hydroxyproline to fasting rats produced extra liver glycogen but the results were variable. Butts, Blunden and Dunn[5] fed the anhydride of glutamic acid, pyrrolidone carboxylic acid, a compound closely related to both proline and hydroxyproline, to fasting rats and found an increase in liver glycogen. McFarlane and Guest[6] and Guest[7] have described quantitative colorimetric methods for the determination of hydroxyproline and proline, and we have applied these methods in an investigation of the rates of absorption of these two amino acids. The amino nitrogen method of Pope and Stevens,[8] which estimates quantitatively the nitrogen in these two amino acids, has been used to confirm the results obtained by the colorimetric methods. The formation of extra liver glycogen was followed simultaneously with the determination of the rates of absorption

Experimental

White rats, fasted for 48 hours, were fed the amino acids by stomach tube. The amino acids, dissolved in water, were administered at levels of 100, 200 and 300 mg. per 100 g. of body weight for the 1-, 2- and 3-hour absorption periods, respectively.[9]

The animals were sacrificed at the end of the period and the entire gastrointestinal tract and the liver removed. Glycogen was determined immediately in a portion of the liver by the method of Good, Kramer and Somogyi.[10] The gastrointestinal tract was ground in a Waring blendor with 50 ml. of a 10% aqueous solution of trichloroacetic acid, the extract filtered through a Celite pad and aliquots of the filtrate taken for analysis. A series of experiments with each of the amino acids was conducted in which the amino acid was neutralized by the addition of the calculated amount of 5 *N* NaOH 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; for L-proline the loss was 4.3% and for L-hydroxyproline 3.8%. 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 L-PROLINE

No. of animals	Time, hours	Rate, mg. per 100 g. per hour Colorimet. method	Amino N method	Glycogen, %
4	1	68.7	66.3	0.45[a]
6	2	71.0	70.0	0.69
6	3	68.8	67.2	1.41
Av. 16		69.6 ± 5.6	68.0 ± 5.8	
4[b]	1	75.0	73.1	0.23
4[b]	2	73.7	73.0	.49
4[b]	3	75.5	74.5	.81
Av. 12		74.7 ± 4.4	73.3 ± 4.9	

[a] The glycogen content of the livers of 12 control rats averaged 0.04%. [b] Fed as the sodium salt.

Discussion and Summary.—When fed to white rats previously fasted for 48 hours, L-proline and L-hydroxyproline are absorbed at substantially the same rate, 69.6 and 68.6 mg. per 100 g. of body weight per hour, respectively. When fed as their sodium salts, the rate of absorption of L-hydroxyproline, but not L-proline, is increased at a rate that is statistically significant ($t = 6.30$) a finding that has been observed with other amino acids.[11] The rates determined from the estimation of the amino nitrogen content of the extracts of the gastrointestinal tracts are in excellent agreement with those calculated from the colorimetric estimations. No significant differences in the rates were found after absorption periods of 1, 2 or 3 hours.

Both L-proline and L-hydroxyproline produced extra liver glycogen within 1 hour following their administration, 0.45 and 0.64% compared with 0.04% in the livers of 12 control rats fasted for 48 hours.

TABLE II

RATE OF ABSORPTION OF AND GLYCOGEN FORMATION BY L-HYDROXYPROLINE

No. of animals	Time, hours	Rate, mg. per 100 g. per hour Colorimet. method	Amino N method	Glycogen, %
6	1	70.3	59.9	0.64
6	2	65.5	67.8	.80
5	3	70.6	68.3	.86
Av. 17		68.6 ± 6.7	65.5 ± 7.2	
5[a]	1	77.5	74.1	.50
5[a]	2	84.0	84.1	.72
5[a]	3	81.8	84.2	.80
Av. 15		81.0 ± 4.6	79.1 ± 6.9	

[a] Fed as the sodium salt.

(5) Butts, Blunden and Dunn, *J. Biol. Chem.*, **119**, 247 (1937).

(6) McFarlane and Guest, *Can. J. Research*, **B17**, 139 (1939).

(7) Guest, *ibid.*, **17**, 143 (1939).

(8) Pope and Stevens, *Biochem. J.*, **33**, 170 (1939).

(9) The L-proline and L-hydroxyproline were obtained from Mann Fine Chemicals, Inc. The purity was determined by amino nitrogen (Method of Pope and Stevens[8]) and total nitrogen estimation; both amino acids were at least 98.4% pure. The L-proline had a specific rotation, in water, of $[-84.2°]^{20}D$ and the L-hydroxyproline, also in water, of $[-73.8°]^{20}D$.

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

(11) Hess, THIS JOURNAL, **72**, 1407 (1950).

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The Enzymatic Resolution of DL-Phenylalanine

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

In principle any one of a number of enzymatic methods may be used for the resolution of a particular α-amino acid.[2] However in practice it has been our experience that no one method is ideal for all amino acids and that in general the most suitable procedure will be determined by the nature of the amino acid to be resolved. For DL-phenylalanine it has been found that from the standpoint of reliability, convenience, cost and yield, the method of choice appears to be that based upon the papain-catalyzed synthesis of acetyl-L-phenylalanin-*p*-toluidide from acetyl-DL-phenylalanine and *p*-toluidine[3] followed by hydrolysis of the L-toluidide and the residual D-acid to the corresponding amino acids. The yields obtained with this procedure are superior to those previously reported for the resolution of DL-phenylalanine by another enzymatic process,[4] and are comparable with those achieved in the resolution of the three isomeric nuclear substituted monofluoro-

(1) To whom inquiries regarding this article should be sent.

(2) For a résumé of available methods *cf.* P. J. Fodor, V. E. Price and J. P. Greenstein, *J. Biol. Chem.*, **178**, 503 (1949); and L. Levinton, V. E. Price and J. P. Greenstein, *ibid.*, **184**, 55 (1950).

(3) *Cf.* C. Niemann and P. L. Nichols, Jr., *ibid.*, **143**, 191 (1942).

(4) J. B. Gilbert, V. E. Price and J. P. Greenstein, *ibid.*, **180**, 473 (1949).

DL-phenylalanines by a method analogous to that described in this communication.[5] The use of acetyl-DL-phenylalanine in preference to other acyl-DL-phenylalanines is based upon previous studies on the role of the acyl group in determining the stereochemical course of the reaction under discussion[5,6] and the fact that acetyl-DL-phenylalanine is sufficiently soluble in water to permit the use of relatively concentrated solutions. *p*-Toluidine was chosen as the base because it is a solid, is available in a relatively pure state, and is relatively stable in contact with air.

The authors wish to acknowledge the assistance of W. H. Schuller who checked the procedure given below.

Experimental[7]

Reagents.—Acetyl-DL-phenylalanine, lustrous plates, m.p. 152–154°, was prepared in yields of 93–96% from DL-phenylalanine (Dow) by acetylation of 0.8 mole of the amino acid with 2.4 moles of acetic anhydride and 1.6 moles of sodium hydroxide at a temperature below 10°. Thirty grams of finely ground papain (Wallerstein) was stirred with 150 ml. of water for 3 hours at 5°, the suspension centrifuged for 15 minutes at 2000 r.p.m. and the supernatant solution reserved for use. *p*-Toluidine (Merck and Co., Inc.) was used without further purification.

Acetyl-L-phenylalanine-*p*-toludide (I).—To 2 l. of a 0.5 *M* acetic acid–0.5 *M* sodium acetate buffer containing 9 g. of L-cysteine hydrochloride was added 155.5 g. (0.75 mole) of acetyl-DL-phenylalanine, the suspension warmed to 50° to effect complete solution, 80 g. (0.75 mole) of *p*-toluidine and the above enzyme solution added, the total volume brought to 3 liters with the acetate buffer, and the clear solution, *p*H 4.6, incubated at 40° for 7 days. The reaction mixture was maintained at 5° for 2 hours prior to the collection of the precipitate which was washed with 1 l. of water and air-dried to give 102–106 g. (92–95%) of I, m.p. 215–217°. This product was dissolved in 1.25 l. of hot 96% ethanol, the hot solution filtered, the filtrate held at 5° overnight, the precipitate collected, washed with 500 ml. of cold 96% ethanol, and air-dried to give 86–89 g. of I, m.p. 219°; $[\alpha]^{25}D$ +35 ± 1° (*c*, 4 in pyridine). *Anal.* Calcd. for $C_{18}H_{20}O_2N_2$ (296): C, 73.0; H, 6.8; N, 9.5. Found: C, 72.8; H, 6.7; N, 9.4. An additional 9–10 g. of I, m.p. 219°, was recovered from the mother liquor to give a total yield of recrystallized I of 86–89%.

L-Phenylalanine (II).—A suspension of 86 g. of recrystallized I in 1100 ml. of 20% hydrochloric acid was heated under reflux for 16 hours, the clear solution evaporated to dryness *in vacuo*, the residue dissolved in 300 ml. of water, again evaporated to dryness, the residue dissolved in 250 ml. of water and 450 ml. of 28% aqueous ammonia cautiously added to this solution. After the reaction mixture was held at 5° for 2 hours the precipitated *p*-toluidine was collected, washed with cold water, the filtrate and washings combined, the solution extracted with two 300-ml. portions of chloroform, the volume of the aqueous phase reduced to *ca.* 500 ml. by boiling, the solution cooled to 5° (2 hours), the precipitate collected, washed successively with 100 ml. of water and 40 ml. of 96% ethanol and air-dried to give 28 g. of II, lustrous flat plates; $[\alpha]^{25}D$ −34 ± 1° (*c*, 2 in water). Concentration of the mother liquor gave two additional crops of II of 6 and 4 g., respectively, or a total yield of 38 g. (82%).

D-Phenylalanine (III).—The filtrate remaining after the collection of I was evaporated *in vacuo* below 50° to one-half of its original volume, acidified with 120 ml. of concentrated hydrochloric acid, stored at 5° overnight, the crystalline precipitate collected, washed with 200 ml. of cold water and recrystallized from 20% aqueous methanol to give 50–65 g. (77–84%) of acetyl-D-phenylalanine (IV), m.p. 162–164°; $[\alpha]^{25}D$ −32 ± 1° (*c*, 2 in methanol). A suspension of 50 g. of IV in 300 ml. of 20% hydrochloric acid was heated under reflux for 5 hours, the clear solution evaporated to dryness, the residue dissolved in 300 ml. of water, 100 ml. of 28% aqueous ammonia added, the solution boiled to remove excess ammonia, decolorized with 5 g. of Norite, the clear colorless filtrate stored at 5° overnight, the crystalline precipitate collected, washed successively with 100 ml. of water and 50 ml. of 96% ethanol, and dried to give 27 g. of III; $[\alpha]^{25}D$ +34 ± 1° (*c*, 1 in water). A second crop of 3 g. of III was obtained from the mother liquor to give a total yield of 30 g. (77%).

The average over-all yields of D and L-phenylalanine from DL-phenylalanine were 59 and 68%, respectively.

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(5) E. L. Bennett and C. Niemann, THIS JOURNAL, **72**, 1800 (1950).
(6) E. L. Bennett and C. Niemann, *ibid.*, **72**, 1798 (1950).
(7) All melting points reported are corrected.

On the Interaction between Hexacyanatoferrate(III) Ions and (a) Hexacyanatoferrate(II) or (b) Iron(III) Ions[1a,2]

BY JAMES A. IBERS AND NORMAN DAVIDSON[1b]

In view of the occurrence of non-additive light absorption ("interaction absorption") in hydrochloric acid solutions containing iron(II) and (III),[3] it was of interest to determine whether the hexacyanatoferrate(II) and (III) ions exhibited this same effect. The present note reports experiments showing that the answer to this question is in the negative. At the same time we have examined the well-known brown coloration of solutions containing iron(III) ions and hexacyanatoferrate(III) ions and have evaluated the equilibrium constant for the formation of the uncharged complex species, $FeFe(CN)_6$, responsible for this color.

Figure 1 shows the absorption spectra of 0.1 *F* $Fe(CN)_6^{-3}$ and 0.1 *F* $Fe(CN)_6^{-4}$ solutions buffered at a *p*H of 6.8 (where the ratio $[HFe(CN)_6^{-3}]/[Fe(CN)_6^{-4}]$ is of the order[4] of 0.003). The data agree fairly well with those given by Kortüm.[5] A 1:1 mixture of these two solutions had optical densities in the wave length range 270–350 mμ which agreed to within 1% with those predicted by Beer's law. No interaction absorption was observed when the ionic strength of the solution was raised from 1.9 to 4.4 by making it 2.5 *F* in potassium chloride. It was thought that such a high ionic strength might diminish the electrostatic repulsion between the $Fe(CN)_6^{-4}$ and $Fe(CN)_6^{-3}$ ions.

It is noteworthy that, by virtue of the short light paths used, the absorption of a moderately concentrated solution of $Fe(CN)_6^{-4}$ and $Fe(CN)_6^{-3}$ has been measured in the wave length region close to the strong ultraviolet "electron-transfer" absorption bands of the components; this is the region where interaction absorption, when it occurs, is most marked.[6,7] One may conclude that there is little or no optical interaction absorption between $Fe(CN)_6^{-4}$ and $Fe(CN)_6^{-3}$ in aqueous solution. It has already been suggested[3] that interaction absorption

(1a) Research supported by the O. N. R. under Contract N6onr-244.
(1b) To whom inquiries concerning this article should be addressed.
(2) For a table of the extinction coefficients of solutions of $K_4Fe(CN)_6$, $K_3Fe(CN)_6$, $Fe(ClO_4)_3$ and $FeFe(CN)_6$ order Document 2944 from American Documentation Institute, 1719 N Street, N. W., Washington 6, D. C., remitting $0.50 for microfilm (images 1 inch high on standard 35 mm. motion picture film) or $0.50 for photocopies (6 × 8 inches) readable without optical aid.
(3) H. McConnell and N. Davidson, THIS JOURNAL, **72**, 5557 (1950).
(4) I. M. Kolthoff and W. J. Tomsicek, *J. Phys. Chem.*, **39**, 955 (1935).
(5) G. Kortüm, *Z. physik. Chem.*, **B33**, 254 (1939).
(6) J. Whitney and N. Davidson, THIS JOURNAL, **71**, 3809 (1949).
(7) H. McConnell and N. Davidson, *ibid.*, **72**, 3168 (1950).