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 pH 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 pH 7.9.

(3) B. M. Iselin, H. T. Huang, R. V. McAllister and C. Niemann, THIS JOURNAL, **72**, 1729 (1950).

(4) S. Kaufman and H. Neurath, *J. Biol. Chem.*, **181**, 623 (1949).

(5) Unpublished experiments of R. V. McAllister, D. W. Thomas and H. T. Huang.

(6) B. M. Iselin and C. Niemann, *J. Biol. Chem.*, **182**, 821 (1950).

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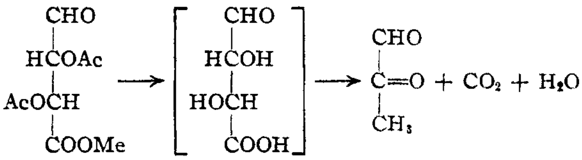
The Decarboxylation of Methyl Diacetyl-L-threuronate[1]

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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(2) Department of Chemistry, California Institute of Technology, Pasadena, California.

(3) Dickson, Otterson and Link, THIS JOURNAL, **52**, 775 (1930).

(4) Nodzu and Goto, *Bull. Chem. Soc. Japan*, **11**, 381 (1936).

(5) Nodzu, *ibid.*, **10**, 122 (1935).

(6) Neuberg, Farber, Levite and Schwenk, *Biochem. Z.*, **83**, 264 (1917).

Experimental[7]

Methyl diacetyl-L-threuronate (I) was prepared according to the directions of Lucas and Baumgarten,[8] and the constants agreed closely with those reported: m. p. 82–83°; $[\alpha]^{20}D$ −55.8° → −39.0° after 5 days (33.9 mg. per ml. in MeOH).

The decarboxylation of I in 12% hydrochloric acid was carried out in a manner similar to that described by Yackel and Kenyon,[9] the evolved carbon dioxide being absorbed in tubes filled with Ascarite and weighed.

Treatment of Methyl Diacetyl-L-threuronate with Sulfuric Acid.—The procedure described by Neuberg, *et al.*,[6] for the conversion of dihydroxyacetone to methylglyoxal was used. To a 100-ml. distilling flask equipped with a dropping funnel were added 1 g. of I and a mixture of 10 ml. of water and 2 g. of concentrated sulfuric acid. The mixture was distilled, water being added through the dropping funnel at such a rate as to maintain a constant liquid volume in the flask. Two hundred and forty ml. of distillate was collected, which yielded 0.567 g. of crude methylglyoxal phenylosazone (52%). Recrystallization from ethanol yielded 0.452 g. of product melting at 143.5–145°.

For the preparation of the semicarbazide derivative[6] 200 ml. of distillate was collected from 0.85 g. of I. One g. of semicarbazide hydrochloride and 1.5 g. of sodium acetate (anhyd.) were added, whereupon the solution quickly became cloudy and a crystalline precipitate formed. The mixture was heated on a steam-bath for half an hour. After standing in a refrigerator for twelve hours, the crystals were filtered off and washed with water. Pure methylglyoxal bis-semicarbazone (0.283 g.) of m. p. 270° (with decomposition) was thus directly obtained. The m. p. remained unchanged on recrystallization from a methanol-water mixture. On concentrating the mother liquor to 20 ml., 0.012 g. of solid melting at 260–262° (with decomposition) was obtained. The total yield was 39%.

Treatment of I with Hydrochloric Acid.—One gram of I in 25 ml. of 12% hydrochloric acid was heated on a steam-bath for one-half hour. A hot solution of 0.5 g. of 2,4-dinitrophenylhydrazine in 25 ml. of 12% hydrochloric acid was then added, whereupon an immediate reddish-orange precipitate was obtained. The solution was filtered while hot and the precipitate washed copiously with hot water, then with a small amount of ethanol and dried (wt., 0.45 g.). Upon recrystallization from dioxane, 0.23 g. of fine reddish-orange needles of methylglyoxal 2,4-dinitrophenylosazone of m. p. 305–306° (with decomposition) were obtained.

(7) All melting points are corrected.

(8) Lucas and Baumgarten, This Journal, **63**, 1653 (1941).

(9) Yackel and Kenyon, *ibid.*, **64**, 121 (1942).

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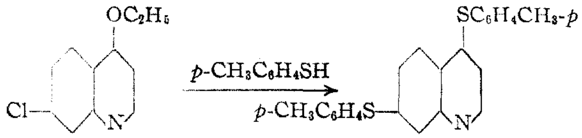
Displacement of Nuclear Hydroxyl in Quinoline Series by Aryl-S Group

By G. Illuminati and Henry Gilman

In connection with studies[1] concerned with the cleavage of alkoxy-substituted heterocycles by thiols, one of the reactions examined was that of 4-ethoxy-7-chloroquinoline with *p*-thiocresol. It was observed that the chief product of this reaction was 4,7-di-(*p*-thiocresoxy)-quinoline.

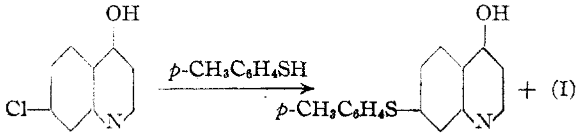
We are now reporting the interesting replacement of a nuclear hydroxyl group, under corresponding conditions, by the *p*-$CH_3C_6H_4S$– group.

(1) Illuminati and Gilman, This Journal, **71**, 3349 (1949); see, also, Hughes and Thompson, *Nature*, **164**, 365 (1949).



(I)

When 4-hydroxy-7-chloroquinoline is heated with *p*-thiocresol, there is obtained a mixture of 4-hydroxy-7-*p*-thiocresoxyquinoline, and 4,7-di-(*p*-thiocresoxy)-quinoline.



Experimental

4-Hydroxy-7-chloroquinoline.—A solution of 30 g. (0.1515 mole) of 4,7-dichloroquinoline (m. p. 84–85°) in 375 ml. of 10% hydrochloric acid was refluxed for ten hours. On allowing the solution to stand for a few hours in a refrigerator, most of the product separated. The filtrate from these white crystals yielded more of the product after making the solution strongly alkaline, filtering, and acidifying with acetic acid. The combined yield of 4-hydroxy-7-chloroquinoline, melting 270–275°, was 26.5 g. (97%). Recrystallization from hot pyridine gave 18 g. (66.1%) of compound melting at 276–280°. The melting point of 4-hydroxy-7-chloroquinoline obtained[2] by decarboxylation of 3-carboxy-4-hydroxy-7-chloroquinoline was 270–272°.

Anal. Calcd. for C_9H_6ONCl: Cl, 19.73. Found: Cl, 19.79.

It should be noted, in connection with the subsequent reactions of this compound, that the 4-hydroxy-7-chloroquinoline could not have been contaminated by any 4-ethoxy-7-chloroquinoline.

Reaction of 4-Hydroxy-7-chloroquinoline with *p*-Thiocresol.—A mixture of 13.3 g. (0.075 mole) of 4-hydroxy-7-chloroquinoline and 27.9 g. (0.225 mole) of *p*-thiocresol was heated at reflux temperature (the initial internal temperature being 190°). The quinoline compound dissolved after heating for a few hours, and the mixture became homogeneous when the temperature was about 210°. The total time of heating was eight hours. The reaction mixture, on cooling, had a glassy appearance. This mixture was resolved into its components by treatment with an ether (100 cc.)–5% sodium hydroxide solution (100 cc.). The shaking was repeated with fresh solvents until complete solution was attained. To the alkaline layer was added petroleum ether (b. p. 63–67°), and this mixture was acidified with acetic acid. The solid which separated on acidification was 4-hydroxy-7-*p*-thiocresoxyquinoline. The crude yield of this compound (m. p. 215–235°) was 6.7 g. Recrystallization from 95% ethanol gave 5.2 g. (26%) of crystals melting at 237–242°. The sample used for analysis melted at 242–245°.

Anal. Calcd. for $C_{16}H_{13}ONS$: S, 12.02. Found: S, 12.15.

The other product, 4,7-di-(*p*-thiocresoxy)-quinoline, was isolated from the ether layer in the usual manner. The crude yield was 18.2 g. (m. p. 90–96°). Recrystallization from 95% ethanol gave 11.5 g. (43.2%) melting at 97–99°. Another crystallization gave the pure compound melting at 100.5–101.5°. This compound was shown to be identical, by the method of mixed melting points, with the compound described previously,[1] which was obtained by reaction of 4,7-dichloroquinoline with *p*-thiocresol.

(2) Price, *et al.*, This Journal, **68**, 1204–1208 (1946). See Bachman and Cooper, *J. Org. Chem.*, **9**, 302 (1944), for the preparation of 4-hydroxy-6-chloroquinoline by the hydrochloric acid hydrolysis of 4,6-dichloroquinoline.