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.

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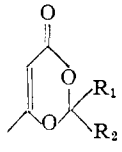
RECEIVED NOVEMBER 10, 1952

THE REACTION OF DIKETENE WITH KETONES

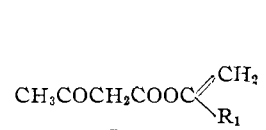
Sir:

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.

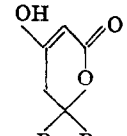


Ia, $R_1 = R_2 = CH_3$
Ib, $R_1 = CH_3$, $R_2 = C_6H_5$

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

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RECEIVED NOVEMBER 20, 1952

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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.

(3) L. W. Cunningham, Jr., F. Tietze, N. M. Green and H. Neurath, *Discussions of the Faraday Society*, in press; F. Tietze, in preparation.

(4) S. M. Partridge, *Nature*, **169**, 496 (1952); A. R. Thompson, *ibid.*, **169**, 495 (1952).

(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.