Nucleosides. III. 1-(2′-Deoxy-3′,5′-epoxy-β-D-*threo*-pentofuranosyl)thymine[1,2]

JEROME P. HORWITZ, JONATHAN CHUA, JOYCE A. URBANSKI, AND MICHAEL NOEL

Roland H. Stevens Laboratory, Detroit Institute of Cancer Research, Detroit 1, Michigan

Received October 22, 1962

Treatment of 5′-*O*-mesyl-2,3′-anhydrothymidine (IIa) with aqueous alkali effects "aryl-oxygen" fission of the anhydro bond with the formation of 1-(2′-deoxy-3′,5′-epoxy-β-D-*threo*-pentofuranosyl)thymine (IV). Cleavage of the epoxide ring in IV with dilute sulfuric acid yields 1-(2′-deoxy-β-D-*threo*-pentofuranosyl)thymine (V). The conversion of V to an isopropylidene derivative (VI) establishes a *cis* configuration for the 3′,5′-hydroxyl groups. An alternative synthetic procedure provided rigid evidence for the structure assigned to V. The interaction of 3′-*O*-mesyl-5′-*O*-tritylthymidine (VII) and excess sodium hydroxide afforded 1-(2′-deoxy-5′-*O*-trityl-β-D-*threo*-pentosyl)thymine (IX) in high yield. Detritylation of IX provided a product identical in every respect with V.

It was shown in a previous study[3] that displacement of a 5′-sulfonyloxy function from uridine and thymidine is readily effected by azide ion and that the azide derivatives, so obtained, are readily transformed, on catalytic reduction, to the corresponding pyrimidine aminonucleosides. An extension of this study required the synthesis of 1-(2′-deoxy-5′-*O*-mesyl-β-D-*threo*-pentosyl)thymine (III) and it was anticipated that 2,3′-anhydro-1-(2′-deoxy-5′-*O*-mesyl-β-D-*threo*-pentosyl)thymine[4] (IIa), on treatment with alkali, would afford the desired intermediate (III).

The interaction of the 3′,5′-di-*O*-mesylthymidine (I)[4] and one equivalent of alcoholic sodium hydroxide provided IIa in 96% yield. This procedure represents an improvement over the previously described method[4] which employs methanolic ammonia to promote the intramolecular displacement of the 3′-mesyloxy group. Contrary to expectations, the 2,3′-anhydro nucleoside (IIa), on treatment with one equivalent of aqueous sodium hydroxide, yielded a sulfur-free crystalline product with an ultraviolet absorption spectrum resembling that of thymidine but distinctly different from that of a 2,3′-[5,6] or 2,5′-anhydro nucleoside.[7,8] The spectral findings and elementary analysis, on the other hand, conformed with the structure 1-(2′-deoxy-3′,5′-epoxy-β-D-*threo*-pentofuranosyl)thymine (IV). A more direct route to the epoxy derivative, (IV), and certainly the method of choice, utilizes I which affords IV in good yield after interactions with two equivalents of aqueous sodium hydroxide.

In 1933 Levene and Raymond[9] described the conversion of 1,2-*O*-isopropylidene-5-*O*-tosyl-D-xylose by sodium methoxide to an epoxide derivative, 1,2-*O*-isopropylidene-3,5-anhydro-D-xylose. It was shown more recently that ring-closure to the same product is obtained from the interaction of 3,5-di-*O*-mesyl-1,2-isopropylidene-D-xylose and alcoholic potassium hydroxide.[10]

A plausible reaction path for the formation of the epoxide derivative, IV, from II initially requires nucleophilic attack by hydroxide ion at C-2 of the aglycon moiety with rupture of the 2,3′-anhydro bond, *i.e.*, "aryl-oxygen" fission.[11] A transient intermediate is thereby obtained in which the conjugate base of the 3′-hydroxyl group has the *up* or 2′-deoxy-*threo* configuration. Intramolecular nucleophilic displacement of the 5′-mesyloxy function by the incipient 3′ alkoxide ion would effect closure of the ring and the completion of a stereochemically controlled sequence of reactions in the conversion of I to IV.

Michelson and Todd[4] reported that alkaline hydrolysis of 2,3′-anhydrothymidine (IIb) yielded a nucleoside (not isolated) with chromatographic and infrared properties closely related to those of thymidine. The product, though not rigidly identified, was believed to be the isomeric thymine 2-deoxy-D-xylofuranoside (V).

Attempts to open the epoxide ring in IV with aqueous alkali, sodium methylate and sodium benzylate all proved unsuccessful. Cleavage, however, was effected in dilute sulfuric acid with the isolation[12] (42% yield) of a crystalline compound (V) whose spectrophotometric (ultraviolet and infrared) and chromatographic

TABLE I

RELATIVE R_f VALUES OF SOME 1-β-D-(2′-DEOXYALDOPENTOFURANOSYL)THYMINES[a]

Thymine derivative	Solvent system[b] A	B	C
Thymidine	0.44	0.73	0.55
V	.45	.74	.59
5′-*O*-Tritylthymidine	.90	.91	.83
IX	.89	.93	.85
IV	.53	.73	.57
VI	.75	.83	.72

[a] Ascending chromatograms were run on Whatman no. 1 paper. Compounds were located on the chromatogram by examination first under ultraviolet light and then the cysteine-spray technique [J. G. Buchanan, *Nature*, **168**, 1091 (1951)]. [b] Three solvent systems were used: (A) *n*-butyl alcohol–water (86:14), (B) isopropyl alcohol–water (7:3), and (C) isopropyl alcohol–ammonia–water (7:1:2). Each chromatogram included thymidine and the recorded values represent an average of several determinations.

---

(1) This work was supported in part by research grants CY-2903 and CY-5943 from the National Cancer Institute, Public Health Service, and in part by an institutional grant from the United Foundation of Greater Detroit allocated through the Michigan Cancer Foundation.

(2) In accord with the suggestion of J. F. Codington, R. Fecher, and J. J. Fox, *J. Org. Chem.*, **27**, 163 (1962), the term "epoxy" is used to refer to an ether linkage in the sugar moiety. The term "anhydro" (as anhydro nucleoside) refers to an oxygen bridge between C-2 of the pyrimidine and C-3 of the sugar moiety.

(3) J. P. Horwitz, A. J. Tomson, J. A. Urbanski, and J. Chua, *J. Org. Chem.*, **27**, 3045 (1962).

(4) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 816 (1955).

(5) R. Fecher, J. F. Codington, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 1889 (1961).

(6) N. C. Yung and J. J. Fox, *ibid.*, **83**, 3060 (1961).

(7) R. Letters and A. M. Michelson, *J. Chem. Soc.*, 1410 (1961).

(8) D. M. Brown, A. R. Todd, and S. Varadarajan, *ibid.*, 868 (1957).

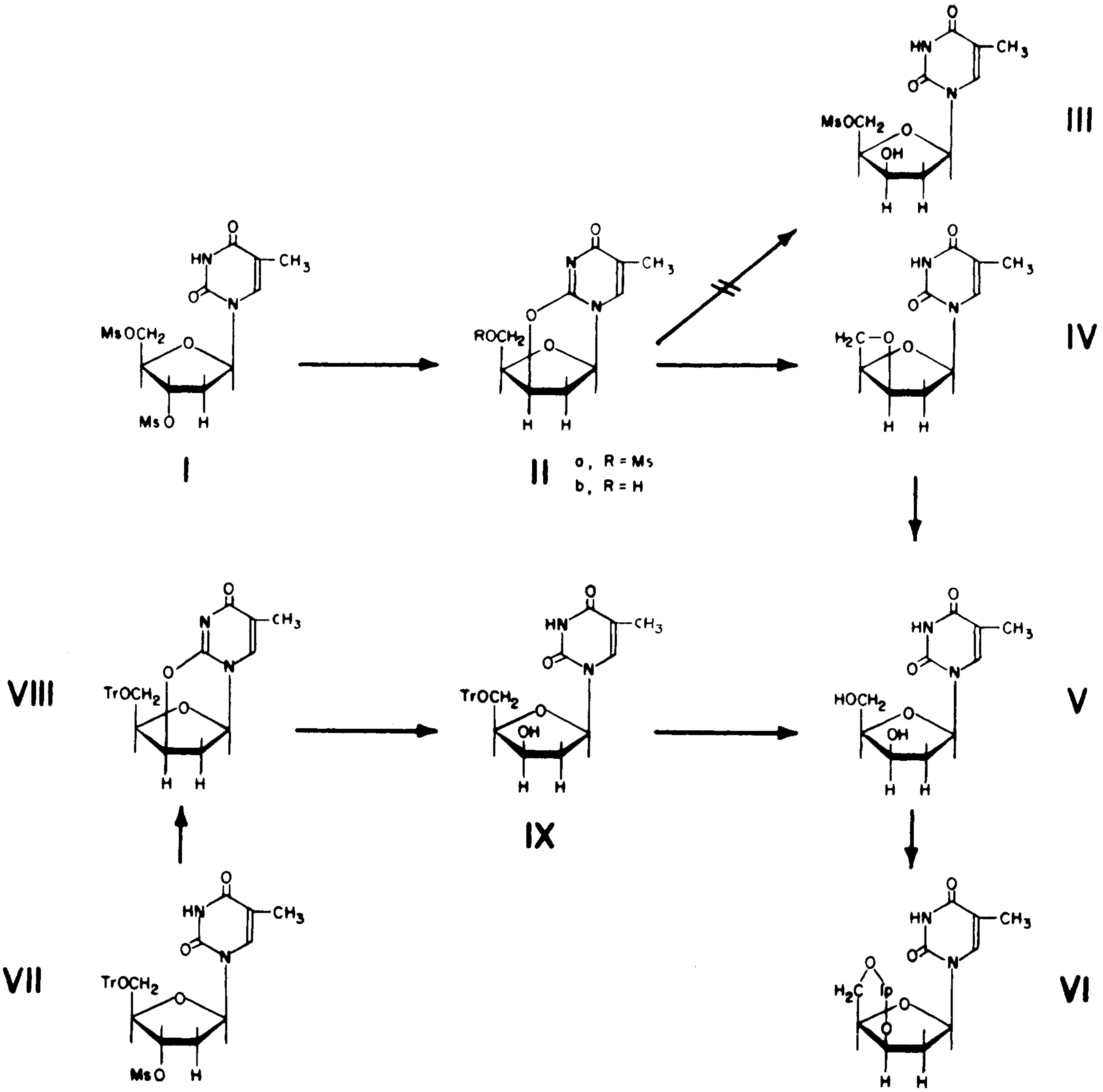
(9) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **102**, 317 (1933).

(10) B. Helferich and M. Burgdorf, *Tetrahedron*, **3**, 274 (1958).

(11) J. J. Fox and I. Wempen, *Advan. Carbohydrate Chem.*, **14**, 283 (1959).

(12) A pair of 2-′deoxypentosylthymines was anticipated since attack (by H_2O) at C-5 and C-3 both appeared sterically possible. However, no thymidine was detected.

(13) J. P. Horwitz, J. A. Urbanski, and J. Chua, *J. Org. Chem.*, **27**, 3300 (1962).



(Table I) properties resembled those of thymidine. On the other hand, the conversion of compound V to an isopropylidene derivative VI establishes a *cis* configuration for the (3',5')hydroxyl groups and the structure 1-(2'-deoxy-β-D-*threo*-pentofuranosyl)thymine for V.

An alternative synthetic approach to V utilized 3'-*O*-mesyl-5'-*O*-tritylthymidine (VII)[4,13] as starting material. The replacement of the mesyloxy function in VII with net inversion to yield (90%) 1-(2'-deoxy-5'-*O*-trityl-β-D-*threo*-pentosyl)thymine (IX) was accomplished with excess sodium hydroxide. The reaction of VII and one equivalent of sodium hydroxide afforded the expected 2,3'-anhydronucleoside, VIII which, on treatment with a second equivalent of base, was converted to IX. Detritylation of IX in 80% acetic acid provided a solid (87% yield) which proved to be identical in every respect with V.

It is expected that the opening of the epoxide ring in IV with anhydrous hydrogen halides will lead to the presently unknown 1-(2',5'-dideoxy-5'-halo-β-D-*threo*-pentosyl)thymines. This expectation is based on the preliminary observation that IV, on treatment with sodium iodide in butanone containing a small amount of acetic acid, yields an apparently new dideoxyiodopentosylthymine.[14] Studies are currently in progress to rigidly establish this structure and, in addition, to utilize the facile conversion of VII to IX as the origin of a new synthetic avenue to III.

Experimental[15]

2,3'-Anhydro-1-(2'-deoxy-5'-*O*-mesyl-β-D-*threo*-pentosyl)thymine (IIa).—To a hot solution of 5.98 g. (15 mmoles) of 3',5'-

---

[14] The product differs in properties from those recorded (see ref. 4) for both 5'-iodo-5'-deoxy- and 3'-iodo-3'-deoxythymidine, the only dideoxyiodopentosylthymines which have been previously described.

[15] All melting points are uncorrected. Analytical determinations were performed by Micro Tech Laboratories, Skokie, Ill. Ultraviolet absorption measurements were made with a Cary recording spectrophotometer, Model 11. Infrared absorption spectra were measured both in chloroform and Nujol using a Perkin-Elmer, Model 21, recording spectrophotometer. Relative R_f values are summarized in Table I.

di-*O*-mesylthymidine[4] (I) in 760 ml. of absolute ethanol was added 15.67 ml. of 0.96 *N* sodium hydroxide (15 meq.) and the solution was refluxed for 1 hr.[16] A colorless crystalline solid was deposited after the reaction mixture was held at room temperature overnight; wt. 4.34 g. (96% yield), m.p. 174–176° dec. (lit.,[4] 176° dec.).

1-(2′-Deoxy-3′,5′-epoxy-β-D-*threo*-pentofuranosyl)thymine (IV). From I.—A solution of 3.74 g. (9.4 mmoles) of I in 250 ml. of water containing 28 ml. of 1 *N* sodium hydroxide was refluxed for 2 hr. The reaction mixture was cooled to room temperature and then neutralized (phenolphthalein) with 9.2 ml. of 1 *N* hydrochloric acid. The neutral solution was evaporated *in vacuo* and the last traces of moisture were removed by several evaporations from absolute ethanol. After removal of chloroform insoluble impurities, the residue crystallized from ethyl acetate; wt. 1.55 g. (74% yield), m.p. 185–189°. Two recrystallizations from ethanol provided an analytical sample; m.p. 193.5–194.5°, $[\alpha]^{24}\text{D}$ −127° (*c*, 1.0, water), $\lambda_{\text{max}}^{\text{CHCl}_3}$ (μ) 2.98 (NH), 5.85, 5.93 (CO), $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (mμ), 267, 208 (ϵ 10,050, 9710), $\lambda_{\text{min}}^{\text{H}_2\text{O}}$ 235 mμ (ϵ 2350).

Anal. Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.56; H, 5.40; N, 12.50. Found: C, 53.74; H, 5.11; N, 12.59.

From IIa.—A solution of 0.15 g. (0.5 mmole) of IIa in 20 ml. of water containing 5.23 ml. of 0.098 *N* sodium hydroxide (0.513 meq.) was refluxed for 7 hr. The cooled solution was neutralized (phenolphthalein) with 0.9 ml. of 0.1 *N* hydrochloric acid and then evaporated to dryness *in vacuo*. The residue was triturated with several portions (5 × 10 ml.) of hot chloroform and the filtered solution, when adjusted to 10 ml. containing 50% carbon tetrachloride, afforded a crystalline solid which was twice recrystallized from absolute alcohol; wt. 0.057 g. (51% yield), m.p. 193–194° alone or when admixed with a sample of product obtained from I.

2,3′-Anhydro-1-(2′-deoxy-5′-*O*-trityl-β-D-*threo*-pentosyl)thymine (VIII).—A solution of 1.37 g. (2.3 mmoles) of 3′-*O*-mesyl-5′-*O*-tritylthymidine[4,13] (VII) in 50 ml. of ethanol containing 2.45 ml. of 1 *N* sodium hydroxide was refluxed for 2.5 hr. The solution was evaporated *in vacuo* and the residue was washed into a filter funnel with cold water. The solid, wt. 1.08 g., was recrystallized first from benzene–methanol, and then twice from methanol to give 0.5 g. of colorless short needles which softened at 145–155°, resolidified at 200° and then melted at 218–222° dec.; $[\alpha]^{25}\text{D}$ −18° (*c*, 0.96, ethanol), $\lambda_{\text{max}}^{\text{EtOH}}$ (mμ) 214, 252 (shoulder), (ϵ 24,640, 9470).

Anal. Calcd. for $C_{29}H_{26}N_2O_4 \cdot 0.5\ CH_3OH$: C, 73.43; H, 5.85; N, 5.81. Found: C, 73.41; H, 5.84; N, 5.87.

1-(2′-Deoxy-5′-*O*-trityl-β-D-*threo*-pentosyl)thymine (IX). From VII.—To a solution of 4.0 g. (7.15 mmoles) of 3′-*O*-mesyl-5′-*O*-tritylthymidine[4,13] (VII) in 100 ml. of ethanol containing 28.5 ml. of 1 *N* sodium hydroxide was added 100 ml. of water and the solution was refluxed for 4 hr. The reaction mixture was concentrated to *ca.* 100 ml. *in vacuo*, chilled by the addition of ice and then carefully acidified (pH 2) with dilute hydrochloric acid. The amorphous colorless material was collected and washed with generous quantities of water. The product crystallized from a large volume of ethanol; wt. 3.11 g. (two crops—90% yield), m.p. 235–240°. A second recrystallization from ethanol provided an analytical sample; m.p. 240–241°, $[\alpha]^{25}\text{D}$ −22.9° (*c*, 0.53, DMF), $\lambda_{\text{max}}^{\text{EtOH}}$ (mμ), 266, 210 (ϵ 11,520, 36,710), $\lambda_{\text{min}}^{\text{EtOH}}$ 244 mμ (ϵ 6210).

Anal. Calcd. for $C_{29}H_{28}N_2O_5$: C, 71.88; H, 5.82; N, 5.78. Found: C, 71.63; H, 6.00; N, 5.97.

From VIII.—A solution of 0.275 g. (0.59 mmole) of VIII in 50 ml. of ethanol containing 0.6 ml. of 1 *N* sodium hydroxide was refluxed for 3 hr. The reaction mixture was concentrated to *ca.* 10 ml., water was added and the solution was acidified (pH 2) with dilute hydrochloric acid. The product was collected and dried in a vacuum desiccator (P_2O_5); wt. 0.25 g. (88%), m.p. 232–235° alone or when admixed with a sample derived from VII.

1-(2′-Deoxy-β-D-*threo*-pentofuranosyl)thymine (V). From IV.—A solution of 0.11 g. (0.49 mmole) of IV in 10 ml. of 0.1 *N* sulfuric acid was refluxed for 1 hr. The cooled solution was neutralized by adding to a column (8 × 1 cm.) of amberlite IR-4B (OH) and the resin was washed with water until the ultraviolet absorption (266 mμ) of the effluent was insignificant (*ca.* 500 ml.). The neutral solution was evaporated to dryness *in vacuo* and the residue was recrystallized first from ethyl acetate (Norit) and then from ether–petroleum ether (30–60°); wt. 0.05 g. (42% yield), m.p. 168–170°. A second recrystallization from ethyl acetate provided an analytical sample; m.p. 170–171°, $[\alpha]^{25}\text{D}$ +12° (*c*, 0.36, water). Spectral properties (water): At pH 6, λ_{max} (mμ) ~210, 269 (ϵ 9580, 10,440), λ_{min} 235 mμ (ϵ 2120). At pH 1, λ_{max} (mμ) ~210, 269 (ϵ 9480, 9980), λ_{min} 235 mμ (ϵ 2120). At pH 12, $\lambda_{\text{max,min}}$ (mμ) 268, 238 (ϵ 7870, 4340).

Anal. Calcd. for $C_{10}H_{14}N_2O_5$: C, 49.58; H, 5.83; N, 11.57. Found: C, 49.28; H, 6.12; N, 11.54.

From IX.—The conversion of IX (1.5 g., 3.1 mmoles) to V was effected according to the procedure described in reference 13 for 3′-*O*-mesylthymidine. Recrystallization of the reaction product from methanol–ethyl acetate gave 0.65 g. (87% yield) of solid, m.p. 169–170°. Admixture with a sample prepared from IV showed no depression in melting point. Infrared measurements (Nujol) on the two samples afforded identical spectra: λ_{max} (μ) 3.02 (NH,OH) 5.87, 5.92 (CO); $[\alpha]^{25}\text{D}$ +12°. Ultraviolet spectral properties (water): λ_{max} (mμ) ~210, 269 (ϵ 9480, 10,370), λ_{min} 235 mμ (ϵ 2390).

1-(2′-Deoxy-3′,5′-*O*-isopropylidene-β-D-*threo*-pentosyl)thymine (VI).—A mixture of 0.2 g. (0.83 mmole) of IV, 0.4 g. of anhydrous copper sulfate and 0.01 ml. of concentrated sulfuric acid in 10 ml. of dry acetone was stirred magnetically at 38° under a stream of nitrogen for 65 hr. The mixture was filtered through Celite and the filtrate was stirred with 0.2 g. of calcium hydroxide at room temperature for 1 hr. The inorganic material was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residue crystallized from acetone–petroleum ether (30–60°); wt. 0.205 g. (88% yield), m.p. 165–167°. A second recrystallization from the same mixture of solvents provided an analytical sample; m.p. 167.5–169°, $[\alpha]^{25}\text{D}$ −11.4°, $\lambda_{\text{max}}^{\text{Nujol}}$ (μ) 5.88, 6.02 (CO), 11.75 (isopropylidene), $\lambda_{\text{max}}^{\text{EtOH}}$ (mμ) 267, 210 (ϵ 9720, 10,250), $\lambda_{\text{min}}^{\text{EtOH}}$ 235 mμ (ϵ 2560).

Anal. Calcd. for $C_{13}H_{18}N_2O_5$: C, 55.31; H, 6.43; N, 9.92. Found: C, 55.14; H, 6.39; N, 10.10.

Acknowledgment.—The authors are indebted to Miss Erika Ziebart for performing the chromatographic studies.

---

(16) The initially pronounced yellow color is gradually discharged during reflux.