detector equipped with a 5 ft × 0.01 mm i.d. back pressure coil (total volume 0.033 cm^3). The solvents used were Burdick and Jackson methanol and glass-distilled water in a ratio of 65:35. Pressures used varied from 1000 to 2500 lb with flow rates (18–50 mL/h) maintained constant in a given determination. Fractions (30 s each) were collected directly into glass liquid scintillation vials and counted with a Beckman Model LS 200 spectrometer employing 15 mL of a fluid containing 8 g of PPO and 0.4 g of POPOP in 1 L of toluene and 100 mL of Scintisol GP. Injected samples (20.0 μL) contained a 1.0-L solution with 5.0×10^{-6} g each of **1a**, **2a,b**, **3a**, **4a,b**, **5**, and 11 in order to compare retention times of standards and ^3H metabolites. Results of the HPLC determinations are given in Table I.

Estrogenicity. The following modification of the estrogen assay described by Dorfman and Dorfman[8] was employed. Twenty-one-day-old female Sprague–Dawley rats of approximately 35 g were ovariectomized. After 7 days and for 7 consecutive days these rats were administered aqueous suspensions of compounds **1a**, **2a,b**, **3a**, and **4a,b** (25 mg/kg) by oral intubation. On day 8 uteri were removed and freed of surrounding tissue. The uteri were weighed after pressing out the intrauterine fluid on blotting paper and results were expressed as weight of the uterus in milligrams per gram of body weight times 100. Additional rates were treated in a similar manner following subcutaneous injection of 17β-estradiol in peanut oil for preparation of a standard curve.

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References and Notes

(1) J. H. Burckhalter, W. D. Dixon, M. L. Black, R. D. Westland, L. M. Werbel, H. A. DeWald, J. R. Dice, G. Rodney, and D. H. Kaump, *J. Med. Chem.*, **10**, 565 (1967).
(2) J. E. Sinsheimer, E. Van den Eeckhout, L. E. Hewitt, Y. Kido, D. R. Wade, D. W. Hansen, Jr., J. C. Drach, and J. H. Burckhalter, *J. Med. Chem.*, **19**, 647 (1976).
(3) D. W. Hansen, J. E. Sinsheimer, and J. H. Burckhalter, *J. Org. Chem.*, 41, 3556 (1976).
(4) M. N. G. James and G. J. B. Williams, *Can. J. Chem.*, **52**, 1880 (1974).
(5) R. R. Ison and A. F. Casy, *J. Pharm. Pharmacol.*, **23**, 848 (1971).
(6) D. W. Hansen, Jr., E. Van den Eeckhout, J. C. Drach, J. H. Burckhalter, and J. E. Sinsheimer, *J. Labelled Compd.*, **10**, 213 (1974).
(7) W. D. Block, K. J. Jarrett, Jr., and J. A. Levine, *Clin. Chem.*, **12**, 681 (1966).
(8) R. I. Dorfman and A. S. Dorfman, *Endocrinology*, **55**, 65 (1954).
(9) J. W. Stanley, Abstracts, 172nd National Meeting of the American Chemical Society, San Francisco, Calif., 1976, MEDI 41.

5-O-Alkylated Derivatives of 5-Hydroxy-2′-deoxyuridine as Potential Antiviral Agents. Anti-Herpes Activity of 5-Propynyloxy-2′-deoxyuridine

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Alkylation of 5-hydroxyuridine or 5-hydroxy-2′-deoxyuridine with various activated alkylating agents in the presence of 1 equiv of NaOH gave a series of new nucleoside analogues which were evaluated for antiviral activity against vaccinia virus, herpes simplex-1 virus, and vesicular stomatitis virus in both primary rabbit kidney cells and human skin fibroblasts. One of these analogues, 5-propynyloxy-2′-deoxyuridine, was a potent inhibitor of herpes simplex virus. Structure–activity considerations suggest that the anti-herpes activity is dependent on the integrity of the acetylene group since substitution of phenyl, *p*-nitrophenyl, vinyl, carboxamido, or carboxyl for the triple bond led to diminished antiviral activity.

Certain 5-substituted pyrimidine deoxyribonucleosides show potent in vitro and/or in vivo antiviral properties. Among the 5-substituents that endow 2′-deoxyuridine with in vitro antiviral activity are iodo, bromo, chloro, fluoro,[2] trifluoromethyl,[3] ethyl,[4] methoxymethyl,[5] methylthio,[6] methylamino,[7] cyano,[8] nitro,[9] thiocyano,[10,11] vinyl,[12] propyl,[12] and allyl.[12] These substituents vary considerably in steric bulk and electronic properties, rendering it difficult to dissociate one effect from the other when attempting to ascertain structure–activity trends that might be used to design other effective nucleoside antivirals. In this study, we have sought to examine the relationship between antiviral activity and the steric bulk and configuration of the 5-substituent. 5-O-Alkylated derivatives of 2′-deoxyuridine provided a useful approach to this problem, since a number of different substitutents could be introduced at the 5 position with relative ease.

Chemistry. The synthetic approach used herein is based on the observations of Otter et al.[13,14] in their approach to 6-substituted pyrimidine nucleosides. They found that 5-hydroxyuridine may, in the presence of base, be selectively 5-O-alkylated by activated alkylating agents. Indeed, compounds **2** and **4** reported here are but 2′-deoxyribonucleoside analogues of the 5-propenyloxy- and 5-propynyloxyuridines reported in their study. Thus, when either 5-hydroxyuridine or 5-hydroxy-2′-deoxyuridine was treated with 1 equiv of NaOH (to form the monoanion of the 5-hydroxyl function) and then reacted with 1.5–2.0 equiv of a properly substituted activated alkyl halide, the corresponding 5-O-alkylated nucleoside was formed in an isolable yield of 40–60%. All products were negative to $FeCl_3$ which gives a blue color with 5-hydroxyuridine or its deoxyribonucleoside analogue. The structures presented in Table I and Chart I were supported by elemental analysis, molecular weight (by chemical ionization mass spectrometry), and ^1H NMR.

Evaluation of Antiviral Activity. Antiviral activity was determined as inhibition of cytopathogenicity induced by three different viruses [herpes simplex-1 (KOS strain), vaccinia, and human skin fibroblasts (VGS strain)]. The

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Table I. Preparation and Characterization of 5-O-Alkylated Pyrimidine Nucleosides

Compd	Prep method	Recrystn solvent	Mp, °C	Analyses	UV_{max}[c]	CI MS (*m/e*)	^1H NMR (δ)
2	A		70 (glass)	*a*	279	285 (P + 1, 80), 302 (P + 18, 12)	7.61 (s, 1, H_6), 6.18 (t, 1, $H_{1'}$), 5.90 (m, 1, vinyl H), 5.34 (d, 2, terminal vinyl CH_2), 4.34 (d, 2, allyl OCH_2)
4	A	CH_3OH	160.5–161.5	C, H, N	275	283 (P + 1, 0.2), 300 (P + 18, 0.25), 282 (P^+, 35), 165 (b + H, 100)	7.71 (s, 1, H_6), 6.18 (t, 1, $H_{1'}$), 4.61 (d, 2, propynyl CH_2)
5	B	H_2O	197–198	C, H, N	276	351 (P + 1, 18), 368 (P + 18, 12)	7.78 (s, 1, H_6), 7.39 (s, 5, benzyl H's), 5.82 (d, 1, $H_{1'}$), 4.86 (s, 1, benzyl CH_2)
6	B	H_2O	132–133	C, H, N	276	335 (P + 1, 100), 352 (P + 18, 85)	7.77 (s, 1, H_6), 7.36 (s, 5-benzyl H's), 6.18 (t, 1, $H_{1'}$), 4.84 (s, 2, benzyl CH_2)
7	B	H_2O	190–191	C, H, N	274	No parent ion obtained	7.96 (q, 4, benzyl H's), 7.82 (s, 1, H_6), 5.78 (d, 1, $H_{1'}$), 5.01 (br s, 3, benzyl CH_2 and 5'-OH)
8	B	H_2O	161–162.5	C, H, N	274	380 (P + 1, 6), 397 (P + 18, 1)	7.96 (q, 4, benzyl H's), 7.76 (s, 1, H_6), 6.17 (t, 1, $H_{1'}$), 5.04 (s, 2, benzyl CH_2)
9	B	H_2O	178.5–179.5	C, H, N	276	365 (P + 1, 50), 382 (P + 18, 10)	7.71 (s, 1, H_6), 7.21 (q, 4, benzyl H's), 5.80 (d, 1, $H_{1'}$), 4.80 (s, 2, benzyl CH_2), 2.30 (s, 3, CH_3)
10	B	H_2O	208–209	C, H, N	277	317 (P + 1, 2.5), 185 (b + H, 100)	7.80 (s, 1, H_6), 7.45 (br s, 2, amide NH_2), 5.75 (d, 1, $H_{1'}$), 4.27 [s, 2, $OCH_2C(=O)$–]
11	B	H_2O	220–221	C, H, N	277	302 (P + 1, 3), 186 (b + 2H, 100)	7.74 (s, 1, H_6), 7.40 (br s, 2, amide NH_2), 6.18 (t, 1, $H_{1'}$), 4.25 [s, 3, $OCH_2C(=O)$– and 3'-H]
12	C		170–175 dec	*b*	277	No parent ion obtained	

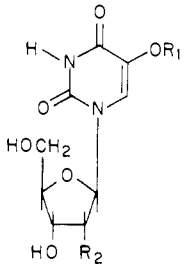
[a] Compound 2 was obtained as an amorphous powder that could not be induced to crystallize. A satisfactory elemental analysis could not be obtained. Calcd: C, 50.70; H, 5.75; N, 9.86. Found: C, 50.01; H, 5.61; N, 9.37. [b] Compound 12 was analyzed as the free acid but could not be obtained crystalline. A satisfactory analysis could not be obtained for the amorphous product. Calcd: C, 43.71; H, 4.67; N, 9.27. Found: C, 43.26; H, 4.45; N, 8.86. [c] In nm. Determined in phosphate buffered saline, pH 7.2.

Table II. Antiviral Activity of 5-O-Alkylated Derivatives of 5-Hydroxy-2'-deoxyuridine[a]

Compd	Vaccinia virus		Herpes simplex virus	
	PRK cells	HSF	PRK cells	HSF
2	7×10^{-5}	3.5×10^{-4}	1.4×10^{-5}	1.4×10^{-4}
4	3.5×10^{-5}	1.4×10^{-4}	2.6×10^{-6}	3.5×10^{-6}
6	$>3 \times 10^{-4}$	$>6 \times 10^{-4}$	$>1.2 \times 10^{-4}$	$>4.5 \times 10^{-4}$
8	$>2.6 \times 10^{-4}$	$>5.2 \times 10^{-4}$	$>2.6 \times 10^{-4}$	$>5.2 \times 10^{-4}$
11	1.3×10^{-4}	1.3×10^{-4}	1.4×10^{-4}	2.6×10^{-4}
12	$>6 \times 10^{-4}$		$>6 \times 10^{-4}$	
5-Hydroxy-2'-deoxyuridine	1.4×10^{-5}	4×10^{-5}	1.6×10^{-5}	3.5×10^{-6}
5-Iodo-2'-deoxyuridine	1.4×10^{-6}	1.4×10^{-6}	1.4×10^{-6}	3.5×10^{-7}
Adenine arabinoside	1.4×10^{-6}	2.6×10^{-5}	1.4×10^{-5}	3.5×10^{-6}

[a] Results expressed as minimum inhibitory concentration (M), i.e., the concentration required to inhibit virus-induced cytopathogenicity by 50%.

Chart I



1, $R_1 = -CH_2CH{=}CH_2$; $R_2 = OH$
2, $R_1 = -CH_2CH{=}CH_2$; $R_2 = H$
3, $R_1 = -CH_2C{\equiv}CH$; $R_2 = OH$
4, $R_1 = -CH_2C{\equiv}CH$; $R_2 = H$
5, $R_1 = -CH_2-C_6H_5$; $R_2 = OH$
6, $R_1 = -CH_2-C_6H_5$; $R_2 = H$
7, $R_1 = -CH_2-C_6H_4-p\text{-}NO_2$; $R_2 = OH$
8, $R_1 = -CH_2-C_6H_4-p\text{-}NO_2$; $R_2 = H$
9, $R_1 = -CH_2-C_6H_4-p\text{-}CH_3$; $R_2 = OH$
10, $R_1 = -CH_2C(=O)NH_2$; $R_2 = OH$
11, $R_1 = -CH_2C(=O)NH_2$; $R_2 = H$
12, $R_1 = -CH_2C(=O)OH$; $R_2 = H$

results are expressed in terms of minimum inhibitory concentration (MIC in M), that is, the concentration of analogue required to effect a 50% reduction in viral cytopathogenicity. Included in these assays were several nucleosides with established antiviral activity.

Of the nucleosides evaluated in this study (Table II), one shows an intriguing antiviral activity; 5-propynyloxy-2′-deoxyuridine (4) possesses an anti-herpes activity that is more potent than that exhibited by adenine arabinoside and nearly comparable (in PRK cells) to that shown by 5-iodo-2′-deoxyuridine. Surprisingly, 4 was significantly less active against another DNA virus (vaccinia). This specific inhibition of different DNA viruses has been noted with other nucleoside analogues; e.g., 5-cyano-[8] and 5-nitro-2′-deoxyuridine[9] block vaccinia virus replication with diminished activity against herpes virus, whereas 5-propyl-2′-deoxyuridine[12] specifically blocks herpes virus multiplication and has little effect on vaccinia virus.[15] The specific antiviral actions of these nucleoside analogues might be related to the nature of the viral-induced enzymes or to other factors such as the cellular site of viral replication.

The data of Table II show clearly that modifications other than the introduction of a propynyloxy substituent have a deleterious effect on antiviral activity. When the triple bond of 4 was replaced by the bulky phenyl or *p*-nitrophenyl groups, the resulting analogues were devoid of significant antiviral activity. Substitution of the vinyl group for the acetylene group of 4 gave **2** which was 10–20 times less active against herpes but retained the moderate activity against vaccinia virus. Replacement of the triple bond of 4 by a carboxamide function (analogue **11**) also led to a significant decrease in antiviral activity. Finally, all antiviral properties were abolished in compound **12** in which the negatively charged (at physiological pH) carboxyl group has replaced the acetylene of 4.

Parenthetically, we noted that all of the ribose analogues (**1**, **3**, **5**, **7**, **9**, and **10**) were devoid of activity [MIC > 200 μg/mL (>6 × 10^{-4} M)] against herpes and vaccinia, and all new analogues, whether ribo- or deoxyribonucleosides, prepared in this study were inactive against an RNA virus (vesicular stomatitis virus).

The possibility that 4 was antiviral simply because of a nonspecific cytotoxic effect was eliminated by the following observations.

(a) Selective activity against one DNA virus (herpes simplex) would not be expected for a substance that acts by blocking host cell macromolecule synthesis. In addition, 4 was inactive against an RNA virus (vesicular stomatitis).

(b) Under conditions where 5-iodo-2′-deoxyuridine (10^{-5} M) completely suppressed the replication of mouse L cells, 4 had no effect even at 10^{-3} M.

(c) The concentration of 4 required to effect a 50% reduction in DNA synthesis in uninfected PRK cells (as measured by [^{3}H]-dT incorporation) was ~150 times that required to effect a 50% reduction in herpes virus cytopathogenicity. For 5-iodo-2′-deoxyuridine, 5-hydroxy-2′-deoxyuridine, and 1-(β-D-arabinofuranosyl)adenine, the corresponding ratios were 12, 6, and 6, respectively.[16]

Conclusion

The data presented here show that 5-propynyloxy substitution at pyrimidine C-5 of 2′-deoxyuridine leads to a nucleoside analogue with a potent in vitro anti-herpes activity. The data further suggest that the high antiviral activity of 5-propynyloxy-2′-deoxyuridine and its selective activity against herpes simplex virus are rather sensitive to the steric and/or configurational aspects of the side chain at C-5. Further modifications at pyrimidine C-5 of 2′-deoxyuridine alone, or possibly in combination with changes in the deoxyribose moiety, may yield new analogues with selective antiviral activity.

Experimental Section

5-Iodo-2′-deoxyuridine was obtained from Sigma (St. Louis, Mo.), and 9-(β-D-arabinofuranosyl)adenine was from P-L Biochemicals (Milwaukee, Wis.). Other chemicals were purchased from Aldrich (Milwaukee, Wis.). 5-Hydroxyuridine,[17] 5-hydroxy-2′-deoxyuridine,[18] and compounds **1**[13] and **3**[14] were prepared according to the literature. Compound **1** was identical with an authentic sample supplied by Dr. Brian Otter (Sloan Kettering). Melting points (uncorrected) were determined on a Thomas-Hoover apparatus and the following spectra determined as indicated: UV on a Cary 15, ^{1}H NMR on a Varian HA-100 or HR-220, and chemical ionization mass spectra (CI MS) on a Finnigan 1015 D gas chromatograph/mass spectrometer using NH_3 or NON_2. Me_4Si was used as the standard for the NMR spectra, and chemical shifts are reported in parts per million (δ). Signals are described as s (singlet), d (doublet), t (triplet), and m (multiplet). Microanalyses were determined by the staff of the microanalytical section of this laboratory. Thin-layer chromatography was performed using silica gel GF plates (Analtech). Methodology used for the assay of antiviral activity has been described earlier.[11]

The procedures for the preparation and isolation of the 5-O-alkylated nucleosides varied among three different methodologies. Rather than describe the procedure for each nucleoside in detail, we describe examples of each procedure, and the specific method is presented with the pertinent characterization data in Table I.

Method A. 5-(2-Propynyloxy)-2′-deoxyuridine (4) [1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-5-(2-propynyloxy)-uracil]. 5-Hydroxy-2′-deoxyuridine (2.44 g, 0.01 mol) was dissolved in a 1:1 mixture of MeOH and H_2O (100 mL) to which had been added NaOH (1 N, 10 mL, 0.01 mol). Propargyl bromide (2.5 g, 1.6 mL, 0.02 mol) was added to the clear solution, and reaction was allowed to proceed for 24 h at room temperature. Solvent was removed by evaporation in vacuo (40 °C), and the residue was dried by coevaporation several times with ethanol. To the oily residue, methanol was added together with 3 g of dry silica gel. Methanol was removed in vacuo, and the resulting powder was applied to the top of a silica gel column made up with $CHCl_3$–MeOH (20:1). The column was eluted with $CHCl_3$–MeOH (15:1). Fractions (10 mL) were collected and checked for homogeneity by TLC [$CHCl_3$–MeOH (7:1)]. The major product (4) had an R_f ~0.6 in $CHCl_3$–MeOH (7:1). A minor product was eluted just before 4 and had an R_f ~0.7 in the same TLC system. Appropriate fractions containing 4 were pooled and evaporated to dryness in vacuo. The residue was dissolved in hot MeOH and filtered to remove foreign material, and the filtrate was kept at

4 °C. After 2 days, the crystals that formed were filtered off, washed with cold MeOH, and dried in vacuo at 80 °C. The yield of 4 was 1.7 g (60%).

Method B. 1-[[1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2,4-dioxopyrimidin-5]oxy]acetamide (11). 5-Hydroxy-2′-deoxyuridine (1 g, 4.1 mmol) was dissolved in 1 N NaOH (4.1 mL, 4.1 mmol) and, to this solution, iodoacetamide (1.52 g, 8.2 mmol) was added. Within a few minutes, a colorless precipitate began to form. The mixture was stirred for a total of 48 h and then filtered. The crystalline precipitate was washed with cold water and then recrystallized twice from hot water. The yield after drying (80 °C, in vacuo; 24 h) was 0.91 g (72%).

Method C. 1-[[1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2,4-dioxopyrimidin-5]oxy]acetic Acid (12). 5-Hydroxy-2′-deoxyuridine (282 mg, 1.15 mmol) was dissolved in 1.16 mL of 1 N KOH (1.16 mmol), and then iodoacetic acid (603 mg, 3.4 mmol) in H_2O (0.84 mL) was added. The solution was allowed to react for 48 h at ambient temperature after which time HCl (3 N, 1.06 mL) was added, and the resulting solution was concentrated to 0.5 mL in vacuo (40 °C). The addition of ethanol (6 mL) produced a precipitate which was filtered off and washed with cold ethanol (4 mL). The yield of product at this point was 150 mg (50%). The product was recrystallized twice from hot EtOH and then dissolved in a minimum of H_2O and applied to a Dowex 50 (H^+) column which was eluted with H_2O. The UV absorbing eluate was pooled and lyophilized. Chromatography on cellulose TLC with *i*-PrOH–1% $(NH_4)_2SO_4$ (2:1) gave but one spot with R_f 0.42.

References and Notes

(1) On leave from the Department of Chemistry, University of Cincinnati, Cincinnati, Ohio.
(2) (a) W. H. Prusoff and B. Goz in "Antineoplastic and Immunosuppressive Agents. Part II. Handbook of Experimental Pharmacology", Vol. XXXVIII, A. C. Sartorelli and D. G. Johns, Ed., Springer-Verlag, New York, N.Y., 1975, pp 272–347; (b) J. Sugar and H. E. Kaufman in "Selective Inhibitors of Viral Functions", W. A. Carter, Ed., CRC Press, Cleveland, Ohio, 1973, pp 295–311.
(3) C. Heidelberger, *Ann N.Y. Acad Sci.*, **255**, 317–325 (1975).
(4) E. De Clercq and D. Shugar, *Biochem. Pharmacol.*, **24**, 1073–1078 (1975).
(5) J. B. Meldrum, V. S. Gupta, and J. R. Saunders, *Antimicrob. Ag. Chemother.*, **6**, 393–396 (1974).
(6) V. S. Gupta, G. L. Bubbar, J. B. Meldrum, and J. R. Saunders, *J. Med. Chem.*, **18**, 973–976 (1975).
(7) T. Y. Shen, J. F. McPherson, and B. O. Linn, *J. Med. Chem.*, **9**, 366–369 (1966).
(8) P. F. Torrence, B. Bhooshan, J. Descamps, and E. De Clercq, *J. Med. Chem.*, **20**, 974–976 (1977).
(9) E. De Clercq, J. Descamps, G. F. Huang, and P. F. Torrence, unpublished observations.
(10) T. Nagamachi, J.-L. Fourrey, P. F. Torrence, J. A. Waters, and B. Witkop, *J. Med. Chem.*, **17**, 403–406 (1974).
(11) E. De Clercq, P. F. Torrence, J. A. Waters, and B. Witkop, *Biochem. Pharmacol.*, **24**, 2171–2175 (1975).
(12) Y.-C. Cheng, B. A. Domin, R. A. Sharma, and M. Bobek, *Antimicrob. Ag. Chemother.*, **10**, 119–122 (1976).
(13) B. A. Otter, A. Taube, and J. J. Fox, *J. Org. Chem.*, **36**, 1251–1255 (1971).
(14) B. A. Otter, S. S. Saluja, and J. J. Fox, *J. Org. Chem.*, **37**, 2858–2863 (1972).
(15) E. De Clercq, J. Descamps, and D. Shugar, unpublished observations.
(16) E. De Clercq, J. Descamps, and P. F. Torrence, unpublished observations.
(17) D. W. Visser in "Synthetic Procedures in Nucleic Acid Chemistry", W. W. Zorbach and R. S. Tipson, Ed., Interscience, New York, N.Y., 1968, pp 428–429.
(18) E. G. Podrebarac and C. C. Cheng in ref 17, pp 412–413.

Conformations of Selected 3-Substituted 4-Hydroxycoumarins in Solution by Nuclear Magnetic Resonance. Warfarin and Phenprocoumon

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The chemical shift position of the benzylic proton, H_x, has been found to be diagnostic in indicating the preferred conformations of selected 3-substituted 4-hydroxycoumarins. In general, the nonrigid open-chain compounds, e.g., the open-chain tautomer of warfarin and phenprocoumon, are found to exist in equal populations of the two conformations in which the benzylic proton is in the plane of the coumarin ring and is either cis or trans to the 3,4 double bond. The cyclic compounds, e.g., cyclocumarol, are constrained to two limiting conformations defined as axial$_2$ or trans or intermediate conformations between these limits. Evidence is presented that suggests that the antivitamin K activity of warfarin is due to its open side-chain tautomeric form.

The oral anticoagulants are known to manifest their biologic activity by inhibiting the synthesis of the calcium binding sites in the vitamin K dependent clotting factors.[1-8] As a class of therapeutic agents they are highly susceptible to the phenomenon of drug interactions[9,10] and as such afford good model compounds to study such phenomena. When specific anticoagulants are chiral, e.g., warfarin, the normal metabolic patterns obtained from the two enantiomers are different[11,12] and the patterns are quantitatively and stereochemically sensitive to the presence of other drugs.[11,13] A knowledge of the structure and conformational preference of these agents in solution would provide a molecular basis for studying their interactions with the specific hemoproteins that lead to their biotransformation or as yet undefined receptors that lead to their inhibition of vitamin K.

The purpose of this report is to present evidence for the preferred conformations of open-chain warfarin, phenprocoumon, and a series of 3-substituted 4-hydroxycoumarins in solution and to demonstrate that the active form of warfarin at the vitamin K dependent site is probably the open-chain tautomer.

Results and Discussion

In the course of studying the ^1H NMR spectra of several anticoagulants and their derivatives, Table I, it became apparent that the range of values observed for the chemical shift of the benzylic proton, H_x, might be a useful probe

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