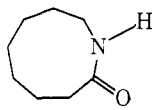
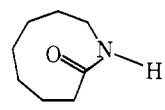
Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy. A Simple Procedure for Determination of the Rates of N–H Proton Exchange of *cis*- and *trans*-1-Aza-2-cyclononanone[1]

Sir:

Several investigations have been made to determine the relative rates of N–H proton exchange in cis and trans isomers of peptide bonds.[2] *N*-Methylacetamide and medium-ring lactams have been traditional models for the cis and trans isomers, respectively. Klotz and Feidelseit[3] have reported that base-catalyzed N–H proton exchange of 1-aza-2-cyclopentanone is much *faster* than that of *N*-methylacetamide. However, Chen and Swenson[4] subsequently found that amide protons of 1-aza-2-cycloheptanone exchange *more slowly* than those of *N*-methylacetamide in deuterium oxide solution. The nine-membered ring lactam, 1-aza-2-cyclononanone (**1**), is well established[5,6] to exist in several solvents as a nearly 1:1 mixture of the cis and trans isomers and, as such, is well suited as a model for both the *cis*- and *trans*-amide bonds. We present here the results of a ^{15}N NMR study of base-catalyzed N–H proton-exchange reactions of **1** at the natural-abundance level of the isotope in dimethyl sulfoxide and in 80% aqueous ethanol.



1a (cis)



1b (trans)

^{15}N NMR spectra[7] of a 2.5 M solution of 1-aza-2-cyclononanone[8] in dimethyl sulfoxide at 25 °C are shown in Figure 1. The proton-noise-decoupled spectrum exhibits two nearly equally intense resonances at 253.4 and 255.9 ppm upfield from an external nitric acid standard. The higher field signal has been previously assigned[6] to the trans isomer. The gated ^{1}H-noise-decoupled ^{15}N spectrum shows two doublets for the cis and trans isomers with the $^{1}J_{^{15}N-^{1}H}$ coupling constants being 89 and 91 Hz, respectively. The larger N–H coupling constant found for the higher field resonance further confirms that this resonance corresponds to the trans isomer.[9] Although the line width at half-peak height in the decoupled spectrum of **1** is <3 Hz, each component of the two doublets in the proton-coupled spectrum has an effective line width[10] of ~6 Hz because of unresolved long-range spin–spin coupling with the ring methylene protons.

When ~0.2 mL of 1 M aqueous sodium hydroxide is added[11] to the dimethyl sulfoxide solution of **1**, the doublet assigned to the cis isomer in the proton-coupled ^{15}N spectrum (Figure 1c) becomes very broad, while that of the trans isomer is only slightly broadened. Further addition of 0.3 mL of the base causes the doublet arising from the cis isomer to collapse and become very broad, centered near the lower component of the other doublet which now also shows appreciable broadening (see Figure 1d). When the amount of the base is increased by a further 0.5 mL, the second doublet also collapses. The proton-decoupled spectrum of **1** now shows two equal-intensity resonances at 253.2 and 255.8 ppm, indicating that the ^{15}N chemical shifts have essentially remained unchanged, and, even under conditions for fast N–H proton exchange, cis–trans isomerization remains slow on the NMR time scale. The two lines remain sharp even when 2 mL of 4 M aqueous sodium hydroxide is added to the ^{15}N NMR tube.

Line-shape calculations for the proton-coupled ^{15}N spectra obtained in the presence of aqueous sodium hydroxide, using an effective line width[10] of 6 Hz and $^{15}N-^{1}H$ spin–spin coupling constants of 89 and 91 Hz for the cis and trans isomers, respectively, showed that the rate of base-catalyzed N–H proton exchange of the cis isomer is 25 ± 3 times faster than for the trans isomer. Similar cis–trans rate ratios were obtained by addition of base to 2 M solutions of **1** in either 80% aqueous dimethyl sulfoxide or 80% aqueous ethanol. These results indicate that rates of N–H proton exchange for amides in cis and trans configurations are likely to be different by one or two orders of magnitude, and it remains to elucidate how effects of strain, solvation, dipole–dipole interactions, steric hindrance, and so on, influence the rates.

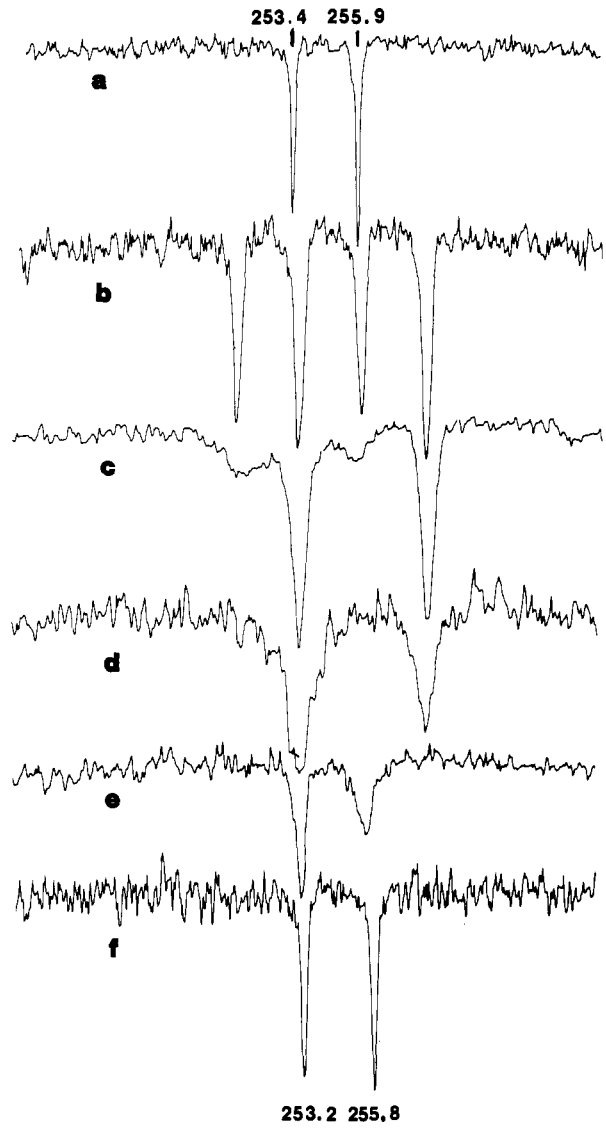


Figure 1. Natural-abundance ^{15}N NMR spectra of 25 mL of a 2.5 M solution of 1-aza-2-cyclononanone in dimethyl sulfoxide at 25 °C: a, with ^{1}H noise decoupling, 200 transients; b, with gated ^{1}H noise decoupling, 2020 transients; c, as b, but after adding 0.2 mL of 1 M aqueous sodium hydroxide, 5000 transients; d, as b, but after further addition of 0.3 mL of the base, 2030 transients; e, as b, but with a total of 1.0 mL of added base, 690 transients; f, as e, but with ^{1}H noise decoupling, 120 transients.

The cis–trans free-energy difference for cyclononene is 2.9 kcal/mol in favor of the cis isomer.[12] The corresponding cis–trans energy difference in 1-aza-2-cyclononanone (**1**) is close to zero. Thus, one might expect that any perturbation of the amide bond, which would increase the double-bond character of the N–C(O) bond, should act to shift the equilibrium in the direction of the cis isomer. Such enhancement of the double-bond character of the N–C(O) bond is expected either on protonation of the amide oxygen[13] or on removal of the N–H proton.[14] For this reason, it is not surprising that **1**, in the

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presence of either base or acid, shows a significant increase in the total amount of cis isomer (un-ionized + ionized).[15]

The procedure used here provides an extraordinarily simple way to determine the rates of proton exchange and cis-trans equilibrium ratios of amides, which should have special utility for peptides. ^{1}H NMR is at best difficultly applicable to simultaneous measurement of exchange rates in systems of this kind because of the broadness of ^{14}N-H proton resonances.

References and Notes

(1) Supported by the National Science Foundation and by the Public Health Service, Research Grant No. GM-11072, from the Division of General Medical Sciences.

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(3) Klotz, I. M.; Feldelseit, P. L. *J. Am. Chem. Soc.*, **1966**, 88, 5103-5105.

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(5) Huisgen, R.; Brade, H.; Wals, H.; Glogger, I. *Chem. Ber.*, **1957**, 90, 1437-1447; Smolíková, J.; Havel, M.; Vašičková, S.; Vítek, A.; Svoboda, M.; Bláha, K. *Collect. Czech. Chem. Commun.*, **1974**, 39, 293-306.

(6) Williamson, K. L.; Roberts, J. D. *J. Am. Chem. Soc.*, **1976**, 98, 5082-5086.

(7) The natural-abundance ^{15}N NMR spectra were obtained at 18.25 MHz with a Bruker WH-180 pulse spectrometer. A 25-mm-o.d. spinning sample tube containing ~25 mL of sample was used. A 5-mm concentric tube containing a 1.0 M solution of 98% ^{15}N-enriched nitric acid in D_2O provided both the external reference standard and the field-frequency lock. The ^{15}N spectra were obtained with a 45° pulse angle, 2K data points, 1200-Hz spectrum width, and a pulse interval of 3 s.

(8) The 1-aza-2-cyclononanone was obtained from Aldrich and recrystallized from benzene-hexane mixture.

(9) The $^1J_{^{15}N-^1H}$ coupling constants for bonds cis and trans to the carbonyl group in formamide have been reported to be 88 and 92 Hz, respectively (Summers, B.; Piette, L. H.; Schneider, W. G. *Can. J. Chem.*, **1960**, 38, 681-688. Levy, G. C.; Holloway, C. E.; Rosanske, R. C.; Hewitt, J. M. *Org. Magn. Reson.*, **1976**, 8, 643-647).

(10) Berger, A.; Loewenstein, A.; Meiboom, S. *J. Am. Chem. Soc.*, **1959**, 81, 62-67 (Appendix A).

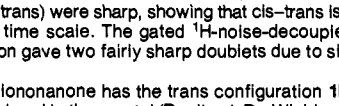
(11) After addition of each portion of the aqueous sodium hydroxide solution to the ^{15}N NMR sample tube at room temperature, argon was bubbled into the solution for a few minutes. The samples were allowed to equilibrate in the probe at ~25 °C for 1 h before taking the spectra.

(12) Turner, R. B.; Meador, W. R. *J. Am. Chem. Soc.*, **1957**, 79, 4133-4136.

(13) When 2 mL of 4.0 M aqueous sodium hydroxide is added to a 2.5 M solution of 1 in dimethyl sulfoxide, the ^{1}H-noise-decoupled ^{15}N NMR spectrum showed two sharp and equally intense resonances at δ 250.0 and 253.8 ppm for the cis and trans isomers, respectively. The ^{15}N spectrum of the same sample after 10 h at room temperature showed an increase in the cis-trans intensity ratio to ~1.3. Similarly, addition of 2 mL of 20% hydrochloric acid to a 2.5 M dimethyl sulfoxide solution of 1 resulted in an increase in the proportions of the cis isomer, the cis-trans intensity ratio becoming ~1.5. For this solution at 25 °C, both ^{15}N resonances (δ 240.4, cis; δ 247.9, trans) were sharp, showing that cis-trans isomerization is slow on the NMR time scale. The gated ^{1}H-noise-decoupled spectrum of the acidic solution gave two fairly sharp doublets due to slow N-H proton exchange.

(14) 1-Aza-2-cyclononanone has the trans configuration **1b** with a rather distorted amide bond in the crystal (Dunitz, J. D.; Winkler, F. K. *Acta Crystallogr., Sect. B*, **1975**, 31, 251-263; Winkler, F. K.; Dunitz, J. D. *Ibid.*, **1975**, 31, 276-278), but when protonated, accepts the proton on oxygen and changes to the cis configuration with a nearly planar amide bond (Winkler, F. K.; Dunitz, J. D. *Ibid.*, **1975**, 31, 278-281). The ^{15}N spectra reported here show that the cis form of protonated **1** is the dominant configuration *in solution* at 25 °C.[13]

(15) Substantial enhancement of the double-bond character of the N-C(O) bond of **1** by removal of the amide proton is expected because delocalization



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of an unshared pair on nitrogen to the oxygen does not involve charge separation.

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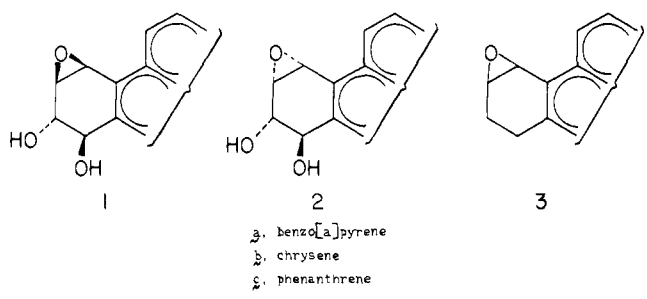
Stereoelectronic Factors in the Solvolysis of Bay Region Diol Epoxides of Polycyclic Aromatic Hydrocarbons

Sir:

Tumor studies have identified (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene[1a] and (±)-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenzo[*a*]anthracene[1b] as ultimate carcinogenic metabolites of their respective hydrocarbons, in accord with the "bay-region" theory[1c-f] and preliminary studies on the binding of benzo[*a*]pyrene (BP) to nucleic acid.[1g,h] Thus, detailed knowledge of the chemistry of diol epoxides on saturated, angular, benzo rings in which the epoxide group forms part of a bay region of the hydrocarbon acquires special significance. Studies of the diastereomeric 7,8-diol 9,10-epoxides of BP have established that (1) they alkylate the exocyclic amino group of guanine[2a,b] and the phosphate backbone[2b,c] of nucleic acid, (2) they hydrolyze in water to form mixtures of tetraols[3] resulting from cis and trans opening of the oxirane ring at C-10 accompanied by a minor amount of 9-keto-7,8-diol,[3e] (3) the isomer in which the benzylic 7-hydroxyl group and the oxirane ring are cis is much more reactive toward nitrothiophenolate, presumably owing to anchimeric assistance,[4] and (4) this same isomer is 30-fold more reactive toward water at neutral to alkaline pH.[3e] The exact role of the hydroxyl groups in determining reaction rates and products remains unknown. To this end, we have prepared the diastereomeric bay-region diol epoxides (**1** and **2**) of phenanthrene and chrysene in order to compare the rates and products of their hydrolysis with those observed for the corresponding derivatives of BP. To help elucidate the role of the hydroxyl groups in the reactions of **1** and **2**, we have also studied the hydrolysis of tetrahydro epoxides (**3**). Rates and



1 2 3

a, benzo[a]pyrene
b, chrysene
c, phenanthrene

product distributions of acid-catalyzed hydrolysis of bay-region epoxides **1-3** in general were found to correlate with the predicted ease of carbonium-ion formation at the benzylic position, and stereoelectronic factors are proposed to account in part for a greater reactivity of the tetrahydro epoxides compared with the diol epoxides for a given hydrocarbon toward acid-catalyzed hydrolysis.

Hydrolyses of the diastereomeric diol epoxides **1** and **2** and the tetrahydro epoxides **3** in water or 25% dioxane-water were fit to the equation

$$k_{obsd} = k_{H^+}a_{H^+} + k_0$$

Values of the rate constants for acid-catalyzed hydrolysis (k_{H^+}) and spontaneous hydrolysis (k_0) pathways are summarized in Table I. In general k_{H^+} for isomer **2** is 2 to 3 times greater than that for isomer **1**, whereas k_0 for isomer **1** is 4 to 30 times greater than that for isomer **2**. Intramolecular hydrogen bonding between the oxirane oxygen and the benzylic hydroxyl group in diol epoxide **1** has been suggested to account for the decreased rate of **1** in acid[3b] and the increased rate of **1** in the k_0 region.[3e] The products of these pathways (Table II) consist of cis and trans hydration of the epoxides at the benzylic position, along with some isomerization to ketones in the k_0 region as illustrated.

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