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Copper-zinc Superoxide Dismutase Stability in Water-miscible Organic Solvent Systems. DARLENE K. TAYLOR and ERIC R. JOHNSON, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—A superoxide dismutase assay system has been adapted for use in aqueous solutions of ethanol and dimethylsulfoxide. The photochemical reduction of nitroblue tetrazolium to formazan in these solvent systems is at least 95% inhibited by copper-zinc superoxide dismutase, indicating that the reaction is mediated by superoxide anion. The observation that copper-zinc superoxide dismutase exhibits its activity in these solvent systems implies an unusual stability against solvent denaturation effects. Prolonged exposure of this enzyme to aqueous solutions of 50% ethanol or 50% dimethylsulfoxide at 25°C results in only small losses of superoxide dismutase activity.

Studies on the Specificity of a Denaturant-stable Protease Isolated from a Commercial Protease Preparation, Pronase. BRENDA R. LINDLEY and ERIC R. JOHNSON, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—A serine protease, with an active site sequence similar to chymotrypsin (S. Siegel, *et al*, *J. Biol. Chem.* 247 4155-4159 (1972)) has been found to rapidly inactivate hen egg lysozyme by proteolysis in the presence of 6.0 M guanidine hydrochloride. The protease is thus stable and active under conditions that denature the protein substrate. No detectable proteolysis of lysozyme is observed in the absence of denaturant, indicating that unfolding of the lysozyme molecules by the addition of denaturant is required to provide access to protease cleavage sites. Thin-layer peptide mapping studies suggest that this proteolysis is reproducible on irreversibly denatured proteins in the absence of denaturant, implying a specificity of cleavage of the protein substrate. This cleavage specificity appears to be retained in the presence of the denaturant, 6.0 M guanidine hydrochloride.

Anion Effects on the Thermal Denaturation of Bovine Copper-zinc Superoxide Dismutase. DOUGLAS B. WILLIAMS and ERIC R. JOHNSON, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—Bovine erythrocyte superoxide dismutase (BESOD) is unusually resistant to irreversible thermal denaturation at temperatures up to 100°C. The unusual stability of this enzyme appears to be dependent on the ionic strength of the medium, the rate of denaturation increasing with increasing ionic strength. The thermal denaturation of BESOD is apparently similar in solutions containing chloride and sulfate anions, while phosphate anions enhance the thermal denaturation of this enzyme markedly. The specific phosphate effect can be explained by the com-

plexation of phosphate anions with Cu^{2+} ions required for BESOD activity or by the conformational instability caused by charge repulsion between phosphate anions bound to the BESOD molecules.

Molar Refractions—A New Look at an Old Term. EUGENE SCHWARTZ, Department of Chemistry, DePauw University, Greencastle, Indiana 46135.—Molar refractions (electronic polarizations) were obtained for a number of conjugated and non-conjugated molecules in several solvents at 25.0°C. A differential refractometer was used to measure the refractive indices of the solutions at the mercury green line. Calculation of the molar refractions was done by the method of Halverstadt and Kumler. Reproducibility of results was better than 0.05 cc in most cases. Molar refractions obtained in this way for non-conjugated molecules in benzene solution were within about 0.4% (0.1 cc) of the refractions for these molecules calculated from the densities and refractive indices of the pure liquids. Deviations of the solution results from the values calculated from the pure liquids were greater for the conjugated molecules, the solution result being larger. Molar refractions of both conjugated and non-conjugated molecules obtained from measurements in cyclohexane were larger than the corresponding results for benzene solution, the difference again being greater for the conjugated systems. Substitution of a chlorine or bromine for hydrogen on benzene produces a considerable enhancement of the molar refraction over that obtained for the analogous substitution on an aliphatic molecule. This enhancement, which is indicative of a loosening of the electronic structure in the conjugated system, decreases as additional halogen is substituted onto the ring. Similar enhancement is shown by substitution on 1,4-benzoquinone.

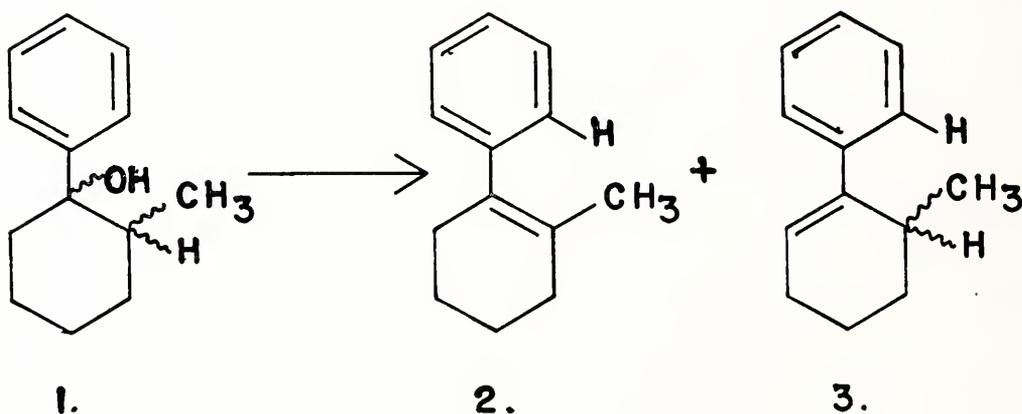
Ligand Steric Effects on Tungsten (0) Substitution Reactions. LOIS M. OUNAPU, JOHN H. RISLEY, J. A. MOSBO, and B. N. STORHOFF, Ball State University.—The *cis-trans* distributions of $\text{W}(\text{CO})_4\text{L}_2$ complexes obtained from the reaction of $\text{W}(\text{CO})_4(\text{tmpa}) + 2\text{L}$ ($\text{tmpa} = \text{N,N,N',N'}$ -tetramethyl-1,3-propanediamine) were studied for two series of electronically similar, but sterically different phosphine ligands. The two series of ligands included methyl-, ethyl-, isopropyl- and tert-butyldiphenylphosphine, and triphenyl-, tri-*p*-tolyl- and tri-*o*-tolylphosphine. The *cis-trans* ratios, obtained from ^{31}P nuclear magnetic resonance chemical shifts, were discussed in terms of the proposed reaction mechanism and ligand size as defined by cone angles.

Equilibria Between Diols and the NMR Shift Reagent $\text{Eu}(\text{fod})_3$. LOIS M. OUNAPU, P. L. ROCK, T. L. KRUGER, and J. A. MOSBO, Ball State University.—The solution equilibria between a difunctional molecule, S, and the lanthanide shift reagent $\text{Eu}(\text{fod})_3$, L, can include three product forms: LS and LS_2 , where the substrate functions as a monodentate ligand, and LS_2 , where a single substrate functions as a bidentate ligand. An iterative computer program was used to fit experimental and calculated NMR proton chemical shifts while optimizing the parameters of equilibrium constants and limiting chemical shifts. Results were compared from fits of (1) LS_2 parameters only, (2)

LS₁, and LS parameters only, and (3) LS₁, LS and LS₂ parameters for the strongly chelating *d,l*-2,4-pentanediol, the more weakly chelating *cis*-1,3-cyclohexanediol, and the non-chelating *trans*-1,3-cyclohexanediol. The viability of single equilibrium constant fits for each type of diol were discussed.

The Dehydration of 2-Methyl-1-Phenylcyclohexanol. HOWARD DUNN, ANDREW JORGENSEN, ROBERT DEWEESE and MICHAEL WALKER, Chemistry Department, Indiana State University-Evansville, Evansville, Indiana 47712.—Competing factors in the placement of a double bond upon dehydration of 2-methyl-1-phenylcyclohexanol, **1**, were studied. Dehydration of **1** resulting in the formation of 1-methyl-2-phenylcyclohexene, **2**, would place the double bond in the most substituted position. This would, however, lock the phenyl group into the same plane with the methyl group and thus lead to steric interactions. A dehydration product of 3-methyl-2-phenylcyclohexene, **3**, would relieve the steric strain but would place the double bond in a less substituted position.

The dehydration reaction was carried out and the products were isolated, quantified, and identified. A discussion of the results and spectral interpretation will be presented.



Phosphorous Ligand Cone Angles from MINDO/3 Optimized Geometries. JANICE T. DESANTO, P. L. BOCK, J. A. MOSBO, and B. N. STORHOFF, Ball State University.—The modified neglect of differential overlap (MINDO/3) technique was used to obtain optimized geometries (bond lengths, bond angles, and dihedral angles) of twelve phosphine compounds. Although computer program limitations precluded inclusion of compounds with greater numbers of atoms, calculations were performed on an additional five phosphines containing phenyl and substituted-phenyl groups by deleting atoms from portions of the phenyl rings. This appears to be a useful procedure, particularly for *o*-tolyl species, as illustrated by the excellent agreement between the calculated geometries of *o*-tolylphosphine obtained when all atoms were included versus that obtained when portions of the *o*-tolyl ring were deleted.

The MINDO/3 atom positions were used to calculate ligand cone angles, a measure of ligand size. The results were compared to previously published values obtained from measurements of CPK models.

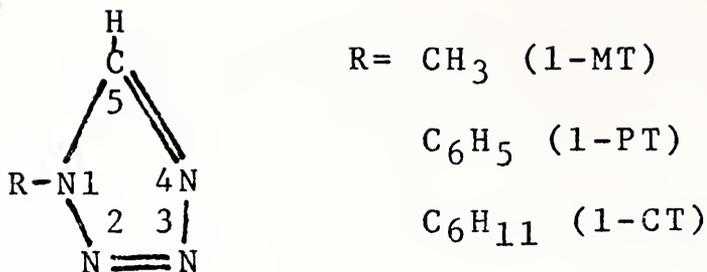
Ambidentate Phosphine Ligands. DANIEL P. HARPER and BRUCE N. STORHOFF, Ball State University.—Synthetic routes to several bidentate ligands, each with two types of donor sites, will be described. The ligands are of the types $R_2P(O)(CH_2)_nCN$, $R_2P(CH_2)_nCN$, $R_2P(CH_2)_nCO_2R$, and $R_2P(CN_2)_nOH$. The cyano-phosphines and oxides are obtained from R_2POCH_3 and $X(CH_2)_nCN$ ($X = Cl, Br$) *via* Arbuzov reactions. Thus, $(C_6H_5)_2P(O)(CH_2)_3CN$ is obtained in *ca.* 70% yield from $Br(CH_2)_3CN$ and $(C_6H_5)_2POCH_3$. The phosphines are obtained by selective reductions of the $P(O)$ function. The other functional groups, such as CO_2R , are subsequently obtained from the nitrile group.

The Chlorination of Several 2-Pyridones. DONALD J. COOK and LARRY BOARDMAN, DePauw University.—In some unreported work done at DePauw University over the past ten years it was noted that the reaction of elemental bromine on 2-quinolones and 2-pyridones in a carbon tetrachloride solution resulted in the formation of what appear to be 1:1 molecular complexes between the quinolones or pyridones and the bromine molecule. These complexes decompose to the starting materials on standing, can be used to brominate an alkene or can enter into a substitution reacting with methyl ketones. The present work was initiated to investigate the possibility that chlorine reacted with 2-pyridones in the same manner.

A study of the chlorination reaction has shown that no molecular complex is formed but the substitution of chlorine in the 3 or 5 position of the pyridone ring occurs and the 3- or 5-chloro-2-pyridone hydrochloride precipitates from the chloroform or carbon tetrachloride solvent. The chlorination of 3-methyl, 4-methyl, 5-methyl, 6-methyl, 1-methyl, 1,3-dimethyl, 1,4-dimethyl, 1,5-dimethyl, 1,6-dimethyl and unsubstituted 2-pyridones was studied.

The Stereospecificity of A New Photochemical Synthesis of β -Lactam Molecules. LYNN SOUSA, KEVIN WILLARD and MARCUS MCKINLEY, Ball State University.—The β -lactam ring is essential to the antibiotic activity of penicillin and cephalosporin medicinals. Generally, β -lactams are not active antibiotics unless substituents at ring carbons 3 and 4 have the *cis* geometry. We have tested the stereo-specificity of a new (Sousa, Johnson, and Fazio-unpublished work) photochemical β -lactam synthesis. Photolysis of *trans*-1,1-dioxo-2-(2-furyl)-1,3-dimethylthiazolidin-4-one produced the *trans*-4-(2-furyl)-1,3-dimethylazetididin-2-one (*trans* β -lactam) in moderate yield. Fortunately, the corresponding *cis* thiazolidinone gives mainly the desired *cis* β -lactam upon photolysis.

The Synthesis and Properties of Tris-(*u*-1-substituted tetrazole) hexa carbonyldimolybdenum Complexes. LAWRENCE L. GARBER, Indiana University at South Bend.—*Tris*-acetonitrilemolybdenum tricarbonyl reacts with various 1-substituted tetrazoles (molar ratio, 1:2) in the solvent methylene chloride at room temperature to form, almost quantitatively, $(CO)_3Mo(l-RT)_3Mo(CO)_3$. The structure of 1-substituted tetrazole is



All of the dimers formed show appreciable stability towards dioxygen in the state solid with the 1-MT complex exhibiting the least stability. These complexes exhibit very limited or no solubility in all common solvents except CH₂Cl₂ and acetone where only limited solubility is exhibited. Solutions of these complexes rapidly decompose upon exposure to dioxygen. The solvent DMSO reacts with these dimers resulting in the replacement of the tetrazole and forming a product believed to be, based upon IR and NMR data, *fac*-Mo(CO)₃(DMSO)₃.

The infrared spectra (solvent, CH₂Cl₂) of the 1-MT and 1-CT complexes each exhibit two intense carbonyl stretching frequencies at 1922 cm⁻¹ and 1805 cm⁻¹ and 1927 cm⁻¹ and 1813 cm⁻¹, respectively. These results suggest that the symmetry about each molybdenum atom is C_{3v} with a facial arrangement of ligands. In the case of the 1-PT complex the infrared spectrum shows considerable more complexity in the carbonyl region. A weak band is observed at 1948 cm⁻¹, an intense band at 1922 cm⁻¹ and a broad, intense, band centered at 1804 cm⁻¹ with shoulders at 1820 cm⁻¹, 1816 cm⁻¹, and 1796 cm⁻¹. This suggests that the symmetry about each molybdenum is less than C_{3v} which indicates possible interaction between the two molybdenum tricarbonyl moieties. Similar results were observed in the solid state for all three complexes.

These tribridging complexes may be treated by the Cotton-Kraihanzel method assuming that the above assignments are correct. The stretching carbonyl force constant and interaction constant for the 1-MT and 1-CT in mdyn/Å are 13.74, 0.59 and 13.85, 0.57, respectively. This result suggests that the interaction between molybdenum and the bridging tetrazoles involves some π-bonding as well as σ-bonding.

All proton NMR signals for the complexes are shifted downfield from those observed for the uncomplexed tetrazoles. A considerable downfield shift (0.66 ppm for 1-CT complex, 0.71 ppm for 1-PT, 0.72 ppm for 1-MT) is observed for the proton bonded to the ring carbon. These shifts indicate an increase in C-H electron densities.

The bonding of the tetrazole to the molybdenum atoms in these dimers most likely involves N(3) and N(4). Reasons for this will be presented.

The Application of Isoelectric pH and Isoelectric Point (*Isokinetic Potential*) for Solids—Liquid Separation. ROBERT H. L. HOWE, West Lafayette, Indiana and ROBERTA C. HOWE, Seattle, Washington.—The principle of applying the isoelectric pH and isoelectric point (*Isokinetic Potential*) to solids—liquid separation in a colloidal system is discussed. Examples of determining the isoelectric point are presented.