[CONTRIBUTION FROM THE DIVISION OF NUCLEOPROTEIN CHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH; SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK 21, N. Y., AND HOFFMANN LAROCHE, INC., NUTLEY, N. J.]

Nucleosides. XII. Direct Synthesis of 2'-Deoxycytidine and its α-Anomer[1,2]

BY JACK J. FOX, NAISHUN C. YUNG, IRIS WEMPEN AND MAX HOFFER

RECEIVED MAY 8, 1961

The direct synthesis of 2'-deoxycytidine (V), a naturally-occurring constituent of deoxyribonucleic acid, was achieved *via* the mercuri process involving the condensation of 3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribosyl chloride (II) with *N*-acetylcytosinemercury (I). The α-anomer (VI) of V also was obtained from this reaction. The synthesis of II from 2-deoxy-D-ribose is described. The optical rotations of this anomeric pair (V and VI) as well as those of their acylated intermediates do not conform to Hudson's rules of isorotation. The synthesis of other fully acylated derivatives of 2-deoxy-D-ribofuranose from pre-formed purine-2'-deoxy-D-ribonucleosides also is described.

In recent years, several papers have appeared dealing with the synthesis of 2'-deoxy-D-ribofuranosyl nucleosides. In earlier papers[3] such syntheses were achieved by conversion of pre-formed 1-β-D-aldopentofuranosyl-pyrimidines or -purines to their corresponding 2'-deoxy derivatives by elaborate, though elegant, reactions. Other reports[4] have dealt with the conversion of 2'-deoxyuridine[4a] and thymidine[4b] to 2'-deoxycytidine and its 5-methyl analog *via* a thiation procedure. More recently, the direct synthesis of 2'-deoxy-D-ribofuranosyl-pyrimidines[2,5] and -purines[6] from suitably-protected derivatives of 2-deoxy-D-ribose has been accomplished. This paper deals with the direct synthesis of 2'-deoxycytidine[7] and its α-anomer by the mercuri process for pyrimidine nucleoside synthesis[8] employed previously in the preparation of cytidine.[9]

Required for this synthesis was a suitably-protected 3,5-di-*O*-acyl-2-deoxy-D-ribosyl halide for condensation with a proper mercuri-pyrimidine. N-Acetylcytosinemercury[9] was employed since mono-mercuripyrimidines[2,5,10] are relatively more reactive in these condensation reactions than monochloromercuripyrimidines or dipyrimidylmercury derivatives. It was found, further, that 3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribosyl chloride (II) served well in this condensation reaction with *N*-acetylcytosinemercury (I, see Fig. 1). The synthesis of II from 2-deoxy-D-ribose[11] was accomplished by a method[2,5] analogous to that employed for the synthesis of the 3,5-di-*O*-(*p*-toluyl) analog of II. 2-Deoxy-D-ribose was converted to sirupy methyl 2-deoxy-α,β-D-ribofuranoside by the procedure of Deriaz, *et al.*[12] Treatment of this sirup with *p*-chlorobenzoyl chloride in pyridine yielded sirupy methyl 3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribofuranoside as a mixture of anomers. This product was treated with anhydrous hydrogen chloride–acetic acid in ether in the cold. 3,5-Di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribosyl chloride (II) crystallized from the reaction solution in 65% yield based upon 2-deoxy-D-ribose.

The crystalline halogenose II was added to an azeotropically-dried, well-stirred mixture of *N*-acetylcytosinemercury in refluxing, anhydrous xylene and the reaction mixture cooled immediately. The reaction mixture was treated in the usual manner[8] and subjected to fractional crystallization. Two anomers[13] were obtained in 32 and 22% yields, respectively. The first of these (III) showed an optical rotation of −19° and the second (IV) exhibited a rotation of −66°. Both compounds exhibited similar ultraviolet absorption spectra and gave similar elemental analyses.

Each of the anomers was deacylated with alcoholic ammonia. The −19° blocked nucleoside (III) yielded a product identical with naturally-occurring 2'-deoxycytidine with regard to melting point, mixed melting point, optical rotation (+78°), infrared (see Fig. 2) and ultraviolet absorption spectra. Synthetic 2'-deoxycytidine (V) formed a picrate and hydrochloride salt with properties also similar to those exhibited by these derivatives of the naturally-occurring nucleoside. Since the configuration of 2'-deoxycytidine (isolated from deoxyribonucleic acid) is now firmly established as beta,[14] it follows that intermediate III is also of

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. 3190).

(2) A preliminary communication has appeared; see M. Hoffer, R. Duschinsky, J. J. Fox and N. C. Yung, *J. Am. Chem. Soc.*, **81**, 4112 (1959).

(3) (a) D. M. Brown, D. B. Parihar, C. B. Reese and A. R. Todd, *Proc. Chem. Soc.*, 321 (1957); *J. Chem. Soc.*, 3035 (1958); (b) G. Shaw and R. N. Warrener, *Proc. Chem. Soc.*, 81 (1958); *J. Chem. Soc.*, 50 (1959); (c) C. D. Anderson, L. Goodman and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 6453 (1958); **81**, 3967 (1959).

(4) (a) I. Wempen, R. Duschinsky, L. Kaplan and J. J. Fox, *ibid.*, in press; (b) J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich and G. B. Brown, *ibid.*, **81**, 178 (1959).

(5) M. Hoffer, *Chem. Ber.*, **93**, 2777 (1960).

(6) R. K. Ness and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **81**, 4752 (1959); **82**, 3434 (1960); C. Pedersen and H. G. Fletcher, Jr., *ibid.*, **82**, 5210 (1960); H. Venner, *Chem. Ber.*, **93**, 140 (1960).

(7) P. A. Levene and E. S. London, *J. Biol. Chem.*, **81**, 711 (1929); **83**, 793 (1929).

(8) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, *J. Am. Chem. Soc.*, **78**, 2117 (1956).

(9) J. J. Fox, N. Yung, I. Wempen and I. L. Doerr, *ibid.*, **79**, 5060 (1957).

(10) As pointed out,[9] these mercuripyrimidines contain pyrimidine and mercury in a 1:1 ratio. Therefore, in the ensuing condensation reaction, two molecular proportions of halogenose per mole of mercury-pyrimidine are needed to strip the pyrimidine-bound mercury from *N*-acetylcytosinemercury.

(11) Prepared at Hoffmann LaRoche, Inc., Nutley, N. J., by modifications of the procedure of J. C. Sowden, *J. Am. Chem. Soc.*, **76**, 3511 (1954).

(12) R. E. Deriaz, W. G. Overend, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 2836 (1949).

(13) The formation of both anomers is to be expected from this condensation reaction due to the absence of an acyloxy function on position 2 of the halogenose. See B. R. Baker in "The Chemistry and Biology of Purines," Ciba Foundation Symposium, Little, Brown and Co., Boston, Mass., 1957, p. 120.

(14) Chemical proof that 2' deoxycytidine is a β-nucleoside rests on the following data: 1-β-D-ribofuranosyluracil (uridine) was converted *via* 2,2'-anhydronucleoside intermediates to 1-(2'-deoxy-β-D-ribofuranosyl)-uracil (2'-deoxyuridine).[3a] 2'-Deoxyuridine has been obtained by nitrous acid deamination of 2'-deoxycytidine (W. W. Mac-

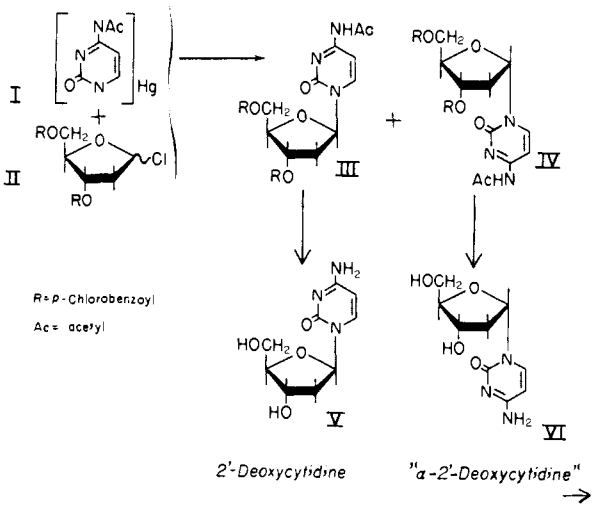

Fig. 1.

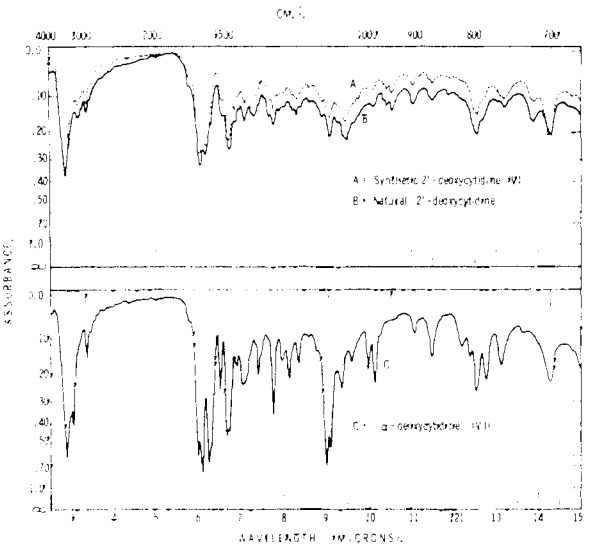

Fig. 2.

the β-configuration. Intermediate IV, therefore, is the α-anomer.

The α-anomer IV was deacylated to VI. The ultraviolet absorption spectrum of VI was similar to that shown by V demonstrating that the sugar in VI was affixed to position 1 of the pyrimidine ring. The elemental analyses of VI were similar to those found for V. Like V, VI failed to consume metaperiodate demonstrating thereby that the furanosyl structure was maintained; VI differed from V in melting point and a mixed melting point produced a depression. The infrared spectrum of VI (Fig. 2) differed from that given by V. Finally, the picrate of VI showed melting point properties different from that given by the picrate of V. These data are consistent with formulation of VI and IV as α-nucleosides.

The optical rotations of the α-nucleosides IV and VI (−66° and −44°, respectively) are less dextrorotatory than their corresponding β-anomers (III and V, −19° and +78°, respectively), a fact which is contrary to the expected optical rotational relationship based upon the isorotation rules of Hudson.[15] A similar situation obtains between thymidine and its α-anomer as well as between the β-nucleoside, 5-fluoro-2′-deoxyuridine and its α-anomer.[2] In the case of the latter two anomeric pairs, there can be no question of the identity of anomers since the configuration of naturally-occurring thymidine has been established and 5-fluoro-2′-deoxyuridine has been reduced catalytically to 2′-deoxyuridine. As concluded previously[16] the isorotation rules of Hudson have only a limited validity in the case of "*N*-glycosides" of 2-deoxy-D-ribose and, further, the assignment of configuration to derivatives of 2-deoxyribose on the basis of optical rotation *only* is certainly unwarranted. Recent studies[17] using nuclear magnetic resonance spectra and optical rotatory dispersion measurements on thymidine and 5-fluoro-2′-deoxyuridine as well as their α-anomers[2,5] support this conclusion.

Like naturally-occurring 2′-deoxycytidine, V was deaminated and cleaved enzymically to uracil[18] by nucleoside deaminase(s) and nucleosidase(s) in resting cell suspensions of *E. coli* B. The α-anomer VI is inert to either deamination or glycosyl cleavage in this system.[19] The α-anomer of thymidine similarly is resistant[5] to glycosyl cleavage by *E. coli* B. nucleosidases. Thus, in addition to the high degree of structural specificity noted previously[8,18,20] with regard to the sugar moiety of pyrimidine nucleosides, these enzymes appear to be specific for the β-anomer of these 2′-deoxy-D-ribofuranosyl nucleosides in these test systems.

Several years ago, this Laboratory attempted the synthesis of pyrimidine-2′-deoxynucleosides *via* the mercuri procedure using 1-*O*-acyl-3,5-di-*O*-benzoyl-2-deoxy-D-riboses (X or XI, see Fig. 3) as an intermediate for the preparation of a 1-halogeno sugar for use in the condensation reaction with mercuripyrimidines. Attempts to use these benzoylated derivatives (*i.e.*, X) in the condensation reaction with dithyminylmercury were repeatedly unsuccessful, due, presumably, to the instability of the sirupy halogenose formed. The synthesis of X and XI from purine-2′-deoxyribonucleosides is of interest. (2-Deoxy-D-ribose was not commercially available at that time.) It was hoped to obtain a fully acylated 2-deoxy-D-ribose derivative in the furanose form without the problem of separation from pyranose isomers. This goal was achieved by adaptation of the procedure of Weygand, *et al.*,[21] who prepared a fully-acylated ribofuranose derivative from guanosine or adenosine. With some modifications of their procedures, X and XI were prepared from 2′-deoxyguanosine

Nutt, *Biochem. J.*, **50**, 384 (1952)). Conversely, 2′-deoxyuridine may be converted *via* the thiation process to 2′-deoxycytidine.[4a]

(15) C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

(16) J. J. Fox and I. Wempen, *Adv. in Carbohydrate Chem.*, **14**, 283 (1959); see p. 340.

(17) R. U. Lemieux and M. Hoffer, *Can. J. Chem.*, **89**, 110 (1961).

(18) I. Wempen, I. L. Doerr, L. Kaplan and J. J. Fox, *J. Am. Chem Soc.*, **82**, 1624 (1960).

(19) The authors are indebted to Dr. Louis Kaplan of the Sloan-Kettering Institute for this enzymic study.

(20) J. J. Fox, J. F. Codington, N. C. Yung, L. Kaplan and J. O. Lampen, *J. Am. Chem. Soc.*, **80**, 5155 (1958).

(21) F. Weygand and F. Wirth, *Chem. Ber.*, **85**, 1000 (1952); F. Weygand and W. Sigmund, *ibid.*, **86**, 160 (1953).

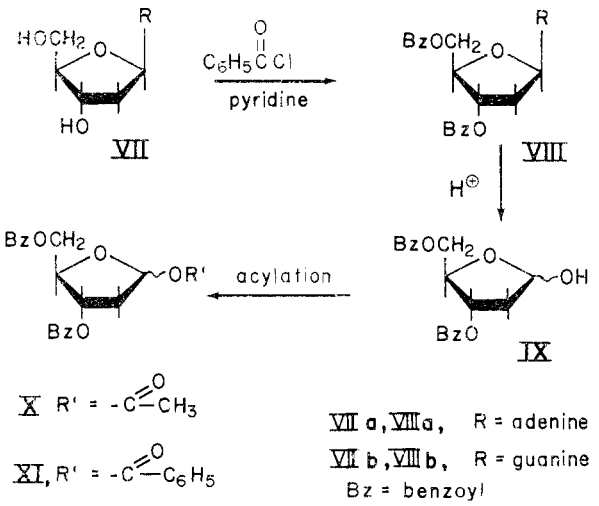

Fig. 3.

or 2′-deoxyadenosine in three steps in 22% overall yield (see Fig. 3). The syntheses of X[22] and XI[23,24] from 2-deoxy-D-ribose have been reported recently.

Benzoylation of 2′-deoxyadenosine (VIIa) or -guanosine (VIIb), followed by acid-induced cleavage of the glycosyl bond of the benzoylated intermediate (VIII), afforded sirupy 3,5-di-*O*-benzoyl-2-deoxy-D-ribose (IX) which, upon treatment with acetic anhydride in pyridine, afforded one product, 1-*O*-acetyl-3,5-di-*O*-benzoyl-2-deoxy-D-ribose (X).[22] Benzoylation of IX under similar conditions afforded *two* crystalline tri-*O*-benzoylated derivatives (XI) which were readily separated by recrystallizations from ethanol. These derivatives are considered to be anomers containing the furanose ring system, since the possibility of rearrangement to a pyranose form would be unlikely under the mild acylating conditions employed. The melting points (83–86° and 110–111°) and the optical rotations of these tri-*O*-benzoates of 2-deoxy-D-ribofuranose agree with those reported by Pedersen, *et al.*,[23] who obtained them by another route. In addition, the higher melting isomer was also obtained by Zinner and Nimz[24] by yet a different method.

In view of the failure of Hudson's rules of isorotation to apply to various anomeric pairs of pyrimidine-2′-deoxy-D-ribonucleosides,[16] we have deliberately omitted assignment of anomeric configuration to either of these tri-*O*-benzoates (XI). As of this writing, there are no data available, other than optical rotations, upon which to base such conclusions. It would be desirable, then, to avoid the assignment of configuration to these anomers (XI) until other criteria for alpha or beta assignments with this class of sugars are forthcoming.

Acknowledgments.—The authors are indebted to Dr. George Bosworth Brown for helpful discussions and continued interest.

(22) M. G. Blair, D. Lipkin, J. C. Sowden and D. R. Strobach, *J. Org. Chem.*, **25**, 1679 (1960).

(23) C. Pedersen, H. W. Diehl and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **82**, 3425 (1960).

(24) H. Zinner and H. Nimz, *Chem. Ber.*, **91**, 1657 (1958).

Experimental[25]

3,5-Di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribosyl Chloride (II).—2-Deoxy-D-ribose[11] (20.0 g.) was dissolved in 380 ml. of anhydrous methanol and treated with 20 ml. of a 1% solution of anhydrous hydrogen chloride in methanol. The reaction solution was allowed to remain at 27° for 20 minutes after which 10.0 g. of silver carbonate was added and the mixture stirred thoroughly. The silver salts were removed by filtration through a pad made of diatomaceous earth and charcoal. The methanolic filtrate was concentrated to dryness and the residue dissolved in 10 ml. of anhydrous pyridine. The pyridine solution was again concentrated *in vacuo* in order to remove traces of methanol. The oily residue (crude α,β-methyl 2-deoxy-D-ribofuranoside)[12] was dissolved in 115 ml. of anhydrous pyridine and treated with cooling with 45 ml. of *p*-chlorobenzoyl chloride (temperature maintained between 20–40°). After 16 hours, water and methylene chloride were added, the layers separated and the organic layer washed successively with potassium bicarbonate solution, then with water, and dried over sodium sulfate. After filtration from salts, the filtrate was concentrated in vacuum to a sirup. This sirupy residue, methyl 3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribofuranoside, was dissolved in 150 ml. of absolute ether, cooled to 0° and treated with (200 ml.) cold acetic acid previously saturated with hydrogen chloride. Additional hydrogen chloride was passed into the cold (below 10°) reaction mixture. Crystallization of 3,5-di-*O*-(*p*-chlorobenzoyl)-2-deoxy-D-ribosyl chloride occurred. After rapid filtration and washing with cold, absolute ether, 28.0 g. (65%) was obtained, m.p. 118–120° (with evolution of hydrogen chloride followed by partial resolidification; the resulting solid was identified as *p*-chlorobenzoic acid). A small sample for analyses was recrystallized from warm carbon tetrachloride.

Anal. Calcd. for $C_{19}H_{16}O_5Cl_2$: C, 53.1; H, 3.5; Cl, 24.8. Found: C, 52.6; H, 3.8; Cl, 25.2, 25.0.

The halogenose may be stored in a vacuum desiccator over soda lime. It is best, however, to use freshly prepared material in the following condensation reaction.

1-[3,5-Di-*O*-(*p*-chlorobenzoyl)-2-deoxy-α,β-D-ribosyl]-4-acetamido-2(1H)-pyrimidinone (III, IV).—Crystalline halogenose (II, 0.005 mole) was added all at once to a vigorously-stirred, refluxing suspension of azeotropically-dried *N*-acetylcytosinemercury[9] (0.0025 mole)[10] in 40 ml. of xylene. Immediately after the addition of the halogenose, the resulting turbid mixture was cooled in an ice-water-bath. The cooled solution was filtered from 0.3 g. of insoluble material and the filtrate treated with 300 ml. of petroleum ether. The precipitate was collected and dissolved in chloroform and the chloroform solution washed with 30% potassium iodide solution, then with water, and the organic layer dried over sodium sulfate. After filtration from salts, the chloroform solution was concentrated *in vacuo* whereupon crystallization occurred. The solid material was dissolved in 15 ml. of ethanol to which ~2 ml. of ethyl acetate was added and cooled; 0.6 g. of colorless crystals was collected. An additional 0.2 g. was obtained from the mother liquor. These solids (0.8 g.) are an *alpha-beta* mixture of the blocked nucleosides III and IV.

Attempts to obtain additional nitrogen-containing products from this mother liquor were unsuccessful. Instead, a white crystalline solid (0.1 g.), m.p. ~ 160°, was isolated which was devoid of nitrogen and lacked selective absorption in the 260–310 mμ range. This compound was not investigated further.

Isolation of the α-Anomer IV.—The combined solids (0.8 g.) were dissolved in *ca.* 20 ml. of hot ethanol and cooled. Crude α-anomer (0.3 g.) was obtained as needles, m.p. (sinters at ~160°) and melts at 200–201° to a clear liquid which resolidifies and melts with decomposition and effervescence at ~230°. Recrystallization of this material from ~25 ml. of boiling ethanol yielded short needles, m.p. 204.5–205°, to a clear liquid which became turbid at ~208°, solidifies at 210° and melted with decomposition and effervescence at ~245° (*p*-chlorobenzoic acid melts at 242–243°), $[\alpha]^{25}D$ −66° (*c* 0.9, chloroform); light absorption properties: in ethanol, maxima at 242, 283 and 299 mμ, minima at 273 and 286 mμ.

(25) Melting points are uncorrected. Analyses performed by Schwartzkopf Microanalytical Laboratory, Woodside, N. Y., and by Dr. A. Steyermark, Hoffmann LaRoche, Nutley, N. J.

Anal. Calcd. for $C_{25}H_{21}Cl_2N_3O_7$: C, 54.96; H, 3.87; N, 7.69. Found: C, 54.23; H, 4.10; N, 7.65.

Isolation of the β-Anomer III.—The β-anomer was obtained from the alcoholic mother liquor of IV. This mother liquor was concentrated to 10 ml. and after cooling 0.3 g. of crude β-anomer was obtained, m.p. (sinters at 128°) and melts to a clear liquid at 130–132° with resolidification and decomposition at 230–240°. An additional 0.14 g. was obtained from the mother liquor. Recrystallization of the β-anomer from 10 ml. of hot ethanol afforded pure crystalline III, m.p. 128–130° to a gel-like liquid with resolidification and melting with decomposition and effervescence at ~240°, $[\alpha]^{25}D$ −19° (*c* 0.9, chloroform); light absorption properties: in ethanol, maxima at 242, 283 and 299 mμ, minima at 278 and 286 mμ.

Anal. Calcd. for $C_{25}H_{21}Cl_2N_3O_7$: C, 54.96; H, 3.87; N, 7.69. Found: C, 55.12; H, 4.09; N, 7.60.

1-(2-Deoxy-α-D-ribofuranosyl)-cytosine (VI, "α-Deoxycytidine").—The α-anomer of the blocked nucleoside (IV, 250 mg.) with 30 ml. of ethanolic ammonia (saturated at 0°) was heated in a sealed tube for 12 hr. at 100°. The tube was cooled, opened and the contents concentrated *in vacuo* to a semi-solid. This residue was dissolved in 10 ml. of water and extracted several times with small portions of chloroform. The aqueous layer was concentrated *in vacuo* to a colorless sirup which was dissolved in absolute ethanol and cooled. After filtration, 100 mg. of "α-deoxycytidine" (VI) was obtained. One recrystallization from ethanol yielded pure material, m.p. 192–193°, $[\alpha]^{25}D$ −44° (*c* 0.7, *N* NaOH). Ultraviolet absorption properties in acid and neutral media were similar to those reported for 2'-deoxycytidine.[25] The infrared absorption spectrum of VI is shown in Fig. 2.

Anal. Calcd. for $C_9H_{13}N_3O_4$: C, 47.57; H, 5.76; N, 18.49. Found: C, 47.76; H, 5.80; N, 18.53.

A mixed melting point with authentic 2'-deoxycytidine was depressed (161–185°).

Picrate Salt of VI.—An ethanolic solution of VI was treated with a saturated solution of picric acid in ethanol. Prisms formed immediately. After filtration, the yellow precipitate was recrystallized from 95% ethanol to yield microscopic prisms, m.p. 173–175° (dec. and efferv.).

Anal. Calcd. for $C_{15}H_{16}N_6O_{11}$: C, 39.51; H, 3.54; N, 18.43. Found: C, 39.96; H, 3.65; N, 18.58, 18.45.

2'-Deoxycytidine (V).—The β-anomer of the blocked nucleoside (III, 300 mg.) was deacylated in a manner similar to that used with the α-anomer (see above). Crystallization of the residue was effected by the use of a minimum volume of methanol and adding ether to faint opalescence and cooling. After two further recrystallizations from the same solvent system, pure material (90 mg.) was obtained, m.p. 199–200°. (Additional material may be obtained from the mother liquor as the picrate salt.) An authentic sample of naturally-occurring 2'-deoxycytidine[27] gave a m.p. of 200–201° and a mixed melting point with the synthetic material gave 199–201°; optical rotation of V, $[\alpha]^{25}D$ +78° (*c* 0.4, *N* NaOH); natural 2'-deoxycytidine showed +78° (*c* 0.9, *N* NaOH).[28] The ultraviolet absorption spectrum of V was identical with that reported for naturally-occurring 2'-deoxycytidine[26] in neutral, acid and basic solutions. The infrared absorption spectrum of V was identical with that for 2'-deoxycytidine[28] (see Fig. 2). The infrared spectrum of the α-anomer VI differed markedly from that for V.

2'-Deoxycytidine Picrate.—An alcoholic solution of V was converted to the picrate salt (yellow needles) following the same procedure used for the α-anomer, m.p. 192–198° dec. A similar melting point was noted for the picrate prepared from naturally-occurring 2'-deoxycytidine and a mixed melting point showed no depression. Manson and Lampen[29] report 191° dec.

(26) J. J. Fox and D. Shugar, *Biochim. et Biophys. Acta*, **9**, 369 (1952).

(27) The authors are indebted to Dr. O. Schindler of the Pharmazeutische Anstalt der Universitat, Basel, Switzerland, for a sample of 2'-deoxycytidine.

(28) Sample purchased from Schwarz Bio-Research, Inc., Mount Vernon, N. Y.

(29) L. A. Manson and J. O. Lampen, *J. Biol. Chem.*, **178**, 431 (1951).

3,5-Di-*O*-benzoyl-2-deoxy-D-ribose (IX). A. From 2'-Deoxyadenosine (VIIa).—Deoxyadenosine (VIIa) (20.1 g., 0.08 mole) was stirred in *ca.* 750 ml. of anhydrous pyridine until complete solution occurred. After chilling in an ice-bath, the stirred solution was treated dropwise with benzoyl chloride (28 ml., 0.24 mole). The final orange colored solution was held at 37–39° for 48 hr. The reaction mixture was concentrated *in vacuo* to *ca.* 200 ml. and poured into a well-stirred, water–ice slurry. The precipitated sirup (or semi-solid) was allowed to settle, the aqueous layer decanted, and the residue extracted thoroughly with hot water on a steam-bath, discarding the decantates. On cooling, the well-washed sirup hardened to a glassy solid (VIIIa), 37 g. (82%). No attempt was made to crystallize this intermediate.

The crude glass was added to a two-phase system consisting of 1700 ml. of 2 *N* sulfuric acid and 500 ml. of di-*n*-butyl ether. The reaction mixture was refluxed on a steam-bath with vigorous stirring for 2 hr. The hot liquids were separated and the aqueous layer returned to a hydrolysis apparatus together with a fresh 500-ml. aliquot of di-*n*-butyl ether, and the heating continued for an additional hour. (The second extraction may be omitted with the loss of *ca.* 5% yield.) The two phases again were separated and the combined organic layers were chilled and filtered to remove any unhydrolyzed starting material. The filtrates were washed acid free with a saturated solution of sodium bicarbonate, then with water, until neutral, and finally dried over sodium sulfate. The solvent was removed *in vacuo* and the residual sirup was azeotroped three times with dried benzene. The sirupy crude 3,5-di-*O*-benzoyl-D-ribose (IX), 19 g. (72% based on deoxyadenosine), was used directly for acylation.

B. From 2'-Deoxyguanosine.—Compound VIII was also obtained from 2'-deoxyguanosine (VIIb) by a procedure essentially similar to that outlined above. Certain modifications were necessitated by the greater insolubility of this starting material and its intermediates in the reaction solvents employed. The initial benzoylation was carried out on a stirred suspension of VIIb in pyridine warmed to 60–65° using a 4:1 ratio of benzoyl chloride to starting material. The stated temperature range was maintained until complete solution had occurred, the heat then was reduced to 40° for an additional 3-hr. period, after which the reaction was allowed to stand overnight at room temperature. The crude benzoylated deoxyguanosine VIIIb was isolated by the procedure analogous to that outlined above.

The intermediate VIIIb was solubilized for the hydrolysis step by taking it up in dioxane, adding an equal volume of di-*n*-butyl ether and refluxing this mixture with an equal volume of 2 *N* sulfuric acid. The rest of the procedure involving the isolation and acylation of the benzoylated ribose (IX) was exactly similar to that already discussed. The yield of product IX averaged 65% based on deoxyguanosine.

1-*O*-Acetyl-3,5-di-*O*-benzoyl-2-deoxy-D-ribose (X).—A solution of IX (0.056 mole) in a mixture of 60 ml. of anhydrous pyridine plus 80 ml. of methylene chloride was treated under anhydrous conditions with acetic anhydride (17.1 g., 0.168 mole). The reaction flask was tightly stoppered and allowed to stand 2 days at room temperature. The solvent was removed *in vacuo* at < 50°. The residual thin sirup was poured into ice-water and rapidly extracted into chloroform. The organic layer was washed acid-free with a saturated solution of sodium bicarbonate, then twice with water and dried over sodium sulfate. After removal of the solvent *in vacuo* at < 50°, the sirup was azeotroped twice with dry toluene and reconcentrated twice with anhydrous ethanol, during which treatment solidification occurred. The solid was dissolved in a minimum amount of hot ethanol–ethyl acetate (5:2), decolorized and chilled. The precipitate was filtered, washed with a minimum quantity of cold solvent; yield of crude product 22% (based on purine-2'-deoxynucleoside). The product recrystallized from ethanol as long feathers, m.p. 85–87°. An aliquot was recrystallized again for analysis, m.p. 86.5–87.5°, $[\alpha]^{26}D$ −23° (*c* 2.0 in chloroform). Blair, *et al.*,[22] report m.p. 88–89°, with $[\alpha]^{27}D$ −23.7° (*c* 2 in chloroform).

Anal. Calcd. for $C_{21}H_{20}O_7$; acyl no., 3.0. Found: acyl no., 2.9.

1,3,5-Tri-*O*-benzoyl-2-deoxy-D-ribose (XI).—Benzoylation of VIII was accomplished under conditions identical

with those used for the acetylation except that one equivalent of benzoyl chloride was used as the acylating agent. The product was crystallized in 15% yield as a brown solid, m.p. 95–102° from the ethanol–ethyl acetate solvent. On recrystallization from ethanol using decolorizing carbon, the pure product was obtained as rosettes of white needles, m.p. 110–111°, $[\alpha]^{25}D$ + 75° (*c* 2.54 in chloroform). Pedersen, *et al.*,[23] report m.p. 110–112°, $[\alpha]^{20}D$ +75.3 (chloroform); Zinner, *et al.*,[24] give 111–111.5°, $[\alpha]^{18}D$ +78° (chloroform).

From the original mother liquor, there was obtained a second crop (7%) of a lower-melting material, m.p. 78–82°. Further recrystallization from ethanol afforded white needles, m.p. 83–86°, $[\alpha]^{25}D$ − 20° (*c* 1.1 in chloroform). Pedersen, *et al.*,[23] found 84–87°, $[\alpha]^{20}D$ − 19.8° (chloroform). This isomer tended to be somewhat hygroscopic, especially in an impure state.

Anal. Calcd. for $C_{26}H_{22}O_7$: acyl. no., 3.0. Found: acyl. no., 3.05.

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF ARIZONA, TUCSON, ARIZ.]

Vinyl Ethers of Carbohydrates. I. Methyl 2-O-Vinyl-α-D-glucopyranoside[1]

BY ARCHIE J. DEUTSCHMAN, JR., AND HENRY W. KIRCHER

RECEIVED MAY 6, 1961

Crystalline methyl 2-*O*-vinyl-α-D-glucopyranoside was isolated as a product of the vinylation of methyl α-D-glucopyranoside with vinyl chloride or with acetylene. The structure of the compound was proved by methylation and hydrolysis to methyl 3,4,6-tri-*O*-methyl-α-D-glucoside and by hydrogenation and hydrolysis to 2-*O*-ethyl-D-glucose.

The preparation of vinyl ethers by the alkali-catalyzed addition of alcohols to acetylene has received considerable study and is the basis for a number of industrial processes. A review of the field[2] lists the preparation and properties of a large number of vinyl ethers derived from primary, secondary and tertiary alcohols.

The preparation of vinyl ethers from polyfunctional alcohols and carbohydrates also has been reported.[2–6] As a rule, the compounds were poorly characterized and, where partial substitution occurred, the positions of the vinyl groups were not established. 3-*O*-Vinyl-1,2;5,6-di-*O*-isopropylidene-D-glucose,[2] 3,5,6-tri-*O*-vinyl-1,2-*O*-isopropylidene-D-glucose[5] and methyl 2,3,4,6-tetra-*O*-vinyl-α-D-glucoside[6] are examples of the vinyl ethers of carbohydrates that have been prepared.

This paper deals with the preparation, isolation and proof of structure of methyl 2-*O*-vinyl-α-D-glucopyranoside (II). The initial vinylations of methyl α-D-glucopyranoside (I) were done with vinyl chloride[2] because facilities for handling acetylene under pressure were not available. Subsequent reactions with acetylene confirmed the results that were observed with vinyl chloride.

The reactions of I with vinyl chloride and sodium hydroxide or with acetylene and catalytic quantities of potassium hydroxide led to water-soluble reaction mixtures. These were mixed with Dry Ice to convert the hydroxides to the carbonates, evaporated to dryness, and extracted with acetone to yield a mixture of several vinylated products. Paper partition chromatography (*vide infra*) showed the presence of at least four vinylated compounds, two of low and two of high R_f. The pattern of spots observed from the acetylene reactions was the same as that observed from the vinyl chloride reactions.

The acetone-soluble portions of several reaction mixtures were redissolved in water and extracted continuously with benzene to yield a fraction that contained the substances of R_f 0.85 and 0.92. Subsequent continuous extraction of the aqueous solution with ether yielded a fraction that contained the components of R_f 0.68 and 0.72. The residual aqueous solution was evaporated to a sirup and extracted with chloroform. The chloroform extract evaporated to a sirup that crystallized upon standing a few days. Nucleation of the ether extract with these crystals caused a portion of it to crystallize. The material melted 122–126.5° after a single recrystallization from acetone, acetonitrile or ether. Recrystallization of this material from acetone gave large prisms, m.p. 126–127.5°, R_f 0.68, $[\alpha]^{25}D$ +136° (water).

The compound had all of the chemical characteristics that one would expect of a methyl mono-*O*-vinyl-α-D-glucopyranoside. It rapidly decolorized bromine and permanganate solutions, it yielded acetaldehyde and methyl α-D-glucopyranoside upon mild acid hydrolysis, and it was readily reduced with hydrogen. The vinyl derivative was methylated to a tri-*O*-methyl derivative (III) whose retention time on gas–liquid partition chromatography was very similar to that of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside[7] (Fig. 1). The product of the methylation was hydrolyzed with dilute acetic acid at room temperature to a methyl tri-*O*-methyl-α-D-glucopyranoside (IV) (Fig. 1).

The four possible methyl tri-*O*-methyl-α,β-D-glucopyranosides were obtained from methylated starch (2,3,6),[7] methylated dextran (2,3,4),[7] 3-*O*-benzyl-D-glucose (2,4,6)[8] and from 3-*O*-methyl-D-glucose (3,4,6).[9] The separation diagrams of the four methyl tri-*O*-methyl-α,β-D-glucopyranosides are shown in Fig. 2.

(1) Presented at the 139th meeting of the A.C.S., St. Louis, Mo., March, 1961.

(2) W. Reppe, *et al.*, *Ann.*, **601**, 81 (1956).

(3) M. F. Shostakovskii, E. N. Prilezhaeva and L. V. Tsymbal, *Doklady Akad. Nauk S.S.S.R.*, **96**, 99 (1954); *C. A.*, **49**, 5312 (1955).

(4) M. F. Shostakovskii, E. N. Prliezhaeva and L. V. Tsymbal, *Zhur. Obshch. Khim.*, **26**, 739 (1956); *C. A.*, **50**, 14564 (1956).

(5) B. I. Mikhant'ev and V. L. Lapenko, *Zhur. Obshch. Khim.*, **27**, 2972, 2840 (1957); *C. A.*, **52**, 8054, 8055 (1958).

(6) S. Kunichika and Y. Sakabibara, *Kogyo Kagaku Zasshi*, **60**, 761 (1957); *C. A.*, **58**, 10009 (1959).

(7) H. W. Kircher, *Anal. Chem.*, **32**, 1103 (1960).

(8) K. Freudenberg and E. Plankenhorn, *Ann.*, **536**, 257 (1938).

(9) R. L. Sundberg, C. M. McCloskey, D. E. Rees and G. H. Coleman, *J. Am. Chem. Soc.*, **67**, 1080 (1945).