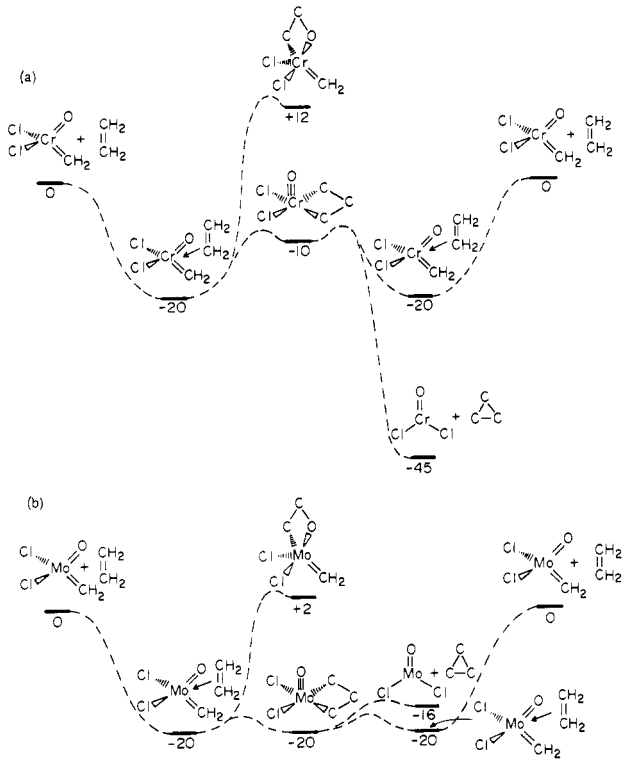


Figure 2. Schematic potential curves for (a) the reaction of $Cl_2Cr(O)(CH_2)$ with C_2H_4 and (b) the reaction of $Cl_2Mo(O)(CH_2)$ with C_2H_4 (energy in kcal/mol).

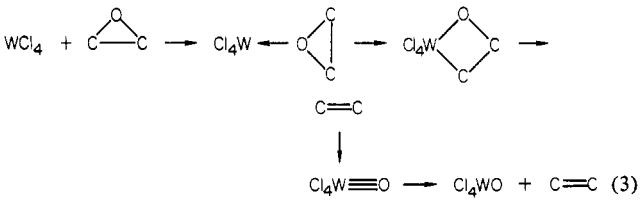
similarity between the two reactions in Figures 1a and 2a.

For Mo, the energies[6b] change as indicated in Figures 1b and 2b, clearly dictating a different chemistry for the molybdenum analogues of Figures 1a and 2a. For example, chromium will epoxidize olefins but should not metathesize olefins (or even produce substantial C–C bond cleavage products) due to the energetic dominance of the reductive elimination pathway c of Scheme I. In contrast, for molybdenum (and tungsten), C–C bond cleavage is competitive with reductive elimination due to the relative disfavor of the reductive elimination pathway for Mo (and W). The origin of this dramatic difference is the increased σ bond strengths in the Mo compounds ($\sim$15 kcal/bond). An obvious prediction is that Cl_2MoO_2 will react with olefins to form substantial C–C bond cleavage products (or decomposition through an alternate pathway involving chlorine).

In considering the role of Cl_2OMCH_2 in metathesis, we must consider the competition of the side reaction b in Scheme I, forming species III with X = CH_2. As shown in Figure 2, this pathway is energetically inaccessible.

Experiments by Muetterties[2b] lead to indirect support of these ideas. He finds that $WCl_6/C_2H_5AlCl_2$ is catalytically inactive for olefin metathesis (a system where Cl_4WCH_2 has been implicated[2c]) unless catalytic amounts of oxygen are present in the reaction mixture. Our conclusion is that Cl_2WOCH_2 is the active catalyst for *stable* W(VI) olefin metathesis catalysts and that a spectator oxo bond is intimately involved in the catalytic process. In addition, Sharpless et al.[3] find that chromyl chloride leads only to epoxidation of olefins,[8] and they have suggested a mechanism essentially identical with that supported by our calculations. Finally, Sharpless and co-workers[4] have developed a synthetically useful deepoxidation process involving the reaction of W(IV) with epoxides, forming the corresponding olefin (and Cl_4WO). We find that this reaction (eq 3) is driven by the formation of a very strong W–O triple bond.

Calculational details are as follows. The basis in the Cr was obtained by optimizing primitive Gaussians so as to have four functions describe each of the 1s, 2p, and 3d atomic orbitals and



two functions describe each of the 2s, 3s, 4s, 3p, and 4p orbitals. The basis was contracted to a split-valence basis (minimal basis for the core orbitals, two contracted basis functions for the 4s, 4p, and 3d orbitals). The oxygen and carbon atoms were described with an analogous basis, but d functions were included on O or C bonded to a metal. The Cl core orbitals were replaced with an effective potential, and the 3s and 3p orbitals were described with a minimum-valence basis.

It was found that uncorrelated wave functions (Hartree–Fock) lead to an extremely bad description of the metal–oxo bond (errors of 150 kcal for species II). Thus, all calculations were carried out with fully correlated metal–oxo bonds. This involved GVB calculations with three correlated pairs per oxygen. In addition, the M–C and M–O bonds of the metallocycle were correlated. Other bonds (C_2H_4 and H_2CO) were correlated so that all processes involved the same level of correlation along the reaction path. After the GVB orbitals were solved, a CI was carried out within the occupied GVB orbitals (GVB–CI).[9]

Summary. Using accurate ab initio methods, we have calculated bond energies and reaction enthalpies for several processes possibly involved in metathesis and epoxidation. These results allow a clearer understanding of the mechanisms and suggest that in activating metal chlorides it is essential to have spectator metal–oxo bonds.

Acknowledgments. The research was partially supported by the Department of Energy Research and Development Administration Grant EX-76-G-03-1305. However, any opinions, findings, conclusions, or recommendations expressed herein are those of the authors and do not necessarily reflect the view of the DOE.

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Resolution of Underivatized Amino Acids by Reversed-Phase Chromatography

Sir:

Enantiomers can be separated by liquid chromatography by using either a chiral support or a chiral mobile phase. The potential of the latter approach has only recently begun to be realized.[1,2]

We report the separation of underivatized amino acid enantiomers by liquid chromatography using a chiral mobile phase and a reversed-phase column. The chiral eluant is a dilute aqueous solution of L-proline (0.017 M) and copper acetate (0.008 M), which can form diastereomeric complexes with the D and L enantiomers of amino acids. Resolution is ascribed to differences in stability and polarity of these diastereomeric species.

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Table I. Separation Factors and Adjusted Retention Times for 19 Pairs of Amino Acid Enantiomers Chromatographed on a Reversed-Phase C-18 Column[a]

amino acid	form	t_R', min	separation factor, $\alpha[t'(2)/t'(1)]$
cysteic acid		3.75	
alanine	D	0.20	2.92
	L	0.58	
α-amino-*n*-butyric acid	D	1.07	3.29
	L	3.52	
valine	D	3.80	4.82
	L	18.3	
norvaline	D	4.3	3.07
	L	13.2	
isoleucine	D	13.7	4.53
	L	62.0	
alloisoleucine	D	13.8	4.38
	L	60.4	
leucine	D	15.9	2.56
	L	40.7	
norleucine	D	17.4	3.51
	L	61.0	
methionine	D	9.4	2.41
	L	22.7	
tyrosine	D	16.3	2.28
	L	37.2	
phenylalanine	D	69.7	1.94
	L	135.0	
3,4-dihydroxyphenylalanine	D	8.7	2.24
	L	19.6	
aspartic acid[b]			
glutamic acid	D	0.46	1.98
	L	0.91	
threonine[b]			
allothreonine	D	0.40	4.50
	L	1.83	
lysine	D	0.14	2.29
	L	0.32	
histidine	D	2.73	0.74
	L	2.02	
arginine	D	1.07	1.85
	L	1.98	

[a] Column, 15 × 0.46 cm (Supelco LC-18); mobile phase, aqueous solution of copper acetate (0.008 M) and L-proline (0.017 M), pH 5; flow rate, 0.5 mL/min; temperature, 25 °C; pump pressure, 30 atm. Retention time of cysteic acid is taken as the approximate column void volume. [b] Aspartic acid and threonine enantiomers are not resolved under the conditions for Table I. Both can be resolved at pH 7 with the L enantiomer eluting before the D enantiomer as for histidine in Table I.

Though there are reports in the literature[3] on relatively large variations in the free-energy values of analogous diastereomeric compounds, it has not been realized that this effect could be utilized in such a simple operational mode. The efficiency of the reversed-phase chiral eluant system far surpasses that of the ion-exchange chiral eluant system reported earlier.[4] Another important difference is that the present system resolves the basic and acidic amino acids in addition to the neutral amino acids.

The basic approach and system are similar to those previously described.[4-6] An important feature of the procedure is the postcolumn derivatization with *o*-phthaldehyde and thioethanol,[7] which leads to fluorescent products with primary, but not with secondary, amino groups, in which picomole amounts of amino acids can be detected without interference by the proline dissolved in the mobile phase. The reversed-phase packing was mainly octadecyl silica gel (C-18), with columns of 15- or 25-cm length and 4.6-mm internal diameter. The results (Table I and Figure 1) show remarkably high resolution factors[8] (α) for most of the amino acids resolved. Factors of the order of 2–4 are common. Valine at 0 °C was separated with a factor as high as 6.5, corresponding to a difference in free energy of solution ($\Delta\Delta G° = -RT \ln \alpha$) of the two enantiomers of 1 kcal/mol (Figure 1).

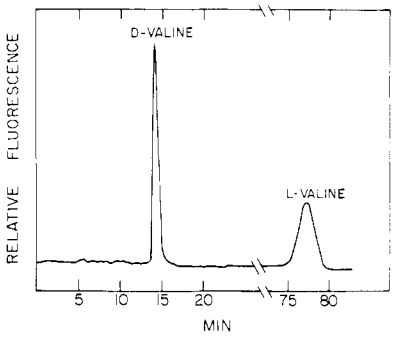

Figure 1. Resolution of DL-valine on a reversed-phase column with an eluant containing $Cu(L\text{-}Pro)_2$. Conditions as in Table I, except that 4×10^{-3} M H_3BO_3 was added, pH was adjusted with NaOH to 8.1, and the temperature was 0 °C.

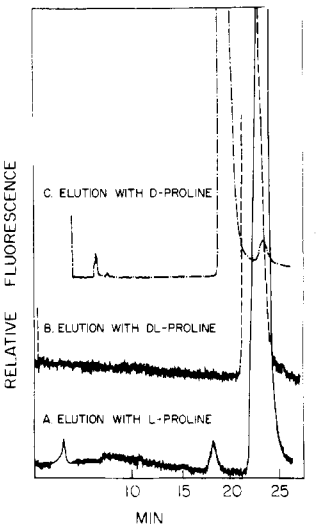

Figure 2. Test of the optical homogeneity of 2-(trifluoromethyl)histidine by reversed-phase chromatography with eluants containing, respectively, L-proline (A), DL-proline (B), and D-proline (C). Column 25 × 0.46 cm self-packed with 10-μm Lichrosorb RP-18 (Merck). Eluant composition: $CuSO_4$ 4×10^{-3} M, proline 8×10^{-3} M; room temperature; flow rate 1 mL/min. The absolute retention times for curve C are somewhat smaller than for curve A, possibly because of incomplete conditioning of the column. However, the relative retentions of the two peaks are the same within experimental errors ($\alpha = 1.30 \pm 0.02$) for both chromatograms.

It has been found in our laboratory that with other cations [Zn(II), Co(II) and Hg(II)] and the ligands L-Pro-Gly, L-Pro-L-Tyr, L-Pro-L-Val, and *N*-methylvaline chiral recognition can also be achieved. Furthermore, it has been established that parameters such as temperature, pH, and ionic strength and the addition of water-miscible organic solvents have strong effects. Thus, under the general conditions of Table I, glutamic acid can be resolved only at pH ≤5 whereas for threonine and aspartic acid a range of 7–8 is required. It seems likely that most of the remaining problems of resolution for protein amino acids will be solved by a suitable adjustment of eluant composition.[9] On the

(3) For example: B. E. Leach and R. J. Angelici, *J. Am. Chem. Soc.*, **91**, 6296 (1969).
(4) P. E. Hare and E. Gil-Av, *Science (Washington, D.C.)*, **204**, 1226 (1979).
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(6) See also captions and legends of Table I and Figure 1.
(7) M. Roth, *Anal. Chem.*, **43**, 880 (1971); J. R. Benson and P. E. Hare, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 619 (1975).

(8) Separation factor (α) = ratio of the capacity factor of the enantiomer emerging last over that of the enantiomer emerging first.
(9) Recently, resolution of amino acids on a reverse-phase column with an eluant containing Zn(II) and L-Asp-L-Phe-*O*-Me has also been observed: C. Gilon, R. Leshem, Y. Tapuhi, and Eli Grushka, *J. Am. Chem. Soc.*, **101**, 7612 (1979).

other hand, for proline and hydroxyproline, the problem is to find a suitable detection method because, as mentioned, the present postcolumn derivatization procedure is not applicable to secondary amines.

A particularly interesting aspect of the method is the possibility of changing the chirality of the system by switching to a mobile phase containing D- or DL-proline, instead of L-proline.[5] Such a change leads to a reversal of the order of emergence with D-proline and to a coalescence of the peaks when a racemic ligand is dissolved in the mobile phase. Figure 2 illustrates the application of this approach to the determination of the enantiomeric composition of a sample of 2-(trifluoromethyl)histidine synthesized for the first time by L. Cohen and co-workers.[10] If no optical impurity is present in a sample, the determination of optical homogeneity can be based on the measurement of retention times.

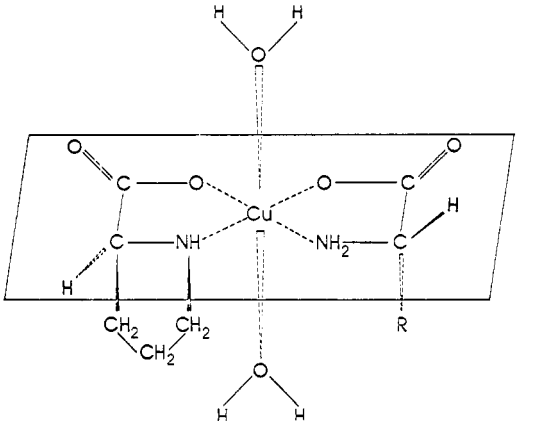
The present results suggest a new approach in amino acid analysis in which reversed-phase columns and chiral mobile phases would be used, possibly in conjunction with the classical ion-exchange resins. Such a combination of chromatographic phases will lead to procedures for rapid quantitative amino acid analysis with simultaneous determination of the enantiomeric composition. In view of the growing interest in D-amino acids in research on antibiotics, cell-wall composition, testing of synthetic peptides having physiological properties, geochemical dating, and food and drug analysis, the development of such a method appears to be timely. It should also be pointed out that the unavailability of an automatic method for enantiomeric analysis has hampered in the past the study of the occurrence and role of D-amino acids in nature.

For resolution to occur in our system, the enantiomers separated must partition between the two phases with involvement of diastereomeric complexes formed by Cu(II) and the amino acids resolved. The amino acids may be present in solution uncomplexed as cations, anions, or zwitterions, depending on the pH, or complexed in the form of different metal-coordinated species. It is, therefore, not a simple matter to recognize the mechanisms by which stereoselectivity is attained. In analogy to ion-pairing chromatography,[11] the following mechanisms could, for instance, be considered.

The amino acid in solution undergoes ligand exchange on the copper–proline complex adsorbed on the surface of the support. The situation is similar to the case of the polymeric stationary phases to which proline is linked covalently,[12,13] and indeed, the order of emergence of the amino acids is the same (the D before the L isomer with L-proline; for the exceptional case of histidine, see below). This mechanism is also supported by the finding that on cyanopropylated silica gel adsorption of Cu(L-Pro)$_2$, if any, seems to be very low, and, in parallel, no resolution is observed. The order of emergence is the inverse of that reported in our previous paper,[5] where an ion-exchange resin was the stationary phase and steric selection is considered to take place in the mobile phase. Ligand exchange will, of course, also occur in the eluant in reversed-phase columns. The resulting chiral discrimination should counteract that obtained on the support, perhaps with varying consequences for different amino acids.

An alternative mechanism is partitioning of the intact metal-coordinated diastereomeric species between the two phases. This mechanism could be superimposed on the above processes and either enhance or reduce their stereoselectivity.

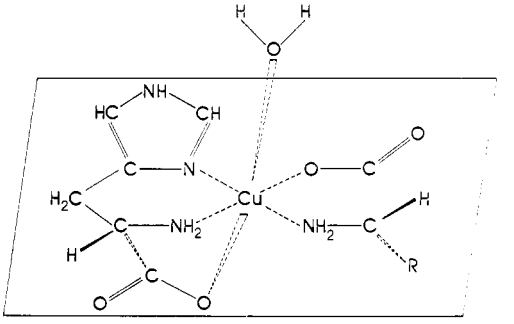
α-Amino acids in many cases chelate with copper(II) by coordinating through the α-amino and carboxylic groups, with the bonds to the metal in a square-planar arrangement. Two weaker apical bonds might be formed with other groups in the ligand or with solvent molecules, as shown in structure **1**. The relative



1

disposition of the asymmetric carbons in the two ligands around the metal will determine the relative stability (and partition coefficient) of the corresponding diastereomeric compounds. Any change in the structure, such as trans instead of cis geometry of the ligands with respect to the metal atom, will drastically affect the stability relationships or might even reverse them. (In many cases, the amino acid ligands are oriented preferentially trans rather than in the configuration illustrated in Figure 1.)

It has been reported[14] that histidine may form strong coordination bonds with the α-amino group and the imidazolidine nitrogen, with the carboxylic acid placed in an apical position, as shown in **2**. It is obvious that such a structure will change the



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relative disposition of the asymmetric center of the histidine with respect to that of L-proline, as compared with other α-amino acids. This behavior of histidine might be related to the reversal of the order of emergence observed.

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