for reactions 13, 14, and 15 of 23.2, 4.6, and 17.8 kcal/mol:

$$C^+(g) + NH_3(g) \rightarrow \mathbf{1a}(g) + NH_4^+(g) \qquad (13)$$

$$D^+(g) + NH_3(g) \rightarrow \mathbf{1a}(g) + NH_4^+(g) \qquad (14)$$

$$E^+(g) + NH_3(g) \rightarrow \mathbf{1a}(g) + NH_4^+(g) \qquad (15)$$

Thus, in the absence of chelation, ring protonation would be vastly preferred and **1** would behave as a much weaker base. These remarkable base strengthening and leveling effects originate in the mutual "solvation"[16] of both moieties of $\mathbf{1}H^+(g)$. Furthermore, this approximately equal basicity of $N_{(1)}$ and N seems to explain the known ability of histamine to form bidentate chelates with metal ions and likely supports Kimura et al.'s model of histamine's H_2 receptor.[4]

4. Based on the ratio of protonation microconstants in D_2O solutions,[8] N protonation is seen to be favored over ring protonation by 3.97 kcal/mol[22] (in Gibbs energy) at 298 K. Clearly, this reversal of the relative basicities of both sites originates in solvation effects, the tremendous influence of water solvent on acid–base equilibria being well documented.[23] It thus seems possible that in a medium less efficient at charge dispersal (through hydrogen bonding) than water, such a stability gap could be strongly reduced. This would favor the prototopic exchange between both moieties of $\mathbf{1}H^+$. This contention is supported by the fact that recent theoretical calculations suggest that $\mathbf{1}H^+$ remains chelated even in aqueous solution.[24]

4. Experimental Section

FT-ICR studies were performed on a modified Brucker CMS-47 mass spectrometer under conditions similar to those used in previous works.[8] In every case, the attainment of equilibrium was established by means of double resonance experiments.

A sample of histamine (Aldrich) was twice sublimed and immediately introduced into the high-vacuum section of the mass spectrometer by means of a direct insertion probe. The nominal cell temperature was 343 K.

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[22] From the effect of temperature on this ratio, it can be deduced that, in D_2O solution at 313–333 K, chain protonation is 1.9 kcal mol^{-1} more exothermal than ring protonation.

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Matrix Isolation of Tricyclo[3.3.1.0^{3,7}]non-3(7)-ene, a Doubly Pyramidalized Alkene Predicted To Have a Nearly Tetrahedral Geometry at the Olefinic Carbons

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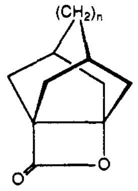
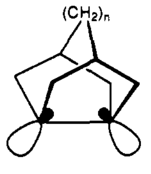
Abstract: The title alkene, which is calculated to have an approximately tetrahedral geometry at each of the doubly bonded carbons, has been generated by dehalogenation of 3,7-diiodotricyclo[3.3.1.0^{3,7}]nonane in the gas phase, using both potassium and cesium vapor, and has been isolated in an argon matrix at 10 K. The IR bands belonging to the alkene have been identified by photobleaching the matrix-isolated material and recording the difference spectrum. A weak band at 1496 cm^{-1} in the IR spectrum is assigned to the double-bond stretch on the basis of both semiempirical and ab initio electronic structure calculations.

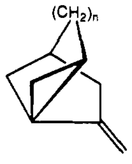
As part of a systematic study of the spectroscopic and chemical effects of enforcing pyramidalized geometries on doubly bonded carbons,[2] we have reported the IR, Raman, and UV spectra of tricyclo[3.3.2.0^{3,7}]dec-3(7)-ene (**2b**) in matrix isolation.[3] Matrix isolation of **2b** was made possible by the availability of β-lactone **1b**[4] as a precursor, from which **2b** was generated by gas-phase pyrolysis. More recently, gas-phase pyrolysis of **1b** has allowed the photoelectron spectrum of **2b** to be obtained.[5]



1a, n = 1
1b, n = 2



2a, n = 1
2b, n = 2



3a, n = 1
3b, n = 2

β-Lactone **1a** has been synthesized as a possible precursor of **2a**.[4] The spectra of alkene **2a** are of even greater interest than those of its homologue (**2b**), since MM2, MNDO, and RHF/3-21G calculations[6] all predict a nearly tetrahedral geometry at each of the doubly bonded carbons of **2a**. Unfortunately, pyrolysis of **1a** did not prove to be a satisfactory method for generating **2a**.[7,8]

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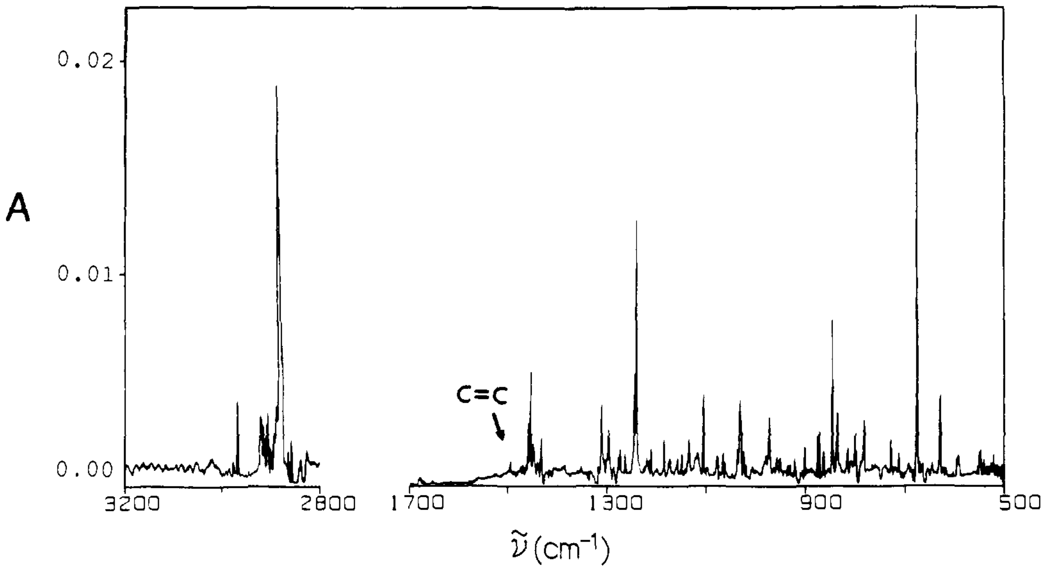

Figure 1. FTIR spectrum of **2a**, obtained as the difference between the spectra of the matrix-isolated material before and after irradiation at 248 nm with a KrF excimer laser.

β-Lactone **1a** is much more resistant to pyrolysis than its homologue (**1b**). Although flash vacuum pyrolysis at temperatures above 550 °C did result in cleavage of the oxetanone ring in **1a**, instead of losing CO_2, cleavage occurred in the undesired sense to afford the ketoketene. On pyrolysis of **1a** at still higher temperatures, however, CO_2 loss was observed, suggesting that ketoketene formation is reversible. Under these conditions a small amount of the dimer of **2a** was formed, but the major product isolated was 2,6-dimethylenebicyclo[2.2.1]heptane.[7,8]

At the temperatures required for loss of CO_2 from **1a**, **2b** was found to undergo a rearrangement in which 2,6-dimethylenebicyclo[2.2.2]octane is the final product.[8] This thermal reorganization was shown to involve the intermediacy of **3b**, formed from **2b** via a retrograde vinylcyclopropane rearrangement. The same highly strained vinylcyclopropane (**3b**) is also formed upon photolysis of **2b**.[3,8]

Presumably, an analogous sequence of thermal rearrangements, involving the even more highly strained **3a**[9] as a putative intermediate, accounts for the fact that pyrolytic loss of CO_2 from **1a** leads to 2,6-dimethylenebicyclo[2.2.1]heptane as the major product. Although the retrograde vinylcyclopropane rearrangement of **2a** and **2b** provides information about the olefin strain energies (OSEs) in these two pyramidalized alkenes,[6,8] the occurrence of this rearrangement at the temperatures necessary to effect loss of CO_2 from **1a** renders this β-lactone unsatisfactory for the generation and matrix isolation of **2a**.

In solution alkene **2a** can be generated by reductive elimination of iodide from 3,7-diiodotricyclo[3.3.1.$0^{3,7}$]nonane.[7,12] This finding led us to investigate the diiodide as a possible precursor for the generation of **2a** in the gas phase. Gas-phase dehalogenation[13] has been used previously to generate adamantene from the corresponding diiodide precursor and to obtain the IR spectrum of this torsionally strained olefin in matrix isolation.[14]

Results and Discussion

A sample of 3,7-diiodotricyclo[3.3.1.$0^{3,7}$]nonane[7] was sublimed at 60 °C into a stream of potassium or cesium vapor, diluted with argon, passed through a hot zone, and condensed on a CsI window that was maintained at 10 K. Although the condensate consisted largely of unreacted diiodide, evidence for formation of **2a** was provided by the detection of the "2 + 2" dimer of **2a** upon thawing the argon matrix. The dimer was identified by comparison of its GC retention time with that of an authentic sample, prepared from reaction of the diiodide with butyllithium in THF.[7]

Alkene **2a** not only is calculated to be more highly pyramidalized and to have a higher OSE than its homologue (**2b**) but also is predicted to absorb light of even longer wavelength.[6] The presence of metal atoms in the matrix prevented us from obtaining a UV spectrum of **2a** to test this prediction. However, evidence for the existence of a long wavelength absorption in **2a** was provided by the observation that, like **2b**, **2a** underwent photobleaching when irradiated with 248-nm light from a KrF excimer laser.

Upon irradiation, IR bands of a photoproduct grew in as those attributed to **2a** disappeared. Prominent in the IR spectrum of the photoproduct were bands at 1666, 1430, 1323, 1284, 1156, 1016, 998, 859, 854, 825, 816, 602, 599, and 586 cm^{-1}. These are very similar to the IR absorptions found in **3b**,[8] and on this basis, we tentatively assign structure **3a** to the photoproduct formed from **2a**.

The IR spectrum, assigned to **2a**, was obtained by photobleaching the matrix-isolated material and recording the difference spectrum. The spectrum is shown in Figure 1. Attempts to obtain the polarized IR (after photoorienting the sample by selective photobleaching with the polarized laser beam)[3] and Raman spectra were unsuccessful. The failure of these attempts was primarily due to the very low concentrations of **2a** that were present in the matrix.

The weak band[15] at 1496 cm^{-1} in the IR spectrum is assigned to the stretching mode of the highly pyramidalized double bond in **2a**. The results of both semiempirical and ab initio calculations support this assignment, when appropriate scaling factors are used.

For example, multiplying the frequency of 1633 cm^{-1}, calculated by MNDO for the C=C stretch in **2a**, by the scaling factor of 0.915, used previously[3] to predict the frequency of the C=C stretch in **2b** from the MNDO value of 1694 cm^{-1}, gives a pre-

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(9) This molecule may be viewed as a derivative of *trans*-bicyclo[4.1.0]heptane. Derivatives of this hydrocarbon have been prepared,[10] and the strain in one of them, relative to its cis isomer, has been calculated.[11]

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(15) Both semiempirical and ab initio calculations predict the IR absorption that corresponds to the C=C stretching mode to be weak, since the IR absorption has intensity only to the extent that the dipole moment, perpendicular to the C—C double bond, changes upon excitation of this vibrational mode.

J. Am. Chem. Soc. 1993, 115, 1456-1460

dicted value in **2a** of 1494 cm^{-1}. A smaller scaling factor of 0.860 is required to fit the calculated RHF/3-21G frequency of 1811 cm^{-1} for **2b** to the experimental value[3] of 1557 cm^{-1}. Using this scaling factor, together with the RHF/3-21G value of 1742 cm^{-1} for the C═C stretch in **2a**, gives a predicted frequency of 1498 cm^{-1}.

The close agreement of the scaled frequencies, obtained from two different types of electronic structure calculations, with that of the weak absorption at 1496 cm^{-1} in the IR spectrum of **2a** provides support for assigning this band to the C═C stretch in **2a**. The frequency of this band is about 60 cm^{-1} lower than that of the corresponding stretching frequency in **2b**[3] and about 185 cm^{-1} lower than the frequencies for the C═C stretch in bicyclo[3.3.0]oct-1(5)-ene[16] and in tetramethylethylene. The highly pyramidalized, nearly tetrahedral geometry,[6] to which the doubly bonded carbons in **2a** are constrained by the rigid tricyclic skeleton, obviously has a substantial effect on lowering the C═C stretching frequency from those found in unconstrained alkenes.

Experimental Section

Synthesis of 3,7-Diiodotricyclo[3.3.1.$0^{3,7}$]nonane.[7] A solution of 200 mg of tricyclo[3.3.1.$0^{3,7}$]nonane-3,7-diol[17] and 3.0 g of sodium iodide in 10 mL of 95% phosphoric acid was heated at 110 °C for 48 h. The purple reaction mixture was then cooled, diluted with 20 mL of water, and extracted with three 20-mL portions of diethyl ether. The combined ether solutions were washed with two 25-mL portions of saturated, aqueous sodium thiosulfate and 20 mL of saturated, aqueous sodium chloride and then dried over magnesium sulfate. Removal of solvent under vacuum gave a yellow solid, which was chromatographed on 5 g of silical gel, using hexane–ethyl acetate (10:1) to elute the column. Isolated was 195 mg (40%) of the diiodide as a white, crystalline solid, mp 130–131 °C and pure by ^{1}H NMR, after sublimation under vacuum. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.76 (d, 4 H, J = 10.3 Hz), 2.43 (d, 4 H, J = 10.3 Hz), 1.90 (s, 2 H), 1.63 (s, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$) 57.79 (t), 53.14 (s), 39.45 (d), 30.78 (t). Exact mass. Calcd for $C_9H_{12}I_2$: 373.9028. Found: 373.9036.

Matrix Isolation Procedure. A sample of 3,7-diiodotricyclo[3.3.1.$0^{3,7}$]nonane was sublimed at 60 °C into a stream of potassium or cesium vapor, diluted with argon, passed through a 2 in. long hot zone that was heated to 180 °C, and condensed on a CsI window that was maintained at 10 K using an Air Products CS-202 Displex closed-cycle helium cryostat. The IR spectra were obtained with a Nicolet 60-SXR FTIR spectrometer. The photobleaching was performed with the 248-nm KrF line of a Lambda Physik EMG 50E excimer laser.

Calculations. Vibrational analyses, using the semiempirical MNDO method,[18] as well as ab initio RHF calculations with the 3-21G basis set[19] were performed with the package of programs in Gaussian 90.[20]

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Slow Amide Proton Exchange Rates from the Line Widths in a Single Two-Dimensional ^{1}H NMR Spectrum

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Abstract: A method is presented that allows a quantitative determination of chemical exchange rates of the order of reciprocal hours from the line widths of the signals in a single two-dimensional ^{1}H nuclear magnetic resonance spectrum. The method is based on a linear prediction analysis of the time domain signal and a nonlinear least squares fit of the entire frequency domain signal. The applicability of the method is demonstrated by the determination of the exchange rates of seven slowly exchanging amide protons in the des-[Phe(B25)] mutant of human insulin.

Introduction

The exchange of amide protons in proteins has attracted much attention over the years due to its close relation to the secondary and tertiary structure of the proteins. In particular NMR spectroscopy has proved valuable in such studies since it allows a determination of the rate of exchange of individual amide protons in the range from reciprocal milliseconds to reciprocal months.

In principle, the rate of exchange of relatively slowly exchanging amide protons in proteins can be determined from series of 1D NMR spectra,[1,2] by following the decay of the intensities of the amide proton signals after dissolution of the protein in D_2O. However, in the case of larger peptides and proteins a determination of the rates from 1D NMR spectra is often prevented by signal overlap and line broadening. This resolution problem can be reduced considerably by using series of 2D NMR spectra.[3] Because of the large amount of time necessary to record such series, the NMR measurements are normally carried out independently of the exchange process in order to ensure a sufficient time resolution for this process. Thus, samples corresponding to the individual 2D NMR spectra are removed from a stock solution of the exchanging protein at different time intervals after the start of the exchange, and the exchange process is quenched prior to the NMR experiment. This procedures ensures a time resolution for the exchange process that is independent of the time interval between consecutive 2D NMR spectra in the series. At the same time, however, it makes considerable demands on the amount of protein. Also a calibration of the individual spectra is normally

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