(26) One possibility suggested in the literature[4] is that His-119 may not be protonated when the substrate is first bound to RNase A. If His-119 is initially neutral and does protonate the leaving group, this suggestion implies that His-119 protonates the leaving group with the hydrogen abstracted from $O_{2'}$. For this step to occur, the abstracted hydrogen must be transferred from the group which deprotonates $O_{2'}$ to His-119. It seems likely from the X-ray structure of RNase A[1,2] that only His-12 could be involved in such a hydrogen transfer. If this is true, it is difficult to account for the enzymatic activity observed when His-12 is blocked[27] or missing.[13] Furthermore, a positive charge on His-119 will aid in stabilizing the negatively charged phosphate group.[4] It also should be noted that a His-12–His-119 proton shuttle is not required for microscopic reversibility.[4] Consequently, we did not consider this possibility when carrying out our calculations.
(27) (a) E. Machuga and M. H. Klapper, *J. Biol. Chem.*, **250,** 2319 (1975); (b) E. Machuga and M. H. Klapper, *Biochim. Biophys. Acta*, **481,** 526 (1977).
(28) Ab initio calculations for this anion have been carried out by M. D. Newton, *J. Am. Chem. Soc.*, **95,** 256 (1973), and D. Perahia, A. Pullman, and H. Berthod, *Theor. Chim. Acta*, **40,** 47 (1975); H. Berthod and A. Pullman, *Chem. Phys. Lett.*, **32,** 233 (1975).
(29) J. L. Sussman, N. C. Seeman, S. H. Kim, and H. M. Berman, *J. Mol. Biol.*, **66**, 403 (1972).
(30) S. Martinez-Carrera, *Acta Crystallogr.*, **20,** 783 (1966); for molecules other than imidazole, see *Chem. Soc.*, *Spec. Publ.*, **11** (1958); **18** (1965).
(31) (a) D. G. Gorenstein, D. Kar, B. A. Luxon, and R. K. Momii, *J. Am. Chem. Soc.*, **98,** 1668 (1976); (b) D. G. Gorenstein and D. Kar, *ibid.*, **99,** 672 (1977); (c) D. G. Gorenstein, J. B. Findlay, B. A. Luxon and D. Kar, *ibid.*, **99,** 3473 (1977); (d) D. G. Gorenstein, B. A. Luxon, J. B. Findlay, and R. Monii, *ibid.*, **99,** 4170 (1977); (e) D. G. Gorenstein, B. A. Luxon, and J. B. Findlay, *ibid.*, **99,** 8048 (1977).
(32) H. W. Wyckoff, D. Tsernolou, A. W. Hanson, J. R. Know, B. Lee, and F. M. Richards, *J. Biol. Chem.*, **245,** 305 (1970).
(33) C. H. Carlisle, R. A. Palmer, S. K. Mazumdar, B. A. Gorinsky, and D. G. R. Yeates, *J. Mol. Biol.*, **85,** 1 (1974).
(34) (a) Two additional combinations were computed for reference: His-119, N–H(back) and Asp-121···His-119,N–H(back) with Asp···His optimized. His-119 and N–H(back) interact electrostatically but are not linked by a hydrogen bond and their positions were those found when substrate is attached. (b) The total charge on the substrate, negative charges on the oxygen, and positive charge on the phosphorus will be reduced in the TBP substrate and connected systems if $O_{2'}$ is not totally deprotonated. However, the direction of change in the substrate interaction with His-119, Asp-121, Lys-41, and 119–120 backbone N–H will not be affected. For example, the His-119–substrate interaction will still reduce the positive charge on P and the negative charge on $O_{5'}$ and $O_{5'}CH_3$ (Table V), though possibly to a lesser extent.
(35) C. A. Deakyne and L. C. Allen, *J. Am. Chem. Soc.*, **98,** 4076 (1976).
(36) Ab initio calculations with the 4–31 G basis.
(37) R. S. Mulliken, *J. Chem. Phys.*, **23,** 1841, 2338, 2343 (1955). In Mulliken population analysis, bond strength is equated to overlap population.
(38) Although it is irrelevant for the mechanistic studies discussed here, it is interesting to note that Gorenstein et al.'s[31d,39] NMR results for dianionic inhibitors indicate a strong hydrogen bond between His-119 and a phosphoryl oxygen but not a transferred hydrogen as found in this work. There are at least two reasonable explanations for this. One is the possibility of a nonlinear hydrogen bond between His-119 and the phosphoryl oxygen in Gorenstein et al.'s work.[39] Another strong possibility is the inadequacy of the CNDO/2 calculations in discriminating between the strong and transferred hydrogen bond cases.
(39) D. G. Gorenstein, A. M. Wyrwicz, and J. Bode, *J. Am. Chem. Soc.*, **98,** 2308 (1976).
(40) L. R. Brown and J. H. Bradbury, *Eur. J. Biochem.*, **68,** 227 (1976).
(41) H. Iijima, H. Patrzyc, and J. Bello, *Biochim. Biophys. Acta*, **491,** 305 (1977).
(42) J. L. Markley, *Biochemistry*, **14,** 3546 (1975).
(43) D. M. Blow, *Acc. Chem. Res.*, **9,** 145 (1976).
(44) M. Hunkapiller, S. H. Smallcombe, D. R. Whitaker, and J. H. Richards, *J. Biol. Chem.*, **248,** 8306 (1973); W. W. Bachovchin and J. D. Roberts, *J. Am. Chem. Soc.*, **100,** 8041 (1978).
(45) M. D. Newton and S. Ehrenson, *J. Am. Chem. Soc.*, **93,** 4971 (1971).
(46) (a) B. M. Dunn, C. Dibello, K. L. Kirk, L. A. Cohen, and I. M. Chaiken, *J. Biol. Chem.*, **249**, 6295 (1974); (b) H. C. Taylor and I. M. Chaiken, *ibid.*, **252,** 6991 (1977).
(47) (a) O. D. van Battenberg, I. Voskuyl-Holtkamp, C. Schattenkerk, K. Hoes, K. E. T. Kerling, and E. Havinga, *Biochem. J.*, **163,** 385 (1977). (b) Although N_1 of the imidazole ring is a better proton acceptor than the oxygen of the carbonyl group, the $O_{2'}$–H···N_1 hydrogen bond is bent and the $O_{2'}$–H···O bond need not be. Hydrogen bond energy falls off as the cosine of the nonlinearity angle and could make the $O_{2'}$–H···Asn-44 bond energy comparable to that of $O_{2'}$–H···His-12; R. C. Kerns and L. C. Allen, *J. Am. Chem. Soc.*, **100,** 6592 (1978).
(48) D. Findlay, D. G. Herries, A. P. Mathias, B. R. Rabin, and C. A. Ross, *Biochem. J.*, **85,** 152 (1962).
(49) A. Deavin, A. P. Mathais, and B. R. Rabin, *Biochem. J.*, **101,** 14c (1966).
(50) R. N. Lindquist, J. L. Lynn, and G. E. Lienhard, *J. Am. Chem. Soc.*, **95,** 8762 (1973).
(51) When the substrate is initially bound to RNase A, P and $O_{2'}$ are too far away (3.35 Å[1]) to interact. Either the phosphate or the 3′-ribose must rotate toward the other group before the nucleophilic addition can occur. There is evidence that the 3′-ribose and 3′-base are rigidly bound,[52] leading to the conclusion that the phosphate rotates toward $O_{2'}$.
(52) D. G. Gorenstein and A. M. Wyrwicz, *Biochem. Biophys. Res. Commun.*, **59,** 718 (1974).
(53) (a) C. H. W. Hirs, *Brookhaven Symp. Biol.*, **15,** 154 (1962); (b) R. P. Carty and C. H. W. Hirs, *J. Biol. Chem.*, **243,** 5244, 5254 (1968); (c) R. L. Heinrikson, *ibid.*, **241,** 1393 (1966).

Communications to the Editor

The Hydronium Ion (H_3O^+). Preparation and Characterization by High Resolution Oxygen-17 Nuclear Magnetic Resonance[1]

Sir:

First postulated[2] in 1907, the existence of protonated water, the parent oxonium ion, gained wider acceptance with the acid-base theory of Brønsted[3] and Lowry.[4] Hund[5] suggested for H_3O^+ a tetrahedral geometry and Hückel[6] provided the first quantitative discussion of the "anomalous" mobility of the hydrogen ion. The lifetime of H_3O^+ in aqueous solution was estimated to be about ten times longer than the duration of molecular vibrations.[7,8] Experimental work (IR,[9] Raman,[10] ^{1}H NMR,[11] X-ray,[12] mass spectrometry[13]) reported to date indicates the presence of the hydronium ion, both in solution and solid phase. The strongest evidence for the existence of the long-lived ion was reported by Gold and co-workers.[11e] They observed in the proton spectrum of the partially deuterated HSO_3F-SbF_5-H_2O system in SO_2 or SO_2ClF between −20 and −60 °C three signals, of which one was a triplet assigned to H_2DO^+, another one, an unresolved multiplet, assigned to HD_2O^+, and the third, a singlet, assigned to H_3O^+. However, no direct proof has yet been obtained to unambiguously demonstrate the structure and, particularly, the geometry of this ion.

We present here such a proof, namely the high resolution ^{17}O NMR spectrum obtained from a 1.5 M solution of ^{17}O-enriched water[14] in SO_2, with a slight excess of HF-SbF_5 (1:1 M). At −15 °C a quartet was observed (Figure 1b), which collapsed into a singlet (56-Hz FWHM) upon proton noise decoupling (Figure 1c). The measured ^{17}O-H coupling constant is 106 ± 1.5 Hz and the chemical shift, 9 ± 0.2 ppm downfield from external H_2O (or 496 ppm upfield from SO_2 which was the solvent).

The H_3O^+ spectrum is to be compared with that of H_2O, dissolved in CCl_4 (Figure 1a), where we observed[15] a very sharp triplet (7.6-Hz fwhm, $J_{O\text{-}H}$ = 79.9 Hz) at 12.6 ppm upfield from the broad signal of bulk water (probably present as microscopic droplets in suspension and/or on the wall of the NMR tube).

Our finding clearly demonstrates the tying of three equivalent protons to an oxygen atom in a bona fide covalent species[17] and allows interesting conclusions regarding its properties.

The ion can have a very long lifetime (which implies a significant proton exchange barrier). In fact, convinced that a nonexchanging species would only be observed at very low temperature, we started the experiment at −70 °C (just above the freezing point of SO_2). A very broad oxygen signal was observed, barely revealing a fine structure. This is probably due to hydrogen bonding and/or viscosity effects. Warming the solution resulted in progressive sharpening of the quartet

0002-7863/79/1501-3959$01.00/0

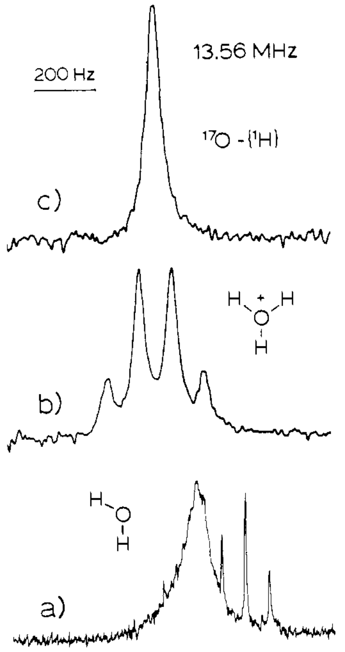

Figure 1. The 0-17 FT NMR spectra (Varian XL-100) of (a) H_2O in CCl_4, 360 000 transients at 0.1-s repetition rate; (b) H_3O^+ in SO_2 at −15 °C, 10 000 transients at 0.1-s repetition rate; (c) H_3O^+ in SO_2 with proton noise decoupling.

lines, with the best spectrum being obtained at a temperature close to the boiling point of SO_2.[18] No alteration of the coupling constant was observed. The relatively narrow line width (34 Hz) indicates that in H_3O^+ the oxygen electric field gradient is small; this is the first experimental result which should provide a better basis for the assessment of theoretical work.[19]

The ion is, at least under our conditions, practically planar. This is supported by two facts associated with the magnitude of the ^{17}O-H coupling constant. First, assuming a linear relation between J_{O-H} and the s character of the oxygen orbital hybridization,[20] the 33% increase in the J_{O-H} in going from H_2O (sp^3 hybridization) to H_3O^+ indicates that in the latter the oxygen hybridization is sp^2, hence its planarity. Second, should the equilibrium conformation of H_3O^+ be pyramidal (i.e., more toward an sp^3 hybridization) and the inversion barrier insignificant,[21] the coupling constant should average to a value *between* 79.9 and 107 Hz, which is not the case.

Our result thus confirms ab initio calculations which in general predict the planar geometry to be the most stable[22] and disagrees with the semiempirical methods which predict a tetrahedral configuration.[23] The planar geometry is also in agreement with the high (>700 cm^{-1}) frequency of the librational mode found in IR spectroscopy.[8b]

We refer now to the linear relation[20] between J_{O-H} and the bond length, r(O-H). Two points, each given by a J_{O-H} and r(O-H) value, are needed to obtain the equation. While there are reliable data for H_2O (−79.9 Hz; 0.958 Å),[24] for H_3O^+ only J_{O-H} (−106 Hz) is now well established. Assuming 0.98 Å for r(O-H) in H_3O^+, the following equation is obtained:

$$r(\text{O-H}) = (0.890\text{-}8.429 \times 10^{-4})J_{\text{O-H}}\ \text{Å}$$

Thus for protonated acetone,[25] where we found $J_{O-H} = 85 \pm 5$ Hz, an O-H bond length of 0.961 ± 0.005 Å is calculated. The measured coupling constant indicates that in protonated acetone the oxygen hybridization is almost sp^3; i.e., the resonance form with a formal positive charge on the carbonyl carbon is dominant.

For an sp hybridization we predict $J_{O-H} \simeq 160$ Hz and $r(\text{O-H}) \simeq 1.02$ Å; it would be of great interest to obtain O-protonated carbon monoxide in order to see whether or not it is linear.

We are presently investigating the kinetic properties of H_3O^+ under various solvent and temperature conditions and are also seeking evidence for the existence of H_4O^{2+}, $H_5O_2^+$, $H_7O_3^+$, $H_9O_4^+$, and OH^-. Details will be reported in a forthcoming full paper.

Acknowledgments. We thank Professors G. Klopman and J. A. Pople for helpful discussions. G.M.B. thanks the Department of Chemistry and the Major Analytical Instruments Facility at Case for financial support during his Ph.D. research. The valuable technical assistance of Messrs. Alan Olson and Nicholas Baldwin is gratefully acknowledged.

References and Notes

(1) (a) This communication, which is part 1 of the series Water and Related Systems, is dedicated to the memory of the late Professor C. D. Nenitzescu. (b) Presented, in part, at the 20th ENC (Experimental NMR Conference), Asilomar, Calif., 1979.

(2) Hantzsch, A.; Caldwell, K. S. *Z. Phys. Chem.* **1907,** *58,* 575. Goldschmidt, H.; Udby, O. *ibid.* **1907,** *60,* 728.

(3) Brønsted, J. N. *Recl. Trav. Chim. Pays-Bas* **1923,** *42,* 718; *J. Phys. Chem.* **1926,** *30,* 777.

(4) Lowry, T. M. *Trans. Faraday Soc.* **1924,** *20,* 13; *Chem. Ind. (London)* **1923,** 1048.

(5) Hund, F. *Z. Phys.* **1925,** *31,* 81; **1925,** *32,* 1.

(6) Hückel, E. *Z. Elektrochem.* **1928,** *34,* 546.

(7) Conway, B. E.; Bockris, J. O'M.; Linton, H. *J. Chem. Phys.* **1956,** *24,* 834.

(8) Eigen, M.; De Maeyer, L. *Proc. R. Soc. London, Ser. A* **1958,** *247,* 505.

(9) (a) Bethell, D. E.; Sheppard, N. *J. Chem. Phys.* **1953,** *21,* 1421. (b) Ferisso, C. C.; Hornig, D. F. *ibid.* **1955,** *23,* 1464. (c) Giguère, P. A. *Rev. Chim. Miner.* **1966,** *3,* 627, and references cited therein. (d) Fournier, M.; Rozière, J. *C.R. Acad. Sci., Ser. C* **1970,** *270,* 729.

(10) (a) Taylor, R. C.; Vidale, G. L. *J. Am. Chem. Soc.* **1956,** *78,* 5999. (b) Giguère, P. A.; Madec, C. *Chem. Phys. Lett.* **1976,** *37,* 569, and references cited therein.

(11) (a) Richards, R. E.; Smith, J. A. S. *Trans. Faraday Soc.* **1951,** *47,* 1261. (b) Kakiuchi, Y.; Shono, H.; Komatsu, H.; Kigoshi, K. *J. Chem. Phys.* **1951,** *47,* 1261. (c) Brookhart, M.; Levy, G. C.; Winstein, S. *J. Am. Chem. Soc.* **1967,** *89,* 1735. (d) Commeyras, A.; Olah, G. A. *ibid.* **1969,** *91,* 2929. (e) Gold, V.; Grant, J. L.; Morris, K. P. *J. Chem. Soc., Chem. Commun.* **1976,** 397, and references cited therein.

(12) (a) Volmer, M. *Justus Liebigs. Ann. Chem.* **1924,** *440,* 200. (b) Luzatti, V., *Acta Crystallogr.* **1953,** *6,* 157. (d) Yoon, Young Ku; Carpenter, G. B. *ibid.* **1959,** *12,* 17.

(13) (a) Kebarle, P.; Haynes, R. M.; Collins, J. G. *J. Am. Chem. Soc.* **1967,** *89,* 5753. (b) Castelman, A. W., Jr.; Tang, N. I.; Munkelwitz, H. R. *Science* **1971,** *173,* 1025.

(14) 28.48 ^{17}O atom % (Merck Sharp and Dohme Co.).

(15) (a) Mateescu, G. D.; Wilson, L. A., 14th ENC (Experimental NMR Conference), Boulder, Colo., 1973. (b) It should be emphasized that the coupling constant of 79.9 Hz found by us in H_2O dissolved in CCl_4 is practically the same as in the vapor phase.[16] This indicates that the narrowing of the ^{17}O lines is due either to a very short correlation time of water monomers or to formation of highly symmetric clusters. (c) Subsequently, even narrower (3.4 Hz) ^{17}O lines were recorded with aqueous sodium molybdate by Vold, R. R.; Vold, R. L. *J. Chem. Phys.* **1974,** *61,* 4360.

(16) Florin, A. E.; Alei, M., Jr. *J. Chem. Phys.* **1967,** *47,* 4268. See also Reuben, J.; Tzalmona, A.; Samuel, D. *Proc. Chem. Soc.* **1962,** 353. Meiboom, S. *J. Chem. Phys.* **1961,** *34,* 375.

(17) It follows that most of what might be considered a very high heat of hydration for the hydrogen ion should be regarded as heat of formation of H_3O^+; cf. Fajans, K. *Naturwissenschaften* **1921,** *9,* 729.

(18) We are presently attempting to obtain spectra at higher temperatures using SO_2 and SO_2ClF in sealed tubes.

(19) Olympia, P. L., Jr.; Fung, B. M. *Chem. Phys. Lett.* **1970,** *6,* 87.

(20) We extend here to the O-H bond the relation between J_{C-H} and both the s character of the carbon atom hybridization and the C-H bond length, found by Muller, N.; Pritchard, D. *J. Chem. Phys.* **1959,** *31,* 768; *31,* 1471. Thus, in the relation $J_{O-H} = J_0 a^2 b^2$, we established that $J_0 = 1.278 \times 10^3\ s^{-1}$. In a simpler way, % s character = $J_{O-H}/3.2$; see also Shoolery, J. N. *ibid.* **1959,** *31,* 1427.

(21) Cherry, W.; Epiotis, N.; Borden, W. T. *Acc. Chem. Res.* **1977,** *10,* 167.

(22) (a) Newton, M. O.; Ehrenson, S. *J. Am. Chem. Soc.* **1971,** *93,* 4971, and references cited therein. (b) Grahn, R. *Ark. Fys.* **1961,** *19,* 147. (c) Whitman, D.; Simon, J., personal communication. (d) Pople, J. A., personal communication.

(23) (a) DePaz, M.; Ehrenson, S.; Friedman, L. *J. Chem. Phys.* **1970,** *52,* 3362. (b) Klopman, G., personal communication.

(24) The sign of J_{O-H} is assumed to be negative; cf. Jenkins, W. B., McFarlane, W. *J. Chem. Soc., Chem. Commun.* **1977,** 922.

(25) ^{17}O-Enriched acetone was prepared by acid-catalyzed exchange with $H_2{}^{17}O$. Protonation was obtained as that for H_3O^+.

Gheorghe D. Mateescu,* George M. Benedikt
Department of Chemistry, Case Western Reserve University
Cleveland, Ohio 44106

Received February 24, 1979