Preparation of Nucleosides *via* Isopropylidene Sugar Derivatives. IV. Synthesis of 9-α- and 9-β-L-Erythrofuranosyladenine[1]

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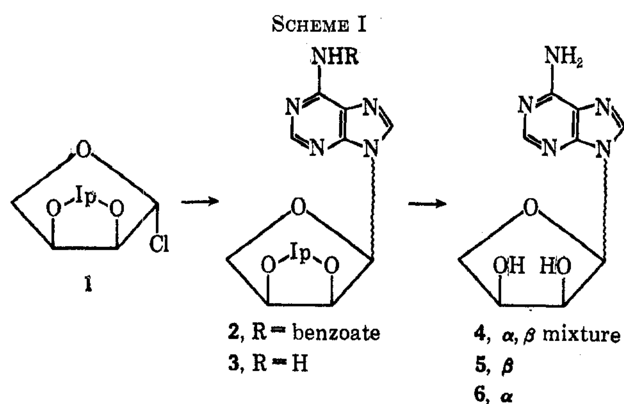
2,3-O-Isopropylidene-β-L-erythrofuranosyl chloride (1) was condensed with 6-benzamidochloromercuripurine and the blocking groups were removed to yield the anomers of 9-L-erythrofuranosyladenine (4) which were separated by column chromatography. In all experiments the β anomer (5) was the main product, leading to the conclusion that this condensation proceeded by an SN1 mechanism.

A common method for the chemical synthesis of the naturally occurring nucleosides and their analogs is by condensation of an appropriate acylated glycosyl halide with the mercuric chloride salt of a purine derivative in a hot inert organic solvent such as xylene or toluene.[2] Such a route has recently been used to prepare adenine nucleosides of D- and L-erythrose as well as D- and L-threose.[3] The reaction pathway consisted of acetylation of the tetrose, condensation with 6-benzamidochloromercuripurine in a refluxing mixture of titanium tetrachloride and ethylene dichloride, followed by deacylation. A 5:1 ratio of β to α anomers of 9-L-erythrofuranosyladenine was obtained.[3]

The present report is the result of an attempt to ascertain the efficacy of synthesis of erythronucleosides using nonparticipating isopropylidene blocking groups and to obtain information about the steric effect of the 2,3-O-isopropylidene group on the anomeric configuration of the product. In previous experiments,[4] the 5,6-O-isopropylidene groups of 2,3:5,6-di-O-isopropylidene-β-D-glucofuranosyl chloride and 2,3:5,6-di-O-isopropylidene-α-D-mannofuranosyl chloride have been implicated as having an important role in the course of the reaction.

The synthetic route used is shown in Scheme I.

SCHEME I



2,3-O-Isopropylidene-β-L-erythrofuranosyl chloride (1)[5] was condensed with 6-benzamidochloromercuripurine to give a crude nucleoside mixture (2). The N-benzoyl group was removed with sodium methoxide and the isopropylidene group was hydrolyzed in hot 25% acetic acid solution. Purification *via* the picrate[6] gave an anomeric mixture (4). The anomers were separated by column chromatography on Dowex 1 (OH) resin by the technique developed by Dekker.[7] In this condensation and others, the β anomers predominated over the α form by a ratio close to 30:1. The yields in all cases were poor, due in part to the unusual stability of the chloride[5] and to adsorption of the nucleosides onto the resin used during the regeneration from the picrate.[6]

In an attempt to isolate the isopropylidene nucleosides (3), a crude picrate was obtained in a separate experiment which upon recrystallization from hot water gave a picrate whose elementary analysis and infrared spectrum indicated that it contained no isopropylidene group. Presumably some hydrolysis of the picrate occurred, liberating sufficient acid to catalyze hydrolysis of the acid labile isopropylidene group to yield **8**. To avoid this deacetonation, **7** was purified by recrystallization from boiling absolute methanol and characterized by melting point, infrared spectrum, and elementary analysis.

Identification of the L-erythrosyl nucleosides was made by comparison of their properties with those reported earlier.[3] A mixture melting point gave no depression in either case and the infrared and ultraviolet spectra were identical. In the earlier experiments[3] the configurational designations were made by referral to the isorotation rules of Hudson. Because some anomeric purine nucleosides do not follow this rule,[4,8] another substantiating proof of configuration was made and this is shown in Scheme II. Determination of the configuration was made by observing the optical rotation of the dialdehyde (**9**) following oxidation with periodate. The reference compound, 9-β-D-xylopyranosyladenine (**10**),[9] yielded a solution of a dialdehyde (**11**) having an optical rotation of equal value but of opposite sign. The single asymmetric carbon atom of each dialdehyde was originally the anomeric carbon atom of the carbohydrate. Compound **5** yielded dialdehyde **9** instead of **11** and therefore must have a β configuration since the carbohydrate is an L sugar.

When a heavy metal salt of a purine or pyrimidine is coupled with a sugar halide containing an acyloxy group at C-2, the predominating nucleoside product will

(1) This work was supported by Grant No. T-442 from the American Cancer Society.

(2) J. A. Montgomery and H. J. Thomas, *Advan. Carbohyd. Chem.*, **17**, 301 (1962).

(3) D. H. Murray and J. Prokop, *J. Pharm. Soc.*, **56**, 865 (1967).

(4) L. M. Lerner, B. D. Kohn, and P. Kohn, *J. Org. Chem.*, **33**, 1780 (1968).

(5) L. M. Lerner, *Carbohydrate Res.*, in press.

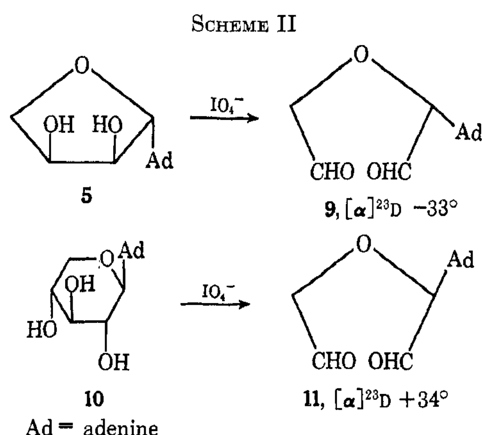
(6) B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 959 (1957).

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

(8) L. Vargha and J. Kuszmann, *Ann.*, **684**, 231 (1965); W. A. Klee and S. H. Mudd, *Biochem.*, **6**, 988 (1967); A. P. Martinez, W. W. Lee, and L. Goodman, Abstracts of Papers, 155th Meeting of the American Chemical Society, San Francisco, Calif., April 1968, N 20.

(9) (a) J. Davoll, B. Lythgoe, and A. R. Todd, *J. Chem. Soc.*, 833 (1946); (b) G. W. Kenner, B. Lythgoe, and A. R. Todd, *ibid.*, 652 (1944).

Scheme II



have a configuration *trans* to the hydroxyl at C-2 (*trans* rule).[10] It has been demonstrated that generation of the chloride with titanium tetrachloride in the presence of the mercuric chloride salt of the base often yields both anomers; however, the *trans* form still predominates.[2,3] The reason for the *trans* predominance has been attributed[10] to the participating nature of the acyloxy group at C-2 in the formation of an orthoester ion involving C-1.

In recent years a number of investigations have been made into the use of nonparticipating groups in nucleoside synthesis. For example, benzyl ethers have been used with arabinose in the synthesis of 9-β-D-arabinofuranosyladenine.[11] In this case a good yield of the *cis* product was obtained but the halogenose was not coupled with a heavy metal salt of the purine. In a separate experiment, details of which were not given, the condensation of the halogenose with 6-benzamidochloromercuripurine yielded both anomers in approximately equal amounts.[11] Lerner and coworkers[4,12] have reported on the use of nonparticipating isopropylidene groups for the facile synthesis of hexofuranosyl nucleosides using the same reaction conditions described in the present paper. In these cases, only the *trans* nucleosides were obtained. These results indicated that there was a steric hindrance to the attack of the incoming base on the *cis* side. Since the chlorides were known to have a *trans* relationship with the oxygen at C-2, it was apparent that a retention of configuration had occurred and that the mechanism of the reaction resembled the SN1 type.

The present experiments with **1** have demonstrated again that the reaction proceeds predominantly with retention of configuration. The main steric hindrance is obviously from the 2,3-*O*-isopropylidene group. The reaction apparently proceeds through loss of a chloride ion, formation of a carbonium ion at C-1, and attack of the nucleophile from the nonhindered side. It has been demonstrated that heterogeneous reactions of alkyl halides in the presence of heavy metal salts resemble homogeneous SN1 reactions.[13] The dipositive mercury removes the expelled chloride ion, thereby preventing ion pair formation with the carbonium ion and permitting nucelophilic attack from the nonhindered β side of the latter. It is of interest to compare the results of this report with that of Murray and Prokop[3] in that more of the α anomer was obtained with a β-directing acyloxy group at C-2 than with a bulky isopropylidene group.

(10) B. R. Baker in Ciba Foundation Symposium, "Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little, Brown, and Co., Boston, Mass., 1957, p 120.

(11) C. R. J. Glaudemans and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 3004 (1963).

(12) L. M. Lerner and P. Kohn, *ibid.*, **31**, 339 (1966).

(13) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., New York, N. Y., 1959, p 273.

Experimental Section[14]

9-α,β-L-Erythrofuranosyladenine (4).—From 3.0 g of 2,3-*O*-isopropylidene-L-erythrose was prepared 2.6 g of the chloride (1).[5] This was allowed to react with 6.6 g of 6-benzamidochloromercuripurine in 400 ml of hot dry xylene containing 6.6 g of Celite 545 and worked up by procedures already described.[4,12] The crude residue (2) was dissolved in 42 ml of warm methanol and 15 ml of a solution of methanolic sodium methoxide, prepared by dissolution of a small chip of sodium in methanol, was added. After 1 hr at room temperature the mixture was neutralized with acetic acid and evaporated to dryness. The residue was partitioned between 50 ml each of chloroform and water. The chloroform layer was removed and the water layer was extracted three times with 50-ml portions of chloroform. The chloroform extracts were combined and the solvent was removed by evaporation. A dark syrup (4.2 g) remained, which was dissolved in 200 ml of 25% acetic acid and heated on a steam bath for 4 hr. Evaporation to a syrup was followed by coevaporation three times with ethanol and three times with toluene to remove traces of acetic acid. The syrup was dissolved in 75 ml of warm methanol, treated with Norit A, and evaporated to a volume of about 50 ml. To this solution was added 50 ml of 10% methanolic picric acid and the flask was kept in an ice bath for 3.5 hr. The yellow precipitate was removed by filtration and washed with cold methanol (40 ml), cold water (25 ml), and cold absolute ethanol (40 ml). The precipitate (1.1 g) was dried *in vacuo* over anhydrous calcium sulfate. This picrate had no melting point but sublimed above 150° and decomposed between 195 and 265°.

The picrate was dissolved in 300 ml of boiling water, the color was discharged with Bio-Rad AG1-X8(CO_3^{2-}) resin,[6] the resin was removed by filtration, and the water was evaporated to yield a white solid (4).

9-α-L-Erythrofuranosyladenine (6).—The white solid was dissolved in 10 ml of water and was applied to the top of a column (13 × 4 cm) of Dowex 1-X2 (OH, 200–400 mesh);[7] fractions (10 ml) were collected. Elution was started with 30% aqueous methanol and after 40 fractions were collected, the eluent was changed to 60% aqueous methanol. Fractions 81–106 were pooled and evaporated to a very small amount of residue which was crystallized from a few drops of water to give 4.5 mg of large crystals, mp 202°. Recrystallization from 95% ethanol gave 2.9 mg of **6** as tiny rods, mp 221.5–222.5°. A sample, kindly supplied by Drs. Murray and Prokop, had mp 221–222.5° (lit.[3] mp 216–218° uncor). A mixture melting point gave no depression and the infrared and ultraviolet spectra were identical.

9-β-L-Erythrofuranosyladenine (5).—Elution of the above resin with 90% aqueous methanol was started with fraction 109. Fractions 110–160 were pooled and removal of the solvents yielded a white solid which was crystallized from water to give 137 mg of white, fine feathery needles, mp 243.5–245° dec. A sample of **5**, kindly supplied by Drs. Murray and Prokop, had the same melting point (lit.[3] 238.5–239.5° uncor). The mixture melting poing gave no depression and the infrared and ultraviolet spectra of the samples were identical.

9-L-Erythrofuranosyladenine Picrate (8).—After removal of the N-benzoyl group of **2** in methanolic sodium methoxide, the picrate was prepared[6] and recrystallized from boiling water. Compound **8** sublimed when heated to 160° and decomposed slowly at a temperature above 180°. The infrared spectrum showed the following peaks: ν_{max}^{KBr} (cm^{-1}) 3360–3100 (broad OH, NH), 1690 ($NH_2C{=}N$), 1615 (phenyl and purine ring), 1565–

(14) Elementary analyses were performed by the Baron Consulting Co., Orange, Conn. Melting points were obtained on a Kofler hot stage and are corrected values. Infrared spectra were recorded on a Perkin-Elmer Model 21 spectrophotometer and ultraviolet spectra were recorded on a Beckman DK-2 spectrophotometer. Evaporations were performed *in vacuo* on a rotary evaporator with a bath temperature of 40–45°.

1545 (broad NO_2, asymmetrical stretching), 1340–1310 (broad NO_2, symmetrical stretching), 1080, 1060 sh (C–O, C–O–C).

Anal. Calcd for $C_{15}H_{14}N_8O_{10}$: C, 38.63; H, 3.03; N, 24.03. Found: C, 38.42; H, 3.21; N, 23.84.

9-(2,3-*O*-Isopropylidene-L-erythrofuranosyl)adenine Picrate (7).—To 2.74 g of 2 in 10 ml of hot ethanol was added 20 ml of 10% ethanolic picric acid, the solution was refluxed for 5 min and chilled.[15] A yellow precipitate (2.28 g) was obtained and recrystallized from boiling methanol to yield 1.26 g of product, mp 230–232° dec, with sublimation beginning near 180°. The infrared spectrum had the following peaks: ν_{max}^{KBr} (cm^{-1}) 3400 (OH, NH), 1690 ($NH_2C{=}N$), 1638, 1610 (phenyl and purine ring), 1570, 1545 (NO_2, asymmetrical stretching), 1362 (*gem*-dimethyl), 1325 (NO_2, symmetrical stretching), 1100–1080, 1055 (C–O, C–O–C).

Anal. Calcd for $C_{18}H_{18}N_8O_{10}$: C, 42.69; H, 3.58; N, 22.13. Found: C, 42.60; H, 3.57; N, 22.44.

9-(2,3-*O*-Isopropylidene-L-erythrofuranosyl)adenine (3).—All attempts to crystallize the regenerated[15] isopropylidene nucleoside from common solvents failed. The anomers were not separated by chromatography on neutral alumina or Dowex 1 (OH) resin, which gave only one peak. Ultraviolet and infrared spectra are as follows: λ_{max}^{EtOH} 258 mμ; ν_{max}^{film} (cm^{-1}) 3300, 3140 (doublet, NH), 1655 sh, 1640, 1600, 1580 ($NH_2C{=}N$, purine ring), 1372 (*gem*-dimethyl), 1110–1090, 1050 (C–O, C–O–C).

(15) J. R. Parikh, M. E. Wolff, and A. Burger, *J. Amer. Chem. Soc.*, **79**, 2778 (1957); M. L. Wolfrom, A. B. Foster, P. McWain, W. von Bebenburg, and A. Thompson, *J. Org. Chem.*, **26**, 3095 (1961).

9-β-D-Xylopyranosyladenine (10) was prepared by condensation of tetra-*O*-acetyl-D-xylopyranosyl bromide with 6-benzamidochloromercuripurine by the method of Davoll and Lowy:[16] mp 302–305° dec; $[\alpha]^{23}D$ −22.5° (*c* 0.62, 1 *N* HCl); lit.[9a] 290° dec; $[\alpha]^{14}D$ −24 ± 4° (*c* 0.17, H_2O). A melting point of 298° has also been reported.[9b]

Polarimetric Studies.—To 14.6 mg of **10** in a 2-ml volumetric flask was added 1.5 ml of 0.08 *M* sodium periodate. The sample was dissolved by heating for several minutes in a steam bath; the flask was stored in the dark for 24 hr. The volume was adjusted to 2 ml with water and the optical rotation obtained—$[\alpha]^{23}D$ +34° based upon the concentration of dialdehyde **11**.

Similarly, 13.9 mg of **5** was treated with periodate and after 24 hr 0.12 ml of 0.503 *M* formic acid was added to adjust for the presence of liberated formic acid in the above experiment. The volume was adjusted to 2 ml and the optical rotation obtained—$[\alpha]^{23}D$ −33° based upon **9**.

Registry No.—3β, 18031-26-0; 3α, 18031-43-1; 4β picrate, 18031-27-1; 4α picrate, 18031-22-6; **5**, 17019-48-6; **6**, 14266-04-7; 7β picrate, 18031-21-5; 7α picrate, 18031-25-9.

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(16) J. Davoll and B. A. Lowy, *J. Amer. Chem. Soc.*, **73**, 1650 (1951).

Reassignment of Configuration to the 22-Hydroxycholesterols. Synthesis of (22*S*)- and (22*R*)-^{3}H-Cholesterols[1]

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Stereospecifically labeled (22*S*)- and (22*R*)-^{3}H-cholesterols were synthesized *via* (22*S*)-hydroxy-22-^{3}H- and (22*R*)-hydroxy-22-^{3}H-cholesteryl 3-benzoates, respectively. The (22*S*)- and (22*R*)-hydroxycholesterols, the (22*S*)- and (22*R*)-hydroxycholesteryl 3-benzoates and the (22*S*)- and (22*R*)-hydroxycholesteryl 3-methyl ethers were interrelated. Assignments of configuration at C-22 were made on the basis of the Horeau and Prelog procedures; the two methods lead to identical assignments. Our results demonstrate that the configurations assigned previously to the 22-hydroxycholesterols and their derivatives are incorrect and should be reversed.

For our continuing studies of the mechanisms of biosynthesis of sterols in plants and animals, samples of cholesterols labeled stereospecifically with a tritium atom at C-22 were required. The synthesis and configurational assignments of the (22*S*)-hydroxy-22-^{3}H- and (22*R*)-hydroxy-22-^{3}H-cholesteryl 3-benzoates and of the (22*S*)- and (22*R*)-^{3}H-cholesterols derived therefrom is the subject of this paper.

The requisite starting material, 22-ketocholesteryl acetate (**1**, Scheme I), was prepared from 3β-acetoxy-23,24-bisnor-5-cholenic acid according to the published procedure.[2] Because the 3-acetoxy group is known not to survive borohydride treatment,[3] it was necessary to replace it by a benzoate prior to introduction of a tritium atom at C-22 by reduction with sodium borotritide. Hydrolysis of the acetate with sodium carbonate was reported to give 22-ketocholesterol without epimerization[2] at C-20, and we have confirmed this by comparison of the nmr spectrum of this product with that of material obtained by potassium hydroxide treatment (see Experimental Section).

(1) The work was supported by Grants GB 5832 from the National Science Foundation, American Cancer Society P-500H and K3 16614 from the National Cancer Institute.

(2) W. Cole and P. L. Julian, *J. Amer. Chem. Soc.*, **67**, 1369 (1945).

(3) K. Tsuda and R. Hayatsu, *ibid.*, **81**, 5987 (1959).

In a cold run, 22-ketocholesteryl benzoate on treatment with a large excess (greater than tenfold) of sodium borohydride gave a high yield of (22*R*)- and (22*S*)-hydroxycholesteryl benzoates (**2a** and **2b**) in a ratio of 1:3. The alcohols were separated by column chromatography followed by repeated thin layer chromatography (tlc) on alumina. The minor alcohol (**2a**, eluted first from the column) was assigned the (*R*) configuration and the major alcohol (**2b**, eluted second) the (*S*) configuration by the Horeau method[4] (Table I).

TABLE I
ASSIGNMENT OF CONFIGURATION TO 22-HYDROXYCHOLESTERYL 3-BENZOATES AND 3-METHYL ETHERS BY THE α-PHENYLBUTYRIC ACID METHOD

Alcohol	Mp, °C	Wt of alcohol, mg	α-Phenylbutyric acid: $[\alpha]D_{obsd}$, deg	α-Phenylbutyric acid: $[\alpha]D$, deg	Optical yield,[a] %	Configuration of alcohol
2a	190	202.2	+0.87	+3.55	15	(22*R*)
2b	162	203.0	−3.28	−13.2	55	(22*S*)
5a	161.5–163.5	164.7	+0.71	+2.81	59	(22*R*)
5a	90–92	167.9	−3.49	−14.2	12	(22*S*)

[a] Calculated as in W. Herz and H. B. Kagan, *J. Org. Chem.*, **32**, 216 (1967).

(4) A. Horeau, *Tetrahedron Lett.*, 506 (1961); 965 (1962); A. Horeau and H. B. Kagan, *Tetrahedron*, **20**, 2431 (1964).