Ammonium 7*H*-Purin-6-yl 1-Thio-β-D-glucopyranosiduronate (1). Compound **3** (1.17 g, 1.99 mmol) was dissolved in 35 mL of MeOH and 10 mL of water to which was added 10 mL of 1.0 M KOH, pH ~10. The mixture warmed intially. After 3 h, Dowex 50W-X8 resin, H^+ form, was added to the solution, and the mixture was swirled. The pH decreased gradually to a minimum pH 2.45, whereupon the mixture was filtered and the resin was rinsed extensively, first with methanol and then with water. The methanol was removed by rotary evaporation, and the water solution was concentrated. NH_4OH (28%, 0.55 mL) was added (pH ~9.5), the solution was kept for 2 h and concentrated, and the salt was precipitated by addition of Me_2CO to the water solution. The salt was removed by filtration, redissolved in water, and again precipitated by the addition of Me_2CO. The white solid was collected and dissolved in water. The solution was frozen and lyophilized. The product (0.480 g, 1.38 mmol, 69% yield) began to darken at 150 °C, softened at 178 °C, and by 185 °C had completely decomposed: pK_a = 3.08 ± 0.02 (25 °C); UV λ_{max} (water) 281.5 nm (log ε 4.12); TLC (cellulose; *n*-BuOH/H_2O/MeOH, 3:1:0.4) R_f 0.18; NMR (D_2O) δ 8.1 (s, 1 H, purine), 7.95 (s, 1 H, purine), 5.6 (m, 1 H, H-1′), 5.0–4.4 (m, H-2′,5′), 4.0–3.4 (m, H_2O). Anal. ($C_{11}H_{15}N_5O_6S\cdot H_2O$) C, H.

Kinetics of Hydrolysis of 1 and 2 Catalyzed by Bovine Liver β-Glucuronidase. Three milliliters of 0.1 M acetate buffer (μ = 0.1 M, KCl) at each of several pH values was pipetted into 1-cm cuvettes and placed in Gilford spectrophotometer cell holder for 20 min at 30 °C. For all pH studies, except that at pH 3.38, 10 μL of the substrate in water was also added at this point. At pH 3.38, spontaneous substrate hydrolysis was appreciable, and the substrate was added after 20 min. The final substrate concentration in each cuvette ranged from 4.0×10^{-4} to 4.8×10^{-3} M. Fifteen microliters of bovine β-glucuronidase (30 FU) in 0.1 M, pH 5.0, acetate buffer was added at the end of 20 min. The change in absorbance per minute at 324 nm was recorded, and initial velocities were computed with $\Delta\epsilon = 2 \times 10^4$ M^{-1} cm^{-1}. At each substrate concentration, duplicate experiments were performed. For hydrolysis of **2**, the protocol was similar except 20 μL of **2** in Me_2SO was added to 2 mL of acetate buffer and 50 μL of bovine β-glucuronidase (42 000 FU) per reaction was used.

A reaction mixture containing 20 μL of **2** (1.1 μmol) in Me_2SO in 2 mL of 0.01 M acetate buffer (pH 4.96) and 14 520 FU of bovine β-glucuronidase was lyophilized after 48 h, and the residue was treated with 50 μL of H_2O. Water-soluble material was spotted on a cellulose plate (Eastman 13254), which was developed in BuOH–pyridine–H_2O (6:4:3), subsequently sprayed with a solution of 1.23% *p*-anisidine and 1.66% phthalic acid in MeOH and dried with a heater–blower gun. A peach-colored spot with R_f 0.22 ± 0.01 corresponded to that of authentic D-glucuronomide. D-Glucuronic acid, which does not migrate in this system, was not detected. Parenthetically, there is no lag phase for hydrolysis of **2**, and purin-6-yl 1-thio-β-D-glucopyranoside is also a poor substrate.

Biological Testing. Ammonium 7*H*-purin-6-yl 1-thio-β-D-glucopyranosiduronate (**1**) was investigated with respect to its effect on Chinese hamster lung fibroblast (V-79) growth.[15] The V-79 cell line is not of tumor origin. It has a doubling time of 9.0 h under normal culture conditions (RPMI-1640, 10% FCS, 1% antibiotics penicillin and streptomycin, 5% CO_2 humidified incubator). This cell line has been actively cultured in Dr. Bardos' laboratory for over 2 years. New cells are acquired from frozen stock approximately every 6 months.

Exponentially growing cultures of asynchronous cells plated 24 h prior to the experiment were treated, as is, with various doses ranging from 10^{-3} to 10^{-5} M compound for 1 h. Following treatment, duplicate plates for each dose were rinsed free of drug-containing media, refed with fresh media, and placed back into the incubator for the short term (24 h) viability study. Twenty–four hours posttreatment, control plates approached confluency. At this time, all plates were trypsinized, and the cells from each plate were counted with an electrozone particle counter.

Compounds **1** and **2** were investigated with respect to their effect on L1210 cell growth.[16] The L1210 line is of tumor origin. One milliliter [RPMI-1640, 20% FCS (HI)] of 20 mM Hepes buffer containing 10^5 L1210 cells was added to 1 mL of RPMI-1640, 20 mM Hepes buffer containing 2×10^{-3} to 2×10^{-6} M **1** or **2** in a tube. Triplicate experiments were done at each drug concentration. The tubes were incubated at 37 °C for 48 h. Forty-eight hours posttreatment, the cells from each tube were counted with a Coulter counter.

Acknowledgment. This work was supported by Grant CA-21755 from the National Institutes of Health. We thank Drs. V. Alks and W. Dunn for cell culture studies.

(15) Testing was done by Dr. Joseph A. Dunn at SUNY/Buffalo, Department of Biochemical Pharmacology, under Dr. Thomas J. Bardos' Direction.

(16) Testing was done by Ms. Patricia Dixon at Roswell Park Memorial Institute.

Crystal Structure and Anti Herpes Simplex Virus Activity of 2,2′-Anhydro-1-β-D-arabinofuranosylthymine

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1-β-D-Arabinofuranosylthymine (aThy; *ara*-T) is a potent selective anti herpes simplex virus drug. Its anhydro analogue, 2,2′-anhydro-aThy, was shown to be 9-fold less active and at least 3-fold less toxic than aThy. This compound was relatively stable at physiological pH and in strong acid but was rapidly hydrolyzed in base with a half-life of 18.3 min. The three-dimensional crystal structure of 2,2′-anhydro-aThy revealed a rigid structure with the arabinose ring in the unusual O1′ endo, pucker, conformation. The trans–gauche conformation along the C4′–C5′ bond permits only intermolecular hydrogen bonding of the 5′-hydroxy and O3′.

Several β-D-arabinofuranosyl nucleosides are potent inhibitors of viral and mammalian cell replication. Prominent examples of this diverse group of compounds, which inhibit herpes simplex viruses (HSV), are the clinically approved drug 9-β-D-arabinofuranosyladenine (*ara*-A; Vidarabine; Vira-A) and the experimental drug 1-β-D-arabinofuranosylthymine (*ara*-T; aThy).[1,2]

We have recently described a rapid and simple method for preparing pyrimidine arabinosyl nucleosides via 2,2′-

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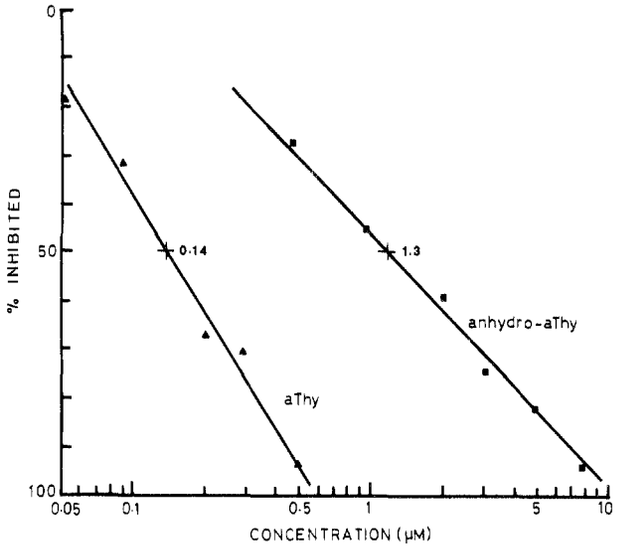

Figure 1. A comparison of the inhibitory effect of aThy (▲) and 2,2′-anhydro-aThy (■) on herpes simplex virus type 1 (strain F) in Vero cells, by plaque reduction. Numbers marked with + on the line represent ED_{50}. Every point is the mean of duplicate experiments. Control = 187 plaques.

anhydro derivatives;[3] the anhydro analogue of aThy can be readily hydrolyzed in base to aThy. If a similar or enzymatic process can occur in cell culture or in vivo, this derivative, then, may be considered a prodrug.[4]

In hamsters, aThy has been shown to be rapidly metabolized and excreted in urine.[2,5,6] For this reason, Aswell and Gentry[7] have studied the antiviral properties of 1-β-D-arabinofuranosyl-5-methylcytosine (5-Me-aCyt), a compound that is converted to aThy in cells with substantial levels of 2′-deoxycytidine deaminase.[5] Similarly, 2,2′-anhydro-1-β-D-arabinofuranosylcytosine (anhydro-aCyt) hydrochloride, a depot form of aCyt, as well as a number of analogues of this compound, has been prepared in an attempt to find more potent antiviral and antitumor agents.[8-11]

The work presented in this paper demonstrates that anhydro-aThy can also act as a repository form of aThy and that it has less anti-HSV activity and lower toxicity than aThy in cell culture. This drug may have an improved physiological distribution, resorption, and activity in vivo. We have determined the crystal structure of 2,2′-anhydro-aThy and compared it to that of 2,2′-anhydro-aCyt and -aUra.[12,13] Information on these rigid fused polycyclic ring systems permits a high degree of confidence in comparing the conformation and molecular strain in the crystalline state and in solution and may give insight into the molecular basis for biological activity.

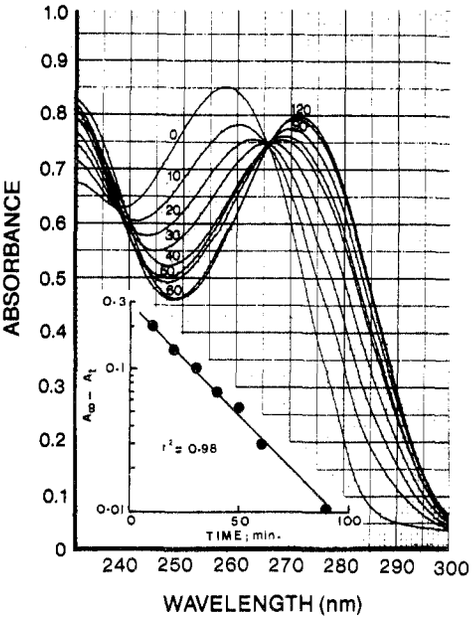

Figure 2. Spectral changes of alkaline-treated (NaOH; 0.1 M at 22 °C) anhydro-aThy. Spectrum plotted every 10 min for 1 h and every 30 min thereafter. The inset is a replot of the $A_\infty - A_t$ vs. time. Absorbance values were measured at 272 nm, where A_∞ is the asymptotic absorbance and A_t is the absorbance at time t.

Results and Discussion

Biologic and Hydrolytic Studies. Figure 1 compares the antiviral effect of aThy and its anhydro analogue on herpes simplex virus type 1 (strain F) in Vero cells. Although both compounds were very active at concentrations greater than 10 μM, aThy was consistently more active at lower doses. The doses that inhibited 50% of the plaques (ED_{50}) were 0.14 and 1.3 μM for aThy and the anhydro compound, respectively. At 100 μM and lower concentrations, both compounds had no apparent toxicity to rapidly dividing Vero cells. However, whereas the toxic effect of aThy plateaus at 200 μM (40% inhibition), the anhydro derivative was not toxic even at a concentration of 600 μM. (aThy inhibition was 59% at 600 μM.)

The anhydro compound did not decompose to aThy in phosphate-buffered saline (PBS; pH 7.2) or cell culture medium and was stable at 22 or 38 °C for at least 8 weeks as determined by monitoring changes in UV absorbance and changes in λ_{max}. There was also no apparent change in UV absorbance and changes in λ_{max} of supernatant from confluent Vero cells exposed to 10 μM anhydro-aThy for 5 days at 38 °C. In alkali (NaOH; 0.1 M), the UV maxima shifted from 258 to 272 nm (coinciding with λ_{max} for aThy[14]) over a period of 2 h at 22 °C. Typical spectra and kinetic data for the alkaline degradation of the anhydro compound are given in Figure 2. From the slope of the plot of $\log_{10}(A_\infty - A_t)$ vs. t, where A_∞ is the final absorbance at 272 nm and A_t is the absorbance at time t, the apparent first-order rate constant (k) for the alkaline degradation

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(3) R. F. Schinazi, M. S. Chen, and W. H. Prusoff, *J. Med. Chem.*, **22**, 1273 (1979).
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Table I. Comparison of Bond Lengths about C2, Glycosidic Torsion Angles, and Pseudorotation Angles for the 2,2′-Anhydro Derivatives of aThy, aCyt, and aUra

anhydro derivative	C2–O2, Å	C2–N1, Å	C2–N3, Å	χ_{CN},[a] deg	P,[b] deg	pucker	ref
aThy	1.33	1.36	1.29	289.0	86	O1′-endo	
aCyt	1.34	1.34	1.24	299.0	233	C4′-endo	13
aUra	1.33	1.35	1.30	294.5	227	C4′-endo,	12
	1.34	1.35	1.29	290.0	213	C3′-exo	

[a] Glycosyl torsion angle, O1′–C1′–N1–C6. [b] Pseudorotation phase angle.[16]

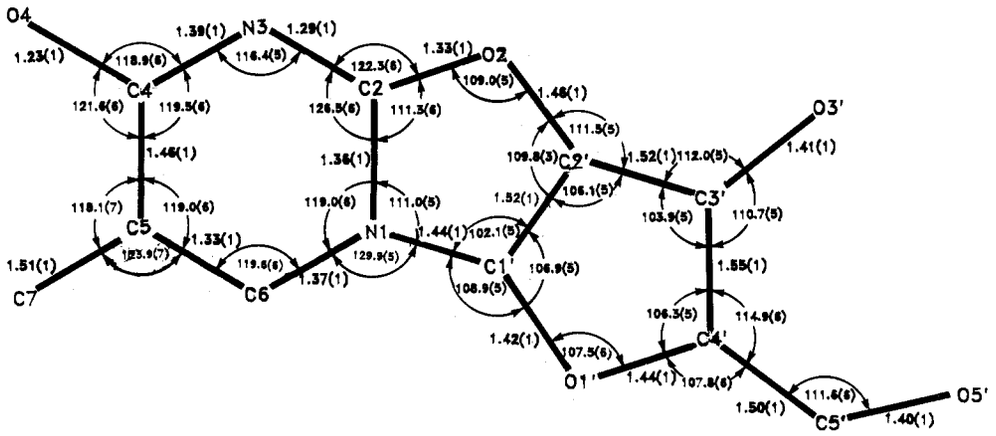

Figure 3. Molecular geometry and numbering system. Bond distances (in angstroms) and bond angles (in degrees) are given. The estimated standard deviations in the last digit of each are the values in parentheses.

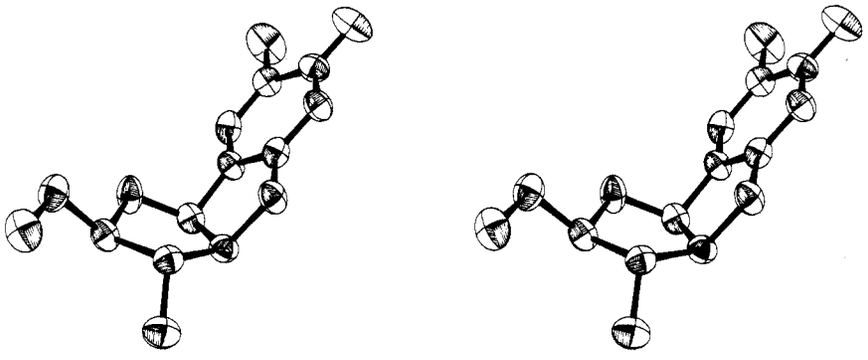

Figure 4. Stereoscopic view of anhydro-aThy; the thermal ellipsoids correspond to 50% probability.

of this compound was found to be 3.8×10^{-2} min^{-1} ($t_{1/2}$ = 18.3 min). The anhydro compound decomposed at a much slower rate in mineral acid (HCl or H_2SO_4; 0.1 M) at room temperature; the UV maxima shifted from 257 to 272 nm over a period of 25 days.

Since anhydro-aThy was not degraded at physiological pH and temperature or by hydrolases originating from the cells, it appears that this compound must be taken up by infected cells and then hydrolyzed under basic conditions either chemically or enzymatically to aThy in order to exert its antiviral activity. We had previously determined that anhydro-aThy was a poor competitive inhibitor of the HSV-encoded thymidine kinase with an inhibition constant (K_i) of 160 μM.[3] However, our results do not preclude the possibility that the anhydro compound is a substrate for this enzyme.

X-ray Characterization. The calculated parameters for the mean plan through atoms N1, C2, N3, C4, C5, C6, O2, C1′, and C2′, as well as the other planar systems in the molecule, have been calculated[15] (see paragraph at the end of paper concerning supplementary material). The root mean squared deviation of these atoms from the calculated plane is 0.049 Å and is not statistically significant. The bond lengths and angles are essentially the same as those determined for similar molecules: 2,2′-anhydro-1-β-D-arabinofuranosyluracil[12] and 2,2′-anhydro-1-β-D-arabinofuranosylcytosine.[13] As indicated in Table I, 2,2′-anhydro-aThy shows the same shortening of the C2–N3 bond and lengthening of the C2–O2 and C2–N1 bonds as do 2,2′-anhydro-aCyt and -aUra.

Unlike anhydro-aCyt or -aUra, the conformation of the arabinose ring in anhydro-aThy has a pseudorotation angle,[16] P = 86°, corresponding to an O1′ endo pucker (Figures 3 and 4). The displacement of O1′ from the mean plane through the five sugar ring atoms is 0.18 Å. The O1′ distance from the best plane through the C1′, C2′, C3′, and C4′ plane is 0.435 Å. Only a few nucleoside structures are known to have this high-energy conformation, and it is of interest that 5-iodo-5′-amino-2′,5′-dideoxyuridine, a specific inhibitor of HSV, also has this unusual sugar conformation.[17] Only intermolecular hydrogen bonding between the O5′ hydrogen and O3′ (HO5′–O3′; 1.98 Å) and the O3′

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(16) C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **94**, 8205 (1972).

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hydrogen and O4 (HO3′–O4; 1.81 Å) is observed and may relate to the difference in sugar conformation in anhydro-aThy and the corresponding cytosine and uracil analogues. The conformation along the C4′–C5′ bond appears trans–gauche, i.e., the O5′ is trans to O1′ and gauche to C3′ (Figure 4). This conformation, coupled with the O1′ pucker, does not permit the formation of a dimer linked by hydrogen bonds as seen in the structure of the uracil analogue.[12]

Experimental Section

Methods and Materials. Compounds. The pure 2,2′-anhydro-aThy and aThy were synthesized and characterized as described by Schinazi et al.[3] For biological evaluations, the filter-sterilized drug stock solutions were prepared immediately before use in distilled sterile water.

Biological Evaluation. aThy and 2,2′-anhydro-aThy were tested simultaneously for antiviral activity against strain F of herpes simplex virus type 1 by a plaque reduction assay in Vero cells as described previously.[18] The toxicity of the drugs was quantitated by measuring their effect on rapidly dividing Vero cells for 3 days as described previously.[18] (The number of cells in control on day 3 was 1.96×10^6/flask.)

X-ray Crystallography. Colorless crystals of anhydro-aThy were grown from aqueous ethanol solution. A crystal with dimensions $0.4 \times 0.3 \times 0.2$ mm was mounted on a quartz fiber with the long axis parallel to the fiber axis. Unit cell dimensions and the orientation matrix were determined at room temperature on a Nicolet R3 four-circle diffractometer (Cupertino, CA) using Ni-filtered Cu Kα radiation. Fifteen reflections whose Bragg angles varied from $2\theta = 12.73°$ to 29.86° were centered by machine and used in an unconstrained least-squares refinement of the lattice parameters and orientation matrix; $a = 7.825$ (1), $b = 9.752$ (2), $c = 13.924$ (3) Å; $V = 1062.4$ (3) Å^3. ω scans of several low 2θ reflections gave peak widths at half-height of less than 0.3°, indicating a satisfactory mosaic spread for the crystals. The crystals' density determined by flotation in benzene/CCl_4 was $\rho_{exp} = 1.496$ (5) g cm^{-3} in agreement with the value $\rho_{calcd} = 1.508$ g cm^{-3}, calculated for four $C_{10}H_{12}N_2O_5$ molecules per unit cell. Axial photographs indicated the crystals were orthorhombic. The absence of $h00$ reflections with $h = 2n + 1$, $0k0$ with $k = 2n + 1$, and $00l$ with $l = 2n + 1$ was consistent only with the space group $P2_12_12_1$.[19] Intensity data were collected by the $\theta - 2\theta$ scan technique, with a scan rate ranging from 5.91 to 29.3° min^{-1}. A scan width of 2° was sufficient to collect all of the peak intensity. Stationary background counts were measured at the beginning and at the end of each scan with a total background to scan time ratio of 1. No significant fluctuations were observed in the intensities of three standard reflections monitored every 82 reflections. Data with 2θ equal to 4–110° (869 total) were corrected for Lorentz and polarization effects and for absorption by an empirical correction,[20] where the minimum transmission factor was $I(\Phi)/I_{max} = 0.91$. The standard deviation for each reflection was calculated on the basis of counting statistics.

X-ray Analysis. The structure was solved by direct methods and refined with SHELX76.[21] Stereo drawings were produced by ORTEP,[22] and distance and angle calculation were carried out with ORFFE.[23] After anisotropic refinement of non-hydrogen atoms, a difference Fourier synthesis revealed the positions of 5 of the 12 hydrogens. The remaining hydrogens were placed in their calculated positions. Refinement cycles of all positional parameters with anisotropic thermal parameters and isotropic thermal parameters for the hydrogen atoms were obtained by using the 851 reflections with $F_{hkl} > \sigma_{hkl}$ until the shift/ESD for each parameter was less than 1. Final R and $R_W = \sum w^{1/2}\Delta / \sum w^{1/2}F_0$ were 0.0441 and 0.0384, respectively.

Hydrolysis Studies. Stock solutions (1 mM) of anhydro-aThy were prepared immediately before use in phosphate-buffered saline (pH 7.2). A portion of that solution (100 μL) was transferred to two UV quartz cuvettes containing PBS or cell culture medium (0.9 mL). The cuvettes were kept either at 22 or 38 °C for 8 weeks, and absorption spectra were obtained every 24 h on a Beckman Model 25 spectrophotometer. Similar experiments were carried out in acid or base (HCl, H_2SO_4, or NaOH; 0.1 M), and the spectral absorbance was monitored in situ every 10 min at 22 °C for 2 h and daily thereafter. The apparent first-order rate constant (k) was derived from the expression:

$$\log_{10}(A_\infty - A_t) = -kt/2.303 + \log_{10} A_\infty$$

where A_t is the absorbance at time t, and A_∞ is the final absorbance.

Acknowledgment. This investigation was supported in part by Grants GM 26905, GM 27907, AI 18600, and DE 07074 from the National Institutes of Health.

Supplementary Material Available: Anisotropic thermal parameters, list of observed and calculated structure factors, and a tabulation of mean planes (2 pages). Ordering information is given on any current masthead page.

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Additions and Corrections

1981, Volume 24

Joseph W. Epstein,* Herbert J. Brabander, William J. Fanshawe, Corris M. Hofmann, Thomas C. McKenzie, Sidney R. Safir, Arnold C. Osterberg, D. B. Cosulich, and F. M. Lovell: 1-Aryl-3-azabicyclo-[3.1.0]hexanes, a New Series of Nonnarcotic Analgesic Agents.

Page 481. In Table IX, the sign of the Z coordinate of atom C-8 is incorrect. The correct value is +0.152590.

Gary L. Anderson, Donald L. Bussolotti, and James K. Coward*: Synthesis and Evaluation of Some Stable Multisubstrate Adducts as Inhibitors of Cathechol *O*-Methyltransferase.

Page 1274. In Scheme I, structures **10**, **11**, **13**, and **5e** should read: