6-Diamino-5-phenylazopyrimidine-4-C^{14} (V)[4]: - IV (1.70 g., 10 mmoles, 8.5 mc.) was placed in a 50 ml. conical flask equipped with a ℞ joint. Absolute butanol (5 ml.), 1.24 g. (15.4 mmoles) of formamidine hydrochloride[14] and 14.0 ml. of a 1.2 F sodium butoxide solution were added. A reflux condenser and drying tube were attached, the contents were mixed by swirling at room temperature for 10 minutes, then refluxed for four hours. After storage at 0° overnight, the product was removed by filtration and washed with small portions of cold ethanol and

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(13) J. B. Conant, R. E. Lutz and B. B. Corson, "Organic Syntheses", Coll. Vol. I, John Wiley & Sons, Inc., New York, New York (1941), P. 49.

(14) Difficulty was experienced in the preparation of ethyl formimino ether hydrochloride on a 4 mole scale by the procedure described in (4) 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[15] yielded a product containing about 65 mole percent of the desired product and 35 mole percent ammonium chloride. The analysis given for the imino ether in (4) (theory 32.4%) indicates that it also may have been impure.

(15) S. M. McElvain and J. W. Nelson, J. Am. Chem. Soc. 64, 1825 (1942).

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.

4,5,6-Triaminopyrimidine Sulfate-4-C^{14} (VI): - V (1.60 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 by filtration 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 F sulfuric acid were added. After storage for 3 hours at 5°, VI was filtered and washed with three 2 ml. portions of cold water; yield 1.23 g. (78%). No further product was obtained even after the addition of inactive carrier. Several trial reductions using Raney nickel on V dissolved in glacial acetic acid gave 65-70% yields, but required a longer time to carry out and were no more satisfactory than the reduction described.

Adenine Sulfate-hydrate-4-C^{14} (VII)[4]: - Several attempts to repeat the procedure described in (4) 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., 2.54 mmoles) was heated in a flask equipped with a reflux condenser 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 F sulfuric acid and the solution was decolorized with a small amount of Norite and allowed to crystallize for

several days 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 containing the adenine were concentrated to 2.5 ml, 60 mg. of inactive adenine sulfate and 0.10 ml. of 9 F sulfuric acid were added, and after treatment with Norite and crystallization at 5°, 84 mg. of VII, (specific activity 0.55 mc/mmole) were 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 pH 2.0 [5,16] 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-amine-1-v-triazolo (d) pyrimidine-3a,7-C^{14}) (VIII)[17]: - VI (608 mg., 2.52 mmoles) was dissolved by warming with 10 ml. of 2 F sodium acetate. The solution was cooled to room temperature, 1.5 ml. of glacial acetic acid added, followed by 213 mg. of sodium nitrite in 5 ml. of water. The solution was allowed to stand at room temperature for 5 minutes during which time a buff colored precipitate formed, subsequently the solution was

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(16) L. F. Cavalieri, A. Bendich, J. F. Tinker, and G. B. Brown, J. Am. Chem. Soc., 70, 3875 (1948).

(17) R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. B. Cole, and J. R. Vaughan, Jr., J. Am. Chem. Soc., 67, 290 (1945).

heated on a steam bath for 15 minutes. After allowing VIII to crystallize at 5°, it was filtered, washed with a small amount of water and alcohol and dried. Yield: 295 mg (86%). The above product was dissolved by warming in 20 ml. of 1 F ammonium hydroxide, decolorized with 50 mg. of Norite, and after filtering glacial acetic acid was added dropwise to the warm filtrate until a precititate 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 adsorption spectra, ε_{max} = 10,100 at 264 mμ, pH 2.0; ε_{max} = 10,850 at 275 mμ, pH 6.5; and ε_{max} = 10,900 at 275 mμ pH 9.0. The position of ε_{max} at pH 2.0 agrees with that reported in (16), but the other values are not in agreement. However, the position of ε_{max} for pH 6.5 and 9.0 are in substantial agreement with those reported in (17).

Chromatography in butanol-propionic acid-water gave a single spot visible under ultraviolet light, R_f = 0.70 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-hydroxy-pyrimidine-4-C^{14} (IX) and 2,4-Diamino-5-isonitroso-6-hydroxy-pyrimidine-4-C^{14} (X) [18,19]: - 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 from moisture by a drying tube). The methanol was removed under reduced pressure to yield a thick brown syrup (IX).[20] This was dissolved in 40 ml. of water; 30 ml. of 2 F sodium acetate, 8 ml. of 6 F hydrochloric acid and 30 ml. of 2 F sodium nitrite were added. After several minutes 4 ml. additional 6 F hydrochloric acid was added and the solution was stored at 0° for several hours; then the rose red precipitate of X was filtered and washed with water. It was used to prepare XI without drying since it was found that it could be more readily suspended while still moist. The filtrate contained about 10% of the starting C^{14} activity.

2,4,5-Triamine-6-hydroxy-pyrimidine sulfate (XI)[18,19]: - Moist X was suspended and partially dissolved in 35 ml. of water and 4 ml. of 5.9 F sodium hydroxide. After several minutes 2.5 ml. of 4.5 F 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 to the clear yellow filtrate was added 6 ml. of 4.5 F sulfuric acid. After storage at 0°, the crystalline, light yellow precipitate was

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(18). W. Traube, Ber. 33, 1371 (1900).

(19) C. K. Cain, M. F. Mallette, and E. C. Taylor, Jr., J. Am. Chem. Soc., 68, 1996 (1946).

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

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 5 ml. of 4.5 F sulfuric acid to the filtrate from the above crystallization, 338 mg. additional XI was obtained, specific activity 0.44 mc./mmole (equivalent to 3% additional yield from sodium cyanide).

Guanine hydrochloride dihydrate-4-C^{14} (XII)[18]:- XII (1.29 g., 5.0 mmoles) was refluxed for 15 hours with 15 ml. of 98-100% formic acid and 357 mg (5.2 mmoles) of sodium formate. The formic acid was removed under reduced pressure, and the light yellow solid was dissolved in 25 ml. of 0.5 F hydrochloric acid and after treatment with Norite, the solution was filtered and stored for several days at 5°. XII was removed by filtration, 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 was shown by paper chromatography using butanol-propionic acid-water as the solvent system; only one spot (R_f = 0.40) visible under ultraviolet light or on making a radioautograph was present. The ultra-violet adsorption spectrum, ε_{max} = 11,000 at 248 mμ, 7200 at 270 mμ, pH 2.0 agreed with an authentic sample[16].

8-Azaguanine-4-C^{14} (5-Amino-7-hydroxy-1-v-triazolo (d) pyrimidine-3a-C^{14}) (XIII)[17]:- XI (1.36 g., 5.3 mmoles) was dissolved by warming to 80° with 30 ml. of 2 F sodium acetate; 1.4 g. (20 mmoles) 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 overnight at 5°, the product was filtered, washed with water, and dissolved by warming in 40 ml. of 1 F ammonium hydroxide, treated with 125 mg. of Norite and filtered. On storage at 5°, 556 mg. of XIII was obtained (69%).

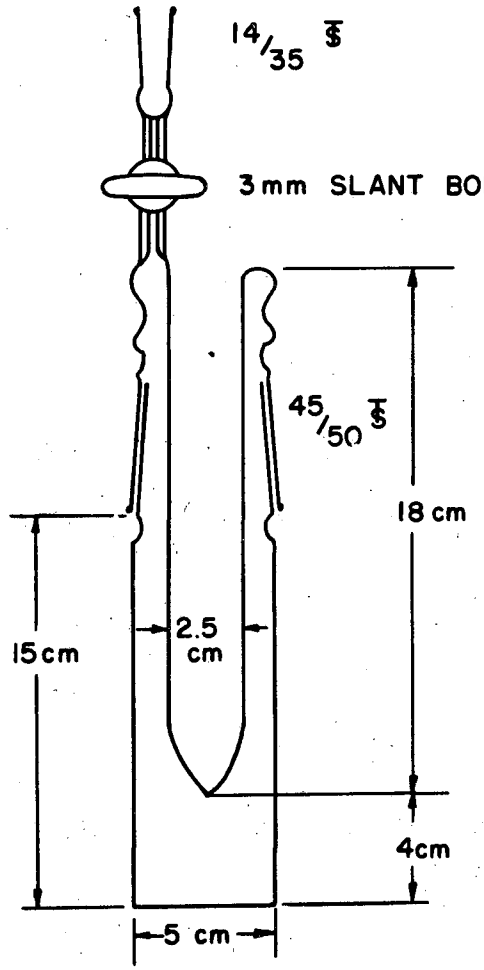
By addition of glacial acetic acid to the warmed filtrate until a precipitate started to form, an additional 194 mg. (24%) of XIII was obtained. Total yield: 93% from XI, 51% from sodium cyanide.

XIII exhibited only one radioactive zone after chromatography on paper using butanol-propionic acid-water as the solvent system, (R_f = 0.30). This spot fluoresced strongly under ultraviolet light. The ultraviolet adsorption spectrum at pH 2.0 indicated a single max. at 247 mμ, ε_{max} = 10,500, and a broad shoulder from 262 mμ to 272 mμ and agrees with that reported in (16).

SUMMARY

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.

The author wishes to express his appreciation to Dr. Melvin Calvin for his guidance and interest in the described work.



REACTION - SUBLIMATION APPARATUS

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FIG 2