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The Synthesis and Antiviral Properties of 8-Amino-3-[(2-hydroxyethoxy)methyl]-1,2,4-triazolo-[4,3-*a*]pyrazine

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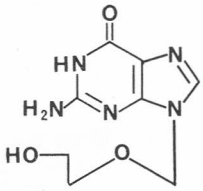
The preparation of 8-amino-3-[(2-hydroxyethoxy)methyl]-1,2,4-triazolo[4,3-*a*]pyrazine (*IV*) as an analogue of 9-[(2-hydroxyethoxy)methyl]guanine and 9-(S)-(2,3-dihydroxypropyl)adenine is described from the reaction of 3-chloro-2-hydrazinopyrazine (*V*) and ethyl 2-(2-acetoxyethoxy)thioacetimidate (*IXg*) followed by treatment with ammonia. Compound *IV* was found to lack antiviral properties towards herpes simplex I and II, vaccinia virus, vesicular stomatitis virus, measles, reovirus type 1, parainfluenza virus type 3, Sindbis virus, Coxsackie type B4 virus, and poliovirus type 1.

9-[(2-Hydroxyethoxy)methyl]guanine (*I*)[1] and 9-(S)-(2,3-dihydroxypropyl)-adenine (*II*)[1c,2] have been shown to possess interesting antiviral activity and, as a consequence, have provided an impetus for the search for other biologically active »partial« nucleosides.[1c,1d,3] Arising from our studies with the nucleoside *III*,[4] this paper reports the synthesis and antiviral activity of 8-amino-3-[(2-hydroxyethoxy)methyl]-1,2,4-triazolo[4,3-*a*]pyrazine (*IV*) which incorporates a base unit isomeric to the base unit of *II* and a side-chain isomeric to the side-chain of *I*.

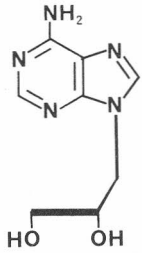
Chemistry

1,2,4-Triazolo[4,3-*a*]pyrazines are most easily constructed[5] by reacting hydrazinopyrazines with ortho esters or thioimidates. Thus, 3-chloro-2-hydrazinopyrazine (*V*)[6] was treated with trimethyl ortho(2-chloroethoxy)acetate (*VI*) to give 8-chloro-3-[(2-chloroethoxy)methyl]-1,2,4-triazolo[4,3-*a*]pyrazine (*VII*). Ammonolysis of *VII* produced *VIII* but numerous attempts at converting *VIII* into *IV* were unsuccessful, often giving extremely hygroscopic products.

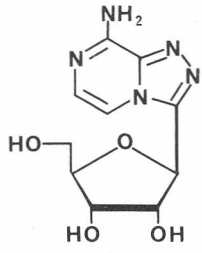
Attention then turned to having the side-chain hydroxyl, or a protected form of it, in place at the time of ring closure with *V*. This necessitated uncovering a route to the appropriate nitrile (*IXa*) for transformation to the



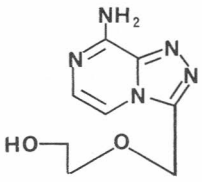
I



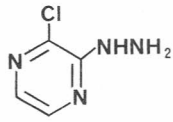
II



III



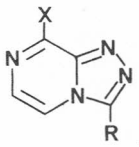
IV



V

$ClCH_2CH_2OCH_2C(OMe)_3$

VI



VII; X=Cl, R=$CH_2OCH_2CH_2Cl$

VIII; X=NH_2, R=$CH_2OCH_2CH_2Cl$

X; X=SEt, R=$CH_2OCH_2CH_2OAc$

$ROCH_2CH_2OCH_2X$

IXa; X=CN, R=Me or Bzl

b; X=Cl, R=Me or Bzl

c; X=I, R=$SiMe_3$

d; X=CN, R=$SiMe_3$

e; X=Cl, R=Ac

f; X=CN, R=Ac

g; X=C(SEt)=NH_2Cl, R=Ac

ortho ester or the thioimidate. In this regard, attempts to displace the chloro substituent of *IXb* (prepared from ethylene glycol monomethyl or monobenzyl ether and paraformaldehyde in the presence of anhydrous hydrogen chloride)[7a,b] with cyanide ion failed under a variety of conditions.[7c] Similar problems were encountered with efforts to react *IXc*[8] with cyanide and upon treatment of 1,3-dioxolane with trimethylsilyl cyanide[9] in hopes of obtaining *IXd*.

Success was achieved, however, when 1,3-dioxolane was reacted with acetyl chloride to give *IXe* which, following reaction with cuprous cyanide, was transformed into *IXf*. The ortho ester derivative of *IXf via* the Pinner procedure could not be achieved. As a consequence, the thioimidate *IXg* was prepared by reacting *IXf* with ethanethiol in the presence of anhydrous hydrogen chloride. Heating *IXg* with *V* in pyridine gave 3-[(2-acetoxyethoxy)--methyl]-8-ethylthio-1,2,4-triazolo[4,3-*a*]pyrazine (*X*). Conversion of *X* into the desired *IV* was accomplished by heating with ammonia at 127 °C for 22 hours (any less reaction time or a lower temperature led to deacetylation of *X* without substitution of the ethylthio group at C-8).

Biological Results

Compound *IV* did not display any antiviral potential against herpes simplex I, herpes simplex II, vaccinia virus and vesicular stomatitis virus, measles, reovirus type 1, parainfluenza virus type 3, Sindbis virus, Coxsackie type B4 virus and poliovirus type 1 as evidenced by a minimum inhibitory concentration[10] of 200 μg/mL or greater.[11]

EXPERIMENTAL

General Methods

All melting points were obtained on a Thomas-Hoover or a Mel-Temp melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman AccuLab 3 spectrophotometer. The ^{1}H NMR spectra were determined at 60 MHz with a Varian EM-360 spectrometer and are reported in parts per million downfield from tetramethylsilane as an internal standard. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). The silica gel used for the separation was Baker 60—200 mesh. Elemental analyses were performed by M-H-W Laboratories, Phoenix, Arizona.

8-Chloro-3-[(2-chloroethoxy)methyl]-1,2,4-triazolo[4,3-a]pyrazine (VII)

Paraformaldehyde (44.8 g) and 2-chloroethanol (100 mL, 120.1 g, 1.49 mol) were cooled in an ice bath. Anhydrous hydrogen chloride was bubbled into the solution for 1.5 h. The organic and aqueous layers were separated, and the organic layer dried over calcium chloride. The organic portion was concentrated by distillation under a stream of dry nitrogen and to the residue was added a mixture of dry cuprous cyanide (150 g, 1.67 mol) in dry benzene (600 mL). This mixture was refluxed for 5 h, filtered, and the benzene removed by evaporation. The dark residue was vacuum distilled through a 10-inch Vigreux column and the second fraction (b. p. 95—97 °C/1 mm) was collected to give 59.31 g (0.496 mol, 33%) of 2-(2-chloroethoxy)acetonitrile: ^{1}H NMR (neat) δ 3.74 (m, 4 H, CH_2CH_2), 4.3 (s, 2H, OCH_2CN).

Anhydrous hydrogen chloride was next bubbled into a solution of dried methanol (5.6 mL), 2-(2-chloroethoxy)acetonitrile (14 g, 0.117 mol), and dry diethyl ether (40 mL) for 15 min under ice water cooling. This mixture was placed in a freezer for one day and the liquid removed by decantation. The solid remaining was washed with anhydrous diethyl ether and the ether removed by evaporation. Dry methanol (50 mL) was added to the residue and the solution allowed to stand for one day. The methanol was removed by evaporation and anhydrous diethyl ether added. This mixture was filtered to remove the ammonium chloride, and anhydrous potassium carbonate and molecular sieves (3 A) were added to the filtrate. After three days, this mixture was filtered and the ether again removed by evaporation. Petroleum ether was added to the residue. The amide by-product was removed by filtration, and the petroleum ether evaporated to give 8.25 g (0.042 mol, 36%) of trimethyl ortho(2-chloroethoxy)acetate (*VI*) as a clear, colorless liquid of sufficient purity for use in the next step: ^{1}H NMR (neat) δ 3.22 (s, 9 H, OCH_3), 3.69 (m, 4 H, CH_2CH_2), 4.12 (s, 2 H, CH_2).

A mixture of *VI* (15 g, 75.5 mmol) and *V*[6] (2.5 g, 17.29 mmol) was refluxed in dry xylene (20 mL) for 3 h. Hexane was added to the solution and the resulting precipitate isolated by filtration. The crude solid (2.6 g) was purified by recrystallization from methylene chloride/pentane (charcoal) to give 2.14 g (8.66 mmol, 50%) of *VII* as yellow plates: m. p. 113—115 °C; IR (KBr) 1610 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.7 (m, 4 H, CH_2CH_2), 5.12 (s, 2 H, CH_2), 7.7 (d, J = 5 Hz, 1 H, H-6), 8.29 (d, J = 5 Hz, 1 H, H-5).

Anal. $C_8H_8Cl_2N_4O$ (247.08) Calc'd.: C 38.89; H 3.26; N 22.68%
Found: C 38.89; H 3.26; N 22.54%

8-Amino-3-[(2-chloroethoxy)methyl]-1,2,4-triazolo[4,3-a]pyrazine (VIII)

A mixture of *VII* (1.56 g, 6.31 mmol) in 70 mL of ethanol containing 20 mL of anhydrous ammonia was heated at 70 °C for 24 h in a stainless steel sealed

reaction vessel. After cooling, the vessel was opened, and the solid obtained by filtration and washed with ethanol and 2-propanol to give 0.78 g of a pale yellow solid. The solid was recrystallized twice from water (charcoal) to give 0.25 g of a white solid which, following recrystallization from 2-propanol, gave 0.22 g (0.996 mmol, 15.3%) of *VIII* as white crystals: m. p. 190—191 °C; IR (KBr) 3280 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.73 (s, 4 H, CH_2CH_2), 5.0 (s, 2 H, CH_2), 7.25 (d, $J = 5$ Hz, 1 H, H-6), 7.44 (br, 2 H, NH_2), 7.68 (d, $J = 5$ Hz, 1 H, H-5).

Anal. $C_8H_{10}ClN_5O$ (227.65) Calc'd.: C 42.21; H 4.43; N 30.76%
Found: C 42.14; H 4.31; N 31.00%

8-Ethylthio-3-[(2-acetoxyethoxy)methyl]-1,2,4-triazolo[4,3-a]pyrazine (X)

Acetyl chloride (44 mL, 48.58 g, 0.619 mol) was added to a mixture of 1,3-dioxolane (48 mL, 50.88 g, 0.687 mol) and dry methanol (0.9 mL, 0.72 g, 0.022 mol). The resultant solution was allowed to stand at room temperature for 10 days. It was then added dropwise into a refluxing mixture of cuprous cyanide (80 g, 0.893 mol) in dry benzene (300 mL). The mixture was then refluxed for 7 h and concentrated under reduced pressure to 62.96 g (0.44 mol, 71%) of *IXf* as a clear colorless liquid which was used directly in the next step: ^1H NMR (neat) δ 2.01 (s, 3 H, CH_3), 3.74 (t, $J = 6$ Hz, 2 H, OCH_2), 4.2 (t, $J = 6$ Hz, 2 H, OCH_2), 4.54 (s, 2 H, OCH_2CN).

To an ice chilled mixture of *IXf* (10 mL, 10.24 g, 72 mmol) in ethanethiol (5.1 mL, 4.28 g, 70 mmol) and dry diethyl ether (90 mL) was added anhydrous hydrogen chloride over a period of 15 min. This mixture was placed in a freezer overnight and then evaporated, under vacuum, to an oil, assumed to be *IXg*. Dry pyridine (100 mL) and *V* (6 g, 41.5 mmol) were added to the oil and the mixture stirred for 6 h at room temperature. The mixture was then filtered, and the solids obtained were washed with pyridine and methylene chloride. The filtrate was evaporated to a black residue and the residue was dissolved in methylene chloride, treated with charcoal, and filtered. The dark filtrate was placed on a silica gel column and found to give a dark oil after eluting with methylene chloride-acetone (9 : 1). The oil crystallized on standing to give 6.35 g of a brown material which was dissolved in hot toluene, treated with charcoal, filtered hot, cooled to room temperature, and treated with hexane which caused precipitation. The resultant precipitate was isolated by filtration and recrystallized from aqueous methanol to give 4.05 g (13.7 mmol, 33%) of *X* as white crystals: m. p. 102—103 °C; IR (KBr) 1700 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (t, $J = 6$ Hz, 3 H, CH_3 of ethylthio), 2.03 (s, 3 H, CH_3 of acetate), 3.35 (q, $J = 6$ Hz, 2 H, CH_2 of ethylthio), 3.76 (m, 2 H, OCH_2 of ethoxy), 4.21 (m, 2 H, OCH_2 of ethoxy), 5.12 (s, 2 H, isolated CH_2 of C-3 side-chain), 7.68 (d, $J = 5$ Hz, 1 H, H-6), 7.9 (d, $J = 5$ Hz, 1 H, H-5).

Anal. $C_{12}H_{16}N_4O_3S$ (296.35) Calc'd.: C 48.64; H 5.44; N 18.91%
Found: C 48.44; H 5.61; N 18.89%

8-Amino-3-[(2-hydroxyethoxy)methyl]-1,2,4-triazolo[4,3-a]pyrazine (IV)

Ethanol (35 mL), liquid ammonia (15 mL), and *X* (1.05 g, 3.54 mmol) were heated together in a 100 mL stainless steel sealed reaction vessel for 32 h at 127 °C. After cooling the vessel, the solvents were evaporated under vaccum to give 0.52 g of a tan powder. Recrystallization of this powder from ethyl acetate gave 0.42 g (2 mmol, 56.5%) of *IV* as pink crystals. After three recrystallizations from ethyl acetate, *IV* was obtained as white needles: m. p. 152—153 °C; IR (KBr) 3250 (OH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.53 (s, 4 H, CH_2CH_2), 4.63 (br, 1 H, OH), 4.98 (s, 2 H, CH_2), 7.36 (br, 3 H, H-6 and NH_2), 7.7 (d, 1 H, $J = 5$ Hz, H-5).

Anal. $C_8H_{11}N_5O_2$ (209.208) Calc'd.: C 45.93; H 5.30; N 33.48%
Found: C 46.03; H 5.18; N 33.71%

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10. Minimum inhibitory concentration is that amount (μg/mL) required to reduce virus-induced cytopathogenicity in either primary rabbit kidney, Vero or HeLa cell cultures by 50%.[4]
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POVZETEK

Sinteza in antivirusne lastnosti 8-amino-3-/(2-hidroksietoksi) metil/-1,2,4-triazolo-/4,3-*a*/pirazola

S. W. Schneller, J. L. May in E. De Clercq

Opisali smo sintezo 8-amino-3-/(2-hidroksietoksi)metil/-1,2,4-triazolo/4,3-*a*/pirazina (*IV*) kot analoga 9-/(2-hidroksietoksi)metil/guanina in 9-(S)-(2,3-dihidroksipropil)adenina iz 3-kloro-2-hidrazinopirazina (*V*) in etil 2-(2-acetoksietoksi) tioacetamida (*IXg*) in sledečo reakcijo z amoniakom. Spojina *IV* ne kaže antivirusne aktivnosti proti herpes simplex I in II, vaccinia virusu in virusu stomatitisa.