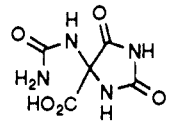
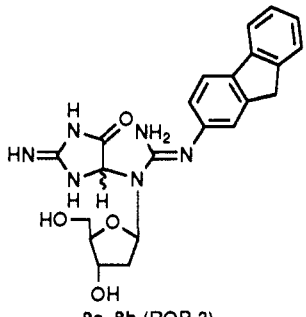
6a and **6b** are quite stable to basic reaction conditions.

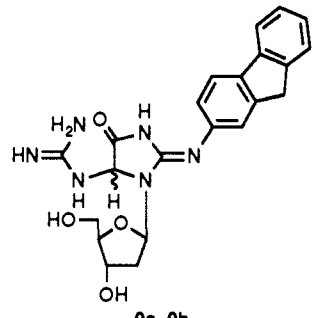


7

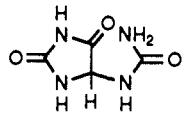
The third compound (ROP-3) isolated by HPLC, although transient at 75 °C, becomes the major product (~60% yield) in 1 N NaOH at 10 °C. This, by ^{1}H NMR spectroscopy, appears to be a 4:1 mixture of inseparable isomers of **8a** and **8b**, although structures **9a** and **9b** cannot be completely excluded. The epimeric hydrogen atoms at C-5 of the imidazolone ring appear as non-exchangeable (D_2O) singlets at δ 5.44 and 5.48 respectively. The positive- and negative-ion FAB-MS again show good correlation, exhibiting parent ions at m/z 437 and 435 respectively, thus identifying the molecular weights as 436 Da. Significantly there is a positive-ion peak at m/z 340 (negative-ion peak at m/z 338) corresponding to the loss of the imidazolone ring. Subsequent loss of the sugar residue to give the fluorenylguanidine ion is indicated by peaks at m/z 207 (positive ion) and 205 (negative ion). The structure postulated for ROP-3 is analogous to allantoin (**10**), again a well-established oxidative degradation product of uric acid.[7,8]



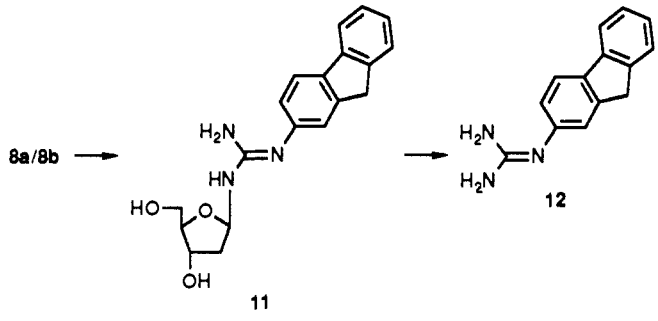
8a, 8b (ROP-3)



9a, 9b



10



11

The further action of base on ROP-3 causes a rapid conversion at pH 13 to *N*-(2-fluorenyl)guanidine (**12**) identical with a synthetic sample.[12] At neutral pH, however, the dominant product becomes the deoxyribofuranoside intermediate **11**. We have also found that **11** can be obtained from **4b** directly by allowing the latter to stand in aqueous buffer at neutral pH (half-life of **4b**: 6.9 days).

Finally the oxidative pathway that leads to the destruction of **4a**/**4b** provides a complete mechanistic explanation for the strand scission and depurination observed by Johnson et al.[13] when dG-(C8)AF- or dG(C8)AAF-modified oligomers were treated with 1 M piperidine at 90 °C. It now appears that an *abasic site* is therefore an intermediate in this strand scission process.

Given the sensitivity to aerial oxidation of dG(C8)AF (**4b**), it seems highly likely that many of the related analogues derived from different carcinogenic amines will be equally susceptible to oxidative degradation. *These findings may have significant implications for the mutagenic profile of* ***4a*** (and for dG(C8) adducts derived from other carcinogenic amines) when present as a residue in DNA. Further studies in this area are being pursued.

Acknowledgment. We thank Mr. Robert Rieger for his expert assistance in obtaining the mass spectral data. We are pleased also to acknowledge the interest and helpful discussions provided by Drs. A. P. Grollman and M. Takeshita. This research was supported by a grant (No ES04068) from the National Institutes of Health, National Institute of Environmental Health Sciences.

Supplementary Material Available: Schemes depicting the synthesis of **13**, **6a,b**, **9a,b**, and **12** and the base-catalyzed equilibration of **8** and **9** (3 pages). Ordering information is given on any current masthead page.

(12) The isolation of *N*-(2-fluorenyl)guanidine as the end product of the degradation of **4a** by alkali in air confirms completely that an oxidative mechanism is involved; otherwise the end product should have been the corresponding fluorenylurea, no trace of which could be discerned in the reaction mixture.

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Reactions of Dimethylamine with Multiply Charged Ions of Cytochrome *c*†

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It has recently been demonstrated, first by Fenn and co-workers[1] and subsequently by other groups,[2,3] that multiply charged ions from high-mass molecules can be formed from electrospray ionization. It is particularly noteworthy that proteins and peptides show a strong tendency for multiple cationization. Thus far, all of the proteins studied are characterized by multiple cationization to the extent that the observed mass/charge ratio is less than 3000. Therefore, these unusual ions, despite masses sometimes in excess of 10^5 daltons (Da),[2c] fall within the mass/charge range accessible to many modern mass spectrometers. We have recently coupled electrospray with a three-dimensional quadrupole[4] (i.e., a Paul trap[5]). This type of mass spectrometer is particularly well-suited for kinetic studies due to its ion-trapping and ion-isolation capabilities.[6] We describe here results of the first systematic study

† Research sponsored by the U.S. Department of Energy, Office of Energy Research, under Contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

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(4) A 4 × 10^{-6} M solution of cytochrome *c* was prepared in a solvent mixture of HPLC grade water, methanol, and glacial acetic acid in relative proportions of 20%, 75%, and 5% by volume, respectively. This solution was passed at a flow rate of 1.0 μL/min through a 120 μm i.d. stainless steel capillary needle held at a potential of +3.5 kV. The outlet of the needle was positioned about 1 cm from a 100-μm inlet aperture into the mass spectrometer. For details of this system, see: Van Berkel, G. J.; Glish, G. L.; McLuckey, S. A. *Anal. Chem.*, in press.

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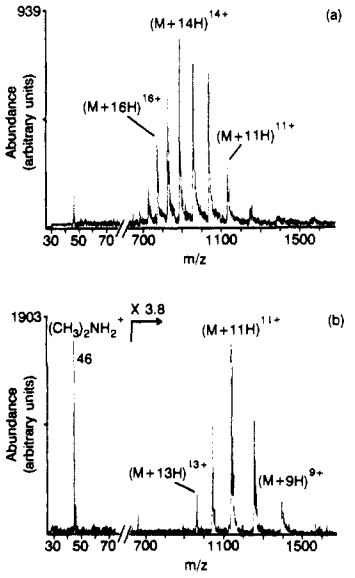

Figure 1. Electrospray ionization mass spectra of horse heart cytochrome *c* acquired by using a three-dimensional quadrupole analyzer. Dimethylamine was present in the analyzer at a pressure of 1.2×10^{-6} Torr, and ions were trapped for (a) 0.02 s and (b) 1.06 s, respectively, prior to acquisition of the mass spectra.

of the near-thermal-energy ion/molecule reactions[7] of a multiply protonated protein. These studies were carried out with a neutral reactant, dimethylamine (proton affinity = 223 kcal/mol[8]), that has proven to be a "selective" base.

Figure 1a shows the electrospray ionization mass spectrum of horse heart cytochrome *c* (MW = 12360 Da) acquired with the quadrupole ion trap at a background pressure of dimethylamine of 1.2×10^{-6} Torr and a reaction time of 0.02 s. Figure 1b shows the spectrum acquired at the same dimethylamine pressure but with a reaction time of 1.06 s. A distribution of charge states is observed in Figure 1a that ranges from +12 to +17. This distribution is largely reduced to the +12 to +10 charge states in Figure 1b by proton-transfer reactions with dimethylamine. The signal due to protonated dimethylamine (see low *m/z* region of Figure 1) is also seen to increase, as expected, in concert with the shift in charge of the peptide cations to the lower charge states. The rate constant associated with the depletion of the ions of each charge state by proton transfer to dimethylamine was measured by isolating ions of the charge state of interest and monitoring the rate of loss of the ions of as a function of time.[9] The measured rate constants are listed in Table I.

Table I

reaction[a]	rate constant, cm^3 molecule^{-1} s^{-1}
$(M + 15H)^{15+} + (CH_3)_2NH \rightarrow (M + 14H)^{14+} + (CH_3)_2NH_2^+$	9.3×10^{-10}
$(M + 14H)^{14+} + (CH_3)_2NH \rightarrow (M + 13H)^{13+} + (CH_3)_2NH_2^+$	8.5×10^{-10}
$(M + 13H)^{13+} + (CH_3)_2NH \rightarrow (M + 12H)^{12+} + (CH_3)_2NH_2^+$	6.3×10^{-10}
$(M + 12H)^{12+} + (CH_3)_2NH \rightarrow (M + 11H)^{11+} + (CH_3)_2NH_2^+$	3.4×10^{-10}
$(M + 11H)^{11+} + (CH_3)_2NH \rightarrow (M + 10H)^{10+} + (CH_3)_2NH_2^+$	1.9×10^{-10}
$(M + 10H)^{10+} + (CH_3)_2NH \rightarrow (M + 9H)^{9+} + (CH_3)_2NH_2^+$	8.5×10^{-11}
$(M + 9H)^{9+} + (CH_3)_2NH \rightarrow (M + 8H)^{8+} + (CH_3)_2NH_2^+$	$<2 \times 10^{-12}$
collision rate constant[b]	$\approx 4 \times 10^{-8}$

[a] Reference 11. [b] Reference 12.

Each rate constant may reflect the reactivity of a mixture of ion structures (i.e., several proton distributions on the molecule may give a stable ion), and the relative contribution of each structure may change during the trapping period due both to intramolecular proton transfer and to different reactivity with dimethylamine. Furthermore, the lower charge states (+11 to +9) show mass shifts which indicate the formation of proton-bound adducts with dimethylamine.[11] We do not see significant adduct formation for the higher charge states. Similar observations have been obtained for multiply protonated myoglobin (horse skeletal muscle).[13]

It is noteworthy that the multiply protonated cytochrome *c* ions are not more extensively deprotonated, even at higher dimethylamine pressures and longer reaction times than were used to acquire the spectrum of Figure 1b. The trend in rate constants probably reflects both the number of weakly basic sites that are protonated in the respective ion populations and the relative basicities of those sites. For example, the $[M + 15H]^{15+}$ population is likely to contain more ions protonated at weakly basic sites than, say, the $[M + 11H]^{11+}$ ion population. Furthermore, the basicity of a given site in the molecule is affected by the Coulombic potential arising from protons attached to other parts of the molecule.[1d] As each proton is removed, all of the remaining protons are held more strongly as the basicity of each site more closely approaches its intrinsic basicity. The reactivities of the lower charge states are also reduced by stabilization from the solvation by dimethylamine. Proton-transfer experiments of this type, involving small peptides with bases of various strengths, can provide information on the degree to which the intrinsic basicity of a residue, or pair of adjacent residues, is affected by the presence of a proton or protons on other parts of the molecule. This kind of information may prove useful in determining the most stable ion structures for a highly charged protein.

The fact that multiply protonated species can be partially deprotonated by using a basic reagent may also prove useful for peptide structure determination. For example, concentrating charge into fewer ions is particularly useful when further stages

(6) For examples of kinetic studies using instrumentation similar to that employed here, see: (a) Brodbelt-Lustig, J. S.; Cooks, R. G. *Talanta* **1989**, *36*, 255. (b) McLuckey, S. A.; Glish, G. L.; Asano, K. G.; Van Berkel, G. J. *Anal. Chem.* **1988**, *60*, 2312. (c) Flurer, R. A.; Glish, G. L.; McLuckey, S. A. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 217. (d) Eckenrode, B. A.; Glish, G. L.; McLuckey, S. A. *Int. J. Mass Spectrom. Ion Processes*, in press.

(7) For discussions of ion "temperatures" in a Paul trap, see: (a) Nourse, B. D.; Kenttamaa, H. I. *J. Phys. Chem.*, in press. (b) Vedel, F.; Andre, J. *Phys. Rev. A* **1984**, *29*, 2098. (c) Vedel, F.; Andre, J. *Int. J. Mass Spectrom. Ion Processes* **1985**, *65*, 1. (d) Alili, A.; Andre, J.; Vedel, F. *Phys. Sci.* **1988**, *T22*, 325.

(8) Based on the proton affinity of ammonia = 207 kcal/mol. Hartman, K. N.; Lias, S.; Ausloos, P.; Rosenstock, H. M.; Schroyer, S. S.; Schmidt, C.; Martinsen, D.; Milne, G. W. A. "A compendium of Gas Phase Basicity and Proton Affinity Measurements"; NBSIR 79-1777, July 1979.

(9) The rate constant for ions of each charge state, *n*, was determined from the slope of the plot of $-\ln [M + nH]^{n+}_t/[M + nH]^{n+}_0$ versus the product of the dimethylamine number density and the reaction time, *t*. The plot was constructed from a series of experiments in which the reaction time was varied systematically. To correct for variations in the number of ions initially admitted into the ion trap between each experiment, $[M + nH]^{n+}_0$ for each reaction time was taken as the total signal due to multiply charged ions observed in the spectrum at the end of each reaction time. Dimethylamine was present in the vacuum system at a constant pressure ($(1.2 \pm 0.1) \times 10^{-6}$ Torr) for all studies. Pressure was measured by an ionization gauge attached to the vacuum housing of the ion trap and was corrected for the ion-gauge sensitivity of dimethylamine.[10] The reaction time, which typically was extended to 300 ms, was determined to within 1 ms.

(10) Bartmess, J. E.; Georgiadis, R. M. *Vacuum* **1983**, *33*, 149.

(11) The reactions as listed are intended to represent all of the ions with the respective charges. For the +15 to +12 charge states, no significant numbers of dimethylamine adduct species are observed at the beginning of the kinetics experiment. However, shifts to higher mass within the charge state become increasingly important as the charge state decreases. These shifts are interpreted as arising from the formation of proton-bound dimethylamine adducts. The measured rate constant, therefore, reflects the reactivity of the ionic mixture at these charge states. The observed mass/charge shifts for the peak maxima for the +11, +10, and +9 charge states at the beginning of the kinetics experiment indicate the presence of one, two to three, and three molecules, respectively, of dimethylamine adducted to the multiply protonated molecule.

(12) The collision rate constant is estimated by using an assumed collision cross section of 10^{-12} cm^2. See: Smith, R. D.; Barinaga, C. J.; Udseth, H. R. *J. Phys. Chem.* **1989**, *93*, 5019.

(13) McLuckey, S. A.; Van Berkel, G. J.; Glish, G. L., Oak Ridge National Laboratory, 1990, unpublished results.

of mass spectrometry are performed. Ion structure determination using mass spectrometry/mass spectrometry[14] can benefit both by providing a larger number of parent ions in a given charge state and by providing parent ions wherein the sites of protonation can be assigned with greater confidence. The latter information may prove to be useful in extracting structural information from the daughter ion spectra of multiply protonated molecules.

Acknowledgment. This research was sponsored by the U.S. Department of Energy, Office of Energy Research, Basic Energy Sciences, under Contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

(14) (a) McLafferty, F. W., Ed. *Tandem Mass Spectrometry*; Wiley and Sons: New York, 1983. (b) Busch, K. L.; Glish, G. L.; McLuckey, S. A. *Mass Spectrometry/Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry*; VCH Publishers: New York, 1988.

Synthesis of $(PMe_3)_4Ru(Me)(OC(CH_2)Me)$ as an Equilibrium Mixture of Oxygen- and Carbon-Bound Transition-Metal Enolates. Thermal Elimination of Methane To Form an η^4-Oxatrimethylenemethane Complex

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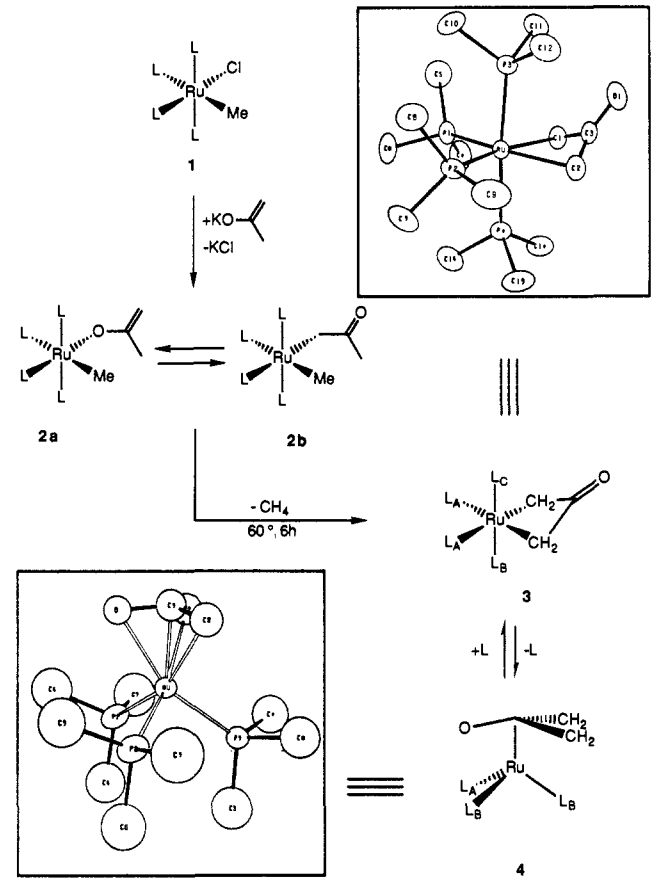
Transition-metal enolate[1] and trimethylenemethane complexes[2] have been used extensively in metal-mediated organic synthesis.[3] Many early-transition-metal enolate complexes of the O-bound form and late-transition-metal complexes of the C-bound form have been synthesized and used either stoichiometrically[1b,4] or catalytically[1a,5] in aldol reactions. Palladium complexes of trimethylenemethane have been generated in solution and used catalytically in 2 + 3 cycloaddition reactions,[2a,b] and other trimethylenemethane complexes have been isolated and structurally characterized.[2c-h] We report the synthesis of a ruthenium enolate that exists in both the C- and O-bound forms, the first direct observation of such a mixture. We also report the reaction of this equilibrium mixture to form a metallacyclobutan-3-one and its subsequent reaction to form the first isolated mononuclear oxatrimethylenemethane transition-metal complex.

Scheme I



The chemistry we have observed is summarized in Scheme I. Treatment of $(PMe_3)_4Ru(Me)Cl$ (**1**)[6] with the potassium enolate of acetone[7a] for 4 h at room temperature in toluene cleanly formed the methyl enolate complex as a 70:30 mixture (C_6D_6) of the O-bound $(PMe_3)_4Ru(Me)(OC(CH_2)Me)$ (**2a**) and C-bound $(PMe_3)_4Ru(Me)(CH_2C(O)Me)$ (**2b**) forms. This mixture was isolated in 37% yield after crystallization from ether and was characterized by conventional spectroscopic techniques, as well as microanalysis.[7b] The ^1H NMR spectrum showed two metal-bound methyl groups at δ 0.40 and −0.26 and two enolate methyl groups at δ 2.06 and 2.00. The inequivalent methylene resonances for the O-bound form appeared at δ 3.50 and 3.96, while the equivalent methylene resonances for the C-bound form appeared as a multiplet at δ 1.90. The methylene of the O-bound form was observed in the $^{13}C\{^1H\}$ NMR spectrum (DEPT) as a singlet at δ 75.68, and the CH_2 of the C-bound form appeared as a multiplet at δ 22.83.

Variable-temperature ^1H NMR spectroscopy of the isomeric mixture in THF-d_8 revealed that the O- and C-bound forms exist in equilibrium. Over the temperature range of 5–60 °C, a significant and *reversible* change in the ratio of isomers was observed. At 5 °C the ratio of O- to C-bound isomers was (2.8 ± 0.2):1, and at 60 °C the ratio was (4.4 ± 0.3):1. Cooling the sample again to 5 °C provided the same 2.8:1 mixture after 15 min. A plot of ln K vs $1/T$, containing six points in the 5–60 °C temperature range, demonstrated that in this solvent ΔH (1.5 ± 0.3 kcal/mol) is positive and favors the C-bound form, while ΔS (7.6

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(3) Davies, S. G. *Organotransition Metal Chemistry: Applications to Organic Synthesis*; Pergamon Press: New York, 1982.

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(5) Ito, Y.; Sawamura, M.; Hayashi, T. *J. Am. Chem. Soc.* **1986**, *108*, 6408.

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(7) (a) The addition of acetone to a stirred solution of $KN(SiMe_3)_2$ at room temperature in toluene/pentane, 1:100, formed $KOC(CH_2)Me$ as a white precipitate, which was filtered and stored at −40 °C as a solid for up to 1 week. (b) The elemental analyses and NMR spectral data of the ruthenium complexes are available as supplementary material.

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