

CHEMISTRY

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ABSTRACTS

The Effects of Phosphorus Ligand Size on the *cis:trans* Distributions in $W(CO)_4LL'$ Complexes. DANIEL V. BROWN, DENNIS A. DRAKE, CONSTANCE A. KIESLER and JOHN A. MOSBO, Department of Chemistry, Ball State University, Muncie, Indiana 47306. — Thirteen phosphines (L') have been reacted with $W(CO)_4L(\text{pyridine})$ complexes ($L = PPhMe_2, PPh_2Et$ or $P(p\text{-tolyl})_3$) to produce $W(CO)_4LL'$ products. The *cis:trans* product ratios, measured by integration of 31-phosphorus nmr spectra, generally decreased as the size of L' increased. Thus, for example, when $L = PPh_2Et$, the *cis:trans* ratios decreased in the L' order $PPhMe_2 > PPh_2H > Pet_3 \sim PBu_3 \sim PPh_2Me > PPh_2Et > PPh_2(\text{iso-Pr}) > PPh_3 \sim P(p\text{-tolyl})_3 \sim PPh_2(\text{tert-butyl})$. These results are consistent with a rapid basal-apical equilibrium of a square pyramidal $W(CO)_4L$ intermediate.

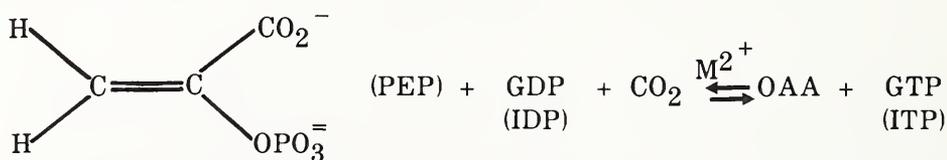
Computer-Controlled Multiple Standard Addition System for Use with Ion Selective Electrodes. STANLEY L. BURDEN and STEPHEN H. FLOWERS, Departments of Chemistry and Computer Science, Taylor University, Upland, Indiana 46989. — An Apple II Plus microcomputer is used to control the delivery of standard solution from a constant rate buret into a solution being analyzed. The computer automatically logs the potential of electrodes immersed in the solution after equilibrium has been reached. The user may specify the number of additions and either the volume of standard to be added for each addition or the magnitude of electrode potential change before the addition is terminated. The user may elect to determine either the electrode slope or the concentration of an unknown solution. In either case the computer computes both the individual results from each addition as well as the average and relative standard deviation of the series. An optional high resolution graphics display of a conventional standard addition plot of the data fit with a least squares line is also available. The system has been tested with both nitrate and chloride ion selective electrodes. Accuracies and precisions comparable to or better than manual standard addition methods are typical but very significant savings in time and analyst effort are realized.

The Isolation and Characterization of a Denaturant-stable Bacterial Protease. NEAL E. COLEMAN and ERIC R. JOHNSON, Department of Chemistry, Ball State University, Muncie, Indiana 47306. — A new protease component that is both stable and active in the presence of 6.0 M guanidinium chloride has been isolated from Pronase, a commercially available mixture of proteases isolated from *Streptomyces griseus*. This protease has exhibited esterase activity against N- α -acetyl-L-tyrosine ethyl ester (ATEE), a synthetic substrate for chymotrypsin, both in

the presence and absence of 6.0 *M* guanidinium chloride. This newly isolated protease comprises approximately 10% of the total denaturant-stable ATEE esterase activity found in the Pronase mixture. The unique ion-exchange chromatographic behavior and electrophoretic mobility suggest that this proteolytic enzyme is different than the two denaturant-stable Pronase proteases that have been previously described (W.M. Awad, Jr., *et al.* (1972) *Proc. Nat. Acad. Sci (USA)* 69 2561-2565).

Stereoselective Ligand Interactions with Phosphoenolpyruvate Carboxykinase.

THOMAS DUFFY, MYOUNG HEE LEE and THOMAS NOWAK, University of Notre Dame, Notre Dame, Indiana 46556. — Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the reversible carboxylation of phosphoenolpyruvate (PEP), with concomitant phosphoryl transfer to a nucleotide acceptor, to form oxaloacetate (OAA) and GTP. Analogues of substrates PEP, GDP and GTP have been used to study



specific ligand-enzyme interactions with this enzyme. Analogues of the substrate PEP include E and Z-2-phosphoenolbutyrate (E-PEB and Z-PEB), E and Z-3-fluorophosphoenolpyruvate (E-F-PEP and Z-F-PEP), and Z-3-bromophosphoenolpyruvate (Br-PEP). Only Z-F-PEP showed substrate activity (42% V_{\max}) with the Mn^{2+} activated enzyme. E-F-PEP was a competitive inhibitor ($K_I = 23 \mu\text{M}$). The PEB diastereomers exhibited stereoselective inhibition (K_I , Z = $32 \mu\text{M}$; K_I , E = $1.5 \mu\text{M}$). PRR titration studies show that $K_I = k_d$ for these ligands and the enzyme-Mn complexes containing Z-PEB and F-PEP resemble the complex containing PEP. Thiol analogues of the nucleotides in which a nonbridge oxygen is substituted by sulfur showed selectivity in interactions. The R and S diastereomers of GTP- α S both demonstrated substrate activity with a V_{\max} ratio (R/S) of 0.5 - 1.8. The GDP- α S pair showed a ratio of 3-5; the ratio was dependent upon the divalent cations used. GTP- β S exhibited dramatic selectivity with a V_{\max} ratio of 0.005 - .01. GDP- β S showed no substrate activity whereas GTP- γ S did. The kinetic studies with PEP analogues suggest steric effects are stereoselective at the C-3 position but are not detrimental to ligand binding but perhaps block catalysis. Results with nucleotide analogues indicate the α -P is not very sensitive to events during catalysis, however the β -P where the reaction occurs shows strict selectivity.

Enthalpy of Nucleophilic Addition to Enenitriles. JANE E. HINNERS and TERRY KRUGER, Ball State University, Muncie, Indiana 47306. — Nucleophilic attack by the 1°, 2°, and 3° amines on cyclohexylidenemalononitrile yields monoadducts. The temperature change accompanying each reaction was recorded in a Parr solution calorimeter. The heat of reaction was calculated. The enthalpies were correlated with the proton affinity of each amine. The structure of the adducts were studied by nuclear magnetic resonance and infrared spectroscopy at different time intervals.

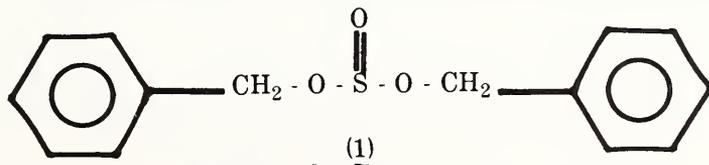
A Spectrophotometric Investigation of the Reaction Between Aqueous Cadmium(II) Ions and Electrolytically Generated Hydroxide Ions. A.J.C.L. HOGARTH, Department of Chemistry, Depauw University, Greencastle, Indiana 46135 and T. S. WEST, Director, Macauley Institute for Soil Research, Craigiebuckler, Aberdeen, Scotland. — Spectroelectrochemical techniques have made a modest impact on the world of analytical chemistry in the last decade-and-a-half. The products of

research have been thin film cells and sometimes valuable observational data relating to electrochemical reactions. This paper considers a somewhat different approach not using optically transparent cells, but a generally simpler experimental arrangement. Hydroxide ions were generated at an anodically pretreated platinum plate electrode, and their reaction with aqueous cadmium(II) ions observed by attenuations to a collimated beam of radiation passing through the solution and over the electrode surface. The instrumentation and experimental results are discussed briefly.

A 4-H Theory of Photosynthesis by Green Plants. ROBERT H. L. HOWE, Eli Lilly and Company, Lafayette, Indiana 47809. — A new interpretation of the chemical mechanism of oxygen production by algae is advanced by the author through his many years of investigation, as based on the accepted theory that H_2O is the H-donor during the process of photosynthesis. Therefore, the 4H will be required and 4OH will be released from water. Then, $2\text{H}_2\text{O}_2$ will be formed and one molecule of O_2 is later generated. The explanation of this interpretation is presented, the peroxide route reaction pathways are discussed, and the significance of the phenomena is related.

The Proteolytic Action of Guanidine-stable Chymoelastase on Low Molecular Weight Peptides. KATHERINE J. JORDAN and ERIC R. JOHNSON, Department of Chemistry, Ball State University, Muncie, Indiana 47306. — Guanidine-stable chymoelastase, a denaturant-stable proteolytic enzyme produced by the K-1 strain of *Streptomyces griseus*, has been reported to preferentially catalyze the hydrolysis of protein substrates at peptide bonds on the C-terminal side of phenylalanine, tyrosine, and leucine in the absence of denaturant (Y. Narahashi and K. Yoda (1973) *J. Biochem. (Tokyo)* 73 831-841). The results of the current study indicate that, in the presence of denaturant, phenylalanyl and tyrosyl peptide bonds of low molecular weight peptide substrates are hydrolyzed preferentially under the catalytic influence of this enzyme. However, leucyl peptide bonds in three different low molecular weight peptides were not hydrolyzed by this enzyme in the presence of denaturant. Although some denaturant-dependent differences in cleavage specificities are apparent, these observations indicate that guanidine-stable chymoelastase does show cleavage specificity for phenylalanyl and tyrosyl peptide bonds in the presence of 6.0 M guanidinium chloride.

Singlet and Triplet Mechanisms in the Photochemistry of Benzyl Sulfite. ROBERT J. OLSEN, BRAD D. MAXWELL and C. ALLEN RIDGEWAY, Department of Chemistry, Wabash College, Crawfordsville, Indiana 47933. — The photorearrangement and photofragmentation reactions of benzyl sulfite (1) have been examined.



Direct irradiation in benzene results in the formation of benzyl alcohol, benzyl ether and benzyl phenylmethanesulfonate as the major products. The quantum yield for disappearance of 1 is 0.39 at 254 nm and the reaction is only weakly quenched by isoprene. A crossover experiment indicates that approximately 85% of the benzyl ether is formed by a recombination reaction occurring within a solvent cage.

Acetone sensitized photolysis in benzene at 300 nm affords benzyl alcohol

and bibenzyl as major products. A crossover experiment indicates that the bibenzyl is formed outside the initial solvent cage.

A mechanism to account for these observations will be presented.

Inhibition of ADP-Induced Aggregation of Human Platelet-Rich Plasma Suspensions by the β,γ -Phosphonic Acid Analog of ATP. B. H. RAGATZ, School of Medicine, Indiana University, Center for Medical Education, Fort Wayne, Indiana 46805, P. G. IATRIDIS, School of Medicine, Indiana University, Center for Medical Education, Gary, Indiana 46408 and S. G. IATRIDIS, School of Medicine, University of Athens, Athens, Greece.—Using washed platelet-rich plasma (PRP) suspensions from rabbit, Packham and coworkers found that various naturally occurring nucleoside triphosphates (NTP) inhibited ADP induced aggregation. They also determined that these compounds served as substrates for platelet nucleoside diphosphokinase (NDK). They postulated that NDK usually catalyzed the transfer of membranbound phosphate to ADP as a primary event in aggregation but that various NTP compounds could substitute as phosphate donors and thus block dephosphorylation of the platelet surface.

To test this hypothesis, Born and Foulks incubated rabbit PRP with β,γ -methylene ATP, an ATP analog which cannot be easily hydrolyzed by IM hydrochloric acid or by various phosphatases. They found good inhibition by β,γ -methylene ATP if ADP was added to the PRP 10 seconds later and concluded that labile terminal phosphate is not required on an NTP for ADP-induced aggregation to be inhibited. They suggested that NTP compounds merely bind competitively to the platelet ADP receptor site.

We have expanded the experiments and confirmed the conclusions of Born and Foulks using human PRP suspensions. The following observations were made:

a) The compound, β,γ -methylene ATP, alone showed no intrinsic effects on the light transmitted through a PRP suspension.

b) High doses of β,γ -methylene ATP inhibited the aggregation of PRP exposed to ADP 30 seconds later, but not as extensively as equimolar amounts of ATP.

c) No time dependent changes were observed in the inhibitory profiles when ATP or β,γ -methylene ATP was preincubated with PRP for 5 minutes before ADP addition.

It remains to be investigated if these observations can be generalized to the platelet ADP receptors of other mammalian species.

Potential Method for the Improved Determination of Lecithin/Sphingomyelin Ratios in Patient Amniotic Fluid Samples. B. H. RAGATZ and B. A. OTFINOSKI, School of Medicine, Indiana University, Center for Medical Education, Fort Wayne, Indiana 46805.—Last year, we reported the optimization of conditions for separation of seven phospholipids by silica gel chromatography and their detection by ten different reagents. Among the seven phospholipids separated, two are of published importance in assessment of fetal lung maturity when extracted from amniotic fluid. These two are lecithin and sphingomyelin.

More recently we have prepared chloroform/methanol extracts of 37 amniotic fluid samples taken from patients at three different hospitals. The concentrated extracts have been spotted on air dried silica gel coated plates, along with lecithin and sphingomyelin standards and developed in CHCl_3 :methanol:30% NH_4OH (68:28:4). Resolved lecithin and sphingomyelin have been detected with iodine vapor, 1-anilino-8-naphthalene sulfonate (ANS) (50 mg/100 ml water), rhodamine B (50 mg/100 ml methanol), and 1, 6-diphenyl-hexatriene (DPH) (10 mg/100 ml dichloromethane).

Standard recoveries of lecithin/sphingomyelin mixtures(2:1) using the various detection systems have been calculated and ANS yields the best recovery observed. Using a subpopulation of calculated ratios from one hospital (determined by the Helena Laboratories method), we have found that our results with ANS detection are not statistically different. Data on subsequent deliveries of three infants from this subpopulation indicate that lung development was normal as predicted by both clinical tests.

Effect of the Presence of a *Clostridium* sp. on Fecal Bile Acids of a Gnotobiotic Gerbil. BERNARD S. WOSTMANN AND MARGARET H. BEAVER, Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556. — The gerbil appears to be a better model for the study of cholesterol and bile acid metabolism than the rat, mainly because its bile acid pattern lacks the muricholic acids and their secondary derivatives. Its body cholesterol pools are sensitive to dietary cholesterol. When fed 0.1% of cholesterol with the diet (average U.S. human intake) serum cholesterol will range between 250 and 400 mg/dl.

The germfree (GF) gerbil would appear to be an ideal baseline for the study of microbial effects on sterol metabolism. However, GF gerbils do not reproduce, due to excessive cecal enlargement. Association with a murine-derived hexaflora consisting of *Lactobacillus brevis*, *Streptococcus faecalis*, *Staphylococcus epidermidis*, *Bacteroides fragilis*, var. *vulgatus*, *Enterobacter aerogenes*, and a *Fusibacterium* sp. reduced cecal size and made reproduction possible. This hexaflora will deconjugate most intestinal BAs, but little further secondary modification occurs. Total fecal BA excretion is, surprisingly, substantially higher than in the conventional (CV) gerbil. Upon introduction of a *Clostridium* sp. this pattern changes. Deconjugation is still almost complete but 1/3 of BA is now modified further, resulting largely in keto-acid formation with limited 7 α -dehydroxylation. The fecal neutral sterol fraction showed only cholesterol. The CV microflora, on the other hand, largely 7 α -dehydroxylates the primary BAs, with limited keto-acid formation. The fecal neutral sterol fraction contained cholesterol and some coprostanol, but no coprostanone.

Total fecal BA excretion of this hepta-flora associated gerbil is now in the CV range. The data indicate that, while in the GF gerbil approximately 1/3 of biliary BA is chenodeoxycholic acid and 2/3 cholic acid, the relative proportion of cholesterol catabolized via chenodeoxycholic acid declines with increasing complexity of the microflora. The addition of the *Clostridium* sp. thus leads to substantial keto-acid formation and "normalization" of the amount of fecal BA excreted. A next step will be to introduce a gerbil-derived *E. coli* in the hope of introducing 7 α -dehydroxylating capacity comparable to that of the CV microflora.

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