helix packed column yielded 21.4 g. (44%) of material; b. p. 181–182° (10 mm.), m. p. 44–48°. Several recrystallizations from benzene–petroleum ether (30–60°) yielded colorless needles; m. p. 51.5–52.5° (reported 51–2°,[10] 53.5°[9]).

The **p-nitrophenylhydrazone of III**, prepared by the method of Mauthner[10] was obtained as orange needles; m. p. 184–184.5° (reported[10] 182–183°).

The **2,4-dinitrophenylhydrazone of III**, prepared in the usual manner, was recrystallized from chloroform to yield small, dark-red prisms; m. p. 203–205°.

Anal. Calcd. for $C_{18}H_{20}N_4O_7$: C, 53.46; H, 4.99. Found: C, 53.50; H, 5.10.

The **semicarbazone of III** was prepared by standard methods and obtained as a colorless powder by recrystallization from ethanol; m. p. 174.5–175.5°.

Anal. Calcd. for $C_{13}H_{19}N_3O_4$: C, 55.50; H, 6.81. Found: C, 55.60; H, 6.75.

(b) **Effect of Reaction Conditions.**—The effects of the amount of diethylcadmium and the time of reflux are shown in Table I.

TABLE I

Moles of EtBr per mole of II	Reflux time, hr.	Crude III, %	V in crude III, %	Unreacted II, %
6	2	..	40.0	1
2	2.5	98.5	27.5	1
1.5	2.5	91.5	18.0	7
2	1	22	2.0	76
2	2	77[a]	5.0	2

[a] This product was obtained from an "inverse" reaction in which the diethylcadmium was added to II. The yield shown in the table is of distilled material.

(c) **Olefin Assay by Hydrogenation.**—A 1.00-g. sample of the crude product was purified by molecular distillation at 0.01 mm., dissolved in ethyl acetate, and added to an equilibrated suspension of 0.200 g. of 10% palladium on charcoal in ethyl acetate. The fast hydrogen uptake, complete within three to five minutes, was taken as a direct index of the amount of V in the product since III under the same conditions absorbed hydrogen extremely slowly.

(d) **Olefin Assay by Fractional Distillation.**—A careful fractional distillation of a sample of crude ketone through a Piros–Glover column yielded 12% of V. An assay by hydrogenation on the same crude sample indicated 14% of olefin.

(e) **Isolation of V from the Product.**—A benzene solution containing 3.17 g. of the product from run 1, Table I was adsorbed on a column of alumina. Elution with benzene produced in the first three fractions 1.5 g. (47%) of a pale yellow oil with $n^{20}D$ of 1.5344, 1.5360 and 1.5362, respectively (reported[9] 1.5360). The middle fraction did not form a *p*-nitrophenylhydrazone or 2,4-dinitrophenylhydrazone. Hydrogenation in the presence of 10% palladium on charcoal resulted in an uptake of 104% of the amount calculated for one double bond.

Anal. Calcd. for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53. Found: C, 71.12; H, 8.14.

β-(3,4,5-Trimethoxyphenyl)-propionic Acid (IV): (a) Optimum Reaction Conditions.—The method employed was patterned after one described by Schwenk and Papa.[11] A 5.00-g. (0.0223 mole) sample of 3,4,5-trimethoxypropiophenone (m. p. 44–48°) was heated for four hours at 110° in an atmosphere of nitrogen with 5.83 g. (0.067 mole) of morpholine and 1.79 g. (0.056 mole) of sulfur. The mixture was cooled, the organic material extracted into chloroform, and the chloroform solution washed with dilute hydrochloric acid and water and then evaporated to leave the crude, oily thiomorpholide. This could not be purified by distillation or crystallization and was saponified directly by refluxing for thirty-six hours with 60 cc. of ethanol containing 12 g. of potassium hydroxide. Although the reaction mixture after this process was still very alkaline, a resaponification was found, in almost every case, to markedly increase the amount of acidic product obtained. The crude acid, obtained by working up in the usual way, was separated into 1.10 g. of bicarbonate-insoluble material and 3.47 g. (65%) of bicarbonate-soluble material. The latter was molecularly distilled at 0.02 mm. and the sublimate recrystallized from benzene–Skellysolve C to yield 2.39 g. (45%) of a light tan powder; m. p. 91–92°. Three recrystallizations from water yielded small, colorless plates, m. p. 96.5–97°, which showed no depression in m. p. when admixed with a sample of IV prepared by an alternate method as described below.

Anal. Calcd. for $C_{12}H_{16}O_5$: C, 59.98; H, 6.71. Found: C, 59.66; H, 6.45.

The **p-toluidide of IV** was prepared in the usual fashion and obtained, after recrystallization from aqueous ethanol, as almost colorless needles; m. p. 101.5–102°.

Anal. Calcd. for $C_{19}H_{23}NO_4$: C, 69.27; H, 7.04. Found: C, 69.41; H, 7.02.

(b) **Effect of Reaction Conditions.**—The reaction proved to be quite insensitive to changes in the ketone to sulfur ratio (1/1.25 to 1/2.5), the temperature (110 to 147°), and the time of heating (two to four hours). The crude yields, which in these cases were 57–65%, dropped significantly only when the time of heating was reduced to one hour (45%), when piperidine instead of morpholine was used (33%), or when the original Willgerodt sealed tube reaction conditions were employed (no product).

(c) **Alternate Preparation of IV.**—β-(3,4,5-Trimethoxyphenyl)-propionic acid was prepared according to a previously described method[2,4] and was obtained as a crystalline product melting, in one experiment, at 97–97.5° and, in another experiment, at 103–103.5°.

Over-all Reaction without Purification of Intermediates.—A 20.0-g. sample of I was converted to II which, without distillation, was subjected to reaction with diethylcadmium as described above to yield 19.0 g. (90%) of crude III. The Willgerodt–Kindler reaction was carried out with 5.00 g. of this material to yield 1.6 g. of once-recrystallized IV (m. p. 91–92°), bringing the over-all yield of IV from I to 28%.

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The Inertness of Crystalline Ovalbumin in Systems Containing α-Chymotrypsin and Hydrolyzable Substrates

BY H. T. HUANG AND CARL NIEMANN[1]

The observation that native crystalline ovalbumin is not hydrolyzed and heat denatured ovalbumin is hydrolyzed by α-chymotrypsin at 25° and *p*H 7.9 may be regarded as a confirmation of earlier reports in respect to the lack of digestability of certain native proteins by the so-called tryptic enzymes.[2] However in view of the fact that the above phenomenon has been interpreted in terms of the non-availability of certain peptide bonds in the native protein which become available when the protein is denatured[2] we believe it important to call attention to the hitherto undisclosed fact that native ovalbumin also has no demonstrable inhibitory properties

(1) To whom inquiries regarding this article should be sent.
(2) For a review of the literature see H. Neurath, J. P. Greenstein, F. W. Putnam and J. O. Erickson, *Chem. Revs.*, **34**, 157 (1944).

when present in systems containing α-chymotrypsin and hydrolyzable substrates such as nicotinyl-L-tryptophanamide and nicotinyl-L-tyrosinamide.[3]

It is known[4,5] that the structural requirements of specific competitive inhibitors of α-chymotrypsin are in a sense less extensive than those of specific substrates of this enzyme and that for both substrate and inhibitor an important structural requirement is an available β-indolylmethyl-, *p*-hydroxybenzyl-, benzyl- or β-thiomethylethyl- side-chain.[3-5] Thus it appears that a substantial number of the tryptophan, tyrosine, phenylalanine and methionine residues which are present in native ovalbumin are unavailable, because of steric or coulombic factors, and cannot function even in an inhibitory process which is clearly associated with the presence of these side-chains in simpler molecules.

Experimental

Reagents.—The nicotinyl-L-tryptophanamide and nicotinyl-L-tyrosinamide used in these studies was prepared as described previously.[3] The α-chymotrypsin and crystalline ovalbumin preparations were obtained from Armour and Company.

Enzyme Experiments.—All hydrolyses were studied at 25° and *p*H 7.9 in the presence of a tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer. In every instance the buffer concentration given is that of the amine. The extent of hydrolysis was determined by a potentiometric formol titration.[6]

One of two solutions of native ovalbumin in 0.04 *M* tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer (protein concn. 4.0 mg. per ml. solution) was gently boiled for twenty minutes to give a finely divided stable suspension of denatured ovalbumin. After both solutions had attained a temperature of 25° sufficient α-chymotrypsin was added to each to give an enzyme concentration of 0.104 mg. of enzyme protein nitrogen per ml. of solution. It was found that in one hour 3.0 μm. per ml. of carboxyl groups was liberated in the case of the denatured ovalbumin and less than 0.3 μm. per ml. of carboxyl groups in the case of the native ovalbumin after allowance was made for the usual blank corrections.

In the inhibition studies conditions were selected so that the reaction was apparently zero order with respect to the substrate concentration for the first 60% of hydrolysis. Under these conditions the apparent initial reaction velocity v_0' affords a convenient measure of enzymatic activity. All hydrolyses were conducted in the presence of a 0.02 *M* tris-(hydroxymethyl)-aminomethane–hydrochloric acid buffer with an enzyme concentration of 0.208 mg. of protein nitrogen per ml. of solution. Blank experiments with native ovalbumin and enzyme indicated negligible blank corrections over that of enzyme alone. The corrected apparent initial reaction velocities, v_0', and other pertinent information are given in Table I.

TABLE I

HYDROLYSIS OF SEVERAL SYNTHETIC SUBSTRATES BY α-CHYMOTRYPSIN IN THE PRESENCE AND ABSENCE OF CRYSTALLINE OVALBUMIN[a]

Substrate, 10 μm. per ml. soln.	Ovalbumin, mg. per ml. soln.	v_0', per ml. μm. per min.
Nicotinyl-L-tryptophanamide	0.0	0.25 ± 0.01
Nicotinyl-L-tryptophanamide	2.1	.25 ± .01
Nicotinyl-L-tyrosinamide	0.0	.41 ± .01
Nicotinyl-L-tyrosinamide	1.4	.40 ± .01
Nicotinyl-L-tyrosinamide	2.8	.41 ± .01

[a] At 25° and *p*H 7.9.

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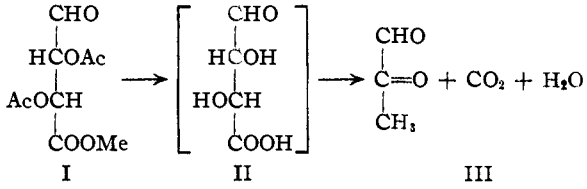
The Decarboxylation of Methyl Diacetyl-L-threuronate[1]

BY MIYOSHI IKAWA[2] AND KARL PAUL LINK

During the course of studies on the decarboxylation of sugar acids[3] the action of sulfuric and hydrochloric acids on the teturonic acid derivative methyl diacetyl-L-threuronate (I) was investigated. When heated with 12% hydrochloric acid



I II III

in a uronic acid determination, I evolved a total of 0.96 mole of carbon dioxide per mole within two hours, decarboxylating at a faster rate than D-galacturonic acid under the same conditions. The fragment remaining after treating I with acid was identified as methylglyoxal (III) through its phenylosazone, 2,4-dinitrophenylosazone and bis-semicarbazone. Acetol forms the same osazones as III, and the formation of methylglyoxal osazones has often, erroneously, been attributed to the presence of III when, in reality, acetol was present.[4] Acetol and methylglyoxal can, however, be distinguished through their semicarbazide derivatives.[5]

Neuberg and co-workers[6] on heating glyceraldehyde and dihydroxyacetone with dilute sulfuric acid obtained methylglyoxal (as the *p*-nitrophenylosazone) in a 70% yield from the former and almost quantitatively from the latter. What the exact sequence of reactions leading to the formation of III may be is obscure. Decarboxylation of L-threuronic acid (II) to glyceraldehyde might occur first or dehydration to perhaps a β-ketonic acid could take place prior to decarboxylation.

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