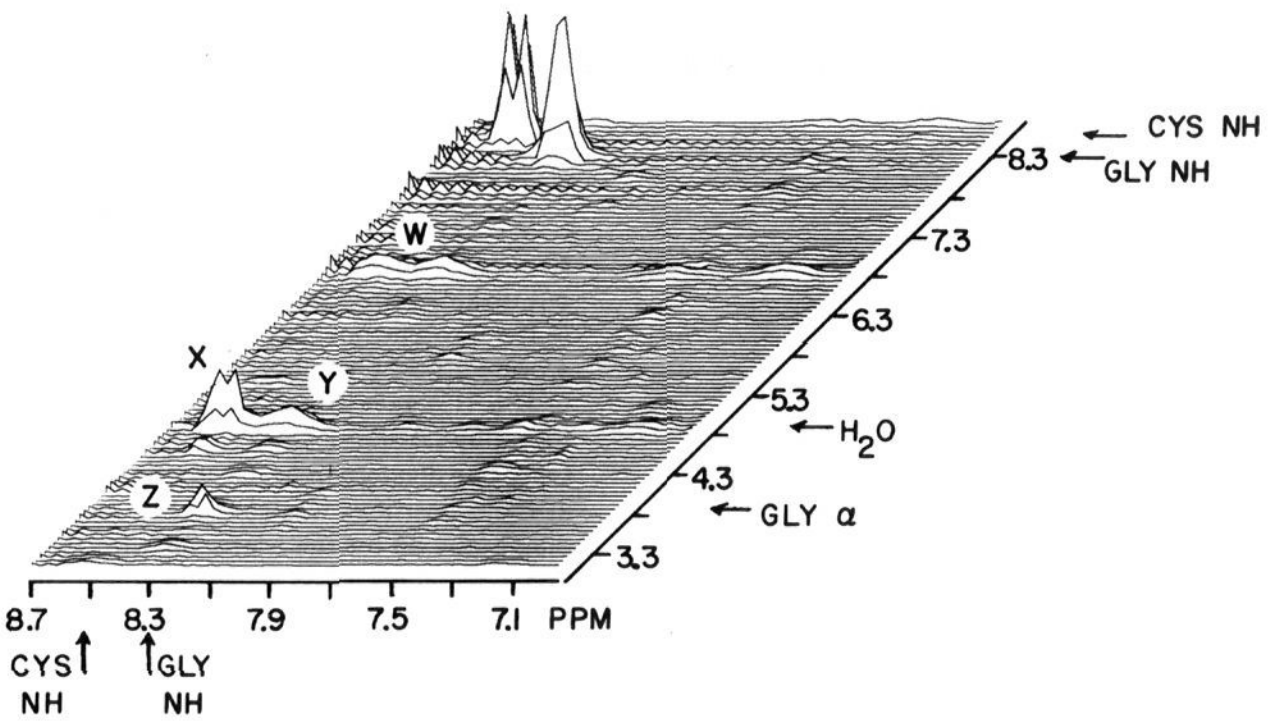

Figure 1. Two-dimensional ^1H NMR spectrum of glutathione (0.14 M, 87% H_2O/13% D_2O, pH_m 5.3, 19 °C). Data were collected as described in the text by utilizing single-phase detection, 1400-Hz bandwidth, 512 data points, and 76 scans per FID. Sine bell apodization was used in each dimension, and the spectrum shown is an absolute value spectrum. Chemical shifts are relative to TSP.

Redfield part of the protocol is designed to minimize stimulation of the H_2O resonance, but the decoupling rf unavoidably stimulates it too much, often making the experiment impossible.[6]

The situation encountered in the 2-D experiment is, in fact, more favorable than that faced in Redfield under-water decoupling for several reasons. Since the acquisition pulse in the 2-D experiment examines the spin system in a nonequilibrium state,[2,3] residual excitation of Z-axis H_2O magnetization by a Redfield pulse nutates proportionately less signal into the transverse plane. In addition, the optimum mixing periods[2] appropriate for observation of amide proton exchange (vide infra) may be long enough to permit significant decay of transverse H_2O magnetization.

Using a $(-90°_x$–evolution–$90°_x$–mixing–$90°_{x,\text{Redfield 2-1-4}}$–detection–preparation–$)_n$ sequence, we have successfully observed NH exchange in 90% H_2O solutions. However, this sequence yields undesirable axial resonances. A phase alternation scheme has been developed[3] for suppressing such resonances as well as reducing unwanted transverse magnetization without a homospoil pulse. Limitations of the pulse programmer in our spectrometer, however, prevent us from conveniently implementing this scheme along with a Redfield 2-1-4 pulse. Therefore, in order to take advantage of the phase alternation procedure, we forego the 2-1-4 modification[1] of the Redfield pulse and use a simple long, soft 90° pulse to excite the amides and suppress the H_2O when acquiring data.

Figure 1 shows the results of this compromise pulse sequence $[(-90°_x$–evolution–$90°_x$–mixing–$90°_{x,\text{soft}}$–detection–preparation–$90°_{-x}$–evolution–$90°_x$–mixing–$90°_{-x,\text{soft}}$–detection–preparation–$)_n]$ for glutathione (γ-Glu-Cys-Gly) in 90% H_2O solution. The portion of the 2-D spectrum near the H_2O resonance is not shown, because it exhibits the usual distortions which characterize Redfield spectra. The effect of these distortions in the amide region of the spectrum is greatly reduced by sine bell apodization[7] in both dimensions.

This work was performed on a NTC-200 spectrometer, utilizing the NTC-1180 computer and NTC-293A′ pulse programmer. Programmable transition between high and low power modes of transmitter operation is possible and required by the experiments discussed above. The 2-D spectrum was accumulated in the single-phase detector mode of receiver operation. Conditions for optimizing the off-diagonal exchange resonances were determined according to the theory of Jeener et al.[2] by using data from one-dimensional spin–lattice relaxation and saturation-transfer experiments for the cysteine amide. Note that the resonances in the top left corner of Figure 1 are the diagonal resonances corresponding to the cys-NH and the gly-NH. Assignments of the NH resonances are obvious from their multiplet structures in a 1-D spectrum of sufficient resolution. The glutamic acid amide resonance is missing in Figure 1, because it is too exchange broadened to survive the sine bell apodization used in processing the data.

The off-diagonal resonances X and Y in Figure 1 indicate magnetization exchange between the cysteine and glycine amide protons of glutathione and the H_2O solvent, for they occur at the expected locations defined by the chemical shifts of these resonances. At present the contributions from chemical exchange and intermolecular cross relaxation have not bee separated, although such differentiation is possible in principle.[2,3] The location of off-diagonal resonance Z indicates that it arises from dipolar and/or spin–spin interactions between the glycine amide and α protons.[8] The resonances labeled W appear to be artifacts related to residual excitation of H_2O by the acquisition pulse. In conclusion, then, the study of amide proton exchange in aqueous solutions by the important 2-D NMR technique appears to be feasible, particularly if advantage can be taken of the more sophisticated modifications[1] of the Redfield H_2O suppression technique.

Registry No. Glutathione, 70-18-8.

(6) Cutnell, J. D.; Dallas, J.; Matson, G.; LaMar, G. N.; Rink, H.; Rist, G. *J. Magn. Reson.* **1980**, *41*, 213–221.

(7) Brereton, R. G.; Garson, M. J.; Staunton, J. *J. Magn. Reson.* **1981**, *43*, 224–233.

(8) Macura, S.; Huang, Y.; Suter, D.; Ernst, R. R. *J. Magn. Reson.* **1981**, *43*, 259–281.

Structure of Ristocetin A: Configurational Studies of the Peptide

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Received August 13, 1981

From studies of a number of examples[1] it is now apparent that the vancomycin-group glycopeptide antibiotics contain heptapeptides in which residues II and IV–VII (numbered from the N terminus) are similar or identical, whereas wide variations occur in residues I and III. In ristocetin A these residues are contained within ristomycinic acid (**1**), which has two substituted phenyl-

(1) For a recent review, see: Williams, D. H.; Rajananda, V.; Williamson, M. P.; Bojesen, G. *Top. Antibiot. Chem.* **1980**, *5*, 119.

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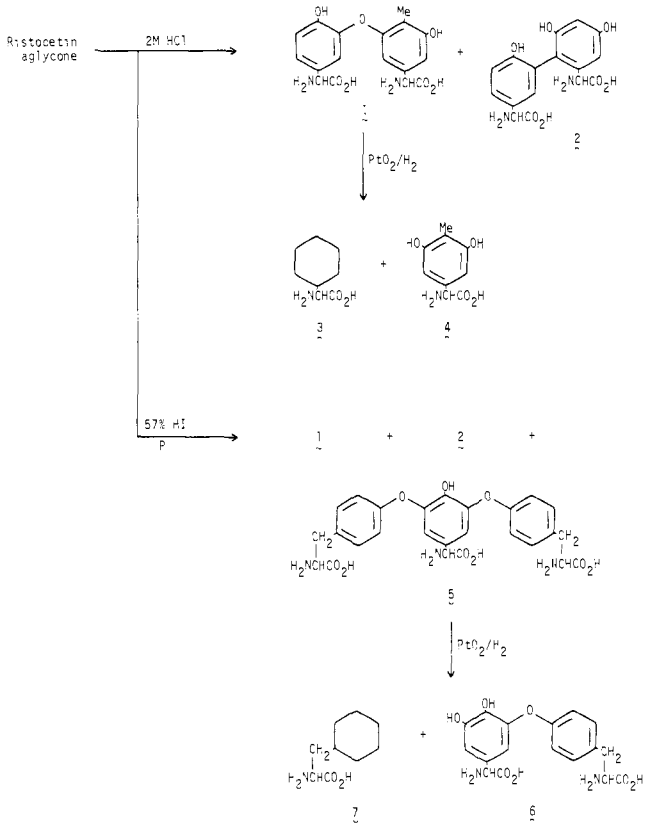
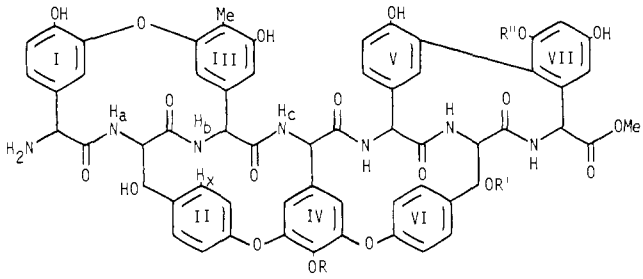
Scheme I



glycine residues joined via an ether linkage. Studies by Williams,[2] Katrukha,[3] Sztaricskai,[4] and us[5] have established the peptide sequence and sites of carbohydrate attachment. In addition, Williams and co-workers[2b,2c] have delineated details of the three-dimensional structure by NMR spectroscopy, showing that residues I and III both have *S* configurations and the remaining chiral centers have configurations identical with those established for vancomycin by X-ray analysis of a partial hydrolysis product.[2e]



Ristocetin A R, R', R'' = glycosides

These configurational assignments attracted our attention because Lomakina and co-workers[6] have assigned an *R* configuration to residue I in ristomycin A on the basis of degradation by hydrogenolysis to give (*R*)-cyclohexylglycine. Ristomycin A is now known to be identical with ristocetin A.[1] It seemed possible to us that the difference could be explained by the Russian workers having inadvertently isolated an inverted product because ristomycinic acid and other phenylglycine-type amino acids can undergo epimerization/racemization during acid-catalyzed peptide hydrolysis.

We have reinvestigated this question by using a degradation sequence (Scheme I) based on the one employed by Lomakina but making provisions to monitor the integrity of the chiral centers in ristomycinic acid (**1**). Initial removal of the sugars (1 M DCl, 45 min, 100 °C) followed by peptide hydrolysis (2 M DCl, 24 h, 105 °C) gave **1** which was isolated by ion-exchange chromatography (Aminex 50W × 2, pH 4.53, 0.1 M pyridine acetate). Only one diastereomer was found.[7] Incorporation of deuterium at the α positions was assayed by comparing NMR signals at δ 4.82 and 4.86 for the α protons with that of the C-methyl group at δ 2.23. Integration showed less than 10% deuteration at either α position. Degradation of **1** by hydrogenolysis gave (*R*)-cyclohexylglycine (**3**), $[\alpha]^{27}_D$ −29° (*c* 0.22, 0.2 M HCl),[8] and (*S*)-amino acid **4**, $[\alpha]^{27}_D$ +77° (*c* 0.69, 0.2 M HCl).[9a] In a parallel experiment (HCl/H_2O hydrolysis), configurations of the amino acids were determined from the CD spectra (Table I) of their *N*-salicylidene derivatives by the method of Smith et al.[10] These data confirm Lomakina's assignment of residue I as having the *R* configuration. Residue III is *S* as has been proposed by Williams.[2b] Confirmation that the two chiral centers of **1** have opposing configurations is obtained from the small specific rotation of **1**, $[\alpha]^{25}_D$ −9.8° (*c* 1.12, 0.2 M HCl),[9b] and from the CD spectrum of its bis(salicylidene) derivative, which lacked discrete Cotton effects with only a broad dip near 280 nm.

Actinoidinic acid (**2**), containing residues V and VII, was isolated from the DCl hydrolysis with less than 10% deuterium incorporation in either of the α positions. A low specific rotation, $[\alpha]^{25}_D$ −14.6° (*c* 0.96, 0.2 M HCl),[11] of **2** and negligible CD spectrum (Table I) indicated that the two residues have opposing configurations, consistent with Williams' assignment of *R* and *S*, respectively, for the two centers.

Residues II, IV, and VI are contained within a tris(amino acid) which is destroyed during acid hydrolysis on account of the lability of the phenylserine fragments. In an attempt to explore the configuration of these residues, ristocetin aglycon was treated with 57% HI and red phosphorus (22 h, 106 °C), conditions that convert phenylserine fragments to phenylalanines during peptide hydrolysis.[12] The resulting tris(amino acid) **5**[12] was cleaved by catalytic hydrogenolysis to give bis(amino acid) **6** and cyclohexylalanine (**7**). A parallel experiment carried out with DI revealed less than 10% D/H exchange at the phenylglycyl α position of **5**; the phenylalanyl α positions had undergone D/H

(2) (a) Williams, D. H.; Rajananda, V.; Kalman, J. R. *J. Chem. Soc., Perkin Trans. 1* **1979**, 787. (b) Kalman, J. R.; Williams, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 897. (c) Williams, D. H.; Rajananda, V.; Bojesen, G.; Williamson, M. P. *J. Chem. Soc., Chem. Commun.* **1979**, 906. (d) Williamson, M. P.; Williams, D. H. *Tetrahedron Lett.* **1980**, 4187. (e) Sheldrick, G. M.; Jones, P. G.; Kennard, O.; Williams, D. H.; Smith, G. A. *Nature (London)* **1978**, *271*, 223.

(3) Silaev, A. B.; Diarra, B.; Trifonova, Zh. P.; Katrukha, G. S. *Chem. Nat. Compd. (Engl. Transl.)* **1979**, *15*, 661.

(4) Sztaricskai, F.; Neszmélyi, A.; Bognár, R. *Tetrahedron Lett.* **1980**, 2983.

(5) (a) Harris, C. M.; Harris, T. M. *Tetrahedron Lett.* **1979**, 3905. (b) Sztaricskai, F.; Harris, C. M.; Neszmélyi, A.; Harris, T. M. *J. Am. Chem. Soc.* **1980**, *102*, 7093.

(6) Lomakina, N. N.; Zenkova, V. A.; Bognár, R.; Sztaricskai, F.; Sheinker, Yu. N.; Turchin, K. F. *Antibiotiki (Moscow)* **1968**, *13*, 675.

(7) Two diastereoisomers of ristomycinic acid have been isolated by ion-exchange chromatography of 6 M HCl hydrolysates (Fehlner, J. R.; Hutchinson, R. E. J.; Tarbell, D. S.; Schenck, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2420.

(8) Cf. $[\alpha]^{20}_D$ −48° (*c* 0.15, CH_3COOH).[6] See also ($[\alpha]^{26}_D$ −35.0° (*c* 1.0, 5 M HCl)): Rudman, D.; Meister, A.; Greenstein, J. P. *J. Am. Chem. Soc.* **1952**, *74*, 551.

(9) (a) Cf. $[\alpha]^{20}_D$ +75° (*c* 0.5, H_2O).[6] (b) $[\alpha]^{20}_D$ −5° (*c* 0.85, 1.2 M HCl).[6]

(10) Smith, H. E.; Burrows, E. P.; Marks, M. J.; Lynch, R. D.; Chen, F.-M. *J. Am. Chem. Soc.* **1977**, *99*, 707. This method depends on determination of the sign of the Cotton effect near 255 and 315 nm in the circular dichroism spectra of the *N*-salicylidene derivatives of α-amino acids.

(11) In the case of actinoidinic acid, the biphenyl linkage contributes an additional site of asymmetry. Lomakina et al. (Lomakina, N. N.; Yurina, M. S.; Sheinker, Yu. N.; Turchin, K. F. *Antibiotiki (Moscow)* **1972**, *17*, 488) discuss the isolation and rotation of two different forms of actinoidinic acid, one with $[\alpha]^{20}_D$ −22° (*c* 1.0, H_2O) and the other with $[\alpha]^{20}_D$ +39° (*c* 1.0, H_2O).

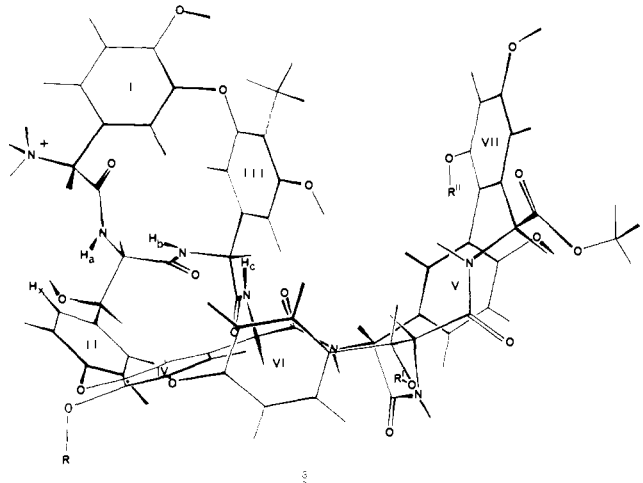
(12) Katrukha, G. S.; Diarra, B.; Silaev, A. B.; Trifonova, Zh. P.; Rozynov, B. V.; Reshetova, O. S. *Antibiotiki (Moscow)* **1979**, *24*, 179. This reduction was used by these authors for the preparation of amino acid **5**. It had been used previously for the proof of configuration at the α position of (*S*)-threonine (McCoy, R. H.; Meyer, C. E.; Rose, W. C. *J. Biol. Chem.* **1935**, *112*, 283. Meyer, C. E.; Rose, W. C. *Ibid.* **1936**, *115*, 721). In this reaction, carried out at 150–155 °C for 7 h, approximately 50% racemization occurred. In a model study done by us, phenylalanine, prepared by reduction of *N*-acetylphenylserine methyl ester in 57% DI (22 h, 106 °C), incorporated approximately 35% D at the α position.

Table I. CD Spectra of *N*-Salicylidene Derivatives[a]

amino acid	λ, nm	[θ]	λ, nm	[θ]
1	*f*			
2	*f*			
3[b]	315	−2100	252	−8000
4[b]	316	−4300	262	−2200
5	315	+12800	255	+17700
6	320	+2100	262	+5000
7[b]	320	nil	252	−1400
(*R*)-phenylglycine[c]	315	+5600	277	+5500
(*R*)-*p*-hydroxyphenylglycine	315	+8900	260	+11700
(*R*)-cyclohexylglycine[b,d]	317	−1900	252	−11000
(*S*)-cyclohexylalanine[b,e]	320	+1100	252	+4900

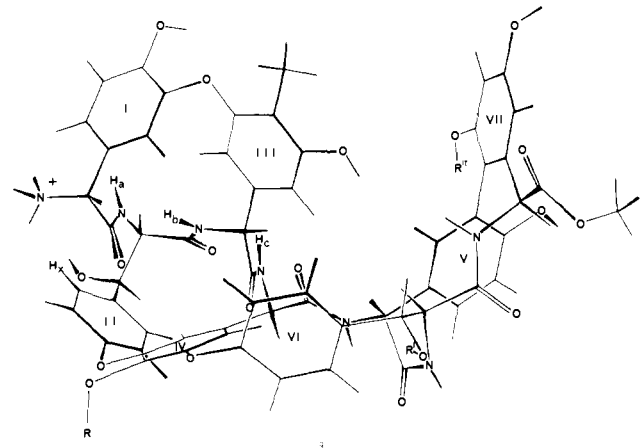
[a] *N*-Salicylidene derivatives were prepared in situ in MeOH by the method of Smith et al;[10] a 15% excess of sodium salicylaldehyde was used. [b] Also showed a band in the 275-nm region ascribed to a quinoid tautomer. [c] Lit.[10] 313 (+6000), 277 nm (+5800). [d] Prepared by reduction (PtO_2/H_2) of (*R*)-phenylglycine. [e] Prepared by reduction (PtO_2/H_2) of (*S*)-phenylalanine. [f] No measurable band in 350–300-nm region; broad band around 280 nm.

exchange to the extent of 70%. As expected the phenylalanyl β positions had each incorporated 1 equiv of D. The optical rotation, $[\alpha]^{28}_D$ −37° (*c* 0.7, 0.2 M HCl), of **5** and CD spectrum of derivatized **5**, dominated by the unracemized residue IV, are in agreement with Williams' *R* assignment. The CD spectrum for derivatized amino acid **6** was similar. The CD spectrum of derivatized **7** was too weak to be of significance, although it showed a slight excess of the *R* configuration. Thus, no independent assignments of residues II and VI can be made on the basis of this study. Further study is needed.



The change of configuration at residue I is the only major change we propose for the structure of ristocetin A. The assignment of the *S* configuration to residue I was made by Williams and co-workers on the basis of two NMR experiments which place the terminal amino group near one proton (H_x) on the aromatic ring of residue II.[2c] Their studies also place amide proton H_a close to H_x.[2b] In ristocetin A, having Williams' three-dimensional structure except that the configuration of I is *R* (i.e., **8**), H_a but not the amino group is close to H_x. If another conformation is chosen in which the I–II amide linkage has undergone a 180° rotation such that the carbonyl group is on the front (interior) side and amide proton H_a is on the back, i.e., structure **9**, then the amino group approaches H_x; H_a still remains reasonably close to H_x. Conformation **9** is analogous to that proposed for vancomycin on the basis of the X-ray structure of degradation product CDP-I[2e] and high-field NMR studies of the parent antibiotic.[13] With the proviso that the absolute configurations of all asymmetric centers are now correctly assigned, we conclude that ristocetin A lies either in conformation **9** or is in mobile equilibrium between **8** and **9**.

(13) Williams, D. H.; Kalman, J. R. *J. Am. Chem. Soc.* **1977**, *99*, 2768.



Ristocetin and related antibiotics exert their antibacterial action by complexing with bacterial cell wall constituents which contain peptides terminating in D-alanyl-D-alanine. Kalman and Williams[14] have proposed that peptide binding involves the cleft on the front face of the molecule and that amide protons (H_a, H_b, and H_c) are involved in intermolecular hydrogen bonds to the carboxyl group of the peptide. Thus, upon binding, ristocetin becomes fixed in conformation **8**. Kalman and Williams have also speculated upon the presence of a salt bridge between the N terminus of the antibiotic (in the ammonium form) and the C terminus of the peptide (as the carboxylate anion). With the reassigned configuration of residue I, the amino group is pointed away from the cleft. Nevertheless, it still lies approximately 5 Å from the peptide carboxyl group and may provide additional stabilization for the peptide–antibiotic complex. A similar but more extensive conformational change upon peptide binding has been proposed for vancomycin.[15,16] The additional degrees of freedom present in vancomycin because residue I is not cross-linked to residue III may allow the terminal ammonium ion to interact more closely with the peptide than in the case of ristocetin.

(14) Kalman, J. R.; Williams, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 906.
(15) Convert, O.; Bongini, A.; Feeney, J. *J. Chem. Soc., Perkin Trans. 2* **1981**, 1262.
(16) Williams, D. H.; Butcher, D. W. *J. Am. Chem. Soc.* **1981**, *103*, 5697.

Preparation and Crystal Structure of $[Mo_3S_8(NNMe_2)_2]^{2-}$, a Trinuclear Sulfido-Bridged Molybdenum Anion with Coordinated Isodiazene Ligands

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Received September 14, 1981

There has been intense recent interest in molybdenum complexes with sulfur ligands stimulated by the EXAFS studies[1] of nitrogenase and other molybdoenzymes. However, none of the complexes so far reported is able to bind or activate dinitrogen. There are very few examples of molybdenum–sulfur complexes which interact with the small molecules that can function as

(1) Cramer, S. P.; Hodgson, K. O.; Gillum, W. O.; Mortenson, L. E. *J. Am. Chem. Soc.* **1978**, *100*, 3398. Cramer, S. P.; Gillum, W. O.; Hodgson, K. O.; Mortenson, L. E.; Stiefel, E. I.; Chisnell, J. R.; Brill, W. J.; Shah, V. K. *Ibid.* **1978**, *100*, 3814.

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