[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, GEORGETOWN MEDICAL SCHOOL]

The Rates of Absorption of and the Formation of Liver Glycogen by DL-Serine and DL-Threonine[1]

BY W. C. HESS

The rate of absorption of DL-serine from the gastrointestinal tract of the white rat was determined by Schofield and Lewis[2] by means of the Van Slyke amino nitrogen method to be 67.1 mg. per 100 g. of body weight per hour. Butts, Blunden and Dunn[3] fed DL-serine to white rats and found that it caused an increase in liver glycogen. No reports were found on the rate of absorption of DL-threonine; however, Hall, Doty and Eaton[4] have reported an increase in liver glycogen following the administration to rats of DL-threonine and also DL-allothreonine. The periodic acid methods of Nicolet and Shinn[5] and of Shinn and Nicolet[6] for the determination of serine and threonine have proven to be exceedingly accurate and they have been applied to an investigation of the rates of absorption of these two hydroxyamino acids. The amino nitrogen method of Pope and Stevens[7] has been used to confirm the results obtained by the periodic acid methods. The formation of extra liver glycogen was followed at the same time that the rates of absorption were determined.

Experimental.—White rats, fasted for forty-eight hours, were fed the amino acids by stomach tube. The amino acids were administered, dissolved in water, at levels of 100, 200 and 300 mg. per 100 g. of body weight for the one-, two- and three-hour absorption periods, respectively.[8] At the end of the period the animal was sacrificed and the entire gastrointestinal tract and the liver were removed. Glycogen was determined immediately in a portion of the liver by the method of Good, Kramer and Somogyi.[9] The gastrointestinal tract was ground with 50 ml. of a 10% solution of trichloroacetic acid in a Waring blender, the extract was filtered through a Celite pad and aliquots of the filtrate taken for analysis. For each amino acid a series of experiments were conducted in which the amino acid was neutralized by the addition of the calculated amount of 5 *N* sodium hydroxide solution to the aqueous solution prior to administration. The results of the experiments are given in Tables I and II. All the results are corrected for the loss occurring in the procedure; the loss being 3.5% for DL-serine and 2.8% for DL-threonine. The loss was determined by adding the amino acid to the isolated gastrointestinal tract of a control rat and carrying out the procedure described above.

TABLE I

RATE OF ABSORPTION OF AND GLYCOGEN FORMATION BY DL-SERINE

No. of animals	Time, hours	Rate Mg. per 100 g. per hour		Glycogen, %
		Periodate method	Amino N method	
4	1	53.1	55.7	0.73[a]
8	2	55.0	54.7	1.08
5	3	57.8	59.5	0.52
Av. 17		55.2 ± 4.0	56.4 ± 4.4	
4[b]	1	84.2	89.2	0.71
5[b]	2	87.1	87.5	0.98
4[b]	3	86.5	85.5	0.50
Av. 13[b]		86.0 ± 5.8	87.4 ± 5.2	

[a] The glycogen content of the livers of 12 control rats averaged 0.02%. [b] Fed as the sodium salt.

TABLE II

RATE OF ABSORPTION OF AND GLYCOGEN FORMATION BY DL-THREONINE

No. of animals	Time, hours	Mg. per 100 g. per hour		Glycogen, %
		Periodate method	Amino N method	
3	1	63.2	64.3	0.88
3	2	64.8	63.5	1.13
6	3	65.0	63.7	1.84
Av. 12		64.5 ± 4.1	63.9 ± 4.5	
2[a]	1	105.3	99.5	0.56
4[a]	2	97.6	96.5	0.43
4[a]	3	91.3	89.6	0.36
Av. 10[a]		96.6 ± 5.7	94.3 ± 4.7	

[a] Fed as the sodium salt.

Discussion and Summary.—The rate of absorption of DL-serine fed as the sodium salt was higher than that for the free amino acid; a similar result was obtained with the sodium salt of DL-threonine. Wilson and Lewis[10] have reported that the rate of absorption of sodium glycinate was higher than that of free glycine and Hess[11] has found that the rate of absorption of sodium cysteinate is higher than that of cysteine hydrochloride. The rates determined from the estimation of the amino nitrogen content of the extracts of the gastrointestinal tracts were in good agreement with those calculated from the periodic acid determinations. No significant differences were found in the rates determined after absorption periods of one, two or three hours.

Both DL-serine and DL-threonine formed considerable extra liver glycogen within one hour after feeding the amino acids.

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(1) Presented in part before the Division of Biological Chemistry of the American Chemical Society, Atlantic City, September, 1949.

(2) Schofield and Lewis, *J. Biol. Chem.*, **168**, 439 (1947).

(3) Butts, Blunden and Dunn, *ibid.*, **124**, 709 (1938).

(4) Hall, Doty and Eaton, *Am. J. Physiol.*, **131**, 252 (1940).

(5) Nicolet and Shinn, *J. Biol. Chem.*, **139**, 687 (1941).

(6) Shinn and Nicolet, *ibid.*, **138**, 91 (1941).

(7) Pope and Stevens, *Biochem. J.*, **33**, 1070 (1939).

(8) The DL-serine and DL-threonine were obtained from Mann Fine Chemicals, Inc. The purity was determined by amino nitrogen analysis and also by estimation with the periodic acid methods. Both amino acids were at least 98.8% pure.

(9) Good, Kramer and Somogyi, *J. Biol. Chem.*, **100**, 485 (1933).

(10) Wilson and Lewis, *ibid.*, **84**, 511 (1929).

(11) Hess, *ibid.*, **181**, 23 (1949).

WASHINGTON, D. C. RECEIVED OCTOBER 1, 1949

Quinolinium Thiocyanate

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When benzoyl chloride, quinoline and potassium cyanide are combined in an aqueous medium, the product obtained[2] is 1-benzoyl-1,2-dihydro-

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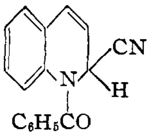
(2) Reissert, *Ber.*, **38**, 1603 (1905).

quinaldinonitrile (I). This crystalline compound yields quinaldinic acid and benzaldehyde on acid hydrolysis.



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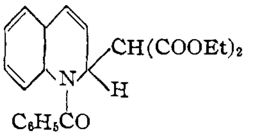
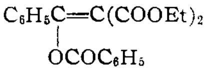


II

The reaction may be used as a method of reducing acyl halides to aldehydes, especially in the aromatic series.[3] Aliphatic acyl halides can be reduced similarly if anhydrous conditions are employed.[4]

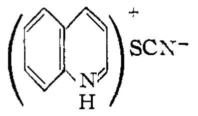
The present investigation was undertaken to determine whether this reaction could be extended by the use of salts other than potassium cyanide. Potassium thiocyanate and ethyl sodiomalonate were selected for preliminary study.

The reaction of benzoyl chloride with quinoline and ethyl sodiomalonate did not yield the desired 1,2-dihydroquinoline derivative (II), since most of the quinoline was recovered. It is known[5] that when benzoyl chloride and sodiomalonic ester react in the absence of quinoline, the products obtained are benzoylmalonic ester and a dibenzoyl derivative, III. These products seemed to be



$$\underset{\displaystyle OCOC_6H_5}{C_6H_5C}{=}C(COOEt)_2$$

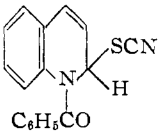
III



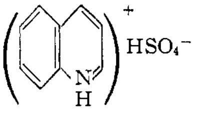
IV

formed in the present investigation as judged by their behavior toward hydrolysis.

The reaction of benzoyl chloride with quinoline and potassium thiocyanate in water gave a good yield of a crystalline product, but it proved to be quinolinium thiocyanate (IV), not V.



V



VI

Oxidation of IV with nitric acid produced quinolinium hydrogen sulfate (VI).

Experimental

Reaction of Benzoyl Chloride with Quinoline and Ethyl Sodiomalonate.—A mixture of 0.28 mole each of quinoline, benzoyl chloride and ethyl sodiomalonate in 215 ml. of benzene was refluxed for one hour. Solvent was removed and 200 g. of ice water was added to the residue. The layers were separated. The non-aqueous layer was washed with two 100-ml. portions of water, dried (Na_2SO_4) and distilled.

Fraction	B. p., °C.	Pressure, mm.	Weight, g.
a	43–83	25	8.5
b	80–100	15	44
c	70–130	2	16.5
d	Residue	..	25

The alkaline hydrolysis of 2 g. of fraction c yielded 0.7 g. of benzoic acid, m.p. 122–123°. Acid hydrolysis of residue d gave rise to a ketone; 2,4-dinitrophenylhydrazone, m.p. 239–240° (uncor.). The reported m.p. of acetophenone 2,4-dinitrophenylhydrazone is 249° (cor.). Fractions a and b were combined and extracted with two 50-ml. portions of 6 *N* hydrochloric acid. Eighteen grams of material was insoluble. The acid solution was made alkaline and extracted with 100 ml. of ether. Evaporation of the ether left 31 g. of quinoline, b.p. 109° at 14 mm. The reactions of fraction c with alkali and of the residue (d) with acid are similar to those reported[6] for benzoylmalonic ester and the dibenzoyl derivative (III), respectively.

Quinolinium Thiocyanate (IV).—To a stirred and cooled mixture of 63 g. of redistilled quinoline and 156 g. of potassium thiocyanate in 600 ml. of water, there was added 148 g. of benzoyl chloride in seven minutes. After one hour of stirring, most of the aqueous solution was removed by decantation and 70 ml. of ether was added to the residue. The insoluble tan solid was collected by filtration, washed with two 100-ml. portions of ether, and dried at 50°. A yield of 71 g. (77%) of quinolinium thiocyanate was obtained. This material was recrystallized from 200 ml. of absolute alcohol yielding 60 g. of light yellow material melting at 140–142°. A sample for analysis was recrystallized from methanol; m.p. 141–141.5°.

Anal. (by M. Ledyard) Calcd. for $C_{10}H_8N_2S$: C, 63.80; H, 4.28; neut. equiv., 188.2. Found: C, 64.04; H, 4.50; neut. equiv., 186.4, 191.2, 189.4.

A qualitative analysis (sodium fusion) indicated the presence of sulfur. No ash was obtained on ignition. A deep red color was produced immediately upon the addition of a few drops of ferric chloride solution to a hot aqueous solution of the product.

Quinolinium Hydrogen Sulfate (VI).—Fourteen grams of quinolinium thiocyanate was added in small portions to 25 ml. of concentrated nitric acid. A vigorous reaction occurred on each addition. The mixture was heated on the steam-bath for one hour and then was evaporated to dryness. The residue was heated with 75 ml. of absolute alcohol, and the mixture was filtered to remove a small amount of insoluble material. The filtrate was cooled. The precipitated solid was collected by filtration and recrystallized five times from absolute alcohol. There was obtained 2.5 g. of material melting at 162–164°. An additional 6.5 g. of material, m.p. 162–166°, was obtained by concentration of the alcohol filtrates from the crystallizations.

Anal. Calcd. for $C_9H_9NO_4S$: neut. equiv., 113.6. Found: neut. equiv., 114.7.

The product was quinolinium hydrogen sulfate. Its water solution caused precipitation of barium ion. Krakau[6] listed its m.p. as 163.5–164.5°. Some of it was made by direct reaction of quinoline and sulfuric acid. Its m.p. (162–163°) was not depressed by admixture with the above material.

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[3] Sugasawa and Tsuda, *J. Pharm. Soc. Japan*, **56**, 103 (1936).

[4] Grosheintz and Fischer, THIS JOURNAL, **63**, 2021 (1941).

[5] Giacalone and Russo, *Gazz. chim. ital.*, **65**, 1126 (1935).

[6] Krakau, *J. Russ. Phys.-Chem. Soc.*, **17**, 364 (1885).

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RECEIVED AUGUST 8, 1949

Spectrophotometric Study of Reactions of Sodium Silicates with Sulfuric Acid and Ammonium Sulfate

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The absorption spectrum of the cationic dye, pinacyanol chloride, in sodium silicate solutions depends on the form in which the silica is present and thus varies with the silica to alkali (Na_2O)