Nitration of Pyrimidine Bases and Nucleotides by Nitronium Tetrafluoroborate. Synthesis of 5-Nitro-2′-deoxyuridine

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The reagent nitronium tetrafluoroborate in sulfolane was investigated as a nitrating agent for pyrimidine bases, nucleosides, and nucleotides. Reaction of uracil (**1**), 1-methyluracil (**3**), or 1,3-dimethyluracil (**5**) with NO_2BF_4 in sulfolane led to 80–90% yields of the corresponding 5-nitro derivatives, 5-nitrouracil (**2**), 1-methyl-5-nitrouracil (**4**), and 1,3-dimethyl-5-nitrouracil (**6**). Uridine (**7**) and 2′-deoxyuridine (**8**) failed to give significant yields of 5-nitro nucleosides, but rather suffered extensive glycoside bond rupture under the conditions employed. However, 2′-deoxyuridine 5′-monophosphate (**12**) was readily nitrated to give two products that accounted for 70% of the starting material, namely, 5-nitro-2′-deoxyuridine 5′-monophosphate (**13**) and its 3′-*O*-nitrate (**14**). These nucleotides **13** and **14** were dephosphorylated by *Escherichia coli* alkaline phosphatase to the corresponding nucleosides, 5-nitro-2′-deoxyuridine (**15**) and 3′-*O*,5-dinitro-2′-deoxyuridine (**16**). In addition, uridine 5′-monophosphate (**10**) was converted by nitronium tetrafluoroborate/sulfolane to 5-nitrouridine 5′-monophosphate (**11**), which was also prepared by the action of $POCl_3$/triethyl phosphate on 5-nitrouridine (**9**).

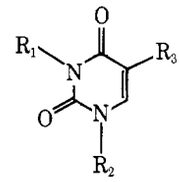
Pyrimidine nucleosides and nucleotides, substituted at the 5 position by various functional groups, are of interest for at least two reasons: first, C-5 substituted nucleosides may possess antiviral and/or antitumor properties;[1-4] second, when incorporated into nucleic acids, the structural modification may provide valuable information for the study of, for instance, the physicochemical properties[5] and interferon-inducing ability[6] of polynucleotides. The nitro group, because of its potent electron-withdrawing properties, would seem a modification of special interest in view of the well-documented antiviral and/or antitumor activities of two deoxyribonucleosides, namely, 5-fluoro- and 5-trifluoromethyl-2′-deoxyuridine.[5]

5-Nitrouridine was first synthesized by Wempen et al.[7] who used nitric acid to nitrate 2′,3′,5′-tri-*O*-(3,5-dinitrobenzoyl)-uridine; later, the nucleoside was prepared by the $Hg(CN)_2$ nitromethane condensation procedure.[8] Prystas and Sorm[9] found that an analogous nitration approach to a similarly protected 2′-deoxyuridine afforded only glycoside bond cleavage. They did, however, obtain a 1% yield of an anomeric mixture of the di-*O*-toluyl derivative of 5-nitro-2′-deoxyuridine when 5-nitrouracilylmercury was reacted with the protected 2′-deoxy-D-ribofuranosyl chloride. An enzymatic synthesis of 5-nitro-2′-deoxyuridine was reported by Kluepfel et al.,[10] but this method cannot be regarded as unambiguous since only UV spectra and elemental analysis were provided to support the structure of the product. Additionally, this approach is inconvenient, since large amounts of crude *trans*-*N*-deoxyribosylase from *Lactobacillus* cultures must be employed.

In view of the report by Kluepfel et al.[10] that this enzymatically prepared 5-nitro-2′-deoxyuridine possessed potent antiviral properties, we considered it imperative to provide a less equivocal synthesis of this nucleoside in order to corroborate their finding. To this end, and to develop a more unified access to nitropyrimidine nucloesides and nucleotides, we investigated the relatively mild nitrating agent, nitronium tetrafluoroborate (NO_2BF_4), introduced by Olah.[11] We report here that excellent yields of nitrated uracil derivatives can be obtained with this reagent, and, furthermore, that nitronium tetrafluoroborate provides a useful access to the 5′-monophosphates of 5-nitrouridine, 5-nitro-2′-deoxyuridine and its 3′-nitrate, as well as the corresponding free nucleosides themselves.[12]

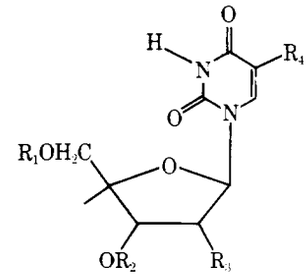
Results

When either uracil (**1**), 1-methyluracil (**3**), or 1,3-dimethyluracil (**5**) was reacted with 2 equiv of NO_2BF_4 in sulfolane for periods up to 24 h at ambient temperature, the corresponding 5-nitro derivative (**2**, **4**, or **6**) was obtained in 80–90% yield.



1, $R_1 = R_2 = R_3 = H$
2, $R_1 = R_2 = H$; $R_3 = NO_2$
3, $R_1 = R_3 = H$; $R_2 = CH_3$
4, $R_1 = H$; $R_2 = CH_3$; $R_3 = NO_2$
5, $R_1 = R_2 = CH_3$; $R_3 = H$
6, $R_1 = R_2 = CH_3$; $R_3 = NO_2$

Under conditions where uracil and its methylated derivatives or nucleotides (vide infra) gave good to excellent yields of nitrated products, both uridine (**7**) and 2′-deoxyuridine (**8**) failed to give the corresponding 5-nitro derivatives. Instead, the major and only identifiable product of the reaction of **7** or **8** with NO_2BF_4 was 5-nitrouracil. The use of a variety of protecting groups (5′-*O*-nitrate, triacetate, isopropylidene and tris(3,5-dinitrobenzoyl)uridines and the diacetate of 2′-



7, $R_1 = R_2 = R_4 = H$; $R_3 = OH$
8, $R_1 = R_2 = R_3 = R_4 = H$
9, $R_1 = R_2 = H$; $R_4 = NO_2$; $R_3 = OH$
10, $R_1 = PO_3H_2$; $R_2 = R_4 = H$; $R_3 = OH$
11, $R_1 = PO_3H_2$; $R_2 = H$; $R_3 = OH$; $R_4 = NO_2$
12, $R_1 = PO_3H_2$; $R_2 = R_3 = R_4 = H$
13, $R_1 = PO_3H_2$; $R_2 = R_3 = H$; $R_4 = NO_2$
14, $R_1 = PO_3H_2$; $R_2 = R_4 = NO_2$; $R_3 = H$
15, $R_1 = R_2 = R_3 = H$; $R_4 = NO_2$
16, $R_1 = R_3 = H$; $R_2 = R_4 = NO_2$

deoxyuridine) did not alter the course of the reaction, yielding either a variety of unidentified products or extensive glycoside bond cleavage. For the reactions with 2′-deoxyuridine and its diacetate, quantitative recoveries of 5-nitrouracil were typical. Alteration of reaction conditions including temperature, the presence or absence of water, and the presence or absence of bases (Et_3N or K_2CO_3) also gave 5-nitrouracil as the major product.

The action of NO_2BF_4 in sulfolane on 2′-deoxyuridine 5′-monophosphate (**12**) gave three products that were separated and purified by preparative paper chromatography, DEAE-Sephadex column chromatography, and crystallization. One of these products, formed in less than 10% yield, was 5-nitrouracil (**2**). The other two products both contained deoxyribose moieties as ascertained by the Dische color reaction[13] (pink), and both were nucleotides, as judged from their chromatographic behavior and elemental analysis. Elemental analysis also suggested that the substance with the greater mobility (**14**) (in isobutyric acid–1 M NH_4OH) contained one additional nitrogen atom compared to the other compound of lower mobility (**13**). Both materials had virtually identical UV spectra, which were characteristic of 1-substituted nitrouracils, in acidic or basic conditions. They both had similar ^1H NMR spectra with one dramatic exception: the product **14** with higher R_f and additional nitrogen atom gave rise to a chemical shift of 5.79 ppm for the C-3′ proton (assigned by decoupling experiments). This was ~1 ppm lower than the chemical shift of the C-3′ proton in the other product (**13**) and is also ~1 ppm lower than the chemical shift of the C-3′ proton in other nucleosides and nucleotides. This observation suggested that an electron-withdrawing substituent may be present on the C-3′ oxygen of **14**. In fact, studies on the nitric acid O-nitration[14] of nucleosides have shown that this downfield shift is a characteristic of a proton proximal to the *O*-nitro function. Consideration of all these data suggested that the two products were 5-nitro-2′-deoxyuridine 5′-monophosphate (R_f 0.36, system A) (**13**) and 3′-*O*-nitro-5-nitro-2′-deoxyuridine 5′-monophosphate (R_f 0.63, system A) (**14**).

A final proof of this contention was obtained when the two nucleotides were dephosphorylated with bacterial alkaline phosphatase. The fact that dephosphorylation did occur is further evidence against the (unlikely) possibility that the additional nitro group resided on the phosphate moiety, since bacterial alkaline phosphatase does not attack phosphodiesters. As with the nucleotides, both dephosphorylated products gave positive Dische tests and had virtually identical UV spectra. The ^1H NMR spectrum of the nucleoside **16** obtained from the purported *O*-nitro nucleotide **14** revealed the marked downfield shift of the C-3′ proton and (in Me_2SO-d_6) the absence of 3′-OH proton. Finally, compound **15** (from the nucleotide of lower R_f) had a UV spectrum and melting point identical with the nucleoside prepared enzymatically by Kluepfel et al.[10] The products from the dephosphorylation of the nucleotides **13** and **14** are, therefore, 5-nitro-2′-deoxyuridine (**15**) and 3′-*O*-nitro-5-nitro-2′-deoxyuridine (**16**), respectively.

The reaction of 5′-UMP (**10**) with NO_2BF_4–sulfolane gave but two major products which could be separated and purified by preparative paper chromatography and ion-exchange chromatography. Negligible quantities of 5-nitrouracil were formed in this reaction. One of the major products (25%) was identified as 5-nitrouridine 5′-monophosphate (**11**) by the following criteria: (1) Its mobility toward the anode on paper electrophoresis at pH 3.5 was the same as UMP and half that of UDP, consistent with the behavior of a monophosphate. (2) The UV λ_{max} values at pH 1 and 12 were those typical of the 1-substituted 5-nitrouracil chromophore. (3) The product gave a positive periodate–benzidine color test, demonstrating the presence of the *cis*-2′,3′-hydroxyl groups. (4) Treatment of this product with alkaline phosphatase gave only 5-nitrouridine (**9**). (5) Phosphorylation of 5-nitrouridine (**9**) by the $POCl_3$–triethyl phosphate method of Yoskikawa et al.[15] gave only one product which was identical with the product (**11**) of the NO_2BF_4 nitration.

Discussion

We cannot completely rationalize the failure of the nucleosides uridine and 2′-deoxyuridine to give even minor quantities of 5-nitro nucleosides under conditions where other uracil derivatives give the desired products in moderate to excellent yield. It is clear, however, that nitronium tetrafluoroborate in sulfolane provides a useful approach to nitrouracils, nitrouracil nucleotides and, from them, nitrouracil nucleosides. It also results in the only successful chemical approach to 5-nitro-2′-deoxyuridine reported to date.

Experimental Section

General Procedures. Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Ultraviolet spectra were obtained on a Cary 15 spectrometer, and ^1H NMR spectra on a Varian A60 or HA 100 spectrometer with solvents as indicated. Standards used were Me_4Si (Me_2SO-d_6) and DDS (D_2O). Chemical shifts are reported in parts per million (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Chemical ionization mass spectra were determined on a Finnigan 1015D gas chromatograph/mass spectrometer using NH_3 or isobutylene as the ionizing gas. Microanalyses were performed by the staff of the microanalytical section of this laboratory. Reagents were obtained as follows: 2′-deoxyuridine 5′-monophosphate, uridine 5′-monophosphate, and bacterial alkaline phosphatase (*E. coli* 3.1.3.1) were from P-L Biochemicals (Milwaukee, Wis.); uridine, uracil, and nitronium tetrafluoroborate in sulfolane were from Aldrich Chemicals (Milwaukee, Wis.); 1-methyluracil[16] and 1,3-dimethyluracil[17] were prepared according to the literature; an authentic sample of 5-nitrouridine was kindly provided by Dr. Harry Wood of the National Cancer Institute and was also synthesized according to the procedure of Wempen et al.[7] For paper chromatography, both Whatman no. 1 and 3MM paper were used with the following solvent systems: system A, isobutyric acid–1 M NH_4OH (5:3, v/v); system B, 95% EtOH–1 M NH_4OAc (adjusted to pH 5 with HOAc; 7:3, v/v); system C, *t*-BuOH (70) and 6 N HCl (13.2) made up to 100 mL with H_2O. Paper electrophoresis was with Whatman no. 1 paper in 0.05 M ammonium formate (pH 3.5) at 30 V/cm. Thin-layer chromatography was performed using silica gel GF plates (Analtech). Silica gel powder (70–230 mesh, ICN, Irvine, Calif.) and DEAE-Sephadex (Pharmacia, Upsalla, Sweden) were used in column chromatography. For DEAE-Sephadex chromatography, fractions were monitored at 260 nm with an LKB Uvicord detector and recorder.

5-Nitrouracil (2). Uracil (1, 224 mg, 2 mmol) was dissolved in a 0.5 M solution of nitronium tetrafluoroborate (NO_2BF_4) in sulfolane (total of 8 mL or 4 mmol of NO_2BF_4), and this solution was left at room temperature for 21 h. Chloroform (8 mL) was added to the reaction mixture, and the resulting precipitate was filtered, washed with ether, and then recrystallized from methanol. This procedure gave 260 mg (83%) of **2** (mp >300 °C) that was identical in all respects (UV, ^1H NMR, chemical ionization mass spectrometry, and TLC) to authentic material. The elemental analysis was also satisfactory (±0.3% of theory).

1-Methyl-5-nitrouracil (4). 1-Methyluracil (**3**, 252 mg or 2 mmol) and 0.5 M NO_2BF_4 in sulfolane (8 mL or 4 mmol) were reacted for 24 h at room temperature. Chloroform (16 mL) and water (16 mL) were added to the above solution, and, after shaking, the water phase was collected and evaporated to ~2 mL. A precipitate formed and was removed by filtration and washed with minimal quantities of absolute ethanol and ether and recrystallized from water. Compound **4** was isolated in 82% yield (280 mg). The melting point (264–265 °C) was in agreement with that reported earlier:[18] ^1H NMR (CF_3COOH) δ 9.2 (s, 1, H-6), 3.78 (s, 3, 1-CH_3); UV λ_{max} pH 1 250 and 300 nm, pH 12 320 nm. Anal. Calcd for $C_5H_5N_3O_4$: C, 35.19; H, 3.03; N, 24.30, Found C, 35.08; H, 2.92; N, 24.56.

1,3-Dimethyl-5-nitrouracil (6). In the same manner as described for **2** and **4**, 1,3-dimethyluracil (**5**, 280 mg, 2 mmol) and 0.5 M NO_2BF_4 in sulfolane (8 mL, 4 mmol) were reacted at ambient temperature for 20 h during which time a deep red color developed. Chloroform (1 mL)

was added, and the mixture was kept at room temperature until no further bubbling occurred and the color became yellow. This mixture was applied directly to a silica gel column (2 × 30 cm) which was washed with chloroform (200 mL) and then eluted with chloroform–methanol (4:1, v/v). The fractions containing UV-absorbing product were pooled and evaporated to give a red-brown syrup which crystallized after several hours at room temperature. The crystals were filtered off with the aid of a small amount of cold methanol and chloroform and were recrystallized from chloroform to yield 352 mg (95%) of **6**. Material prepared in this manner has a melting point of 158–159 °C, the same as the literature value.[19] Spectral data (UV, ^{1}H NMR, chemical ionization mass), elemental analysis (±0.3%), and TLC were consistent with the structure **6**.

Attempted Nitration of Uridine by Nitronium Tetrafluoroborate. Uridine (**7**, 291 mg, 1.19 mmol) and NO_2BF_4 in sulfolane (5.96 mL of 0.5 M solution, 2.98 mmol) were allowed to react at ambient temperature for 16 h. The UV of an aliquot of this mixture showed the disappearance of the λ_{max} at 260 nm but failed to show the typical 321 nm λ_{max} (pH 12) of the nitrouracil chromophore. In addition, silica gel TLC in $CHCl_3$–MeOH revealed a large number of products. This reaction mixture was diluted with 3 volumes of $CHCl_3$ and applied to a silica gel column (1.5 × 18 cm) and washed with $CHCl_3$ to remove the sulfolane. The column was then eluted with $CHCl_3$–MeOH (4:1). The UV-absorbing fractions were combined and evaporated, and the resulting residue was rechromatographed on silica gel with $CHCl_3$–MeOH (4:1) as eluant. The major product of these procedures was 5-nitrouracil (30 mg, 0.19 mmol, 16%) (identified by TLC comparison with authentic material). There was no evidence for any 5-nitrouridine.

1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-nitrouracil 5′-monophosphate (13, 5-Nitro-2′-deoxyuridine 5′-monophosphate) and 1-(2-Deoxy-3-*O*-nitro-β-D-*erythro*-pentofuranosyl)-5-nitrouracil 5′-monophosphate (14, 3′-*O*-, 5-dinitro-2′-deoxyuridine 5′-monophosphate). 2′-Deoxyuridine 5′-monophosphate (**12**, disodium salt, dihydrate, 4 g, 10.3 mmol) was dried at 110 °C in vacuo for 6 h before use. This procedure removed ~92% of the water of hydration (w/w). Depending on the major product desired (**13**, or **14**), an appropriate quantity[20] of water was then added to **12** followed by the addition of NO_2BF_4 in sulfolane (0.5 M, 52 mL, 25.8 mmol). The reaction mixture was warmed at 37 °C for 16 h. After this time, the UV of an aliquot of the reaction mixture showed the disappearance of the 260-nm λ_{max}. Chloroform (300 mL) was added and the resulting precipitate was collected by centrifugation and washed with chloroform (150 mL). The precipitate was dissolved in absolute EtOH (250 mL), and to this solution concentrated NH_4OH was added dropwise until the pH was 7.5. During this procedure a fluffy white precipitate appeared, and this was collected by centrifugation, washed with absolute EtOH (150 mL), and dried.

This product was dissolved in a minimum amount of H_2O and applied to Whatman 3MM paper which was then developed in solvent system A (descending). Ten sheets of 18 × 57 cm paper were used in the separation. The developed chromatogram showed three UV-absorbing bands with R_f values of 0.54, 0.39, and 0.29.[20] Each band was extracted with dilute NH_4OH (pH 8). After reduction (in vacuo) of the volume of each eluant, each band was applied to a column of DEAE-Sephadex (A-25, 1.6 × 24 cm), and the column was eluted with a linear gradient (0.01–0.2 M) of ammonium bicarbonate (pH 7.8) (total volume 2 L). Fractions of 15 mL were collected. UV-absorbing fractions were checked for homogeneity by paper chromatography (system A), appropriate fractions were pooled, and solvent was removed (in vacuo, <40 °C). Ammonium bicarbonate was removed by repeated addition and evaporation of H_2O.

The product of R_f 0.39 (system A) was determined to be 5-nitrouracil by comparison with authentic material (R_f, UV, ^{1}H NMR) and elemental analysis. Consistently, the yield of 5-nitrouracil was less than 10% even when 2 molar equiv of H_2O was present in the initial reaction mixture.

The product **13** of R_f 0.29 (system A) was deionized by a Dowex 50 (H^+) column, and the eluted solution was concentrated in vacuo to a small volume. Refrigeration at 4 °C for 1 day gave white needles (~1.1–1.6 g, ~28–42%) of **13**, mp 129–130 °C (dec). An analytical sample was prepared by recrystallization from water. **13** gave a positive Dische reaction[13] (pink color with cysteine–H_2SO_4) indicative of a deoxyribose residue: R_f (Whatman no. 1) system A 0.36, system B 0.53, system C 0.77; UV λ_{max} (ϵ) pH 1 239 nm (7490) and 303 (9000), pH 12 321 nm (12 170); ^{1}H NMR (D_2O) δ 2.54 (dd, 2, H2′), 4.07 (dd, 2, H5′), 4.31 (m, 1, H4′), 4.60 (m, 1, H3′), 6.23 (t, $J_{1',2'}$ = 6 Hz, 1, H1′), 9.25 (s, 1, H6). Anal. Calcd for $C_9H_{10}N_3O_{10}P\cdot H_2O$: C, 29.11; H, 3.77; N, 11.32; P, 8.36. Found: C, 28.87; H, 4.06; N, 11.27; P, 7.85.

The product **14** of R_f 0.54, after purification by DEAE-Sephadex as described, was freeze-dried to give ~1.2–1.9 g, ~28–42%. To obtain analytical sample, the product was dissolved in a minimum amount of H_2O and refrigerated at 4 °C for 24 h. Light-yellow prisms formed which were filtered off and washed with a small quantity of ice-cold H_2O. After drying, the product **14** had mp 136–138 °C (dec) and also gave a positive Dische color reaction[13] (deoxyribose): R_f (Whatman no. 1) system A 0.63, system B 0.62, system C 0.88; UV λ_{max} (ϵ) pH 1 237 (8370) and 301 (10 090), pH 12 321 nm (13 980); ^{1}H NMR (D_2O) δ 2.80 (m, 2, H2′), 4.17 (dd, 2, H5′), 4.66 (m, 1, H4′), 5.79 (m, 1, H3′), 6.25 (t, $J_{1',2'}$ = 7 Hz, 1, H1′) 9.28 (s, 1, H6). Anal. Calcd for $C_9H_{10}N_4O_{12}P\cdot H_2O\cdot NH_4$: C, 24.94; H, 3.70; N, 16.17; P, 7.16. Found: C, 24.64; H, 4.09; N, 16.20; P, 7.06.

5-Nitro-2′-deoxyuridine (15, 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-nitrouracil) by Bacterial Alkaline Phosphatase Treatment of 13. 5-Nitro-2′-deoxyuridine 5′-monophosphate (**13**, 120 mg, 0.34 mmol) was dissolved in Tris-HCl buffer (0.5 M, pH 8.7, 8 mL). To this solution, concentrated NH_4OH (85 μL) and then 200 μL of *E. coli* alkaline phosphatase (28.3 units/mL) were added. The mixture was incubated at 37 °C overnight. Solvent was removed in vacuo at <40 °C, and the residue was extracted with three 80-mL portions of MeOH. The methanol-soluble product was applied to a silica gel column (1.5 × 30 cm) and eluted with MeOH–$CHCl_3$ (1:4). UV absorbing fractions were pooled and evaporated, and the residue was crystallized from MeOH–EtOH (1:1) to give colorless needles of **15** in a yield of 54 mg (58%) with mp 149–150 °C (dec) (lit.[10] 152–153 °C). Compound **15** gave a positive Dische color test: R_f system A 0.56, system B 0.74, system C 0.77; UV λ_{max} (ϵ) pH 1 239 (8050) and 304 (10 070), pH 12 321 nm (14 140); ^{1}H NMR (Me_2SO-d_6) δ 2.36 (t, $J_{2',3'}$ = 5.0 Hz, 2, H2′), 3.64 (dd, 2, H5′), 3.89 (q, $J_{4',5'}$ = 4.0 Hz, 1, H4′), 4.27 (q, $J_{3',4'}$ = 4.0 Hz, 1, H3′), 6.06 (t, $J_{1',2'}$ = 5.8 Hz, 1, H1′), 9.47 (s, 1, H6), ~5.25 (br, 2, 3′, and 5′ OH's). Anal. Calcd for $C_9H_{11}N_3O_7$: C, 39.56; H, 4.08; N, 15.38. Found: C, 39.42; H, 4.21; N, 15.41.

3′-*O*,5-Dinitro-2′-deoxyuridine (16, 1-(2-Deoxy-3-*O*-nitro-β-D-*erythro*-pentofuranosyl)-5-nitrouracil) by Bacterial Alkaline Phosphatase Cleavage of 14. Compound **14** (200 mg, 0.46 mmol) was dissolved in Tris-HCl buffer (10 mL, 0.5 M, pH 8.7), 330 μL of *E. coli* alkaline phosphatase (28.3 units/mL) was added, and the solution was incubated overnight at 37 °C. The reaction mixture was then treated the same as for **15**. Crystallization (EtOH) of the residue from silica gel chromatography gave light-yellow prisms of **16** of mp 167–169 °C (dec) in a yield of 65% (95 mg). **16** gave a positive Dische test:[13] R_f system A 0.78, system B 0.84, system C 0.88; UV λ_{max} (ϵ) pH 1 236 (8790) and 301 (10 400), pH 12 321 nm (15 260); ^{1}H NMR (Me_2SO-d_6) δ 2.67 (dd, $J_{2',3'}$ = 3.0 Hz, 2, H2′), 3.72 (dd, 2, H5′), 4.40 (m, $J_{4',5'}$ = 1.8 Hz, 1, H4′), 5.45 (t, 1, 5′OH), 5.63 (m, 1, H3′), 6.12 (t, $J_{1',2'}$ = 6.8 Hz, 1, H1′), 9.38 (s, 1, H6); 12.07 (s, 1, 3NH). Anal. Calcd for $C_9H_{10}N_4O_9$: C, 33.96; H, 3.14; N, 17.61. Found: C, 33.69; H, 3.33; N, 17.39.

5-Nitrouridine 5′-Monophosphate (11, 1-(β-D-ribofuranosyl)-5-nitrouracil 5′-monophosphate). The disodium salt of uridine 5′-monophosphate (**10**, 1.5 g, 4.07 mmol, commercial dihydrate, dried 3 h at 110 °C in vacuo) was mixed with a solution of nitronium tetrafluoroborate (0.5 M, 20.4 mL, 10.2 mmol) in sulfolane, and the homogeneous solution was maintained at 37 °C for 16 h. At this time, the UV spectrum of an aliquot of the reaction mixture showed that no starting material remained. Chloroform (160 mL) was added to the reaction mixture, and the resulting precipitate was centrifuged down, washed with chloroform (160 mL), and then dissolved in absolute ethanol (300 mL) at room temperature. Ammonium hydroxide (concentrated) was added dropwise to the solution until the pH reached 7.5 and a fluffy precipitate appeared. This precipitate was harvested by centrifugation, washed with absolute ethanol, and dried in vacuo at ambient temperature.

The above precipitate was dissolved in a minimum quantity of water, the solution was applied to Whatman 3MM paper (ten sheets), and the chromatogram was developed in solvent system A (descending). After development, two bands (R_fs of 0.64 and 0.43) were visualized under UV light. The lower band (**11**) (R_f 0.43) was cut out and extracted with water. This eluted solution was concentrated and then rechromatographed on Whatman 3MM in solvent system C, and the major band was extracted as above. This product was then applied to a DEAE-Sephadex column (A-25, 1.6 × 16 cm), and the column was eluted by a linear gradient (0.01–0.2 M) of ammonium bicarbonate (pH 7.8, total 2 L). Fractions (14 mL) were collected, and fractions 97–151 were combined and evaporated to dryness at 40 °C in vacuo. Ammonium bicarbonate was removed by repeated addition and evaporation of water to give 410 mg (~25% yield) of chromatographically pure (systems A, B and C) product **11**.

To obtain an analytical sample, the above product was applied to a column of Dowex 50 (H^+) which was eluted with water. The eluant

was concentrated to a minimum volume in vacuo, and then the solution's pH was adjusted to 7.5 with a solution saturated with barium hydroxide. Ethanol (2 volumes) was added, and the resulting precipitate was filtered off and washed with ethanol and then ether. This barium salt was deionized with Dowex 50 (H^+) to give the free acid. However, under no conditions could the free acid (or its ammonium salt) be induced to crystallize.

The R_f values of product 11 were (Whatman no. 1, descending): system A 0.24; system B 0.45; system C 0.64. The relative mobility on paper electrophoresis [Whatman no. 1, 0.05 M $HCOONH_4$ (pH 3.5), 30 V/cm] was: UDP/UMP/11 = 1:0.59:0.57. UV λ_{max} (ϵ) pH 1 237 (9380) and 302 (10 580), pH 12 321 nm (14 560). Extinction coefficients were based on inorganic phosphate determination after washing with mixed acids.

This product gave a positive periodate–benzidine color test (yellow-white spot on blue background): ^1H NMR (D_2O) 5.81 (1, d, H1'); 9.20 (1, s, H6). A satisfactory C, H, and N analysis cound not be obtained for the barium salt. Anal. Calcd for $C_9H_9N_3O_{11}PBa_{1.5}\cdot 2H_2O$: N, 6.91; P, 5.10. Found: N, 6.51; P, 5.19.

Bacterial Alkaline Phosphatase Treatment of 11. 11 (1 mg) was dissolved in Tris-HCl buffer (50 μL, 0.5 M, pH 8.7), and to this solution bacterial alkaline phosphatase (1 μL of a solution of 5 mg of enzyme/mL, 48 units/mg) was added. This solution was incubated overnight at 37 °C. The reaction mixture was split into three parts and chromatographed on Whatman no. 1 paper in solvent systems A, B, and C. The product of this alkaline phosphatase cleavage had R_fs identical to that of authentic 5-nitrouridine (**9**) (R_fs: system A, 0.41; system B, 0.68; system C, 0.63).

Preparation of 5-Nitrouridine 5'-monophosphate (11) by Phosphorylation with $POCl_3$. The method of Yoshikawa et al.[15] was followed. 5-Nitrouridine (**9**, 72.3 mg, 0.25 mmol) was dissolved in a solution of triethyl phosphate (0.63 mL) and phosphoryl chloride (0.05 mL, 0.5 mmol). The mixture was left at room temperature overnight. Ether (25 mL) and water (1 mL) were added, and the resulting mixture was vigorously stirred for 0.5 h in an ice bath. The water phase was separated, neutralized with concentrated NH_4OH, and then applied to Whatman 3MM paper, and the chromatogram developed in solvent system B (descending). Two bands were visualized under UV light. The faster band (R_f 0.74) was determined to be unreacted starting material **9**. The lower band (R_f 0.41) was cut out and extracted with water, and the aqueous solution was evaporated to give a 24% yield of **11**. This product showed a positive periodate–benzidine test and a UV typical of 5-nitrouridine. Hydrolysis of this product with bacterial alkaline phosphatase under conditions described above gave only 5-nitrouridine (R_fs in systems A, B, and C identical to authentic **9**). This product, prepared by phosphorylation of **9**, had identical R_fs to **11**, prepared with NO_2BF_4, in solvent systems A, B, and C and, in addition, cochromatographed with 11 in systems A, B, and C.

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Registry No.—**1,** 66-22-8; **3,** 615-77-0; **4,** 28495-88-7; **5,** 874-14-6; **7,** 58-96-8; **9,** 3106-03-4; **10** 2Na, 3387-36-8; **11,** 23568-00-5; **11** Ba, 63689-78-1; **12** 2Na, 42155-08-8; **13,** 63689-79-2; **14** ammonium salt, 63689-80-5; **15,** 3106-01-2; **16,** 63689-81-6; NO_2BF_4, 13826-86-3.

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Stereostructures of the Macrocyclic Diterpenoids Ovatodiolide and Isoovatodiolide[1]

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The stereostructures of the 14-membered carbocyclic diterpenoids ovatodiolide (**3**) and isoovatodiolide (**4**), isolated from *Anisomeles indica* (Labiatae), have been established by x-ray crystallographic analyses. Acid treatment of **3** led to facile transannular ring closure to give **5** or **6,** depending on the acid used. The carbobicyclic system present in **5** and **6** occurs in the Gorgonian metabolite eunicellin (**9**).

Since the isolation of the 14-membered macrocyclic diterpene cembrene (**1**), a number of diterpenoids possessing the cembrane ring system (cembrenoids) have been isolated, many of which have shown pronounced biological activities.[2]

Our interest in the cembrenoids stems, in part, from the recognition that **1** and its congener casbene (**2**) are the biogenetic precursors of a variety of transannularly cyclized diterpenoids which exhibit a wide range of biological activities.[3] Prominent among such compounds are the cytotoxic principles of the Euphorbiaceae.[4] Also of some interest, is the in vitro transannular cyclization that the cembrenoids are expected to undergo. Somewhat surprisingly, there is a dearth of information on such cyclizations. In this paper we describe the stereostructures of the crystalline cembrenoids ovatodi-