The above observations are consistent with the mechanism shown in Scheme I.[16] A key feature of this mechanism is the intermediacy of oxidized species **3** in which the C–H bond is activated for deprotonation by an agostic interaction. The dependence on L can be rationalized by considering the conditions necessary for an agostic interaction; specifically, an unfilled metal orbital directed at the C–H bond is needed, which in this case is the d_{z^2} orbital. Although almost all octahedral Co(III) (d^6) complexes are diamagnetic (low spin), and thus the d_{z^2} orbital is normally assumed to be empty, there are paramagnetic square-pyramidal pentacoordinate Co(III) complexes,[7c,18] with weak axial ligands such as Cl^-, Br^-, or I^- where presumably the d_{xy} and d_{z^2} orbitals contain a single electron each. If this were the case in $[Co^{III}(dacoda)L]^+$, where L is a weak field ligand, the d_{z^2} orbital would be occupied and there would be no agostic interaction to promote deprotonation of the C–H bond. The nature of L would also affect the redox potential of the oxidation depicted in Scheme I, with stronger field ligands increasing the oxidation potential. The loss of L in the last step of Scheme I is consistent with the strong trans influence of alkyl ligands (which is evidenced here by the long Co–OH_2 bond, 2.15 Å in **5**).

The overall reaction shown in Scheme I represents an electrophilic attack of low-spin Co(III) on the C–H bond of the coordinated dacoda ligand. There is precedence for electrophilic attack on aliphatic C–H centers by Co(III) species, as exemplified by the $Co(OAc)_3$ and $Co(OAc)_2/O_2$ systems[3b,19] which oxidize alkanes (although organometallic intermediates have not been isolated in this case). In addition, a number of metal complexes that are capable of C–H activation by electrophilic pathways have recently been described.[3b,20-24]

The results presented here further illustrate the importance of agostic interactions in promoting C–H reactivity.[2a,3b] This system demonstrates that the essential requirements of the agostic interaction, heretofore satisfied only by organometallic-type systems, can be met in an (aqueous) Werner-type complex with similar results in reactivity. Thus, activation of C–H bonds in a biological system in a similar fashion would appear to be plausible.

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Supplementary Material Available: Details of the X-ray structure solutions for **5**, with listings of coordinates, thermal parameters, bond distances, and bond angles (5 pages); table structure factors for **5** (8 pages). Ordering information is given on any current masthead page.

(15) The structure of $K[Co^{III}dacoda(SO_3)]\cdot 5H_2O$ has been refined to an unweighted *R* value of 0.051. Some disorder exists in the waters of crystallization. The Co–"agostic" H and C distances are 2.26 (5) and 2.524 (4) Å, respectively, while the Co–H–C angle is 93 (3)°. The "agostic" C–H distance is 0.98 (5) Å. Full details will be described elsewhere.

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Jack Bean Urease (EC 3.5.1.5). 8. On the Inhibition of Urease by Amides and Esters of Phosphoric Acid

Robert K. Andrews, Annette Dexter, Robert L. Blakeley,* and Burt Zerner*

Department of Biochemistry, University of Queensland St. Lucia, Queensland, Australia 4067

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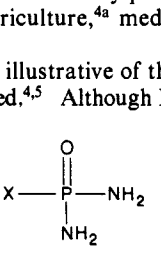
Our idea that phosphoramidate (**1**, $H_3N^+-PO_3^{2-}$) might be a substrate for urease led to the discovery in 1975 that it inhibits urease by virtue of stoichiometric coordination to active-site nickel ion.[1,2] Further, a large fraction of **1** is released intact upon reactivation of the inhibited enzyme under appropriate conditions.[3] Since that time, the inhibition of urease by amides and esters of phosphoric acid has been extensively pursued because of its potential application in agriculture,[4a] medicine,[4b] and veterinary science.[4c]

General structure **2** is illustrative of the range of compounds which has been investigated.[4,5] Although Kobashi and co-workers



2a, X = ArO
b, X = HO
c, X = H_2N
d, X = AlkC(O)NH
e, X = ArC(O)NH

have reported that *N*-acylphosphoric triamides are degraded by urease to the corresponding carboxamide, ammonia, and inorganic phosphate,[6] no details of this work have been published and the chemistry of the urease inhibition has remained obscure.

We now adduce evidence that diamidophosphate [**2b**, $(H_2N)_2PO_2^-$] is the inhibitory species and that its interaction with the active site of urease must involve two isomeric complexes with active-site nickel ion. Moreover, we establish that phosphoramidate is a very poor substrate for the enzyme.

Phenyl phosphoramidate (**2a**, Ar = Ph, PPD), *N*-(3-methyl-2-butenyl)phosphoric triamide [**2d**, Alk = $(H_3C)_2C{=}CH$, MBPT], and phosphoric triamide (**2c**) each stoichiometrically inactivates urease, since the residual specific activity after reaction with excess urease in 0.05 M *N*-ethylmorpholinium chloride (NEM) buffer at pH 7.0 is within 5% or better of the predicted value $[(1 - [I]_0/[E]_0) \times$ initial specific activity]. The inactivation is rapid,[7] and the inactive enzyme slowly regains full activity.

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Table I. Reactivation of Inhibited Urease at 38 °C

inhibitor	$10^4 k_{react}$, s^{-1}
PPD[a]	0.36 ± 0.03
MBPT[a]	0.34 ± 0.04
2c[a]	0.36 ± 0.03
2b (1:1 complex)[b]	7 ± 1
1 (1:1 complex)[c]	8.2 ± 0.5

[a] In oxygen-free NEM buffer (pH 7.0; 1 mM in EDTA). [b] In oxygen-free NEM buffer, pH 7.01. [c] In oxygen-free NEM buffer (pH 7.11; 1 mM in EDTA, 5 mM in 2-mercaptoethanol, 0.1 M in KCl).[3]

Relevant first-order rate constants for reactivation are included in Table I, about which the following points are made: (i) the carboxamide from MBPT is released, while the inactivated urease contains intact **2b**; (ii) PPD and **2c** are initially hydrolyzed to diamidophosphate; (iii) the consonance of the first three rate constants in Table I identifies a diamidophosphate–nickel complex as the species responsible for inhibition; (iv) a different, significantly more labile diamidophosphate–nickel complex is formed when the inhibitor is not enzymatically derived from a substrate; (v) the consonance of the last two rate constants strongly suggests that **2b** is itself a substrate for the enzyme. The isomeric diamidophosphate–nickel complexes presumably differ in strength by virtue of N- vs. O-coordination to the metal ion and constitute the first such enzymatic data.

These results may be compared with the *N*-hydroxyurea inhibition of the enzyme where isomeric *primary substrate–nickel complexes* are responsible for inhibition and substrate activity, respectively.[8]

We earlier reported that approximately 50% of the phosphoramidate is released intact after reactivation of the isolated phosphoramidate–urease complex at 38 °C for 50 min but cautioned that catalysis of phosphoramidate decomposition by the tertiary amine buffer[9] or protein side chains may not be sufficient to account for the kinetics observed. Further work under conditions where the nonenzymatic decomposition of **1** is not catalyzed by buffer salts or other additives clearly demonstrates that **1** is itself a very poor substrate for urease. At 38 °C, $k_{cat}/K_m = 8 \pm 2$ M^{-1} s^{-1} ($[\mathbf{1}]_0 = 8.4$ mM), and this value may be compared with those for other substrates which range from 2.0×10^6 M^{-1} s^{-1} for urea to 0.34 M^{-1} s^{-1} for *N*-methylurea.[10] That this is a genuine substrate activity has been confirmed by its abolition after preequilibration of the enzyme with 0.9 mM acetohydroxamic acid.

Compounds **1**,[11] **2b**,[12] and **2c**[13] were prepared by published procedures. The value of k_{obsd} for the spontaneous hydrolysis of **1** (1.6 mM) in 0.3 M NEM (pH 7.0) at 38 °C was 6.5×10^{-5} s^{-1} (cf. 6.6×10^{-5} s^{-1} in water[14] at pH 7.0 and 36.8 °C). The concentration of **1** (elution time, 5.5 min) and/or of ammonia (elution time, 15 min) was measured in a single assay with an LKB Alpha-Plus amino acid analyzer using 0.2 M borate (pH 9.7, 0.05 M in NaCl) at 40.9 mL/h. Protein was removed from enzymatic reactions with a Centricon 30 microconcentrator (Amicon). Other enzymatic experiments used procedures similar to those previously reported.[1,3]

We have investigated the reaction of these inhibitors with urease from *jack beans* for the following reasons: (1) This nickel(II) metalloenzyme is readily available in high purity,[2] and the concentration of its active sites is easily measured. (2) Its reversible inhibition by 2-mercaptoethanol, fluoride, and hydroxamic acids involves direct coordination to active-site nickel ion.[2] (3) Nickel nutrition and/or inhibition studies strongly indicate that the active site of urease is essentially the same regardless of its source (mycoplasma, bacteria, fungi, algae, higher plants, invertebrates, and soil).[2c] The findings of this research can therefore be expected to be of general validity.

All aspects of this work continue under active investigation.

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(7) With PPD at 1.86×10^{-5} M and urease at 2.0×10^{-5} N, inhibition was virtually complete after 1 min at pH 7.0 and 38.0 °C. A second-order rate constant of $(4 \pm 0.5) \times 10^4$ M^{-1} s^{-1} was measured for the reaction between urease and MBPT at pH 7.0 and 38.0 °C, while the release of ammonia from **2c** (2.47×10^{-5} M) in the presence of urease (2.68×10^{-5} N) was complete within the time of mixing at pH 7.0 and 25.0 °C.

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Generation and Trapping of Alkynolates from Alkynyl Tosylates: Formation of Siloxyalkynes and Ketenes

Peter J. Stang* and Kenneth A. Roberts

Chemistry Department, The University of Utah
Salt Lake City, Utah 84112

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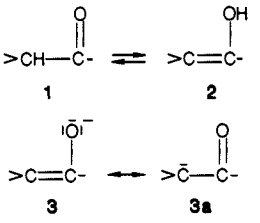
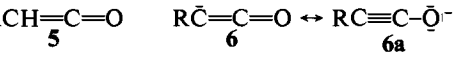
Alcohols and alkoxides are well-known organic compounds and reagents. Likewise, carbonyls, **1**, and their enols, **2**, are well-known and important species.[1] Yet despite the importance and wide spread use of enolates, **3**, simple enols, **2**, have only recently been prepared and fully characterized.[2]



With the exception of the recent observation[3] of the parent hydroxyacetylene, HC≡COH, by tandem mass spectrometry in the gas phase, hydroxyalkynes **4** are not known and outside of some theoretical calculations[4] little is known about the alkynol ketene **4–5** tautomerism of alkynolates, **6**, and their O-trapping in particular.



Alkynolates **6** have been generated previously by Rathke et al. via ketene **7**, by Hoppe and Schöllkopf[6] via isoxazoles **10**, and by Kowalski and co-workers[7] by rearrangement of α-keto dianions **14**, as shown in Scheme I.

However, in all previous cases trapping with a variety of electrophiles, including silicon in the case of **8** and **11**, gave only carbon-silylated (alkylated) ketenes **9** and **12** or ketene-derived products **16**.

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