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Nucleosides. 100. General Synthesis of Pyrimidine C-5 Nucleosides Related to Pseudouridine. Synthesis of 5-(β-D-Ribofuranosyl)isocytosine (Pseudoisocytidine), 5-(β-D-Ribofuranosyl)-2-thiouracil (2-Thiopseudouridine) and 5-(β-D-Ribofuranosyl)uracil (Pseudouridine)[1]

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A general procedure for the synthesis of pyrimidine C-5 nucleosides related to pseudouridine was developed. 5-(β-D-Ribofuranosyl)isocytosine (**7**, pseudoisocytidine), the first chemotherapeutically active synthetic C nucleoside, was prepared from readily available ethyl 2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)acetate (**3**). Compound **3** was formylated with ethyl formate and sodium hydride to the corresponding formylacetate sodium enolate **4** and methylated with methyl iodide in DMF to give 3-methoxy-2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)acrylate (**5**). Cyclization of **5** with guanidine afforded the protected isocytosine C-5 nucleoside **6**. Treatment of **6** with methanolic hydrogen chloride gave the desired crystalline β nucleoside, pseudoisocytidine (**7**). From the mother liquor, the α isomer **8** was obtained. Compound **8** can be epimerized effectively to **7** in methanolic hydrogen chloride so that a very high yield of the desired isomer **7** from **6** is readily achieved. The general applicability of this method to the syntheses of other C nucleosides was demonstrated by the synthesis of 2-thiopseudouridine (**10**) and pseudouridine (**13**). Condensation of the acrylate derivative **5** with thiourea followed by deblocking of the product afforded **10**. When **5** was treated with urea, the protected pseudouridine derivatives (**11** and **12**) were obtained. After deprotection of **11**, pseudouridine (**13**) was obtained.

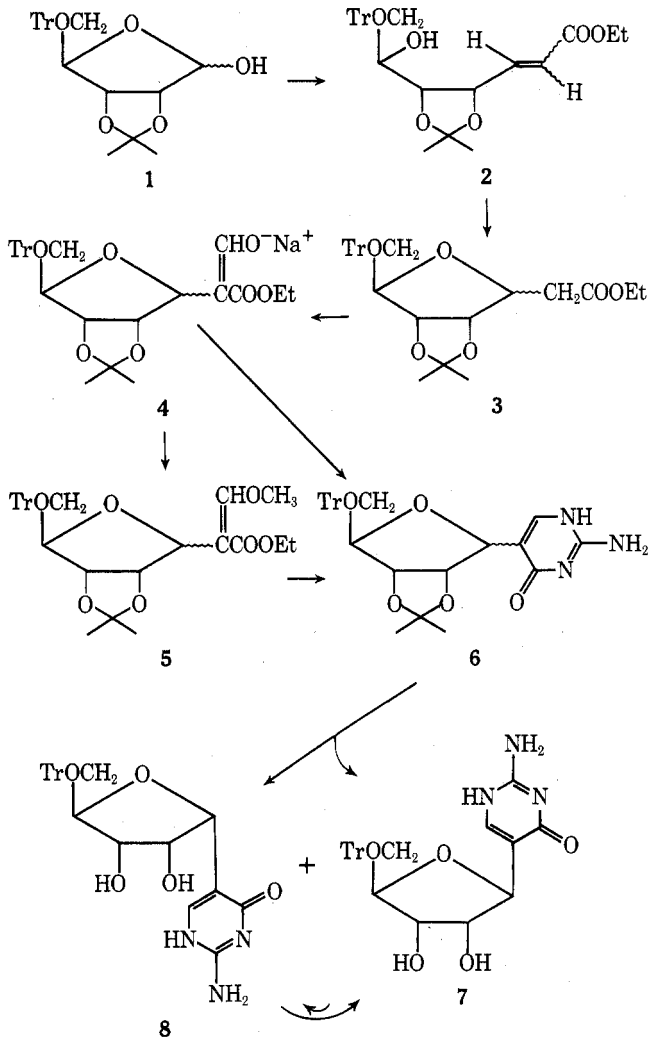
Pseudouridine, the first C nucleoside found in nature, has attracted the interest of organic chemists and biochemists since its discovery in 1957.[2] Recently, other C nucleosides have also been isolated as nucleoside antibiotics from the culture filtrates of various *Streptomycetes*.[3] The unique structural characteristic of C nucleosides which distinguishes them from the ordinary nucleosides is the presence of a carbon to carbon linkage instead of a carbon to nitrogen bond between the aglycon and sugar moieties. This structural feature renders traditional approaches[4] for nucleoside synthesis of limited value.

Although several reports have appeared on the synthesis of pseudouridine[5] and pseudocytidine,[6] these methods involve the condensation of a suitably protected sugar with a preformed pyrimidine-5-yllithium derivative. These procedures are difficult to perform and are not suitable for large-scale preparations. More importantly these methods are specific for each C nucleoside, i.e., for the synthesis of a modified base analogue preparation of a particular pyrimidine 5-lithio derivative is required individually.

As a part of our efforts to develop a *general* method for the synthesis of pseudouridine and analogues thereof we reported in a recent communication[7] a synthesis of 5-(β-D-ribofuranosyl)isocytosine (**7**, pseudoisocytidine) in four steps from 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranose (**1**) via intermediates **3** → **4** → **6** (see Chart I).

Owing to the potential clinical importance[8] of pseudoisocytidine as an antileukemic agent, we now report our synthetic

Chart I



procedures including modifications which result in improved yield of pseudoisocytidine. Further, the general applicability of our methods for the synthesis of pyrimidine C nucleosides is exemplified by the synthesis of 2-thio-pseudouridine (**10**) and pseudouridine (**13**).

Treatment of **1** with (ethoxycarbonylmethylene)triphenylphosphorane according to Ohrui et al.[9] afforded ethyl 2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)acetate (**3**). This reaction is often accompanied by the formation of significant amounts of a more polar by-product as observed by thin layer chromatography (TLC) in addition to the desired product **3**. The by-product was isolated by silica gel column chromatography and was shown by ^{1}H NMR to be a mixture of cis and trans olefins **2** (see Experimental Section). Buchanan et al.[10] had reported the isolation of an olefin analogous to **2** after treatment of tri-*O*-benzyl-D-ribofuranose with the same Wittig reagent and showed that their olefin could be converted into a cyclic derivative analogous to **3** by base catalysis. Thus, after the reaction of **1** with the Wittig reagent, the product which contained the open-chain intermediate **2** was treated with alkoxide and the desired intermediate **3** was obtained in high yield. The epimeric configuration at C-1 of the "ribosyl" derivative **3** was not determined, and indeed is not crucial because epimerization occurs in subsequent steps (vide infra).

The key step in the synthesis of pseudoisocytidine and related C nucleosides is the formylation of **3**. Compound **3** was treated with ethyl formate and sodium hydride in a mixture of anhydrous ether and absolute ethanol. Without purification, the product sodium enolate **4** was treated with guanidine in ethanol in the presence of sodium ethoxide. Protected pseudoisocytidine (**6**) was obtained in 5% yield as colorless crystals after silica gel column chromatography. The reproducibility of the yield of **6**, however, was inconsistent. Owing to the low acidity of the α hydrogens of the ester **3**, formylation did not go to completion resulting in an intractable mixture.

Base-catalyzed cyclization of crude **4** with guanidine to the protected nucleoside **6** proceeded poorly owing to the preponderance of the enolate form **4** in base. The attack by the nitrogen nucleophile on the aldehydic (enolic) carbon atom would be electrostatically hindered by the adjacent negatively charged oxygen. Removal of the negative charge from the enolic oxygen by alkylation should therefore favor the cyclization reaction. Thus, the crude sodium enolate **4** was methylated with methyl iodide in DMF and the desired β-methoxyacrylate derivative [ethyl 3-methoxy-2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)acrylate] (**5**) was isolated in crystalline form after column chromatography in ~25% yield from **1**.

Attempts to separate **5** on a large scale were found not practical owing to appreciable decomposition of this product on the silica gel column. Although the protected C nucleoside **6** could be obtained in ~90% yield from crystalline β-methoxyacrylate **5**, it was found more practical to use crude **5** directly for cyclization with guanidine. Under these conditions, pure compound **6** was isolated in crystalline form in ~15% overall yield from **1**.

The α configuration is assigned to crystalline **6** on the basis of Imbach's rule[11] and deblocking experiments. The difference in chemical shifts of the methyl signals of the isopropylidene group (11 Hz) falls into the α-nucleoside range (<15 Hz). Brief treatment of crystalline **6** with 10% methanolic hydrogen chloride gave predominantly the α isomer **8**, whereas prolonged treatment afforded predominantly the desired β isomer **7**. Compound **7** crystallized from the reaction mixture as its hydrochloride salt while the α isomer **8** remained in solution. Thus, the yield of the desired pseudoisocytidine **7** was readily raised to ~80%.

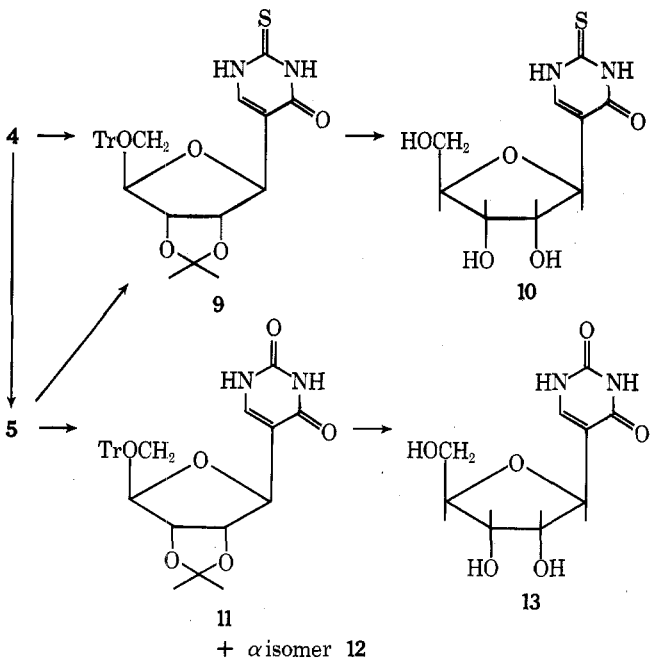
The assignment of configuration at C-1′ of pseudoisocytidine (**7**) and the α isomer **8** is based on ^{1}H NMR studies. The ^{1}H NMR spectrum of **7** is almost identical with that of pseudouridine[12] and quite different from the α-pseudouridine spectrum,[12] the latter of which is almost identical with the ^{1}H NMR spectrum of **8**. The chemical shift of H-1′ of **7** (δ 4.72) is higher than that of **8** (δ 5.04) and the chemical shift of H-6 of **7** (δ 7.75) is lower than that of **8** (δ 7.65). Furthermore, the allylic coupling between H-1′ and H-6 in **7** (0.78 Hz) is smaller than that of **8** (1.27 Hz). All of these NMR characteristics are also observed for the "anomeric" pseudouridines.[13] Moreover, the difference in chemical shifts for H-1′ (0.32) and H-6 (0.10) for the pseudoisocytidine isomers **7** and **8** are identical with the corresponding values for the isomeric pseudouridines.[12] The pK_a value for the β isomer **7** (8.97) is smaller than that of the α isomer **8** (9.12). Chambers[14] also observed that the pK_a value of pseudouridine (9.1) is smaller than that of α-pseudouridine (9.5) and suggested that the reason for this difference is that the monoanion of the β isomer is stabilized by a hydrogen bond between the 2-carbonyl and 5′-hydroxyl groups.

The pure α isomer **8** slowly underwent epimerization at C-1′ in dilute deuterium chloride to pseudoisocytidine **7** as indicated by ^{1}H NMR. The H-6 signal of **8** decreased slowly with concomitant increase of a new signal corresponding to the H-6 signal of **7**. Also, pure **7** epimerized to **8** under the same conditions, but the rate of epimerization was significantly lower than that of **8** to **7**. At the equilibrium point the α:β ratio was approximately 1:4. The formation of pyranosyl isomers, as

found in the case of pseudouridine,[14] was not observed. Pseudoisocytidine 7 was stable at pH 7.2 at 38 °C for at least 7 days. The mechanism for the interconversion (7 ⇌ 8) is probably akin to that proposed[15] for the isomerization of pseudouridines involving protonation of the sugar ring oxygen followed by opening of the furanoid ring. To our knowledge the synthesis of pseudoisocytidine is the first example of exploitation of epimerization at C-1′ to obtain the desired nucleoside isomer.

The versatility of intermediates 4 and 5 was further demonstrated (Chart II) by the synthesis of 5-(β-D-ribofurano-

Chart II



syl)-2-thiouracil (10). Treatment of 4 with 1.5 equiv each of thiourea and sodium ethoxide afforded the protected 2-thiopseudouridine 9 which was isolated in crystalline form after column chromatography. When the acrylate 5 was cyclized with thiourea, the same product 9 was obtained in higher yield. The large difference in chemical shifts of the two isopropylidene methyl signals in the ^{1}H NMR ($\Delta\delta$ 22.8) suggests that the crystalline product 9 is the β isomer. Brief treatment of 9 with methanolic hydrogen chloride afforded the pure β isomer 10, the ^{1}H NMR spectrum of which was almost identical with that of pseudouridine, whereas longer treatment gave an α,β mixture of isomers. The epimerization occurred much more rapidly than that noted above for pseudoisocytidines. At equilibrium, the α/β ratio was almost 1. Prolonged treatment of 10 with methanolic hydrogen chloride produced a complicated mixture, as indicated by the ^{1}H NMR spectrum, owing probably to the formation of pyranosyl isomers as well.

A total synthesis of pseudouridine (13) was also achieved from the common β-methoxyacrylate intermediate 5. Treatment of pure 5 with urea and sodium ethoxide in ethanol afforded an isomeric mixture of the protected C nucleosides (11 and 12, β:α ≃ 3:1) in 57% yield after chromatographic purification. A partial separation of the mixture of 11 and 12 was achieved on a thick layer plate using a multidevelopment technique. The β isomer 11 was isolated in pure form, but the minor nucleoside 12 was not obtained in pure state. When compound 11 was treated with methanolic hydrogen chloride, crystalline unprotected nucleoside 13 precipitated from the reaction mixture. Compound 13 was identical with natural pseudouridine.

Extensions of these syntheses to other pyrimidine C nucleosides with modifications in the aglycon and in the sugar moiety are underway in our laboratory.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are corrected. ^{1}H NMR spectra were obtained on a JEOL J1M-PET-100 spectrometer, and Me_4Si was the internal standard for organic solvents and $Me_3Si(CH_2)_3SO_3Na$ for D_2O; chemical shifts are reported in parts per million (δ) and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet); δ and J values are first order. TLC was performed on microscope slides coated with silica gel GF_{254} (Merck), and column chromatography on silica gel G or silica gel G60 (70–230 mesh, ASTM, Merck). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and by Spang Microanalytical Laboratory, Ann Arbor, Mich. Optical rotations were measured on a Rudolf polarimeter with a photomultiplier attachment.

Ethyl 2-(2,3-*O*-Isopropylidene-5-*O*-trityl-D-ribofuranosyl)acetate (3). 2,3-*O*-Isopropylidene-5-*O*-trityl-D-ribofuranose[16] (43.2 g, 0.1 mol) and (ethoxycarbonylmethylene)triphenylphosphorane (37.3 g, 0.11 mol) were dissolved in dry acetonitrile (500 ml, dried over 4-Å molecular sieves) and the solution was heated under reflux for 6 h. The mixture was allowed to cool to room temperature. If two sugar spots (R_f 0.45 for 2 and R_f 0.5 for 3) were found in the mixture on TLC (petroleum ether–ethyl acetate, 5:1), solid potassium *tert*-butoxide (~0.5 g) was added and the mixture was stirred at room temperature until the lower spot on TLC disappeared. The solvent was removed in vacuo and the residue was dissolved in ether (~500 ml). On cooling the solution in an ice bath, triphenylphosphine oxide precipitated which was removed by filtration and the filtrate was evaporated to dryness. This procedure was repeated three times to remove most of triphenylphosphine oxide. The yield of crude product 3 was 45 g (90%) which was sufficiently pure to be used in the formylation reaction to obtain the sodium enolate 4.

Separation of Ethyl 2,3-Dideoxy-4,5-*O*-isopropylidene-7-*O*-trityl-D-*ribo*-sept-2-enonates (2) and Their Conversion into 3. Occasionally in the reaction of 1 with the Wittig reagent, two product spots were observed by TLC. In one experiment a crude syrup (10 g), after removal of triphenylphosphine oxide, was chromatographed on a column of 250 g of silica gel G60 in nylon tube (2.25-cm diameter) using petroleum ether–ethyl acetate (5:1) as the eluent and *p*-dimethylaminoazobenzene (butter yellow) as the marker. After the yellow band of the marker came off, the column was cut up into 14 fractions. Each fraction was extracted with ethyl acetate and checked by TLC. From the 6th fraction from bottom, 490 mg of a colorless syrup was obtained after evaporation of the ethyl acetate extracts. ^{1}H NMR spectrum of the syrup showed complex olefinic signals that integrated for two protons at δ 5.51–6.18 in $CDCl_3$ (indicating a mixture of olefins 2).

To a solution of 2 (~100 mg) in acetonitrile (10 ml) was added potassium *tert*-butoxide (~5 mg) and the mixture was stirred for 1 h at room temperature. TLC showed a single spot corresponding to 3 in the mixture and complete disappearance of the spot for 2. After evaporation of the solvent, the residual syrup showed no olefinic signals at δ 5.51–6.18 in NMR spectrum which, in turn, showed the syrup to be an isomeric mixture of the acetates 3.

Ethyl 2-Formyl-2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)acetate Sodium Enolate (4). To a suspension of sodium hydride (18 g, 50% in mineral oil) in absolute ether (300 ml, distilled over $LiAlH_4$) was added 2 ml of absolute ethanol followed immediately by dropwise addition of a mixture of compound 3 (155 g, 0.31 mol), ethyl formate (100 ml, distilled over K_2CO_3) and anhydrous ether (200 ml). The mixture was stirred overnight at room temperature, and then the solvent was removed by evaporation in vacuo at room temperature. The residue, 155 g of crude 4, was not purified but directly used in the next step.

Ethyl 3-Methoxy-2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)acrylate (5). The crude sodium enolate 4 (155 g) was dissolved in DMF (745 ml, dried over 4-Å molecular sieves). Methyl iodide (75 ml) was added dropwise to the solution over a period of 1 h. The mixture was stirred for 4 h at room temperature and then poured into a mixture of ice and water (3 l.). The supernatant was removed by decantation and the residual syrup was dissolved in CH_2Cl_2 (1 l.), washed with water, dried over sodium sulfate, and evaporated to a brown syrup comprising crude 5 (149 g) which was

used directly in the cyclization reaction with guanidine to **6**.

TLC (benzene–ethyl acetate, 9:1) of crude product showed that it contained at least four components [R_f 0.3, 0.35, 0.4 (major), 0.5]. About 1.2 g of the crude material was chromatographed over a silica gel G column (20 g) using benzene as the eluent. The first compound eluted (203 mg, corresponding to R_f 0.5 on TLC) was compound **3**. The second fraction (402 mg, corresponding to R_f 0.4) was obtained after evaporation of the solvent. The residue, which solidified on standing, was recrystallized twice from ethanol; 135 mg of **5** (mp 161.5–162 °C) was obtained. ^{1}H NMR ($CDCl_3$) δ 1.17 (t, 3 H, CH_3CH_2), 1.32 (s, 3 H, isopropylidene CH_3), 1.56 (s, 3 H, isopropylidene CH_3), 3.23 (m, 2 H, H-5′), 3.81 (s, 3 H, OCH_3), 3.96–4.20 (m, 3 H, CH_3CH_2 and H-4′), 4.90 (t, 1 H, H-3′, $J_{2',3'} \simeq J_{3',4'}$ 6.0 Hz), 4.92 (q, 1 H, H-2′, $J_{1',2'} \simeq 3.6$, $J_{2',3'} \simeq 6.0$ Hz), 5.10 (d, 1 H, H-1′, $J_{1',2'} \simeq 3.6$ Hz).

Anal. Calcd for $C_{33}H_{36}O_7$: C, 72.79; H, 6.62. Found: C, 72.92; H, 6.68.

5-(2,3-*O*-Isopropylidene-5-*O*-trityl-D-ribofuranosyl)isocytosine (6). Method A. From the Sodium Enolate 4. Guanidine hydrochloride (15.0 g) was added to ethanolic sodium ethoxide (prepared by dissolving 4.6 g of sodium in 200 ml of absolute ethanol) and the mixture was stirred for 10 min at room temperature, then the mixture was filtered through Celite and the filtrate was added to a solution of 55.2 g of the crude enolate **4** in absolute ethanol (100 ml). The reaction mixture was heated under reflux for 24 h and cooled in an ice bath, the insolubles were removed by filtration, and the filtrate was carefully neutralized with 1 N hydrochloric acid (to pH 6.8–7). During the addition of 1 N hydrochloric acid, a small amount of product precipitated. Water was added to complete precipitation. The supernatant was decanted and the residual brown syrup was dissolved in benzene (100 ml), dried over sodium sulfate, and chromatographed on a column of silica gel G (700 g) using benzene–ether (30:1) as the eluent to remove all the unknown by-products. One-liter fractions were collected and each fraction was checked by TLC. Finally, the column was washed with benzene–methanol (10:1). The crude syrup (~7 g), which was obtained after evaporation of the solvent, solidified when refluxed in methanol for ~10 min. Colorless crystals of **6** after cooling, were collected by filtration, 2.7 g, mp 251–253 °C.

Anal. Calcd for $C_{31}H_{31}N_3O_5$: C, 70.86; H, 5.90; N, 8.00. Found: C, 70.85; H, 5.81; N, 7.78.

Method B. From Crude 5. Cyclization of crude **5** (55 g) instead of **4** with guanidine under the same condition and procedure as method A afforded 6.6 g (13%) of **6**, mp 251–253 °C.

Method C. From Pure 5. Guanidine hydrochloride (950 mg, 0.01 mol) was added to ethanolic sodium ethoxide solution (prepared by dissolving 230 mg of metallic sodium in 30 ml of ethanol) and the mixture was stirred for 10 min at room temperature. Crystalline **5** (2.72 g, 0.005 mol) was added and the mixture was refluxed for 24 h, concentrated to ~15 ml, then neutralized with 1 N HCl. Compound **6** precipitated as colorless crystals which were collected by decantation of the supernatant and washed with methanol, mp 251.5–253 °C (2.32 g, 88%).

5-(β-D-Ribofuranosyl)isocytosine (7, Pseudoisocytidine). Method A. Compound **6** (525 mg) was dissolved in 10% methanolic hydrogen chloride (20 ml) and the solution was stirred for 6 h at room temperature. Compound **7** precipitated as colorless crystals which were filtered and washed with ethanol: 53 mg, mp 215–216 °C dec; $[\alpha]^{25}D$ +120° (*c* 0.1, water); uv[17] λ_{max} (pH 13) 277 nm (ϵ 7340), 232 (9700), λ_{min} (pH 13) 249 (2710); λ_{max} (pH 7.2) (5700), λ_{min} (pH 7.2) 248 (2750); λ_{max} (pH 1) 262 (7820), 221 (10 520), λ_{min} (pH 1) 241 (4920); pK_a 3.72 ± 0.05 and 8.97 ± 0.05.

Anal. Calcd for $C_9H_{13}N_3O_5$·HCl: C, 38.64; H, 5.02; N, 15.02; Cl, 12.70. Found: C, 38.74; H, 5.10; N, 14.91; Cl, 12.82.

The filtrate was evaporated in vacuo at room temperature and the residue was triturated with 5 ml of a mixture of ether and ethanol (1:1). Compound **8** precipitated as colorless crystals: 182 mg, mp 182–183°C dec; $[\alpha]^{25}D$ −164 (*c* 0.1, water); uv[17] λ_{max} (pH 13) 277 nm (ϵ 7170), 232 (9300), λ_{min} (pH 13) 253 (2640); λ_{max} (pH 7.2) 290 (3980), 270 (3890), λ_{min} (pH 7.2) 288 (3810), 249 (2900); λ_{max} (pH 1) 262 (7470), 222 (10 106), λ_{min} (pH 1) 242 (4800); pK_a 3.92 ± 0.05 and 9.12 ± 0.05.

Anal. Calcd for $C_9N_{13}N_3O_5$·HCl: C, 38.64; H, 5.02; N, 15.02; Cl, 12.70. Found: C, 38.81; H, 5.12; N, 14.88; Cl, 12.91.

Method B. A mixture of **6** (1.5 g) and 10% methanolic hydrogen chloride (50 ml) was stirred for 2 weeks at room temperature. Compound **7** (651 mg, 81%) precipitated and was collected by filtration, mp 215–216 °C dec.

Method C. For a large-scale preparation this method was found to be convenient. Crude syrup of **5** (149 g, 0.27 mol) was dissolved in 300 ml of absolute ethanol and the solution was added to ethanolic guanidine [prepared from guanidine hydrochloride (49 g, 0.47 mol), ethanol (300 ml), and metallic sodium (12 g, 0.52 mol)]; the mixture was heated under reflux for 24 h and cooled in an ice bath, and the insoluble materials were removed by filtration. The filtrate was carefully neutralized with 1 N hydrochloric acid (to pH 6.8–7), then the mixture was diluted with water (600 ml) and extracted with methylene chloride (500 ml × 3). The combined extracts were dried over sodium sulfate and evaporated to dryness to a dark syrup which was dissolved in 10% methanolic hydrogen chloride (300 ml) and the solution was stirred for 1 week at room temperature. Crude **7** (14.0 g) precipitated, and was collected by filtration and recrystallized from water–ethanol to give pale yellow microcrystals, 12.6 g, mp 215–216 °C dec.

5-(2,3-*O*-Isopropylidene-5-*O*-trityl-D-ribofuranosyl)-2-thiouracil (9). Method A. From the Sodium Enolate 4. A mixture of **4** (55.2 g of crude syrup, 0.1 mol) and thiourea (11.4 g, 0.15 mol) in ethanolic sodium ethoxide (prepared from 4.6 g of metallic sodium and 200 ml of absolute ethanol) was refluxed for 15 h. The reaction mixture was allowed to cool to room temperature and neutralized with 1 N hydrochloric acid, and the brown syrup which precipitated was collected by decantation of the supernatant. The syrup was dissolved in ether (~200 ml), washed with water, and dried over sodium sulfate. The ether was evaporated to a syrup which was purified by alumina column chromatography (400 g, Bio-Rad neutral alumina AG-7 100–200 mesh) using chloroform–methanol (4:1) as the eluent. Fractions with a uv absorbing spot and a positive sulfuric acid spray test were collected and evaporated to dryness to give a foam (2.5 g) which was crystallized from ethanol. Compound **9** was obtained as colorless needles: mp 82–83 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 3 H, isopropylidene CH_3), 1.55 (s, 3 H, isopropylidene CH_3), 3.24 (q, 1 H, H-5′, $J_{5',5''} \simeq 10.3$, $J_{4',5'} \simeq 10.3$, $J_{4',5'} \simeq 6.6$ Hz), 3.41 (q, 1 H, H-5″, $J_{5',5''} \simeq 3.1$), 4.24 (m, 1 H, H-4′), 4.65 (m, 2 H, H-2′, 3′), 4.89 (q, 1 H, H-1′, $J_{1',2'} \simeq 2.8$, $J_{1',6} \simeq 0.9$), 7.24–7.48 (m, 16 H, trityl H and H-6), 9.79 (d, 1 H, N1-H, exchangeable), 9.94 (s, 1 H, N3-H, exchangeable).

Anal. Calcd for $C_{31}H_{30}N_2O_5S$: C, 68.63; H, 5.54; N, 5.17; S, 5.90. Found: C, 68.69; H, 5.65; N, 5.05; S, 5.77.

Method B. From Compound 5. A mixture of crude **5** (54.4 g, 0.1 mol) and thiourea (11.4 g, 0.15 mol) was treated with methanolic sodium ethoxide as described above. Compound **9** (3.5 g) was obtained as colorless needles, mp 83–84 °C.

5-(β-D-Ribofuranosyl)-2-thiouracil (10, 2-Thiopseudouridine). A mixture of **9** (542 mg, 0.001 mol) and 10% methanolic hydrogen chloride (25 ml) was stirred for 10 min at room temperature. The solvent was evaporated in vacuo (below 25 °C) to give a syrup which was triturated with 10 ml of water and filtered. The filtrate was decolorized with charcoal and evaporated to dryness in vacuo. The residue was triturated with cold ethanol and the white precipitate was collected by filtration. The ^{1}H NMR spectrum (D_2O) of this sample was very similar to that of pseudouridine.[12] The signals for H-6 and H-1′ occurred at δ 7.65 and 4.69 with $J_{1',6} \simeq 0.91$ Hz. Uv λ_{max} (pH 1–7) 276 nm, 290 (shoulder), λ_{min} (pH 1–7) 242, λ_{max} (pH 12) 313, 263, 235 (shoulder), λ_{min} (pH 12) 298. This compound was so hygroscopic that optical extinction (ϵ value) could not be obtained, and it was best analyzed as a partially hydrated foam.

Anal. Calcd for $C_9H_{12}N_2O_5S$·½ H_2O: C, 39.70; H, 4.94; N, 10.29; S, 11.78. Found: C, 39.55; H, 4.99; N, 8.90; S, 11.44.

Epimerization of **10** in ~1 N DCl was observed by ^{1}H NMR. Two new signals for H-6 and H-1′ appeared at δ 7.55 and 4.98 ($J_{1',6} \simeq 1.21$ Hz) with concomitant decrease of the corresponding signals of **10**. The epimerization occurred faster than that of pseudoisocytidine, and the α/β ratio reached almost 1. In addition, further DCl treatment of **10** gave a more complicated mixture as observed by NMR probably owing to the formation of the pyranosyl isomers.

5-(2,3-*O*-Isopropylidene-5-*O*-trityl-β-D-ribofuranosyl)uracil (11). A mixture of pure **5** (544 mg, 0.001 mol) and urea (120 mg, 0.002 mol) in ethanolic sodium ethoxide (prepared by dissolving 46 mg of sodium in 15 ml of ethanol) was refluxed for 3 days. The mixture was concentrated to ~8 ml in vacuo and, after cooling, the concentrated solution was neutralized with 1 N HCl to give a white precipitate which was chromatographed on a silica gel G 60 column (50 g, 40 × 2.5 cm diameter) using benzene–methanol (19:1) as the eluent. Each fraction was checked by TLC. Appropriate fractions were combined and evaporated to dryness to give 300 mg (57%) of a mixture of the α and β isomers (**11** and **12**). This mixture showed only a single spot on TLC in various solvent systems: ^{1}H NMR ($CDCl_3$) δ 1.27 and 1.39 (2 s, isopropylidene CH_3 for **12**, $\Delta\delta$ 12 Hz), 1.32 and 1.56 (2 s, isopropylidene CH_3 for **11**, $\Delta\delta$ 24 Hz). The relative intensity of the isopropylidene methyl signals of **11** to those of **12** was approximately 3 to 1.

Thick layer chromatography (2 mm, 20 × 20 cm) on silica gel GF_{254} by multidevelopment technique (five developments) using ben-

zene–methanol (19:1) gave a partial separation in the form of an elongated band. Removal of the lower portion of this band followed by extraction with methanol–chloroform (1:1) and evaporation of the solvent gave pure β isomer **11** as colorless microcrystals: 95 mg, mp 125–140 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.31 and 1.55 (2 s, 6 H, isopropylidene CH_3), 3.32 (m, 2 H, H-5′,5″), 4.22 (m, 1 H, H-4′), 4.67–4.93 (m, 2 H, H-2′, H-3′), 4.99 (d, 1 H, H-1′).

Anal. Calcd for $C_{31}H_{30}N_2O_6$: C, 70.71; H, 5.74; N, 5.32. Found: C, 70.58; H, 5.80; N, 5.15.

From the upper layer portion of the elongated band in the thick layer chromatogram, only a mixture of the α and β isomers (**12** and **11**) was obtained.

5-(β-D-Ribofuranosyl)uracil (13, Pseudouridine). A mixture of **11** (105 mg, 0.2 mmol) and 10% methanolic HCl (2 ml) was stirred at room temperature for 15 min. During this time, a clear solution was obtained and then crystalline product **13** (20 mg) precipitated. The crystals were collected by filtration and washed with ether, mp 221–222 °C (lit.[15] mp for pseudouridine 220–221 °C). ^{1}H NMR spectrum (D_2O) of this product was identical with that of pseudouridine.[12]

From the filtrate a further quantity of **13** (26 mg) having the same melting point and ^{1}H NMR spectral characteristics was obtained upon dilution with 20 ml of ether. The combined yield was 92%.

Registry No.—**1**, 55726-19-7; **3**, 57100-24-0; **4**, 59464-13-0; **5**, 59464-14-1; **6**, 57100-19-3; **7**, 57100-18-2; **7** HCl, 59464-15-2; **8** HCl, 59464-16-3; **9**, 59464-17-4; **10**, 59464-18-5; **11**, 59464-19-6; **12**, 59464-20-9; **13**, 1445-07-4; (ethoxycarbonylmethylene)triphenylphosphorane, 1099-45-2; guanidine HCl, 15827-40-4; thiourea, 62-56-6.

References and Notes

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Synthesis and Absolute Configuration of Multistriatin

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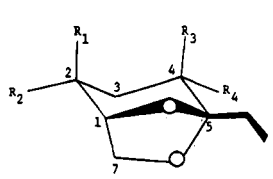
Multistriatin, 2,4-dimethyl-5-ethyl-6,8-dioxabicyclo[3.2.1]octane (**1**), was synthesized as a mixture of the four diastereomers **1**α–δ. The key step was the formation of 4,6-dimethyl-7-octen-3-one (**10**) by the alkylation of 3-pentanone with the tosylate (**7**) of 2-methyl-3-buten-1-ol via the metalloenamine synthesis. Epoxidation of **10** with *m*-chloroperoxybenzoic acid and intramolecular ketalization of the 4,6-dimethyl-7,8-epoxyocten-3-one (**11**) with $SnCl_4$ gave **1**, whose **1**α content was maximized by equilibration of **1**γ to **1**α with $SnCl_4$. Acid equilibration of **1** in the presence of excess peroxide leads to the formation of side products at the expense of the multistriatin isomers. Synthesis of **1** from (*S*)-(+)-2-methyl-3-butenoic acid gave (2*R*)-(−)-**1**α, which established the absolute configuration of natural (−)-**1**α as 1*S*:2*R*:4*S*:5*R*. The enantiomeric composition of synthetic (−)- and (+)-**1**α was determined by ^{13}C NMR with the chiral shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato]europium(III). Natural (−)-**1**α consisted of a single enantiomer.

α-Multistriatin, 2-*endo*,4-*endo*-dimethyl-5-ethyl-6,8-dioxabicyclo[3.2.1]octane (**1**α), is a novel component of the aggregation pheromone of the European elm bark beetle, *Scolytus multistriatus*.[1] In a previous publication,[2] we have determined the relative stereochemistry for each of the four possible pairs of multistriatin stereoisomers. We report here a synthesis of racemic **1**α–δ designed to confirm the gross structure of multistriatin and provide quantities of **1** sufficient

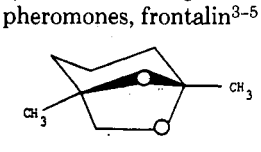


1

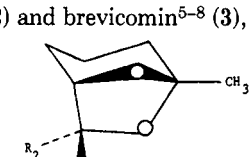
	R_1	R_2	R_3	R_4
1α :	H	CH_3	H	CH_3
1β :	CH_3	H	CH_3	H
1γ :	H	CH_3	CH_3	H
1δ :	CH_3	H	H	CH_3

for field tests. In addition, optically active (+)- and (−)-multistriatin stereoisomers (**1**α–δ) of known absolute configurations were synthesized by inclusion of a chiral intermediate (**5**) of known configuration into the synthetic scheme.

Previous syntheses of the 6,8-dioxabicyclo[3.2.1]octane ring system, including the synthesis of two other bark beetle pheromones, frontalin[3–5] (**2**) and brevicomin[5–8] (**3**), have been



(1S) **2**



(1S) **3**

	R_1	R_2
exo :	CH_2CH_3	H
endo :	H	CH_2CH_3

accomplished by two main routes, Schemes I and II. Scheme I involves the Diels–Alder addition of an α,β-unsaturated carbonyl either to an α,β-unsaturated alcohol,[3,4,9] or to another α,β-unsaturated carbonyl that acts as the dienophile.[5,10,11] The first variation of this route (Scheme Ia), is thought to occur via a hydroxy dihydropyran intermediate[9] that cyclizes to the desired product under the conditions of the initial addition.