$COOCH_3$), 2.12 (3 H, s, H_3C—C(=O)–), and 3.69 ppm (3 H, s, H_3COOC–).

Anal. Calcd for $C_{18}H_{28}O_4$: C, 70.10; H, 9.15. Found: C, 70.25; H, 9.22.

4α,10β-Dimethyl-4β-carbomethoxy-1,2,3,4,5α,6,7,8β,10,12,13,14-dodecahydrophenanthrone-12 (37).—A cold solution of 0.061 g (2.65×10^{-3} g-atom) of sodium in 10 ml of dry methanol was added dropwise over 10 min to a solution of 0.365 g (1.18×10^{-3} mole) of Michael adduct **39**, mp 101–103°, in 15 ml of methanol immersed in an ice–ethanol bath. When the addition was complete, the bath was removed, the system was flushed with nitrogen several times and the mixture was refluxed for 8 hr. It was then cooled, diluted with brine, and extracted with ether. The ethereal extract was washed with brine, dried over magnesium sulfate, filtered, and evaporated to give 0.365 g of crude enone **37**, mp 146–159°. One recrystallization from hexane gave 0.287 g (84%) of **37**, mp 159–164°. Further recrystallization from hexane did not sharpen the melting point. A specimen of **37** was purified by elution chromatography on alumina to mp 158.5–160.5°; it had the following spectral properties: λ_{max}^{KBr} 5.80, 6.00, and 6.16 μ; λ_{max}^{MeOH} 239 mμ (ε 22,000); $\delta_{TMS}^{CCl_4}$ 0.94 (3 H, s, H_3C—C<), 1.17 (3 H, s, >C(CH_3)—$COOCH_3$), 3.64 (3 H, s, H_3COOC–), and 5.72 ppm (1 H, d, J = 1.7 Hz, H—C(=)–).

A specimen of authentic naturally derived **37**,[19] mp 115.5–116.0° (lit.[19] mp 116–118°) had λ_{max}^{KBr} 5.80, 5.95, and 6.19 μ; the nmr spectrum of this material was identical with that of the 158.5–160.5° material.

Methyl *dl*-Podocarpate (40).—To a refluxing solution of 0.100 g (3.45×10^{-4} mole) of enone **37**, mp 159–164°, in 25 ml of dry carbon tetrachloride was added, at one time, 0.096 g (5.39×10^{-4} mole) of freshly recrystallized N-bromosuccinimide. The mixture was refluxed for 50 min, cooled, and partitioned between water and ether, whereupon crystals which had appeared during cooling dissolved. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and evaporated to give 0.166 g of brown oil which was chromatographed on 10 g of acid-washed alumina. Two fractions, eluted successively with 8:1 and 9:1 ether–pentane, furnished 0.034 g (34%) of **40**, mp 172–180°. Several recrystallizations of **40** from hexane containing a little acetone gave an analytical sample which crystallized as long, white needles: mp 184.5–185° (lit.[18c] mp 193°); λ_{max}^{KBr} 2.86, 5.83, 6.18 μ. The infrared spectrum of **40** in chloroform solution was identical with that of an authentic sample of methyl *d*-podocarpate, mp 210–211.5°, prepared by treatment of *d*-podocarpic acid with diazomethane.

Anal. Calcd for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 75.03; H, 8.20.

Registry No.—**2**, 15292-89-4; **3**, 15292-90-7; **12**, 15292-91-8; **13**, 15292-92-9; **14**, 15292-93-0; **15**, 15292-50-9; **17**, 15292-95-2; **19**, 15268-85-6; **20**, 15268-86-7; **23**, 15268-87-8; **24**, 15268-88-9; **25**, 15268-89-0; **26**, 15268-90-3; **27**, 15285-90-2; **28**, 15268-91-4; **29**, 15268-92-5; **30**, 15268-93-6; **31**, 15268-94-7; **32**, 15268-95-8; **33**, 15268-96-9; **34**, 15268-97-0; **35**, 15268-98-1; **37**, 15268-99-2; **38**, 15269-00-8; **39**, 15269-01-9; **40**, 15292-67-8.

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7-Deazaadenine Ribonucleosides. The Use of Periodate Oxidation in Degradation Studies[1a]

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A number of purine and pyrimidine ribonucleoside analogs have been synthesized in the search for purines possessing antitumor and antibacterial activity. Three naturally occurring, biologically active 7-deazaadenine ribonucleosides have been recently isolated from *Streptomyces*.[2,3] Two of these nucleosides are tubercidin (**1**) and toyocamycin (**3**).

The structure of tubercidin and toyocamycin have been reported,[4,5] as well as the synthesis of the aglycon of toyocamycin.[6] The proof of structure of toyocamycin did not proceed *via* the intact aglycon since the 7-deazaadenine nucleosides are not as easily hydrolyzed as purine nucleosides.[5] Ohkuma[5] and Suzuki and Maruma[7] reported the isolation of ribose from toyocomycin and tubercidin. The yields of ribose were very low, and the intact aglycon was not isolated. Refluxing 7-deazaadenine ribonucleosides in acidic phenylhydrazine solution does not cleave the N-ribosyl bond. A careful examination of the chemical reactions of the purine ribonucleosudes[8,9] indicated that the stability of the N-glycosyl bond of 7-deazaadenine ribonucleosides might be significantly lowered by altering the ribosyl portion of the nucleoside. This study reports on the periodate oxidation of the 7-deazaadenine ribonucleosides, tubercidin and toyocamycin, the isolation of the aglycones, and the isolation of carbon atoms 1 and 2 of the ribose moiety as glyoxal bisphenylosazone by employing the methods as described by Khym and Cohn[9] and Barry.[10] By using this procedure, 4-hydroxy-5-cyanopyrrolo-[2,3-*d*]pyrimidine (**8**) has also been isolated by two methods from toyocamycin. Method A (Scheme I, compounds **3**, **4**, **5**, **8**) affords a 71% yield compared to 19% for method B (Scheme I, compounds **3**, **7**, **8**). Compound **8** had not been previously reported in the degradation studies on toyocamycin as described by Ohkuma.[5] Subsequent treatment of **8** with hydriodic acid and red phosphorus allows the isolation of 4-hydroxypyrrolo[2,3-*d*]pyrimidine (**9**). The product is identical with chemically prepared 4-hydroxypyrrolo-

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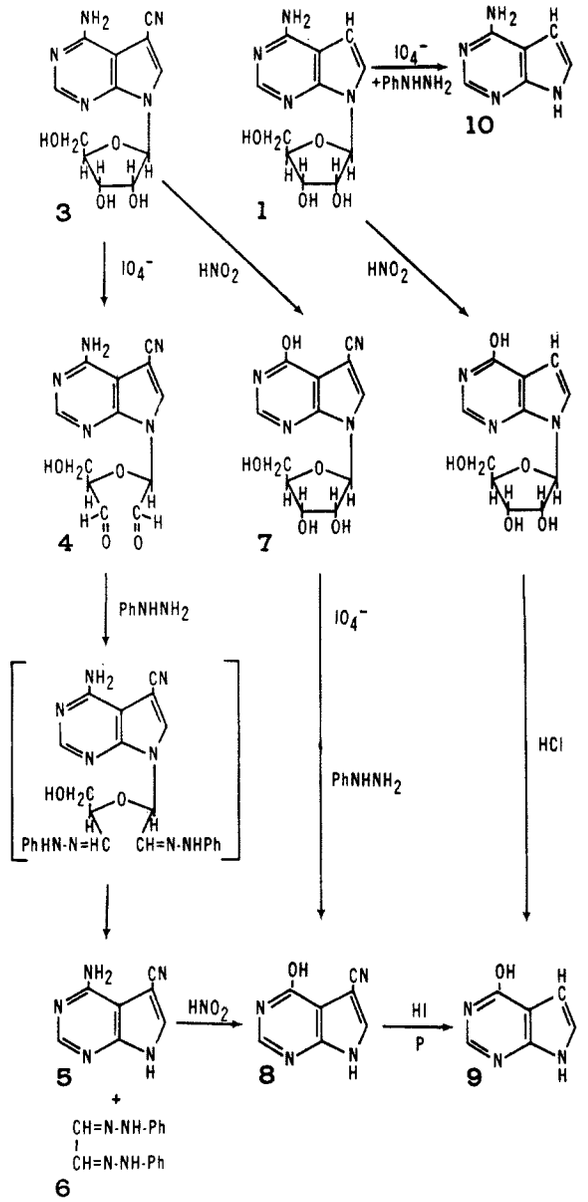
SCHEME I
DEGRADATION OF THE 7-DEAZAADENINE RIBONUCLEOSIDES, TUBERCIDIN (1) AND TOYOCAMYCIN (3)



[2,3-*d*]pyrimidine as judged by their ultraviolet absorption spectra and their chromatographic mobilities.

The oxidation of tubercidin and toyocamycin and the isolation of the aglycones, 4-aminopyrrolo[2,3-*d*]-pyrimidine (**10**) and 4-amino-5-cyanopyrrolo[2,3-*d*]-pyrimidine (**5**), provides a general method for the isolation and proof of structures of the aglycones of synthetic and naturally occurring 7-deazaadenine ribonucleosides. This report also provides a simple degradation procedure useful in studying the biosynthesis of this group of nucleosides.

Experimental Section[11]

Physical Methods and Materials.—Ultraviolet spectra were recorded on a Cary Model 14 M spectrophotometer. Infrared spectra were taken on a Perkin-Elmer 337 spectrophotometer with potassium bromide pellets. Avicel plates (250 μ thick) used for thin layer chromatography were purchased from Analtech, Inc., Wilmington, Del. The solvent used was 95% ethanol–chloroform–concentrated ammonium hydroxide—water (6:4:0.5:0.5). The compounds were visualized with ultraviolet light.

Glyoxal Bisphenylosazone (Carbon Atoms 1′ and 2′ of Toyocamycin) (6).—To a suspension of toyocamycin (170 μmoles) in 4 ml of water was added sodium periodate (230 μmoles). After stirring at room temperature for 30 min, the reaction mixture was added to a Dowex-1-acetate column (8 cm × 1 cm) and then washed with 25 ml of 0.02 *M* acetic acid. The effluent was added to a solution containing 30 ml of ethanol, 60 ml of 4 *M* acetic acid, and 0.02 ml of phenylhydrazine. The mixture was refluxed for 5 min on a steam bath and added to 180 ml of ice water. After 3 hr, the insoluble glyoxal bisphenylosazone was filtered and recrystallized from ethanol–water (1:1) to give 110 μmoles (65%) of pale yellow needles, mp 168–170° (lit.[9] 170°). The isolated compound showed no melting point depression when mixed with authentic glyoxal bisphenylosazone. The ultraviolet spectra of the glyoxal bisphenylosazone isolated from toyocamycin and authentic glyoxal bisphenylosazone were identical. The infrared spectrum had maxima at 3290 (NH) and 1680, 1600, and 1575 cm^{-1} (C=N- NH–Ph). The aqueous filtrate was used to isolate 4-amino-5-cyanopyrrolo[2,3-*d*]pyrimidine (**5**) described in the following procedure.

4-Amino-5-cyanopyrrolo[2,3-*d*]pyrimidine (5) (from Toyocamycin).—The aqueous filtrate was extracted with ether to remove the excess glyoxal bisphenylosazone. The aqueous fraction was then evaporated to dryness *in vacuo* and the residue was dissolved in 0.1 *M* hydrochloric acid and crystallized by the addition of 1 *M* ammonium hydroxide. The crystalline aglycon **5** was removed by filtration. This procedure was repeated three times. The white crystalline compound did not melt at a temperature above 300° (lit.[6] mp >360°). The yield was 99 μmoles (52%). The infrared spectra of the isolated and the chemically synthesized compounds were superimposable: $\lambda_{max}^{0.1\,N\,HCl}$ 234 mμ (log ϵ 4.17), 274 (4.06); $\lambda_{max}^{0.1\,N\,NaOH}$ 245 mμ (log ϵ 4.25), 287 (4.01). The chemically synthesized aglycon, obtained from Dr. Roland K. Robins, had the same ultraviolet spectra.

4-Hydroxy-5-cyanopyrrolo[2,3-*d*]pyrimidine (8). Method A.—4-Amino-5-cyanopyrrolo[2,3-*d*]pyrimidine (**5**) (62 μmoles) was suspended in 4 ml of 5 *M* acetic acid with sodium nitrite (786 μmoles) and stirred at 60–70° for 2.5 hr. The reaction mixture was cooled, the insoluble compound **8** was separated by filtration and recrystallized from ethanol–water (1:1) affording 44 μmoles (71%) of product as white needles, mp >300°. The infrared spectrum exhibited a nitrile band at 2240 cm^{-1} and an imide band at 1,700 cm^{-1}; the ultraviolet spectrum exhibited $\lambda_{max}^{0.1\,N\,HCl}$ 221 mμ (log ϵ 4.15), 262 (4.07); $\lambda_{max}^{0.1\,N\,NaOH}$ 241 mμ (log ϵ 4.20), 282 (4.10).

Anal. Calcd for $C_7H_4N_4O$: C, 52.50; H, 2.50; N, 35.00. Found: C, 52.20; H, 2.39; N, 34.30.

Method B.—7-(D-Ribosyl)-4-hydroxy-5-cyanopyrrolo[2,3-*d*]-pyrimidine (**7**) was prepared from 342 μmoles of toyocamycin;[5] 104 μmoles of **7** was added to 140 μmoles of sodium periodate in 3 ml of 5 *M* acetic acid. After stirring at room temperature for 30 min, the solution was added to a Dowex-1-acetate column (6 cm × 1 cm). The 4-hydroxy-5-cyanopyrrolo[2,3-*d*]pyrimidine was displaced from the column with 20 ml of 0.02 *M* acetic acid. The eluent was added to a solution of 15 ml of ethanol, 0.1 ml of phenylhydrazine, and 30 ml of 4 *M* acetic acid, refluxed for 15 min on a steam bath, and poured into 100 ml of ice water. The insoluble glyoxal bisphenylosazone was removed by filtration after 3 hr. The aqueous filtrate was extracted with ether to remove the excess glyoxal bisphenylosazone. The aqueous fraction was evaporated to dryness *in vacuo*. The 4-hydroxy-5-cyanopyrrolo[2,3-*d*]pyrimidine was recrystallized from ethanol–water (1:1). The yield of colorless needles was 20 μmoles (19%) based on 104 μmoles of **7**. The melting point was greater than 300°. A thin layer chromatogram showed that the R_f of 4-hydroxy-5-cyanopyrrolo[2,3-*d*]pyrimidine was identical with the R_f of the same compound isolated and characterized in method A. The ultraviolet spectra was the same as that reported in method A.

4-Hydroxypyrrolo[2,3-*d*]pyrimidine (9).—The 4-hydroxy-5-cyanopyrrolo[2,3-*d*]pyrimidine (**8**) (3.7 μmoles), obtained by the degradation of toyocamycin (method A), was heated in a sealed tube with 0.1 ml of hydriodic acid (sp gr 1.7) and 2 mg

(11) All melting points were determined on a Thomas-Hoover silicone bath apparatus and are uncorrected. Microanalyses were performed by F. & M. Scientific Corp., Avondale, Pa.

of red phosphorus at 150° for 1 hr. The reaction mixture was cooled, neutralized with concentrated ammonium hydroxide, and centrifuged. The supernatant, containing **9**, was chromatographed on Whatman No. 1 paper (ammonia–water solvent (pH 10.0)). The ultraviolet spot corresponding to the chemically synthesized 4-hydroxypyrrolo[2,3-*d*]pyrimidine was cut out and eluted with water. The yield was 1.5 μmoles (40%). The R_f and the ultraviolet spectra of the 4-hydroxypyrrolo-[2,3-*d*]pyrimidine were identical with those of the chemically synthesized compound supplied by Dr. G. H. Hitchings and reported by Ohkuma:[5] $\lambda_{max}^{0.1\,N\,HCl}$ 263 mμ; $\lambda_{max}^{0.1\,N\,NaOH}$ 265 mμ.

The isolation of 4-hydroxypyrrolo[2,3-*d*]pyrimidine (**9**) from tubercidin was accomplished by treatment of the nucleoside with nitrous acid[12] followed by a 5-hr reflux with 1 *N* HCl.[7] The reaction mixture was cooled to room temperature and barium carbonate was added to neutralize the sulfuric acid. The barium sulfate was removed by filtration. The aqueous fraction was evaporated to a small volume, applied to a Whatman No. 1 paper chromatogram, and developed in 1-butanol–1 *N* NH_4OH (86:14). The R_f value of the 4-hydroxypyrrolo-[2,3-*d*]pyrimidine (**9**), detected by ultraviolet light, was identical with that of authentic compound. The area was cut out and eluted with water. The ultraviolet spectra was the same as that reported for the synthetic compound.

4-Aminopyrrolo[2,3-*d*]pyrimidine (10) (from Tubercidin).—Tubercidin (**1**) (150 μmoles) was oxidized with periodate in exactly the same manner as described above for the oxidation of toyocamycin. The aglycon **10** was crystallized from water. The yield was 31 μmoles (21%). There was one ultraviolet spot as determined by a thin layer of chromatography. The infrared spectra of the isolated and chemically synthesized 4-aminopyrrolo[2,3-*d*]pyrimidine were the same (3400 and 3100 cm^{-1} (NH), 1650 and 1600 cm^{-1} (C=N)). The ultraviolet spectra was the same as that reported above for the synthesized compound.

Registry No.—**1**, 69-33-0; **3**, 1414-35-3; **5**, 1500-90-9; **6**, 1534-21-0; **8**, 15023-88-8; **9**, 3680-71-5; **10**, 1500-85-2.

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Extension of Sugar Chains through Acetylenic Intermediates. IV. Derivatives of 1-Pentyne-D-*erythro*(and D-*threo*)-3,4,5-triol[1-3]

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Ethynylation of 2,3-*O*-isopropylidene-*aldehydo*-D-glyceraldehyde (**1**) gives a 44:56 mixture of 4,5-*O*-isopropylidene-1-pentyne-D-*erythro*(and D-*threo*)-3,4,5-triol (**2** and **7**), separable by glpc as their 3-acetates **3** and **8**. Hemihydrogenation of **3** and **8** gave the derived pentenes **4** and **9**, also obtainable, in admixture, by vinylation of **1** and acetylation of the product. The epimers were individually identified by degradation; the acetates **3** and **8** were ozonized and the products were hydrolyzed to give an erythronolactone and a threonolactone, respectively, and the pentenes **4** and **9** were successively ozonized, reduced, and hydrolyzed to give erythritol and a threitol, respectively. Saponification of **3** and **8** gave the separate epimers **2** and **7**, which were converted into their crystalline 3-(3,5-dinitrobenzoates) **17** and **18**. The esters (**3** and **17**) having the D-*erythro* configuration showed spin–spin couplings between H-3 and H-4 smaller than those of the D-*threo* analogs (**8** and **18**), indicating that the most populated rotamer state of these acetylenic derivatives is that having the 3-acyloxy group antiparallel, and the ethynyl group *gauche*, to the C-5 carbon atom.

In an earlier paper[4] in this series, the ethynylation of 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-L-arabinose to give the 4,5:6,7-diisopropylidene acetal of 1-heptyne-L-*gluco*(and L-*manno*)-pentol was described. The epimers were separated by glpc, and also by fractional crystallization of suitable derivatives, and their structures were proved by two independent degradative routes. It was shown that the epimers could be differentiated readily by nmr spectroscopy of various 3-*O*-acyl derivatives; the coupling of H-3 with H-4 was correlated with the relative configuration at C-3 and C-4. In the present report, the ethynylation of 2,3-*O*-isopropylidene-*aldehydo*-D-glyceraldehyde (**1**) to give a mixture of 4,5-*O*-isopropylidene-1-pentyne-D-*erythro*-3,4,5-triol[5] (**2**) and 4,5-*O*-isopropylidene-1-pentyne-D-*threo*-3,4,5-triol[5] (**7**) is described, together with the separation of the epimers, structural proofs by degradative methods, and characterization of the acetylenic compounds and their derived alkenes by nmr spectroscopy and mass spectroscopy.

2,3-*O*-Isopropylidene-*aldehydo*-D-glyceraldehyde (**1**) was prepared by oxidation of 1,2:5,6-di-*O*-isopropylidene-D-mannitol[6] with lead tetraacetate by the procedure of Baer and Fischer,[7] and azeotropic coevaporation of the crude product with carbon tetrachloride was employed to remove all acetic acid before vacuum distillation of the product. Omission of this step gave **1** containing some acetic acid, detected by nmr spectroscopy, which could not readily be removed by distillation and which led to diminution of yield in the ethynylation step. The nmr spectrum of the freshly prepared aldehyde **1** showed the anticipated low-field signal for the aldehyde proton at τ 0.35 as a one-proton, narrow doublet, $J_{1,2}$ = 1.7 Hz. On storage at room temperature, substance **1** polymerized,[7] as manifested in observed changes in the nmr and ir spectra of the material.

(1) Previous paper in this series: J. L. Godman, D. Horton, and J. M. J. Tronchet, *Carbohydrate Res.*, **4**, 392 (1967).

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(3) Supported by the National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Md. 20014; Grant No. GM-11976-02 (The Ohio State University Research Foundation Project 1820). Funds for the nmr spectrometer were provided by the National Science Foundation, Washington, D. C.

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(5) Compounds **2** and **7** are strictly named as 1,2-*O*-isopropylidene-4-pentyne-L-*erythro*- and -D-*threo*-1,2,3-triol, respectively. In this paper, as in preceding ones,[1,4] the alkyne terminus is considered to be C-1, so that configurational relationships are readily apparent between the acetylenic derivatives, their precursors, and their degradation products.

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