Table I. Gradient Plate Assay, Minimum Inhibitory Concentrations (MIC) Expressed in μg/ml

Compound	*Shigella* sp. N9	*Escherichia coli* N10	*Klebsiella pneumoniae* X26	*Aerobacter aerogenes* X68	*Salmonella heildelberg* X514	Penicillin resistant *Staphylococcus* V41	Penicillin resistant *Staphylococcus* V84
7a	14	15	1.0	3.0	1.0	0.5	0.5
4b	17.5	21.2	0.8	0.9	0.9	11.4	0.5
5b	19.8	20.8	1.0	1.0	1.0	12.0	0.5
6c	49.0	57.5	11.2	9.9	9.8	>20	4.0
2c	120	116	10	30	29	10	3.3

way the 3-methoxy nucleus ester (**6a**, R = H, R′ = *p*-NB) was obtained in 60% yield, mp 163–164°. The uv spectrum of **6a** (EtOH) showed λ_{max} 268 nm (ϵ 14,600). The nmr spectrum (DMSO-d_6) showed in addition to signals expected tor a 3-cephem, a 3-proton singlet at τ 6.20 for a methoxyl grouping at C3.

The 3-methoxy nucleus ester (**6a**) provided a variety of 7-acylamido derivatives[6] upon acylation. With thiophene-2-acetyl chloride for example, in aqueous acetone containing excess $NaHCO_3$, **6a** converted to **6b** (R = thiophen-2-ylacetyl, R′ = *p*-NB), mp 171–172°, in 60% yields. Compound **6b** crystallized from EtOAc and gave satisfactory physical data and elemental analysis.

The *p*-nitrobenzyl ester at C4 in **4a**, **5a**, and **6b** was removed by hydrogenolysis using an equal weight of 5% palladium on carbon in CH_3OH solution, at 50–60 psi and room temperature. Products **4b** (R = thiophen-2-ylacetyl, R′ = H), **5b** (R = thiophen-2-ylacetyl, R′ = H), and **6c** (R = thiophen-2-ylacetyl, R′ = H) crystallized upon trituration with ether. Physical data and elemental analyses for **4b**, **5b**, and **6c** were in agreement with the proposed structures. Compound **3b** could not be isolated as a 4-carboxylic acid due to spontaneous decarboxylation upon ester removal.

Table I compares the antimicrobial activity of compounds **4b**, **5b**, and **6c** with that of cephalothin (**7a**, R = thiophen-2-ylacetyl, R′ = H) and (**2c**, R = thiophen-2-ylacetyl, R′ = H). All three new compounds are especially more potent than the deacetoxy derivative **2c** with regard to inhibiting gram negative bacteria.

Since irreversible acylation of a transpeptidase enzyme important to bacterial cell wall synthesis[7] has been implicated as the mode of action of β-lactam antibiotics, it follows that the chemical reactivity of the β-lactam is an important parameter of biological effectiveness.[8] Reactivity of the penicillin β-lactam has been attributed to the strain caused by the 4–5 ring system.[9] In cephalosporanic acids, such as cephalothin, **7a**, reactivity of the β-lactam is enhanced by stabilization of the possible "enamine" resonance forms of the molecule.[9] The deacetoxy cephalosporins generally possess reduced antibiotic activity.

This new group of antibiotics with direct attachment of an electronegative substituent at C3 has equivalent biological activity to cephalothin (**7a**) and increased activity over the deacetoxy compound (**2c**). This activity can be attributed to an inductive effect on the β-lactam nitrogen causing a weakening of the β-lactam carbonyl nitrogen bond.

The potential utilization of these new cephalosporins for the treatment of human infections depends on many factors in addition to antibacterial potency and these are currently under investigation.

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Role of Acids in Reduction of Acetylene Catalyzed by Molybdenum–Thiol Complexes

Sir:

Recently Schrauzer, *et al.*, discovered that systems which consist of $NaBH_4$, molybdate, and a thiol compound (cysteine, glutathione, or thioglycerol) (designated as Mo–SH systems) can reduce unsaturated compounds such as C_2H_2, N_3^-, and N_2O.[1–6]

These systems were proposed[1–6] as close models of nitrogenase although they are much less active toward N_2[3] than are simpler heterogeneous[7] and homogeneous[8] Mo- or V-containing systems which reduce nitrogen to hydrazine and ammonia in water and water–alcohol solutions. (In agreement with the claims of ref 9, we were also unable to detect any reduction of N_2 to NH_3 with the Mo–SH systems.)

One characteristic feature of the Mo–SH systems is the stimulating effect of adenosine triphosphate (ATP) and other nucleoside phosphates on the reaction rate and yields of products. ATP is known to be a necessary participant of nitrogenase function, the hydrolysis of the macroergic O–P bond being coupled with electron transfer from reducing agent to a substrate. The mechanism of ATP function in nitrogenase is still obscure. Therefore Schrauzer's papers attracted much

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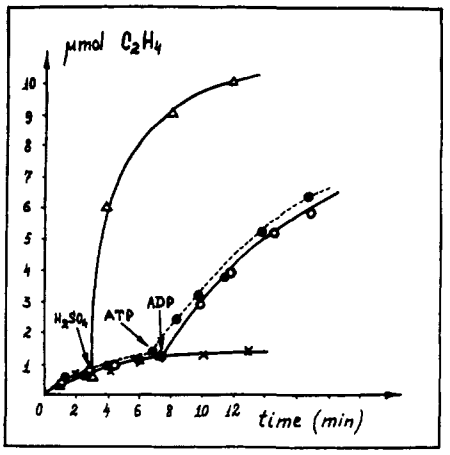

Figure 1. Influence of acids on the reduction by Mo-Glu complex in the presence of $NaBH_4$. The conditions are similar to those in the table, initial pH 9.5–9.6: ×, no acids added; △, with H_2SO_4, pH in ~1 min after H_2SO_4 addition reaches 8.3; ●,○ with ATP and ADP, respectively, pH in ~1 min after addition reaches 9.1. Arrows show the time of addition.

interest and provoked new investigations.[9,10] It was found[9] that Mo–glutathione complex is remarkably specific to ATP (there is 100-fold increase in reaction rate in the presence of ATP and no effect in the case of ADP).

Some doubts were expressed[10] about the interpretation of the mechanism of ATP action suggested earlier[2] which included the phosphorylation of Mo complex, since there is virtually no hydrolysis of ATP under the conditions in which the Mo–SH systems function; that is, there is no use of the energy of hydrolysis of the O–P macroergic bond (in contrast to the biological systems).

It was concluded[10] that ATP in Schrauzer's experiments behaves as merely a protonic acid changing pH at the moment of ATP addition to reaction mixture.

In the recent work by Schrauzer it was found that adenosine monophosphate (AMP), which contains no macroergic O–P bond, activates the reduction of substrate as do ATP and ADP. It was concluded[6] that the mechanism of ATP action does not include the O–P bond hydrolysis but that the phosphate part of ATP plays an important part in activation of Mo–SH systems.

In this communication we present the evidence for a determining role of the initial pH change in stimulation by ATP of the Mo–SH systems toward acetylene reduction and some information concerning the reaction mechanism.

The Mo–glutathione complex which was used in our experiments was prepared according to ref 11. The reaction was performed in a circulating vacuum system which permitted one to take samples for chromatographic analysis during the reaction. For mass spectral analysis of deuterated products, the gaseous mixture was separated by preparative chromatography. As reported,[2] the reaction products are ethylene and some ethane, formed along with the ethylene.

The stimulating effect of ATP (as well as ADP) was found to depend very strongly on the pH of the solution of ATP which is added to the reaction mixture. It may be seen in Figure 1 that ATP and ADP produce the same effect if they are added to the reaction mixture in solutions with equal pH. Moreover, if the initial solution of ATP or ADP is equalized in pH with the reaction mixture before the addition, there is no effect whatsoever on the reaction rate. Presumably the reported[9] specificity of ATP is in fact due to the difference in pH of initial solutions of ATP and ADP (*e.g.*, ATP could be introduced in the form of acid salt and ADP as a neutral salt).[12] It can also be seen in Figure 1 that the effect of added H_2SO_4 is similar to that of ATP and ADP. Therefore stimulation of the activity of the $NaBH_4$–Mo–SH system seems to be due to a nonspecific effect of the protic acid addition.

Acid catalysis in various redox reactions is known. The closest example is the catalysis by H^+ of the reduction of C_2H_2 to C_2H_4 by Cr^{2+} ions.[13] Proton action in the presence of $NaBH_4$ is, however, peculiar since there is simultaneous acid decompositon of BH_4^- with formation of H_2 and borate; therefore the solution can remain alkaline after the acid addition.

The isotopic composition of the ethylene formed in D_2O in the presence of $NaBH_4$ shows much C_2H_4 together with C_2H_3D. Similarly $C_2H_2D_2$, C_2H_3D, and C_2H_4 are formed in H_2O in the presence of $NaBD_4$ (see Table I). These results show that the hydridic mechanism is at least partially operative in the reduction of acetylene. The formation of C_2H_4 in the reaction with $NaBD_4$ seems to be the result of an isotopic exchange with H_2O during the reaction.

Table I

Deuterated ethylenes	Content %			
	A[a]	B[b]		C[c]
C_2H_4	26.7	25	12	17
C_2H_3D	26.8	56.5	59.5	62
$C_2H_2D_2$	33.6	16.5	26.8	18
C_2HD_3	8.9	2.0	1.7	3.0
C_2D_4	4.0	0	0	0

[a] A: Mo-Glu complex ($C_{Mo} = 9 \times 10^{-4} M$) $NaBH_4$ (0.23 *M*), 0.5 atm of C_2H_2 D_2O with addition of D_2SO_4, 25°. [b] B: Mo-Glu complex ($C_{Mo} = 9 \times 10^{-4} M$) $NaBD_4$ (0.25 *M*), 0.5 atm of C_2H_2 H_2O with addition of H_2SO_4, 25° (two experiments). [c] C: Same as B but without H_2SO_4.

According to Schrauzer and Doemeny[2] *cis*-$C_2H_2D_2$ is formed in D_2O ($NaBH_4$) and no deuterated ethylenes form in H_2O ($NaBD_4$) in the case of the Mo–cysteine complex. It was proposed[2] that no hydride transfer takes place. We believe, however, that the Mo–Glu and Mo–Cys systems are very similar and that the absence of deuterated ethylenes in the presence of Mo–Cys ($NaBD_4$, H_2O) complex is due to increased exchange rather than a drastic change in the mechanism.

It is probable that Mo–H species are formed at first from $NaBH_4$, and the reaction with substrate includes H atom transfer from Mo–H to the complexed acetylene molecule. The stimulating effect of acids may be explained if some intermediate $NaBH_4$ decomposition

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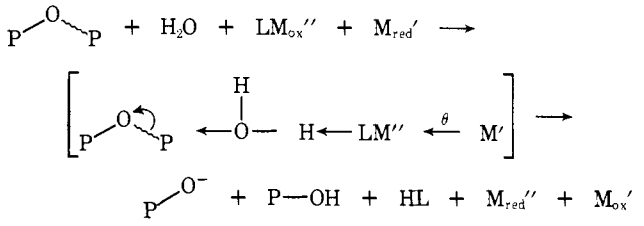
product (*e.g.*, BH_3, $BH(OH)_3^-$ etc.[14]) is more active. than the initial BH_4^- in reaction with Mo–SH complexes.

Another possibility is that the protonation of the ligand, connected with the Mo atom by the acid added or formed during BH_4^- decomposition, could facilitate hydride transfer from BH_4^- to the Mo–SH complex and the following reaction with the complexed C_2H_2.

We believe that, if the latter possibility is the case, the mechanism of acid action in stimulation of Mo–SH systems bears in fact some resemblance with ATP action in enzymes. ATP, when hydrolyzed, can also provide an active proton to a substrate.[15] One may visualize the following enzymatic mechanism of ATP hydrolysis coupled with electron (or H^-) transfer from the M′ atom to the M″ atom (M″ then can reduce a substrate).



It is evident that this mechanism requires a very specific spatial arrangement of substrates which has not been achieved so far in model experiments.

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Spin Delocalization in 7-Norbornenyl-Type Radicals

Sir:

The 7-norbornenyl radical poses some interesting problems regarding its structure,[1,2] stereoselective reactions,[1,3] and possible nonclassical nature.[3,4] The recent studies on this [1–3] and related[5,6] radicals prompt us to make a preliminary report on the systems we have been studying.

The work reported here involves an examination of the nmr paramagnetic contact shifts induced in 7-azabenzonorbornene (I) and 7-azabenzonorbornadiene (II) and derivatives by the addition of bis(acetylacetonato)nickel (II).[7] These shifts, resulting from positive spin density in the nitrogen nonbonding orbital, have been shown to be accurate probes of the spin distributions in the corresponding hydrocarbon radicals.[8] The present study, however, offers several advantages over the conventional esr experiments; these are (a) unequivocal assignment of the spin densities to specific atoms, (b) direct determination of the *sign* of the spin density, and (c) the determination of spin densities which normally would give rise to unobservably small esr hyperfine splitting constants.

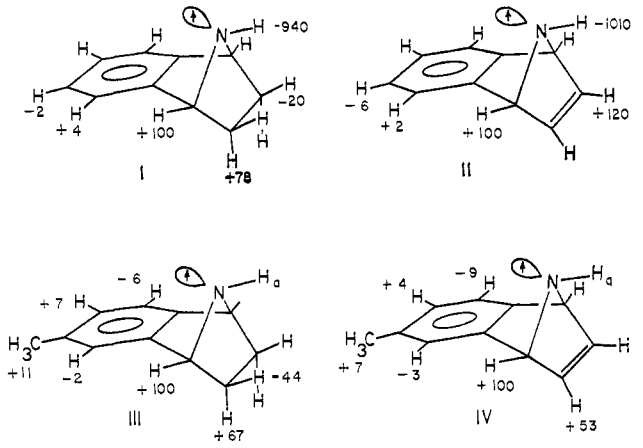

Figure 1. Nickel-induced contact shifts in the 7-azanorbornenes and -norbornadienes. The signs given refer to the signs of the spin densities at the protons in question. The numbers given are obtained by a least-squares analysis of data obtained over at least six different concentrations. (a) Insufficient data could be obtained for these –NH absorptions to allow reliable analyses. (b) The assignments of the aromatic protons are based upon the general observation that alkyl substitution causes a slight upfield shift for protons ortho to the substitution site: L. M. Jackman, Q. N. Porter, and G. R. Underwood, *Aust. J. Chem.*, **18**, 1221 (1965). The assignments of the exo and endo protons are made by direct comparison with the nmr spectra of the carbocyclic analogs: K. Tori, K. Aono, Y. Hata, R. Muneyuki, T. Tsuji, and H. Tanida, *Tetrahedron Lett.*, 9 (1966); N. Inamoto, S. Masuda, K. Tori, K. Aono, and H. Tanida, *Can. J. Chem.*, **45**, 1185 (1967); K. Tori, Y. Yoshimura, and R. Muneyuki, *J. Amer. Chem. Soc.*, **93**, 6324 (1971). (c) The contact shifts obtained for the bridgehead protons of the above molecules were as follows: (I, 20.5; II, 78.1; III, 689; IV, 71.7 ppm)/[Ni]/[amine].

The results for the molecules under investigation are given in Figure 1 in which all values are normalized with respect to the spin densities at the bridgehead protons.[9] Several interesting points can be noted from these data. First, all –NH protons exhibit upfield shifts resulting from negative (or β) spin densities. This is a typical result for cyclic amines, although in this case, where substantial nonplanarity at the radical site is expected,[10] the result was not a foregone conclusion. That the radical site is in fact nonplanar can be deduced from the

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(9) We prefer this reference to the more frequently used –NH protons which often cannot be monitored over a sufficiently wide range of concentrations and which may be subject to substantial medium and concentration effects.

(10) For the 7-norbornenyl radical[2] and for the analogous nitroxides,[5,6] ring strain is proposed to cause substantial nonplanarity at the radical site.