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Synthesis of Branched-Chain Apiosylpyrimidines and Their Inhibition of Lymphocyte Proliferation

Dilipkumar K. Parikh and Ronald R. Watson*

Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana 46202.
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Branched-chain nucleosides containing β-D-apio-L-furanose were synthesized by condensation of the bis(trimethylsilyl) derivative of uracil (**11**), thymine (**12**), 5-bromouracil (**13**), and 5-iodouracil (**14**) with the protected 1-*O*-acetylapiose in the presence of a Friedel–Crafts catalyst. D-Apio-L-furanosyluracil (**11**) and D-apio-L-furanosylthymine (**12**) show immunosuppressive activity for rat T-lymphocytes stimulated to grow by phytohemagglutinin but exhibit no inhibitory activity against herpes simplex virus. Compounds **11**–**14** did not inhibit herpes simplex virus replication, while low inhibition was obtained with the one nucleoside containing D-apio-D-furanose, D-apio-D-furanosyladenine. D-Apio-L-furanosyl-5-bromouracil (**13**), D-apio-L-furanosyl-5-iodouracil (**14**), bromouridine, and iodouridine suppressed growth of human lymphoblasts significantly more than the nonhalogenated apiosylpyrimidines.

Purine and pyrimidine nucleosides containing the sugar analogue arabinose have been reported to elicit a variety of biological effects, including antiviral and antilymphocyte activities. At the clinical level the suppression of herpes simplex and of lymphocyte replication has been realized.[1,2] In order to ascertain whether the introduction of sugar analogues other than arabinose likewise might lead to therapeutically useful agents, the synthesis and biological evaluation of D-apio-L-furanosylpyrimidines appeared worthy of investigation.

The chemistry and biochemistry of the branched-chain, naturally occurring sugar D-apiose (3-*C*-hydroxymethyl-aldotetrose) have recently been reviewed.[3] The presence of asymmetric centers at carbon 1, 2, and 3 results in eight possible apiose isomers. Apiosyl nucleosides with the L configuration at carbon atom 2 would have the stereochemistry which provides antiherpes and immunosuppressive activity of 1-β-D-arabinofuranosylcytosine (*ara*-C) and 9-β-D-arabinofuranosyladenine (*ara*-A).[1,2] The relationship of structure to function such as inhibition of viral growth in L-apiosyl nucleosides could not be attributed a priori to the L configuration at carbon 2. Structure–function relationships would be complicated by any effects on their biological activity of the hydroxymethyl group at carbon atom 3 and the D or L stereochemistry of the hydroxyl group on carbon atom 3. On the other hand, D-apiosyl nucleosides would differ structurally from ribosyl nucleosides only in the location and stereochemistry at carbon atom 3 of the hydroxymethyl group. Hence, any biological activity could be attributed to the constituents on carbon atom 3, their stereochemical location, and any effects on the overall configuration of the nucleoside. Other than slight bacteriostatic effects on the growth of the two bacteria tested, *Escherichia coli* and *Staphylococcus aureus*, by 9-[3-*C*-(hydroxymethyl)-β-L-threofuranosyl]adenine, D-Api-L-A, nothing is known of the effects of D-Api-L-A or D-Api-D-A, 9-[3-*C*-(hydroxymethyl)-β-D-erythrofuranosyl]adenine, on the replication of viruses, bacteria, lymphocytes, or other mammalian cells (Figure 1).[3,4] Therefore, we have synthesized several D-apiosyl nucleosides as potential inhibitors of lymphocyte proliferation or growth of herpes simplex virus, bacteria, and fungi.

Chemistry. Silylation of the corresponding pyrimidine base was performed by refluxing the base with hexamethyldisilazane (HMDS).[5,6] Excess hexamethyldisilazane was removed by vacuum distillation and the residue was used for condensation. 1,2-Di-*O*-acetyl-3,3′-di-*O*-benzyl-D-apio-L-furanose (**5**) and 1,2-di-*O*-acetyl-3,3′-*O*-carbonyl-D-apio-L-furanose (**6**) were prepared by published procedures.[7,8] Condensation of bis(trimethylsilyl)pyrimidine with **5** or **6** in the presence of stannic chloride according to the procedure of Niedballa and Borbruggen[9] gave blocked nucleosides. Condensation was monitored by TLC in ethyl acetate and showed the absence of anomeric nucleosides in each case. Removal of the blocking groups gave free nucleosides **11**–**14** (see Figure 2). Assignment of the anomeric configuration was made by NMR, mainly on the basis of the $J_{1',2'}$ coupling constant. The small coupling constant ($J_{1',2'}$) of about 2.0 Hz was indicative of a β-nucleoside,[10] which would be expected to result from 2-*O*-acyl group participation.[11]

Biological Activity. Lymphocyte viability was measured by trypan blue dye exclusion after 5 days of incu-

* Address correspondence to this author at the Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana 47907.

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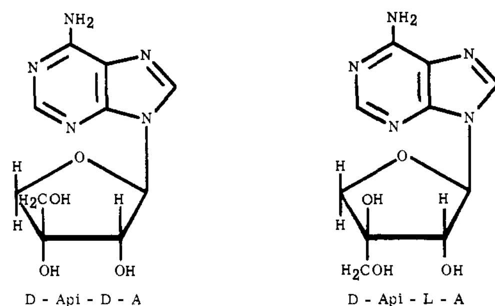

Figure 1.

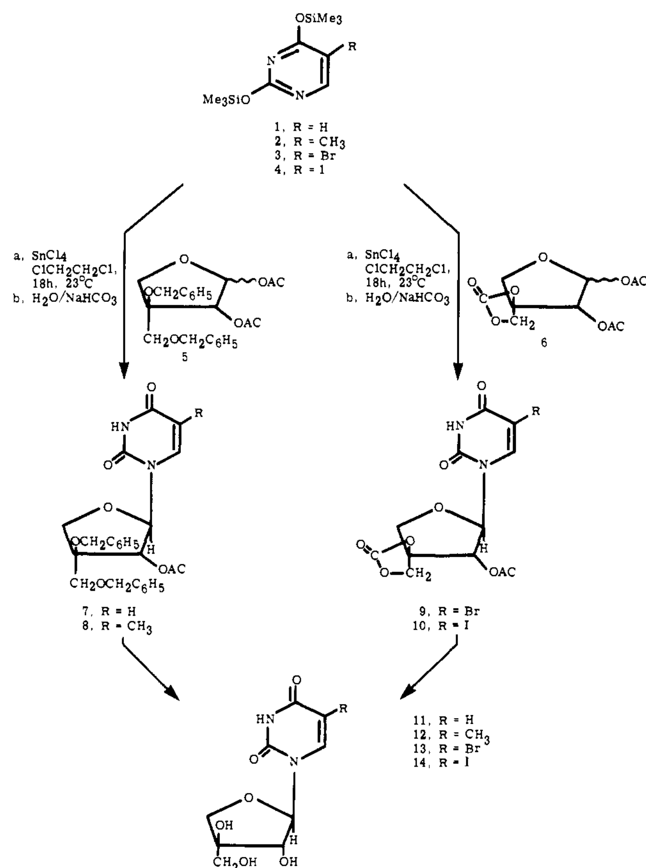

Figure 2.

bation of the nucleoside analogue in whole, heparinized rat blood, stimulated to divide by phytohemagglutinin (PHA). D-Api-L-A and D-Api-D-A did not suppress lymphocyte viability at 100 μM (Table I). Rat lymphocytes were stimulated by PHA to proliferate as described for primate lymphocytes. The assay was slightly modified by incubating 0.05 mL of heparinized rat blood in 10% fetal calf serum in 1 mL of RPMI 1640 culture media (Microbiological Associates) supplemented with herpes buffer, penicillin, and streptomycin at pH 7.35. The nucleosides and PHA were added on day 0 and cultured for 5 days at 37 °C. On day 4 tritiated thymidine containing 0.5 μCi (specific activity 20 mCi/μmol) was added and 24 h later DNA was collected from the T-lymphocytes in the culture which had been preferentially stimulated to divide by PHA. The DNA was washed and counted, and stimulation or suppression was determined as compared to controls which contained PHA but no nucleosides.[12] Cultures containing D-Api-L-A and D-Api-D-A had increased incorporation of [^{3}H]thymidine into DNA of rat T-lymphocytes. In contrast, nucleosides formed from coupling the sugar D-apio-L-furanose to pyrimidine bases, 11 and 12, as well as *ara*-C significantly inhibited T-lymphocyte

Table I. Effect of Nucleoside Analogues on [^{3}H]Thymidine Incorporation into DNA in Rat T-Lymphocytes Induced to Mitosis in Vitro by Phytohemagglutinin

Compd[a]	% of control, 1 μM
Control (no nucleoside addition)	100
ara-A	71
Ribavirin	127
9-β-D-Apio-L-furanosyladenine	14[b]
9-β-D-Apio-D-furanosyladenine	159[c]
ara-C	18[c]
11[c]	16[c]
12[c]	56[c]

[a] The percentage of viable lymphocytes after 6 days of incubation at 37 °C and 100 μM of each compound were as follows: *ara*-C (76%), *ara*-A (94%), 11 (90%), 12 (89%), control (93%), D-Api-L-A (85%), and D-Api-D-A (91%). [b] Geometric mean value which was significantly different from control ($p < 0.05$). DPM of [^{3}H]thymidine in control were 4808 ± 455. Tritiated thymidine containing 0.5 μCi (specific activity 20 mCi/μmol) was added to each culture 24 h before lymphocyte harvesting. [c] No significant inhibition was noted at 0.1 and 0.01 μM. 13 and 14 were not tested.

Table II. Growth Inhibition of Human MGL8 Lymphoblasts by Apiosylpyrimidines and Halogenated Ribonucleotides

Compd[a, b]	% of control	
	50 μM	500 μM
Control	100	100
11	100	72[c]
12	95	83[c]
13	52[c]	13[c]
5-Bromouridine	40[c]	11[c]
14	24[c]	6[c]
5-Iodouridine	32[c]	9[c]

[a] None of the compounds showed inhibition of growth at 5 μM. [b] Cultures were begun with 22 × 10^4 cells/mL and incubated for 48 h reaching 89 × 10^4 cells/mL in the control. [c] Mean value was significantly different from control ($p < 0.01$).

proliferation at 1 μM as shown in Table I.

Since the apiosylpyrimidines inhibited lymphocyte proliferation, inhibition of human lymphocyte division by compounds 11–14 was investigated in a lymphoblast cell line MGL-8. Stationary suspension cultures of 22 × 10^4 cells/mL were initiated in RPMI 1640 media containing 10% fetal calf serum, penicillin, and streptomycin. Cells were counted in a Coulter counter after 48 h of incubation at 37 °C. As shown in Table II, compounds 11–14, as well as two halogenated analogues of D-ribosylpyrimidines (5-iodouridine and 5-bromouridine), caused significant reduction in cell growth at 50 and 500 μM. At each concentration, the halogenated apiosyl compounds, 13 and 14, showed greater inhibition of cell growth than the nonhalogenated ones, 11 and 12.

Baby hamster kidney (BHK) cells were cultured with herpes simplex virus type 2 in confluent monolayers in 24 well culture trays.[1] After removal of the supernatant fluid, 50 μL of the appropriate dilution of virus was added to each well. The plates were incubated for 2 h with intermittent agitation at 37 °C in 5% CO_2–95% air and 100% humidity. One milliliter was added to each well of Eagle's medium containing tryptose phosphate broth and calf serum. After 24 h viral plaques were counted. D-Api-L-A (4 μM) significantly reduced the herpes simplex plaques (42%). The nucleosides 11–14 and D-Api-L-A which all contained the sugar, D-apio-L-furanose, did not

even at concentrations up to 400 μM.

Gram-negative and gram-positive bacteria as well as fungi were grown on agar plates at 37 °C.[13] Compounds **9–12** were diluted to 1 mg/mL of methanol and placed on a 2-cm disk which was deposited on an organism streaked on the agar plate. None of the samples tested suppressed growth of the gram-positive organisms (*Staphylococcus aureus*, *Bacillus subtilis*, and *Sarcina lutea*), the gram-negative organisms (*Proteus vulgaris*, *Salmonella gallinarum*, *Escherichia coli*, *Pseudomonas aeruginosa*, *P. solanocearum*, and *Serratia marcencens*), or the fungi (*Saccharomyces pastorionus*, *Neurospora Crassa*, and *Candida albicans*) studied.

Since all D-apio-L-furanosylpyrimidines tested (Tables I and II) inhibited mammalian cell division, the location of the hydroxymethyl group and/or the stereochemical configuration at carbon atom 3 of apiose was associated with growth reduction. However, as both D-apio-D-furanose and D-apio-L-furanose attached to adenine did not inhibit rat T-lymphocyte division, the importance of the configuration at carbon atom 3 is unclear in apiosylpyrimidines which inhibit mammalian cell division. A significant difference in herpes simplex virus growth inhibition by these adenosine analogues was observed, so this inhibition may be attributed to the placement of the hydroxymethyl group on carbon atom 3.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. The proton magnetic resonance spectra were obtained on a 60-MHz Perkin-Elmer R-24B NMR spectrometer and on a 100-MHz Varian HA-100 spectrometer with Me_4Si as an internal standard. UV spectra were measured on a Beckmann Model 24 spectrophotometer. Mass spectra were obtained on a LKB-9000-S. The infrared spectra were determined with a Perkin-Elmer infrared spectrophotometer. The elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, Ind.

1-*O*-β-D-Apio-L-furanosyluracil (11). To a solution of 1,2-di-*O*-acetyl-3,3′-di-*O*-benzyl-D-apio-L-furanose (5, 6.22 g, 0.015 mol) in 1,2-dichloroethane (50 mL) was added a solution of bis(trimethylsilyl)uracil (1, 5.08 g, 0.0195 mol) in 1,2-dichloroethane (50 mL) with stirring and exclusion of moisture. The reaction mixture was stirred at room temperature for 18 h, then treated with saturated aqueous sodium hydrogen carbonate (300 mL) and extracted with chloroform (3 × 100 mL). The combined extracts were washed with water, dried (Na_2SO_4), and concentrated under reduced pressure to give 5.92 g (85%) of 7 as an oil, $[\alpha]^{20}_D$ −47° (*c* 2.1, $CHCl_3$).

To a solution of 7 (5.56 g, 0.012 mol) in 200 mL of dry methanol was added 1 g (0.02 mol) of NaOMe. The reaction mixture was refluxed for 3 h and then neutralized with Dowex 50W-X8 (H^+) resin. Solvent was removed by vacuum distillation to give 4.2 g (83%) of 3,3′-di-*O*-benzyl-D-apio-L-furanosyluracil. Hydrogenolysis of 3,3′-di-*O*-benzyl-D-apio-L-furanosyluracil gave the free nucleoside 11. Palladium chloride (1.5 g) was suspended in 50 mL of methanol and reduced by shaking with hydrogen at room temperature and 3 atm of pressure. A solution of 3,3′-di-*O*-benzyl-D-apio-L-furanosyluracil (4.2 g, 0.01 mol) in 100 mL of methanol was added to the acidic suspension. The reaction mixture was reduced with hydrogen at 3 atm of pressure for 3 h and neutralized with Dowex 2-X8 (HCO_3^-) after removal of the catalyst. The solvent was removed by vacuum distillation to give 2.0 g (84%) of 11 in a crystalline form. 11 was recrystallized twice from ethanol–ethyl acetate to give the analytical sample: mp 176–178 °C; $[\alpha]^{20}_D$ +73° (*c* 1.0, H_2O); UV λ max (EtOH) 261 nm (ε 10770); NMR (Me_2SO-d_6) δ 5.65 (d, 1, $J_{1',2'}$ = 1.5 Hz, H-1′), 3.94 (d, 1, H-2′), 3.56 (d, 2, H-3″), 3.9 and 4.03 (2 d, 2, $J_{4',4'}$ = 9.5 Hz, H-4′), 5.6 (d, 1, $J_{5,6}$ = 9.0 Hz, H-5), 7.75 (d, 1, *J* = 9.0 Hz, H-6). Anal. ($C_9H_{12}N_2O_6$) C, H, N.

1-*O*-β-D-Apio-L-furanosylthymine (12). To 5 (5.49 g, 0.132 mol) in 1,2-dichloroethane (50 mL) was added bis(trimethylsilyl)thymine (2, 4.05 g, 0.0149 mol) in 1,2-dichloroethane (50 mL) and $SnCl_4$ (5 mL) in 1,2-dichloroethane (50 mL). After 13 h at room temperature and a workup similar to the preparation of 7, 5.12 g (81%) of 8 was obtained as an oil: $[\alpha]^{20}_D$ −48° (*c* 1.9, $CHCl_3$).

The protecting groups were removed from 8, following the same procedure as deblocking of 7, to give 2.160 g (90%) of 12. The crude product was recrystallized twice from ethanol–ethyl acetate to give the analytical sample: mp 199–200 °C; $[\alpha]^{20}_D$ +26° (*c* 1.0, H_2O); UV λ max (EtOH) 266 nm (ε 9700); NMR (Me_2SO-d_6) δ 5.68 (d, 1, $J_{1',2'}$ = 2.0 Hz, H-1′), 3.95 (d, 1, H-2′), 3.57 (d, 2, H-3″), 3.9 and 4.03 (2 d, 2, $J_{4',4'}$ = 9.0 Hz, H-4′), 1.78 (s, 3, CH), 7.63 (d, 1, H-6). Anal. ($C_{10}H_{14}N_2O_6$) C, H, N.

1-*O*-β-D-Apio-L-furanosyl-5-bromouracil (13). To a solution of 1,2-di-*O*-acetyl-3,3′-*O*-carbonyl-D-apio-L-furanose (6, 1.301 g, 0.005 mol) in 1,2-dichloroethane (50 mL) was added a solution of bis(trimethylsilyl)-5-bromouracil (3, 2.0 g, 0.006 mol) in 1,2-dichloroethane (50 mL). To the mixture stannic chloride (1 mL) in 1,2-dichloroethane (20 mL) was added dropwise and stirred at room temperature for 20 h; then the mixture was treated with saturated aqueous sodium hydrogen carbonate (100 mL). The reaction mixture was filtered through Celite, the organic layer was dried (Na_2SO_4), and evaporation of the solvent afforded 0.815 g (42%) of 9. To a solution of 0.8 g (0.002 mol) of 9 in 200 mL of dry methanol was added 0.5 g of NaOMe. The reaction mixture was refluxed for 2 h with the exclusion of moisture and then neutralized with Dowex 50W-X8 (H^+) resin. Solvent was removed by vacuum distillation to give 0.6 g (93%) of 13. It was recrystallized from ethanol to give the analytical sample: mp 203–205 °C; $[\alpha]^{20}_D$ −10° (*c* 0.5, H_2O); UV λ max (EtOH) 275 nm (ε 8600); NMR (Me_2SO-d_6) δ 5.64 (d, 1, $J_{1',2'}$ = 1.5 Hz, H-1′), 3.94 (d, 1, H-2′), 3.57 (d, 2, H-3″), 3.9 and 4.05 (2, d, 2, $J_{4',4'}$ = 9.0 Hz, H-4′), 8.08 (s, 1, H-6). Anal. ($C_9H_{11}N_2O_6Br$) C, H, N.

1-*O*-β-Apio-L-furanosyl-5-iodouracil (14). To a solution of 5 (1.22 g, 0.003 mol) in 1,2-dichloroethane (50 mL) was added a solution of bis(trimethylsilyl)-5-iodouracil (4, 1.22 g, 0.0032 mol) in 1,2-dichloroethane (50 mL) and $SnCl_4$ (0.5 mL) in 1,2-dichloroethane (10 mL). After 20 h at room temperature and workup similar to 13, 0.720 g (54%) of 10 was obtained. Deblocking of 10 with NaOMe in methanol gave 0.560 g (92%) in 14, which was recrystallized from ethanol to give an analytical sample: mp 203–204 °C; $[\alpha]^{20}_D$ −37° (*c* 0.5, H_2O); UV λ max (EtOH) 280 nm (ε 6600); NMR (Me_2SO-d_6) δ 5.65 (d, 1, $J_{1',2'}$ = 1.5 Hz, H-1′), 3.94 (d, 1, H-2′), 3.57 (d, 2, H-3″), 3.9 and 4.05 (2 d, 2, $J_{4',4'}$ = 9.0 Hz, H-4′), 8.12 (s, 1, H-6). Anal. ($C_6H_{11}N_2O_6I$) C, H, N.

9-*O*-β-D-Apio-L-furanosyl- and 9-*O*-β-D-Apio-D-furanosyladenine. β-D-Apio-L-furanose was coupled to adenine by procedures described by Tronchet and Tronchet.[14] Authentic apiosylpurines were kindly supplied by Dr. J. M. Tronchet.

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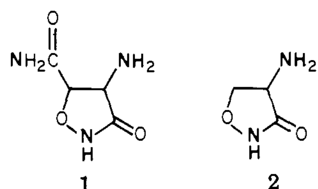
5-Carboxamido-4-amino-3-isoxazolidone, an Asparagine Analogue

Charles H. Stammer* and Masayuki Sato

Department of Chemistry, University of Georgia, Athens, Georgia 30602. *Received December 27, 1977*

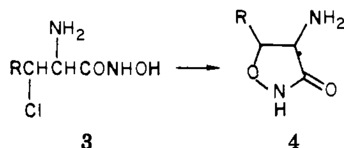
trans-Aziridine-2,3-dicarboxylic ester was used to prepare the required β-chlorohydroxamic acid used in the synthesis of the title compound. The trans configuration of the asparagine analogue was established by hydrogenolysis to *erythro*-β-hydroxyasparagine amide. Neither the title compound nor the intermediate aziridinehydroxamic acid (**8**) showed significant activity against the L1210 and P-388 tumors. The title compound was inactive as an inhibitor of asparagine synthetase from Novikoff hepatoma and did not inhibit the growth of some 25 bacteria and fungi.

The structural analogy among the title compound **1**,



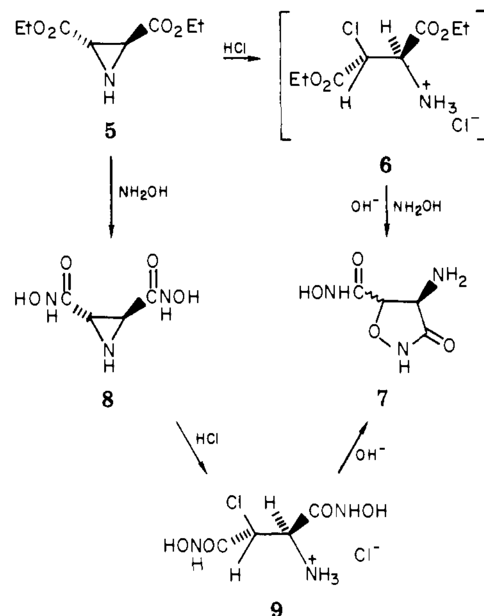
asparagine, and cycloserine (**2**) led us to prepare **1** and to investigate its antibacterial and anticancer activities.

The key intermediates in the synthesis of 5-substituted 3-isoxazolidones (**4**) are β-chlorohydroxamic acids such as



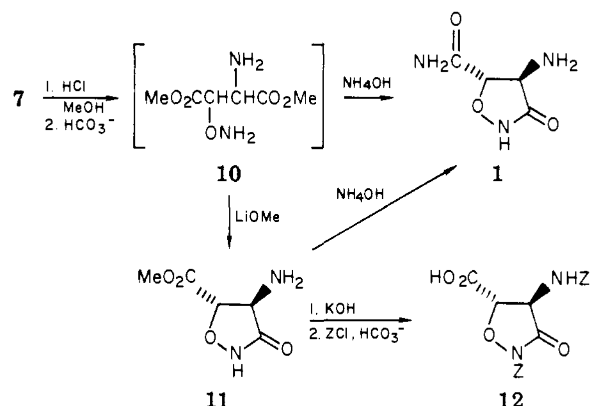
3 which can be cyclized[1-3] readily under basic conditions. Chloro compounds of this type have been previously obtained from oxazolines,[1] β-hydroxyamino acids,[2] and aziridines.[3] In our work toward **1**, we examined both a route using β-hydroxyaspartic acid and an oxazoline-4,5-dicarboxylic ester[4] as approaches to **3** without success. Since the aziridine-2,3-dicarboxylic ester **5** required in the Smrt[3] synthesis has recently been reported,[5] albeit without experimental details, this approach was examined more recently. Scheme I outlines the reaction sequence which yielded the desired product **1**.

Scheme I



The crystalline *trans*-aziridine (**5**) was obtained in 18% yield (from diethyl maleate) by the procedure of Berlin[5] et al., who obtained it as an oil having spectroscopic properties similar to our pure material. This intermediate was converted into the hydroxamic acid **7** by two methods. Excess hydroxylamine in the presence of methoxide reacted with **5** to form the dihydroxamic acid **8** in 83% yield. Dry hydrogen chloride in dimethoxyethane (DME) converted **8** into a chloro compound **9** which crystallized as a DME complex and was characterized spectroscopically. Treatment of **9** with aqueous base gave the cyclic hydroxamic acid **7**. This substance, probably a mixture of stereoisomers, was an amorphous solid which showed positive ninhydrin and ferric chloride tests and, more importantly, a positive nitroprusside test which is specific[6] for the isoxazolidone ring. Alternatively, the aziridine **5** could be converted as shown in Scheme I, without isolation of the intermediate oily chloro compound **6**, directly into **7**. The overall yields of **7** by the two processes were comparable and we felt that the route through **8** and **9** served to confirm the structure of **7** since we were unable to get satisfactory elemental analyses for it.

Scheme II



The hydroxamic acid **7** readily underwent methanolysis (Scheme II) giving an equimolar mixture of the aminooxy diester **10** dihydrochloride and hydroxylamine hydrochloride. A cold aqueous solution of this mixture was neutralized and **10** was quickly extracted into chloroform. The oily **10** could then be converted directly into the desired amide **1** in approximately 50% yield by treatment with concentrated ammonium hydroxide. Complete

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