895.
(34) Booth, H.; Everett, J. R. *J. Chem. Soc., Chem. Commun.* **1976**, 278.
(35) Booth, H.; Bailey, J. M. *J. Chem. Soc., Perkin Trans. 2* **1979**, 510.
(36) Eliel, E. L.; Knoeber, M. C. *J. Am. Chem. Soc.* **1968**, *90*, 3444. Nader, F. W.; Eliel, E. L. *Ibid.* **1979**, *92*, 3050.
(37) For a similar situation in 1,2,3-trimethyl-1,3-diazane, see: Eliel, E. L.; Kopp, L. D.; Dennis, J. E.; Evans, S. A. Jr. *Tetrahedron Lett.* **1971**, 3409.
(38) Profeta, S. Jr. Ph.D. Dissertation, University of Georgia, Athens, Ga., 1978.
(39) Lecomte, C.; Protas, J.; Bianchin, B.; Delpuech, J. J. *Cryst. Struct. Commun.* **1975**, *4*, 477.
(40) Kashino, S. *Acta Crystallogr., Sect. B* **1973**, *29*, 1836; Fukuyama, K.; Kashino, S.; Haisa, M. *Ibid.* **1973**, *29*, 2713.
(41) McDowell, J. J. H. *Acta Crystallogr., Sect. B*, **1975**, *31*, 2256.
(42) Kawazoe, Y.; Tsuda, M.; Ohnishi, M. *Chem. Pharm. Bull.* **1967**, *15*, 51. Kawazoe, Y.; Tsuda, M. *Ibid.* **1967**, *15*, 1405.
(43) Ma, J. C. N.; Warnoff, E. W. *Can. J. Chem.* **1965**, *43*, 1849.
(44) Delpuech, J. J.; Deschamps, M. N. *Chem. Commun.* **1967**, 1188.
(45) Booth, H.; Little, J. H.; Feeney, J. *Tetrahedron* **1968**, *24*, 279.
(46) Because of Me/Me eclipsing in the transition state, the nitrogen inversion of **11m** is relatively slow and can be frozen out at −100 °C in ^{13}C NMR [cf. Anet, F. A. L.; Yavari, I.; Ferguson, I. J.; Katritzky, A. R.; Moreno-Mañas, M.; Robinson, M. J. T. *J. Chem. Soc., Chem. Commun.* **1976**, 399]. When a solution of **11m** is cooled to −95 °C, there is first broadening of the N-Me resonance and then sharpening again, but with no second signal emerging. Evidently the signal for axial N-Me is too small to measure, which we take to mean that there is less than 2% of axial isomer at that temperature; hence $\Delta G^\circ > 1.3$ kcal/mol.
(47) Trotman-Dickenson, A. F. *J. Chem. Soc.* **1949**, 1293.
(48) Cf. Hirsch, J. A. *Top. Stereochem.* **1967**, *1*, 199.
(49) Cf. "Tables of Interatomic Distances and Configuration of Molecules and Ions," Supplement 1956–1959, *Chem. Soc., Spec. Publ.* **1965**, *No. 18*, S 16.
(50) See also: Hargrave, K. D.; Eliel, E. L. *Isr. J. Chem.*, in press.
(51) Eliel, E. L.; Willer, R. L. *J. Am. Chem. Soc.* **1977**, *99*, 1936.
(52) For comparison, we cite here the gauche OH/CH_3 interactions reported by Sicher, J.; Tichy, M. *Collect. Czech. Chem. Commun.* **1967**, *32*, 3687: e,e, 0.38; e-Me, a-OH, 0.66; e-OH, a-Me, 0.83 kcal/mol.
(53) Dalling, D. K.; Grant, D. M. *J. Am. Chem. Soc.* **1972**, *94*, 5318.
(54) The parameters here cited originate from different sources and are taken from ref 23 and 55.
(55) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; p 49.
(56) E.g., Vierhapper, F. W.; Eliel, E. L. *J. Org. Chem.* **1975**, *40*, 2729.
(57) Vierhapper, F. W.; Eliel, E. L. *J. Org. Chem.* **1975**, *40*, 2734.
(58) E.g., Clarke, H. T.; Gillespie, H. B.; Wiesshaus, S. Z. *J. Am. Chem. Soc.* **1933**, *55*, 4571.
(59) Cf. Booth, H.; Little, J. H.; Feeney, J. *Tetrahedron* **1968**, *24*, 279. However, these investigators effected complete reduction with Na/EtOH and obtained only ca. 6% each of the two minor isomers.
(60) Marvel, C. S.; Lazier, W. A. "Organic Syntheses", Collect. Vol. I; Wiley: New York, 1932; p. 99. Braun, J. v. *Ibid.*, p 428.
(61) Leonard, N. J.; Wicks, Z. W. *J. Am. Chem. Soc.* **1946**, *68*, 2402.
(62) Whitehead, E. V.; Dean, R. A.; Fidler, F. A. *J. Am. Chem. Soc.* **1951**, *73*, 3632.
(63) Hanschke, E. *Chem. Ber.* **1955**, *88*, 1048.
(64) The value in *N*-methylpiperidine also includes a vicinal effect. Unfortunately, the values for $\alpha_a(2)$ in these two cases (as well as in the oxanes[23]) depend on measurements on compounds in which the Me-2a conformer is the minor component and the values are therefore not very accurate.

Influence of Substituents at the 5 Position on the Structure of Uridine

Ernst Egert,* Hans Jörg Lindner, Wolfgang Hillen,† and Michael C. Bohm

Contribution from the Institut für Organische Chemie und Biochemie, Technische Hochschule Darmstadt, D-6100 Darmstadt, West Germany.
Received July 26, 1979

Abstract: The conformations of 5-substituted uridines, some of which are found in the first anticodon position of tRNAs, have been studied by X-ray crystallography, ^1H NMR spectroscopy, and quantum-chemical methods. The results clearly demonstrate that the electronic effect of a substituent X at the 5 position influences not only the base moiety, but also (1) the N(1)–C(1′) and C(1′)–O(1′) bonds in opposite ways and (2) the conformation of the ribose via the dihedral angle χ about the glycosidic bond. This can be rationalized in simple MO schemes using the concepts of "through-bond" and "through-space" interaction which result in an electron transfer from O(1′) to X. EH and MINDO/3 calculations suggest that the ribosyl moiety acts as a variable donor whose strength depends on χ and X. The favored χ values of C(2′)-*endo*- and C(3′)-*endo*-uridine predicted by MINDO/3 are in line with the experimental data.

Introduction

In addition to the four standard bases A, U, C, and G there are a remarkable number of modified nucleosides present in tRNAs.[1] 5-Substituted uridines (x^5U) and 2-thiouridines (s^2x^5U) occur in the first anticodon ("wobble")[2] position (Figure 1), where uridine itself is only found exceptionally. Although 5 substituents do not directly participate in the formation of hydrogen bonds, they strongly influence the codon recognition of urdine and effect a significant modulation of the base pairing between mRNA and tRNA.[3] The three-dimensional structure of yeast $tRNA^{Phe}$ [4,5] shows the "wobble" nucleoside in a sterically exposed position being the terminator of the anticodon helix.[6] The codon-anticodon interaction is likely to be influenced by the conformations of the two trinucleoside diphosphates involved. Substituents at the "wobble" base could modify the exact conformation and hence the base-pairing properties of the anticodon. We have studied the influence of substituents at the 5 position on the structure of uridine with the aid of X-ray crystallography, ^1H NMR spectroscopy, and quantum-chemical calculations.

Results and Discussion

A. Hypotheses Arising from Crystal Data. The replacement of the hydrogen atom at the 5 position of uridine by a substituent with different electronic properties will change the electron distribution within the π system and hence the geometry of the heterocyclic base, especially if the 5 substituent has not only an inductive but also a mesomeric effect. π-Accepting and π-donating substituents have opposing effects on bond lengths which can be predicted by a simple scheme (Figure 2). This agrees well with the bond lengths observed in crystal structures.[7]

A detailed analysis of published strcutres indicates that 5 substituents influence not only the π system, but also the glycosidic bond N(1)–C(1′) and, to a lesser degree, C(1′)–O(1′). These bonds change gradually going from 5-nitrouridine to 5-aminouridine (Table I[8–14]). Electron-withdrawing 5 substituents lead to a lengthening and electron-donating groups to a shortening of the N(1)–C(1′) bond with reverse effect on

* University Chemical Laboratory, Cambridge, England.
† Department of Biochemistry, University of Wisconsin, Madison, Wis. 53706.

0002-7863/80/1502-3707$01.00/0

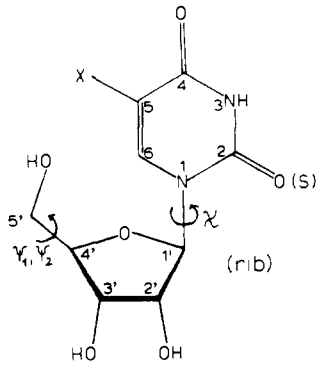


Figure 1. Structural formula of a uridine derivative x^5U (s^2x^5U) showing the numbering of the atoms and the characteristic dihedral angles $\chi \equiv$ C(6)-N(1)-C(1′)-O(1′), $\psi_1 \equiv$ O(1′)-C(4′)-C(5′)-O(5′), and $\psi_2 \equiv$ C(3′)-C(4′)-C(5′)-O(5′).

Table I. Some Bond Lengths of 5-Substituted Uridines x^5U (Å)

x	N(1)-C(1′)	C(1′)-O(1′)	C(4′)-O(1′)
NO_2[8]	1.504	1.390	1.461
Cl[9]	1.474	1.410	1.448
H[10,a]	1.490	I.413	1.454
CH_3[11]	1.481	1.410	1.460
OCH_3[12]	1.490	1.395	1.463
OH[13]	1.467	1.416	1.460
$N(CH_3)_2$[14]	1.464	1.422	1.465
NH_2[7]	1.427	1.432	1.460

[a] Mean values from the two independent molecules found in the asymmetric unit.

the C(1′)-O(1′) bond. The latter distance is always shorter than C(4′)-O(1′), but the difference between these two bond lengths depends on the specific 5 substitution. It is 0.071 Å in 5-nitrouridine, 0.041 Å in uridine, and 0.028 Å in 5-aminouridine.

The 5 substituents also influence the dihedral angle $\chi \equiv$ C(6)-N(1)-C(1′)-O(1′) (see Figure 1): 5-substituted uridines with electron-donating groups generally have larger χ values than those with electron-accepting substituents (Table II[15-21]). There is a definite relationship between the puckering of the

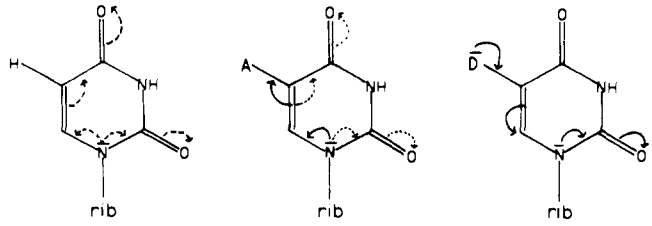


Figure 2. The predicted influence of a π-accepting (A) and a π-donating (D) 5 substituent on the bond lengths within the base moiety of uridine (rib ≡ ribosyl moiety). Extensive π-electron delocalization is indicated by full, moderate by dashed, and weak by dotted arrows.

ribose and the orientation of the base:[22] in almost all pyrimidine nucleosides studied by crystallographic methods[23,34] those with C(3′)-endo conformation have χ values of between 0 and 35° and those with C(2′)- endo conformation 40-70°. A similar relation seems to hold also in solution.[35] Model-building studies indicate that in the case of C(3′)-endo conformation a large χ value is prevented by steric interaction between H(C6) and the axial H(C3′). In the case of C(2′)-endo conformation low dihedral angles are unlikely because of repulsive forces between O(2) and O(2′) (Figure 3). 2-Thiouridine and its derivatives exhibit exclusively the C(3′)-endo conformation with χ values between 0 and 20° as a result of the enhanced Coulomb interaction between O(2′) and the sulfur atom S(2).

Within these rather narrow limits for the orientation of the base, the H atom at C(6) is next to O(5′) of the ribose if the conformation at C(4′)-C(5′) is gauche-gauche with O(5′) above the five-membered ring, the preferred orientation both in crystals and in solution.[36-38] In this conformation the great majority of uridine derivatives exhibit a C(6)-H···O(5′) interaction resulting in a shortened C···O distance (compared with the sum of the van der Waals radii of 3.6 Å) and a C-H···O angle of 145-180° (see Table II). The characteristic intramolecular "hydrogen bond"[39] is responsible for the precise orientation of the base moiety within the range set by the ribose puckering. In compounds like 6-azauridine[40] and 6-azacytidine[41] the replacement of such a stabilizing interaction by the N(6)···O(5′) repulsion gives rise to unusually high χ values of 75-100° (in spite of the C(3′)-endo conformation in the crystal) and to a destabilization of the gauche-gauche arrangement in solution.[42]

Table II. Structural Parameters of Some Uridine Derivatives Listed in Order of Increasing Dihedral Angle χ[a,b]

nucleoside	χ, deg	conformation of the ribose	C(6)···O(5′), Å	<C(6)-H···O(5′), deg
s^2cm^5U[6]	3	C(3′)-endo	C(4′)-C(5′)→gauche-trans	
i^5U I[15]	13.2	C(3′)-endo	3.353	
s^2mnm^5U[16]	15	C(3′)-endo	3.27	161
s^2mcm^5U[6]	16	C(3′)-endo	3.41	146
s^2U[17]	17	C(3′)-endo	C(4′)-C(5′)→gauche-trans	
U I[10]	18.3	C(3′)-endo	3.112	148
s^2s^4U[18]	19.5	C(3′)-endo	3.419	151
U II[10]	24.3	C(3′)-endo	3.548	169
mo^5U[12]	25.4	C(3′)-endo	3.242	158
no_2^5U[8]	25.6	C(3′)-endo	3.333	160
m^5U[11]	29.4	C(3′)-endo	3.429	171
$mcmo^5U$[19]	34.3	C(3′)-endo	3.128	157
o^5U[13]	42.1	C(2′)-endo	C(6)-H···O(2) interaction	
m_2n^5U[14]	51.0	C(2′)-endo	C(4′)-C(5′)→gauche-trans	
cl^5U[9]	51.4	C(2′)-endo	3.288	165
*[20]	52.3	C(2′)-endo	3.406	162
**[21]	53.5	C(2′)-endo	3.297	166
i^5U II[15]	58.7	C(2′)-endo	C(4′)-C(5′)→trans-gauche	
n^5U[7]	61.0	C(2′)-endo	3.539	158

[a] Abbreviations: cl ≡ -Cl, cm ≡ $-CH_2COOH$, i ≡ -I, m ≡ $-CH_3$, mcm ≡ $-CH_2COOCH_3$, mcmo ≡ $-OCH_2COOCH_3$, $m_2n \equiv -N(CH_3)_2$, mnm ≡ $-CH_2NHCH_3$, mo ≡ $-OCH_3$, n ≡ $-NH_2$, $no_2 \equiv -NO_2$, o ≡ -OH, s ≡ =S, * ≡ 3-deazauridine, ** ≡ 3-deaza-4-deoxyuridine. [b] If no C(6)···O(5′) interaction is observed, the conformational abnormality preventing it is listed in columns 4 and 5.

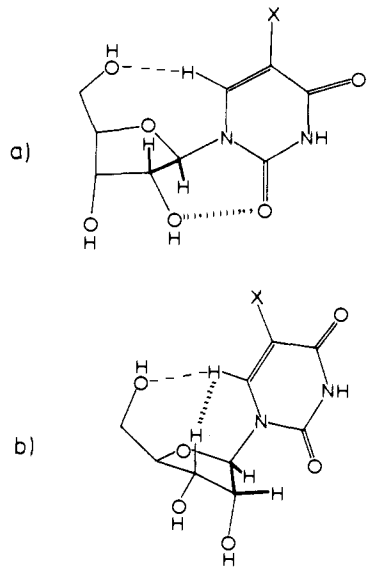


Figure 3. Schematic drawings of a uridine derivative with (a) C(2′)-endo and (b) C(3′)-endo puckering of the ribose showing the stabilizing C(6)-H···O(5′) hydrogen bond, the conformation at C(1′)-C(2′), and the interactions which prevent a free rotation about the glycosidic bond.

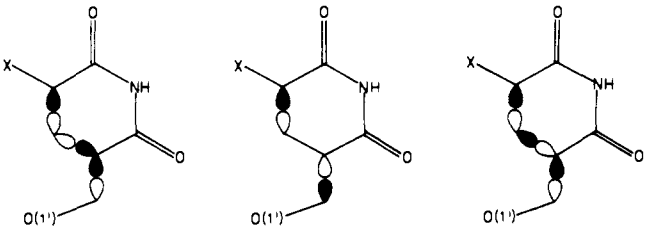


Figure 4. Semilocalized σ^* orbitals between C(1′) and C(5).

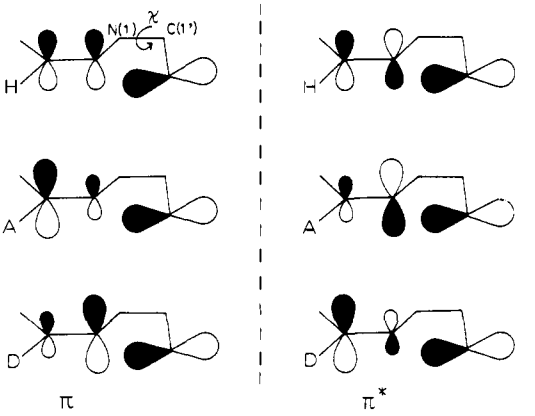


Figure 5. "Through-space" interaction between the n orbital at O(1′) and the C(5)=C(6) double bond at small χ values.

The interaction between 5 substituents and the ribosyl moiety leading to the specific bond-length alterations found by X-ray analyses can be explained by a simple MO scheme assuming a charge transfer from a lone pair at O(1′) to the 5 substituent X via three semilocalized σ^* orbitals which arise from suitable combinations of those localized between C(1′) and C(5) (Figure 4). There will be a noticeable charge transfer only for a group X with low unoccupied levels, i.e., for an electron-accepting group, because otherwise a significant interaction is not possible. In the case of an electron-accepting 5 substituent the partial population of the σ^* MOs will weaken, and hence elongate, the three σ bonds C(5)-C(6)-N(1)-C(1′). In the base moiety this effect will be dominated by the marked π-electron delocalization. Therefore, the charge transfer will mainly influence the N(1)-C(1′) bond length but also the C(1′)-O(1′) distance, which will be shortened owing to the enhanced Coulomb interaction.

In addition to this "through-bond" coupling a "through-space" interaction[43-45] has to be considered (Figure 5): a small dihedral angle χ favors the overlap between a lone pair at O(1′) with the π and π^* orbitals of the C(5)=C(6) double bond. The energy of the n orbital is lowered by the interaction with the unoccupied π^* MO in contrast to the n-π overlap which is destabilizing as a result of the occupation of both levels. An electron acceptor at the 5 position leads to an increase of the π coefficient at C(5) and the π^* coefficient at C(6) compared with the unperturbed π bond. An electron-donating group causes a converse polarization.[46] Thus, electron-attracting 5 substituents weaken the antibonding (n-π) and strengthen the bonding (n-π^*) interaction. They will favor a small C(6)···O(1′) distance and thus a small χ value. Electron-donating groups at the 5 position will produce a net destabilization of this conformation. This can be avoided by an increased dihedral angle.

The values of χ as measured in the crystal (see Table II) support the predictions by the qualitative MO arguments. For example, χ is considerably larger for X = NH_2 or $N(CH_3)_2$ than for X = NO_2. The data of the two independent molecules of 5-iodouridine and a comparison between X = Cl and X = OCH_3, however, show that this intramolecular effect can be compensated by intermolecular interactions.

B. ^{1}H NMR Results. From the results obtained in the previous chapter it can be predicted that substituents at the 5 position of uridine should exert influence on the conformation of the furanose ring, too. Since an electron-accepting group favors a low χ value, C(3′)-endo rather than C(2′)-endo puckering will be preferred. On the other hand, an electron-donating 5 substituent favors a greater dihedral angle χ, which will result in a C(2′)-endo conformation.

Table III. Chemical Shifts and Coupling Constants of 5-Substituted Uridines x^{5}U[a]

x	$\delta_{H(C6)}$, ppm	$\delta_{H(C1')}$, ppm	$J_{1',2'}$, Hz
H	7.87	5.91	4.6
OH	7.45	5.94	4.5
OCH_3	7.56	5.96	3.6
NH_2	7.32	5.95	4.7
NH_2 (pD ≈ 1)	8.32	5.90	3.5
$N(CH_3)_2$	7.52	5.96	3.7
$N(CH_3)_2$ (pD ≈ 1)	8.75	5.87	2.1
Cl	8.24	5.89	3.5
CN	8.71	5.87	2.8
CHO	8.90	5.92	2.5
NO_2	9.66	5.91	1.7

[a] The signals are given relative to 3-(trimethylsilyl)propionate-*2,2′,3,3′-d*$_4$.

^{1}H NMR spectra of different uridine derivatives measured under identical conditions agree well with these predictions (Table III). The chemical shift of H(C6) reflects the electronic influence of the 5 substituent, whereas the δ values for H(C1′) are almost identical. In solution, there is a rapid equilibrium between all possible conformations, the most stable ones of which are C(2′)-endo and C(3′)-endo. A sensitive indicator of the shift of the equilibrium C(2′)-endo ⇌ C(3′)-endo should be the coupling constant between H(C1′) and H(C2′). Since these hydrogen atoms are nearly trans diaxial ($\phi \approx 160°$) in a C(2′)-endo but approximately diequatorial ($\phi \approx 90°$) in a C(3′)-endo ribose (see Figure 3), one would expect $J_{1'2'}$ to be considerably larger in C(2′)-endo ($J_{1'2'} \approx 8$ Hz) than in C(3′)-endo ($J_{1'2'} \approx 0$ Hz) puckering according to the Karplus equation.[47] The observed $J_{1'2'}$ may therefore be considered to be an average value which is indicative of the C(2′)-endo:

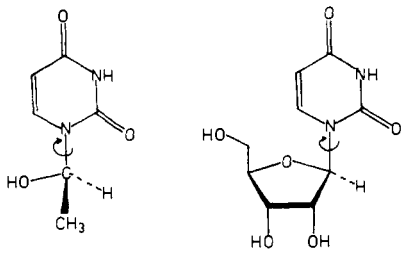


Figure 6. Uridine and the uridine model 1-(α-hydroxyethyl)uracil.

C(3′)-endo ratio. The 5 substituents are so remote from C(1′) and C(2′) that they should have no measurable effect on the coupling of the individual conformations. Table III shows that the electron-donating 5 substituents OH and NH_2 have no significant effect compared with uridine ($J_{1'2'}$ = 4.6 Hz). In contrast electron-withdrawing groups give rise to considerably lower $J_{1'2'}$ values according to their acceptor capability: Cl (3.5), $^+NH_3$ (3.5), CN (2.8), CHO (2.5), and NO_2 (1.7 Hz). Thus, these results confirm the postulated influence of 5 substituents on the conformation of uridine. The shifts in the equilibrium constants correspond to a maximum change of the relative free energy of about 3-4 kJ/mol. Since there is no reason for the entropy of the two conformations to differ significantly, this reflects mainly energy differences. The remarkable difference between 5-aminouridine, 5-hydroxyuridine, and their methylated derivatives may be explained by a steric effect of the methyl groups which gives rise to a twist of the substituent out of the base plane and hence to a reduced electron-donating ability.[14]

Since the base stacking in polyribonucleotide chains is correlated with large C(3′)-endo populations in solution studies,[48-52] uridines with an electron-withdrawing group should have an enhanced stacking tendency compared to those with an electron-donating 5 substituent. This prediction has also been verified experimentally by the UV, CD, and ^1H NMR spectra of a series of dinucleoside phosphates.[53,54]

C. Extended Hückel (EH) Calculations. The validity of the simple MO schemes given above was examined using the EH method.[55,56] This allows for the study of specific orbital interactions by comparing the results of a complete Hückel-Hamiltonian calculation with those of a second calculation where the matrix elements between the interacting orbitals have been omitted. The calculations were based on a standard geometry identical for all derivatives. This was necessary in spite of the previous analysis of bond-length effects because different geometries are the result of different electron distributions and therefore would have implied the electronic effects which we wanted to study with the EH method. The bond lengths and angles of the base moiety have been taken from the crystal structure of uridine;[10] the ribose was replaced by the 1-hydroxyethyl group, $-CH(OH)CH_3$, which should be a suitable model for its electronic influence on the base (Figure 6). All bond lengths to hydrogen atoms were given a standard value of 1.08 Å.

In order to study the "through-space" interaction C(6)···O(1′) all resonance integrals between the p_z orbital at C(6) and the orbitals at O(1′) were set to zero. The comparison of the results obtained with and without this decoupling at various dihedral angles χ yields the interaction energy between C(6) and O(1′) (Figure 7). It is found that all uridine derivatives with $0° < \chi < 90°$ are stabilized below and destabilized above $\chi = 20°$ by this "through-space" effect; its exact amount depends on the electronic properties of the 5 substituent. At low χ values an electron-withdrawing group gives rise to greater n-π* and less n-π overlap (see Figure 5), but with increasing χ values the destabilizing interaction of π with the sp^n hybrid lone pair becomes more and more important. Since the energy difference between these two levels is considerably reduced by electron-accepting groups, the order of the stabilizing power of 5 substituents is inverted when proceeding to greater dihedral angles χ. Thus, the EH calculations confirm the postulated conformation preferences of electron-accepting and electron-donating 5 substituents. The variation in charge transfer is indicated by the gradual change of the charge densities at C(6) and O(1′) as a consequence of "through-space" interaction (Table IV).

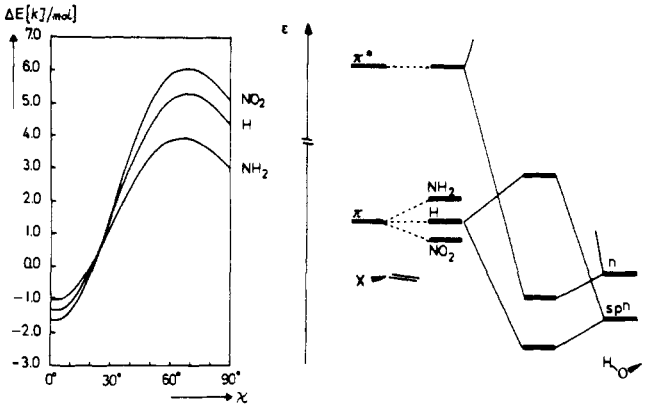


Figure 7. The difference between the C(6)···O(1′) two-center energy of a complete EH calculation and that of a "through-space" decoupled one vs. χ; a qualitative interaction diagram is given on the right side. The nitro and the amino group have been chosen as representative 5 substituents which are compared with the unsubstituted model system.

The effect of "through-bond" coupling on the N(1)-C(1′) bond length (see Figure 4) has also been verified using the EH method. In the calculations the interaction of O(1′) with C(1′) and the sp^2 hybrid orbitals of the ring atoms was suppressed. As expected, the charge transfer from O(1′) to the 5 substituent by a "through-bond" mechanism causes a weakening of the N(1)-C(1′) bond owing to the partial occupation of σ* levels. This is evident from inspection of the corresponding reduced overlap population data (see Table IV) which indicate that electron-withdrawing 5 substituents lead to a more efficient interaction.

D. MINDO/3 Calculations. The EH studies as discussed above gave some insight into the principal features of the various interaction mechanisms. Semiempirical all-valence methods which agree well with the experimental data for purine and pyrimidine nucleosides were thought to be exact enough to produce quantitative values for both molecular geometries and energies. They predict the gauche-gauche arrangement as the most stable conformation at C(4′)-C(5′)[57,58] and also give different χ values for C(2′)-*endo*- and C(3′)-*endo*-uridine.[59,60] We have used the SCF-MO method MINDO/3,[61,62] which minimizes the total energy by optimization of the geometrical variables (bond lengths, bond angles, and dihedral angles). In order to get the "best" molecular structure within a reasonable computer time the π system was held planar and the X-H bond lengths were fixed at a distance of 1.08 Å. Thus, it was possible to calculate, with the variation of up to 75 parameters, the complete nucleoside. Each calculation was carried out with the 5 substituents NO_2, CN, H, OH, and NH_2 in order to amplify the weak effects by selecting groups with extreme electronic properties.

As a first step the influence of 5 substituents on the geometry of the base was investigated (Figure 8). Although the absolute values of the calculated bond lengths of uracil derivatives correspond only approximately with X-ray data, the differences between them agree well with qualitative predictions (see Figure 2). The bond-length alterations induced by 5 substitution show clearly that this perturbation of the π system affects the pyrimidine ring much more than the carbonyl groups.

Table IV. EH Results Calculated at $\chi = 0°$

X	NO_2	CN	H	OH	NH_2
a $\Delta q_{C(6)}$	−0.001 97	−0.001 75	−0.001 30	−0.000 62	−0.000 22
$\Delta q_{O(1')}$	0.000 83	0.000 69	0.000 55	0.000 47	0.000 44
b $\Delta ROP_{N(1)-C(1')}$	−0.1548	−0.1598	−0.1518	−0.1454	−0.1433

[a] Change of the net charges at C(6) and O(1′) as a result of "through-space" interaction. [b] Change of the reduced overlap population (ROP) at N(1)-C(1′) by "through-bond" coupling.

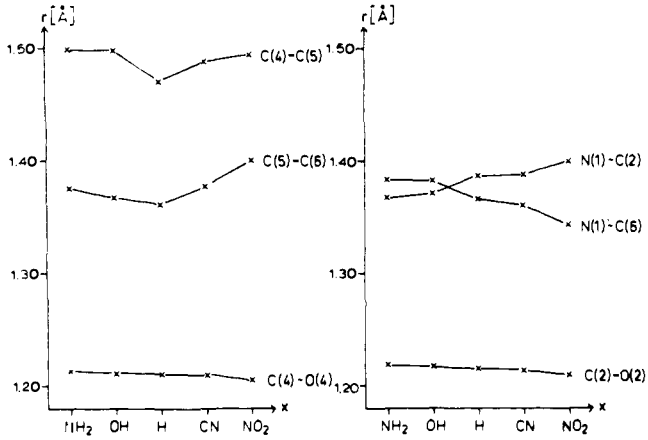


Figure 8. The influence of 5 substituents on the bond lengths of uracil calculated by MINDO/3.

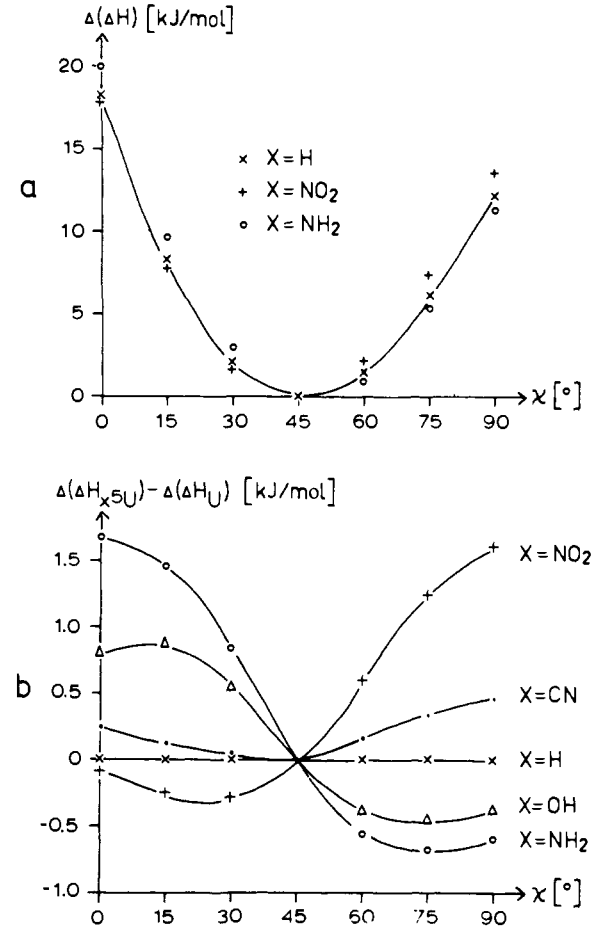


Figure 9. MINDO/3 energy of 5-substituted uridines vs. χ, calculated with the nucleoside model 1-(α-hydroxyethyl)uracil (a); (b) shows energy difference curves with respect to X = H vs. χ.

Proceeding from the base to the nucleoside, the 1-hydroxyethyl group was used again as a model for the electronic influence of the ribosyl moiety (see Figure 6). The rotation about the glycosidic bond is represented by an energy curve with a minimum at $\chi = 45°$, which in the case of X = H is 18.2 and 12.1 kJ/mol lower than the energy at $\chi = 0$ and 90°, respectively (Figure 9a). According to the MINDO/3 results, a 5 substituent does not shift the position of the minimum but modifies the shape of the potential curve as a function of its electronic properties. An electron-withdrawing group causes a flattening of the curve at smaller χ values and a steepening at greater dihedral angles; electron-donating groups show the converse effect. For example, X = NH_2 at $\chi = 0°$ and X = NO_2 at $\chi = 90°$ give rise to a destabilization of 1.6 kJ/mol compared with X = H (Figure 9b). Thus, these results confirm the hypothesis that electron-attracting 5 substituents should favor small dihedral angles, whereas electron-donating groups, on the other hand, should act in the opposite direction.

The 1-hydroxyethyl substituent operates as a rotation-dependent donor which is of maximum efficiency at $\chi = 0°$ and shows minimum interaction at $\chi = 60°$. This variable function results in a continual alteration of the distances N(1)-C(1′) and C(1′)-O(1′) from 1.491 and 1.364 Å at χ = 0° to 1.479 and 1.368 Å at $\chi = 60°$. These bond lengths are also influenced by the 5 substituent. In agreement with the experimental results (see Table I), electron acceptors elongate N(1)-C(1′) and shorten C(1′)-O(1′), whereas electron-donating 5 substituents lead to the opposite effects.

The postulated influence of 5 substituents on the conformation of the ribosyl moiety cannot, of course, be covered completely by such a ribose model. We have therefore searched for the energy minimum of uridine and its derivatives having C(2′)-endo as well as C(3′)-endo conformation. The only restriction was that one dihedral angle in the furanose ring was fixed in order to define the ribose conformation by keeping four atoms in a plane. Beginning with χ values of 15 (C(3′)-endo) and 50° (C(2′)-endo) and an ideal gauche-gauche conformation with $\psi_1 \equiv$ O(1′)-C(4′)-C(5′)-O(5′) = −60° (see Figure 1) the total energy was minimized until the sum of all gradients had been reduced to 5.0. Other local minima were also examined to confirm that the true energy minimum was found.

The results clearly demonstrate the variable electron-donating function of the ribose, which is more effective in the C(3′)-endo than in the C(2′)-endo conformation and is enhanced by electron-attracting 5 substituents (Table V). The bond lengths N(1)-C(1′) and C(1′)-O(1′) show the same dependence on the donor strength (and thus on the conformation) of the ribose and on 5 substitution as that calculated with the ribose model. The C(5′)-O(5′) bond remained at the gauche-gauche arrangement with $-66.4° > \psi_1 > -83.7°$. The well-known relationship between the orientation of the base and the puckering of the ribose is verified by the calculated χ values which span a range between 29.6 and 48.6° (C(3′)-endo) and between 63.2 and 71.6° (C(2′)-endo). The large χ values calculated for the C(3′)-endo conformation, especially for 5-cyanouridine, are due to the known tendency of the MINDO/3 method to smooth puckered ring systems. Thus, the steric hindrance between H(C6) and H(C3′) is reduced and the energy minimum is shifted to larger χ values. During the rotation of the base about the glycosidic bond, the atom O(5′)

Table V. MINDO/3 Results for the Completely Geometry Optimized Uridine Derivatives[a]

	X = NO_2	X = CN	X = H	X = OH	X = NH_2
ΔH, kJ/mol	−1119.8 −1109.7	−975.1 −968.6	−1050.6 −1040.6	−1220.5 −1208.2	−1050.0 −1036.6
$\Delta(\Delta H)$, kJ/mol	10.1	6.5	10.0	12.3	13.4
N(1)-C(1′), Å	1.479 1.487	1.468 1.474	1.464 1.474	1.462 1.472	1.460 1.471
C(1′)-O(1′), Å	1.378 1.376	1.382 1.381	1.383 1.381	1.384 1.380	1.386 1.382
χ, deg	71.6 37.3	71.3 48.6	71.5 35.3	63.2 29.6	68.6 30.8
ψ_1, deg	−75.2 −79.3	−72.0 −83.7	−70.3 −74.2	−79.9 −74.1	−66.4 −68.4
charge of the ribosyl moiety	+0.18 +0.20	+0.14 +0.16	+0.13 +0.15	+0.13 +0.15	+0.12 +0.14

[a] The upper value of a column holds for C(2′)-endo, the lower one for C(3′)-endo conformation.

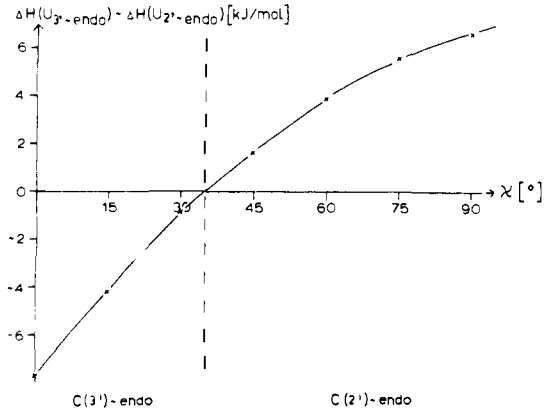


Figure 10. MINDO/3 energy difference between C(3′)-*endo*- and C(2′)-*endo*-uridine vs. χ.

remained distant from the only slightly positive hydrogen atom at C(6) since MINDO/3 considers such a short O---H distance as a repulsive interaction and not as a hydrogen bond.[63]

All five uridine derivatives are calculated to be more stable with C(2′)-endo than with C(3′)-endo puckering, but the energy difference depends on the 5 substituent and is larger for X = OH (12.3 kJ/mol) and X = NH_2 (13.4 kJ/mol) than for unsubstituted uridine (10.0 kJ/mol). This result is in line with the ^{1}H NMR spectra, which showed the preference of the C(2′)-endo conformation in uridines with electron-donating 5 substituents. The reason for the rather marked energy difference between the ribose conformations seems to be the flattening of the furanose ring in MINDO/3 calculations. Thus, the repulsive interaction between O(2) and O(2′) is underestimated in the case of C(2′)-endo puckering.

We have also calculated the energy of C(2′)-*endo*- and C(3′)-*endo*-uridine at several χ values using the already determined geometries except N(1)-C(1′), which was the only parameter to be optimized. The resultant energy-difference curve is clearly separated into two regions (Figure 10). The C(3′)-endo conformation should be favored below χ = 35°, the C(2′)-endo puckering above. This result is in exact agreement with the experimental data discussed previously (see Table II).

Conclusions

The combination of X-ray, ^{1}H NMR, and theoretical studies indicates that substituents at the 5 position of uridine not only lead to a perturbation of the pyrimidine ring but also show a definite influence on the structure of the whole nucleoside molecule. They are able to shift the equilibrium between the ribose conformations and to modify the stacking tendency of the bases in oligonucleotides. Thus, if the conformation of a free nucleoside is maintained in larger nucleic acid fragments, the ability of an oligonucleotide to form a complex with a second oligonucleotide can be modified by substituents which specifically alter the stacking geometry without direct intervention with the system of hydrogen bonds. This is of significance for the codon–anticodon interaction, where the base-pairing properties are certainly influenced by the exact conformation of the two trinucleoside diphosphates involved. If one takes into account the fact that the precise orientation of the base is determined by the C(6)-H---O(5′) interaction, the most stable conformation of a 5-substituted uridine can be predicted in spite of the large number of spatial arrangements possible.

Experimental Section

The ^{1}H NMR spectra were recorded at 100 MHz on a Varian XL 100-15 spectrometer in the FT mode using a 620L 16K computer (sample temperature 28 °C); 25–100 inteferograms were accumulated with a spectral width of 1000 Hz and an acquisition time of 4 s corresponding to a digital resolution of 0.25 Hz/point. For 5-nitrouridine the spectral width was 1500 Hz. The nucleosides were dissolved in D_2O (isotope purity 99.96%), which was also used for the internal field-frequency lock. The measurements at pD ≈ 1 were done after addition of a drop of trifluoroacetic acid.

Acknowledgments. We thank Mrs. P. Hofmann and Miss Angela Brown for their help in preparing the manuscript. This work was supported by the Fonds der Chemischen Industrie and the Deutsche Forschungsgemeinschaft.

References and Notes

(1) J. A. McCloskey and S. Nishimura, *Acc. Chem. Res.*, **10**, 403–410 (1977).
(2) F. H. C. Crick, *J. Mol. Biol.*, **19**, 548–555 (1966).
(3) W. Hillen, E. Egert, H. J. Lindner, and H. G. Gassen, *FEBS Lett.*, **94**, 361–364 (1978).
(4) S. H. Kim, F. L. Suddath, G. J. Quigley, A. McPherson, J. L. Sussman, A. H.-J. Wang, N. C. Seeman, and A. Rich, *Science*, **185**, 435–440 (1974).
(5) J. D. Robertus, J. E. Ladner, J. T. Finch, D. Rhodes, R. S. Brown, B. F. C. Clark, and A. Klug, *Nature (London)*, **250**, 546–551 (1974).
(6) W. Hillen, E. Egert, H. J. Lindner, and H. G. Gassen, *Biochemistry*, **17**, 5314–5320 (1978).
(7) E. Egert, H. J. Lindner, W. Hillen, and H. G. Gassen, *Acta Crystallogr., Sect. B*, **34**, 2204–2208 (1978).
(8) E. Egert, H. J. Lindner, W. Hillen, and H. G. Gassen, *Nucleic Acids Res.*, **4**, 929–937 (1977).
(9) S. W. Hawkinson and C. L. Coulter, *Acta Crystallogr., Sect. B*, **27**, 34–42 (1971).
(10) E. A. Green, R. D. Rosenstein, R. Shiono, D. J. Abraham, B. L. Trus, and R. E. Marsh, *Acta Crystallogr., Sect. B*, **31**, 102–107 (1975).

(11) D. J. Hunt and E. Subramanian, *Acta Crystallogr., Sect. B*, **25**, 2144–2152 (1969).
(12) W. Hillen, E. Egert, H. J. Lindner, H. G. Gassen, and H. Vorbrüggen, *J. Carbohydr. Nucleosides Nucleotides*, **5**, 23–32 (1978).
(13) U. Thewalt and C. E. Bugg, *Acta Crystallogr., Sect. B*, **29**, 1393–1398 (1973).
(14) E. Egert, H. J. Lindner, W. Hillen, and H. G. Gassen, *Acta Crystallogr., Sect. B*, **35**, 920–923 (1979).
(15) A. Rahman and H. R. Wilson, *Acta Crystallogr., Sect. B*, **26**, 1765–1775 (1970).
(16) E. Egert, H. J. Lindner, W. Hillen, H. G. Gassen, and H. Vorbrüggen, *Acta Crystallogr., Sect. B*, **35**, 122–125 (1979).
(17) S. W. Hawkinson, *Acta Crystallogr., Sect. B*, **33**, 80–85 (1977).
(18) G. H.-Y. Lin and M. Sundaralingam, *Acta Crystallogr., Sect. B*, **27**, 961–969 (1971).
(19) K. Morikawa, K. Torii, Y. Iitaka, and M. Tsuboi, *Acta Crystallogr., Sect. B*, **31**, 1004–1007 (1975).
(20) C. H. Schwalbe and W. Saenger, *Acta Crystallogr., Sect. B*, **29**, 61–69 (1973).
(21) E. Egert, H. J. Lindner, W. Hillen, and H. G. Gassen, *Acta Crystallogr., Sect. B*, **33**, 3704–3707 (1977).
(22) M. Sundaralingam, *Biopolymers*, 7, 821–860 (1969).
(23) B. Kojić-Prodić, R. Liminga, M. Šljukić, and Ž. Ružić-Toroš, *Acta Crystallogr., Sect. B*, **30**, 1550–1555 (1974).
(24) B. Kojić-Prodić, A. Kvick, and Ž. Ružić-Toroš, *Acta Crystallogr., Sect. B*, **32**, 1090–1095 (1976).
(25) G. H.-Y. Lin, M. Sundaralingam, and S. K. Arora, *J. Am. Chem. Soc.*, **93**, 1235–1241 (1971).
(26) M. Sundaralingam, S. T. Rao, and J. Abola, *J. Am. Chem. Soc.*, **93**, 7055–7062 (1971).
(27) D. Suck, W. Saenger, and K. Zechmeister, *Acta Crystallogr., Sect. B*, **28**, 596–605 (1972).
(28) B. Hingerty, P. J. Bond, R. Langridge, and F. Rottman, *Acta Crystallogr., Sect. B*, **33**, 1349–1356 (1977).
(29) W. L. B. Hutcheon and M. N. G. James, *Acta Crystallogr., Sect. B*, **33**, 2224–2228 (1977).
(30) J. J. Guy, L. R. Nassimbeni, G. M. Sheldrick, and R. Taylor, *Acta Crystallogr., Sect. B*, **32**, 2909–2911 (1976).
(31) R. A. G. de Graaff, G. Admiraal, E. H. Koen, and C. Romers, *Acta Crystallogr., Sect. B*, **33**, 2459–2464 (1977).
(32) H. M. Berman, W. C. Hamilton, and R. J. Rousseau, *Biochemistry*, **12**, 1809–1814 (1973).
(33) P. Narayanan and H. M. Berman, *Acta Crystallogr., Sect. B*, **33**, 2047–2051 (1977).
(34) A. H.-J. Wang, J. R. Barrio, and I. C. Paul, *J. Am. Chem. Soc.*, **98**, 7401–7408 (1976).
(35) H. Follmann, R. Pfeil, and H. Witzel, *Eur. J. Biochem.*, 77, 451–461 (1977).
(36) T. Schleich, T. R. Lusebrink, B. P. Cross, and N. P. Johnson, *Nucleic Acids Res.*, **2**, 459–467 (1975).
(37) D. B. Davies and S. S. Danyluk, *Biochemistry*, **13**, 4417–4434 (1974).
(38) D. B. Davies and S. S. Danyluk, *Biochemistry*, **14**, 543–554 (1975).
(39) (a) W. Saenger, *Angew. Chem.*, **85**, 680–690 (1973); (b) G. R. Pack and G. H. Loew, *Int. J. Quantum Chem., Quantum Biol. Symp.*, **4**, 87–96 (1977).
(40) C. H. Schwalbe and W. Saenger, *J. Mol. Biol.*, **75**, 129–143 (1973).
(41) P. Singh and D. J. Hodgson, *Biochemistry*, **13**, 5445–5452 (1974).
(42) D. J. Wood, F. E. Hruska, R. J. Mynott, and R. H. Sarma, *Can. J. Chem.*, **51**, 2571–2577 (1973).
(43) R. Hoffmann, A. Imamura, and W. J. Hehre, *J. Am. Chem. Soc.*, **90**, 1499–1509 (1968).
(44) R. Hoffmann, *Acc. Chem. Res.*, **4**, 1–9 (1971).
(45) R. Gleiter, *Angew. Chem.*, **86**, 770–775 (1974).
(46) L. Libit and R. Hoffmann, *J. Am. Chem. Soc.*, **96**, 1370–1383 (1974).
(47) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).
(48) C.-H. Lee, F. S. Ezra, N. S. Kondo, R. H. Sarma, and S. S. Danyluk, *Biochemistry*, **15**, 3627–3638 (1976).
(49) F. S. Ezra, C.-H. Lee, N. S. Kondo, S. S. Danyluk, and R. H. Sarma, *Biochemistry*, **16**, 1977–1987 (1977).
(50) F. E. Hruska and S. S. Danyluk, *J. Am. Chem. Soc.*, **90**, 3266–3267 (1968).
(51) P. N. Borer, L. S. Kan, and P. O. P. Ts'o, *Biochemistry*, **14**, 4847–4863 (1975).
(52) C. Altona and M. Sundaralingam, *J. Am. Chem. Soc.*, **95**, 2333–2344 (1973).
(53) W. Hillen and H. G. Gassen, *Biochim. Biophys. Acta*, **518**, 7–16 (1978).
(54) W. Hillen and H. G. Gassen, unpublished results.
(55) R. Hoffmann and W. N. Lipscomb, *J. Chem. Phys.*, **37**, 2872 (1962).
(56) R. Hoffmann, *J. Chem. Phys.*, **39**, 1397–1412 (1963).
(57) A. Saran, B. Pullman, and D. Perahia, *Biochim. Biophys. Acta*, **287**, 211–231 (1972).
(58) A. Saran, H. Berthod, and B. Pullman, *Biochim. Biophys. Acta*, **331**, 154–164 (1973).
(59) A. Saran, D. Perahia, and B. Pullman, *Theor. Chim. Acta*, **30**, 31–44 (1973).
(60) H. Berthod and B. Pullman, *Biochim. Biophys. Acta*, **232**, 595–606 (1971).
(61) R. C. Bingham, M. J. S. Dewar, and D. H. Lo, *J. Am. Chem. Soc.*, **97**, 1285–1293 (1975).
(62) P. Bischof, *J. Am. Chem. Soc.*, **98**, 6844–6849 (1976).
(63) G. Klopman, P. Andreozzi, A. J. Hopfinger, O. Kikuchi, and M. J. S. Dewar, *J. Am. Chem. Soc.*, **100**, 6267–6268 (1978).

Origin of the Differential Acidity of Diastereotopic Protons in Sulfonium Salts

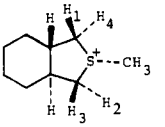
Samuel Lindsay Graham and Clayton H. Heathcock*

Contribution from the Department of Chemistry, University of California, Berkeley, California 94720. *Received April 12, 1979*

Abstract: A semiquantitative model for prediction of the relative acidities of diasterotopic protons in sulfonium salts is presented. The model is based upon a reevaluation of the pertinent literature coupled with the insight provided by ab initio molecular orbital methods. In summary, the proton that lies most nearly perpendicular to the axis of the sulfur lone pair will undergo abstraction by base at the greatest rate. Minimization of line pair–lone pair repulsions in the intermediate ylide is postulated as the prime determinant of this behavior.

Introduction

Carbanions stabilized by heteroatoms are widely known in organic chemistry and are well established as useful reagents in synthesis. The ylides of phosphorus[2] and sulfur[2a,3] have proved especially useful and have thus been the object of many experimental and theoretical investigations. In particular, the unique zwitterionic structure of these ylides has posed the theoretician with the difficult problem of devising a model for the bonding in such molecules, including a mechanism for stabilizing the negative charge at carbon.[4] One intriguing observation that has yet to be explained adequately is the difference in acidity of diastereotopic protons in the deprotonation reactions of trivalent sulfur compounds. The effect has been noted in the formation of sulfinyl carbanions[5] as well as in the ylide-forming reaction of sulfonium salts.[6] Fava and coworkers have measured the rate of deuterium incorporation into a variety of sulfonium salts under conditions of base catalysis. One such system studied is the thioniabicyclo[4.3.0]nonane **1**.[6e,f] The four unique protons in **1** undergo exchange with relative rates of $H_1:H_2:H_3:H_4 = 1:90:700:90$.



1

0002-7863/80/1502-3713$01.00/0