atives of II. Furthermore, the infrared spectra (Nujol mulls) of closely related acyl derivatives of II exhibit differentiating absorption in the 12 to 14 micron region. Thiolutin is characterized by two similar, sharp bands at 12.1 and 12.5 microns, a dominantly, strong band at 13.45 microns and a partially resolved band at 13.6 microns. Aureothricin exhibits two similar, sharp bands at 12.2 and 12.6 microns, a partially resolved band at 13.45 microns and a dominantly strong band at 13.6 microns.

Structural studies are in progress and will be reported in subsequent publications.

RESEARCH LABORATORIES
CHAS. PFIZER AND CO., INC.
BROOKLYN 6, NEW YORK

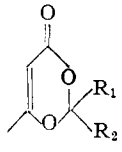
WALTER D. CELMER
FRED W. TANNER, JR.
M. HARFENIST
T. M. LEES
I. A. SOLOMONS

RECEIVED NOVEMBER 10, 1952

THE REACTION OF DIKETENE WITH KETONES

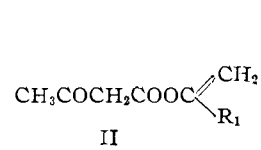
Sir:

Although the preparation of diketene in acetone is a commercial process, its reactions with ketones to form compounds formulated as 2,2-disubstituted-4-methyl-6-keto-1,3-dioxenes, I, have escaped observation.

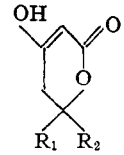


Ia, $R_1 = R_2 = CH_3$
Ib, $R_1 = CH_3$, $R_2 = C_6H_5$

The *p*-toluenesulfonic acid catalyzed reaction of diketene with acetone at 90° yields Ia (91%), b.p. 61–64° (5 mm.), $n^{20}D$ 1.464, d^{20}_4 1.079, $\lambda^{EtOH}_{max.}$ 247.5 mμ, log ε 3.9; *Anal.* Calcd. for $C_7H_{10}O_3$: C, 59.14; H, 7.09; Found: C, 59.20; H, 7.15. The product from acetophenone, Ib, is crystalline, m.p. 93–94°, $\lambda^{EtOH}_{max.}$ 247.5 mμ, log ε 3.86, $\lambda^{isooctane}_{max.}$ 240 mμ, log ε 3.85; *Anal.* Calcd. for $C_{12}H_{12}O_3$: C, 70.57, H, 5.92; Found, C, 70.21; H, 5.81. These ketodioxenes are pleasant smelling liquids or crystalline solids easily handled in the absence of alkali. As many of their reactions parallel those of diketene they may conveniently be used in its place. Thus I reacts with alcohols, aniline and with mild alkali to yield acetoacetates, acetoacetanilide and dehydracetic acid, respectively. I does not react with carbonyl reagents and this and the ultraviolet spectra rule out structures II and III also considered.



II



III

RESEARCH LABORATORIES
A. BOAKE, ROBERTS AND CO. LTD.
LONDON, E. 15, ENGLAND

MICHAEL F. CARROLL
ALFRED R. BADER[1]

RECEIVED NOVEMBER 20, 1952

(1) The Research Laboratories, The Pittsburgh Plate Glass Co., Milwaukee, Wisconsin.

C-TERMINAL GROUPS OF TRYPSINOGEN, DFP-TRYPSIN AND CARBOXYPEPTIDASE[1]

Sir:

A recent study of the effect of carboxypeptidase on chymotrypsinogen and DFP-α-chymotrypsin has led to the conclusion that the zymogen contains no C-terminal groups, in contrast to two such groups, leucine and tyrosine, in DFP-α-chymotrypsin.[2] In the present study, the same experimental technique was employed to investigate similarly the changes involved in the activation of trypsinogen and to test for the autolysis of carboxypeptidase.

Trypsinogen was crystallized in the presence of DFP[3] and residual trypsin (less than 0.01%) was inactivated by the addition of a 2-fold excess of crystalline soybean trypsin inhibitor (Worthington). Twice recrystallized DFP-trypsin,[3] containing less than 0.1% trypsin, was similarly inactivated. Seven times recrystallized carboxypeptidase was freed from residual amino acids (which previously were found to form a background on paper chromatograms[2]) by washing of the crystals with distilled water, and residual tryptic activity (less than 0.04%) was removed by the addition of a 100-fold molar excess of DFP to a solution of the enzyme in 10% LiCl.

Trypsinogen or DFP-trypsin was incubated with carboxypeptidase (substrate/enzyme mole ratio 11/1) at *p*H 7.5, 25°, and aliquots were removed at various time intervals up to 3 hours. Any free amino acids were absorbed onto and eluted from Dowex 50 resin[2,4] and subjected to one-dimensional paper chromatography (butanol–acetic acid–water, 4:1:5 or phenol–*m*-cresol, 1:1). No amino acids whatsoever could be detected. Negative results were obtained also when a 2% solution of carboxypeptidase was similarly tested after it was allowed to autolyze up to 3 hours at 25°, *p*H 7.5.

These negative results suggest that (1) the protein substrates have no free C-terminal groups or (2) that these groups are not reactive toward carboxypeptidase either because they do not conform to the specificity requirements of the enzyme or because they are sterically inaccessible. The first explanation is rendered unlikely by the recent findings of Rovery, Fabre and Desnuelle[5] that trypsinogen and DFP-trypsin each contain one N-terminal group, valine and isoleucine, respectively, suggesting that these proteins are composed of at least one open polypeptide chain. The second interpretation receives support from the observation that following denaturation by acid, DFP-trypsin becomes reactive toward carboxypeptidase (substrate/en-

(1) DFP denotes di-isopropylfluorophosphate. This work was performed under contract No. Nonr-477-04 between the University of Washington and the Office of Naval Research, Department of the Navy, and was supported also by funds made available by the people of the State of Washington, Initiative 171.

(2) J. A. Gladner and H. Neurath, *Biochim. et Biophys. Acta*, **9**, 335 (1952), and unpublished experiments.

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(5) M. Rovery, C. Fabre and P. Desnuelle, *Biochim. et Biophys. Acta*, in press. We are indebted to Professor Desnuelle for informing us of these results prior to publication.

zyme mole ratio 10/1), yielding lysine, and to a lesser extent leucine, methionine, glutamic and aspartic acids, threonine, serine and glycine.

Denaturation was effected by 48 hours of exposure to HCl, *p*H 1, at 0°, followed by 24 hours of dialysis against HCl, *p*H 3.

The present results are compatible with the suggestion that activation of trypsinogen is accompanied by the splitting of a peptide from the amino end of the polypeptide chain[5] but it remains to be seen whether in analogy with the activation of chymotrypsinogen to α-chymotrypsin,[2] a peptide is split from the carboxyl end of the chain as well. Further quantitative studies of these phenomena are in progress and will be published at a later time.

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF WASHINGTON — EARL W. DAVIE
SEATTLE, WASHINGTON — HANS NEURATH

RECEIVED NOVEMBER 24, 1952

IONIZATION OF ORGANOMETALLIC HALIDES

Sir:

Although trimethyltin iodide[1] and the triethyllead halides[2] have long been known to be soluble in water and to undergo metathetic reactions in aqueous solution, the rapid and complete hydrolysis of organosilicon halides[3] precludes such reactions. The similar alcoholysis[4] of organosilicon halides, and their related reaction with ammonia[5] and amines[6] prevent the use of these polar liquids as ionizing solvents.

For much the same reasons, the electrochemistry of organometallic compounds has been concerned chiefly with the electrolytic behavior of organomercury compounds in water,[7] the conductivity of trimethyltin iodide in acetone, alcohols, and pyridine,[8] and of Grignard reagents[9] in ether and related solvents. The organosilicon halides exhibit no conductivity in ether.

We now find that a considerable number of methyl-, ethyl-, dodecyl-, octadecyl- and phenyl chlorosilanes are soluble in anhydrous dimethylformamide, and that such solutions are highly conducting. For example, dimethyldichlorosilane exhibits an equivalent conductance of 18 $ohms^{-1}$ at a concentration of 0.004 equivalent per liter, and 0.5 ohm^{-1} at one equivalent per liter, both at 30°. The conductances of eleven organochlorosilanes are being studied, and from these and the colligative properties of the solutions the degrees of ionic dissociation are being sought. Preliminary results indicate that the dissociation constants are relatively small, in the range 10^{-2} to 10^{-4}.

The chemical significance of these results lies in the fact that the concentrations of organosilicon ions of the types R_3Si^+, R_2Si^{++}, and RSi^{+++} are amply sufficient to permit ionic oxidation-reduction and metathetic reactions in dimethylformamide. Thus dichromate ion is reduced by dimethyldichlorosilane in this solvent to several lower oxidation states of chromium; ammonium thiocyanate undergoes metathetic reaction with the same chlorosilane to precipitate ammonium chloride; boric acid reacts to form a silicone polymer of the "bouncing putty" variety; phosphotungstic acid precipitates a dimethylsilyl phosphotungstate; *para*nitrobenzoic acid dissolves in a solution of dimethyldichlorosilane in dimethylformamide, but not in the amide itself nor in a mixture of the amide with hydrogen chloride.

In similar vein, we have found that some organogermanium halides form conducting solutions in dimethylformamide and undergo ionic metatheses. While it may prove possible to employ concentrated hydrochloric acid as a dissociating solvent for organogermanium compounds because of an observable reversibility,[10] the preparative utility of such a solvent is more limited. However, we have found that dimethyltin dichloride may readily be handled in acidic aqueous solution; it hydrolyzes in pure water only to the extent of 10.5% in 0.064 molal concentration at 25°. Cryoscopic measurements in water give a van't Hoff *i* factor of 2.60 at 0.1693 molal concentration, 2.69 at 0.1041 molal, and 2.86 at 0.0639 molal. The $(CH_3)_2Sn^{++}$ ions may be retained on a cation exchange resin and eluted with various acids. By elution and by metathesis we have prepared dimethyltin tungstate, molybdate, sulfide, oxalate, succinate, naphthionate, salicylate, phthalate, benzoate, ferricyanide, ferrocyanide, iodate, arsenate, vanadate, cyanate and antimonate. A report on the preparation, purification, and properties of these compounds is being prepared.

The financial assistance of the Mallinckrodt Fund and the Office of Naval Research is greatly appreciated.

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(4) C. A. Burkhard, *J. Org. Chem.*, **15**, 106 (1950).

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(9) W. Evans and F. Lee, *ibid.*, **55**, 1474 (1933); **56**, 654 (1934).

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KURT GINGOLD
MALLINCKRODT LABORATORY — EUGENE G. ROCHOW
HARVARD UNIVERSITY — DIETMAR SEYFERTH
CAMBRIDGE 38, MASS. — ALBERT C. SMITH, JR.
ROBERT WEST

RECEIVED OCTOBER 27, 1952

ISOTOPE EFFECTS IN THE IONIZATION OF ALKYL CHLOROSULFITES

Sir:

Examples occur frequently in the literature of marked changes in the rate of reactions which break bonds to hydrogen, when deuterium or tritium is substituted for this hydrogen. No appreciable effects have heretofore been observed when the reactant and product have all bonds to hydrogen intact. Such studies have in fact become a standard tool for the detection of bond-breaking in the rate determining steps of reactions.[1,2] In an experiment designed to study by this technique the mechanism of the elimination reaction which ac-

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