J. Med. Chem. 1985, 28, 1376–1380

Replacement of the Ribofuranose Oxygen of 2-5A Derivatives by Methylene: Synthesis of an Aristeromycin Analogue of 2-5A Core 5′-Monophosphate (5′-*O*-Phosphoryladenylyl)(2′→5′)adenylyl(2′→5′)adenosine

Hiroaki Sawai,[†] Krystyna Lesiak,[‡] Jiro Imai,[‡] and Paul F. Torrence*[‡]

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-Ku, Tokyo 113, Japan, and Laboratory of Chemistry, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205. *Received November 26, 1984*

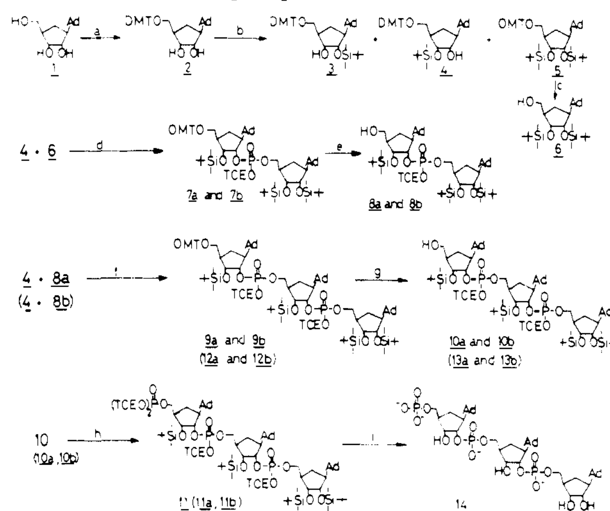
The ribofuranose oxygens of the the three adenosine residues of the 5′-monophosphate of the 2-5A core [adenylyl(2′→5′)adenylyl(2′→5′)adenosine] were replaced by methylenes through the synthesis of an aristeromycin [9-[(1*R*,2*S*,3*R*,4*R*)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]adenosine] analogue. In the synthetic approach, the chlorophosphite triester procedure was employed together with the use of dimethoxytrityl and *tert*-butyldimethylsilyl protecting groups. The final product 14 was bound to the 2-5A-dependent endonuclease of mouse, rabbit, or human cells 100–300 times less effectively than parent p5′A2′p5′A2′p5′A. In extracts of human Daudi cells where the monophosphate p5′A2′p5′A2′p5′A was able to effect ribosomal RNA cleavage at 2×10^{-7} M, 14 required a concentration of 2×10^{-5} M to bring about discernible rRNA cleavage.

2-5A is a unique 2′,5′-linked oligonucleotide[1] that is believed to be a mediator of at least some of the antiviral actions of interferon and may also be involved in other aspects of cellular regulations.[2,3] To explore the relationship between the structure of 2-5A and its ability to activate its target enzyme, a 2-5A-dependent endonuclease or RNase L, a number of analogues of 2-5A have been synthesized.[3,4] While several studies have dealt with the alteration of the bases,[5,7] 5′- or 2′-terminus,[8–10] the 3′-substituent on the sugar,[10–12] or the phosphate,[13] no analogues have been reported in which the basic structure of the ribofuranose ring has been altered in all three nucleotide residues. That the ribose–phosphate backbone of the oligonucleotide must be an important recognition area for the endonuclease interaction is suggested by the relative inactivity of 3′,5′-linkage isomers such as ppp5′A3′p5′A3′p5′A[14] and cordycepin analogues such as ppp5′(3′dA)2′p5′(3′dA)2′p5′(3′dA).[10,11] To further extend this lead, we have prepared and evaluated the biological activity of a carbocyclic 2-5A analogue, that is, an analogue in which the ribofuranose oxygen of adenosine is replaced by methylene, giving the antibiotic aristeromycin.

Results and Discussion

Chemistry. In analogy to previous efforts,[5,6,15–18] lead ion catalyzed polymerization of aristeromycin 5′-phosphoroimidazolidate was first attempted but, in this case, provided no oligomeric products. Thus, the chlorophosphite[19–21] approach using silyl protecting groups was undertaken to prepare the 2′,5′-linked aristeromycin trimer (Scheme I). Aristeromycin (1) was reacted with dimethoxytrityl chloride in pyridine to afford 5′-*O*-(dimethoxytrityl)aristeromycin (2), which was converted to a mixture of 5′-*O*-(dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)aristeromycin (3), 5′-*O*-(dimethoxytrityl)-3′-*O*-(*tert*-butyldimethylsilyl)aristeromycin (4), and 5′-*O*-(dimethoxytrityl)-2′,3′-*O*-bis(*tert*-butyldimethylsilyl)aristeromycin (5) by reaction with 1.3 mol equiv of *tert*-butyldimethylsilyl chloride in the presence of imidazole in DMF. The isolated yields of **3**–**5** were 25%, 24%, and 11%, respectively. The 5′-*O*-dimethoxytrityl group of **5** was removed by acetic acid (80%) at room temperature to give 2′,3′-*O*-bis(*tert*-butyldimethylsilyl)aristeromycin (**6**) in 87% yield. Compound 4 was phosphorylated with (2,2,2-trichloroethyl)phosphorodichloridite followed by coupling with compound **6** in dry THF at −78 °C. After the mixture was warmed to room temperature, the iodine oxidation of the intermediate phosphite gave a diastereomeric mixture of the protected dimers, **7a** and **7b**, in 50%

Scheme I. Synthesis of the Aristeromycin Analogue (14) of 2-5A Core 5′-Monophosphate[a]



[a] Reagents: a, dimethoxytrityl chloride; b, *tert*-butyldimethylsilyl chloride; c, 80% HOAc; d, (1) trichloroethyl phosphorodichloridite and (2) I_2; e, 80% HOAc; f, (1) trichloroethyl phosphorodichloridite and (2) I_2; g, 80% HOAc; h, (1) trichloroethyl phosphorodichloridite, (2) trichloroethanol, and (3) I_2; i, (1) Zn-Cu couple and (2) tetra-*n*-butylammonium fluoride.

(1) Kerr, I.; Brown, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 256–260.
(2) Lengyel, P. "Interferon 3"; Gresser, I., Ed.; Academic Press: New York, 1980; pp 77–99.
(3) Johnston, M. I.; Torrence, P. F. In "Interferon: Mechanisms of Production and Action"; Friedman, R. M., Ed.; Elsevier, Amsterdam, in press.
(4) Torrence, P. F.; Imai, J.; Lesiak, K.; Jamoulle, J.-C.; Sawai, H.; Warinnier, J.; DeClercq, E. In "Targets for the Design of Antiviral Agents"; DeClercq, E., Walker, R., Eds.; Plenum Press: New York, 1984; pp 259–285.
(5) Torrence, P. F.; Imai, J.; Lesiak, K.; Jamoulle, J.-C.; Sawai, H. *J. Med. Chem.* **1984**, *27*, 726–733.
(6) Jamoulle, J.-C.; Imai, J.; Lesiak, K.; Torrence, P. F. *Biochemistry* **1984**, *23*, 3063–3069.
(7) Lesiak, K.; Torrence, P. F. *FEBS Lett.* **1983**, *151*, 291–296.
(8) Imai, J.; Torrence, P. F. *Biochemistry* **1984**, *23*, 766–774.
(9) Imai, J.; Johnston, M. I.; Torrence, P. F. *J. Biol. Chem.* **1982**, *257*, 12739–12745.
(10) Haugh, M. C.; Cayley, P. J.; Serafinowska, H. T.; Norman, D. G.; Reese, C. G.; Kerr, I. M. *Eur. J. Biochem.* **1983**, *132*, 77–84.
(11) Sawai, H.; Imai, J.; Lesiak, K.; Johnston, M. I.; Torrence, P. F. *J. Biol. Chem.* **1983**, *258*, 1671–1677.

[†] University of Tokyo.
[‡] National Institutes of Health.

This article not subject to U.S. Copyright. Published 1985 by the American Chemical Society

Table I. R_f Values of the Compounds on TLC

compd[a]	R_f value[b] C	D	E
2′-5′ (pAris)$_3$ (**14**)	0.29	0.17	0.47
pAris	0.41	0.37	0.58
2′-5′ (pA)$_3$	0.30	0.24	0.48
pA	0.43	0.42	0.58

[a] pAris, aristeromycin 5′-phosphate; pA, adenosine 5′-phosphate. [b] Key: c, Cellulose F, 1-propanol–concentrated ammonia–water (55:10:35); D, Cellulose F, saturated ammonium sulfate–0.1 M sodium acetate–2-propanol (79:19:2); E, PEI-Cellulose F, 0.2 M ammonium bicarbonate.

and 33% isolated yields, respectively. The two diastereomers readily were separated by column chromatography on silica gel. There was no evidence for phosphorylation at the N6 amino group of adenosine when the reaction was carried out at −78 °C. Both dimers, **7a** and **7b**, were treated with 80% acetic acid to remove the dimethoxytrityl group and gave **8a** and **8b** in high yield. The dimer **8a** then was coupled with **4** by the use of (2,2,2-trichloroethyl)-phosphorodichloridite in dry THF at −78 °C followed by iodine oxidation. The diastereomeric mixture of the fully protected trimers, **9a** and **9b**, was obtained in 81% yield. The trimer mixture, **9a** and **9b**, was treated with 80% acetic acid to remove the 5′-dimethoxytrityl group, giving **10a** and **10b** in 56% and 22% yields, respectively. The resulting two diastereomers were separated by preparative TLC. The 5′-termini of **10a** and **10b** were phosphorylated with (2,2,2-trichloroethyl)phosphorodichloridite at −78 °C followed by reaction with 2,2,2-trichloroethanol. The fully protected trimers, **11a** and **11b**, were obtained after iodine oxidation. Likewise, **8b** was coupled with **4** to give a mixture of two diastereomers, **12a** and **12b**, which were detritylated and phosphorylated to yield the protected trimers, **13a** and **13b**. The mixture of **11a** and **11b** was treated with a zinc-copper couple in DMF and acetylacetone to remove the trichloroethyl group. Subsequent removal of the *tert*-butyldimethylsilyl protecting group was accomplished by treatment with tetra-*n*-butylammonium fluoride in THF. Purification on DEAE column chromatography and paper chromatography afforded the 5′-phosphorylated 2′,5′-linked aristeromycin trimer (**14**).

The structure of **14** was substantiated by several enzyme digestions. The trimer **14** was degraded by snake venom phosphodiesterase to give aristeromycin 5′-monophosphate as the sole product; however, **14** was totally resistant to the action of nuclease P_1 and RNase T_2, enzymes that act on 3′,5′-phosphodiester bonds only. Alkaline phosphatase digestion gave a new product presumed to be the 5′-dephosphorylated trimer core. Treatment of **14** with 0.5 N NaOH gave three degradation products in a nearly 1:1:1

(12) Baglioni, C.; D'Alessandro, S. B.; Nilsen, T. W.; de Hartog, J. A. J.; Crea, R.; Van Boom, J. H. *J. Biol. Chem.* **1981**, *256*, 3253–3257.
(13) Nelson, P. S.; Bach, C. T.; Verheyden, J. P. H. *J. Org. Chem.* **1984**, *49*, 2314–2317.
(14) Lesiak, K.; Imai, J.; Floyd-Smith, G.; Torrence, P. F. *J. Biol. Chem.* **1983**, *258*, 13082–13088.
(15) Sawai, H.; Shibata, T.; Ohno M. *Tetrahedron Lett.* **1981**, *37*, 481–485.
(16) Sawai, H.; Ohno, M. *Bull Chem. Soc. Jpn.* **1981**, *54*, 2752–2754.
(17) Sawai, H.; Ohno, M. *Chem. Pharm. Bull.* **1981**, *29*, 2237–2245.
(18) Sawai, H.; Shibata, T.; Ohno, M. *Tetrahedron Lett.* 1979, *47*, 4573–4576.
(19) Ogilvie, K. K.; Therianlt, N. Y. *Tetrahedron Lett.* **1979**, 2111–2114.
(20) Ogilvie, K. K.; Beancage, S. L.; Schifman, A. L.; Therianlt, N. Y.; Sandro, K. L. *Can. J. Chem.* **1978**, *56*, 2768–2780.
(21) Imai, J.; Torrence, P. F. *Methods Enzymol.* **1891**, *79*, 233–244.

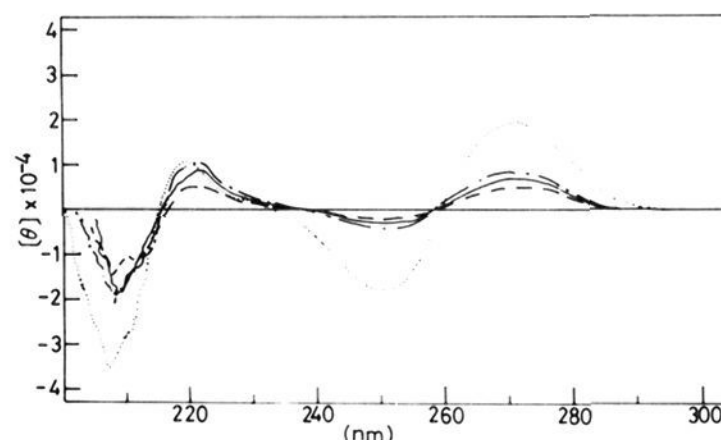


Figure 1. CD spectra of compound **14** and 2′-5′-linked triadenylate in 0.1 M phosphate buffer (pH 6.7). **14**: –·–, at 5 °C; —, at 25 °C; - - -, at 55 °C. 2′-5′(pA)$_3$: ···, at 25 °C.

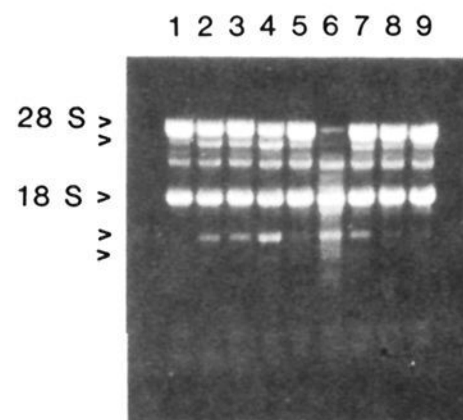


Figure 2. Activation of the 2-5A-dependent nuclease of human Daudi lymphoblastoid cells as monitored by rRNA cleavage. Incubation of the extract from Daudi cells was for 2 h at 30 °C in the absence of any added oligonucleotides (track 1), in the presence of ppp5′A2′p5′A2′p5′A at 2×10^{-7} or 2×10^{-9} M (tracks 2 and 3, respectively), with p5′A2′p5′A2′p5′A at 2×10^{-7} or 2×10^{-9} M (tracks 4 and 5, respectively), or with the aristeromycin analogue **14** at 2×10^{-4}, 2×10^{-5}, 2×10^{-6}, or 2×10^{-7} M (tracks 6–9, respectively). Positions of the 28S and 18S rRNA's and the nuclease cleavage fragments are indicated by arrows on the photograph.

Table II. Interaction of the Aristeromycin Analogue of 2-5A with RNase L as Determined by Radiobinding Assay

compd	IC_{50},[a] M mouse L	Daudi	rabbit reticulocyte
p5′A2′p5′A2′p5′A	9.5×10^{-10}	1.1×10^{-9}	2×10^{-8}
14	3.2×10^{-7}	2.1×10^{-7}	2.3×10^{-6}

[a] Molar concentration of oligomer needed to prevent binding of 50% of the added ppp5′A2′p5′A2′p5′A2′p5′A3′[^{32}P]p5′ (C3′p label).

ratio, and these corresponded to aristeromycin, aristeromycin 3′(2′)-phosphate and aristeromycin 5′,3′(2′)-diphosphate. Finally, the chromatographic behavior of **14** was similar to that of the parent oligoadenylate (Table I).

The CD spectrum of **14** at 4, 25, and 55 °C is given in Figure 1 together with the corresponding spectrum of p5′A2′p5′A2′p5′A at 25 °C. The major CD bands of **14** were significantly less intense than those of p5′A2′p5′A2′p5′A; in addition, the UV hypochromicity of **14** was 12%, which also was small compared to that of the corresponding 2′,5′-oligoadenylate.[15,22] It is likely that base stacking is less pronounced in the aristeromycin analogue **14** than in the 2′,5′-oligoadenylate.

Biological Assays. The binding of **14** to the 2-5A-dependent endonuclease was examined in three different systems: extracts of mouse L cells, human Daudi lym-

(22) Ikehara, M.; Oshie, K.; Hasegawa, A.; Ohtsuka, E. *Nucleic Acids Res.* **1981**, *9*, 2003–2020.

phoblastoid cells, and rabbit reticulocyte lysates. In each case, the ability of 14 to compete with the radiolabeled probe ppp5′A2′p5′A2′p5′A2′p5′A3′[^{32}P]p5′C3′p was determined by using the nitrocellulose filter assay distributed by Knight et al.[23] In all cases (Table II), the aristeromycin analogue 14 was at least 2 orders of magnitude less effective than the parent p5′A2′p5′A2′p5A in displacing the radiolabeled probe. Thus, it required 330, 190, and 115 times as much carbocyclic analogue 14 as it did parent p5′A2′p5′A2′p5′A to prevent binding of 50% of the probe of the RNase L's of mouse L cells, Daudi cells, and rabbit reticulocytes, respectively.

Although the 5′-triphosphate of the aristeromycin analogue 14 was not prepared, it was nonetheless possible to obtain an estimate of the ability of the analogue 14 to activate RNase L since in extracts of Daudi lymphoblastoid cells, oligonucleotide 5′monophosphates are capable of nuclease activation.[6,10] Thus, Figure 2 presents the results of a ribosonal RNA cleavage assay conducted in Daudi cell extracts. In these experiments ppp5′A2′p5′A2′p5′A gave rRNA cleavage at a concentration as low as 2×10^{-9} M (track 3) whereas the 5′-monophosphate, p5′A2′p5′A2′p5′A, was significantly less active, showing clear rRNA degradation at 2×10^{-7} M. The aristeromycin analogue 14, on the other hand, while showing discernible cleavage at 2×10^{-4} and 2×10^{-5} M, was inactive at 2×10^{-6} M. Thus, 14 was approximately 100 times less active than its parent p5′A2′p5′A2′p5′A as far as activation of RNase L. This result was in basic agreement with the RNase L radiobinding assay that showed about a 200-fold drop in binding affinity for the aristeromycin analogue as compared to 2-5A core 5′-monophosphate.

Using HPLC, we also examined the degradation of 14 under conditions of protein synthesis (20% mouse L cell extract, 130 mM KCl, 3 mM $Mg(OAc)_2$, 30 mM Hepes, pH 7.5, 30 °C, no ATP) and found that its half-life (~30 min) was similar to that of parent p5′A2′p5′A2′p5′A under the same conditions (data not illustrated).

Experimental Section

Aristeromycin was supplied by Takeda Chemicals Co. (2,2,2-Trichloroethyl) phosphorodichloridite was prepared from 2,2,2-trichloroethanol and phosphorus trichloride according to the published procedure.[24] Dimethoxytrityl chloride, *tert*-butyldimethylsilyl chloride, and imidazole were obtained commercially. Bacterial alkaline phosphatase and venom phosphodiesterase were from Worthington and nuclease P_1 from Yamasa Co.

Paper chromatography was carried out on Whatman 3MM paper by the descending technique using 1-propanol-concentrated ammonia-water (55:10:35). Thin-layer chromatography (TLC) was performed with silica gel $60F_{254}$ plates (Merck) [using the solvent systems (A) chloroform-methanol (92.5:7.5) and (B) chloroform-methanol (85:15)], with cellulose F_{254} plates (Merck) [using the solvent systems (C) 1-propanol-concentrated ammonia-water (55:10:35) and (D) saturated ammonium sulfate-0.1 M sodium acetate (pH 6.5)-2-propanol (79:19:2)], and with PEI-cellulose F_{254} plates (Merck) [using the solvent system (E) 0.25 M ammonium bicarbonate].

Enzyme digestion and hypochromicity determination were carried out as described previously.[15] CD spectra were measured by a Jasco J-40 spectrometer using a 2-mm cell in 0.1 M phosphate buffer (pH 6.7). UV spectra were recorded by a Shimazu MPS-5000 spectrophotometer in 0.1 M phosphate buffer (pH 6.7). NMR spectra at 400 MHz were taken by a JEOL GX-400 instrument.

Synthesis of 2. Aristeromycin (270 mg, 1 mmol) was dried over phosphorus pentoxide under vacuum and reacted with dimethoxytrityl chloride (370 mg, 1.1 mmol) in dry pyridine (4 mL) at 90 °C for 2 h with stirring. After the solvent was evaporated under vacuum, the oily residue was dissolved in ethyl acetate (150 mL), washed with saturated sodium bicarbonate solution followed by water, then dried over anhydrous sodium sulfate, and concentrated under vacuum. The concentrated solution was slowly poured into *n*-hexane to give a white precipitate of 2 (545 mg, 96%), TLC (system B) R_f 0.56. Compound 2 was used without further purification.

Syntheses of 3-5. Dried 2 (428 mg, 0.75 mmol) was dissolved in dry DMF (4 mL) and the resultant mixture reacted with *tert*-butyldimethylsilyl chloride (150 mg, 0.975 mmol) in the presence of imidazole (155 mg, 2.4 mmol) at room temperature for 2.5 h with stirring. The solvent was concentrated under vacuum to give an oily residue that was dissolved in ethyl acetate (30 mL) and was washed with saturated sodium bicarbonate solution (20 mL) and then with water (20 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was applied to a column of silica gel (11 mm × 30 cm) and eluted with methylene chloride-ethyl acetate (9:1) to give 5 (65 mg, 0.082 mmol, 11%) as the first product; TLC (system A) R_f 0.76. Compound 3 was eluted second with methylene chloride-ethyl acetate (3:2): yield 126 mg (0.183 mmol, 25%); TLC (system A) R_f 0.56; NMR ($CDCl_3$) δ -0.45 (s, 3 H, $SiCh_3$), -0.20 (s, 3 H, $SiCH_3$), 0.76 (s, 9 H, $SiC(CH_3)_3$), 1.76 (s, 1 H, 3′-OH), 2.31 (m, 1 H, 5′-CH), 2.36 (m, 1 H, 4′-CH), 2.58 (m, 1 H, 5′-CH), 3.27 (d, 2 H, 6′-CH_2), 3.81 (s, 6 H, OCH_3), 3.98 (dd, 1 H, 3′-CH), 4.75 (dd, 1 H, 2′-CH), 4.82 (m, 1 H, 1′-CH), 6.80-7.50 (m, 13 H, Ar), 7.76 (s, 1 H, 2-CH), 8.25 (s, 1 H, 8-CH).

Compound 4 was eluted third with methylene chloride-ethyl acetate (1:1): yield 123 mg (0.180 mmol, 24%); TLC (system A) R_f 0.53; NMR ($CDCl_3$) δ -0.01 (s, 3 H, $SiCH_3$), 0.02 (s, 3 H, $SiCH_3$), 0.82 (s, 9 H, $SiC(CH_3)$), 2.04 (m, 1 H, 5′-CH), 2.26 (m, 1 H, 4′-CH), 2.50 (m, 1 H, 5′-CH), 3.06 (s, 1 H, 2′-OH), 3.14 (dd, 1 H, 6′-CH), 3.25 (dd, 1 H, 6′-CH), 3.82 (s, 6 H, OCH_3), 4.15 (dd, 1 H, 3′-CH), 4.39 (dd, 1 H, 2′-CH), 4.62 (m, 1 H, 1′-CH), 5.60 (s, 2 H, -NH_2), 6.75-7.40 (m, 13 H, Ar), 7.86 (s, 1 H, 2-CH), 8.27 (s, 1 H, 8-CH).

Synthesis of 6. Compound 5 (150 mg, 0.19 mmol) was dissolved in 80% acetic acid (2 mL) and the resultant mixture stirred at room temperature for 30 min. Ethyl acetate (80 mL) was added to the mixture, and the resulting solution was washed with saturated sodium bicarbonate solution (100 mL) and then with water (100 mL). The organic layer was dried over anhydrous sodium sulfate, evaporated under vacuum, and subjected to column chromatography on silica gel (11 mm × 30 cm). Compound 6 was eluted with methylene chloride-ethyl acetate (1:1): yield 82 mg (0.166 mmol, 87%); TLC (system A) R_f 0.47; NMR ($CDCl_3$) δ -0.72 (s, 3 H, $SiCH_3$), 0.19 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$), 0.75 (s, 9 H, $SiC(CH_3)_3$), 0.93 (s, 9 H, $SiC(CH_3)_3$), 1.97 (s, 1 H, 6′-OH), 2.21 (m, 1 H, 5′-CH), 2.36 (m, 1 H, 4′-CH), 2.60 (m, 1 H, 5′-CH), 3.82 (m, 2 H, 6′-CH_2), 4.08 (dd, 1 H, 3′-CH_2), 4.70 (dd, 1 H, 2′-CH), 5.04 (s, 2 H, NH_2), 7.78 (s, 1 H, 2-CH), 8.31 (s, 1 H, 8-CH).

Coupling of 6 with 4. Compound 4 (112 mg, 0.164 mmol) in dry THF (0.5 mL) was added to a stirred solution of collidine (0.1 mL, 0.73 mmol), 2,2,2-trichloro ethyl phosphorodichloridite (27 μL, 0.17 mmol), and dry THF (0.25 mL) at -78 °C. The reaction mixture was stirred for 30 min, and 6 (70 mg, 0.14 mmol) in dry THF (1.0 mL) was added dropwise. The reaction mixture was stirred for 1 h at -78 °C. After the mixture was warmed to room temperature, iodine (93 mg, 0.36 mmol) in aqueous THF (0.75 mL) was added to the mixture and stirring was continued for 30 min at room temperature. The solvent was evaporated under vacuum. The residue was dissolved in ethyl acetate (25 mL), washed with aqueous sodium thiosulfate solution (10 mL) and then with water (10 mL × 2), dried over anhydrous sodium sulfate, and evaporated under vacuum. The residue was applied to a silica gel column (11 mm × 30 cm) and eluted with methylene chloride-methanol (99:1 → 97.5:2.5 → 95:5). Diastereomer **7a** was eluted first, followed by diastereomer **7b**. The yield of **7a** was 95 mg (0.07 mmol, 50%): TLC (system A) R_f 0.41; NMR ($CDCl_3$) δ -0.67 (s, 3 H, $SiCH_3$), -0.22 (s, 3 H, $SiCH_3$), 0.06 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$), 0.69 (s, 9 H, $SiC(CH_3)_3$), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 3.79 (s, 6 H,

(23) Knight, M.; Cayley, P. J.; Silverman, R. H.; Wreschner, D. H.; Gilbert, C. S.; Brown, R. E.; Kerr, I. M. *Nature (London)* 1980, *288*, 189-192.
(24) Gerrard, W.; Green, W. J.; Phillips, R. J. *J. Chem. Soc.* 1954, 1148-1150.

OCH_3), 4.00 (d, 2 H, OCH_2CCl_3), 5.09 (m, 1 H, 1'-CH_A), 5.29 (m, 1 H, 1'-CH_B), 6.56 and 6.63 (s, 2 H, NH_2), 6.84–7.50 (m, 13 H, Ar), 7.72 and 7.88 (s, 1 H, 2-CH_A and 2-CH_B), 8.17 and 8.21 (s, 1 H, 8-CH_A and 8-CH_B).

The yield of **7b** was 58 mg (0.043 mmol, 33%): TLC (system A) R_f 0.38; NMR ($CDCl_3$) δ –0.59 (s, 3 H, $SiCH_3$), –0.19 (s, 3 H, $SiCH_3$). 0.06 (s, 6 H, $SiCH_3$), 0.08 (s, 3 H, $SiCH_3$), 0.13 (s, 3 H, $SiCH_3$), 0.71 (s, 9 H, $SiC(CH_3)_3$), 0.90 (s, 9 H, $SiC(CH_3)_3$), 0.91 (s, 9 H, $SiC(CH_3)_3$), 3.79 (s, 6 H, OCH_3), 3.95 (d, 2 H, OCH_2CCl_3), 5.15 (m, 1 H, 1'-CH_A), 5.38 (m, 1 H, 1'-CH_B), 6.37 (s, 4 H, NH_2), 6.84–7.74 (m, 13 H, Ar), 7.74 and 7.77 (s, 1 H, 2-CH_A and 2-CH_B), 8.16 and 8.26 (s, 1 H, 8-CH_A and 8-CH_B).

Synthesis of 8a by Removal of the 5'-Dimethoxytrityl Group from 7a. Compound **7a** (92 mg, 0.068 mmol) was kept in 80% acetic acid (2 mL) at room temperature for 1 h. The reaction mixture was concentrated under vacuum, and the residue was dissolved in ethyl acetate (30 mL). This solution was washed with saturated sodium bicarbonate solution (50 mL) and then with water (20 mL) and dried over anhydrous sodium sulfate. After the solvent was evaporated under vacuum, the residue was applied to a silica gel column (11 mm × 30 cm), which was eluted with methylene chloride–methanol (97.5:2.5 → 95:5). The yield of **8a** was 60 mg (0.057 mmol, 84%): TLC (system A) R_f 0.15; NMR ($CDCl_3$) δ –0.59 (s, 3 H, $SiCH_3$), –0.19 (s, 3 H, $SiCH_3$). 0.08 (s, 3 H, $SiCH_3$), 0.09 (s, 3 H, $SiCH_3$), 0.16 (s, 3 H, $SiCH_3$), 0.18 (s, 3 H, $SiCH_3$), 0.71 (s, 9 H, $SiC(CH_3)_3$), 0.92 (s, 9 H, $SiC(CH_3)_3$), 0.94 (s, 9 H, $SiC(CH_3)_3$), 2.12 (m, 1 H, 5'-CH), 2.34 (m, 3 H, 4'-CH and 5'-CH), 2.46 (m, 1 H, 5'-CH), 2.69 (m, 1 H, 5'-CH), 3.83 (d, 2 H, OCH_2CCl_3), 4.01 (d, 1 H, 6'-CH), 4.12 (m, 1 H, 6'-CH), 4.21 (m, 1 H, 3'-CH), 4.32 (m, 1 H, 6'-CH), 4.39 (m, 1 H, 3'-CH), 4.48 (d, 1 H, 6'-CH), 4.59 (dd, 1 H, 2'-CH), 4.72 (dd, 1 H, 2'-CH), 5.05 (m, 1 H, 1'-CH), 5.50 (m, 1 H, 1'-CH), 6.87 (s, 2 H, NH_2), 7.04 (s, 2 H, -NH_2), 7.77 and (7.95 (s, 1 H, 2-CH), 8.26 (s, 2 H, 8-CH).

Compound **7b** (50 mg, 0.37 mmol) was unmasked in the same procedure, giving **8b** (29 mg, 0.028 mmol, 75%), TLC (system A) R_f 0.13.

Coupling of 8a with 4. Compound **4** (15 mg, 0.022 mmol) in dry THF (0.4 mL) was added to a solution of collidine (14 μL, 0.10 mmol), 2,2,2-trichloroethyl phosphorodichloridite (4 μL, 0.024 mmol), and dry THF (0.2 mL) at –78 °C with stirring. The reaction mixture was stirred at 78 °C for 30 min, and the compound **8a** (14 mg, 0.013 mmol) in dry THF (0.4 mL) was added dropwise. The solution was stirred for 1 h at –78 °C. After the mixture was warmed to room temperature, iodine (14 mg, 0.055 mmol) in aqueous THF (0.4 mL) was added and the mixture was stirred for 30 min at room temperature. The solvent was evaporated under vacuum, and the residue was dissolved in ethyl acetate (25 mL) and washed with sodium thiosulfate solution (10 mL), sodium bicarbonate solution (10 mL), and finally water (10 mL). The organic layer was dried over anhydrous sodium sulfate, evaporated under vacuum, and applied to a silica gel column (11 mm × 30 cm), which was eluted with methylene chloride–methanol (20:1 → 10:1). A diastereomeric mixture (**9a** and **9b**) was obtained in 81% yield (20 mg, 0.01 mmol): **9a**, TLC (system B) R_f 0.57; **9b**, TLC (system B) R_f 0.55.

Synthesis of 10a and 10b by Removal of the 5'-Dimethoxytrityl Group from 9a and 9b. The diasteromeric mixture of **9a** and **9b** (20 mg, 0.01 mmol) in 80% acetic acid (1 mL) solution was kept at room temperature for 1 h. Ethyl acetate (25 mL) was added to the mixture, and the solution was washed with saturated sodium bicarbonate solution (10 mL × 2) and then with water (10 mL), dried over anhydrous sodium sulfate, and evaporated under vacuum. The residue was applied to a preparative silica gel TLC plate (20 × 20 cm) and developed twice with methylene chloride–methanol (92:8). Two diastereomers **10a** and **10b** thus separated were eluted with methylene chloride–methanol (9:1). Data for **10a**: yield 9.5 mg (0.0058 mmol, 55%); TLC (system B) R_f 0.47; NMR ($CDCl_3$) δ 3.88 (d, 2 H, OCH_2CCl_3), 4.05 (s, 2 H, OCH_2CCl_3), 7.87, 7.98 and 8.00 (s, 1 H, 2-CH), 8.26, 8.30 and 8.30 (s, 1 H, 8-CH). Data for **10b**: yield 3.8 mg (0.0023 mmol, 22%); TLC (system B) R_f 0.44; NMR ($CDCl_3$) δ 3.87 (d, 2 H, OCH_2CCl_3), 4.04 (s, 2 H, OCH_2CCl_3), 7.78, 8.02 and 8.04 (s, 1 H, 2-CH), 8.29, 8.29 and 8.32 (s, 1 H, 8-CH).

Synthesis of 11a and 11b by 5'-Phosphorylation of 10a and 10b. Compounds **10a** (9.5 mg, 5.8 μmol) and **10b** (3.8 mg, 2.3 μmol) were combined and dissolved in dry THF (0.4 mL). The solution was added to a mixture of collidine (12 μL, 86 μmol) and 2,2,2-trichloroethyl phosphorodichloridite (3 μL, 19 μmol) in dry THF (0.2 mL) at –78 °C. The mixture was stirred for 30 min at –78 °C, and then 2,2,2-trichloroethanol (5 μL, 46 μmol) in dry THF (0.4 mL) was added to the mixture. After stirring for 30 min at –78 °C, the reaction mixture was warmed to room temperature. Iodine (14 mg, 0.055 mmol) in aqueous THF (0.5 mL) was added, and stirring was continued for 30 min at room temperature. Ethyl acetate (25 mL) was added to the mixture, and the solution was washed with sodium thiosulfate solution (10 mL), saturated sodium bicarbonate solution (10 mL), and then with water (10 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated under vacuum. The residue was applied to a preparative silica gel TLC plate (20 × 20 cm) and developed with methylene chloride–methanol (92.5:7.5). The yield of the diastereomeric mixture, **11a** and **11b**, was 6.8 mg (3.1 μmol, 38%): **11a**, TLC (system B) R_f 0.50; **11b**, TLC (system B) R_f 0.48.

Synthesis of 14 by Deblocking of 11a and 11b. The Zn–Cu couple (13 mg) and acetylacetone (70 μL) were added to a solution of the mixture **11a** and **11b** (6 mg, 3.1 μmol) in dry DMF (0.2 mL). The reaction mixture was stirred for 4 h at 65 °C. Methanol (15 mL) was added to the solution that was then treated with Chelex resin (10-mL wet volume) to remove zinc and copper ion. After the mixture was stirred for 30 min, Chelex was removed by filtration and the filtrate was evaporated under vacuum. The residue was dissolved in 0.5 mL of THF followed by addition of 1 M tetra-*n*-butylammonium fluoride in THF (0.5 mL), and the solution was kept at room temperature for 20 h. After removal of THF by evaporation, water (15 mL) was added to the residue and the solution was washed with ether (10 mL). The aqueous layer was concentrated under vacuum and applied to a DEAE-Toyopearl 650 M column (12 mm × 25 cm). The column was eluted with a linear gradient of triethylammonium bicarbonate buffer (0–0.3 M; 500/500 mL). Fractions of 5 mL were collected every 6 min. Fractions 25–31 were pooled and evaporated under vacuum and repeatedly evaporated with water to remove triethylammonium bicarbonate. The residue was purified by paper chromatography on Whatman 3 mM to give **14** (18.1 OD_{260} units). The biological activities reported in Table II and Figure 2 were determined with this material. Additionally, however, a portion was further purified on HPLC using a 9.4 mm × 25 cm Zorbax ODS column and an electron program of 0–50% MeOH–H_2O (1:1) in 0.05 M ammonium phosphate (pH 7.0). Under these conditions, the retention time of the aristeromycin analogue was 22 min. It was collected (total volume 1 mL, ~25% B corresponding to a concentration of ~40 mM ammonium phosphate) and the solvent removed in vivo. The residue was dissolved in 0.5 mL of water; yield of 0.6 A_{260} unit. Binding was then redetermined by using the radiobinding assay and mouse L cell extracts. In this case, the IC_{50} value was 8×10^{-7} M.

Biological Assays. The preparations of mouse L cell extracts and human Daudi cell extracts have been described elsewhere.[11,25] Radiobinding assays were performed according to Knight et al.[23] with ppp5'A2'p5'A2'p5'A2'p5'A3'[^{32}P]p5'C3'p of specific activity 3000 Ci/mmol; Amersham, Chicago, IL). Ribosomal RNA cleavage assays were carried out by using the basic procedure of Wreschner et al.[26] Specifically, for rRNA cleavage assays in extracts of mouse L_K cells, conditions for protein synthesis were employed except that ATP, phosphocreatine kinase, and the amino acids were omitted. After a 2-h incubation period, at 30 °C, the samples were diluted 10-fold with SDS buffer (50 mM NaOAc, 10 mM EDTA, 0.5% sodium dodecyl sulfate, pH 5.0) and then extracted twice with water-saturated phenol/chloroform (1:1). Before the second extraction, the water phase was made 100 mM in sodium acetate. The RNA was precipitated with 2.5 volumes of ethanol at –20 °C. These RNA samples were denatured with glyoxal and electrophoresed on 1.8% Agarose gels in a buffer consisting of 40 mM Tris acetate, 1 mM EDTA, pH 7.2 at 110 V for 2.5 h. The gel was stained with ethidium bromide (1 μg/mL) and photographed under ultraviolet light (302 nm).

(25) Torrence, P. F.; Friedman, R. M. *J. Biol. Chem.* **1979**, *254*, 1259–1267.
(26) Wreschner, D. H.; James, T. C.; Silverman, R. H.; Kerr, I. M. *Nucleic Acids Res.* **1981**, *9*, 1571–1581.

Conclusion

The above results clearly show that replacement of the ribofuranose oxygen of all three adenosine residues of 2-5A core 5′-monophosphate results in a major reduction in ability to interact with the 2-5A-dependent endonuclease from a variety of sources. The decreased binding activity may be related to a loss of interaction of one or more of the ribofuranose oxygens with some site on thee endonuclease. Certainly the methylene group would introduce considerable hydrophobicity into a previously hydrophilic site. Alternatively, the conversion of oxygen to methylene may bring about a conformational change in the 2-5A molecule, and this could be detrimental to optimal binding interactions. In this regard, both UV and CD spectra suggested that the aristeromycin analogue 14 possesses less base stacking than the parent p5′A2′p5′A2′p5′A.

Acknowledgment. We are grateful to Dr. M. Nishikawa (Takeda Chemical Industries) for a supply of aristeromycin, to Professor H. Hirai, Y. Gotob, and M. T. Kawamura for their assistance in determining 400-MHz NMR spectra, and to Dr. S. Higuchi for his help in measuring CD spectra.

Book Reviews

The Alkaloids. Volume 22. Chemistry and Pharmacology. Edited by Arnold Brossi. Academic Press, Orlando, FL. 1983. xviii + 342 pp. 16 × 23.5 cm. ISBN 0-12-469522-1. $50.00.

This well-established series of volumes on the most beautiful of all natural products, the alkaloids, continues to go from strength to strength. Since the publication of Volume 1, edited by R. H. F. Manske and H. L. Holmes, in 1950, the series has been devoted to a series of reviews on the different classes of alkaloids which are again reviewed when sufficient new data has been accumulated. Volume 21 saw a change in editor and in Volume 22 we see also a marked change in two of the five chapters presented. One chapter is devoted to one of the sophisticated physical techniques which has been used for the determination of novel structures of alkaloids and one concentrates on a specific chemical method which has proved valuable in alkaloid synthesis. Further reviews of this type are promised for future volumes, and hence it is of interest at this early stage to note the scope and contribution of these new departures.

The chapter on "Elucidation of Structural Formula, Configuration and Conformation of Alkaloids by X-Ray Diffraction" briefly outlines the experimental procedure, interpretation of data, and evaluation of the results. Applications to the problems of establishing structure, configuration, and conformation are described and then a series of examples are given. Among the examples used are a series of alkaloids from neotropical poison frogs, morphine agonists and antagonists, and some bisindole and bisditerpene alkaloids. The chapter illustrates quite clearly the dramatic effect which X-ray diffraction techniques have had on natural product chemistry by examples in the alkaloid field.

"Application of Enamide Cyclizations in Alkaloid Synthesis" is the title of the second departure from traditional "Manske and Holmes" chapters. This versatile synthetic method is discussed in detail, and the two techniques of photocyclization and thermalcyclization are described. Applications of the method to the synthesis of numerous alkaloids are given and include the Amaryllidaceae alkaloids, benzophenanthridines, protoberberines, yohimbines, and ergot alkaloids.

For those who may think that all is not well with the world, I should add that tradition has not been thrown completely out of the window and that their chapters on "golden oldies" such as the "Ipecac Alkaloids" and the imidazole alkaloids of the pilocarpine-type are still there. Both these areas continues to be the subject of considerable research and are of interest to medicinal chemists because of the range of pharmacological properties shown by these compounds.

The remaining chapter reviews in some detail the putrescine, spermidine, spermine, and related polyamine alkaloids. Such alkaloids occur as mixtures and are very difficult to separate and even when separated the results obtained from spectral or chemical analyses are equivocal. This timely review is a valuable contribution to our understanding of these alkaloids.

What of the change of approach? I welcome the new emphasis and the two new chapters bring the promise of further interesting reviews on the physical and chemical techniques which are so important in alkaloid chemistry.

School of Pharmacy
University of London
J. David Phillipson

Allenes in Organic Synthesis. By Herbert E. Schuster and Gary M. Cappola. Wiley-Interscience, New York. 1984. xvi + 358 pp. 17 × 24 cm. ISBN 0-471-87284-9. $47.50.

The purpose of the book, as stated by the authors in the preface, "is to describe the synthesis of a variety of functionalized allenes and their application to the preparation of a variety of interesting chemical intermediates and natural products", a coverage that is not adequately indicated by the title of the book. Overall, the authors have done an excellent job in accomplishing the stated purpose of the book.

Chapter 1, "Allenes: An Introduction", is a very short chapter (seven pages) that includes very brief sections on ^{1}H and ^{13}C chemical shifts and optical properties and chirality.

Chapter 2, "Alkyl, Aryl and Cyclic Allenes", describes the many methods of syntheses of these classes of substituted allenes.

Chapter 3, "Additions to Allene Hydrocarbons", describes reduction (hydrogenation) and electrophilic, nucleophilic, and miscellaneous additions. Interestingly, free-radical additions are not discussed. Cycloaddition and dipolar cycloaddition reactions are covered later in a separate chapter. Most of the references cited in this chapter are rather old (only a few being post-1980) and have been reviewed previously. The inclusion of this material, however, does provide for a complete and comprehensive review of the synthesis of substituted allenes.

Chapters 4–8 cover the syntheses and reactions of various types of substituted allenes: "Allenes Containing Unsaturated Substituents", Chapter 4; "Hydroxy and Oxo-Substituted Allenes", Chapter 5; "Allenic Acids and Their Derivatives", Chapter 6; "Hetero-Substituted Allenes", Chapter 7, including alkoxy- and acetoxy-substituted allenes, sulfur-substituted allenes having sulfur in various oxidation states, and nitrogen-, phosphorus-, silicon- and boron-containing functions; "Haloallenes", Chapter 8. The syntheses of many natural products containing these functions, or in which these functionalized allenes were used as synthetic intermediates, are described.

Chapter 9 is devoted to a discussion of cycloaddition reactions of substituted allenes, including allene–allene dimerizations, allene–ketene and allene–olefin cycloadditions, and allene–enone photochemical cycloadditions. The chapter concludes with a discussion of the synthesis of heterocyclic systems via dipolar cycloaddition reactions. These areas are the subject of active research today, and some of the statements appearing in this chapter are not correct in terms of results published in late 1983 and 1984. For example, in the section on allene–olefin cycloaddition reactions it is stated that "the stereochemistry about the alkene is retained in the cycloaddition". This is not universally