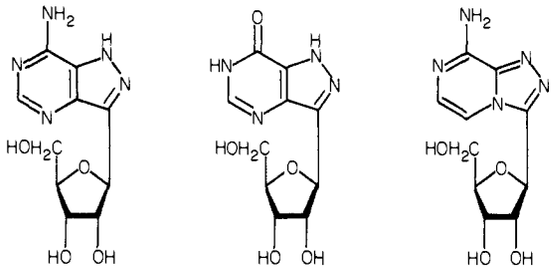
Biological Activity and a Modified Synthesis of 8-Amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-a]pyrazine, an Isomer of Formycin

Stewart W. Schneller,*,† Robert D. Thompson,† Joseph G. Cory,‡ Ray A. Olsson,§ Erik De Clercq,⊥ In-Kyung Kim,‖ and Peter K. Chiang#

Department of Chemistry, University of South Florida, Tampa, Florida 33620, Departments of Biochemistry and Internal Medicine, University of South Florida College of Medicine, Tampa, Florida 33612, The Rega Institute, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium, Department of Biochemistry, Catholic Medical College, Seoul 110, Korea, and Division of Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20012. *Received December 1, 1983*

A two-step synthesis of 8-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-a]pyrazine (3), which is an isomer of formycin that resembles 3-deazaadenosine, is reported. Compound 3 is also described as being a very poor substrate for adenosine deaminase and to be both a competitive and an irreversible inhibitor of S-adenosylhomocysteinase in the synthesis direction. L1210 cell growth in culture was inhibited by 3. Compound 3 was not converted to the nucleotide level in erythrocytes but was found to inhibit both the cellular uptake of nucleic acid precursors and their incorporation into the nucleic acids of L1210 cells. Finally, 3 was found to be a weak antiviral agent and coronary vasodilator.

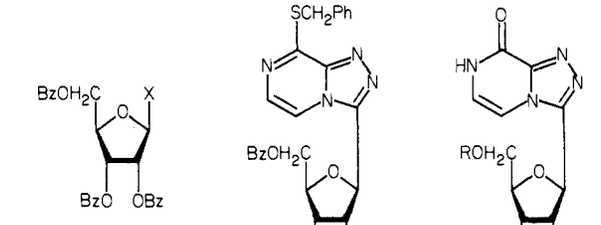
Since few molecules are more pervasive in biochemical processes than adenosine derivatives,[1,2] it is not surprising that many efforts toward uncovering new medicinal agents have concentrated on adenosine metabolism.[3] One molecule that has emerged from this search is the naturally occurring *C*-nucleoside formycin (1), an isomer of adenosine that has exhibited antitumor, antiviral, antibacterial, and antifungal activities.[4] These effects seem to be due to the ability of 1 to biologically replace adenosine and, as a result, affect protein and purine nucleoside, nucleotide, and nucleic acid metabolism.[4]



1 2 3

Unfortunately, from a chemotherapeutic standpoint, included in this metabolic similarity to adenosine is the catabolism of 1 by adenosine deaminase to formycin B (2), an isomer of inosine that is of little medicinal potential. In an effort to uncover metabolically more stable derivatives of formycin, one of our target analogues was 8-amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-a]pyrazine (3),[5] which was viewed not only as an isomer of formycin (and, in turn, adenosine) but also structurally related to the biologically potent 3-deazaadenosine.[3b,6-8] In this direction, a modified high-yielding synthesis and the biological properties of 3 are described herein.

Chemistry. Even though a synthesis of 3 had previously been reported,[9] we sought a less tedious, higher yielding route to it. Thus, treating 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl cyanide (4)[10] with benzyl mercaptan in the presence of anhydrous hydrogen chloride led to 5. Compound 5 was reacted with 3-chloro-2-hydrazinopyrazine[5] in pyridine to yield 8-(benzylthio)-3-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-a]pyrazine (6). Conversion of 6 into the desired 3 was then accomplished with anhydrous ammonia.[11] The overall recrystallized



4, X = CN
5, X = $C(SCH_2Ph)=NH_2^+Cl^-$

6 7, R = H 8, R = Bz

† University of South Florida.
‡ University of South Florida College of Medicine, Department of Biochemistry.
§ University of South Florida College of Medicine, Department of Internal Medicine.
⊥ The Rega Institute.
‖ Catholic Medical College.
Walter Reed Army Institute of Research

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(3) Selected examples relevant to this study include (a) 1-deazaadenosine (Itoh, T.; Sugawara, T.; Mizuno, Y. *Heterocycles* **1982**, *17*, 305-309), (b) 3-deazaadenosine (Chiang, P. K. *Science* **1981**, *211*, 1164-1165), (c) 7-deazaadenosine (tubercidin) (Suhadolnik, R. J. "Nucleosides as Biological Probes"; Wiley-Interscience: New York, 1979; pp 158-166), and (d) 9-deazaadenosine (Lim, M.-I.; Klein, R. S. *Tetrahedron Lett.* **1981**, *22*, 25-28).
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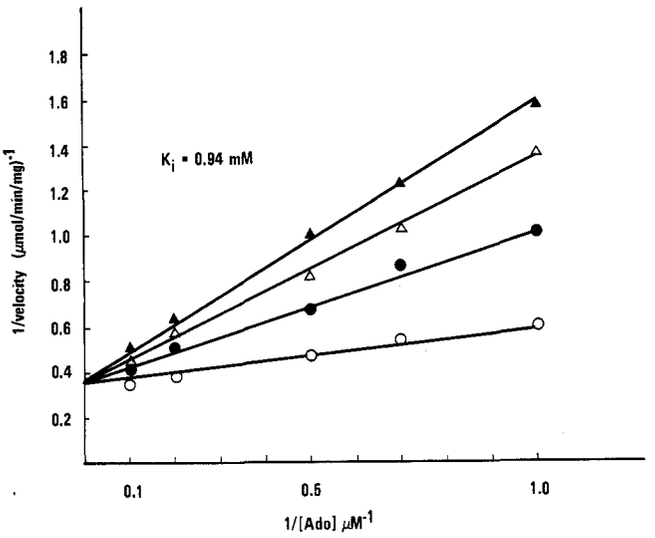

Figure 1. Competitive inhibition of hamster liver AdoHcyase by **3**. The following concentrations of **3** were used: (○) none; (●) 0.1 mM; (△) 0.5 mM; (▲) 1 mM.

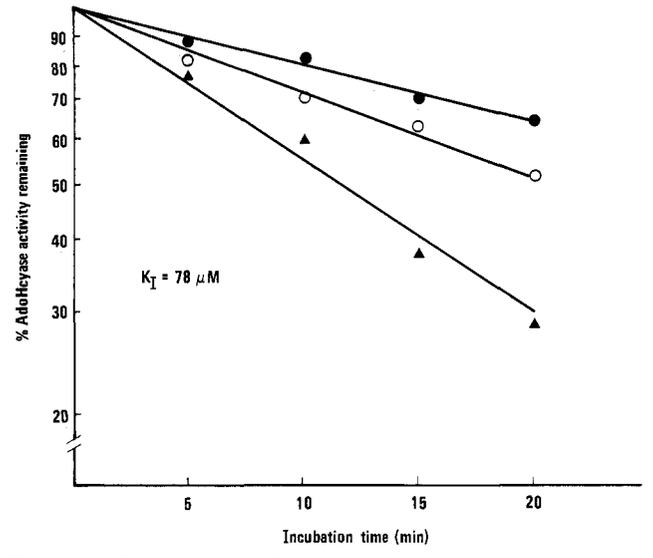

Figure 2. Irreversible inhibition of hamster liver AdoHcyase by **3**. The following concentrations of **3** were used: (●) 100 μM; (○) 300 μM; (▲) 600 μM.

yield of **3** in this two-step process beginning with **4** was 47.3%, whereas the literature method,[9] commencing with **4**, gave **3** in seven steps in a 2.5% overall yield, with some of the steps reported only in chromatographic yields.

To assist in monitoring the effect of adenosine deaminase on **3**, the expected product of this transformation (the formycin B isomer **7**) was also desired. As with **3**, this compound was synthesized in a more convenient manner than that previously described[9] by first treating **6** with hydrogen peroxide in acetic acid to produce **8**. Ammonolysis of **8** then yielded **7**.

Biological Results

Both 3-deazaadenosine and (±)-3-deazaaristeromycin (carbocyclic 3-deazaadenosine) have been shown to be potent inhibitors of *S*-adenosylhomocysteinase (AdoHcyase).[6,8] These 3-deaza analogues also exhibit potent antiviral activities, which have been attributed to their ability

Table I. Antiviral Activity of Nucleoside Derivatives

	minimum inhibitory concentration,[a] μg/mL												
	in primary rabbit kidney cells				in Vero cells					in HeLa cells			
compd	HSV-1 (KOS)[b]	HSV-2 (G)[b]	vaccinia virus	VSV[b]	measles virus	reovirus type 1	parainfluenza virus type 3	Sindbis virus	Coxsackie virus type B4	VSV[b]	Coxsackie virus type 4	polio virus type 1
3	>400	200	100	100	200	200	100	300	200	150	>400	>400
3-deazaadenosine	≥40 (40)[c]	10 (40)	7 (40)	7 (40)	≥40 (40)	4 (40)	2 (40)	≥40 (40)	4 (40)	10 (40)	≥40 (40)	≥40 (40)
formycin (1)	4 (40)	4 (40)	4 (40)	2 (40)	≥40 (40)	≥40 (40)	≥40 (40)	≥40 (40)	≥40 (40)	10 (200)	≥200 (200)	10 (200)
(*S*)-DHPA	>400	400	40	20	20	20	10	>400	40	100	>400	>400
ribavirin	>400	>400	20	20	20	100	10	40	200	20	20	10

[a] Required to reduce virus-induced cytopathogenicity by 50%. [b] Abbreviations used are: HSV-1 (KOS), herpes simplex virus type 1 (strain KOS); HSV-2 (G), herpes simplex virus type 2 (strain G); VSV, vesicular stomatitis virus. [c] The minimum cytotoxic concentration (micrograms per milliliter) causing a microscopically detectable alteration of normal cell morphology is given in parentheses. For compound **3**, (*S*)-DHPA, and ribavirin, no such alteration was observed at the highest concentration tested (400 μg/mL).

(11) For comparative purposes, an authentic sample of **3** was supplied by Dr. Jean Igolen, Institut Pasteur, Paris, France.

Table II. Coronary Vasoactivity of Adenosine Analogues

nucleoside	MPR[a]
3-β-D-ribofuranosyladenine	0.15
2-azaadenosine	0.15
7-deazaadenosine (tubercidin)	0.071
formycin (1)	0.043
8-azaadenosine	0.042
compound **3**	0.0015
3-deazaadenosine	none
lin-benzoadenosine	none
1,N^6-ethenoadenosine	none

[a] MPR = molar potency ratio, the quotient of the ED_{50} of adenosine divided by that of the test nucleoside. Dog to dog variations in MPR are about ±10%.

Table III. Effect of Compound **3** on the Incorporation of Nucleic Acid Precursors into L1210 Cells (cpm × 10^{-3})[a]

precursor[b]	total uptake	acid soluble fraction	RNA fraction	DNA fraction
[^{14}C]Cyd, control	128.8[c]	40.2	82.7	5.7
[^{14}C]Cyd + **3**[d]	50.4 (39)[e]	24.0 (60)	24.0 (29)	2.4 (42)
[^{14}C]Urd, control	193.0	121.2	66.6	5.2
[^{14}C]Urd + **3**[d]	128.8 (67)	91.1 (75)	34.4 (52)	3.3 (63)
[^{14}C]Ado, control	203.0	151.3	46.0	5.7
[^{14}C]Ado + **3**[d]	161.7 (80)	138.4 (91)	19.8 (43)	3.5 (62)
[^{14}C]Thd, control	98.1	27.4		70.8
[^{14}C]Thd + **3**[d]	60.4 (62)	18.0 (66)		42.5 (66)

[a] L1210 cells were incubated in culture for 90 min in the absence (control) and presence of compound **3** (1 mM). The ^{14}C-labeled precursor was added to each flask (0.04 μCi/mL), and the cells were incubated for an additional 30 min. The cells were collected by centrifugation, and the cell pellet was subjected to the Schmidt–Thannhauser[19] procedure to separate the acid-soluble, RNA, and DNA portions. Each flask was set up in duplicate. [b] The ^{14}C-labeled precursors were added to reach a final concentration of 0.04 μCi/mL of culture medium. The specific activities of the compounds used were as follows: [^{14}C]cytidine, 485 mCi/mmol; [^{14}C]uridine, 522 mCi/mmol; [^{14}C]adenosine, 45.5 mCi/mmol; [^{14}C]thymidine, 55 mCi/mmol. A Searle Mark III scintillation counter was used in these experiments. [c] Does not equal the sum of the acid-soluble, RNA, and DNA fractions due to rounding off. [d] The concentration of **3** was 1 mM. [e] Percentage of control value.

to inhibit the activity of AdoHcyase.[8,12] Therefore, in view of the structural similarity between **3** and 3-deazaadenosine, compound **3** was tested for its ability to inhibit AdoHcyase. When the enzyme isolated from hamster liver was used, **3** was not a substrate for AdoHcyase but it functioned both as a competitive inhibitor (K_i = 0.94 mM, Figure 1) and as an irreversible inhibitor (K_I = 78 μM, Figure 2). (For comparative purposes, 3-deazaadenosine was found to have a K_i = 0.07 mM while lacking irreversible inhibitory properties toward hamster liver AdoHcyase). Thus, it appeared that **3** was a more effective irreversible inhibitor than a competitive inhibitor, which is not surprising in light of a recent report[13] describing other nucleosides that irreversibly inhibited AdoHcyase.

When tested for antiviral activity (Table I), **3** was only weakly active in comparison to several other established antiviral agents, including 3-deazaadenosine,[12] formycin (1),[14] (*S*)-DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine],[15] and ribavirin.[16] The poor antiviral activity of **3** might be related to its weak inhibitory potency toward AdoHcyase rather than any metabolic instability. In the latter regard, **3** was only a very poor substrate of adenosine deaminase from calf intestine. When assayed spectrophotometrically as a substrate for adenosine deaminase under conditions for measuring adenosine deamination, **3** did not appear to be a substrate. However, incubation of **3** with a high concentration of adenosine deaminase resulted in a 70% conversion to **7** (by HPLC) after 13 h. Compound **3** did not inhibit the deamination of adenosine.

In view of the coronary vasoactivity of a variety of adenosine derivatives[17] and the hypotensive effect of 3-deazaadenosine,[18] **3** was assessed in this regard and found to be a very weak coronary vasodilator (Table II). Even at the highest infusion rate (ca. 1 mL/min), **3** did not produce maximum coronary vasodilation. Attempts to improve the vasodilation with higher concentrations of **3** were precluded by its limited solubility in coronary plasma water.

Table II also points out that the hypotensive effect of 3-deazaadenosine[18] is not due to a vasoactivity effect but is, probably, related to its ability to inhibit AdoHcyase and the associated biological methylation reactions.

Finally, at 1 and 3 mM, **3** was found to cause 40 and 100%, respectively, growth inhibition of L1210 cells in culture after 3 days. By day 5 of the growth curve, the cells in the presence of 1 mM **3** had recovered to approximately 90% of control, while there was no growth in the cells incubated with 3 mM **3**. The reason for this is uncertain, since **3** was not subject to conversion to its nucleotide derivatives in mouse erythrocytes. However, two interesting effects were observed for **3** in L1210 cells (Table III) that should be mentioned as possible causes for its biological effects on the L1210 cells. In one case, **3** caused a decrease in the cellular uptake of the labeled nucleic acid precursors, indicating that **3** interfered with or competed for the transport sites. In the other case, it appeared there was a differential effect on the incorporation of the precursors into RNA. For cytidine, uridine, and adenosine, there was a greater inhibition of incorporation of these precursors into RNA than into the acid-soluble and DNA fractions. For thymidine, the inhibition of uptake was similarly reflected in the levels of labeled thymidine in the acid-soluble and DNA fractions. It is also possible, however, that the aforementioned effects of **3** on AdoHcyase may also account for the growth inhibition of L1210 cells by **3**.

Experimental Section

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 chemical shifts are reported in parts per million downfield from Me_4Si as an internal standard.

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The spin multiplicites are indicated by the symbols s (singlet), d (doublet), t (triplet), and m (multiplet). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. The microanalyses are indicated only by symbols of the elements, which indicates that the analytical results obtained for those elements were within ±0.4% of the theoretical values.

For analytical TLC, Eastman precoated silica gel Chromagram sheets with fluorescent indicator (100-μm thick) were used; the spots were detected by irradiation with a Mineralight UVS-11 and by charring after spraying with MeOH–H_2SO_4 solution (9:1, v/v). Baker silica gel (60–200 mesh) was used for the column chromatographic separations.

The ^{14}C-labeled precursors were purchased from New England Nuclear, Boston, MA (uridine, adenosine, and thymidine), and Amersham, Arlington Heights, IL (cytidine).

8-(Benzylthio)-3-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*a*]pyrazine (6). A solution of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl cyanide (**4**;[10] 7 g, 0.015 mol), benzyl mercaptan (3.5 mL, 0.03 mol), and 700 mL of anhydrous Et_2O was saturated at 0 °C with anhydrous HCl for 2 h (after 45 min, a white precipitate formed). The mixture was then kept at −10 °C for 20 h. The white precipitate was isolated by filtration and washed with anhydrous Et_2O. The thioimino ether hydrochloride (**5**, white precipitate) and 3-chloro-2-hydrazinopyrazine[5] (2.2 g, 0.015 mol) in 200 mL of dry pyridine were stirred for 3 h at room temperature and then refluxed for 1.5 h. The pyridine was removed in vacuo to give a dark syrup, which was purified by column chromatography (silica gel; toluene–AcOEt, 9:1, v/v) to give 8 g (79%) of a white foam (R_f 0.79; toluene–AcOEt, 4:1, v/v). Recrystallization of this material from C_6H_6–MeOH gave 7.5 g (74%) of **6** as white prisms: mp 143–144 °C; IR (KBr) 1720 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 4.45 (s, 2 H, CH_2), 4.47–5.05 (m, 3 H, H-4′, H-5′), 5.80 (d, 1 H, H-1′, $J_{1',2'}$ = 6.5 Hz), 6.03 (dd, 1 H, H-3′), 6.50 (dd, 1 H, H-2′), 7.2–8.1 (m, 22 H, H-5, H-6, and phenyl). Anal. ($C_{38}H_{30}N_4O_7S$) C, H, N.

8-Amino-3-β-D-ribofuranosyl-1,2,4-triazolo[4,3-*a*]pyrazine (3). A mixture of **6** (2 g, 2.9 mmol) and 70 mL of dry MeOH was added to a stainless-steel reaction vessel (125 mL) and then saturated with anhydrous NH_3 at 0 °C. The vessel was heated at 110 °C for 48 h and then cooled to 0 °C, opened, and resaturated with NH_3 at 0 °C. The vessel was heated again for an additional 24 h at 100 °C and cooled, and the MeOH was removed in vacuo to give a light yellow solid. Water (40 mL) was added to the residue, and the mixture was extracted with $CHCl_3$ (3 × 50 mL). The aqueous layer was evaporated in vacuo to give a white solid, which was purified by column chromatography (silica gel; $CHCl_3$–EtOH, 2:1, v/v) to give 500 mg (64%) of **3** as a white solid: mp 202–203 °C (lit.[9] mp 207 °C); R_f 0.44 ($CHCl_3$–EtOH, 1:1, v/v) (lit.[9] R_f 0.38). A mixture melting point determination and NMR spectral comparison indicated that the **3** obtained by this method was identical with that reported earlier.[11]

3-(2′,3′,5′-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,4-triazolo[4,3-*a*]pyrazin-8(7*H*)-one (8). A mixture of **6** (2.4 g, 3.5 mmol), 20 mL of AcOH, and 2 mL of 30% H_2O_2 was heated to 50 °C and then stirred for 20 h at room temperature. The AcOH was removed in vacuo to a light yellow syrup to which H_2O (70 mL) was added. The pH of this solution was brought to 6 with 3 N NaOH solution. The resulting precipitate was isolated by filtration and recrystallized from MeOH to give 1.2 g (60%) of **8** as white needles: mp 231–232 °C; IR (KBr) 1730 (ester C=O), 1680 (C=O), 1610 (C=N) cm^{-1}; NMR ($CDCl_3$) δ 4.55–5.05 (m, 3 H, H-4′ and H-5′), 5.77 (d, 1 H, H-1′, $J_{1',2'}$ = 6.2 Hz), 6.01 (dd, 1 H, H-3′), 6.46 (dd, 1 H, H-2′), 7.2–8.02 (m, 17 H, H-5, H-6, and phenyl), 11.42 (br s, 1 H, NH). Anal. ($C_{31}H_{24}N_4O_8$) C, H, N.

3-β-D-Ribofuranosyl-1,2,4-triazolo[4,3-*a*]pyrazin-8(7*H*)-one (7). A mixture of **8** (1 g, 1.72 mmol) and 100 mL of MeOH was saturated at 0 °C with NH_3. The solution was stirred for 72 h at room temperature, and the MeOH was then removed in vacuo. The resulting light yellow solid was isolated by filtration and washed with MeOH. Recrystallization of this material from H_2O–EtOH (charcoal) gave 300 mg (65%) of **7** as colorless plates: R_f 0.16 ($CHCl_3$–EtOH, 1:1, v/v) (lit.[9] R_f 0.38); mp 242–244 °C (lit.[9] mp 244 °C); NMR (Me_2SO-d_6) δ 3.4–4.6 (m, 8 H, ribosyl H), 5.15 (d, 1 H, H-1′, $J_{1',2'}$ = 6.8 Hz), 6.87 (d, 1 H, H-6, $J_{5,6}$ = 5.7 Hz), 7.72 (d, 1 H, H-5, $J_{5,6}$ = 5.7 Hz), 11.45 (s, 1 H, NH), which is identical with that reported for **7**.[9]

***S*-Adenosylhomocysteinase (AdoHcyase) Assay.** *S*-Adenosylhomocysteinase purified from hamster liver was used for assaying the inhibitory potency of **3**. The activity of the AdoHcyase was assayed in 0.5 mL containing 50 mM potassium phosphate (pH 7.6), 5 mM dithiothreitol, 1 mM EDTA, 10% glycerol, 10 mM DL-Hcy, and [^{14}C]Ado. The assay time was 10 min at 37 °C. The reaction was stopped by the addition of 1 mL of 50 mM HCl, and the mixture then poured onto a column (0.8 × 2.5 cm) of SP-Sephadex C-25, equilibrated in 10 mM HCl. Each column was next washed with 30 mL of 50 mM HCl. The [^{14}C]AdoHcy formed was subsequently eluted into a scintillation vial with 10 mL of 1 N HCl. The radioactivity was counted after adding 10 mL of scintillation fluid to the vial. When the K_I for the irreversible inhibition of AdoHcyase was determined, 20 μM of [^{14}C]Ado was used in the assay after incubation with **3**. A Beckman LS-9000 scintillation counter was used in this assay.

Antiviral Activity. The antiviral assays were based upon the inhibition of virus-induced cytopathogenicity. The method for measuring this inhibition has been described previously.[20,21] The origin of the viruses was as follows: HSV-1 (KOS) (herpes simplex virus type 1, strain KOS) and HSV-2 (G) (herpes simplex virus 2, strain G), see ref 21; vaccinia virus, vesicular stomatitis virus (VSV), measles virus, Sinbis virus, Coxsackie virus type B4, and polio virus type 1, see ref 20; reovirus type 1 (ATCC VR-230) and parainfluenza virus type 3 (ATCC VR-93), obtained from the American Type Culture Collection (Rockville, MD).

The virus stocks were grown in either primary rabbit kidney cells (HSV-1, HSV-2, VSV), Vero cells (measles, reo, parainfluenza, Coxsackie), HeLa cells (polio), chick embryo cells (Sindbis), or chorioallantoic membrane cells (vaccinia). The antiviral assays were run in primary rabbit kidney, Vero, or HeLa cells as shown in Table I.

Adenosine Deaminase Assay. For adenosine as the substrate, the method of Chilson and Fisher[22] was employed by monitoring the change in absorbance at 265 nm on a Beckman CV Acta recording spectrophotometer. The utilization of **3** as a substrate for adenosine deaminase was determined by two methods, depending on the level of adenosine deaminase. In the first method, **3** (0.55 mM) in Tris-HCl buffer, 0.02 M, pH 7.0, was incubated with adenosine deaminase (0.04 unit) in a final reaction volume of 3 mL. Ultraviolet spectra were taken at 0, 10, 20, 30, and 60 min. In the second method, **3** (0.55 mM) in Tris-HCl buffer, 0.02 M, pH 7.0, was incubated with adenosine deaminase (4 units) in a final reaction volume of 0.1 mL. A control was set up that contained no adenosine deaminase. Analysis of the reaction mixtures was conducted by HPLC in a Partisil SCX column using ammonium phosphate, pH 2.77, 0.1 M, as the solvent. The flow rate was 2 mL/min. The eluting times were 1.87 and 3.24 min for **7** and **3**, respectively.

Calf intestinal mucosa, type I adenosine deaminase (EC 3.5.4.4) was purchased from Sigma Chemical Co., St. Louis, MO, and used in this assay.

Coronary Vasoactivity. The coronary vasoactivity of **3** was assessed in morphone–thiamylal–fluothane anesthesized dogs by the reported method.[17]

Growth of L1210 Cells. The L1210 cells were grown in suspension culture in RPMI 1640 medium that was supplemented with 10% horse serum, sodium bicarbonate (2 g/L), and gentamicin sulfate (50 mg/L). Cells were grown at 37 °C. Each group, control or experimental, was set up in triplicate. Drugs were added at day 0 of the growth curve. Duplicate aliquots were taken daily for cell counts in a Coulter ZBI counter.

Assay Method for the Nucleotide Formation from Compound 3. Attempts to measure the formation of nucleotides of **3** in mouse erythrocytes was carried out by the HPLC method of Crabtree et al.,[23] except that the wavelength used for **3** and the adenine nucleotides was 310 and 254 nm, respectively.

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Separate samples were injected and measured either at 310 or 254 nm.

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Registry No. **3**, 68797-11-5; **4**, 23316-67-8; **5**, 38716-29-9; **6**, 89578-31-4; **7**, 68797-10-4; **8**, 89578-32-5; benzyl mercaptan, 100-53-8; 3-chloro-2-hydrazinopyrazine, 63286-28-2; *S*-adenosylhomocysteinase, 9025-54-1; adenosyl deaminase, 9026-93-1.

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Steroidogenesis Inhibitors. 1. Adrenal Inhibitory and Interceptive Activity of Trilostane and Related Compounds†

Robert G. Christiansen,*‡ Helmut C. Neumann,‡ U. Joseph Salvador,‡ Malcolm R. Bell,‡ H. Phillip Schane, Jr.,*§ John E. Creange,§ Gordon O. Potts,§ and A. J. Anzalone§

Departments of Medicinal Chemistry and Endocrinology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144.
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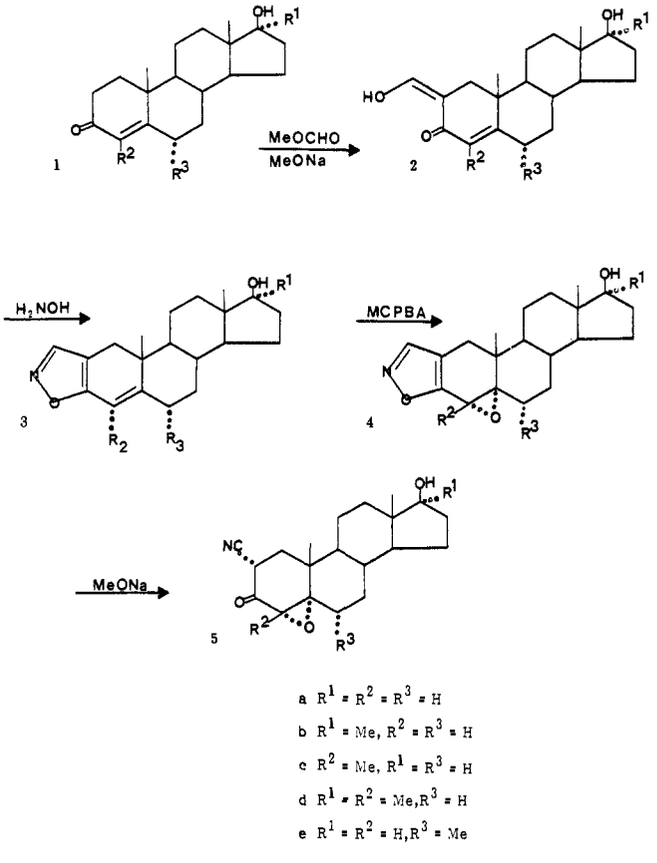
Several methylated derivatives of trilostane were prepared. Methylation of C-4 or C-4 and C-17 changes this relatively selective adrenal inhibitor to compounds with increased ovarian/placental inhibitory activity with decreased adrenal inhibitory activity.

Interceptives are agents that interrupt pregnancy after implantation,[1] a definition that qualifies trilostane (**5a**) as an interceptive agent in the rat and monkey,[2,3] The interceptive activity and adrenal inhibitory properties of trilostane result from inhibition of 3β-hydroxysteroid dehydrogenase.[2,3] An advantage of blockade at the 3β-hydroxy dehydrogenase step is that relatively innocuous precursors accumulate while inhibition at later stages of steroidogenesis results in the accumulation of or shunting to physiologically active intermediates. A further advantage of trilostane and its congeners is that they are devoid of hormonal or antihormonal activities.[2] At the clinical level, trilostane is effective in the treatment of primary aldosteronism,[4] reverses diuretic-induced hypokalemia,[5] decreases blood pressure in some patients with low renin hypertension,[6,7] and is useful in the treatment of Cushing's syndrome.[8] These results establish trilostane as an important therapeutic agent and confirm the revelance of the laboratory models used to develop the compound. Trilostane does not appear to quality as a useful interceptive agent, since much higher doses are required to terminate pregnancy in the rhesus monkey than are required to reduce adrenal steroidogenesis.[3]

This report and those to follow will relate the effect of molecular modification of trilostane on adrenal inhibitory activity and interceptive activity, with particular attention to (1) the identification of selective inhibitors of adrenal vs. ovarian/placental steroidogenesis and (2) the structural features required for carry-over of activity from the rat to the rhesus monkey. This report describes the synthesis and identification of compounds and compares the doses needed for interceptive activity with those that inhibit ACTH-stimulated glucocorticoid production.

Chemistry. The synthetic route to the three new methylated trilostane derivatives **5c–e** from the known testosterone derivatives **1c–e** is the same as that reported for trilostane (**5a**) and the 17-methyl analogue **5b**[9] (Scheme

Scheme I



a $R^1 = R^2 = R^3 = H$
b $R^1 = Me, R^2 = R^3 = H$
c $R^2 = Me, R^1 = R^3 = H$
d $R^1 = R^2 = Me, R^3 = H$
e $R^1 = R^2 = H, R^3 = Me$

Table I. Chemical Shifts of C-19 Protons at 100 MHz in Me_2SO-d_6

no.	δ, 19-H
5a	0.97
5e	0.97
14	1.06

† This paper was presented in part. See "Abstracts of Papers", 179th National Meeting of the American Chemical Society, Houston, TX, Mar 23-28, 1980; American Chemical Society, Washington, DC, 1981; Abstr MEDI 010.

‡ Department of Medicinal Chemistry.

§ Department of Endocrinology.

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