Evaluation of 1,3-Diaxial Steric Hindrance to Proton Abstraction Alpha to Carbonyl Groups

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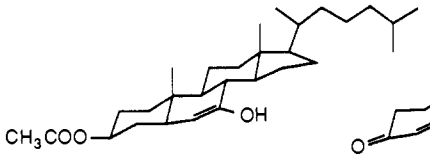
Abstract: An assessment of the magnitude of steric hindrance by axial substituents to abstraction of syn-axial protons alpha to carbonyl groups in trans-fused decalones has been made by a comparison of the rates of reactions of beta acetoxy ketones **7**, **4**, **8**, and **9** to form enones **18**, **5**, **26**, and **27**, respectively, in the presence of four bases (hydroxide ion, morpholine, *N*-methylmorpholine, and *N*-ethylmorpholine). For all the reactions of **7** and **4**, it was shown that C-1 β (axial) proton abstraction is the rate-limiting step by observation of large primary kinetic isotope effects when the appropriately monodeuteriated **10** and **6** were used. The results obtained permit the following conclusions concerning the rate of C-1 β proton abstraction: (1) a C-10 (angular) methyl group slows reaction by a factor of ca. 5 regardless of the size of the base, (2) a second axial alkyl group at C-8 further slows reaction by a factor that does depend on the size of the base, (3) axial methyl and ethyl groups at C-8 have the same effect, and (4) after correction for the difference in base strength, *N*-methylmorpholine is less effective than morpholine for C-1 β proton abstraction by a factor that increases as the substrate is changed from **7** to **4** to **8** or **9**.

Although axial substituents, such as the ubiquitous angular methyl group, are generally assumed to hinder reactions at the other axial positions on the same side of a cyclohexane ring, there is little direct quantitative evidence of this effect. In their development of the principle of stereoelectronic control, Corey and Sneen[1] had to estimate the influence of an angular methyl group on the rate of axial deprotonation to form enol **1**, and they assumed a 10-fold retardation.[1] Bordwell and Scamehorn,[2] however, found that the axial methyl or phenyl groups in 4,4-disubstituted cyclohexanones had only a very small retarding effect (ca. a factor of 2 for CH_3) on proton abstraction alpha to the carbonyl group. The only other experimental measurement of such an effect that we are aware of was made by Ringold and co-workers,[3] who found that potassium *tert*-butoxide catalyzed abstraction of the gamma[4] axial proton from **2** was 5 times faster than from **3**.



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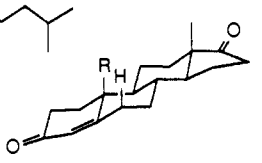
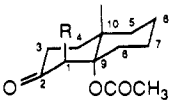


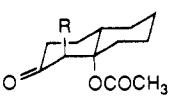
2, R = H
3, R = CH_3

In our studies[5] of proton abstraction alpha to carbonyl groups and derived iminium ions, we have made extensive use of beta acetoxy ketone **4**,[6] which, after it undergoes alpha deprotonation, inevitably expels acetate ion to afford enone **5**, whose formation can readily be monitored by ultraviolet spectroscopy. Observation of large, invariant primary kinetic isotope effects (KIE's) when the reactivity of **4** was compared with that of its specifically monodeuteriated analogue **6** established that alpha proton abstraction was indeed the rate-determining step in these elimination reactions (irreversible ElcB mechanism)[6] and that the C-1 β[4] (axial) proton was being selectively abstracted.[7]

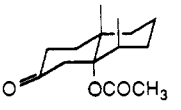


4, R = H
6, R = D

5



7, R = H
10, R = D



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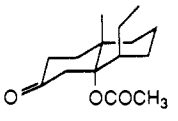

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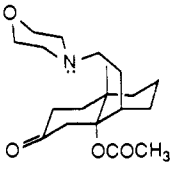

11

With a convenient method for measuring the rate of alpha axial proton abstraction thus available, it seemed that a comparison of the rates of elimination reactions of **4** with those of analogues having different β substitution patterns could provide a means of assessing the magnitude of steric hindrance by axial substituents. This paper describes the results of such a study. The rates of enone formation from the four beta acetoxy ketones **4**, **7**, **8**, and **9**, as catalyzed by hydroxide ion and three general bases of differing steric requirements, have been determined and compared. As had previously been demonstrated in the case of **4**,[6] it has now been established for **7** that alpha proton abstraction is rate-limiting by the observation of large invariant KIE's when the reactivity of **7** was compared with that of the monodeuteriated **10**. Morpholine, *N*-methylmorpholine, and *N*-ethylmorpholine were chosen as the three general bases because they have pK_a's that make rate measurements convenient and because their reactions represent intermolecular analogues of the intramolecular elimination reaction of compound **11**, which is currently under investigation in our laboratory.[8]

Synthesis of Substrates

The angularly unsubstituted **7** was synthesized from ketol **12**, which was prepared by condensation of cyclohexanone with methyl vinyl ketone according to the procedure of Marshall and Fanta.[9]

(1) Corey, E. J.; Sneen, R. A. *J. Am. Chem. Soc.* **1956**, *78*, 6269-6278.
(2) Bordwell, F. G.; Scamehorn, R. G. *J. Am. Chem. Soc.* **1968**, *90*, 6749-6751.
(3) Subrahmanyam, G.; Malhotra, S. K.; Ringold, H. J. *J. Am. Chem. Soc.* **1966**, *88*, 1332-1333.
(4) To avoid confusion, the words alpha and beta are used to denote the positions of carbons relative to the carbonyl or derived functional group, and the symbols α and β are used to denote substituents respectively underneath and above the steroid or decalin ring system.
(5) Review: Spencer, T. A. *Bioorganic Chemistry*; van Tamelen, E. E., Ed.; Academic Press: New York, 1977; Vol. 1, Chapter 13, pp 313-337.
(6) Roberts, R. D.; Ferran, H. E., Jr.; Gula, M. J.; Spencer, T. A. *J. Am. Chem. Soc.* **1980**, *102*, 7054-7058.
(7) Ferran, H. E., Jr.; Roberts, R. D.; Jacob, J. N.; Spencer, T. A. *J. Chem. Soc., Chem. Commun.* **1978**, 49-50. More recently we have obtained evidence that the high selectivity for abstraction of the C-1 β axial proton (k_{ax}/k_{eq} = 130) observed with **4** does not obtain in the absence of the beta acetoxyl group, which reduces the value of k_{eq} (Schach von Wittenau, A. E.; Abbott, R. E.; Vitale, D. E.; Spencer, T. A., unpublished results).
(8) Jacob, J. N.; Nelson, J. A.; Spencer, T. A. *J. Org. Chem.* **1980**, *45*, 1645-1650.

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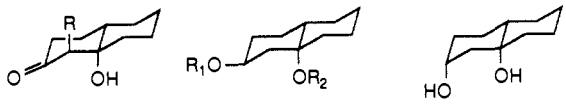
Since direct acetylation of beta ketols had proved unsuccessful in attempts to synthesize **4**[6] and its cis-fused isomer,[10] owing to concomitant enone formation, the indirect route developed in this earlier work[10] was adopted. Reduction of **12** with sodium borohydride afforded, after chromatographic separation, 46% of diol **13** and 40% of diol **14**. The identity of the two diols was established on the basis of the NMR signals of their C-2 protons: **13**, C-2 α-H, δ 3.97, $W_{h/2} \sim 12$ Hz; **14**, C-2 β-H, δ 4.14, $W_{h/2} \sim 3$ Hz.[11] Diol **14** has much greater chromatographic mobility than its isomer, presumably owing to intramolecular hydrogen bonding.



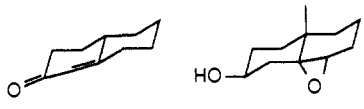
12, R = H
17, R = D

13, $R_1 = R_2 = H$
15, $R_1 = R_2 = COCH_3$
16, $R_1 = H$, $R_2 = COCH_3$

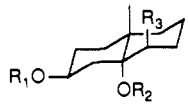
14

Diol **13** was bisacetylated with isopropenyl acetate to give **15**. Selective hydrolysis of **15** in dilute methanolic base yielded **16**, which was oxidized with Jones reagent[12] to afford **7**. Diol **14**, on the other hand, was not used in such a sequence because we had previously found[13] during synthesis of **4** that acetyl transfer from the C-**9** α to the C-**2** α hydroxyl group occurred during attempted selective hydrolysis of an analogous 1,3-diaxial diacetoxy intermediate. Accordingly, **14** was instead oxidized back to **12**. One attempt to effect conversion of **12** to **13** more selectively by use of lithium in ammonia reduction[14] failed.

The axially monodeuteriated analogue **10** was prepared in the same manner from deuteriated ketol **17**, which was obtained from $\Delta^{1(9)}$-octal-2-one (**18**) by, successively, reduction with $NaBH_4$, oxidation with *m*-chloroperoxybenzoic acid, reduction with $LiAlD_4$, Jones oxidation,[12] and chromatographic separation from the isomeric cis ketol. This sequence of reactions was essentially the same as that used by Marshall and Fanta[9] as part of an alternate synthesis of **12**.



18 **19**

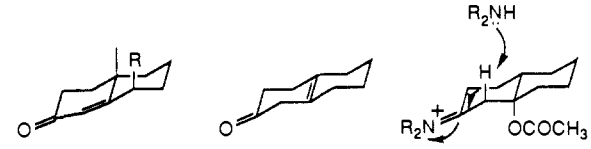


20, $R_1 = R_2 = H$, $R_3 = CH_3$
21, $R_1 = R_2 = H$, $R_3 = CH_2CH_3$
22, $R_1 = R_2 = COCH_3$, $R_3 = CH_3$
23, $R_1 = H$, $R_2 = COCH_3$, $R_3 = CH_3$
24, $R_1 = R_2 = COCH_3$, $R_3 = CH_2CH_3$
25, $R_1 = H$, $R_2 = COCH_3$, $R_3 = CH_2CH_3$

Synthesis of compounds **8** and **9** possessing two β axial alkyl groups was accomplished by using the strategy previously employed[8] for preparation of **11**, namely, nucleophilic attack on oxide **19**. Reaction of **19** with excess methyl or ethyl Grignard reagent afforded, respectively, **20** or **21** in good yield. Each of these diols was then converted by bisacetylation, selective hydrolysis, and Jones oxidation,[12] as described above, to the desired substrates through the sequences **20** → **22** → **23** → **8** or **21** → **24** → **25** → **9**. Like the other beta acetoxy trans-fused decalones we had previously prepared,[8,6] **7**–**9** showed NMR signals for their C-1 β protons at δ 2.70 ± 0.04 (d, J = 15 Hz) and for their C-1 α protons at δ 3.37 ± 0.03 (d, J = 15 Hz).

Kinetic Results

As expected, beta acetoxy ketones **7**–**9** are converted to unsaturated ketones **18**, **26**, and **27** essentially quantitatively upon treatment with aqueous base, just as **4** is cleanly converted to **5**. All three enones, the previously reported **18**[15] and **26**[16] and the new **27**, show $\lambda_{max}^{H_2O}$ 247.5 nm and ϵ 15 250 ± 250. That the enone from **8** was **26** rather than its isomer with an equatorial C-8 methyl group was demonstrated by comparison of the ^1H NMR spectrum of our elimination product with those of authentic **26** and its C-8 epimer, kindly furnished by Professor Drury Caine.[16] The β configuration was assigned to the ethyl group of **27** by analogy to the isolation of **26** and on the basis of our previous demonstration[8] that epimerization at C-8 of such enones requires much more vigorous conditions than those employed for their formation.



26, R = CH_3
27, R = CH_2CH_3

28 **29**

In the case of **7**, the possibility exists for subsequent conversion of some of **18** to its unconjugated isomer **28**, since a significant amount of **28** is known to be present at equilibrium.[15] However, the isomerization to **28** is apparently considerably slower than formation of **18**,[17] because monitoring of the latter process at 247.5 nm gave clean pseudo-first-order kinetics, and the product isolated from a reaction of **7** showed essentially no unconjugated carbonyl stretching band in its IR spectrum.[18]

The formation of enone from beta acetoxy ketones like **4** in the presence of a tertiary amine buffer has been shown[10] to be described by the kinetic expression given in eq 1, where $[OH^-]$ is K_w/a_H (a_H as measured by pH meter) and [AM] is the concentration of free amine.

$$\frac{d(\text{enone})}{dt} = (k_{OH}[OH^-] + k_B[AM])[\text{reactant}] \qquad (1)$$

This equation was found to hold for the reactions **7** → **18**, **8** → **26**, and **9** → **27** from the results of numerous kinetic runs with various concentrations of *N*-methylmorpholine and *N*-ethylmorpholine at differing buffer ratios. Values of k_{OH} were determined by extrapolation to zero buffer concentration.

In the case of morpholine, the only nontertiary amine studied, the possibility exists for catalysis via iminium ion formation, as shown in **29** for substrate **7**. However, on the basis of our previous studies of such nucleophilic catalysis,[6,10] the contribution of the

(9) Marshall, J. A.; Fanta, W. I. *J. Org. Chem.* **1964**, *29*, 2501–2505.
(10) Hupe, D. J.; Kendall, M. C. R.; Spencer, T. A. *J. Am. Chem. Soc.* **1972**, *94*, 1254–1263.
(11) Bhacca, N. S.; Williams, D. H. *Applications of NMR Spectroscopy in Organic Chemistry*; Holden-Day: San Francisco, 1964; pp 47–52.
(12) Bowers, A.; Halsall, T. G.; Jones, E. R. H.; Lemin, A. J. *J. Chem. Soc.* **1953**, 2548–2560.
(13) Ferran, H. E., Jr.; Spencer, T. A., unpublished results.
(14) Huffman, J. W.; Charles, J. T. *J. Am. Chem. Soc.* **1968**, *90*, 6486–6492.
(15) Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrell, R. *J. Am. Chem. Soc.* **1963**, *85*, 207–222. See also: Baisted, D. J.; Whitehurst, J. S. *J. Chem. Soc.* **1961**, 4089–4091.
(16) Caine, D.; Boucugnani, A. A.; Pennington, W. R. *J. Org. Chem.* **1976**, *41*, 3632–3634.
(17) There is good evidence that gamma protonation of dienolate anions is significantly slower than alpha protonation (see, e.g., Perera, S. K.; Dunn, W. A.; Fedor, L. R. *J. Org. Chem.* **1980**, *45*, 2816–2821. Pollack, R. M.; Mack, J. P. G.; Eldin, S. *J. Am. Chem. Soc.* **1987**, *109*, 5048–5050.) The reverse action, gamma deprotonation of an alpha, beta unsaturated ketone thus is slow compared to alpha deprotonation of a beta, gamma unsaturated ketone, and probably is also slow compared to alpha deprotonation of **7**, which is accelerated by the inductive effect of the beta acetoxy group by a factor of ca. 10^2 compared to *trans*-decal-2-one (Ahlberg, P. *Chem. Scr.* **1973**, *3*, 183–189. More O'Ferrall, R. A.; Warren, P. J. *J. Chem. Soc., Chem. Commun.* **1975**, 483–484. More O'Ferrall, R. A., private communication).
(18) Schuster, D. I.; Brizzolara, D. F. *J. Am. Chem. Soc.* **1970**, *92*, 4357–4365 report ν = 1710 cm^{-1} for **28**. We observed ν = 1720 cm^{-1} in the mixture of **18** and **28** obtained by the procedure of Augustine, R. L.; Caputo, J. A. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, pp 869–871.

Table I. Rate Constants for the Formation of Enones **18**, **5**, **26**, and **27** from Beta Acetoxy Ketones **7**, **4**, **8**, and **9** in H_2O at 25 °C with μ = 0.4

catalyst (pK_a)	k_{OH} or k_B, M^{-1} s^{-1} [no. of runs]			
	7	**4**	**8**	**9**
hydroxide ion (15.74)	8.2 ± 2.3 [4][a]	1.5[b]	0.33 ± 0.04 [4][a]	0.37 ± 0.03 [4][a]
morpholine (8.58)[c]	(1.6 ± 0.10) × 10^{-2} [11]	(6.5 ± 0.08) × 10^{-3} [10]	(4.6 ± 0.82) × 10^{-4} [8]	(3.8 ± 0.18) × 10^{-4} [6]
N-methylmorpholine (7.46)[c]	(7.9 ± 0.57) × 10^{-4} [10]	(1.6 ± 0.17) × 10^{-4} [9]	(1.9 ± 0.28) × 10^{-6} [7]	(1.8 ± 0.24) × 10^{-6} [6]
N-ethylmorpholine (7.82)[c]	(1.9 ± 0.18) × 10^{-4} [15]	(5.1 ± 0.92) × 10^{-5} [14]	(2.7 ± 0.91) × 10^{-6} [8]	(2.1 ± 0.39) × 10^{-6} [11]

[a] For hydroxide ion catalysis the number of runs is the number of extrapolations of series of buffer concentrations. [b] Determined in ref 6. [c] Determined by the half-neutralization method with ionic strength μ = 0.4.

Table II. Values of Observed Kinetic Isotope Effects (k_H/k_D) for the Elimination Reactions of **7** versus **10** and **4** versus **6** in H_2O at 25 °C with μ = 0.4

catalyst	**7** vs **10**	**4** vs **6**
hydroxide ion	8.4	7.1[a]
morpholine	7.2	7.4
N-methylmorpholine	6.0	9.8
N-ethylmorpholine	7.0	8.1

[a] A value of k_H/k_D = 6.2 was previously obtained for the reactions of **4** versus **6** (ref 6).

process depicted in **29** would be expected to be relatively small with an amine like morpholine having a $pK_a > 8$. At the pH's and buffer concentrations used in the present work, less than 2% of the observed rate was attributable to a kinetic term k_{AB}[AM][AMH^+], corresponding to catalysis as shown in **29**,[10] and this small contribution was neglected.[19]

The values of k_{OH} and k_B, the rate constants for hydroxide ion and general base catalyzed enone formation, were determined for each of the four substrates, and these are shown in Table I. The values of k_{OH} and k_B for the two monodeuteriated substrates **6** and **10** were also determined, and the values of KIE_{obsd} obtained by comparing these rate constants with those for the reactions of **4** and **7** are shown in Table II.

Discussion

Before the kinetic results are discussed, certain assumptions made in this research should be delineated. First, compounds **8** and **9** have been described as having two "axial" substituents, without acknowledging the possibility of cyclohexane distortion to relieve 1,3-diaxial interaction. Evidence that this description is essentially correct has been found in the recently determined[20] X-ray crystal structure of **11**, which shows very little distortion from a normal chair conformation for the ring bearing the two β substituents, which are indeed axial. Second, it is assumed that in the transition state for alpha deprotonation extensive rehybridization will have occurred at C-1 and that the C-H bond will be significantly elongated. The large values of KIE_{obsd} (Table II), suggesting that the proton is approximately half transferred in the transition state, are reasonably consistent with this view.[21] Third, it is assumed that the bases react directly with the alpha proton, rather than through one or more water molecules. It has been argued that direct abstraction is generally true for removal of protons from carbon.[22]

The kinetic results obtained upon introduction of the angular methyl group of **4** will be considered first. The observation of large primary KIE's with each of the four catalysts employed for both **7** vs **10** and **4** vs **6** (Table II) establishes that abstraction of the axial alpha proton is the dominant process in all cases (i.e., $k_{obsd} = k_{ax} + k_{eq}$ and $k_{ax} > k_{eq}$). It should be noted that the values of KIE_{obsd} ($= k_{ax}^H + k_{eq}^H/k_{ax}^D + k_{eq}^H$) in Table II are in several cases the result of only one or two determinations. There is sufficient uncertainty in some of the values of KIE_{obsd} that it is unlikely that any reliable inferences can be drawn from the rather random variation among them. The substantial values of KIE_{obsd} do establish, however, that there is highly selective axial proton abstraction and that the rate constants for the reactions of **7** vs **4** should indeed reveal the steric effect of the angular methyl group on axial alpha proton abstraction.

In each case the value of k_{OH} or k_B for **4** is slightly smaller (by a factor of 2.5–5.5) than that for **7**. Even with *N*-ethylmorpholine, the most hindered base used,[23] the angular methyl group makes only this modest difference in the rate of alpha axial proton abstraction. These results are consistent with the observation of Ringold[3] that potassium *tert*-butoxide catalyzed proton abstraction from **2** is 5 times faster than from **3**.

Interpretation of the results of introduction of the second axial substituent in **8** and **9** is not as straightforward, because isotope effects are not available. Measurement of these KIE's was clearly desirable, but the synthesis of **8** and **9** selectively monodeuteriated in the same manner as **6** and **10** appeared so formidable a task that it was not undertaken. Nonetheless, in certain instances interpretation of the kinetic results with **8** and **9** can reliably be made.

The second axial substituent, either a methyl group (**8**) or an ethyl group (**9**), causes k_{OH} to be reduced by about another factor of 5. It is not surprising that the methyl and ethyl groups have essentially the same effect. There is a negligible[24] or very small[25] difference in the free energy required to place these substituents axially rather than equatorially, and it can reasonably be assumed[25] that a C-8 axial ethyl group would have its methyl moiety oriented away from the C-1 β proton.

It seems safe to conclude that the values of k_{OH} for **8** and **9** also reflect predominantly axial alpha proton abstraction. We have determined previously that for k_{OH} in the case of **4** the ratio k_{ax}/k_{eq} = 130.[7] It seems reasonable to assume that k_{eq}, which presumably reflects alpha proton abstraction from a twist-boat conformation of the cyclohexanone ring,[26] will be essentially unaffected by the presence of a C-8 axial alkyl group, so that the influence of such a substituent will be on k_{ax}. Accordingly, the effect of introduction of the C-8 axial groups in **8** and **9** will be to reduce k_{ax} by about a factor of 5, meaning that the contribution of k_{eq} to k_{obsd} should still be essentially negligible (ca. 4%). Therefore the values of k_{OH} in Table I should provide a reasonably reliable assessment of the relative ease of C-1 axial proton abstraction by hydroxide ion from the four substrates.

The second axial alkyl substituent does make a distinctly greater difference when the bulkier amines are the proton acceptors rather than hydroxide ion. With morpholine, **8** and **9** react ca. 15 times slower than **4**, and with *N*-methylmorpholine, the difference is a factor of over 80. With each of the three amines, as with hydroxide ion, substrates **8** and **9** react at essentially identical rates.

(19) Another previously demonstrated[6,10] kinetic term, k_A[AMH^+], representing catalysis via iminium ion formation in which water acts as the proton acceptor, is ignored here because it is insignificant at any buffer concentrations with amines as basic as morpholine.

(20) Onan, K. D.; Nelson, J. A.; Spencer, T. A. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1986**, *42*, 1782–1785.

(21) For a recent discussion of transition states for such proton transfers, see: Zimmerman, H. E. *Acc. Chem. Res.* **1987**, *20*, 263–268.

(22) Hibbert, F. *Comprehensive Chemical Kinetics*; Bamford, C. H., Tipper, C. F. H., Eds.; Elsevier: Amsterdam, 1977; Vol. 8, Chapter 2, p 175. Bednar, R. A.; Jencks, W. P. *J. Am. Chem. Soc.* **1985**, *107*, 7126–7134.

(23) In the only study we are aware of concerning general base proton abstraction from carbon by *N*-methyl- versus *N*-ethylmorpholine, L. R. Fedor (*J. Am. Chem. Soc.* **1967**, *89*, 4479–4482.) observed a rate slowed by steric hindrance with *N*-methylmorpholine and "no catalysis detectable above the hydroxide ion catalyzed reaction" with *N*-ethylmorphine, implying greater steric hindrance.

(24) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A. *Conformational Analysis*; Wiley-Interscience: New York, 1966; p 44.

(25) Dale, J. *Stereochemistry and Conformational Analysis*; Verlag Chemie: New York, 1978; p 154.

(26) Lamaty, G. *Isotopes in Organic Chemistry. Isotopes in Hydrogen Transfer Processes*; Buncel, E., Lee, C. C., Eds.; Elsevier: Amsterdam, 1976; Vol. 2, Chapter 2, pp 63–66.

Table III. Rate Constants (k_B^{corr}) for the Elimination Reactions of **7**, **4**, **8**, and **9** Adjusted to Those for a General Base with $pK_a = 7.46$

	$10^4 k_B^{corr}$, M^{-1} s^{-1}			
catalyst	**7**	**4**	**8**	**9**
morpholine	61	25	1.8	1.5
N-methylmorpholine	7.9	1.6	0.019	0.018
N-ethylmorpholine	1.2	0.31	0.016	0.013

Since the value of the ratio k_{ax}/k_{eq} are not known for these reactions, it is not possible to say in which of them axial proton abstraction is still dominant, but it is likely that k_{eq} makes at least a significant contribution to k_{obsd} for the reactions of **8** or **9** with *N*-methyl- and *N*-ethylmorpholine, so that the values of k_b in Table I probably do not reveal the full extent of hindrance to axial deprotonation in these cases.

In order to compare the steric effects of the three morpholine bases in their reactions with a given substrate, it is necessary to take into account the difference in base strengths among these amines. We have previously found that general base catalyzed proton abstractions from **4**[6] and its cis-fused analogue[10] have Brønsted β's of 0.62 and 0.59, respectively. Accordingly, it seems reasonable to use a value for β of 0.6 to correct the observed rate constants given in Table I to rate constants that would have been found if all three amines had the same pK_a [i.e., $\log k_B^{corr} = \log k_B - 0.6(\Delta pK_a)$]. It also seems appropriate to take into account the fact that tertiary amines are more effective alpha proton abstractors than secondary amines with the same pK_a. For example, in the case of the cis-fused analogues of **4**, tertiary amines give a Brønsted plot with $\beta = 0.59$ having an intercept 0.25 log k_B unit greater than the parallel Brønsted plot for secondary amines,[10] and this value has been used to make an additional correction in the values of k_B^{corr} for morpholine. The rate constants in Table III have been thus adjusted to represent catalysis by a tertiary amine general base with $pK_a = 7.46$, the value determined under the reaction conditions for *N*-methylmorpholine.

It is clear that switching from morpholine to *N*-methylmorpholine makes a larger difference in k_B as the number of axial substituents is increased, with the ratio of the values of k_B^{corr} for the two catalysts increasing from 7.7 with **7** to 16 with **4** and to 95 and 83 with **8** and **9**, respectively. Such a trend is not seen, however, upon changing from *N*-methylmorpholine to *N*-ethylmorpholine. Again, an unequivocal interpretation of these results is not possible in the absence of isotope effect data, but the fact that the values of k_B^{corr} for the reactions of **8** and **9** with *N*-methyl- and *N*-ethylmoprholine are all virtually the same could lead one to speculate that these are really values of k_{eq}, which is less sensitive than k_{ax} to the size of the base.

The following conclusions may be drawn from the results described herein. A C-10 angular methyl group exerts a modest (ca. fivefold) hindering effect on axial proton abstraction from C-1 regardless of the steric requirements of the proton-accepting base. Introduction at C-8 of a second axial methyl group results in a further retardation of C-1 β proton removal, which does depend on the size of the base. An axial ethyl group at C-8 has the same effect as an axial methyl group at that position. When the base is changed from morpholine to the more hindered *N*-methylmorpholine, the rate of C-1 β proton abstraction is decreased by a factor which increases as the substrate is changed from **7** to **4** to **8** or **9**. It is hoped that this information will prove useful in interpreting and predicting cyclohexanone chemistry.

Experimental Section

General Methods. Melting points were determined in a Thomas-Hoover apparatus in unsealed capillaries and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 137, 257, or 599 spectrophotometer. IR spectra were taken as KBr pellets for solids or as neat liquids on NaCl plates. ^1H nuclear magnetic resonance (NMR) spectra were recorded at 60 MHz in $CDCl_3$ solution, unless otherwise indicated, on a Perkin-Elmer R-24 instrument or on a JEOL FX-60Q multinuclear Fourier transform NMR spectrometer. Chemical shifts are reported in ppm downfield from Me_4Si. Low-resolution mass spectra (MS) were determined on a Finnigan Model 4000 gas chromatograph–mass spectrometer. The high-resolution MS was determined at the MIT mass spectrometry facility, sponsored by NIH Division of Research Resources Grant PR00317. Preparative thin-layer chromatography (TLC) was performed on 20 × 20 cm plates coated with 1.45 mm thick layers of silica gel $PF_{(254+366)}$ (Brinkman Instruments, Inc., Westbury, NY). Ultraviolet (UV) light was used to visualize TLC plates. Qualitative TLC plates were coated with 0.25 mm thick layers of silica gel $PF_{(254+366)}$ and were sprayed with a 5% isopropyl alcohol solution of phosphomolybdic acid (Eastman Kodak) and heated briefly at 110 °C. Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI. Brine refers to saturated aqueous sodium chloride solution.

Synthesis. 2β,9α-Dihydroxy-*trans*-decalin (13) and 2α,9α-Dihydroxy-*trans*-decalin (14). To a solution of 2.00 g (11.9 mmol) of 9-hydroxy-*trans*-decal-2-one (**12**), mp 146.5–148 °C, prepared by the method of Marshall and Fanta,[9] in 100 mL of methanol was added 1.25 g (~30 mmol) of $NaBH_4$ in portions over 1.5 h. The reaction was stirred with protection from moisture overnight. The solvent was removed under reduced pressure, and the residue was added to 50 mL of water. The aqueous solution was acidified with concentrated hydrochloric acid, saturated with NaCl, and extracted with ether (4 × 50 mL). The combined ether extracts were washed with brine (2 × 50 mL), dried over $MgSO_4$, and concentrated to afford 2.00 g (99%) of white solid, which was chromatographed on 40 g of activity I basic alumina with ether–ethyl acetate as eluent. There was eluted 0.81 g (40%) of **14** followed by 0.93 g (46%) of **13**. Analytical samples of both diols were prepared by recrystallization from ether. Pure **14**: mp 98–99 °C; IR 3500–3100 cm^{-1}; NMR δ 4.14 (m, 2β-H). Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66. Found: C, 70.46; H, 10.73. Pure **13**: mp 135–137 °C; IR 3480 and 3400 cm^{-1}; NMR δ 3.97 (br m, 2α-H). Anal. Calcd: C, 70.55; H, 10.66. Found: C, 70.45; H, 10.74.

Reduction of **12** with $LiAlH_4$ gave a 3:7 ratio of **13** to **14**. Reduction of **12** by the procedure of Huffman and Charles[14] gave a solid, mp 223–225 °C, after recrystallization from ether: IR 3300 cm^{-1}. This could be a pinacol product.

2β,9α-Diacetoxy-*trans*-decalin (15). A mixture of 0.249 g (1.46 mmol) of **13**, 25 mL of freshly distilled isopropenyl acetate (bp 92.5 °C), and a few small crystals of *p*-toluenesulfonic acid were stirred at reflux for 22 h. After being cooled to room temperature, the reaction mixture was diluted with an equal volume of benzene, and the solvents were removed under reduced pressure. The residue was dissolved in 100 mL of ether, washed with brine (2 × 30 mL), dried over $MgSO_4$, and concentrated to afford 0.450 g of pale yellow oil. This material was homogeneous by TLC (1:1 ethyl acetate–hexane) except for some residual isopropenyl acetate and was used without further purification for the preparation of **16**. An analytical sample of **15** was obtained by column chromatography on activity I basic alumina with hexane–ethyl acetate as eluent, which afforded **15** as a clear oil: IR 1730 cm^{-1}; NMR δ 4.76 (br m, 1), 2.03 (s, 3), 1.97 (s, 3). Anal. Calcd for $C_{14}H_{22}O_4$: C, 66.12; H, 8.72. Found: C, 65.94; H, 8.81.

9α-Acetoxy-*trans*-decal-2β-ol (16). To a solution of 0.34 g (1.34 mmol) of **15** (contaminated with a small amount of isopropenyl acetate) in 15 mL of methanol was added 5 mL of 5% NaOH solution, and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with 150 mL of ethyl acetate, washed with brine (3 × 40 mL), dried over $MgSO_4$, and evaporated. Preparative TLC with 1:1 ethyl acetate–hexane afforded 0.24 g (85%) of **16** as a pale yellow oil. Crystallization from ether afforded 0.13 g (46%) of **16** as feathery white crystals: mp 60–62.5 °C. An analytical sample was obtained by recrystallization from hexane: mp 62–63 °C; IR 3500–3200 cm^{-1}; NMR δ 3.60 (br m, 2α-H), 2.02 (s, 3). Anal. Calcd for $C_{12}H_{20}O_3$: C, 67.89; H, 9.50. Found: C, 68.00; H, 9,56.

9α-Acetoxy-*trans*-decal-2-one (7). To a solution of 0.132 g (0.62 mmol) of **16**, which was homogeneous by TLC but not crystalline, in 20 mL of acetone was added 20 drops of Jones reagent.[12] The reaction mixture was stirred at room temperature for 10 min. The excess reagent was consumed with isopropyl alcohol and the solvents were removed under reduced pressure without heating. The residue was partitioned between 70 mL of ether and 20 mL of brine. The ether layer was washed with brine (2 × 20 mL), dried over $MgSO_4$, and concentrated to afford 0.108 g (83%) of pale yellow oil, which was crystallized twice from hexane to afford 0.072 g (55%) of **7** as clear prisms: mp 58–61 °C. An analytical sample of **7** was obtained by recrystallization from hexane: mp 59–60 °C; IR 1750–1720 cm^{-1}; NMR δ 3.41 (dd, 1, J = 15 and 1 Hz), 2.66 (d, 1, J = 15 Hz), 1.97 (s, 3). Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.55; H, 8.63. Found: C, 68.52; H, 8.62.

1β-Deuterio-9α-acetoxy-*trans*-decal-2-one (10). According to a slight modification of the sequence of reactions used by Marshall and Fanta[9] to prepare ketol **12** from $\Delta^{1(9)}$-octal-2-one (**18**), 1β-deuterio-9α-hydroxy-*trans*-decal-2-one (**17**) was prepared from **18**. A methanolic solution of **18**, which had been purified by low-temperature crystalliza-

tion, was treated with excess $NaBH_4$ overnight at room temperature to afford a mixture of allylic alcohols, bp 85–91 °C (1.25 Torr). This material was oxidized with *m*-chloroperbenzoic acid by the procedure of Marshall and Fanta,[9] and the crude epoxide mixture was reduced with excess $LiAlD_4$ (Stohler) in ether at room temperature for 6 h, again as previously described, to afford a mixture of deuteriated diols. This crude product was oxidized, as described, with Jones reagent to afford a mixture of **17** and 1α-deuterio-9β-hydroxy-*cis*-decal-2-one. Pure **17** was obtained from column chromatography on Florisil. From a 5.93-g sample of Jones oxidation[12] product, elution with 2:3 ether–hexane afforded 2.93 g, which was recrystallized from ether several times to afford a total of 1.11 g of **17**: mp 143–148 °C. Further elution with ether afforded fractions rich in the cis isomer. Conversion of **17** to **10** was accomplished by the same sequence as the conversion of **12** to **7**.

8β,10β-Dimethyldecalin-2β,9α-diol (20). Epoxide **19** was prepared as previously described,[7] except that isolation of **19** by direct crystallization from its mixture with its β-epoxy isomer was accomplished in 54% crude yield. The **19** used in the following experiments was recrystallized from ether–hexane and had mp 71–72 °C.

An ethereal solution of methylmagnesium iodide was prepared by adding a solution of 7.8 g (55 mmol) of freshly distilled methyl iodide in 40 mL of ether dropwise over 1 h to 1.60 g (66 mmol) of magnesium in 20 mL of ether. The Grignard reagent was kept at reflux for an additional h and then a solution of 1.00 g (5.49 mmol) of **19** in 50 mL of ether was added dropwise over 1 h. The mixture was refluxed overnight, cooled to 0 °C, and cautiously decomposed by addition of 50 mL of saturated NH_4Cl solution. The ether layer was decanted, and the gelatinous residue was extracted with ether (3 × 25 mL). The combined ether extracts were washed with brine (2 × 25 mL), dried over $MgSO_4$, and concentrated to afford 1.00 g (92%) of gummy orange solid. Recrystallization from ether gave 0.707 g (65%) of **20** as clear prisms: mp 162–164 °C. An analytical sample was prepared by recrystallization from ether: mp 167–168 °C; IR 3500–3200 cm^{-1}; NMR δ 1.02 (s, 3) 0.94 (d, J = 7 Hz); MS, m/e M^+ 198 (calcd for $C_{12}H_{22}O_2$, 198). Anal. Calcd: C, 72.68; H, 11.18. Found: C, 72.81; H, 11.14.

8β,10β-Dimethyl-2β,9α-diacetoxydecalin (22). A mixture of 0.700 g (3.53 mmol) of **20**, 30 mL of freshly distilled isopropenyl acetate, and 0.800 g (4.21 mmol) of *p*-toluenesulfonic acid was stirred at reflux for 20 h and stored at room temperature for 18 h. The reaction was diluted with an equal volume of benzene, and the solvents were removed under reduced pressure. The residue was dissolved in 125 mL of ether, washed with 5% $NaHCO_3$ solution (3 × 45 mL) and brine (2 × 30 mL), dried over $MgSO_4$, and evaporated to yield 1.45 g of dark brown oil. Column chromatography on 25 g of Florisil with hexane–ether as eluent afforded 0.251 g of material tentatively identified as product formed by elimination of acetic acid: IR 1730 and 1640 cm^{-1}. Further elution gave 0.637 g (64%) of **22** as a colorless oil. An analytical sample was prepared by distillation: bp 105–115 °C (0.1 Torr); IR 1725 cm^{-1}; NMR δ 4.8 (m, 1), 2.02 (s, 3), 1.98 (s, 3). Anal. Calcd for $C_{16}H_{26}O_4$: C, 68.06; H, 9.28. Found: C, 67.97; H, 9.22.

8β,10β-Dimethyl-9α-acetoxydecal-2β-ol (23). To a solution of 0.440 g (1.56 mmol) of **22** in 12 mL of methanol was added 2.2 mL of 5% NaOH solution, and the mixture was stirred at room temperature for 1 h. The mixture was diluted with 100 mL of ethyl acetate, washed with brine (3 × 25 mL), dried over $MgSO_4$, and evaporated to afford 0.411 g of white solid: mp 125–127 °C. Recrystallization from ether afforded 0.224 g (60%) of **23** as white needles: mp 131–132.5 °C; IR 3500 and 1710 cm^{-1}; NMR δ 2.01 (s, 3), 1.13 (s, 3), 1.01 (d, 3, J = 7 Hz). Anal. Calcd for $C_{14}H_{24}O_3$: C, 69.96; H, 10.07. Found: C, 69.93; H, 10.06.

8β,10β-Dimethyl-9α-acetoxydecal-2-one (8). As in the preparation of **7**, treatment of 0.050 g (0.21 mmol) of **23** with Jones reagent[12] afforded a colorless oil, which crystallized upon addition of a few drops of ether to afford 0.049 g (99%) of **8**: mp 89–91 °C. An analytical sample was obtained as colorless prisms from ether: mp 91–92.5 °C; IR 1735 and 1720 cm^{-1}; NMR δ 3.38 (dd, 1, J = 15 and 1.6 Hz), 2.72 (d, 1, J = 15 Hz), 2.32 (m, 2), 1.95 (s, 3). Anal. Calcd for $C_{14}H_{22}O_3$: C, 70.56; H, 9.30. Found: C, 70.69; H, 9.28.

8β-Ethyl-10β-methyldecalin-2β,9α-diol (21). Exactly as in the preparation of **20**, 1.00 g (5.49 mmol) of **19** was treated with ethylmagnesium bromide to afford 1.11 g (95%) of pale yellow **21**: mp 114–119 °C. Recrystallization from ether afforded, in two crops, 0.669 g (57%) of **21**: mp 133–135 °C. An analytical sample was prepared by recrystallization from ether: mp 138.5–139.5 °C; IR 3500–3200 cm^{-1}; NMR δ 1.05 (s); MS, m/e M^+ 212 (calcd for $C_{13}H_{24}O_2$, 212). Anal. Calcd: C, 73.54; H, 11.39. Found: C, 73.44; H, 11.37.

8β-Ethyl-10β-methyl-2β,9α-diacetoxydecalin (24). Exactly as in the preparation of **15**, treatment of 0.300 g (1.41 mmol) of **21** with 20 mL of isopropenyl acetate and 0.32 g of *p*-toluenesulfonic acid afforded 1.07 g of orange oil. Column chromatography on 25 g of silicic acid with hexane-ether as eluent afforded 0.359 g (86%) of **24**, which was homogeneous by TLC (1:1 ether–hexane). An analytical sample was prepared by distillation: bp 107–111 °C (0.1 Torr); IR 1720 cm^{-1}; NMR δ 2.05 (s, 3), 1.99 (s, 3), 1.08 (s, 3). Anal. Calcd for $C_{17}H_{28}O_4$: C, 68.89; H, 9.52. Found: C, 68.89; H, 9.45.

8β-Ethyl-10β-methyl-9α-acetoxydecal-2β-ol (25). Exactly as in the preparation of **16**, treatment of 0.329 g (1.11 mmol) of **24** in 10 mL of methanol with 1.6 mL of 5% NaOH solution afforded 0.241 g (85%) of **25**: mp 132–137 °C. Recrystallization from ether yielded 0.185 g (65%) of **25**: mp 143–144.5 °C; IR 3500 and 1710 cm^{-1}; NMR δ 2.01 (s, 3). Anal. Calcd for $C_{15}H_{26}O_3$: C, 70.83; H, 10.30. Found: C, 70.80; H, 10.26.

8β-Ethyl-10β-methyl-9α-acetoxydecal-2-one (9). Exactly as in the preparation of **7**, treatment of 0.085 g (0.33 mmol) of **25** with Jones reagent[12] afforded a clear oil, which crystallized upon addition of a few drops of ether to give 0.083 g (100%) of **9**, mp 98–102 °C. An analytical sample of **9** was obtained by recrystallization from ether: mp 103.5–104.5 °C; IR 1735 and 1710 cm^{-1}; NMR δ 3.34 (dd, 1, J = 14.6 and 1.6 Hz), 2.73 (d, 1, J = 14.6 Hz), 2.29 (m, 2), 1.94 (s, 3). Anal. Calcd for $C_{15}H_{24}O_3$: C, 71.39; H, 9.59. Found: C, 71.51; H, 9.58.

Identification of $\Delta^{1(9)}$-Octal-2-one (18) as the Product from 7. To a solution of 0.041 g (0.194 mmol) of **7**, mp 59.5–60.5 °C, in 10 mL of methanol was added 0.25 mL of 1 M sodium hydroxide solution. Progress of reaction was monitored at $\lambda_{max}^{CH_3OH}$ = 238 nm. Increase in absorption ceased after ca. 3 min at a value corresponding to ε = 14 000 for **18**. After 10 min, the reaction mixture was concentrated under reduced pressure at room temperature, diluted with ether, and extracted with brine until the extracts were neutral. The ether layer was dried over $MgSO_4$ and evaporated to afford 0.025 g (86%) of colorless oil: IR 1680 and 1625 cm^{-1}, with absorbance at 1720 cm^{-1}[18] no greater than that of enones **26** and **27**; NMR δ 5.85 (s, 1).

Identification of 8β,10β-Dimethyl-$\Delta^{1(9)}$-octal-2-one (26) as the Product from 8. To a solution of 0.040 g (0.168 mmol) of **8**, mp 89–91 °C, in 9 mL of methanol was added 1 mL of 2 M aqueous sodium hydroxide solution. This mixture was stirred at room temperature for 30 min, concentrated under reduced pressure, diluted with ether, washed with water until the aqueous phase was neutral, washed with brine, dried over $MgSO_4$, and evaporated to afford 0.027 g of oil: IR 1670 and 1610 cm^{-1}; NMR (in $CDCl_3$ and CCl_4 respectively) δ 1.23 and 1.25 (d, 3, J = 8 Hz, C-8 H_3C), 1.32 and 1.34 (s, 3, C-10 H_3C), 5.78 and 5.62 (s, 1, C-1 H). NMR data for authentic samples[16] of **26** and 8α,10β-dimethyl-$\Delta^{1(9)}$-octal-2-one, respectively, are as follows: **26** (CCl_4) 1.26 (d, 3, J = 7 Hz), 1.32 (s, 3), 5.60 (s, 1); 8α,10β-dimethyl-$\Delta^{1(9)}$-octal-2-one ($CDCl_3$) 1.04 (d, 3, J = 7 Hz), 1.23 (s, 3), 5.71 (d, 1, J = 2 Hz). On the basis of these NMR data, particularly the distinctly different vinyl proton resonances for **26** and its epimer, it was clear that the product from **8** was essentially pure **26**.

8β-Ethyl-10β-methyl-$\Delta^{1(9)}$-octal-2-one (27). To a solution of 0.058 g (0.23 mmol) of **9**, mp 103–104 °C, in 9.5 mL of methanol was added 0.2 mL of 2 aqueous sodium hydroxide solution at room temperature. The reaction was monitored by UV and was complete in 10 min. Workup as in the preceding experiment afforded 0.040 g (90%) of **27** as an oil: IR 1675 and 1605 cm^{-1}; NMR δ 0.87 (t, 3, J = 7 Hz), 1.25 (s, 3) 5.60 (s, 1). This product was purified by preparative TLC with 1:1 ether–hexane and by bulb-to-bulb distillation to afford 0.033 g (75%) of **27**: bp 95–105 °C (0.5 Torr); MS, m/e 192.1517 (M^+) (calcd for $C_{13}H_{20}O$, 192.1514).

After treatment of **27** with a solution of 0.020 g of sodium in 5 mL of methanol at room temperature for 3 h, the NMR spectrum of the product showed δ 5.58 (s) and 5.65 (d, J = 2 Hz), presumably as a consequence of partial epimerization of the 8β ethyl group of **27**.

Kinetics. Ultraviolet (UV) spectra were recorded in 1-cm quartz cuvettes on a Varian Cary 219 spectrophotometer equipped with an automatic cell changer. The temperature of all kinetic runs was maintained at 25.0 ± 0.1 °C with a Tamson or a Forma-Temp Model 2095 thermostated bath to circulate water through the cuvette housings in the spectrophotometer. Measurements of pH were made with a Corning Model 130 pH meter equipped with a Fisher glass-body E-5A electrode or a Markson Poly Mark combination semimicro electrode.

Laboratory distilled water was redistilled from potassium permanganate before use. Commercially available amines were refluxed over and distilled from barium oxide before use.

Amine pK_a's were determined automatically at 25.0 ± 0.1 °C and at ionic strength μ = 0.4 with a Fisher Auto-Burette Model 390 and an Orion Research Model 601A pH meter interfaced to a New England Digital Corporation mini-computer. Data acquisition and analyses of the pK_a's were conducted with an existing software package written by D. Deuring and D. Saucy by using the method described by Gran.[24]

All reactions were conducted under pseudo-first-order conditions with ca. 10^{-5} M substrate solutions. Whenever practical, values of A_∞ were determined by allowing reactions to go essentially to completion; in other

cases, A_∞ could be calculated by use of a known weight of reactant or determined by catalyzing the reaction to completion by addition of a small amount of hydroxide ion. Amine buffer solutions used ranged from 0.05 to 0.40 M. Reaction progress was monitored in a scanning mode or at 247 nm.

Reaction rates for the pseudo-first-order reactions were determined by using a least-squares computer plotting program written in SBASIC for the Dartmouth Time-Sharing System. Use of the program required the input of A_∞ and absorbances with their respective times as determined experimentally. The observed reaction rate, k_{obsd}, is given by the least-squares slope of the line determined by plotting ln $(A_\infty - A_{time})$ versus time.

For a series of reactions at differing buffer concentrations but at a constant pH, the hydroxide-catalyzed rate of reaction, $k_{OH}[OH^-]$, was obtained by extrapolation to zero-buffer concentration, and division by $[OH^-]$ gave the value of k_{OH}. The rate of the amine-catalyzed reaction was obtained by subtracting $k_{OH}[OH^-]$ from k_{obsd}. The concentrations of free and protonated amine, [AM] and $[AMH^+]$, were calculated from the expressions [AM] = [total buffer] $\times k_a/(k_a + a_H)$ and $[AMH^+]$ = [total buffer] - [AM]. Values of k_B were calculated from $k_B = (k_{obsd} - k_{OH}[OH^-])/[AM]$.

With morpholine as a catalyst, the contribution of the $k_{AB}[AM][AMH^+]$ term was evaluated by using the expression $(k_{obsd} - k_{OH}[OH^-])/[AM] = k_{AB}[AMH^+] + k_B$ and iteratively fitting data to it, starting with an appropriate value of k_B determined where $k_B[AM]$ accounts for >90% of k_{obsd}.

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Registry No. 4, 66875-62-5; **7**, 114200-69-0; **8**, 114200-76-9; **9**, 114200-80-5; **10**, 114200-72-5; **12**, 20721-86-2; **13**, 114200-65-6; **14**, 114200-66-7; **15**, 114200-67-8; **16**, 114200-68-9; **17**, 114200-70-3; **18**, 1196-55-0; **19**, 72938-41-1; **20**, 114200-73-6; **21**, 114200-77-0; **22**, 114200-74-7; **23**, 114200-75-8; **24**, 114200-78-1; **25**, 114200-79-2; **26**, 64281-61-4; **27**, 114200-81-6; 1α-deuterio-9β-hydroxy-*cis*-decal-2-one, 114200-71-4; morpholine, 110-91-8; *N*-methylmorpholine, 109-02-4; *N*-ethylmorpholine, 100-74-3; deuterium, 7782-39-0.

Selective Deuteriation as an Aid in the Assignment of ^1H NMR Spectra of Single-Stranded Oligodeoxynucleotides

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Abstract: The thymidine C5 methyl and C6 proton resonances in the ^1H NMR spectrum of the tetradeoxynucleotide TpTpTpT were assigned with the aid of selective deuteriation. Hydrogen was replaced with deuterium in the methyl group of thymidine by platinum-catalyzed exchange in D_2O. The trideuteriothymidine was converted to the protected mononucleotide for either solution- or solid-phase phosphotriester oligodeoxynucleotide synthesis chemistry. Two tetradeoxynucleotides TpTpTpT were prepared with defined percentages of [*methyl*-$^1H_3$]thymidine and [*methyl*-$^2H_3$]thymidine at each position in the sequence. Assignment of the methyl signals was accomplished by integration and confirmed by examination of the aromatic region of the spectrum. The thymine H6 protons were assigned by homonuclear decoupling experiments and inspection of the ^1H NMR spectrum to determine the relative ratio of each resonance that appeared as a quartet and as a singlet as a result of the isotopic substitution at the adjacent methyl group. The catalyzed exchange of deuterium for hydrogen in the thymidine methyl group was found to proceed efficiently with a modest amount of catalyst. The partial deuteriation method described in this paper should be useful for the assignment of single-stranded oligodeoxynucleotides, duplexes in which B-DNA geometry is not followed, and novel oligodeoxynucleotide structures.

Much attention has been directed recently to the ^1H NMR spectral assignment of Watson-Crick oligodeoxynucleotide duplexes by use of two-dimensional nuclear Overhauser effect techniques (NOESY).[1a-f] These methods assume that the oligodeoxynucleotide is in the B-DNA conformation and rely on the nucleotides being held in relatively constrained positions so that dipolar coupling may be observed between protons on the heterocyclic bases, the corresponding deoxyribose moiety, and the neighboring 5'-deoxyribose. The connectivity between bases and the neighboring 5' deoxyribose sugars may be followed through the sequence and the assignments deduced.

Less attention has been paid to the spectral assignment of single-stranded oligomers and duplex oligomers containing nucleotide sequences not in the Watson-Crick conformation. In these situations, there is often insufficient conformational stability to observe specific intra- and internucleotide NOEs necessary to derive sequence information. The two-dimensional nuclear Overhauser effect experiment, which gives good results when applied to a Watson-Crick duplex, often fails with a single-stranded oligomer.

Borer et al.[2] developed and used the method of incremental analysis to assign the ^1H NMR resonances of oligonucleotides. They synthesized a series of short oligomers and analyzed the NMR spectrum of each at high temperature. When the spectrum of the smaller oligomer from the next larger in the series was compared, the resonances of the added nucleotide were assigned. Recently, several promising NMR methods for the sequence assignment of single-stranded oligonucleotides have been devel-

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