Table I. Comparative Gas Phase Acidities and Estimates (kcal/mol) of Bond Dissociation Energies and Electron Affinities

	Bond energies	Electron affinities[a]
	Lower Acidity than HO_2	
Phenol	$[C_6H_5O-H] = 88.3$[b]	$[C_6H_5O]$ 55[c]
	Higher Acidity than HO_2 but Lower than HCl	
Benzoic acid	$[C_6H_5COO-H] = 110$[d]	$75 < [C_6H_5COO] < 90$
Acetic acid	$[CH_3COO-H] = 110$[d]	$75 < [CH_3COO] < 90$[e]
	Higher Acidity than HO_2 and HCl but Lower than HBr	
Malononitrile	$[(CH)_2CH-H] = 80$[f]	$60 < [(CN)_2CH] < 70$
p-Nitrophenol	$[NO_2C_6H_4O-H] = 88.3$[b]	$68 < [NO_2C_6H_4O] < 78$
2,4,6-Trinitrotoluene	$[(NO_2)_3C_6H_2CH_2-H] = 85$[g]	$65 < [(NO_2)C_6H_2CH_2] < 75$
	Higher Acidity than HO_2, HCl, HBr, and HI	
Picric acid	$[(NO_2)_3C_6H_2O-H] = 88.3$[b]	$88.3 < [(NO_2)_3C_6H_2O]$

[a] The limits of the electron affinities were estimated using D(H-X) and EA(X) for X = Cl, Br, and I from R. S. Berry and C. W. Riemann, *J. Chem. Phys.*, **38**, 1540 (1963). [b] Accurate values for phenol and several substituted phenols were recently determined (Lee R. Mahoney, private communication) to be 84–90 kcal/mol. These results indicate that the bond energies of phenol, *p*-nitrophenol, and picric acid will not be identical. However, the same value (88.3 kcal/mol) is used here in order to define the order of electron affinities. [c] Determined to be 55.0 kcal/mol, from recent studies of $D(H - A) - EA(A)$[8] and bond energy (L. R. Mahoney, private communication). [d] Accurate values for aromatic and aliphatic carboxylic acids are not available. The same value of 110 kcal/mol was used for both types of acids, from ref 5a. [e] Now known to be 79 kcal/mol.[1] [f] Estimated on basis of $D(Cl_2CH-H) = 80$ kcal/mol from ref 5a. [g] Estimated on the basis of $D(C_6H_5CH_2-H) = 85$ kcal/mol, from R. Walsh, D. Golden, and S. Benson, *J. Amer. Chem. Soc.*, **88**, 650 (1966).

The gas phase acidity of an acid, HA, will be higher the smaller the heterolytic dissociation energy, $HA = H^+ + A^-$. Therefore, acidity increases as the difference between the bond dissociation energies and electron affinities, $D(H-A) - EA(A)$, decreases. The $D(H-A) - EA(A)$ for HO_2 is estimated on the basis of $D(H-O_2) = 47$ kcal/mol[5] and $EA(O_2) = 11.4$ kcal/mol[6] to be 35.6 kcal/mol. This can be compared with the corresponding value for CH_3COOH which is 31.8 kcal/mol.[1] The fact that acetic acid is a stronger gas phase acid than HO_2 was verified experimentally by the formation of acetate ions in the API source through reaction with O_2^-.

Compounds whose acidities were examined are listed in Table I. The acidities of these compounds, with the exception of acetic acid, have not been reported previously. The H–A bond energies, and the estimated limits which must hold for $EA(A)$ values, are also in Table I. All experiments were carried out at atmospheric pressure with a source temperature of 200°, with air as the carrier gas, and with low concentrations (picogram samples) of acids to avoid cluster ion formation.

The strongest gas phase acid found in this study was picric acid (2,4,6-trinitrophenol). This was ionized by O_2^-, Cl^-, Br^-, and I^-. *p*-Nitrophenol also ionized readily; both O_2^- and Cl^- led to phenolate ion formation. The relatively high acidity of malononitrile (ionized by O_2^- and Cl^-) was not unexpected; carbanion formation through gas phase ionization was encountered earlier for *N*,*N*′-dimethylbarbituric acid.[7] 2,4,6-Trinitrotoluene was also found to be a strong gas phase acid (ionized by O_2^- and Cl^-). The acidity of hydrogen atoms attached to a benzylic carbon atom was noted by Brauman and Blair[2a] in work with toluene. The acidity is greatly increased in this instance by nitro groups in the ortho and para positions.

Benzoic and acetic acids are stronger acids than HO_2 in the gas phase, so that the electron affinities of the corresponding radicals are above 75 kcal/mol (based on an estimate of 110 kcal/mol for the dissociation energy of the RCOO–H bond). The value found by Yamdagni and Kebarle[1] for the acetate radical was 79 kcal/mol.

Phenol was not ionized by O_2^-. The recently measured value of $D(H-A) - EA(A)$ for phenol is 33.3 kcal/mol.[8] Since the corresponding value for HO_2 is 35.6 kcal/mol, phenol was expected to be a stronger acid than HO_2. This observation indicates that the actual value for $D(H-O_2) - EA(O_2)$ is probably a few kcalories per mole lower than that estimated from available data.[5,6] In a previous study[1] ordinary aliphatic and aromatic carboxylic acids were ionized by F^- ions. The use of O_2^- instead of F^- has the advantage that its production does not require the use of highly corrosive gases.

Acknowledgment. This work was aided by Grants 13901 and 16216 of the National Institute of General Medical Sciences, Grant 05435 of the National Heart and Lung Institute, and Grant Q-125 of the Robert A. Welch Foundation.

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Crystallographic Studies on Manganese Hemoglobin

Sir:

The role of the heme iron in hemoglobin may be examined through the effects on structure and function of replacement of the iron by other metals. The functional properties of coboglobin (CoHb),[1] in which

(1) Abbreviations: CoHb, cobaltohemoglobin; MnHb, manganohemoglobin; $Mn^{III}Hb$, manganihemoglobin; Hb, ferrohemoglobin; $Fe^{III}Hb$, ferrihemoglobin.

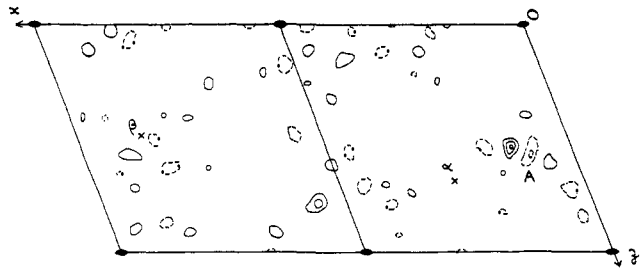

Figure 1. Difference Fourier projection of $Mn^{III}Hb$–$Fe^{III}Hb$, plotted from 0–$\frac{1}{2}$ in x and 0–$\frac{1}{2}$ in z. Solid lines denote positive contours; dashed lines denote negative. The contour interval is one standard deviation. The zero contour and the first positive and negative contours have been omitted for clarity. The points marked α and β are the projected positions of the iron atoms in the α and β chains of $Fe^{III}Hb$, respectively. The group of positive and negative features denoted A probably arise from differences in protein conformation near the sulfhydryl group of cys (93)β. The conformation of this region appears to be closely linked to the state of ligation of the hemoglobin and the conformation of the heme itself.[10]

cobalt replaces iron, have been extensively studied.[2] Oxygen binding to CoHb is accompanied by allosteric effects which are very similar to those of native hemoglobin. Thus, such effects are not dependent on properties exclusive to iron. However, the ability to bind oxygen reversibly is only exhibited by CoHb initially prepared with the cobalt in the divalent state, Co^{II}. Oxidation to $Co^{III}Hb$ leads irreversibly to a partially denatured state[3] in which both the proximal and distal histidines are covalently bonded to the metal, an internal hemichrome.[4] The redox properties of CoHb[2c] are therefore not directly comparable to those of native hemoglobin.

Recently, manganese hemoglobin (MnHb) has been prepared, and its functional properties have been investigated.[5] MnHb also exhibits allosteric effects, as shown by the change in proton affinity upon NO binding, kinetics of ligand binding by stopped-flow and flash photolysis, subunit dissociation, and redox properties. However, manganese hemoglobin is initially prepared as $Mn^{III}Hb$. In order to compare these redox and other functional properties with those of native hemoglobin, a structural characterization of $Mn^{III}Hb$ is essential.

We have prepared crystals of $Mn^{III}Hb$, and here report the first crystallographic studies on metal-substituted heme proteins. These show that $Mn^{III}Hb$ resembles $Fe^{III}Hb$ very closely, that it undergoes a similar change in quaternary structure on reduction to MnHb, and that manganese hemoglobin thus retains both the major functional[5] and structural properties of Hb.

$Mn^{III}Hb$ was prepared from horse hemoglobin as described previously.[5a] Crystallization was carried out according to the scheme of Perutz[6] originally devised for horse $Fe^{III}Hb$. The crystals of $Mn^{III}Hb$ grow within a gel-like matrix of amorphous material, which appears within a day or so of setting up the crystallization vials. $Mn^{III}Hb$ was found to be considerably more soluble than $Fe^{III}Hb$. The best crystals were grown from solutions about 2.4 *M* in Perutz's solution B,[6] though the dependence of crystal quality on salt concentration was not as marked as with $Fe^{III}Hb$. Any contaminating $Fe^{III}Hb$ would thus have been immediately precipitated at these salt concentrations; further, the $Mn^{III}Hb$ crystals were clearly distinguishable by their visible spectra from $Fe^{III}Hb$ crystals, grown in separate vials. Individual $Mn^{III}Hb$ crystals were mounted in capillaries in the usual way, and X-ray photographs were taken on a Supper precession camera, with Cu Kα radiation. The photographs were densitometered on an Optronix P1800 Photoscan interfaced to a Digital Equipment Corporation PDP-11 computer.

The crystals of $Mn^{III}Hb$ are isomorphous with those of $Fe^{III}Hb$, space group $C2$; the cell dimensions found ($Fe^{III}Hb$ values in parentheses) are 108.6 (108.9) × 63.5 (63.5) × 54.6 (54.9) Å; β = 110°50′ (110°50′). A difference Fourier projection was calculated at 2.8 Å resolution from the centrosymmetric *h*0*l* zone, using the diffraction amplitudes measured photographically on $Mn^{III}Hb$ and $Fe^{III}Hb$ crystals and the signs obtained for $Fe^{III}Hb$.[7] Such a difference Fourier gives directly the difference in electron density between $Mn^{III}Hb$ and $Fe^{III}Hb$, projected down the molecular twofold axis, and is very sensitive to small structural differences.[8]

The most striking aspect of the calculated projection (Figure 1) is the almost complete *absence* of significant features; $Mn^{III}Hb$ is thus a close structural analog of $Fe^{III}Hb$. In particular, partial denaturation to the internal hemichrome (which would require extensive structural changes around the heme groups) does not occur in $Mn^{III}Hb$, in contrast to $Co^{III}Hb$.

The sixth metal ligand in $Fe^{III}Hb$ is H_2O at neutral pH and OH^- at alkaline pH; the transition occurs with a $pK_a \sim 8$. However, electrophoretic and optical studies of $Mn^{III}Hb$ fail to show any such ionization,[5c] which is consistent with the suggestion[9] that Mn^{III} porphyrins prefer five-coordination, rather than binding H_2O or OH^- as a sixth ligand. The absence of significant negative features on the difference Fourier projection in the position of the ligands rules out the loss of the sixth ligand in $Mn^{III}Hb$. Thus the sixth ligand in $Mn^{III}Hb$ is H_2O at both neutral and alkaline pH. These results validate the method of preparation of MnHb and $Mn^{III}Hb$ and provide a firm structural basis for interpreting their redox and other functional properties.

Oxidation or ligation of the ferrous heme in Hb induces a motion of the iron atom relative to the mean porphyrin plane and a spin state change. It has been

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proposed[10] that these changes "trigger" the protein conformational changes associated with cooperativity. The adequacy of this model is now in question, however, for neither the cooperative oxygenation of CoHb nor the cooperative oxidation of MnHb is accompanied by a spin state change.[2a,5a,9]

Unliganded hemoglobin (Hb) has a different quaternary (and tertiary) structure from that of its liganded forms, and the Hb linkage effects arise from a reversible transition between the two structures.[10] If crystals of $Fe^{III}Hb$ are reduced with dithionite, their crystalline order and diffraction pattern are rapidly lost, as the change in quaternary structure of the hemoglobin on reduction disrupts the crystal lattice. When crystals of $Mn^{III}Hb$ are reduced with dithionite, a similar loss of diffraction pattern is noted, though this occurs more slowly than with $Fe^{III}Hb$. This decreased rate of crystalline transformation is consistent with results in solution, where the rate of reduction of $Mn^{III}Hb$ by dithionite is much less than that of $Fe^{III}Hb$. Evidently $Mn^{III}Hb$ undergoes a substantial change in quaternary structure on reduction. We conclude from this observation, from the similarity of the structures of $Fe^{III}Hb$ and $Mn^{III}Hb$, and from the close functional analogies between Hb and MnHb that the quaternary structures of Hb and MnHb are probably identical.

Functional studies gave rise to the original prediction that CoHb[2b] and MnHb[5] undergo quaternary structure transitions similar to that of Hb. The demonstration that a quaternary structure change occurs upon reduction of $Mn^{III}Hb$ to MnHb tends to confirm the prediction of such a change upon deoxygenation of CoHb.

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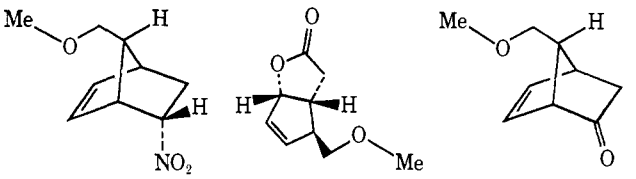
Nitroethylene as a Versatile Ketene Equivalent. Novel One-Step Preparation of Prostaglandin Intermediates by Reduction and Abnormal Nef Reaction

Sir:

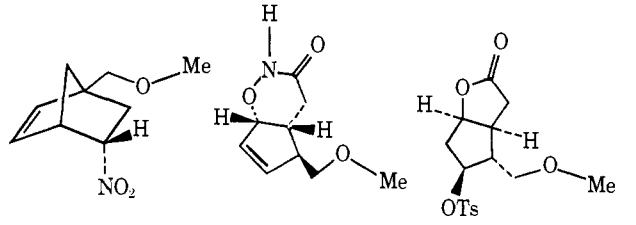
This communication presents, *inter alia*, the novel, convenient, facile one-step preparation of prostaglandin intermediates II and III from the nitroethylene adduct I. The I → II change represents the simplest entry to the prostanoid A nucleus and the lactone II is a key intermediate in the recently developed synthesis of A



I II III

prostaglandins.[1] Compound III is a well-recognized prostaglandin intermediate[2] and the I → III change not only constitutes a highly competitive route to III but also a general method for the preparation of the otherwise difficultly accessible bicyclo[2.2.1]heptenones.

5-Methoxymethylcyclopentadiene,[2] prepared, *in situ*, from thallium cyclopentadienide and 1.5 equiv of chloromethyl methyl ether (ether, −20°), was subjected to cycloaddition with nitroethylene[3] (1 equiv, ether, −60°, 100%) to give adducts I[4] and IV[4] in the ratio 9:1. In contrast to other ketene equivalents,[5] which are reactive only above 0°, nitroethylene undergoes addition even at −100° and this is particularly advantageous in dealing with the highly sensitive 5-substituted cyclopentadienes; further, this high reactivity of nitroethylene has made it possible to replace the undesirable thallium cyclopentadienide with the corresponding sodium derivative.[2] The preparation of I represents a new route to 7-substituted 2-functionalized norbornenes. The sodium salt of I, prepared *in situ* (14 equiv, 20% aqueous NaOH, 0°) on treatment with HCl (28 equiv, 18% aqueous HCl, 10°), gave the lactone II[4] (32%) and the cyclic hydroxamic ester V[4] (33%). Compound V could be quantitatively transformed to



IV V VI

the lactone II with nitrous acid (1, 36% aqueous $NaNO_2$ (2.8 equiv), 18% aqueous HCl (2.4 equiv), −5°; 2, Et_2O extract; 3, EtOH, reflux). Consequently, the I → II change can be effected in 60–70% yields. The I → II + V change involves the cyclization of the common hydroxamic acid intermediate utilizing either of

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