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SYNTHESIS OF 2'-O-NITRO-9-β-D-ARABINOFURANOSYLADENINE AND 2'-O-NITRO-9-β-D-ARABINOFURANOSYLHYPOXANTHINE

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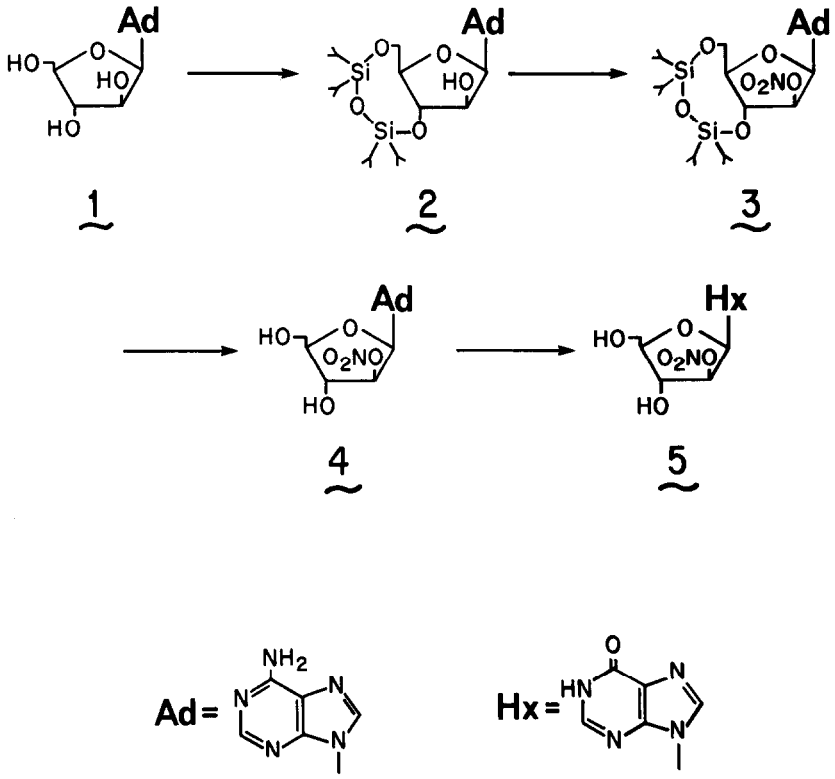
Summary: Nitration of 9-[3,5-O-(tetraisopropyldisiloxanyl)-β-D-arabinosyl]adenine, followed by removal of the protecting function, affords 2'-O-nitro-9-β-D-arabinofuranosyladenine. The latter has been deaminated enzymatically to give 2'-O-nitro-9-β-D-arabinofuranosylhypoxanthine.

The arabinonucleosides, including 1-β-D-arabinofuranosylcytosine (ara-C)[1,2] and 9-β-D-arabinofuranosyladenine (ara-A, 1)[3], comprise a class of anticancer drugs that are rapidly inactivated by enzymatic deamination. Attempts to circumvent the problem of inactivation have included structural modification of the two drugs[4-13] as well as development of deaminase inhibitors to be used in combination with these drugs.[14-17] Substitution with certain functional groups at the 2'-position of ara-C[5,6] and ara-A[11,12] not only alters the susceptibility of these compounds to enzymatic deamination but also modifies their antitumor activity. To this end, we recently introduced a neutral O-nitro group at the 2'-position of ara-C.[4] The resulting compound, 2'-O-nitro-1-β-D-arabinofuranosylcytosine (nitrara-C), not only is resistant to degradation by enzymatic deamination but also has antileukemic activity as measured in cell cultures and in animal models.[4] We now report the synthesis of 2'-O-nitro derivatives of ara-A (1) and 9-β-D-arabinofuranosylhypoxanthine (ara-H): 2'-O-nitro-9-β-D-arabinofuranosyladenine (nitrara-A, 4) and 2'-O-nitro-9-β-D-arabinofuranosylhypoxanthine (nitrara-H, 5).

We described previously that O-nitro groups can be introduced, by nitration at low temperature, to a selective position in the sugar moiety of a nucleoside in which other hydroxyl groups of the sugar moiety have been protected by acyl groups.[4,18] Mild alkali solvolysis will then remove the acyl groups without affecting the O-nitro function.[4,18] The report that O-nitration of 2',3'-O-isopropylidene derivatives of nucleosides gave exclusively 5'-O-nitronucleosides[19] suggested to us that O-nitration of arabinosides, with 3'- and 5'-hydroxyl functions simultaneously protected, could, more efficaciously, yield the desired 2'-O-nitro products. Recently, a new method was developed for the simultaneous protection of 3'- and 5'-hydroxyl functions in several ribonucleosides[20-23] and cytosine arabinoside.[24] The novel aspect of this method is based on the use of a hindered bifunctional disiloxane reagent, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, which reacts very rapidly with the primary 5'-hydroxyl function and then, intramolecularly, more slowly with the secondary 3'-hydroxyl group to form a ring structure.[20-24] Accordingly, it was hoped that this new procedure of simultaneously protecting 3'- and 5'-hydroxyl functions would be applicable to purine arabinonucleosides, thus enabling us to selectively nitrate the 2'-hydroxyl function of ara-A (1).



Ara-A (1) was reacted with 1.10 molar equivalents of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in anhydrous pyridine at room temperature for 3 h. After work-up, the product was purified by "flash" chromatography[25] over silica gel with CH_2Cl_2:i-PrOH (4:1) at a relatively high eluant flow rate of ≈65 ml/min. The product, 9-[3,5-O-(tetraisopropyldisiloxanyl)-β-D-arabinofuranosyl]adenine (2), was crystallized from 95% EtOH-water (82% yield): mp 97-98.6°C; UV λ_{max} (95% EtOH) 259 nm (ε 15100); ^{1}H NMR (Me_2SO-d_6 + D_2O) δ 1.08 (m, 28, i-Pr), 4.55 (m, 1, H-2'), 6.19 (d, $J_{1',2'}$ = 5.9 Hz, 1, H-1'), 7.98 (s, 1, H-2), 8.03 (s, 1, H-8). IR (KBr) ν 1250 cm^{-1}.

The N-glycosidic bond in 2 is unaffected by fuming nitric acid at -70°C, but the 3',5'-O-tetraisopropyldisiloxanyl functional group is unstable to even very brief exposure to such conditions. Hence, acetic nitric anhydride, generated in a 4:1 volume mixture of acetic anhydride and nitric acid as described by Lichtenthaler[19], was used as the nitrating agent. Reacting 2 with 4:1 acetic anhydride-60% nitric acid at 0°C for 30 min in the presence of urea (to protect the amino group of the adenine moiety against the effect of nitrous acid) gave 9-[2-O-nitro-3,5-O-(tetraisopropyldisiloxanyl)-β-D-arabinofuranosyl]adenine (3), which was purified by "flash" chromatography[25] over silica gel using CH_2Cl_2-CH_3COCH_3 (10:1). Crystallization of the

product from MeOH gave 3 (34% yield): mp 76.5-78°C; UV λ_{max} (95% EtOH) 259 nm (ε 13580); ^{1}H NMR (Me_2SO-d_6 + D_2O) δ 1.08 (m, 28, i-Pr), 6.01 (m, 1, H-2'), 6.58 (d, $J_{1',2'}$ = 6.7 Hz, 1, H-1'), 8.03 (s, 1, H-2), 8.10 (s, 1, H-8). IR (KBr) ν 1665, 1610, 1285, 1250 cm^{-1}.

The disilyl protecting group in 3 was then removed by the action of tetra-n-butylammonium fluoride in tetrahydrofuran (THF) at room temperature for 30 min. After purification by short column chromatography over silica gel (THF followed by ethyl acetate) and then crystallization from water, pure nitrara-A (4) was obtained (60% yield): mp >175°C (dec.); UV λ_{max} (0.1 N HCl) 256 nm (ε 14440), (0.1 N NaOH) 258 nm (ε 14160); ^{1}H NMR (Me_2SO-d_6 + D_2O) δ 5.83 (dd, $J_{1',2'}$ = 6.2 Hz, $J_{2',3'}$ = 7 Hz, 1, H-2'), 6.63 (d, $J_{1',2'}$ = 6.2 Hz, 1, H-1'), 8.21 (s, 1, H-2), 8.40 (s, 1, H-8). IR (KBr) ν 1640, 1610, 1290, cm^{-1}.

A suspension of nitrara-A (4) in triethylamine bicarbonate buffer (pH = 7.4) was treated at 37°C for 24 h with an excess amount of adenosine deaminase, type I, from calf intestinal mucosa (Sigma Chemical Co.). After evaporation, the residue was reconstituted in water and treated with cation exchange resin (BioRad AG 50W x 8/H^+) and then with anion exchange resin (BioRad AG 3/OH^-). Crystallization from EtOH gave nitrara-H (5) (75% yield): mp 124.9-125.9°C; UV λ_{max} (0.1 N HCl) 248 nm (ε 10570), (0.1 N NaOH) 252 nm (ε 11910); ^{1}H NMR (Me_2SO-d_6 + D_2O) δ 5.83 (dd, $J_{1',2'}$ = 6.1 Hz, $J_{2',3'}$ = 7 Hz, 1, H-2'), 6.60 (d, $J_{1',2'}$ = 6.1 Hz, 1, H-1'), 8.12 (s, 1, H-2), 8.28 (s, 1, H-8). IR (KBr) ν 1655, 1280 cm^{-1}.

All new compounds gave satisfactory elemental analyses for carbon, hydrogen, and nitrogen, and silicon when applicable. ^{1}H NMR spectra of the nitrate esters of nucleosides are, in many instances, amenable to unambiguous interpretation. As expected, and as previously reported,[4,19,26,27] O-nitration causes a downfield shift (about 1.5 ppm), with respect to the parent OH-compound, of the signal of the proton attached to the same carbon as the nitrate group.

Nitrara-A (4) is substantially less susceptible to enzymatic deamination than ara-A (1).[28] Interestingly, both nitrara-A (4) and its deaminated product, nitrara-H (5), appreciably inhibited the proliferation of human leukemic lymphoblasts and their arabinonucleoside-resistant variants.[28] Further evaluations of the biologic properties of these new compounds are in progress.

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