

CHEMISTRY

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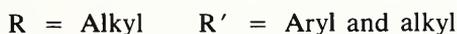
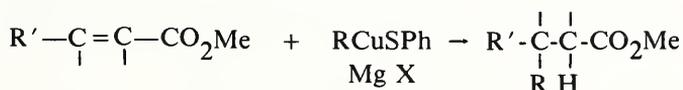
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ABSTRACTS

Modification of the Classical Preparative Method of Carbonyl 2,4-Dinitrophenylhydrazones. JOSEPH L. BOLAN, MICHAEL FLYNT and MOHAMMAD BEHFOROZ, Department of Chemistry and Burriss Laboratory School, Ball State University, Muncie, Indiana 47306.—Frequently, the existing classical method for the preparation of 2,4-dinitrophenylhydrazones of Carbonyl compounds gives lower melting derivatives, even after recrystallization of the products. We found that by a slight modification of the general procedure this problem can be solved, and the results can be reproducible and improved appreciably. The modification simply involves a wash of the 2,4-DNP crystals with a 5.0% sodium bicarbonate solution. A series of aldehydes and ketones were selected and their 2,4-DNP derivatives were prepared by both methods and their melting points were compared. It was found that: a) in nearly all cases the melting points of the derivatives obtained by the modified method were higher than those obtained by the old method and b) the modified method gave more reproducible results.

Regiospecific Addition of Organocuprate Reagents to α , β -unsaturated Esters. JOSEPH L. BOLAN and MOHAMMAD BEHFOROZ, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—A series of organocuprate complexes were made *in situ* by the reactions of the corresponding Grignard reagents with cuprous thiophenoxide (PhSCu) and the reactions of these complexes with, α , β -unsaturated esters were studied:



High yields of saturated esters when obtained when R' is aryl. No detectable amounts of 1,2-addition products even with a hindered alkyl group such as *t*-butyl were obtained. This indicates the regiospecificity of this reaction and its potential application in organic synthesis. The result of the studies on the addition of cuprate complexes to aliphatic esters (R = alkyl) also will be reported.

On-line Data Acquisition and Reduction System for Lineweaver-Burk Enzyme Kinetics Studies Using a U.V.-Visible Spectrophotometer and an Apple II Plus Microcomputer. STANLEY L. BURDEN, Department of Chemistry, Taylor University, Upland, Indiana 46989, and STEVEN E. NYGREN, NALCO Chemical Company, Chicago, Illinois 60638.—Software has been written and hardware configured to interface an Apple II Plus microcomputer to a Coleman 124 u.v.-visible spectrophotometer and log absorbance vs. time data for enzyme kinetics experiments as well as do a Lineweaver-Burk analysis. The software was written to utilize the commercially "ADALAB" and

“ADA AMP” but all software was written to optimize the enzyme kinetics studies. No commercial software was used. The system is menu driven and provides options to plot A vs. t data in real time and store this data as a binary file on a floppy disk. The data can then be rescaled, labeled and replotted on the monitor and/or printer. The user can then request a least squares fit to be made of any desired segment of the data to determine the best approximation of the initial slope. Initial slope and substrate concentration data is then entered and a Lineweaver-Burk analysis is performed. The constants of interest are determined from a least squares fit to this data and a plot is made using high resolution color graphics. The system was tested using the ADH - methanol system and the results were superior to those from hand computations.

Concentration and pH Dependent Stability of Sodium Thiosulfate Solutions. SHRIKRISHNA W. DHAWALE and BRIAN SHORT, Department of Chemistry, Indiana University East, Richmond, Indiana 47374.—In the present investigation, the effect of various factors such as concentration, pH, and presence of some metal on the stability of $S_2O_3^{--}$ ion in aqueous solutions has been studied.

Sodium thiosulfate slowly changes in titer with lapse of time. Thiosulfate solutions at various concentrations and pH values were titrated at time intervals. Some short term pH dependent stability experiments were performed. Effect of the presence of metal was examined in a similar way.

Some of the general conclusions of the present work are:

- (1) At lower concentrations, $S_2O_3^{--}$ ions are destabilized to greater extent.
- (2) At about the same pH range, less concentrated solutions are more unstable.
- (3) Amongst metals or 304 stainless steel, copper has more destabilizing effect on $S_2O_3^{--}$.
- (4) The lower the concentration of solution, the greater is the effect of copper.
- (5) pH titration of $S_2O_3^{--}$ against HCl or H_2SO_4 show buffering action. The pH range of strong buffering action depends on $S_2O_3^{--}$ concentration.
- (6) Metals like Ni and Zinc dissolve, forming their ions. Some crevice corrosion in 304 stainless steel was detected.

Further studies are in progress.

The Reaction of Aroyl Chlorides with 1,1-Dichloroethene: The First Reported Synthesis of β,β,β -Trichloropropiophenones. H. E. DUNN, B. G. ADDISON, P. A. PETERS, and M. J. PHILLIPS, Department of Chemistry, Indiana State University Evansville, Evansville, Indiana 47712, and C. Y. Meyers and T. N. Schmitz, Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Illinois 62901.—It has been reported in the literature that β,β -dichloroacrylophenones can be prepared by the reaction of the appropriate aroyl chloride with 1,1-dichloroethene in the presence of aluminum chloride. By a slight modification of this procedure we were able to prepare a variety of ring substituted β,β,β -trichloropropiophenones, a previously unreported class of compounds. The β,β,β -trichloropropiophenones can be converted into the corresponding β,β -dichloroacrylophenones by heating, or by treatment with triethylamine. When stored in a glass container at room temperature in the laboratory, β,β,β -trichloropropiophenone, an oil, was found to slowly emit hydrogen chloride and convert to β,β -dichloroacrylophenone. On the other hand, p - β,β,β -tetrachloropropiophenone, a white crystalline solid, appears to be quite stable under the same storage conditions. The synthesis, spectra, and reactions of this new class of compounds will be discussed.

The Design of a High Pressure Liquid Chromatography-based System for the Quantitative Determination of Denaturant-stable Protease Cleavage Specificities. JEFFREY

A. GORDON and ERIC R. JOHNSON, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—High Pressure Liquid Chromatography was utilized in a system designed to obtain quantitative data for the hydrolytic cleavage of small peptides catalyzed by guanidine-stable chymoelastase. This system is based on the fact that, each time a peptide of known amino acid sequence is cleaved, a free alpha-amino group is produced. This N-terminal amino acid can be identified and directly related to the site of cleavage within the peptide. Upon cleavage of the peptide by guanidine-stable chymoelastase, the newly produced N-terminal groups were then labelled with dansyl chloride and the remaining peptide bonds were hydrolyzed with 6.0 *M* hydrochloric acid. The dansylated amino acids were then separated by reverse-phase isocratic high pressure liquid chromatography and detected with a fluorescent spectrophotometer. By comparison of the separated dansylated amino acids with standards, it was possible to identify where the peptide cleavage had occurred. Furthermore, the chromatographed peaks for the dansylated amino acids were integrated in order to quantitate the extent of cleavage occurring at the hydrolysis sites. It was also found that, without any adverse effects to the dansylation process or to the HPLC separation of the dansylated amino acids, this system can operate efficiently with the presence of 6.0 *M* guanidinium chloride in the peptide hydrolysate. These observations show that the system can be used for the identification of peptide cleavage sites for denaturant-stable proteolytic enzymes functioning in the presence of denaturant.

A Comprehensive One-year Chemical Study of Acid Precipitation across Central Indiana. WILLIAM GROSS and JAMES P. RYBARCZYK, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—Rain events from October 1982 to October 1983 were collected throughout central Indiana by volunteer members of Rotary International District 656. The area of interest encompassed 25 separate sampling sites and extended from West Lebanon and Rockville in the west to Union City and Richmond in the east. Over 1100 samples were collected and analyzed from 115 individual rain events. The rain water was analyzed for pH, ion conductivity, Ca ion, Mg ion, Na ion, K ion, sulfate ion, and nitrate ion. Each sample was also categorized by such meteorological conditions as wind direction, pressure movements, temperature, and time. The "average" pH across this region was found to be in the 4.0 - 4.3 range, with a seasonal change to a 3.2 - 3.5 average in hot summer thunderstorms. Little pH or ion concentration variations were noted across the state, suggesting that Indiana neither enhances nor decreases the acid content of already acidic rain that enters its borders.

Exploratory Experiments in Thermometric Titrimetry Using Hypohalites as Oxidimetric Reagents. DEBORAH L. GUTTMAN, Eli Lilly Company, Indianapolis, Indiana 46296 and A. J. C. L. Hogarth, Department of Chemistry, DePauw University, Greencastle, Indiana 46135.—Since the early fifties thermometric titrimetry has been shown to be a useful method for quantitative analysis, and many chemical systems have been investigated. One type of chemical reaction that has been neglected until recently is the REDOX system, and only a few workers have reported results. This is a peculiar oversight since it is known that considerable heats of reaction may be involved, in some cases as many as 20 Kcal/electron.

This paper deals with results obtained in a preliminary investigation of the reaction between selected ammonium salts and urea, and alkaline solutions of hypohalites. It is shown that the method, even with the use of classical apparatus, may be used to determine the percentage of nitrogen in certain fertilizers. It is clear that the method is limited by the sensitivity of the thermometer and that it is not selective, *ie*: if a mixed fertilizer were to be analyzed, the two or more components could not be determined individually. Precision for the method was found to be better than 3.0% (rsd)

for single compounds, and a single analysis was found to take no more than about one minute.

Permanganate Oxidations of Aldehydes in Non-aqueous Media. F. J. HADLEY, J. BONDI and I. SCHWARTZMAN, Department of Chemistry, Wabash College, Crawfordsville, Indiana 47933.—Permanganate oxidations have seen infrequent use as a synthetic tool because of solubility problems necessitating two phases. The recent use of crown ethers as phase transfer catalysts for solubilizing potassium permanganate in benzene and other non-polar solvents allows oxidation reactions to proceed in a single organic phase. We have investigated the kinetics of aldehyde oxidations in chlorobenzene. In particular, rate laws and thermodynamic parameters were determined for a series of aliphatic aldehydes. Also, Taft plots were constructed, and ESR spin-trapping studies were undertaken in order to identify the nature of the transition stage. Finally, a kinetic isotope study showed that C-H bond fragmentation was involved in the rate step. Comparisons were made with the accepted mechanism in aqueous acidic solution.

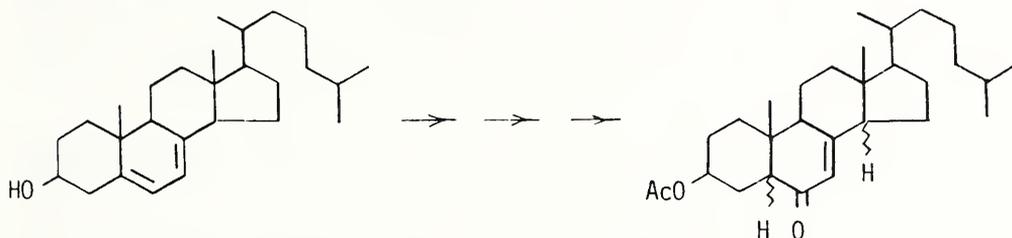
A Kinetic Study of the Reaction of $W(CO)_4(\text{phosphine})(\text{pyridine})$ Complexes with Phosphines. CHERYL K. HOSTETLER and JGHN A. MOSBO, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—The reactions of $W(CO)_4(L)(\text{pyridine})$ complexes with L' (L and L' are phosphines) produce *cis* and *trans* $W(CO)_4(L)(L')$ products plus pyridine. Two kinetic studies of this reaction have been performed with $L = PPh_2Et$. In the first, the rates of reaction were determined as a function of ligand concentrations. Complex to reactant ligand ($L' = PPh_2Et$) ratios of 1:2, 1:4, 1:8 and 1:16 were utilized. In the second study, three different reactant ligands ($L' = P(n-Bu)_3$, $PPhMe_2$, or PPh_2Et) were employed using identical complex to ligand ratios of 1:4. Concentrations of the tungsten starting materials and products as a function of time were determined by periodic integration of ^{31}P nmr spectra. The rate data obtained from both studies were consistent with a first order reaction. The results were discussed in terms of a proposed mechanism.

Quantitative Assessment of the Cleavage Preference of Guanidine-stable Chymoelastase in the Presence of Denaturant. REBECCA M. LAMKIN and ERIC R. JOHNSON, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—Guanidine-stable chymoelastase, a bacterial protease known to be stable and active in denaturant, was studied with respect to its cleavage preference in 6.0 *M* guanidinium chloride. Peptides of known amino acid sequence were incubated with the stable protease in the presence and absence of 6.0 *M* guanidinium chloride. The new N-terminal peptide groups produced by the protease action were then labelled with dansyl chloride. Following hydrolysis, the dansylated amino acids were identified and quantitated by reverse phase high pressure liquid chromatography. In the absence of the denaturant, specific cleavage occurred on the C-terminal side of phenylalanyl, tyrosyl, and leucyl peptide bonds, which is in agreement with previous studies. In the presence of denaturant, the rate of peptide bond cleavage was found to be substantially lower. However, the peptide substrates were hydrolyzed at the same positions, which indicates that this stable enzyme retains its cleavage preference for phenylalanyl, tyrosyl, and leucyl peptide bonds in the presence of denaturant.

Rapid Colorimetric Estimation of Acetate in Commercial Vinegar. PATRICIA L. LANG and ROBERT E. VAN ATTA, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—The modified basic ferric acetate qualitative test for acetate ion has been adapted to the quantitative estimation of that anion in commercial vinegars. A 5-ml sample of centrifuged vinegar is roughly neutralized with ammonia, then diluted to 10 ml. A 5-drop (0.25-ml) portion of this prepared solution is treated with 5 drops of 25.0% ferric nitrate, diluted with 5 ml of water, mixed and allowed to stand for

2 minutes for color development. The red-brown color is compared at 430-450 nm with that from a single standard, similarly prepared from a 3.0 volume percent acetic acid solution. The solution absorbance is linear with concentration from 0.5 to 3.5 volume percent acetate and is stable for at least an hour after color development. Results for a variety of commercial vinegars generally agree with pH-potentiometric titration results within 2-3 relative percent. The colorimetric procedure requires less than 10 minutes for completion and is quite appropriate for high school laboratory operations in limited-time laboratory sessions with inexpensive equipment.

Isomerization of the 5- and 14- Positions of Δ^7 -6-Oxo Steroids. JOHN McCUNE and BAHMAN NASSIM, Department of Chemistry, Indiana University Southeast, New Albany, Indiana 47150 and JERRY R. DIAS, Department of Chemistry, University of Missouri-Kansas City, Kansas City, Missouri 64110.—Cholest-7-en- 3β -ol-6-one acetate was synthesized from cholest-5, 7-dien- 3β -ol in three steps. The 5- and 14- positions of the enone were then isomerized by two different methods, each showing a high degree of regiospecificity. One method employed an acidic catalyst, p-toluene sulfonic acid; the other a basic catalyst, potassium tertiary butoxide. The acid catalyzed reaction favored 14α and 14β epimers while the base catalyzed reaction selectively isomerized the 5- position. Synthesis, separation and spectral measurements of the isolated isomers will be discussed.



Mass Spectral Fragmentation of Novel Steroidal Ketones and Ketals. BAHMAN NASSIM, Department of Chemistry, Indiana University Southeast, New Albany, Indiana 47150 and PIERRE CRABBÉ, Division of Scientific Research and Higher Education, UNESCO, 7, Place de Fontenoy, 75700 Paris, France.—Electron impact induced fragmentation of several A-nor, A-nor-18-homo and A-nor-D-homo steroidal ketones and their synthetic precursors are examined and mechanistic interpretation of some of the processes involved in their molecular breakdown are presented. As has been described by Djerassi and co-workers, extrusion of C_3H_4O and C_3H_5O from D-ring and usually the expulsion of the alkyl group from the 13-position of 17-oxosteroids are initiated by a preliminary ionization of the 17-ketone. It will be shown that the integrity of this initial charge development is dependent on the existence and nature of other functional groups present in the molecule. Therefore, the abundance of ions generated from these ketones can vary in a wide range. In the 17-oxo-D-homo steroids, a similar loss of 13-alkyl is observed, but the two losses seen from the 5-membered 17-oxo-D-ring are replaced by losses of C_2H_3O and C_4H_7O from the 6-membered 17-oxo-D-ring. 13-Ethyl-17-oxo steroids, in addition to extrusion of 13-alkyl group, lose ethylene through a McLafferty rearrangement, initiated by ionization of 17-keto group. Among other things, structural requisite for elimination of $C_2H_4O_2$ from odd electron ketal ions and generation of acetylacetone from a β -diketone steroid derivative, via two consecutive McLafferty rearrangements, will be defined.

Evaluation of Fluorescent Membrane Probes as Potential Detectors of Resolved Amniotic Fluid Phospholipids. BARTH H. RAGATZ and GINA M. MODRAK, Fort Wayne Center for Medical Education, Indiana University School of Medicine, Fort Wayne, Indiana 46805.—We have evaluated ten fluorescent membrane probes as potential

detectors of important phospholipids [phosphatidyl choline (LEC), sphingomyelin (SPH), and phosphatidyl glycerol, (PG)] which can be separated by thin layer chromatography from organic solvent extracts of human amniotic fluid. Detection of these compounds remains important in the assessment of fetal lung development.

We have evaluated the following parameters associated with these fluorescent sprays: sensitivity, color, stability, linearity of response, and limit of compound detection. Considering all these parameters, we have determined that 1-anilino-8-naphthalene sulfonate is the most useful fluorescent compound for this purpose. Other probes with some desirable characteristics are 5-dimethylamino-1-naphthalenesulfonyl-N-phosphatidyl ethanolamine and N-(5-dimethylamino-1-naphthalenesulfonyl)-L- α -phosphatidyl ethanolamine. Among the probes which are of no value in the present application are 5-carboxyfluorescein, 6-carboxyfluorescein, and 3-azido-2,7-naphthalene disulfonate (which must be photochemically activated and is quite unstable).

Hydrolysis of the Adenine Nucleotides, AMP and ADP, and Selected Purine and Phosphate Modified Analogs, BARTH H. RAGATZ and GINA M. MODRAK, Fort Wayne Center for Medical Education, Indiana University School of Medicine, Fort Wayne, Indiana 46805.—Using a Varian 5000 high pressure liquid chromatograph, we have determined the susceptibility to metal ion catalyzed hydrolysis of the following compounds: adenosine-5'-monophosphate (AMP); adenosine-5'-diphosphate (ADP); 1, N⁶ etheno AMP (E-AMP); 1, N⁶ etheno ADP (E-ADP); N⁶ (γ , γ -dimethylallyl) ADP (DMA-ADP); α , β -methylene ADP (m-ADP); and adenosine-5'-phosphoramidate (AMP-NH₂).

At 56 °C in pH5.0 sodium acetate buffer, 0.1M, with metal ion present in excess of the nucleotide, we have observed the following;

a) Virtually no ADP or AMP hydrolysis occurs over a six hour period in metal ion free-or Cu⁺⁺ containing solutions. Dramatic hydrolysis (AMP ADP) occurs when Cu⁺⁺ is present.

b) Cu⁺⁺ catalyzed hydrolysis of E-ADP progresses more slowly than hydrolysis of ADP, whereas E-AMP is not hydrolyzed under present conditions.

c) The Cu⁺⁺ catalyzed hydrolysis of DMA-ADP is comparable to that of ADP.

d) With the phosphate modified analogs, m-ADP and AMP-NH₂, hydrolysis does not occur under the stated conditions.

We conclude that charge distribution and size of substituents attached to the purine ring are both important determinants of the rates of hydrolysis of ADP-like compounds. Furthermore, phosphonate or phosphoramidate linkages are considerably more stable to hydrolysis at pH5 and 56 °C than the corresponding diphosphate.

The Synthesis and Study of Functionalized Phosphines: Phosphine-nitrile and Phosphine-amine Ligands with Long Spacer Chains. PATRICK J. SELBY and BRUCE N. STORHOFF, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—A series of mono- and bidentate phosphines which also contain nitrile or amine groups have been synthesized and subsequently used to prepare metal complexes. Monodentate phosphine-nitrile ligands of the type Ph₂P(CH₂)_nCN (n = 1,3,4,10) were obtained by reducing (Ph₂SiH₂) the Arbuzov products (Ph₂POC₂H₅ + X(CH₂)_nCN). Bidentate phosphine-nitrile ligands were obtained from [Ph₂P(CH₂)₃P(Ph)]⁻ and X(CH₂)_nCN (n = 4,10). The nitrile groups in these ligands were reduced to amines by AlH₃, LiAlH₄, or BH₃·THF. These new functionalized phosphines have been characterized by multinuclear nmr studies of the free ligands and of metal derivatives. A tungsten complex of the stoichiometry W(CO)₄L (L = Ph₂P(CH₂)₃P(Ph)(CH₂)₁₁NH₂) was attached

to aldehyde-functionalized glass beads. Fourier transform ir studies of the derivatized beads clearly showed the presence of the expected CO and CN stretching bands.

Detection of Trace Substances by Gas Chromatography—Mass Spectrometry. JOSEPH R. SIEFKER and BRADLEY K. YOWELL, Department of Chemistry, Indiana State University, Terre Haute, Indiana 47809.—Water samples were collected from selected wells in Vigo County and Parke County, from the Wabash River at Terre Haute, from the Terre Haute Wastewater Treatment Plant, and from a natural spring in Parke County. After concentration, trace substances were separated with gas chromatograph and introduced into a mass spectrometer. Mass spectra were obtained for numerous trace substances. Dibromochloromethane and 1-octadecanamine were possibly indicated in some wastewater samples. Other spectra gave no clear match with library reference spectra.

The Synthesis of a Crown Ether Designed to Exhibit Alkali Metal Cation Enhancement of Crown Ether Fluorescence. LYNN R. SOUSA, TODD E. TREHEARNE and ROBERT W. STEVENSON. Department of Chemistry, Ball State University, Muncie, Indiana 47306.—A bis(crown ether) is being synthesized that is likely to exhibit enhanced fluorescence in the presence of alkali metal cations. Such a "fluorogenic" crown ether could prove useful for the quantitative analysis of alkali metal cations such as K^+ or Na^+ or Li^+ . The synthesis of the potential analytical reagent involves the production of azacrown ethers and their subsequent coupling to form the desired bis(crown ether)s. A progress report will be given.

Sample Introduction Effects on the Excitation Mechanism in the Inductively Coupled Plasma. NORMAN SPROCH and JAMES P. RYBARCZYK, Department of Chemistry, Ball State University, Muncie, Indiana 47306.—An arc/sparks emission spectrograph was locally adapted as a scanning monochromator/photomultiplier detection system for a Plasma Therm ICP. This system was utilized to measure changes in the excitation region of the ICP as the sample aerosol composition was altered. The vertical location of the various sample excitation zones of the ICP, from the preheat zone to the normal analytical zone, is directly related to aerosol composition. Aerosol modifications included smaller particle size, chemical composition, and desolvation effects. Effects of sample introduction changes on relative atom and ion populations also were studied.

Fragmentation and Isomerization of Trimethylene Sulfites. JEFFREY G. STACK and ROBERT J. OLSEN, Department of Chemistry, Wabash College, Crawfordsville, Indiana 47933.—Trimethylene sulfites 1 and 2 were found to undergo thermal and photochemical fragmentation reactions to produce olefins and carbonyl compounds.

