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BETHESDA 14, MD.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

2-Deoxy-D-ribose. IV.[1] A Direct Synthesis of 2′-Deoxyadenosine and its Anomer through 2-Deoxy-D-ribose Derivatives[2]

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Mono-*p*-nitrobenzoylation of 2-deoxy-D-ribose diisobutyl dithioacetal affords a 5-*O*-*p*-nitrobenzoyl derivative. Demercaptalation of this, followed by further *p*-nitrobenzoylation, gives the two anomeric 2-deoxy-D-ribofuranose tri-*p*-nitrobenzoates. Conversion of these esters to amorphous 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride, followed by condensation with chloromercuri-6-benzamidopurine and removal of the protecting groups, has led to the isolation of 2′-deoxyadenosine, its anomer 9-(2-deoxy-α-D-ribofuranosyl)-adenine and a third nucleoside, probably 7-(2-deoxy-α-D-ribofuranosyl)-adenine. A direct preparation of 5-*O*-trityl-2-deoxy-α-D-ribose from 2-deoxy-D-ribose is described.

Chemical syntheses of nucleosides containing the 2-deoxy-D-ribofuranosyl moiety have heretofore relied on indirect pathways, preformed pentofuranosylpyrimidines[3] and purines[4] being reduced at carbon two of the sugar moiety through various ingenious transformations. In view of the relative accessibility of 2-deoxy-D-ribose,[5] the direct synthesis of such nucleosides through suitably substituted 2-deoxy-D-ribofuranosyl halides offers a number of attractive features. The present paper will describe such a direct synthesis.[6]

Zinner and co-workers[7] have shown that pentose dialkyl dithioacetals may be readily *p*-nitrobenzoylated at carbon five and that, through demercaptalation, 5-*O*-acylpentose dialkyl dithioacetals may be converted to 5-*O*-acylpentofuranoses. This method of obtaining pentofuranose derivatives was used in the course of the present research. 2-Deoxy-D-ribose was converted to its known diisobutyl dithioacetal[8] and mono-*p*-nitrobenzoylated to yield the crystalline 5-*O*-*p*-nitrobenzoyl derivative I. Demercaptalation with mercuric chloride and mercuric oxide then gave an amorphous 2-deoxy-5-*O*-*p*-nitrobenzoyl-D-ribose (II) which was further *p*-nitrobenzoylated, affording the two anomeric 2-deoxy-D-ribofuranose tri-*p*-nitrobenzoates (III and IV).

Either of these esters or a mixture of the two was dissolved in methylene chloride and treated with a very slight excess of hydrogen chloride.[9] *p*-Nitrobenzoic acid, which was precipitated in nearly quantitative yield, was removed, leaving a solution of 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride (V).[10] The amorphous, solvent-free halide was then condensed with chloromercuri-6-benzamidopurine in dimethyl sulfoxide solution. Protecting groups were stripped off with alkali and the product chromatographed on a cellulose column. 2′-Deoxyadenosine (VI, 9-(2-deoxy-β-D-ribofuranosyl)-adenine) was isolated in 10% yield. Its melting point, rotatory dispersion and ultraviolet absorption spectrum clearly showed it to be identical with the natural nucleoside.

A second crystalline nucleoside, isomeric with the natural product, was isolated through chromatography in 19% yield. In contrast with the first product, it was markedly dextrorotatory in the wave length range measured (589 to 340 mμ). In the ultraviolet it showed an absorption peak at 260 mμ characteristic of a 9-substituted adenine.[11] Hydrolysis with weak acid revealed only adenine, 2-deoxy-D-ribose and unchanged nucleoside. It appears highly probable that the substance is, therefore, the anomer of the natural nucleoside, namely, 9-(2-deoxy-α-D-ribofuranosyl)-adenine (VII).

Finally, the chromatography yielded a small amount (1%) of a third, isomeric nucleoside. From the dextrorotation of this substance and its absorption at 273 mμ (characteristic of 7-substituted adenines[11]) it may tentatively be assigned the structure 7-(2-deoxy-α-D-ribofuranosyl)-adenine.

Zinner, Nimz and Venner[12] have described an indirect method of preparing 2-deoxy-5-*O*-trityl-α-D-ribose; in the course of the present work the same substance was prepared rather more conveni-

(1) 2-Deoxy-D-ribose. III, C. Pedersen, H. W. Diehl and H. G. Fletcher, Jr., THIS JOURNAL, **82**, 3425 (1960).

(2) For a preliminary communication describing this work see R. K. Ness and H. G. Fletcher, Jr., *ibid.*, **81**, 4752 (1959).

(3) D. M. Brown, D. B. Parihar, C. B. Reese and A. Todd, *J. Chem. Soc.*, 3035 (1958); G. Shaw and R. M. Warrener, *ibid.*, 50 (1959).

(4) C. D. Anderson, L. Goodman and B. R. Baker, THIS JOURNAL, **81**, 3967 (1959).

(5) H. W. Diehl and H. G. Fletcher, Jr., *Biochem. Preparations*, **8**, in press (1960); *Arch. Biochem. Biophys.*, **78**, 386 (1958).

(6) Subsequent to the completion of this work we were informed of the successful synthesis of several pyrimidine 2′-deoxynucleosides by a direct procedure which has some features similar in principle to that described here; see M. Hoffer, R. Duschinsky, J. J. Fox and N. Yung, THIS JOURNAL, **81**, 4112 (1959).

(7) H. Zinner, K. Wessely, W. Bock, K. Rieckhoff, F. Strandt and W. Nimmich, *Chem. Ber.*, **90**, 500 (1957).

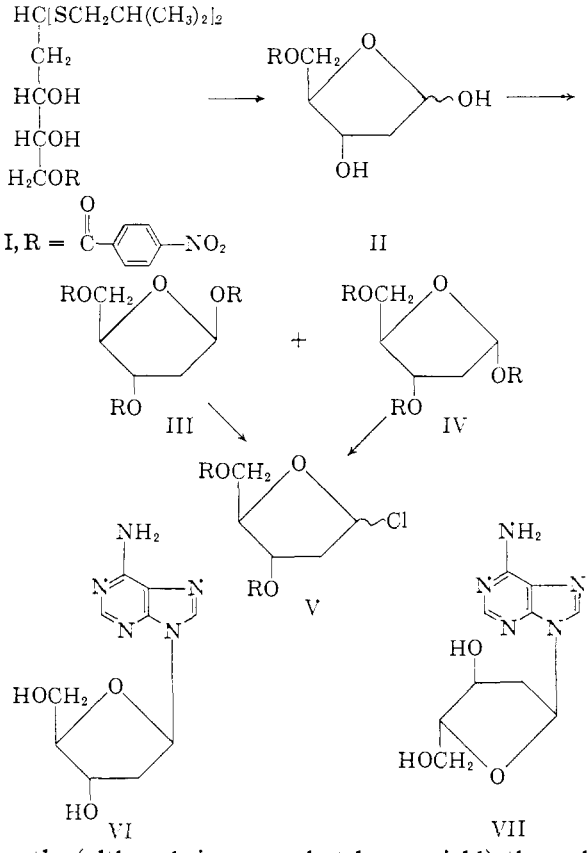
(8) H. Zinner, H. Nimz and H. Venner, *Chem. Ber.*, **90**, 2696 (1957).

(9) This technique is similar to that employed by W. W. Zorbach and T. A. Payne, Jr., THIS JOURNAL, **80**, 5564 (1958).

(10) One of the anomeric forms of this substance has since been obtained in crystalline form; it and some of its reactions will be described in a future communication.

(11) J. M. Gulland and L. F. Story, *J. Chem. Soc.*, 259 (1938).

(12) H. Zinner, H. Nimz and H. Venner, *Chem. Ber.*, **91**, 638 (1958).



ently (although in somewhat lower yield) through the direct tritylation of the sugar as described in the Experimental portion of this paper.

Experimental[13]

2-Deoxy-5-*O*-*p*-nitrobenzoyl-D-ribose Diisobutyl Dithioacetal (I).—2-Deoxy-D-ribose diisobutyl dithioacetal was prepared by a modification[14] of the method of Zinner, Nimz and Venner.[8] To a mixture of 20.0 g. of 2-deoxy-D-ribose and 35.0 ml. of isobutyl mercaptan which had been cooled to 0° was added, while stirring, 30.0 ml. of concentrated hydrochloric acid. After 2 min. the reaction mixture was allowed to warm to room temperature, stirring being continued. After 0.5 hr., methylene chloride (200 ml.) and water (200 ml.) were added, and the non-aqueous layer separated. The aqueous layer was re-extracted with methylene chloride and the combined extracts washed with 200 ml. of water. The aqueous washing was extracted with methylene chloride and the extract added to the combined non-aqueous extracts, which were then washed with saturated aqueous sodium bicarbonate and dried with magnesium sulfate. Removal of the solvent *in vacuo* (45° bath) gave a sirup which was diluted with toluene and reconcentrated *in vacuo*. Repetition of this procedure using benzene and then ether afforded 2-deoxy-D-ribose diisobutyl dithioacetal as a solvent-free sirup (40.0 g.) which was mono-*p*-nitrobenzoylated in a manner similar to that described by Zinner and co-workers.[7] A solution of the sirup in 100 ml. of dry pyridine and 100 ml. of methylene chloride was cooled for 5 min. in a Dry Ice–acetone-bath and, while the solution was stirred, 25.4 g. (1.01 molar equivalents) of *p*-nitrobenzoyl chloride was added. The reaction mixture was slowly brought to −5° and, after 16 hr., warmed at 60° for 30 min. Water and methylene chloride were added and the non-aqueous layer was successively washed, twice with 3 *N* sulfuric acid and once with saturated aqueous sodium bicarbonate. Moisture was removed with magnesium sulfate and the solution concentrated *in vacuo*. The residual sirup was dissolved in 200 ml. of isopropyl ether and the solution filtered through decolorizing carbon. Addition of 150 ml. of pentane afforded 39.0 g. of crude crystalline product melting at 64–70°. Recrystallization from a mixture of 390 ml. of isopropyl ether and 375 ml. of pentane gave 29.5 g. (44% based on 2-deoxy-D-ribose) of practically pure product melting at 73–74°. Further recrystallization from the same solvent mixture raised the melting point to 74–75° and afforded material showing $[\alpha]^{20}D$ −11.4° in chloroform (*c* 0.81).

Anal. Calcd. for $C_{20}H_{31}NO_6S_2$: C, 53.91; H, 7.01; N, 3.14. Found: C, 53.68; H, 6.91; N, 3.33.

The Anomeric 2-Deoxy-D-ribofuranose Tri-*p*-nitrobenzoates (III and IV).—2-Deoxy-5-*O*-*p*-nitrobenzoyl-D-ribose diisobutyl dithioacetal (8.00 g.) was dissolved in a mixture of 80 ml. of acetone and 2.5 ml. of water. Yellow mercuric oxide (8.0 g.) was added and then 8.0 g. of mercuric chloride added to the stirred solution. The reaction mixture was stirred at 50° for 2 hr., cooled and dehydrated through the addition of 23 g. of anhydrous magnesium sulfate. Methylene chloride (200 ml.) was added, the solid material removed by filtration and the solvent evaporated under reduced pressure. The sirupy residue was dissolved in 50 ml. of dry pyridine and the solution treated at 0° with 8.00 g. of *p*-nitrobenzoyl chloride. After standing 2.5 hr. at 0° and 16 hr. at room temperature, the reaction mixture was treated with a relatively large volume of water and the products extracted with methylene chloride. The combined extracts were washed successively with water, twice with 3 *N* sulfuric acid and, finally, with saturated aqueous sodium bicarbonate. Moisture was removed with magnesium sulfate and the solution concentrated to give a sirup which, when dissolved in 55 ml. of ethyl acetate and cooled to −5°, yielded a crude product. Dried *in vacuo* (0.03 mm.) at 100°, it weighed 4.78 g. (46%) and rotated $[\alpha]^{20}D$ +23.8° in chloroform (*c* 1.03). The material was dissolved in a mixture of methylene chloride and 10 parts of ethyl acetate, the methylene chloride then being distilled off to give (with 30% loss) material rotating $[\alpha]^{20}D$ +17.5° in chloroform. Further recrystallization from benzene yielded pure **2-deoxy-β-D-ribofuranose tri-*p*-nitrobenzoate (III)** melting at 172–173° and rotating $[\alpha]^{20}D$ +17.1° in chloroform (*c* 0.36). Solvated forms of the substance were obtained from ethyl acetate and from benzene. These melted at 125–130° (gas evolution) and then resolidified; in both cases, heating at 105° and 0.03 mm. for 2 hr. gave the higher-melting, unsolvated form.

Anal. Calcd. for $C_{26}H_{19}N_3O_{13}$: C, 53.71; H, 3.29; N, 7.23. Found: C, 53.98; H, 3.57; N, 7.15.

Addition of pentane to the original ethyl acetate mother liquor and gradual cooling to −5° afforded 3.39 g. (32%) of vacuum-dried material which melted at 155–157° (after sintering at 144°) and rotated $[\alpha]^{20}D$ +59.1° in chloroform (*c* 2.75). Recrystallization from 11 parts of butanone gave 1.85 g. of product melting at 162–164°; further recrystallization from butanone and benzene led to pure **2-deoxy-α-D-ribofuranose tri-*p*-nitrobenzoate (IV)** melting at 165–166° and rotating $[\alpha]^{20}D$ +70.7° in chloroform (*c* 0.33).

Anal. Calcd. for $C_{26}H_{19}N_3O_{13}$: C, 53.71; H, 3.29; N, 7.23. Found: C, 53.99; H, 3.56; N, 7.21.

9-(2-Deoxy-α-D-ribofuranosyl)-adenine (VII) and 9-(2-Deoxy-β-D-ribofuranosyl)-adenine (VI).—A crystalline mixture (3.04 g.) of the anomeric 2-deoxy-D-ribofuranose tri-*p*-nitrobenzoates (dried 27 hr. at 80° and 0.05 mm.) was treated with 21.0 ml. of 0.252 *N* hydrogen chloride in methylene chloride (1.01 equiv.). Crystallization of *p*-nitrobenzoic acid began before the starting material had completely dissolved. After 45 min. at room temperature and 30 min. at −5°, 0.8324 g. (95%) of *p*-nitrobenzoic acid was removed by filtration at −5° and washed with methylene chloride, care being taken to avoid exposure of the solution to moisture. The filtrate was concentrated *in vacuo* to a light, nearly solvent-free mass of sirupy material to which was added a solution of 3.60 g. of chloromercuri-6-benzamidopurine[15] in 30 ml. of dry dimethyl sulfoxide.[16] The re-

(13) All melting points are corrected.

(14) We are indebted to Dr. D. L. MacDonald of this Laboratory for the development of this modification.

(15) In the present work this substance was made by a modification of the method of J. Davoll and B. A. Lowy [THIS JOURNAL, **73**, 1650 (1951)] which was devised by Dr. J. J. Fox and his co-workers. *Cf.* B. R. Baker, K. Hewson, H. J. Thomas and J. A. Johnson, Jr., *J. Org. Chem.*, **22**, 954 (1957).

(16) This solvent was dried immediately before use by distilling 150 ml. of benzene from 90 ml. of redistilled dimethyl sulfoxide.

action was observed polarimetrically in a 0.5-dm. all-glass tube at 20°: $\alpha^{20}D$ −0.22° (10 min.), −0.34° (35 min.), −0.38° (50, 70 min.). After 2.3 hr. the reaction mixture was cooled while *ca.* 100 ml. of water was slowly added. The material thus precipitated (5.15 g.) was treated with 53 ml. of 0.36 *N* barium methoxide in methanol and the solution refluxed for 5.5 hr. The solution was then neutralized with carbon dioxide, filtered and concentrated under reduced pressure to dryness. Methyl *p*-nitrobenzoate was removed by leaching with methylene chloride and the residue was then extracted with boiling absolute ethanol (2 × 60 ml.). The ethanol solution was partially concentrated and placed on a column (6 × 62 cm.) of 700 g. of standard grade Whatman powdered cellulose which had been washed with 4 l. of water, 1 l. of absolute ethanol and 200 ml. of isopropyl ether–absolute ethanol–water (16:4.5:1). Employing the latter mixture as an eluant and collecting the effluent in 20-ml. fractions, the crude product was chromatographed.

Fractions 283 to 332, containing a total of 226 mg. of material, represented essentially pure adenine.

Paper chromatography, using isopropyl ether–ethanol–water (16:4.5:1) and isopropyl alcohol–ammonia–water (7:1:2), showed that the only ultraviolet-absorbing material in fractions 453 to 553 migrated at a rate similar to that of authentic 9-(2-deoxy-β-D-ribofuranosyl)-adenine. These fractions were then pooled, concentrated to dryness and the residue (284 mg.) extracted with three 10-ml. portions of methylene chloride. The residue was then dissolved in *ca.* 1.5 ml. of water containing a trace of ammonia, a very small amount of insoluble material removed by centrifugation, and the solution seeded. The **9-(2-deoxy-β-D-ribofuranosyl) adenine** thus obtained was dried at 110° and 0.05 mm. pressure for 5 hr.: 116 mg. It melted partially at *ca.* 155° and completely at 183–187°; it rotated $[\alpha]^{20}D$ −27° in water (*c* 0.58). Chromatography of the material in the methylene chloride extracts on 18-cm. strips of Whatman 3 MM paper using isopropyl alcohol–ammonia–water (7:1:2) led to the isolation of a further 15.0 mg. of the same nucleoside, raising the total yield of this substance to 10%. Several recrystallizations of the synthetic material from water afforded the pure nucleoside. After drying at 110° and 0.02 mm. for 16 hr., it melted at 187–189° and showed in water (*c* 0.47) the rotations: $[\alpha]^{24}_{589}$ −25°, $[\alpha]^{24}_{450}$ −59°, $[\alpha]^{24}_{400}$ −72°, $[\alpha]^{24}_{360}$ −104°, $[\alpha]^{24}_{340}$ −127°.

Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21; N, 27.88. Found: C, 47.64; H, 5.15; N, 27.36.

A sample of natural 9-(2-deoxy-β-D-ribofuranosyl)-adenine[17] which had been dried overnight at 100° and 0.01 mm. showed in water (*c* 0.49) the rotations: $[\alpha]^{25}_{589}$ −26°, $[\alpha]^{25}_{400}$ −71°, $[\alpha]^{25}_{360}$ −103°, $[\alpha]^{25}_{340}$ −128°, $[\alpha]^{25}_{330}$ −150°, $[\alpha]^{25}_{320}$ −173°, $[\alpha]^{25}_{310}$ −206°.

Both natural and synthetic VI showed absorption peaks at 260 mμ, A_M for natural VI being 15,900 and A_M for synthetic VI being 16,600. The infrared spectra of both were never found to be completely identical using the KBr-plate technique. However, we have observed that 2'-deoxyadenosine crystallizes with varying amounts of water and possibly in dimorphic forms; both natural and synthetic specimens, even after rigorous drying, often showed a m.p. of *ca.* 160–170° before finally remelting at *ca.* 189°.

Fractions 603 to 733 were pooled and concentrated to dryness. The residue (359 mg.) was dissolved in 35 ml. of hot absolute methanol and the solution filtered to remove a small amount of insoluble material. Upon cooling, the filtrate deposited 250.2 mg. (19%) of **9-(2-deoxy-α-D-ribofuranosyl)-adenine** melting at 206–211° and rotating $[\alpha]^{20}D$ +68.0° in water (*c* 0.48). After recrystallization from ethanol and from methanol, the pure nucleoside melted at 209–211° and showed in water (*c* 0.54) the rotations: $[\alpha]^{25}_{589}$ +71°, $[\alpha]^{25}_{450}$ +132°, $[\alpha]^{25}_{400}$ +173°, $[\alpha]^{25}_{360}$ +220°, $[\alpha]^{25}_{340}$ +258°.

Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21; N, 27.88. Found: C, 48.01; H, 5.49; N, 27.74.

The α-nucleoside shows an absorption maximum at 260 mμ, the molar absorbancy (A_M) being 15,900 at that wavelength.

A sample of the 9-(2-deoxy-α-D-ribofuranosyl)-adenine was hydrolyzed with 1% acetic acid at room temperature for 4 days. Chromatography on paper, using four different solvent systems, then showed only 2-deoxy-D-ribose, adenine and unchanged nucleoside.

Fractions 783 to 863 were pooled and freed of solvent to give 48.5 mg. of material showing an absorption maximum at 273 mμ. Successive recrystallization from 0.5 ml. of absolute alcohol and then from ethanol–pentane (1:1) afforded 14.2 mg. (1% of crystalline product melting at 153–156° and showing the rotations in water (*c* 0.25): $[\alpha]^{23}_{589}$ +7°, $[\alpha]^{23}_{400}$ +12°, $[\alpha]^{23}_{400}$ +23°, $[\alpha]^{23}_{360}$ +51°, $[\alpha]^{23}_{350}$ +65°, $[\alpha]^{23}_{340}$ + 75°. At 273 mμ the substance had a molar absorbancy (A_M) of 14,300. The rotatory dispersion and absorption spectrum of this substance indicate that it is probably **7-(2-deoxy-α-D-ribofuranosyl)-adenine.**

Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21. Found: C, 47.69; H, 5.04.

2-Deoxy-5-*O*-trityl-D-ribose.—A solution of 5.36 g. (0.040 mole) of 2-deoxy-D-ribose and 15.80 g. (0.057 mole) of trityl chloride in 125 ml. of dry pyridine was left at room temperature for 3 days; 2 ml. of water was then added and the solution partially concentrated. Methylene chloride was added and the solution washed successively with water, cold 3 *N* sulfuric acid and saturated aqueous sodium bicarbonate solution. After drying with magnesium sulfate, the solution was concentrated *in vacuo* and the residual sirup dissolved in 40 ml. of benzene and 60 ml. of pentane. On seeding with 2-deoxy-5-*O*-trityl-α-D-ribose[18] and standing for a day the solution deposited 4.19 g. of crude product. More pentane was then added to the filtrate which was reseeded and stirred with a glass-covered magnetic bar for 2 more days, giving a second crop of 3.10 g. Recrystallization from benzene–pentane of the crops of crude material thus obtained afforded 6.01 g. (40%) of product melting at 108–110° and rotating in anhydrous pyridine (*c* 3.9) $[\alpha]^{20}D$ +39.6° (extrapolated)→ +12.4° (equilibrium, 15 hr.). Zinner, Nimz and Venner[12] observed that pure 2-deoxy-5-*O*-trityl-D-ribose melted at 111.5–112.5° and rotated in pyridine $[\alpha]^{19}D$ +25.1° (4 min.)→ +12.0° (2 hr.).

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(17) Nutritional Biochemicals Corp., 21010 Miles Ave., Cleveland 28, Ohio.

(18) Seeds were initially obtained following the procedure of Zinner, Nimz and Venner (ref. 12).