[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION, CORNELL UNIVERSITY MEDICAL COLLEGE]

Nucleosides Labeled with Tritium in the Ribosyl Group[1]

BY MILTON P. GORDON,[2] ORSALIA M. INTRIERI AND GEORGE BOSWORTH BROWN

RECEIVED FEBRUARY 15, 1958

1,2,3,5-Tetra-*O*-acetyl-D-ribofuranose has been tritiated by exposure to tritium gas and converted to 9-β-D-ribofuranosyladenine-*ribosyl-t*. The material was found to be radiochemically pure. A new electrophoretic technique for the separation of the four 1-β-D-aldopentofuranosylthymines has been developed.

Studies of the metabolism of 2′- and 3′-phospho-9-β-D-ribofuranosylpurines have shown that, during the course of their incorporation into nucleic acids, some cleavage of the purine–ribose bond occurs.[3] This process has been termed "transpurination."[4,5] Studies of the mechanism of this process would be facilitated by the use of a purine nucleotide in which the purine and ribose moieties were labeled with different radioactive isotopes, each of which emitted distinctive radiation. The present investigation is concerned with the preparation of a nucleoside in which the ribose is specifically labeled with tritium.

The first trial experiments utilized tritium-recoil labeling based upon the slow neutron reaction, $Li^6(n,\alpha)T$.[6] This procedure involved the irradiation of mixtures of D-ribose and lithium carbonate by thermal neutrons. This method has been used successfully to produce glucose-*t* and galactose-*t* of moderate specific activities.[7] In addition, many other compounds have been labeled by this procedure.[8,9] An excellent review of this technique has appeared.[8]

Preliminary application of this method to D-ribose yielded poor results, Table I, and these attempts were abandoned in favor of labeling 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose by exposure to tritium gas.[10] The latter method has been reported to give specific activities[10] sufficiently high to be of use in the contemplated metabolic experiments.

The mechanism by which compounds are labeled in the presence of tritium gas is not known in detail.[11] In view of the ultimate application of the products of this reaction to the study of biological phenomena, care had to be exercised to detect and, if possible, to eliminate impurities arising by fission of carbon chains and by inversion of asymmetric atoms.

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. CY-3190), and from the Atomic Energy Commission (Contract No. AT. (30-1), 910).

(2) Virus Laboratory, University of California, Berkeley 4, Calif.

(3) P. M. Roll, H. Weinfeld, E. Carroll and G. B. Brown, *J. Biol. Chem.*, **220**, 439 (1956).

(4) G. B. Brown, "Proc. Intl. Conf. Peaceful Uses of Atomic Energy," New York, United Nations, Vol. 12, 485 (1956).

(5) G. B. Brown, *Federation Proc.*, **15**, 823 (1956).

(6) R. Wolfgang, F. S. Rowland and C. N. Turton, *Science*, **121**, 715 (1955).

(7) F. S. Rowland, C. N. Turton and R. Wolfgang, THIS JOURNAL, **78**, 2354 (1956).

(8) F. S. Rowland and R. Wolfgang, *Nucleonics*, **14**, No. 8, 58 (1956).

(9) Å. Hanngren and J. Rydberg, *Acta Chem. Scand.*, **11**, 202 (1957).

(10) K. E. Wilzbach, Abstr. 130th Meeting, Am. Chem. Soc., Atlantic City, N. J., p. 22-O (1956); THIS JOURNAL, **79**, 1013 (1957).

(11) R. W. Ahrens, M. C. Saver, Jr., and J. E. Willard, *ibid.*, **79**, 3285 (1957).

TABLE I

PROPERTIES OF D-RIBOSE IRRADIATED WITH THERMAL NEUTRONS[a]

Sample	Total time of irrad., hr.	Appearance of mixt. after irradiation	Yield of de-ionized solids, g.	Pentose in de-ionized solids, %	Radio-activity of de-ionized solids, c.p.m./mg.
1	3	Light brown crystn.	0.70	78	3,400
2	6	Light brown crystn.	.69	36	3,800
3	12	Brown sticky cryst.	.58	42	11,000
4	24	Brown sticky mass	.54	14	10,000
5	48	Dark brown thick sirup	.42	5	19,000

[a] Samples consisting of intimately ground mixtures of 1 g. of D-ribose and 1 g. of lithium carbonate were irradiated in water-cooled holes in the atomic pile at the Brookhaven National Laboratory, Upton, Long Island, N. Y. The thermal neutron flux was estimated to be *ca.* 3.4×10^{12} NV (neutrons/cm.²/min.)

Results and Discussion

Labeling by the $Li^6(n,\alpha)T$ Reaction.—Very finely ground mixtures of lithium carbonate and D-ribose were irradiated by thermal neutrons in water-cooled holes in the atomic pile at the Brookhaven National Laboratory, Associated Universities, Inc., Upton, Long Island, N. Y. Five samples were irradiated for different periods. The mixtures began to turn dark and sirupy with increasing periods of radiation. The irradiated mixtures were thoroughly extracted with hot water, and cations were removed from the extracts by treatment with Dowex-50 (H^+).[6] Evaporation of the de-ionized solutions yielded brown, glassy residues. The yields of solids at this stage decreased with increasing time of radiation. These residues tended to be more insoluble in water with increasing periods of irradiation. Chromatography of the residues in a solvent system that partially separates ribose, xylose and arabinose,[12] and treatment of the chromatograms with aniline phthalate spray[13] indicated that the major reducing component in all of the irradiated samples was probably D-ribose. Quantitative pentose determinations were carried out on the residues,[14] and the percentage of pentose present in the samples decreased with increasing time while the radioactivity present per unit weight of residue increased (Table I).

(12) E. L. Hirst and J. K. N. Jones, *Disc. Faraday Soc.*, **7**, 268 (1949).

(13) S. M. Partridge, *Nature*, **164**, 443 (1949).

(14) Z. Dische and K. Schwarz, *Mikrochim. Acta*, **2**, 13 (1937).

In view of the extensive decomposition of the ribose, this procedure does not appear to be a suitable method for the preparation of tritium labeled D-ribose; however, it is possible that less decomposition might be obtained in the presence of a smaller proportion of lithium carbonate.[8]

Labeling by Exposure to Tritium Gas.—A sample of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose which had been exposed to tritium gas underwent slight decomposition. The material had a faint odor of acetic acid, and the melting point was depressed *ca.* 1 to 2°; however, the material assayed 98 ± 2% of the expected ribose content. The radioactivity of this material was *ca.* 6×10^6 c.p.m./μmole.

The conversion of the tritium labeled material to 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride occurred readily, but the solution of the product was slightly brown. The chloro compound was converted to 6-methylmercapto-9-β-D-ribofuranosylpurine-*ribosyl-t*.[15] The several treatments with aqueous solvents in the course of the synthesis are assumed to have eliminated labile tritium atoms.[8] The radioactivity of this material assayed *ca.* 1.4×10^6 c.p.m./μmole of 24% that of the starting material; a value of 33% would have resulted if the distribution of the tritium were completely random in both the acetyl groups and the ribose. The distribution of tritium in the ribofuranosyl group is unknown.

The 6-methylmercapto substituted intermediate was chosen because its configuration has been established[15] by conversion to the known 9-β-D-ribofuranosylpurine[16] and it can be converted to a variety of 6-substituted purine nucleosides of biological interest.[15,17] The 6-methylmercapto-9-β-D-ribofuranosyl-*purine-t* was aminated with hot methanolic ammonia to give 9-β-D-ribofuranosyladenine-*ribosyl-t*. The latter product was identical with natural adenosine, and no contaminants were visible when paper chromatograms or ionophoretic papers were examined under ultraviolet light. The specific activity of the radioactive adenosine was 85–88% of that of the 6-methylmercapto derivative. This loss of tritium may reflect the rigorous conditions required for the amination.

Radiochemical Purity.—The chemical properties of radioactive impurities which would result from the cleavage of carbon bonds in the 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose should differ greatly from the properties of the parent sugar derivative; consequently, such fragments should not have been carried through the several steps which culminated in the synthesis of adenosine.

A replacement by tritium of a hydrogen atom bound to an asymmetric carbon atom could possibly lead to the inversion of the configuration of that carbon. Inversion of carbon I would lead to an anomer, but the 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride derived therefrom is thought to be a mixture of anomers. The generally observed *trans* relationship[18,19] between the 2′-hydroxyl and the entering aglycone indicates that during the condensation of chloromercuri-6-methylmercaptopurine and 2,3,5-tri-*O*-acetopentofuranosyl chloride, the group entering at carbon 1 will always be *trans* to the acyl substituent at carbon 2; consequently, inversion at carbon-1 would not affect the configuration of the final product.

Inversion of carbon-2 *and subsequent entrance of the purine in a trans position* would yield 9-α-D-arabofuranosyladenine-*t*.[20] Similarly, inversion at carbon atom 3 would result in the formation of 9-β-D-xylofuranosyladenine-*t*.[21] Inversion at carbon-4 would result in 9-α-L-lyxofuranosyladenine-*t*. Thus, a single inversion occurring at any asymmetric center in the molecule of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose would have given rise to isomers which, if not eliminated by crystallization, would have been detected in the electrophoresis experiments described below.

Since the atom per cent. of tritium in the molecule is extremely small, *ca.* 0.0005%, and since no single inversions were apparent, the possibility of the inversion of two asymmetric centers per molecule of final product is unlikely. However, inversions at both carbons 2 and 3 would lead to 9-α-D-lyxofuranosyladenine-*t*, and at 2 and 4, or at 3 and 4, would lead to the β-L-xylo- or α-L-arabo- isomers, all three of which should be distinguishable by the electrophoresis. For the β-L-ribo-isomer, enantiomorphic to the main product, to arise, inversions would be required at carbons 2, 3 and 4.

TABLE II

IONOPHORETIC SEPARATION OF PENTOFURANOSYL NUCLEOSIDES IN BORATE BUFFERS[a]

Compound	Relative mobilities anodic migration, cm. pH 9.2	pH 6.0
9-β-D-Ribofuranosyladenine-*ribosyl-t*	...	5.2
9-β-D-Ribofuranosyladenine	4.7	5.2
9-α-L-Arabofuranosyladenine	−1.5	−3.3
9-β-D-Xylofuranosyladenine	4.0	0.0
1-β-D-Ribofuranosylthymine	11.1	7.3
1-β-D-Xylofuranosylthymine	10.2	2.9
1-β-D-Arabofuranosylthymine	0.2	−5.0
1-β-D-Lyxofuranosylthymine	11.2	12.5

[a] The adenine nucleosides were run simultaneously with the use of a current of 30–35 milliamp. at 500 volts for 180 minutes. The thymine nucleosides also were run simultaneously with the use of a current of 50 milliamp. at 600–700 volts for 180 minutes.

Electrophoretic Studies.—The availability of all four of the possible 1-β-D-aldopentofuranosylthymines (Table II)[22] made possible an investigation of ionophoretic separation of the isomeric nucleosides. This method was applicable for testing the radiochemical purity of the 9-β-D-ribofuranosyl-

(15) A. Hampton, J. J. Biesele, A. E. Moore and G. B. Brown, THIS JOURNAL, **78**, 5695 (1956).

(16) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(17) A. Hampton, THIS JOURNAL, **79**, 503 (1957).

(18) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954).

(19) Fox and Goodman condensed α- and β-2,3,4,6-tetra-*O*-acetylglucopyranosyl chloride with 2,4-diethoxypyrimidine and obtained the same β-nucleoside in both cases; J. J. Fox and I. Goodman, THIS JOURNAL, **73**, 3256 (1951).

(20) N. W. Bristow and B. Lythgoe, *J. Chem. Soc.*, 2306 (1949).

(21) P. Chang and B. Lythgoe, *ibid.*, 1992 (1950).

(22) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, THIS JOURNAL, **78**, 2117 (1956); J. J. Fox, N. Yung and A. Bendich, *ibid.*, **79**, 2775 (1957); J. J. Fox, J. F. Codington, N. Yung, L. Kaplan and J. O. Lampen, *ibid.*, **80**, 5155. (1958).

adenine-*t*. Extensive studies of the ionophoretic behavior of carbohydrates and carbohydrate derivatives have been carried out by Foster and coworkers,[23] while Burke has reported the ionophoretic mobilities of several glycosylpurines and pyrimidines.[24] Both investigations utilized borate buffer at *p*H 10.

Ionophoresis of 1-β-D-xylofuranosyl- and 1-β-D-ribofuranosylthymine in borate buffers at *p*H values from 6 to 9.2 showed that the mobilities of these compounds were virtually identical at high *p*H values, but that the separations were pronounced at lower *p*H values. At *p*H 6.0 the separation of all four of the thymine nucleosides was very definite (Table II).

The negligible mobility of the arabofuranosyl derivative in borate buffers is probably a reflection of the difficulty of formation of a distorted six-membered ring bridging the 3′- and 5′-hydroxyl groups.[23] In the xylofuranosyl derivatives the *cis* configuration of the 3′-hydroxyl and the 4′-methylol groups should lead to the ready formation of a six-membered ring[23] with the 3′- and 5′-hydroxyl groups. The 2′,3′-*cis*-hydroxyl groups in the ribofuranosyl derivatives are ideally situated to form a stable five-membered cyclic complex with borate ions,[25] whereas the lyxofuranosyl structure should be capable of forming either 3′ to 5′ or 2′ to 3′ cyclic complexes. There is also some possibility of the formation of a tridentate borate complex in the case of the lyxofuranosyl compounds.[26] From these considerations, the ionophoretic mobility in borate buffers would be expected to increase in the order: arabofuranosyl, xylofuranosyl, ribofuranosyl and lyxofuranosyl derivatives. A decrease in the *p*H of the buffer would affect the mobilities of the initial members of this series more than the latter members, either by a decrease in the stability of the complex formation and/or a decrease in the ionization of the complexes.[27] The experimental results are in accord (Table II).

Of the more probable contaminants of the 9-β-D-ribofuranosyladenine-*t*, the 9-β-D-xylofuranosyladenine was available.[28] In addition, 9-α-L-arabofuranosyladenine,[29] which is an enantiomorph of, and must exhibit the same mobility as, the possible 9-α-D-arabofuranosyladenine, was available. The electrophoretic mobilities of these compounds are given in Table II. The 9-α-L-lyxo-isomer is unavailable, but by analogy to the behavior of 1-β-D-lyxofuranosylthymine, would be readily separable. Although a *cis* relationship of the adenine moiety of the cyclic borate complex on the 2′,3′-hydroxyls might be expected to decrease the mobility by some interference with complex formation, a *trans* relationship prevails in the 9-α-L-lyxo-isomer which must be considered.

In borate buffer at *p*H 6.0, the electrophoretic mobility of the 9-β-D-ribofuranosyladenine-*t* was identical to that of adenosine, and no experimentally significant amounts of radioactivity could be found except in that area of the chromatogram (Table III). Thus, the product is essentially radiochemically pure.

TABLE III

RELATIVE MOBILITY OF RADIOACTIVE MATERIAL[a]

Distance from origin,[b] cm.	Reference adenine derivative	Radioactivity,[c] c.p.m.
−8.6 to −7.0		<1
−5.6 to −4.2		1
−4.2 to −2.8	9-α-L-Arabofuranosyl at −3.0 cm.	<1
−2.8 to −1.5		<1
−1.5 to 0.0		1
0.0 to 1.6	9-β-D-Xylofuranosyl at 1.0 cm.	2
1.6 to 3.2		2
3.2 to 4.8		<1
4.8 to 6.5		3
6.5 to 8.8	9-β-D-Ribofuranosyl at 7.9 cm.	108
8.8 to 10.4		2
10.4 to 12.1		<1
12.1 to 13.7		<1
13.7 to 15.2		<1
15.2 to 16.8		<1

[a] The ionophoretic separation was carried out at 700 volts using a current of 21–25 milliamp. for 240 minutes. [b] Anodic migration is considered positive. [c] Strips of the indicated width and 5.5 cm. long were eluted overnight with 4.0-ml. portions of water. Then 2.0-ml. aliquots were plated and assayed for radioactivity. The decrease in counting efficiency due to the presence of salts is assumed to be uniform. The error in radioactivity determinations is *ca.* ±2 c.p.m.

Experimental

All m.p.'s are corrected. Radioactivity determinations were carried out on samples (<2 μg./cm.^{2} unless otherwise noted) plated on 10 cm.^{2} aluminum planchets with an internal Geiger–Müller flow counter (Radiation Counter Laboratories, Mark 12, model 1, helium–isobutane gas, efficiency *ca.* 50%) probable error ±5% unless otherwise noted. Ultraviolet spectra were determined by the use of a Beckman DK2, and O.D. values with a Beckman DU spectrophotometer. Paper chromatograms, unless otherwise specified, were descending and developed on Schleicher and Schuell No. 597 paper with the use of 1-butanol saturated with water.

Li^6(n,α)T Reaction.—A 6.0-g. sample of D-ribose[30] and 6.0 g. of lithium carbonate were intimately ground. Samples (2.00 g.) were irradiated in water-cooled holes under the conditions indicated in Table I. After the period of irradiation each of the samples was extracted with 5.0 ml. of warm water and filtered by gravity. The residues of lithium carbonate and the original containers were washed four times with a total of 10 ml. of hot water. The combined filtrates from each sample were each treated with *ca.* 0.5 ml. of Dowex-50 (H+) until the evolution of carbon dioxide had ceased. Then the solutions were passed through small columns of Dowex-50 (H+) each containing *ca.* 5 ml. of resin to remove cations and the columns washed four times with small portions of water. The eluates were each collected in tared flasks and evaporated to incipient dryness *in vacuo*, temperature *ca.* 50°. The samples were dried *in vacuo* over phosphorus pentoxide. The weights obtained at this stage decreased with increasing time of radiation (Table I), and the residues tended to become more insoluble.

Quantitative pentose[14] and radioactivity determinations were performed on the residues (Table I). Paper chromatographic examination (descending) of the residues with the use of a solvent system composed of the upper layer of a mixture of five parts of 1-butanol, four parts of water[12] and one part of ethanol, indicated that the only aniline phthalate positive component[13] present was D-ribose.

(23) A. B. Foster, *J. Chem. Soc.*, 1395 (1957), and previous papers.
(24) D. C. Burke, *Chemistry & Industry*, 1510 (1954).
(25) J. Böeseken, *Adv. in Carbohydrate Chem.*, **4**, 189 (1949).
(26) S. J. Angyal and D. J. McHugh, *Chemistry & Industry*, 1147 (1956).
(27) R. Consden and W. M. Stanier, *Nature*, **169**, 783 (1952).
(28) D. A. Clarke, J. Davoll, F. S. Philips and G. B. Brown, *J. Pharm. Exptl. Therap.*, **106**, 291 (1952).
(29) J. Davoll and B. A. Lowy, THIS JOURNAL, **74**, 1563 (1952).
(30) Obtained from Hoffman-La Roche, Inc., Nutley, N. J.

Labeling by Exposure to Tritium Gas.[31]—A 0.997-g. sample of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose[32] (m.p. 83.5–84°) was sealed in a vial with 1.2 curies of carrier-free tritium gas at a pressure of *ca.* 340 mm. After 15 days at room temperature the vial was opened and the tritium gas removed. The resulting material (m.p. 81.5–83°) had a faint odor of acetic acid and assayed 5.9×10^6 c.p.m./μmole (0.0014 atom % tritium). The pentose content of the material was determined to be 98 ± 2% of the theoretical by the use of a standard procedure,[14] except that either aqueous ribose or ethanolic solutions of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose were used as standards. When the latter was used, and the last traces of ethanol were removed *in vacuo* over phosphorus pentoxide before the addition of further reagents, there was no experimental difference between the intensities of the final colors produced from the two standards.

Preparation of 6-Methylmercapto-9-β-D-ribofuranosylpurine-ribosyl-*t*.—A 452-mg. sample of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose-*t* (1.42 mmoles) was converted to 2,3,5-tri-*O*-acetyl-D-ribofuranosyl-*t*-chloride.[33] The solution of the product was slightly brownish in contrast to the colorless product usually obtained. The material was condensed with 620 mg. of chloromercuri-6-methylmercaptopurine (1.55 mmoles) in the presence of 500 mg. of Celite, and the reaction mixture was worked up[15] to yield 6-methylmercapto-9-β-D-ribofuranosylpurine-ribosyl-*t*. The product was recrystallized from water and then alcohol to give 38.5 mg. (9%) of material of m.p. 158–161° (reported[34] 163–163.5°). The radioactivity of the material was determined to be 1.4×10^6 c.p.m./μmole (with the use of A_M values of 12,100 at 221 mμ and 18,900 at 289 mμ for water at *p*H 6). The spectrum was identical with that of an authentic sample of 6-methylmercapto-9-β-D-ribofuranosylpurine.

Preparation of 9-β-D-Ribofuranosyladenine-*ribosyl-t*.—Solutions of inert and radioactive 6-methylmercapto-9-β-D-ribofuranosylpurine-*ribosyl-t* were mixed to give material of specific activity 25,900 c.p.m./μmole. This material (205 mg., 0.69 mmole) was heated in a sealed tube with 15 ml. of methanolic ammonia (saturated at 0°) at 100° for 10 hr. The solution was then evaporated *in vacuo*. Chromatography of the residue indicated that the conversion was incomplete. The material was reheated at 125° for 4 hr. with 5 ml. of methanolic ammonia. Chromatographic examination of the residue indicated virtually complete conversion. The solution was treated with charcoal, concentrated to *ca.* 2 ml. and allowed to crystallize. The product was recrystallized twice from ethanol, from water, and then in two crops from water (27.5 and 8.0 mg. respectively) to give 35.5 mg. of product (19%), m.p. (first crop) 235–236.5°, mixed m.p. with adenosine 234–236°. All further studies were done on the first crop of crystals. The spectrum of the material was identical with that of adenosine, and no impurities were visible on paper chromatograms.

Anal.[35] Calcd. for $C_{10}H_{13}N_5O_4$ (267.2): N, 26.21. Found: N, 26.16.

The specific activities of the material after the indicated re-crystallization steps are as follows: third ethanol crystallization, 22,700 c.p.m./μmole; first water crystallization, 22,800 c.p.m./μmole; second water crystallization 22,400 c.p.m./μmole.

Electrophoresis Experiments.—The studies were done by the use of the E. C. Paper Electrophoresis Apparatus, E. C. Apparatus Co., Walnut Lane, Swarthmore, Penna., with Whatman 3 MM paper. The borate buffers were prepared by adjusting the *p*H of saturated aqueous boric acid with 10 *N* sodium hydroxide, except that the *p*H 9.2 borate buffer was a 0.05 *M* solution of sodium tetraborate.

Acknowledgment.—The authors wish to thank Mrs. Dina Van Praag for assistance with the initial phases of this investigation. The receipt of samples of the four 1-β-D-pentofuranosylthymines from Drs. J. J. Fox and J. F. Codington, and the coöperation of Dr. Seymour Rothchild, Technical Director, New England Nuclear Corporation, are gratefully acknowledged. We wish to thank Dr. David I. Magrath of the Australian National University, Canberra, Australia, for the suggestions concerning the theoretical basis of the electrophoretic separations, and Dr. Fox for discussions of the stereoisomerism involved.

(31) The exposure to gaseous tritium was performed by the New England Nuclear Corporation, 575 Albany Street, Boston 18, Mass.

(32) G. B. Brown, J. Davoll and B. A. Lowy, "Biochemical Preparations," Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 70.

(33) J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 967 (1948).

(34) A. Hampton, unpublished results.

(35) Analysis by J. F. Alicino, Metuchen, N. J. The sample was dried *in vacuo* at 100° for 4 hr. over phosphorus pentoxide.

NEW YORK 21, N. Y.

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION, CORNELL UNIVERSITY MEDICAL COLLEGE]

The Synthesis of Cytidine-2-C^{14}-ribosyl-*t*[1]

BY JOHN F. CODINGTON, RONALD FECHER, M. HELEN MAGUIRE, R. Y. THOMSON AND GEORGE BOSWORTH BROWN

RECEIVED FEBRUARY 15, 1958

The synthesis of cytidine has been adapted to the use of tetra-*O*-acetylribofuranose, and a synthesis from cytosine-2-C^{14} and tetraacetylribofuranose-*t*-has been carried out.

Studies with cytidine[2,3] and with cytidylic acid,[4] labeled with C^{14} in both the cytosine and ribosyl moieties, have demonstrated in the rat that the base–ribose bond remains intact during the incorporation of the cytidine unit into ribonucleic acids. Those studies also have confirmed the earlier deductions[5] that the ribosyl derivative can be converted to the deoxyribosyl derivative without cleavage of the glycosyl bond. In *Escherichia coli*,[2] however, the ribosyl bond is extensively cleaved and the pyrimidine is utilized independently. The use of cytidine labeled with C^{14} and tritium in the aglycone and glycosyl moieties, respectively, would simplify surveys of the type of reaction which takes place in various tissues and species. It should also permit studies of the mechanism by which ribosyl derivatives are "deoxygenated" to deoxyribosyl derivatives.

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. CY-3190), and from the Atomic Energy Commission (Contract No. AT. (30-1), 910).

(2) I. A. Rose and B. S. Schweigert, *J. Biol. Chem.*, **202**, 635 (1953).

(3) P. Reichard, *Acta Chem. Scand.*, **11**, 11 (1957).

(4) P. M. Roll, H. Weinfeld and E. Carroll, *J. Biol. Chem.*, **220**, 455 (1956).

(5) E. Hammarsten, P. Reichard and E. Saluste, *ibid.*, **183**, 105 (1950).