of *o*-hydroxyphenylmercuric chloride in 25 cc. of alcohol. The product melted at 126°; yield 79.3%. The analytical data indicate that the hydrochloride is formed and that treatment with potassium hydroxide gives an *o*-hydroxyphenylmercuric hydroxide derivative. It is not stable to acetic acid, probably forming *o*-hydroxyphenylmercuric acetate.

o-Hydroxyphenylmercuric Hydroxide.—Ten grams of *o*-hydroxyphenylmercuric chloride in 25 cc. of alcohol was treated with 17 cc. of 10% alcoholic potassium hydroxide, warming slightly and stirring vigorously. The product is insoluble.

Anal. Calcd. for $C_6H_6O_2Hg$: Hg, 64.5. Found: Hg, 64.7.

o-Hydroxyphenylmercuric Fatty Acid Compounds.—The calculated quantity of *o*-hydroxyphenylmercuric hydroxide was suspended in warm alcohol and the fatty acid added and stirred with warming until solution resulted. On cooling the fatty acid derivative precipitated. It was recrystallized from alcohol and dissolved in dilute alcohol or alkaline solution for bacteriological study.

Summary

A number of imide derivatives of *o*-hydroxyphenylmercuric chloride have been prepared, one of which has bacteriostatic properties similar to the parent compound.

Some fatty acid compounds with *o*-hydroxyphenylmercuric hydroxide have been made which may promise to give enhanced *in vivo* activity.

KALAMAZOO, MICH. RECEIVED APRIL 21, 1937

[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND CHEMISTRY, UNIVERSITY OF NORTH CAROLINA]

The Identification of the Amino Acids: *p*-Toluenesulfonyl[1] Chloride as a Reagent

BY EVAN W. MCCHESNEY AND WM. KIRK SWANN, JR.

I. Introduction

Numerous reagents have been employed for the identification of the amino acids. Among these perhaps the most important group are those reagents which conjugate readily with the amino group, such as benzoyl chloride, β-naphthalenesulfonyl chloride, benzenesulfonyl chloride, *p*-nitrobenzoyl chloride, and phenyl isocyanate. With the passing of years, however, and the discovery of new amino acids, there have come to be gaps in our knowledge such that the derivatives of all of the known amino acids with any one of these reagents do not seem to have been described. Also, in some cases, the derivatives have been prepared, but it has not been possible to crystallize them. The result of this situation is that an investigator who has at hand a small amount of an unknown amino acid cannot rely safely on one derivative for the identification, since it may not crystallize, or it may not have been described. The object of this work has been to find, if possible, one derivative which would be satisfactory for all of the amino acids, and to describe it for those which are at present recognized as products of protein hydrolysis and are commercially available.

For this work two reagents not extensively used thus far in amino acid work were selected for study: *p*-toluenesulfonyl chloride and *p*-bromobenzenesulfonyl chloride. It was soon found that the derivatives obtained with the former crystallized much more readily; therefore it was selected to complete the study. The idea of using *p*-toluenesulfonyl chloride as a reagent for the amino acids is not a new one, Oseki[2] having prepared about a dozen of the compounds, and several other investigators having prepared one or more of them (see references to Table I). A considerable number of the amino acids have not been included in these studies, however, and in some cases there are serious discrepancies in the melting points recorded (see, for example, *d*-alanine, *d*-valine, and *l*-leucine in Table I). It therefore seemed worth while to try to repeat all of the work previously done and, if possible, to complete the list.

II. Materials.—The amino acids used in this work were the highest grade products of the Eastman Kodak Co., the Pfanstiehl Chemical Co., and Hoffman–La Roche, Inc., except those which are commonly prepared in the laboratory (glycine, *d,l*-alanine, *l*-tyrosine, *l*-cystine, and *d*-glutamic acid). The latter were prepared by the standard methods, crystallized from the appropriate solvents, and their purity was demonstrated by nitrogen analyses. The *p*-toluenesulfonyl chloride was an Eastman product, m. p. 67–69° (m. p. in the literature is 69°[3]).

III. Method.—The method used[4] is such a familiar one that it requires little comment. The amino acid, 0.01 equivalent as to combining power, is dissolved in 20 cc. of *N* sodium hydroxide, an ethereal solution of 2 g.

(1) Presented at the Chapel Hill meeting of the American Chemical Society, April 14, 1937.

(2) Oseki, *J. Tokyo Chem. Soc.*, **41**, 8 (1920).

(3) *Cf.* Beilstein, "Handbuch der organischen Chemie," 4th ed., Vol. XI, p. 103.

(4) Fischer and Bergell, *Ber.*, **35**, 3779 (1902).

(0.01 mole) of *p*-toluenesulfonyl chloride is added, and the mixture is shaken mechanically for three to four hours. The ethereal solution is then separated off and the aqueous solution is acidified to congo red with hydrochloric acid. The derivative begins to crystallize at once (or separates as an oil which, on standing in the refrigerator, will eventually crystallize except in special cases to be discussed later). The crystals are collected on a filter and are recrystallized from a very small amount, usually 5 cc. or less, of 60% alcohol. The yield at this point is about 50% of the theoretical. Two or three crystallizations give a constant melting point.

Exceptions.—In the case of phenylalanine and tyrosine, the sodium salt of the derivative is sparingly soluble in water and separates out during the reaction. The semi-emulsion which results is acidified to congo red, whereupon the sodium salt goes into solution and the mixture separates into two layers. The derivative passes into the ethereal layer from which it begins to crystallize spontaneously in a few minutes; it may be collected on a filter. With these amino acids the yield is nearly quantitative and more of the solvent, up to 25 cc., is needed for crystallization. There is a second exception in the dicarboxylic acids, the derivatives of which are very soluble in water, therefore do not precipitate on acidification. They are converted to their dibutyl esters by the method of Gurin and Clarke[5] as described later.

IV. Experimental Data

The amino acid derivatives which could be crystallized were dried *in vacuo* over boiling ethyl alcohol in a drying pistol. They were analyzed for nitrogen by a semi-micro Kjeldahl method in order to demonstrate their purity; in the cases of cystine and methionine, sulfur was also determined, the Parr bomb method being used. The data are shown in Table I, which includes derivatives reported by other investigators to crystallize, but found in this work, however, to form persistent oils.

The *p*-toluenesulfonyl derivatives of the amino acids formed colorless crystals which usually separated in the form of needles or rods. The only important exceptions were the derivatives obtained from cystine and histidine; the former crystallized in the form of prisms of a gritty character, while the latter crystallized as rosets of needles. Of the various solvents used for recrystallization, only 60% alcohol seemed suitable for all of the derivatives. A few (notably those from glycine and alanine) crystallize from water, a few crystallize from benzene (only those from leucine and phenylalanine were tried), and a large number crystallize from β,β'-dichloroethyl ether (those from cystine, serine and histidine were the only exceptions found). However, the question of solubility has been studied extensively by others, notably Oseki, and no discussion seems called for in this paper.

In addition to the melting points, further evidence as to the nature of the compounds is obtained readily by analyzing for nitrogen or sulfur, or by titrating the carboxyl group in alcoholic solution, using phenolphthalein as an indicator. This gives results which agree exactly with the nitrogen determinations.

V. Treatment of Non-Crystalline Derivatives.—The derivatives of glutamic and aspartic acids, arginine, lysine, tryptophane and proline were found not to crystallize. The same experience was reported by Oseki as regards arginine and lysine, but the other four derivatives have been crystallized by various investigators. In this work, for lack of material, tryptophane was not studied further; the preparation of a crystalline derivative from it would be of little practical value, however, since it is readily recognized by other means. The other derivatives were taken up in butyl alcohol if they precipitated as oils, or were extracted from aqueous solution by butyl alcohol if they did not precipitate. They were then converted

TABLE I

DATA ON THE *p*-TOLUENESULFONYL DERIVATIVES OF SOME AMINO ACIDS

Amino acid	Melting point of derivative, °C. In literature	Found (uncorr.)	N, % Calcd.	Found
d,l-Alanine	138–139[6]	138–139	5.76	5.79
d-Alanine	92– 94[7] 131–132[8]	132–133	5.76	5.62
l-Aspartic acid	126–128[9] 139–140[7]	Oil		
l-Cystine (di-subs.)	204–205[2]	201–203 (dec.)	5.11	5.08
d-Glutamic acid	115–117[8]	Oil		
Glycine	146[2] 147[9] 149–150[10]	147	6.11	6.04
l-Histidine		202–204 (dec.)	13.59	13.45
l-Hydroxyproline[11]		153	4.91	4.97
d,l-Isoleucine	141 (corr.)[12]	139–140	4.91	4.86
d-Isoleucine		130–132	4.91	4.83
l-Leucine	111.5[9] 113.5[13] 124 (corr.)[14]	121–122	4.91	4.91
d,l-Methionine		104–105	4.62	4.78
d,l-Norleucine		124	4.91	4.88
d,l-Phenylalanine	134–135[15]	134–135	4.39	4.40
l-Phenylalanine	164–165 (corr.)[16]	161	4.39	4.36
l-Proline	130–133[17]	Oil		
d,l-Serine[11]		212–213 (dec.)	5.41	5.25
l-Tryptophane	176[8]	Oil		
l-Tyrosine (di-subs.)	117–119[9,18]	113–114	2.86	2.84
d-Valine	110–111[9] 147[19]	147	5.17	5.08

Sulfur analyses: For di-*p*-toluenesulfonyl-*l*-cystine calcd., 23.35%; found, 23.58%. For *p*-toluenesulfonyl-*d,l*-methionine, calcd., 21.14%; found, 21.36%.

(5) Gurin and Clarke, *J. Biol. Chem.*, **107**, 403 (1934).

(6) Gibson and Simonsen, *J. Chem. Soc.*, **107**, 799–802 (1915).

(7) Freudenberg and Noe, *Ber.*, **58B**, 2407 (1925).

(8) Bergell, *Z. physiol. Chem.*, **104**, 185 (1919).

(9) Wallin, Dissertation, *Acta Universitatis Lundensis*, **28**, 3 (1892).

(10) Fischer and Bergmann, *Ann.*, **398**, 117 (1913).

(11) Enough reagent was used to give the disubstituted derivative, but only the monosubstituted derivative was obtained. It is therefore undoubtedly the N-derivative.

(12) Bouveault and Locquin, *Compt. rend.*, **141**, 116 (1905).

(13) Karrer and Kehl, *Helv. Chim. Acta*, **13**, 57 (1930).

(14) Fischer and Lipschutz, *Ber.*, **48**, 365 (1915).

(15) In Oseki's work it apparently is not stated what form of the amino acid he had in hand. Hence except in this one case it has been assumed that he used the naturally occurring form. Here the coincidence of melting points would seem to indicate that he used the racemic substance. Translation of Oseki's work was by Dr. A. R. Merz.

(16) Ref. 14, p. 374.

(17) Kapfhammer and Eck, *Z. physiol. Chem.*, **170**, 306 (1927).

(18) The N-*p*-toluenesulfonyl derivative has been described by Fischer and Lipschutz, ref. 14, p. 376. M. p. 187–188°C. The derivative reported here is N,O-di-*p*-toluenesulfonyl-*l*-tyrosine.

(19) Karrer and van der Sluys Veer, *Helv. Chim. Acta*, **15**, 750 (1932).

to their butyl esters by the method of Gurin and Clarke.[5,20] After evaporation of the butyl alcohol as directed by these authors, the oil remaining was taken up in ether and precipitated by petroleum ether. The precipitate, on standing in the refrigerator, crystallized and was recrystallized from petroleum ether with alcohol added. The derivatives of glutamic and aspartic acids crystallized as silky needles (yield nearly quantitative), lysine also as silky needles (yield 20%), and proline as clear prisms (yield 50%). The arginine derivative was soluble in alcohol but insoluble in ether or petroleum ether. It has not been possible to cause this derivative to crystallize. The characteristics of these compounds are shown in Table II.

TABLE II

Amino acid	M. p. of de. iv., °C.	Analyses, % Calcd.	Found	Calcd.	Found
d-Glutamic acid[21]	61–62	N 3.39	3.19	S 7.76	7.89
l-Aspartic acid[22]	64–65	N 3.51	3.35	S 8.03	8.17
d,l-Lysine[23,24]	111–113	C 56.42	56.28[25]	H 6.71	6.50[25]
l-Proline[26]	53–55	C 59.03	58.85[25]	H 7.13	6.90[25]
d-Arginine	Oil				

(20) *Cf.* also Gurin, THIS JOURNAL, **58**, 2104 (1936).

(21) That is, di-*n*-butyl [N-*p*-toluenesulfonyl]-*d*-glutamate.

(22) That is, di-*n*-butyl [N-*p*-toluenesulfonyl]-*l*-aspartate.

(23) That is, *n*-butyl [α,ϵ-di-*p*-toluenesulfonylamino]-*n*-caproate.

(24) Product contained a trace of ash; analytical data corrected for this.

(25) Analysis made under the direction of Dr. O. P. Wintersteiner.

(26) That is, *n*-butyl [N-*p*-toluenesulfonyl]-*l*-pyrrolidine-α-carboxylate.

Acknowledgment.—One of us (E. W. M.) acknowledges with pleasure his indebtedness to: (1) Dr. H. B. Lewis, for the privilege of working in his laboratories at the University of Michigan, and for the gift of samples of several of the amino acids; (2) The Rockefeller Fund, an appropriation from which partly supported this investigation; (3) Dr. Wm. de B. MacNider, for the gift of a part of his research funds; (4) Dr. O. P. Wintersteiner, through whose courtesy certain of the analyses reported in Table II were made.

Summary

The *p*-toluenesulfonyl derivatives of nineteen amino acids have been prepared of which six have not been described previously. The *p*-toluenesulfonyl derivatives of aspartic acid, *d*-glutamic acid, *d,l*-lysine and *l*-proline did not crystallize. The non-crystalline derivatives, except tryptophane, were converted to their butyl esters and these crystallized, with the exception of arginine. The butyl esters of the derivatives of the four remaining amino acids are described for the first time in this paper.

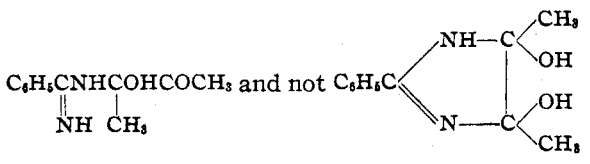
CHAPEL HILL, N. C. RECEIVED JANUARY 28, 1937

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF COLORADO]

The Structure of Benzamidine-glyoxal and of its Compounds with Aromatic Aldehydes

BY JOHN B. EKELEY AND ANTHONY R. RONZIO

In former papers[1] it has been shown that in alkaline solution aromatic amidines form addition products with glyoxal which can be isolated as the free bases or as their hydrochloride salts. In water solution the addition products are dissociated into amidine and glyoxal since from the solutions the osazone and dioxime of glyoxal can be obtained. We now have absorption spectra evidence which confirms this conclusion. We have exactly the same kind of evidence that the addition product from benzamidine and diacetyl



obtained by Diels and Schleich[2] is, like that of benzamidine-glyoxal, an open chain compound as they assumed.

Inspection of the absorption spectra curves for benzamidine hydrochloride, benzamidine-glyoxal and benzamidine-diacetyl confirms the above statements.

There has been described[1] a long series of compounds which were obtained by the action of aromatic aldehydes upon aromatic amidines and glyoxal in alkaline solution. From the evidence then at hand we assumed them to contain either a pyrimidine or a glyoxaline ring. Ruhemann and Cunnington[3] prepared derivatives from benzamidine and phenylpropiolic ester, one of which, diphenylglyoxalidone, a yellow compound, pos-

(1) Ekeley and Ronzio, THIS JOURNAL, **57**, 1353 (1935); Ekeley and Elliott, *ibid.*, **58**, 163 (1936).

(2) Diels and Schleich, *Ber.*, **49**, 1713 (1916).

(3) Ruhemann and Cunnington, *J. Chem. Soc.*, **75**, 954 (1899).