# The Synthesis of Ribose and of Adenine Nucleotides Containing Oxygen-18[1]

**H. Follmann and H. P. C. Hogenkamp**

*Contribution from the Department of Biochemistry, College of Medicine, University of Iowa, Iowa City, Iowa 52240.* *Received June 19, 1969*

**Abstract:** Oxygen-18 labeled nucleotides permit biochemical studies involving the hydroxyl functions of their sugar moiety. We have synthesized and compared a series of $^{18}O$-containing hexoses and pentoses suitable for conversion to D-ribose-$^{18}O$. The key intermediates in these syntheses are the keto sugars 1,2:5,6-di-O-isopropylidene-$\alpha$-D-*ribo*-hexofuranos-3-ulose (**2**), benzyl 3,4-O-isopropylidene-$\alpha$-D-*erythro*-pentopyranos-2-uloside (**11**), and 1,2-O-isopropylidene-5-O-benzoyl-$\alpha$-D-*erythro*-pentofuranos-3-ulose (**15**). The heavy isotope was introduced by an uncatalyzed exchange reaction with oxygen-18 enriched water; crystalline hydrates were obtained from the sugar ketones. Reduction of these hydrates yielded predominantly 1,2:5,6-di-O-isopropylidene-$\alpha$-D-allofuranose-3-$^{18}O$ (**4**), benzyl 3,4-O-isopropylidene-$\beta$-D-ribopyranoside-2-$^{18}O$ (**13**), and 1,2-O-isopropylidene-5-O-benzoyl-$\alpha$-D-ribofuranose-3-$^{18}O$ (**17**). Compound **4** could be converted to D-ribose-3-$^{18}O$ in good yield and to D-ribose-2-$^{18}O$ in poor yield. Both compounds **13** and **17** were readily converted to D-ribose-2-$^{18}O$ and D-ribose-3-$^{18}O$, respectively. Attempts to prepare D-ribose-4-$^{18}O$ in a similar reaction sequence have been unsuccessful. Adenosine-2'-$^{18}O$ and adenosine-3'-$^{18}O$ were synthesized by first condensing the appropriately labeled 1-chloro-2,3,5-tri-O-acyl-D-ribofuranose with 6-benzamidochloromercuripurine, and then removing the protecting groups with ammonia. Both nucleosides were converted to their corresponding triphosphates by published procedures. A comparatively simple route to adenosine-5'-$^{18}O$ involves $H^{18}OH$ exchange into adenosine-5'-aldehyde and subsequent sodium borohydride reduction. The position of the heavy isotope in the specific hydroxyl functions has been verified by mass spectrometry.

Nucleosides and nucleotides bearing oxygen isotopes are necessary for investigations on the origin and fate of oxygen atoms during the enzymatic formation and conversions of their sugar moiety. We desired to synthesize ribonucleoside-5'-diphosphates and -triphosphates specifically labeled in the 2' and 3' hydroxyl groups in order to study the mechanism of the enzymatic reduction of these compounds to 2'-deoxyribonucleotides by ribonucleotide reductases.[2] Moreover, isotope-labeled nucleosides and other sugar derivatives are valuable for direct confirmation of the mode of fragmentation which has been deduced from the mass spectra of the unlabeled materials.[3]

Adenosine-5'-$^{18}O$, adenosine-2',4'-$^{18}O$, deoxyadenosine-5'-$^{18}O$, deoxyadenosine-4'-$^{18}O$, and some other nucleosides with the same labeling pattern have been isolated from the nucleic acids of *Escherichia coli* grown on media containing glucose-$^{18}O$ or fructose-$^{18}O$ as the sole carbon source,[4] and the preparation of 2',3'-isopropylideneuridine-5'-$^{18}O$ from 5'-*p*-toluenesulfonyl-2',3'-isopropylideneuridine and sodium acetate-$^{18}O$ has recently been reported.[4c] However, direct introduction of isotopic oxygen into the 2' or 3' position of preformed nucleosides appears less feasible because addition or substitution reactions at anhydro- or cyclonucleosides which could be carried out with $^{18}O$ enriched water, acetic acid, or similar agents do not usually lead to formation of ribonucleosides but rather to arabino- or xylonucleosides.[5] Only in one specific case does hydrolytic cleavage of acetylated O$^2$,2'-cyclouridine yield uridine.[6] Another method for the introduction of $^{18}O$ into hydroxyl functions is the isotope exchange between water-$^{18}O$ and aldehydes or ketones, followed by a reduction of the carbonyl group. In the nucleoside series, this reaction sequence is limited to nucleoside-5'-aldehydes,[7] since the corresponding 2'- and 3'-ketonucleosides are not generally accessible, are very unstable, and upon reduction yield arabino- or xylonucleosides as the main products.[8] Direct introduction of $^{18}O$ into the 4' position of ribonucleosides would not be possible without prior cleavage of the glycosidic bond. We therefore decided to use a more general approach for the preparation of $^{18}O$-labeled nucleosides by introducing the label *via* water-$^{18}O$ exchange into keto sugars, conversion of the latter to D-ribose and subsequent condensation with the base.

This paper describes a series of $^{18}O$-containing hexose and pentose derivatives suitable for conversion to D-ribose-$^{18}O$, and the preparation of adenosine and adenosine phosphates labeled with oxygen-18 in the 2', 3', or 5' position. D-Ribose-3-$^{18}O$ could be synthsized in good yield from two intermediates, 1,2-O-isopropylidene-5-O-benzoyl-$\alpha$ D-*erythro*-pentofuranos-3-ulose (**15**) and 1,2:5,6-di-O-isopropylidene-$\alpha$-D-*ribo*-hexofuranos-3-ulose (**2**), with removal of carbon-6 from the latter hexose. In contrast, removal of carbon-1 from the same intermediate did not present a satisfactory route to D-ribose-2-$^{18}O$; this sugar was obtained in good yield by using benzyl 3,4-O-isopropylidene-$\beta$-D-*erythro*-pentopyranos-2-uloside (**11**) as starting material.

(1) This work was supported by Grant AM 08627 from the National Institutes of Health, U. S. Public Health Service.

(2) (a) R. L. Blakley, *Fed. Proc.*, **25**, 1633 (1966); (b) P. Reichard, *Eur. J. Biochem.*, **3**, 259 (1968); (c) H. Follmann and H. P. C. Hogenkamp, *Biochemistry*, **8**, 4372 (1969).

(3) (a) K. Biemann and J. A. McCloskey, *J. Amer. Chem. Soc.*, **84**, 2005 (1962); (b) K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, Chapter 10.

(4) (a) R. Caprioli and D. Rittenberg, *Proc. Nat. Acad. Sci. U. S.*, **60**, 1379 (1968); (b) R. Caprioli and D. Rittenberg, *ibid.*, **61**, 1422 (1968); (c) R. Caprioli and D. Rittenberg, *Biochemistry*, **8**, 3375 (1969).

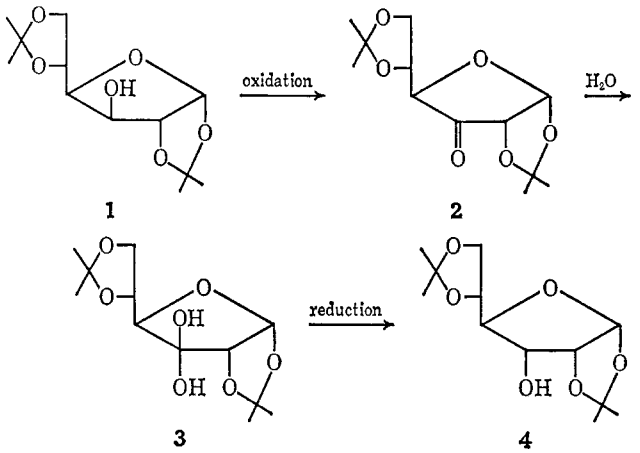
(5) A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press, New York, N. Y., 1963, Chapter 2.

(6) D. M. Brown, D. B. Parihar, and A. R. Todd, *J. Chem. Soc.*, 4242 (1958).

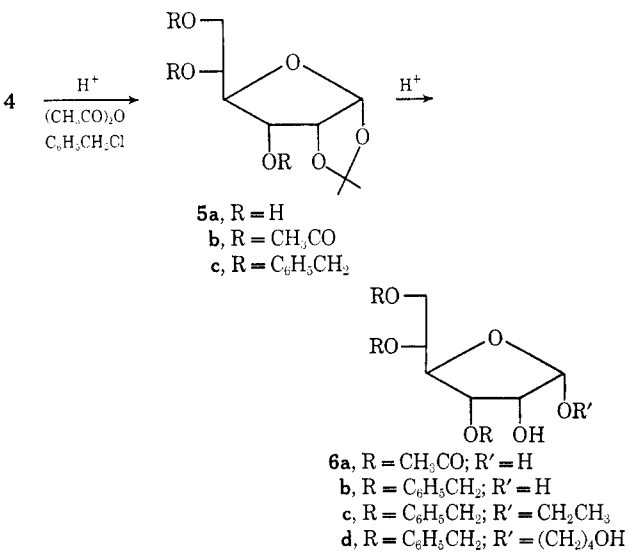
(7) (a) K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, **85**, 3027 (1963); (b) K. E. Pfitzner and J. G. Moffatt, *ibid.*, **87**, 5661 (1965).

(8) A. F. Cook and J. G. Moffatt, *ibid.*, **89**, 2697 (1967).

**Preparation of $^{18}O$-Labeled D-Ribose from D-Allose-3-$^{18}O$.** For the synthesis of 2′ and 3′ labeled nucleosides, we initially focused our attention on the use of 1,2:5,6-di-O-isopropylidene-α-D-allofuranose (**4**) as a common precursor of D-ribose-2-$^{18}O$ and D-ribose-3-$^{18}O$. This derivative of D-allose is readily obtained from diisopropylidene-D-glucose (**1**) *via* reduction of the oxidized sugar, 1,2:5,6-di-O-isopropylidene-α-D-ribo-hexofuranos-3-ulose (**2**).[9] Shortening the allose chain by oxidative removal of either carbon atom 1 or 6 results in a five-carbon precursor of D-ribose. The latter pathway has been successfully applied for the synthesis of D-ribose-3-$^{3}H$.[10]




For the incorporation of heavy oxygen, the keto sugar **2** was especially attractive because it easily forms the stable hydrate **3**.[11] Using $^{18}O$-enriched water (96.5%) a labeled hydrate carrying one heavy oxygen in the *gem*-diol group was obtained in quantitative yield, and its reduction with sodium borohydride in tetrahydrofuran afforded 1,2:5,6-di-O-isopropylidene-α-D-allofuranose-3-$^{18}O$ in 59% yield, containing 41.8% excess $^{18}O$ in the 3-hydroxyl group. The degradation of **4** to D-ribose-3-$^{18}O$ by means of selective acid hydrolysis of the 5,6-isopropylidene group and periodate oxidation of 1,2-O-isopropylidene-α-D-allofuranose (**5a**)




is completely analogous to the synthesis of D-ribose-3-$^{3}H$, as described previously.[10] However, the conversion of **4** into D-ribose-2-$^{18}O$ presents serious difficulties.

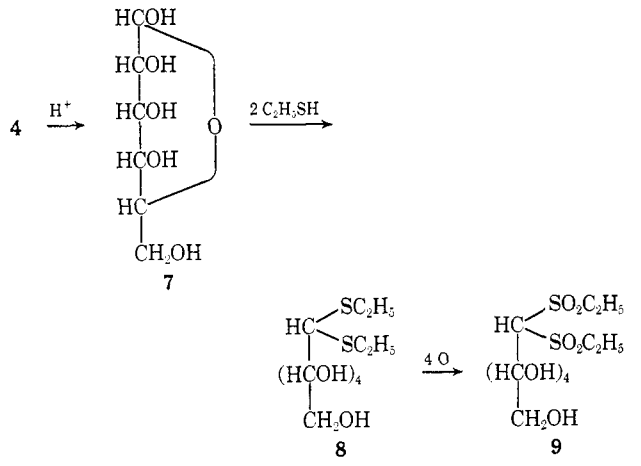
Derivatives of **5a** in which the 3, 5, and 6 hydroxyl groups had been protected with acid-stable acetyl or benzyl residues (**5b**, **5c**) were first subjected to acid hydrolysis to remove the 1,2-isopropylidene group and then oxidized with periodate to remove carbon-1. However, neither 1,2-O-isopropylidene-3,5,6-tri-O-acetyl-α-D-allofuranose (**5b**) nor 1,2-O-isopropylidene-3,5,6-tri-O-benzyl-α-D-allofuranose (**5c**) was suitable for this degradation to D-ribose. From an acid hydrolysate of the triacetate **5b**, no specific product such as 3,5,6-tri-O-acetyl-D-allofuranose (**6a**) could be isolated; subsequent treatment of the reaction mixture with sodium periodate resulted in a nonstoichiometric and irreproducible consumption of the oxidant. Even simultaneous hydrolysis and oxidation of **5b** in aqueous periodic acid, followed by alkaline removal of the acetyl groups, gave only small and varying amounts of ribose. Under these acidic conditions extensive acyl migration may have occurred causing oxidation of the hexose at more than one point.

The isopropylidene group was readily removed from the water-insoluble tribenzyl derivative **5c** by acid hydrolysis in aqueous–organic solvents, but again the expected sugar, 3,5,6-tri-O-benzyl-D-allofuranose (**6b**) was not obtained. Instead, hydrolysis in 60–80% ethanol produced ethyl 3,5,6-tri-O-benzyl-D-allofuranoside (**6c**) in quantitative yield, whereas hydrolysis in tetrahydrofuran yielded a highly viscous product to which the structure of (4-hydroxybutyl)-3,5,6-tri-O-benzyl-D-allofuranoside (**6d**) has been assigned by nmr spectroscopy. Periodate does not affect these compounds, and the substituent on carbon-1 cannot be removed by further treatment with aqueous acids. In another unexpected reaction, the benzyl ether linkages of **5c** and **6c** were readily cleaved in boiling acetic acid containing aqueous hydrochloric acid; here benzyl acetate was the predominant product while most of the sugar decomposed.

The free sugar, D-allose (**7**) proved to be better starting material for the synthesis of D-ribose. D-Allose was obtained by complete hydrolysis of **4**[12] and then subjected to Fischer–MacDonald degradation.[13] In the first reaction step D-allose diethyl dithioacetal (**8**) was prepared in high yield; however, unlike the other hexose dithioacetals, **8** is difficult to crystallize. Oxidation of crude **8** with perpropionic acid in dioxane[14] yielded the disulfone **9** which was finally degraded in dilute ammonia to bis(ethylsulfonyl)methane and D-ribose. Although by this procedure the labeled sulfone, 1-deoxy-1,1-bis(ethylsulfonyl)D-allitol-3-$^{18}O$ was obtained in crystalline form, the low yield of this intermediate (15% from D-allose-3-$^{18}O$) precluded its use for further work in a multistep synthesis of nucleosides with a costly isotope.

**Preparation of D-Ribose-$^{18}O$ from Other Pentoses.** More efficient routes to D-ribose-2-$^{18}O$ and D-ribose-3-$^{18}O$ were found by employing different substituted

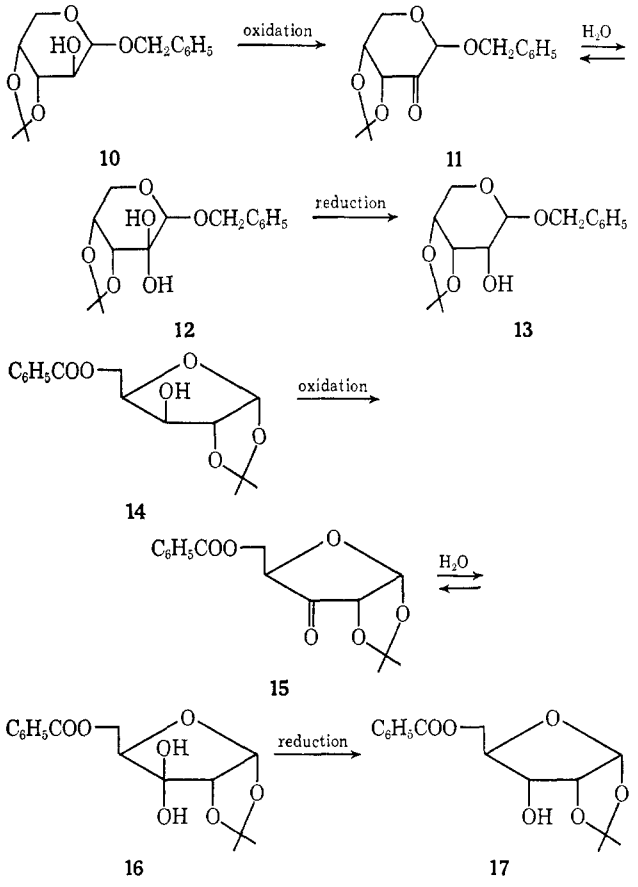
(9) W. Sowa and G. H. S. Thomas, *Can. J. Chem.*, **44**, 836 (1966).
(10) H. P. C. Hogenkamp, *Carbohydrate Res.*, **3**, 239 (1966).
(11) R. J. Beynon, P. M. Collins, P. T. Doganges, and W. G. Overend, *J. Chem. Soc.*, 1131 (1966).
(12) In our hands, this synthesis of D-allose from D-glucose *via* the ketone **2** was superior to the one starting from D-ribose (F. Humoller in "Methods in Carbohydrate Chemistry," Vol. 1, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press, New York, N. Y., 1952, p 102).
(13) D. L. MacDonald and H. O. L. Fischer, *J. Amer. Chem. Soc.*, **74**, 2087 (1952).
(14) R. Barker and D. L. MacDonald, *ibid.*, **82**, 2297 (1960).



pentoses as precursors. Among pentose derivatives that have a free hydroxyl group on carbon atoms 2 or 3, benzyl 3,4-O-isopropylidene-β-D-arabinopyranoside (**10**)[15] and 1,2-O-isopropylidene-5-O-benzoyl-α-D-xylofuranose (**14**)[16] are readily available. Oxidation to the corresponding keto sugars, benzyl 3,4-O-isopropylidene-β-D-*erythro*-pentopyranos-2-ulose (**11**)[17] and 1,2-O-isopropylidene-5-O-benzoyl-α-D-*erythro*-pentofuranos-3-ulose (**15**)[18] was accomplished by the sodium periodate–ruthenium dioxide procedure[19] or by the dimethyl sulfoxide method.[7]

On treatment with water in tetrahydrofuran solution the ketones **11** and **15** undergo reversible hydration. This is evident not only from the exchange with $H^{18}OH$ as described below, but crystalline hydrates (**12, 16**) can be isolated if solvent and excess water are removed carefully. As in the case of the ketohexose **2** and its hydrate **3**, the hydrates **12** and **16** differ from their parent ketones by exhibiting higher melting points and having no carbonyl band in their ir spectra. While stable at room temperature, they slowly lose water upon heating or during recrystallization. This hydrate formation which requires no catalyst appears to be a general characteristic of substituted sugar ketones.

Incubation of the protected ketones **11** and **15** in tetrahydrofuran with $^{18}O$-enriched water (97%) for 6–16 hr at room temperature and subsequent sodium borohydride reduction or catalytic hydrogenation led to benzyl 3,4-O-isopropylidene-β-D-ribopyranoside-2-$^{18}O$ (**13**) and 1,2-O-isopropylidene-5-O-benzoyl-α-D-ribofuranose-3-$^{18}O$ (**17**) in 66 and 70% yield, respectively. An isotope enrichment of more than 50% in the specified hydroxyl group was achieved under these conditions. The preferred formation of the *ribo* configuration during these reductions and in the reduction of **2** is in agreement with earlier observations on the stereochemistry of sugar ketones:[20] an isopropylidene residue adjacent to the carbonyl group renders access of the reductant from the protected side more difficult. In contrast, reduction of the dityrityl-2′- or -3′-ketouridines yields predominantly the *arabino* and *xylo* configurations, respectively.[8]



Attempts have been made to prepare D-ribose-4-$^{18}O$ in a similar reaction sequence starting from a derivative of L-lyxose protected in the 1, 2, and 3 positions. Oxidation and reduction of such a compound can also be expected to produce ribose in view of the observations mentioned above and because these reactions have been successfully applied to the homologous sugar, L-rhamnose.[20] However, methyl L-lyxoside in acetone under a variety of conditions failed to form the desired methyl 2,3-O-isopropylidene-L-lyxopyranoside in satisfactory yield and purity.[21]

**Nucleosides and Nucleotides Labeled with $^{18}O$.** Adenosine-2′-$^{18}O$ and adenosine-3′-$^{18}O$ have been prepared from the labeled ribose derivatives **13** and **17**, respectively. Benzyl and isopropylidene protecting groups were removed from **13** by acid hydrolysis and the resulting free ribose-2-$^{18}O$ was converted to 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose-2-$^{18}O$[22] while **17**, in a one-step reaction, yielded 1,2,3-tri-O-acetyl-5-O-benzoyl-D-ribofuranose-3-$^{18}O$.[23] Condensation of the corresponding 1-chloro sugars with $N^6$-benzoyl-9-chloromercuriadenine[24] and removal of the acyl groups afforded the labeled nucleosides. To phosphorylate the 5′ hydroxyl function, the method of Darlix, *et al.*,[25] was used with intermediate formation of

(15) C. E. Ballou, *J. Amer. Chem. Soc.*, 79, 165 (1957).
(16) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, 102, 317 (1933).
(17) W. Gleason and R. Barker, Abstracts of Papers, 157th National Meeting of the American Chemical Society, Minneapolis, Minn., 1969. We wish to thank these authors for making experimental details available to us.
(18) G. L. Tong, W. W. Lee, and L. Goodman, *J. Org. Chem.*, 32, 1984 (1967).
(19) V. M. Parikh and J. K. N. Jones, *Can. J. Chem.*, 43, 3452 (1965).
(20) P. M. Collins and W. G. Overend, *J. Chem. Soc.*, 1912 (1965).
(21) Examination of the reaction products by nmr indicated the presence of a mixture of isomers which could not be resolved by column chromatography or vacuum distillation. Methyl L-lyxoside has been prepared in analogy to the D isomer as described by P. W. Kent and P. F. V. Wood, *J. Chem. Soc.*, 416 (1953).
(22) E. F. Recondo and H. Rinderknecht, *Helv. Chim. Acta*, 42, 1171 (1959).
(23) G. M. Kamer, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 957 (1948).
(24) J. Davoll and B. A. Lowy, *J. Amer. Chem. Soc.*, 73, 1650 (1951).

2′,3′-cyclic orthoformates in order to avoid losses of the nucleosides usually associated with introduction and removal of isopropylidene protecting groups. In this procedure, unreacted nucleoside was easily recovered during purification of the labeled adenosine-5′-phosphates on an anion exchange column. Finally, the 5′-monophosphates were converted to ATP-2′-$^{18}$O and ATP-3′-$^{18}$O by previously described methods.[26]

The overall yield of nucleoside starting from labeled keto sugars is approximately 10%. This is due mainly to the difficulties of converting small quantities of sugar into the fully acylated intermediates required for nucleoside synthesis, and to the moderate yield in the condensation reaction. However, in the exchange reaction 1 g of highly enriched water–$^{18}$O will suffice for the hydration of at least 25 mmol of keto sugar which in turn will yield 15 mmol of ribose approximately 50% enriched in $^{18}$O in one hydroxyl function. This high isotope content will normally allow dilution with unlabeled intermediates or products in later stages of the synthesis.

The preparation of nucleosides-5′-$^{18}$O by isotope exchange into nucleoside derivatives is comparatively simple. For example, adenosine-5′-aldehyde in tetrahydrofuran solution undergoes $^{18}$O exchange with water–$^{18}$O, and sodium borohydride reduction in the same solvent yields adenosine-5′-$^{18}$O. Because adenosine-5′-aldehyde is only sparingly soluble and is difficult to prepare free from adenosine, the isotope enrichment in the 5′ position of the product did not exceed 50% after incubating the aldehyde in 97% H$^{18}$OH for 16 hr at room temperature.

As expected, physical properties such as melting points, uv spectra, and chromatographic behavior of the $^{18}$O-containing nucleosides do not differ from those of the unlabeled compounds. Mass spectrometry provided evidence for the position and the percentage of heavy oxygen in the nucleosides, indicating that no loss or migration of the isotope had occurred during the various reaction steps.

Because acylated ribofuranosides are universal intermediates for nucleoside syntheses, the procedures described above are readily applicable to the preparation of all the purine or pyrimidine ribonucleosides. Furthermore, they are not limited to the introduction of oxygen isotopes, since reduction of the ketone or aldehyde intermediates by reducing agents that contain deuterium or tritium would yield ribose and nucleosides labeled with isotopic hydrogens in position 2, 3, or 5. A few tritiated compounds of this type have been synthesized.[10,15,27]

## Experimental Section

**General Methods.** Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Melting points are not corrected. Ultraviolet spectra were recorded on a Cary Model 15 spectrophotometer and infrared spectra were obtained from KBr pellets on a Perkin-Elmer Model 521 grating spectrophotometer. Nuclear magnetic resonance spectra were determined using a Varian A-60 spectrometer and tetramethylsilane as reference. Descending paper chromatography on Whatman No. 1 paper and thin layer chromatography on Eastman Chromagram sheets coated with silica gel was carried out in the following solvent systems: solvent I, 1-butanol–acetic acid–water, 4:1:5; solvent II, 1-butanol–water, 86:14; solvent III, ethanol–1 *M* ammonium acetate, 5:2; solvent IV, 2-propanol–water–2 *N* ammonium hydroxide, 7:2:1; solvent V, isopropyl ether. Carbohydrates on chromatograms were detected with 0.3% ammoniacal silver nitrate or with 0.5% sodium periodate and 0.5% benzidine. Benzylated sugars, nucleosides, and nucleotides were located by their absorption of ultraviolet light.

**$^{18}$O Analysis.** The oxygen-18 content of the sugar derivatives was determined by West Coast Technical Service, San Gabriel, Calif., using the $HgCl_2$ oxidation method followed by mass spectrometric analysis of the labeled carbon dioxide formed.[28] These values represent the atom-% $^{18}$O of the entire molecule. Correction for the natural abundance of $^{18}$O (0.204%) and multiplication by the number of oxygen atoms present in the molecule gave the per cent excess $^{18}$O in one particular oxygen atom. Mass spectra of the labeled nucleosides were determined using an Atlas CH-4 mass spectrometer with direct inlet system. The fragmentation pattern observed at 11 or 15 eV electron energy agreed with the reported data.[3,4] The position of isotope could readily be seen from the presence or absence of significant (fragment + 2)$^+$ ions as specified below. The $^{18}$O content of the nucleosides was calculated from the peak heights of the molecular ions $M^+$ and $(M + 2)^+$ respectively, and of the suitable fragments $F^+$ and their $(F + 2)^+$ counterparts. After subtraction of calculated values for the small naturally occurring isotope peaks, atom-% excess $^{18}$O were obtained by using the equation % $^{18}O = (M + 2) \times 100/M + (M + 2)$. Average values from 6–10 different spectra are given.

**1,2:5,6-Di-O-isopropylidene-α-D-allofuranose-3-$^{18}$O (4).** To 1.20 g (4.65 mmol) of 1,2:5,6-di-O-isopropylidene-α-D-*ribo*-hexafuranos-3-ulose (**2**)[9] was added 1.0 g $^{18}$O enriched water (Miles Laboratories, 96.5% $^{18}$O) and the resulting emulsion was stirred rapidly until it solidified under formation of the hydrate **3**, usually after 5–10 min. The excess labeled water (0.91 g) was recovered by condensation into a trap cooled with Dry Ice–acetone under moderate vacuum at room temperature, leaving the hydrate **3** as a white solid in 1.29 g yield (100%). The hydrate was dissolved in 10 ml of anhydrous tetrahydrofuran, 1.9 g (50 mmol) of sodium borohydride was added and the mixture stirred for 1 hr at room temperature. After addition of 50 ml of water, seven 15-ml portions of ethyl acetate were used to extract the product. Evaporation of the combined ethyl acetate solutions *in vacuo* gave a colorless syrup that crystallized from benzene–petroleum ether, mp 73–75°. The yield was 0.71 g (59%) and the $^{18}$O content 7.17 atom-% or 41.8% excess specific label in hydroxyl group 3. The smaller than maximum incorporation (47%) is probably due to incomplete hydrate formation in the heterogeneous medium. From the combined mother liquors of several preparations, a small crop (10%) of crystalline 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-3-$^{18}$O (**1**) was obtained, mp 106–109°.

**1,2-O-Isopropylidene-3,5,6-tri-O-acetyl-α-D-allofuranose (5b).** Diisopropylidene-D-allose (**4**) was hydrolyzed in methanol–0.3 *N* sulfuric acid (1:1) for 2 hr at room temperature[10] and after neutralization with barium carbonate crystalline 1,2-isopropylidene-D-allofuranose (**5a**) was obtained in 80% yield; mp 130–132° (from methanol–ether), $R_f$ 0.70 in solvent I. A solution containing 0.63 g (2.85 mmol) of **5a** in 10 ml of pyridine was treated with 5 ml of acetic anhydride at room temperature for 2 ½ hr. The mixture was then evaporated to dryness; traces of acetic anhydride were removed by several coevaporations with ethanol. The residue crystallized from methanol at −10° in short prisms, mp 79–80°: yield, 1.1 g (58%); ir 1740 cm$^{-1}$ (C=O), no OH absorption.

*Anal.* Calcd for $C_{15}H_{22}O_9$: C, 52.01; H, 6.40. Found: C, 51.81; H, 6.46.

**Degradation Studies.** Hydrolysis of **5b** (0.35 g, 1 mmol) in 25 ml of methanol–0.3 *N* sulfuric acid (1:1) for 2 hr at reflux temperature yielded a compound which precipitated with ether as an amorphous solid but did not crystallize. This material reduced Fehling's solution and had a broad OH absorption in the ir spectrum at 3450 cm$^{-1}$ besides the C=O band at 1745 cm$^{-1}$. Paper chromatography in solvent I showed streaking with a main spot at $R_f$ 0.42. However, treatment with 1 or 2 equiv of sodium periodate did not yield the expected acetylated ribose. Treatment of 1 mmol of **5b** with 25 ml of 0.2 *N* periodic acid in methanol–water (2:3) at reflux temperature likewise led to overoxidation and complete loss of the sugar. When **5b** was treated with periodic acid at room tempera-

(25) J. L. Darlix, H. P. M. Fromageot, and P. Fromageot, *Biochim. Biophys. Acta*, **145**, 517 (1967).
(26) (a) J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 649 (1961); (b) J. G. Moffatt, *Can. J. Chem.*, **42**, 599 (1964).
(27) H. P. C. Hogenkamp, R. K. Ghambeer, C. Brownson, R. L. Blakley, and E. Vitols, *J. Biol. Chem.*, **243**, 799 (1968).
(28) D. Rittenberg and L. Ponticorvo, *Intern. J. Appl. Radiation Isotopes*, **1**, 208 (1956).

ture for 3 days, hydrolysis and subsequent periodate consumption could be detected by following the decrease in absorbancy at 260 mμ. After hydrolysis of the acetyl groups in 1 *N* KOH at 0° for 1 hr, paper chromatography in solvent I revealed the presence of a reducing sugar with the $R_f$ value of ribose (0.30). However, when this degradation was applied to a sample of 3 mmol of **5b** and the resulting material directly used for methylation, benzoylation, and acetylation,[22] the desired 1-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose could not be isolated.

**1,2-O-Isopropylidene-3,5,6-tri-O-benzyl-α-D-allofuranose (5c).** To a suspension of 1.0 g (4.5 mmol) of **5a** in 15 ml of tetrahydrofuran were added 4.5 g of finely powdered KOH and 7 ml of benzyl chloride and the mixture was heated under reflux with stirring for 24 hr. KOH and KCl were then removed by filtration and washed with tetrahydrofuran. The solvent was removed from the filtrate under vacuum, and volatile benzyl compounds were distilled off using an oil pump and 200° bath temperature. The remaining brown oil was applied onto a column of Florisil (1 × 40 cm) which was first eluted with benzene; the first 50 ml of benzene was discarded and 10% ethyl acetate in benzene was then used to elute the product. **5c** was obtained after evaporation as a colorless, viscous syrup which did not crystallize: yield, 1.58 g (72%); homogeneous on tlc, $R_f$ 0.80 in solvent II; nmr ($CDCl_3$) τ 2.70 (s, 15, $C_6H_5$), 4.36 (d, 1, $J$ = 4 Hz, anomeric C–H), 5.15–6.35 (m, 12, $CH_2$ and CH), 8.41 and 8.66 (2 s, 6, $CH_3$).

**Ethyl 3,5,6-Tri-O-benzyl-α-D-allofuranoside (6c).** A solution of 0.35 g (0.7 mmol) of **5c** in a mixture of 4 ml of ethanol and 1 ml of 6 *N* HCl was heated under reflux for 2 ½ hr. After 1 and 2 hr, 1 ml of 1 *N* HCl was added; additional aqueous acid could not be added without precipitating the benzyl compound. Evaporation *in vacuo* left a yellow syrup (0.29 g, 85%) which was homogeneous on tlc ($R_f$ 0.65 in solvent II). In addition to the presence of an ethyl group, the nmr spectrum ($CDCl_3$) showed an OH signal which disappeared upon treatment of the material in ethanol–$D_2O$; τ 2.75 (s, 15, $C_6H_5$), 5.08 (d, $J$ = 2 Hz, 1, anomeric C–H), 5.28–6.62 (m, 14, $CH_2$ and CH), 6.90 (s, 1, O–H), and 8.88 (t, 3, ethyl–$CH_3$).

**(4-Hydroxybutyl)-3,5,6-tri-O-benzyl-α-D-allofuranoside (6d).** A 0.69 g (1.4 mmol) portion of **5c** was boiled for 2 hr in a mixture of 5 ml of tetrahydrofuran and 1 ml of 6 *N* HCl and the resulting solution evaporated to dryness *in vacuo*. The brown residue was chromatographed over Florisil (1 × 30 cm) in benzene, the benzene eluate discarded, and the column then eluted with 10% ethyl acetate in benzene. Upon evaporation, 0.65 g (89%) of a colorless viscous syrup was obtained, homogeneous on tlc ($R_f$ 0.58 in solvent II). The nmr spectrum ($CDCl_3$) exhibited two multiplets typical of tetrahydrofuran or 1,4-butanediol and a signal of two OH protons disappearing after $D_2O$ treatment; τ 2.72 (s, 15, $C_6H_5$), 5.10 (d, $J$ = 2 Hz, 1, anomeric C–H), 6.50 (m, 4, 1,4-butane–$CH_2$), 5.28–6.70 (m, 12, all other $CH_2$ and CH), 6.95 (s, 2, O–H), and 8.32 (m, 4, 2,3-butane–$CH_2$).

**Reactions of 6c and 6d.** These two glycosides could be converted into each other by treating them with aqueous HCl in the required solvent. They remained completely unchanged in 0.17 *M* $HIO_4$, dissolved in ethanol–water, 5:1, for 24 hr. Boiling of either compound in acetic acid with 0.5 volume of 3 *N* HCl immediately led to a deep brown solution; after 0.5 hr, benzyl acetate (60%) was the only product that could be isolated by chromatography on Florisil in ethyl acetate.

**D-Allose-3-$^{18}$O (7).** To 2.20 g (8.5 mmol) of diisopropylidene-D-allose-3-$^{18}$O (**4**) in 100 ml of water–methanol (1:1) were added 100 ml of 0.3 *N* $H_2SO_4$ and the resulting solution was heated under reflux for 2 hr. The mixture was neutralized by addition of solid $BaCO_3$, the barium salts removed by filtration, and washed with 500 ml of water. Evaporation of filtrate plus washings *in vacuo* left a colorless syrup which solidified after repeated evaporation with absolute ethanol. D-Allose-3-$^{18}$O was obtained as a dry white powder in a 1.53 g yield (100%) and was homogeneous as judged by paper chromatography in solvent I ($R_f$ 0.23). The labeled sugar was not crystallized, but from a preparation with unlabeled material 20% of the sugar could be obtained as needles, mp 128° (lit.[12] 128 or 141°) from water–ethanol 1:50.

**D-Allose Diethyl Dithioacetal and D-Allose 3-$^{18}$O-Diethyl Dithioacetal (8).** A solution of 1.50 g of D-allose or D-allose-3-$^{18}$O, respectively (8.3 mmol), in 3 ml of concentrated HCl was stirred vigorously for 2 days at 5° with 8 ml of ethanethiol. After evaporation of the unreacted thiol *in vacuo*, the reaction mixture was diluted with 20 ml of ethanol–water (1:1) and neutralized by addition of solid lead carbonate. The lead salts were filtered off, washed with the same solvent, the filtrate was concentrated to a small volume, taken up in ethanol, and filtered again. The final evaporation *in vacuo* gave 2.17 g (92%) of a yellow syrup which could be decolorized by treatment with charcoal in alcohol or by extraction with ether. The resulting colorless product occasionally crystallized in rosettes after storage for several days at 5°, but separation of the crystals from the adhering syrup or crystallization from solvents could never be achieved. Evidence for the identity of this compound rests upon the mode of preparation, its oxidation to the sulfone **9** as described below, and the identical $R_f$ values of **8**, D-glucose- and D-mannose diethyl dithioacetal in four solvent systems (on paper I, $R_f$ 0.80; II, $R_f$ 0.74; IV, $R_5$ 0.86; on tlc in V, $R_f$ 0.05).

Paper chromatography showed that this preparation contained no allose; however it did ascertain a trace of impurity ($R_f$ 0.57 in solvent I).

**1-Deoxy-1,1-bis(ethylsulfonyl)-D-allitol and 1-Deoxy-1,1-bis(ethylsulfonyl)-D-allitol-3-$^{18}$O (9).** A 1.3 g (4.5 mmol) portion of crude **8** was dissolved with warming in 20 ml of dioxane, the solution brought to boiling, and perpropionic acid (20 mmol, 4 ml of a 5 *M* solution)[14] was added rapidly, causing superheating. After 5 min, the solution was cooled and evaporated *in vacuo*. After removal of acids by repeated evaporation with ethanol, a white product began to crystallize; it was isolated from cold ethanol and washed with ether: yield of crystalline material, 0.35 g (22%), mp 150–156°, from ethanol; the melting point did not change during repeated recrystallizations. The product was homogeneous on paper chromatography ($R_f$ 0.68 in solvent I); it developed a characteristic ochre color when sprayed with the $NaIO_4$–benzidine reagent.

*Anal.* Calcd for $C_{10}H_{22}O_9S_2$: C, 34.28; H, 6.28; S, 18.30. Found: C, 34.53; H, 6.42; S, 18.12.

The mother liquors of crystalline **9** contained more product, but column chromatography over Florisil in various solvents failed to separate this from several by-products. No starting material was left, however. Variation of the oxidation time had no effect on the yield.

**Degradation of 1-Deoxy-1,1-bis(ethylsulfonyl)-D-allitol (9) to D-Ribose.** When 0.35 g (1 mmol) of **9** was treated with 5 ml of 0.3% ammonium hydroxide, the compound dissolved rapidly. In contrast to isomeric hexitol sulfones,[14] degradation of **9** was only complete after 5 days at room temperature as determined by chromatography. The mixture was then extracted twice with chloroform and the aqueous layer concentrated to dryness: D-ribose ($R_f$ 0.30 in solvent I) was the sole product: yield, 0.12 g (80%) as an alcohol-dried powder. Bis(ethylsulfonyl)methane, mp 100–102°, was obtained from the chloroform extract in 75% yield.

**Benzyl 3,4-O-Isopropylidene-β-D-*erythro*-pentopyranos-2-uloside (11).**[17,19] To a solution of 1.4 g (5 mmol) of **10**[15] in 25 ml of carbon tetrachloride was added 25 mg of ruthenium dioxide; a 0.25 *M* solution of sodium periodate was then added dropwise with vigorous stirring at room temperature. The pH of the aqueous mixture was controlled using a pH meter and was maintained between 6 and 7 by addition of small quantities of dilute sodium bicarbonate solution. Twenty-seven milliliters (6.7 mmol) of the oxidant was consumed during 90 min before the end of the reaction was indicated by appearance of the yellow ruthenium tetroxide color. Excess oxidant was destroyed with a few drops of 1-propanol, and the organic layer was separated, filtered, and washed with water. Evaporation *in vacuo* afforded 1.11 g (80%) of a colorless syrup, homogeneous on tlc ($R_f$ 0.80 in solvent V): ir 1750 $cm^{-1}$ (C=O), no OH absorption.

**Benzyl 3,4-O-Isopropylidene-β-D-ribopyranoside-2-$^{18}$O (13).** Because the ketone **11** is insoluble in water and does not form a hydrate in heterogeneous medium as described for **3**, $H^{18}OH$ exchange had to be accomplished in tetrahydrofuran solution, using several small batches of **11**. In a typical preparation, 0.45 g (1.6 mmol) of **11** was treated with 0.200 g of $H^{18}OH$ (97.9%) and 1.4 ml of tetrahydrofuran and the solution was kept at room temperature for 16 hr. During evaporation *in vacuo*, part of the tetrahydrofuran was lost, but the water was condensed quantitatively in a cold trap as determined by fractionated distillation of the recovered solvent mixture. This mixture was used directly for the next incubation of **11**, but it was replenished with 0.060 g of fresh $H^{18}OH$ and sufficient tetrahydrofuran was added to give a clear solution of the ketone. Recovery of the labeled ketone **11**, obtained as a semisolid mixture with its hydrate **12** was also quantitative. For reduction to **13**, this material was dissolved in 4.0 ml of tetrahydrofuran containing 0.050 g of $H^{18}OH$ (recovered from exchange reactions) and the solution was stirred with 0.45 g of sodium borohydride for 45 min. Water was added and the product extracted with five 15-ml portions of ether. The ether layer was concentrated to a small volume and upon addition of petroleum ether and cooling coarse crystals

formed, mp 92–94°;[17] yield, 0.30 g (66%); $R_f$ 0.40 on tlc in solvent V. The $^{18}O$ content of **13** (average value from a mixture of five preparations) was 10.49 atom-% or 51.4% excess in the 2 hydroxyl group. When ketone **11** was incubated for 6 hr only 8.73 atom-% $^{18}O$ was found in **13**. Concentration of the combined mother liquors of **13** gave 18% of the epimer, benzyl 3,4-O-isopropylidene-β-D-arabinopyranoside-2-$^{18}O$ (**10**), mp 55–58[6] (lit.[15] mp 55–58°), $R_f$ 0.50 on tlc in solvent V.

**Benzyl 3,4-O-Isopropylidene-β-D-*erythro*-pentopyranos-2-uloside Hydrate (Benzyl 2-Hydroxy-3,4-O-isopropylidene-β-D-ribopyranoside) (12).** A solution of 0.77 g (2.75 mmol) of **11** in 3.0 ml of tetrahydrofuran and 0.5 ml of water was kept at room temperature for 16 hr, and the solvents were then evaporated *in vacuo*. The residue crystallized in the cold; it was redissolved in ether and precipitated by addition of petroleum ether to give big plates, mp 90–93°: yield of crystalline material, 0.35 g (43%); ir 3500 $cm^{-1}$, sharp, and 3356 $cm^{-1}$, broad (OH), no C=O absorption.

*Anal.* Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 60.76; H, 6.98. Tlc in solvent V showed the same $R_f$ value (0.80) as the ketone. Drying at 50° *in vacuo* also resulted in loss of water and yielded the syrupy ketone **11**.

**1,2-O-Isopropylidene-5-O-benzoyl-α-D-*erythro*-pentofuranos-3-ulose Hydrate (1,2-O-Isopropylidene-3-hydroxy-5-O-benzoyl-α-D-ribofuranose) (16).** A solution of 0.29 g (1 mmol) of **15** in 1 ml of tetrahydrofuran and 0.3 ml of water was kept for 16 hr at room temperature. The solvents were then removed *in vacuo* at 30°. The white amorphous residue (0.31 g, 100%) was dissolved in ether saturated with water, and, upon addition of petroleum ether in the cold, **16** crystallized as prisms, mp 107–111°: ir 1695 $cm^{-1}$ (benzoate), 3430 $cm^{-1}$, sharp, and 3385 $cm^{-1}$, broad (OH).

*Anal.* Calcd for $C_{15}H_{18}O_7$: C, 58.06; H, 5.84. Found: C, 58.73; H. 5.83. Repeated recrystallization from anhydrous ether resulted in loss of water and yielded the ketone **15**.

**1,2-O-Isopropylidene-5-O-benzoyl-α-D-*erythro*-pentofuranos-3-ulose-3-$^{18}O$ (15) and Hydrate-3-$^{18}O$ (16).** $H^{18}OH$ exchange and recovery were carried out in a similar fashion as described for **11**. Portions of 0.25 g (0.85 mmol) of ketone **15** (needles of mp 94–96°)[18] were incubated in 0.6 ml of tetrahydrofuran containing 0.290 g of $H^{18}OH$ (96.5%) for 6 hr at room temperature and evaporated to give 0.265 g (100%) of labeled material. An additional 0.040 g of $H^{18}OH$ was added to the recovered tetrahydrofuran–$H^{18}OH$ mixture prior to incubation of another ketone sample. The white solid residue was recrystallized from ether and had an $^{18}O$ content of 17.14 atom-%. It was a mixture of **15** and its hydrate **16**, as judged by the broad melting range, 90–108°, and by its ir spectrum: 3385 and 3430 $cm^{-1}$ (OH) as in **16** (isotope splitting could not be observed due to the band widths); 1695 $cm^{-1}$ (benzoate), 1702 $cm^{-1}$ (C=$^{18}O$) and 1727 $cm^{-1}$ (C=$^{16}O$). The isotope shift of 25 $cm^{-1}$ for the carbonyl group is similar to those previously reported.[29]

**1,2-O-Isopropylidene-5-O-benzoyl-α-D-ribofuranose-3-$^{18}O$ (17).** A. Following the literature procedure,[18] catalytic hydrogenation of 1.56 g (5.3 mmol) of $^{18}O$ labeled **15** and **16** in 40 ml of absolute ethanol containing 0.15 g of platinum dioxide at atmospheric pressure and room temperature for 20 hr afforded 1.10 g (70%) of protected ribose **17**, mp 78–79% (long prisms, from benzene–cyclohexane): $^{18}O$ content 9.56 atom-% or 56.2% excess label in the 3 hydroxyl group; ir 1720 $cm^{-1}$ (benzoate), 3500 $cm^{-1}$ (OH). No isotope shift could be observed in the OH absorption due to the band width.

B. For the reduction of unlabeled material, 0.50 g (1.7 mmol) of **15** was dissolved in 7 ml of tetrahydrofuran plus 3 ml of water and stirred with 0.35 g of sodium borohydride for 20 min at room temperature. During this time, the pH of the solution was maintained in the range 7–9 by addition of 10% acetic acid. The mixture was then diluted with water and the product extracted with ether to yield 0.31 g (62%) of crystalline **17**, mp 78–80°; the melting point was undepressed in a mixture with authentic **17**, but lowered to 60–65° when mixed with the epimeric xylose derivative **14**.

**1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose-2-$^{18}O$.** D-Ribose-2-$^{18}O$ was obtained from 1.85 g (6.8 mmol) of labeled **13** by hydrolysis in 50 ml of boiling 1 *N* HCl for 75 min. The cooled solution was then extracted with ether and the aqueous phase was passed rapidly through a column (1.5 × 30 cm) of Dowex 1 × 8 ($OH^-$). The column was eluted with water until the eluate ceased to react with Fehling's solution. (To avoid possible epimerization, the quantity of resin should not be larger than required for complete neutralization of the acid present.) The sugar solution was evaporated *in vacuo* to give 0.90 g (6 mmol, 88%) of a colorless glass ($R_f$ 0.30 on paper chromatography in solvent I) which was dried over $P_2O_5$ and directly used for the acylation.[22] The labeled sugar was diluted by adding 0.30 g (2 mmol) of unlabeled D-ribose. Methyl riboside was formed by 5 hr treatment with 25 ml of anhydrous methanol containing 1% of HCl; after neutralization with anhydrous pyridine a yellow syrup was obtained. This material was kept overnight at 5° in a mixture of 7 ml of chloroform, 17 ml of pyridine, and 5 ml of benzoyl chloride. Dilution with water and extraction of the product with chloroform yielded a yellow syrup which was dried *in vacuo*. In the cold, 2 ml of acetic acid and 6 ml of acetic anhydride were added, followed by 0.5 ml of concentrated sulfuric acid, and the mixture kept overnight at 5°. The brown solution was worked up with water and five 20-ml portions of ether. The ether layers were evaporated to dryness and a small volume of cold methanol added to the residue; 1.20 g of crude product precipitated which after recrystallization from ether gave 0.96 of crystals, mp 127–129°, undepressed with an authentic sample (lit.[22] mp 131–132° after repeated crystallization). The mother liquors in ether were extracted with aqueous bicarbonate solution, concentrated, and applied to a column of Florisil (2 × 45 cm) in chloroform; benzoates were first removed with 300 ml of chloroform, while a second fraction of product was eluted with 100 ml of chloroform–methanol, 9:1 (0.32 g after recrystallization): total yield, 1.28 g (32%).

**1,2,3-Tri-O-acetyl-5-O-benzoyl-D-ribofuranose-3-$^{18}O$.** To a cooled solution of 1.20 g (4.0 mmol) $^{18}O$ labeled **17** in 17 ml of acetic acid and 3 ml of acetic anhydride was added dropwise 1.0 ml of concentrated sulfuric acid and the darkening solution was then allowed to reach room temperature. After 16 hr it was diluted with ice cold water and the product was extracted with four 30 ml portions of ether. The ether layer was washed twice with water. Ether was removed *in vacuo*, the residue coevaporated with alcohol to remove acetic acid, dissolved in alcohol and decolorized with charcoal. After concentration of the alcohol solution, 0.39 g of crystals were obtained, mp 116–118° (lit.[23] 117–118°). The mother liquor after evaporation and drying gave an amorphous white solid containing additional 0.22 g of product: total yield, 40%. When **17** was first hydrolyzed in boiling 0.15 *N* sulfuric acid for 2 hr and the resulting syrup acetylated with acetic anhydride in pyridine, the yield of solid product dropped to only 18% while most of the material was obtained as an impure gum not suitable for nucleoside synthesis.

**Adenosine-2′-$^{18}O$.** A suspension of 1.26 g (2.5 mmol) of 1-acetyl-2,3,5-tribenzoylribose-2-$^{18}O$ (0.8 mmol unlabeled plus 1.7 mmol with 38 atom-% specific label) in 100 ml of anhydrous ether saturated with HCl gas at 0° was kept at 5° for 2 1/2 days. The clear solution was then evaporated under exclusion of moisture and coevaporated twice with anhydrous toluene, until a colorless gum resulted. A suspension of 1.40 g (3 mmol) of $N^6$-benzoyl-9-chloromercuriadenine in 120 ml of xylene was dried by slow distillation of one-half of the solvent. The chloro sugar was added to the cooled suspension in two 10-ml portions of dry xylene. The reaction mixture was heated under reflux with stirring for 2 hr (bath, 160°), after which time most of the xylene was allowed to distill off. The cooled residue was poured into 4 vol of petroleum ether and the precipitate (2.7 g) collected. It was extracted with 120 ml of warm chloroform which in turn was first washed with 100 ml of a 30% potassium iodide solution and then with water. The chloroform layer upon evaporation gave 1.15 g of a yellow froth which was kept overnight in 50 ml of methanol saturated with ammonia. The solution was evaporated to dryness, the residue taken up in water and extracted with ether. From the aqueous layer 0.72 g of solid material was obtained which was dissolved in 2 ml of methanol and kept in the cold. Chromatographically pure adenosine-2′-$^{18}O$ (0.24 g) separated; $R_f$ 0.30 in solvent II, $R_f$ 0.68 in solvent III. Further crops (0.11 g) were obtained by adding increasing amounts of ether to the methanolic mother liquor until adenine ($R_f$ 0.42 in solvent II) also precipitated: total yield, 0.35 g (1.3 mmol, 52%); mass spectrum (15 eV) *m/e* 269 and 267 ($M^{18}O^+$, $M^+$), 239 and 237 (loss of 5′-$CH_2O$), 180 and 178 (adeninyl-$CH_2$-$CH_2{}^{18}O^+$, adeninyl-$CH_2CH_2{}^{16}O^+$), 164 (adeninyl-$CH_2O^+$), 135 (adenine$^+$) and 134 (adeninyl$^+$); $^{18}O$ content, 26.9 atom-%.

**Adenosine-3′-$^{18}O$.** This nucleoside was prepared analogous to the adenosine-2′-$^{18}O$ synthesis from 1.025 g (2.7 mmol) of 1,2,3-triacetyl-5-benzoylribose-3-$^{18}O$ (1.6 mmol of 56.2 atom-% specific label diluted with 1.1 mmol unlabeled material) and 1.85 g (4 mmol) of $N^6$-benzoyl-9-chloromercuriadenine, yielding 0.320 g (1.2 mmol, 45%) of chromatographically pure product: mass spectrum (11

(29) M. Byrn and M. Calvin, *J. Amer. Chem. Soc.*, **88**, 1916 (1966).

eV) *m/e* 269 and 267 ($M^{18}O^+$, $M^+$), 239 and 237 (loss of 5′-$CH_2O$), 178 (adeninyl-$CH_2CH_2O^+$), 164 (adeninyl-$CH_2O^+$), 135 (adenine$^+$), 134 (adeninyl$^+$); $^{18}O$ content, 32.7 atom-%.

**Adenosine-5′-$^{18}O$.** Adenosine-5′-aldehyde[7] (50 mg, 0.19 mmol) slowly dissolved in a mixture of 0.6 ml of tetrahydrofuran and 0.250 g of $H^{18}OH$ (97%) when stirred for 16 hr at room temperature. $H^{18}OH$ was recovered as described above and the residue was suspended in 1 ml of tetrahydrofuran containing 0.050 g of $H^{18}OH$. The mixture was stirred for 1 hr with 0.10 g of $NaBH_4$; 0.5 ml of water was then added and stirring continued for 20 min when a clear solution had formed. Tetrahydrofuran was removed *in vacuo*, the aqueous solution neutralized with acetic acid and concentrated. The nucleoside was freed from salts by paper chromatography in butanol–water or by passage over a Dowex 1 × 2 ($OH^-$) column (1 × 30 cm) and elution with 60% methanol, according to the method of Dekker;[30] yield, 34 mg (68%); mass spectrum (11 eV) *m/e* 269 and 267 ($M^{18}O^+$, $M^+$), 237 (loss of 5′-$CH_2{}^{18}O$ and $CH_2{}^{16}O$), 164 (adeninyl-$CH_2CH_2O^+$), 135 (adenine$^+$), 134 (adeninyl$^+$); $^{18}O$ content 50.0 atom-%.

**Adenosine-2′-$^{18}O$ and -3′-$^{18}O$-5′-monophosphates** were prepared by identical procedures.[25] A 3.23-g portion (10 mmol) of barium cyanoethyl phosphate was dissolved in water under addition of some Dowex 50 ($H^+$) and passed through a column of the same resin. The acidic eluate was freeze-dried and the colorless viscous product was further dried *in vacuo* over $P_2O_5$. The cyanoethyl phosphoric acid, dissolved in 4 ml of anhydrous dimethylformamide was added to a stirred suspension of 0.29 g (1.1 mmol) of adenosine-$^{18}O$ and 8 ml of trimethyl orthoformate. After 2 hr at room temperature, a clear solution had resulted which was neutralized with pyridine and evaporated using an oil pump. The residue was dissolved in 10 ml of anhydrous pyridine and 2.1 g (10 mmol) of dicyclohexylcarbodiimide in 5 ml of pyridine was added. The reaction mixture was stirred overnight; dicyclohexyl urea was then removed by filtration and washed with pyridine–water (1:1). The filtrate was evaporated to dryness and treated with 100 ml of 9 *N* ammonia for 90 min at 60°, after which time the solution was filtered, extracted with ether and concentrated to half its volume. The aqueous solution was adjusted to pH 2.2 with concentrated formic acid (about 8 ml), kept for 4 hr at room temperature and lyophilized. The brown residue was redissolved in water and applied to a column (1 × 30 cm) of Dowex 1 × 2 (formate) and unreacted adenosine-$^{18}O$ plus impurities were washed through with water. (The adenosine could best be recovered from the eluate by concentration and chromatography on two sheets of Whatman paper No. 3MM in butanol–water, yielding 0.30 mmol of nucleoside.) AMP-$^{18}O$ was then eluted in a sharp peak with 0.1 *M* formic acid (pH 2.4) and the solution lyophilized; yield, 0.55 mmol (50%, determined optically) as 260 mg of a white solid with unknown water content; $R_f$ 0.20 in solvent III.

**Adenosine-2′- and -3′-$^{18}O$-5′-Triphosphates.** By a published procedure,[26] 0.5 mmol of AMP-2′-$^{18}O$ were converted in quantitative yield to the 5′-phosphoromorpholidate ($R_f$ 0.58 in solvent III) which after drying over $P_2O_5$ was treated with 2.1 mmol of tri-*n*-butylammonium pyrophosphate in 8.1 ml of anhydrous dimethyl sulfoxide. After 2 days no more morpholidate could be detected by chromatography and the mixture was diluted with water and applied to a column (2 × 50 cm) of DEAE-cellulose ($HCO_3^-$). Elution with a triethylammonium bicarbonate gradient (0.05–0.45 *M*) gave 0.030 mmol of AMP-morpholidate, 0.065 mmol of AMP, 0.070 mmol of ADP, and 0.27 mmol (54%) of ATP-2′-$^{18}O$ which was chromatographically pure ($R_f$ 0.08 in solvent III). Similarly, 0.30 mmol of AMP-3′-$^{18}O$ was converted to 0.19 mmol (63%) of ATP-3′-$^{18}O$. The triphosphates were rendered salt free by repeated evaporation *in vacuo* under addition of methanol and after drying over $P_2O_5$ were finally obtained as solid triethylammonium salts.

**Acknowledgment.** We wish to thank Dr. K. L. Rinehart and Mr. J. Wrona of the Department of Chemistry, University of Illinois, Urbana, Illinois, for their assistance with the mass spectrometry of the nucleosides.

(30) C. A. Dekker, *J. Amer. Chem. Soc.*, **87**, 4027 (1965).

# The Synthesis of [1-Deamino,4-L-leucine]-oxytocin and [1-Deamino,4-L-isoleucine]-oxytocin and Some of Their Pharmacological Properties[1,2]

**Herbert Takashima, Victor J. Hruby, and Vincent du Vigneaud[3]**

*Contribution from the Department of Chemistry, Cornell University, Ithaca, New York. 14850.* *Received July 24, 1969*

**Abstract:** [1-Deamino,4-L-leucine]-oxytocin and [1-deamino,4-L-isoleucine]-oxytocin have been synthesized by the solid phase method, and some of their pharmacological properties have been determined. [1-Deamino,4-L-leucine]-oxytocin was also synthesized by the *p*-nitrophenyl ester stepwise procedure and compared to that synthesized by the solid phase method. The two preparations were found to be identical. The oxytocic and avian vasodepressor potencies of [1-deamino,4-L-leucine]-oxytocin and [1-deamino,4-L-isoleucine]-oxytocin are two- to threefold higher than those of [4-L-leucine]-oxytocin and [4-L-isoleucine]-oxytocin respectively. The two deamino analogs also exhibit milk-ejecting activities of approximately 150 units/mg. Neither analog possesses appreciable pressor or antidiuretic activity. A similar enhancement of oxytocic and avian vasodepressor activities had been observed when the free amino group of [4-L-valine]-oxytocin was replaced by hydrogen.

When the amino group of [4-valine]-oxytocin,[4] an analog of oxytocin (Figure 1), was replaced by hydrogen with the formation of [1-demino,4-valine]-oxytocin,[4] it was found that the deamino compound was strikingly more potent than [4-valine]-oxytocin, possessing more than three times the avian vasodepressor potency and more than twice the oxytocic potency of the parent compound. [1-Deamino,4-leucine]-oxytocin and [1-deamino,4-isoleucine]-oxytocin have now been prepared in order to compare their pharmacological properties with those of [4-leucine]-oxytocin and [4-

(1) This work was supported in part by Grant No. HE-11680 from the National Heart Institute, U. S. Public Health Service.

(2) All optically active amino acid residues are of the L variety.

(3) To whom correspondence and reprint requests should be addressed.

(4) V. du Vigneaud, G. Flouret, and R. Walter, *J. Biol. Chem.*, **241**, 2093 (1966).