STUDIES IN THE SYNTHESIS AND HYDROLYSIS OF 2,5-DIKETOPIPERAZINES.

by

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Abstract.

Five simple diketopiperazines have been prepared in good yield in extending a similar investigation made by Sannié. They were obtained in a satisfactory state of purity, indicating the suitability of the method for such purposes.

Fifteen mixed diketopiperazines have been prepared by a similar reaction, using different methods in general for isolation of final product. The yields obtainable are reasonably satisfactory. Purity of products in some cases, as judged by colour and melting point, is open to improvement by much more exhaustive investigation, but generally has been of a reasonable order. It has certainly been high enough to demonstrate that the method of carrying out the reaction is suitable for the preparation of mixed diketopiperazines.

Dielectric constants of acetic acid solutions of three simple diketopiperazines have been measured. The data obtained show that the compounds studied are sufficiently polar to form solutions of higher dielectric constant than the solvent, which itself had a fairly high value. It has also been shown that with increasing molecular weight, the effect diminishes due to the increased influence of the larger alkyl groups.

Investigations have been made of the hydrolysis of a number of mixed diketopiperazines by N/HCl and by N/NaOH. In some

cases hydrolysis was not accomplished, and in one other case success in accomplishing the reaction was variable. In other cases, hydrolysis either gave much more of one dipeptide than the other, or both possible products were formed in more nearly equal quantities.

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The author wishes to acknowledge the parts played by each of the following in making the prosecution of the research possible:-

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CONTENTS.

Index of Figures.

Index of Tables.

INTRODUCTION

2,5-diketopiperazines have attracted considerable attention in past years as derivatives of amino acids and dipetides, being capable of synthesis from both types of compound. Amino acids and peptides generally are known to contribute to the building up of protein molecules. The question was investigated by many workers as to whether diketopiperazines also played such a part, since various members of the series have been isolated along with amino acids and peptides in the degradation of proteins by acids and alkalis.

It is possible to use many simple diketopiperazines to prepare the corresponding dipeptides by hydrolysis. In certain instances workers in the field have published accounts of the hydrolysis of mixed diketopiperazines to dipeptides. The theory has generally been held that a mixture of two possible dipeptides is inevitably formed. It was with the object of determining whether hydrolysis of mixed diketopiperazines derived from aliphatic monamino-monocarboxylic acids was in any way selective that this part of the work was undertaken.

It has long been known that amino acids, peptides and proteins form solutions in aqueous solvents in which the dielectric constant is not only higher than that of water, but also that the increase follows a linear relation to concentration of solution.

Amino acids and peptides are known to give solutions of lower dielectric constant than solvent when the latter has a dielectric constant less than 20. Although contemporary opinion does not seem to regard diketopiperazine rings as such to be present in the structure of protein molecules, it was considered worth while to determine what information might be derived from measurement of dielectric constants of solutions of some members of the series.

REVIEW OF THE LITERATURE

THE CHEMISTRY OF 2,5-DIKETOPIPERAZINES.

2,5-Diketopiperazines are mentioned throughout the literature with respect to their methods of preparation, their possible relationship to proteins, their chemical reactions (most particularly hydrolysis), and instances where they have been formed, either as by-products or as intermediates in some reaction not necessarily designed for their preparation. In this review, attention will be directed mainly to:-

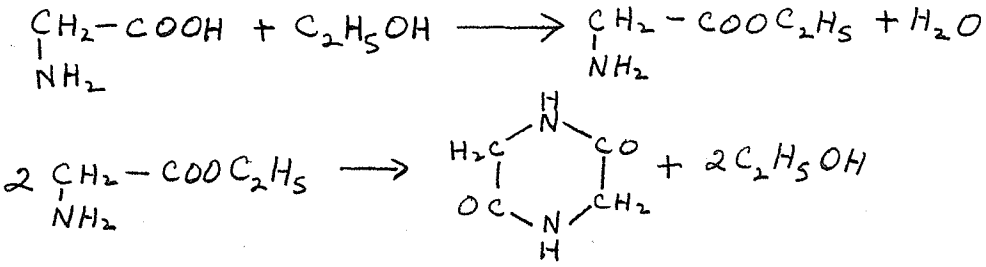
I - Their methods of preparation, with some mention of other cases where they have reported as being formed as intermediates or as by-products in the preparation of other substances.

II - Their hydrolysis.

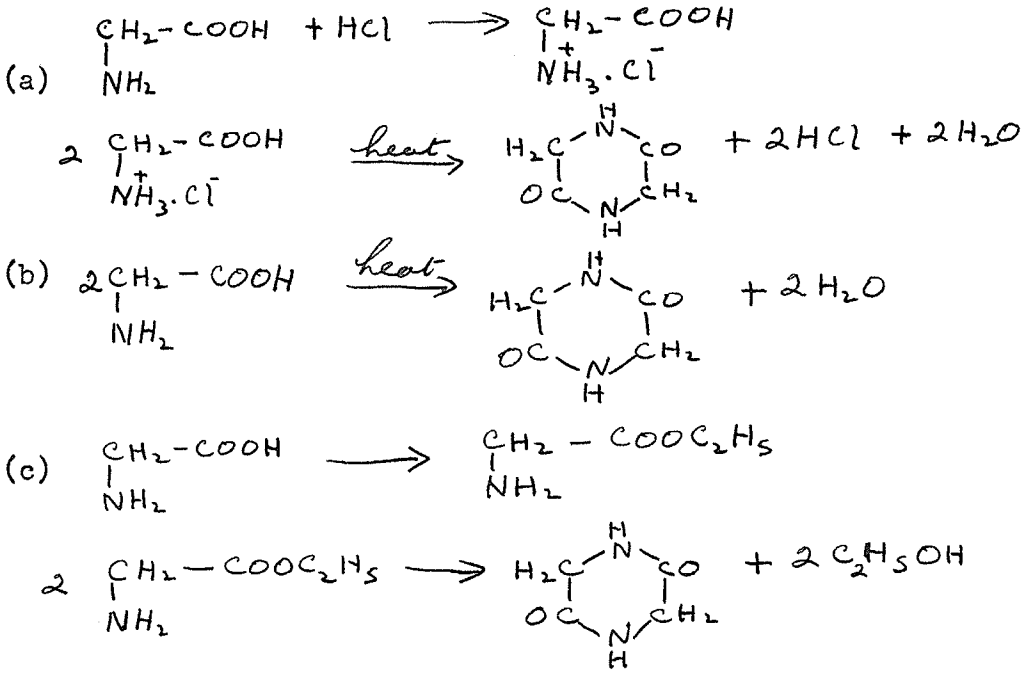
III- Their possible importance to the study of proteins.

I. Preparation of 2,5-Diketopiperazines.

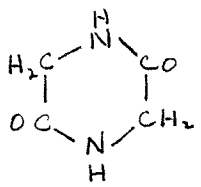
The simplest substance in this class is 2,5-diketopiperazine, frequently called glycine anhydride. The first report of preparation of this substance was published by Curtius and Goebel, who reported that it was formed by the treatment of glycine with ethyl alcohol and sulphuric acid (1).



Other methods reported by Curtius and Goebel for conversion of glycine were (a) heating glycine in a current of hydrogen chloride, (b) by heating glycine at 150°-170° with glycerine, (c) converting glycine to its ethyl ester and leaving in contact with water.

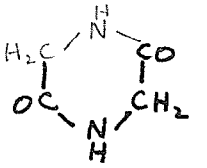


Emil Fischer and Fourneau also synthesised the substance by method (c) above (2), and Fischer with his associates carried out a long series of researches into methods of synthesising it and other members of the series. They found that 2,5-diketopiperazine could also be formed from glycylglycine ethyl ester by (a) heating to 190° (b) standing in contact with water (c) treating with a saturated alcoholic solution of ammonia, or (d) treating with sodium ethoxide solution (2).

(a) $CH_2(NH_2)-CO-NH-CH_2-COOC_2H_5 \xrightarrow[\text{or } H_2O]{\text{heat}}$  $+ C_2H_5OH$

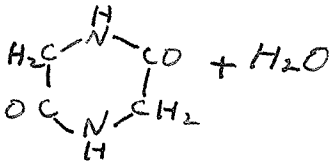
(b) NH_2

(c) $CH_2(NH_2)-CO-NH-CH_2-COOC_2H_5 + NH_3 \longrightarrow (CH_2(NH_2)-CO-NH-CH_2-CO-NH_2) + C_2H_5OH$

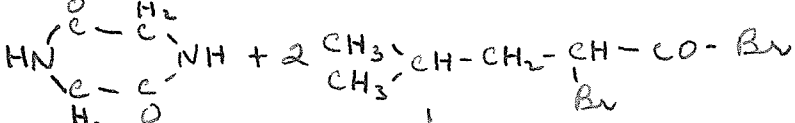
$(CH_2(NH_2)-CO-NH-CH_2-CO-NH_2) \longrightarrow$  $+ NH_3$

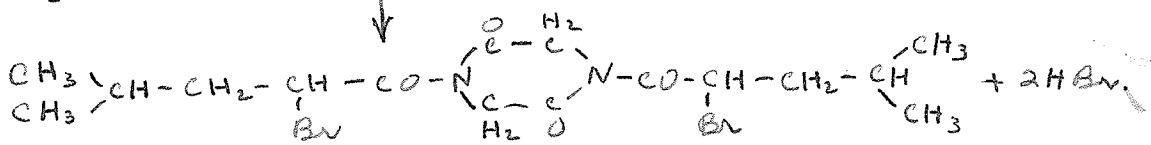
Fischer also obtained the substance from chloracetylglycine or chloracetylglycylglycylglycine by warming with concentrated ammonia solution (3).

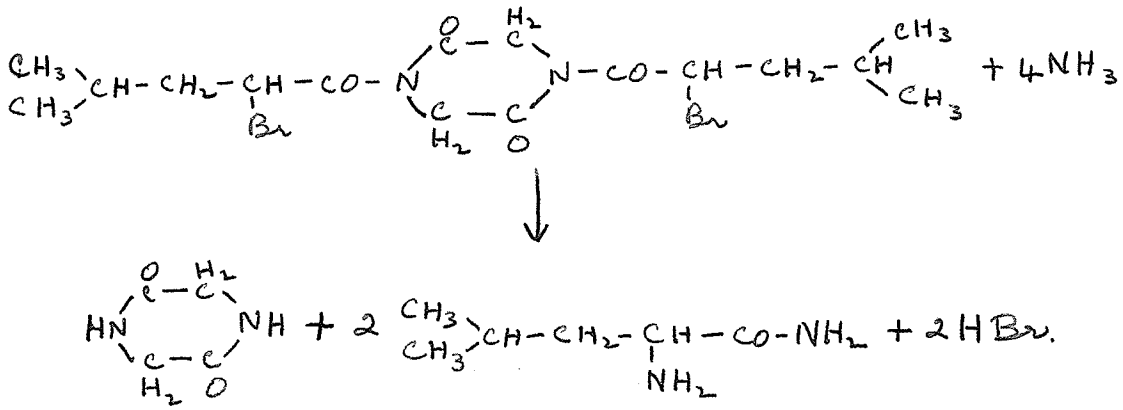
$CH_2(Cl)-CO-NH-CH_2-COOH + NH_3 \longrightarrow CH_2(NH_2)-CO-NH-CH_2-COOH + HCl$

$CH_2(NH_2)-CO-NH-CH_2-COOH \longrightarrow$  $+ H_2O$

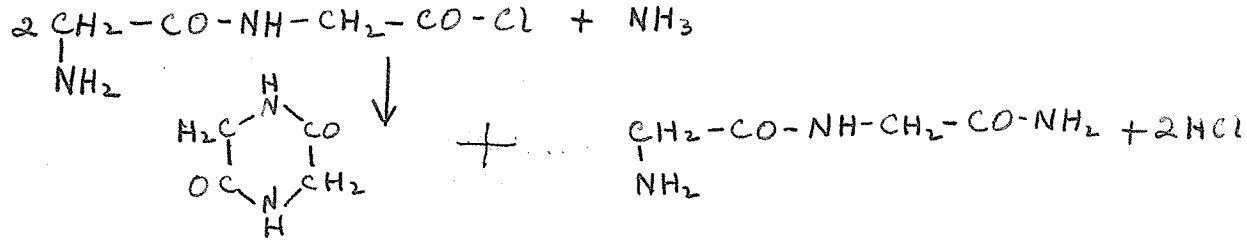
In a later investigation, Abderhalden and Klarmann condensed the substance with ∝-bromoisocaproyl chloride, by heating with thionyl chloride, possibly in an attempt to build up more complex ring systems. The product, 1,4-di (∝-bromoisocaproyl)-2,5-diketopiperazine, on treatment with alcoholic ammonia, merely reverted to a mixture of the original glycine anhydride and leucinamide (4).



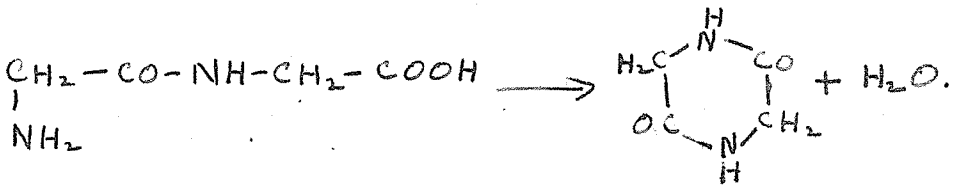




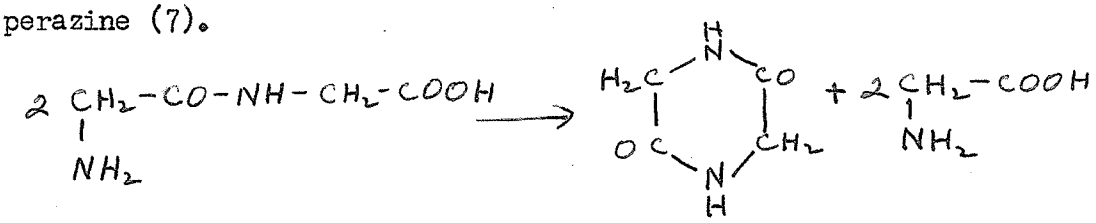
Bergell, in an attempt to convert glycylglycine chloride to glycylglycinamide by the action of aqueous ammonia, obtained instead 2,5-diketopiperazine as main product and only traces of the desired amide in a state of impurity (5).



Abderhalden and Komm also succeeded in preparing the substance from glycylglycine by each of the following procedures (a) heating to 150-160° for several hours with water in a sealed tube (b) heating with dilute hydrochloric acid or sulphuric acid or (c) by refluxing for several days with water (6).



Levene and his associates carried out extensive investigations in the chemistry of 2,5-diketopiperazines, and reported that when studying the catalytic effect of the enzyme erepsin (nowadays regarded as a mixture of dipeptidases) on the hydrolysis of glycylglycine, this dipeptide formed glycine and 2,5-diketopiperazine (7).



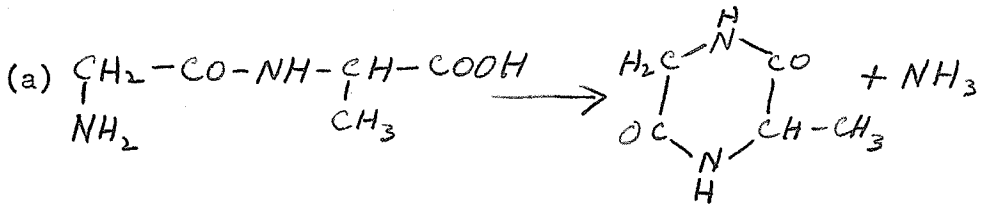
Lichtenstein in a study of conversion of amino acids and dipeptides to diketopiperazines by heating in α-naphthol to 135°-140°, found that glycylglycine could not be converted to the anhydride (8).

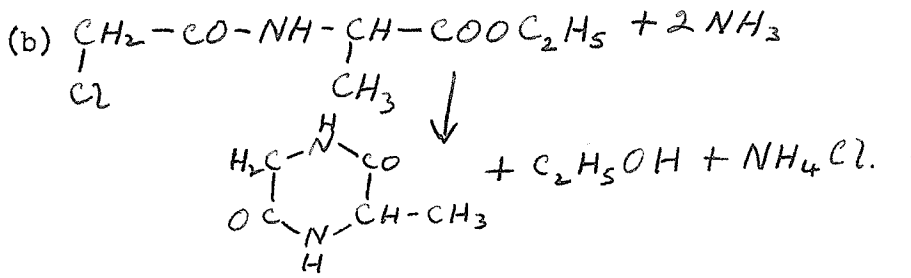
A German patent describes the preparation of the substance by passing ammonia into a benzine-benzene solution of glycine ethyl ester hydrochloride at low temperature (9).

Sannié succeeded in preparing the substance by heating glycine with ethylene glycol (10). Schott, Larkin, Rockland and Dunn (84) describe a procedure similar to that of Sannié, in which they stirred glycine with hot ethylene glycol for about one hour, but the yield of product is much lower than that obtained by Sannié's method (less than 40%, compared to 45-67%).

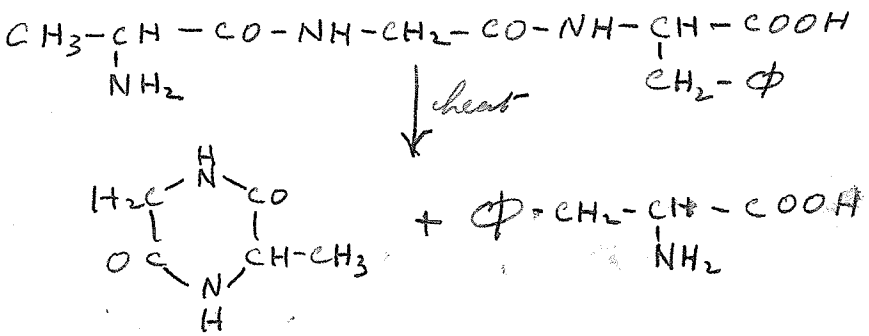
3-Methyl-2,5-diketopiperazine, or glycylalanine anhydride.

Fischer and his co-workers prepared this member of the series by an extension of the methods established earlier for the synthesis of the first member. The reactions were (a) treatment of glycyl-D-alanine or its hydrochloride with saturated alcoholic ammonia solution at low temperature, and (b) heating chloracetyl-DL-alanine ethyl ester with a saturated alcoholic ammonia solution to 100° (11,12,13).

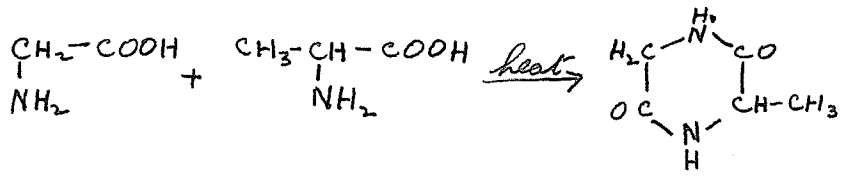




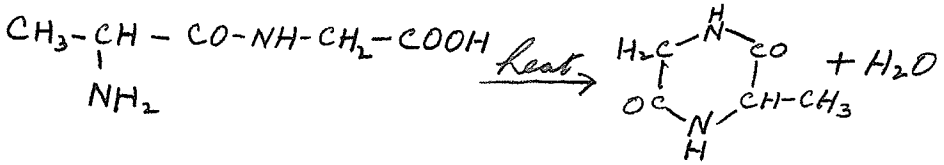
Lichtenstein, in his study of the effect of heating amino acids with α-naphthol found that DL-alanylglycyl-DL-phenylalanine formed 3-methyl-2,5-diketopiperazine along with DL-phenylalanine (8).



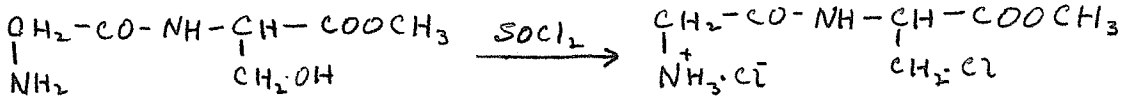
Sannié's method of heating amino acids in ethylene glycol (10) resulted in formation of the compound when a mixture of glycine and DL-alanine was so treated.

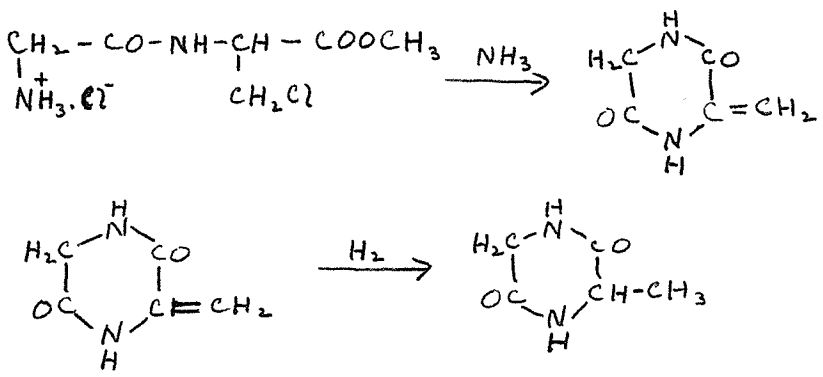


Abderhalden and Komm also prepared it by several methods, for example, they isolated it from the hydrolysates when dog hair had been heated for several hours at 150°-160° with 1% hydrochloric acid (14), and when silk fibroin had been subjected to prolonged action of concentrated hydrochloric or 70% sulphuric acid below 25° (15). He also prepared it from alanylglycine by heating to high temperature with (a) water, (b) dilute acids (6).



Bergmann described an indirect preparation of the compound from glycylserine methyl ester. The latter, with thionyl chloride, formed ∝ -glycylamino-β -chloropropionyl chloride hydrochloride methyl ester, which in turn was converted to 3-methylene-2,5-diketopiperazine by the action of concentrated aqueous ammonia. Catalytic reduction of this anhydride gave the 3-methyl compound (16).



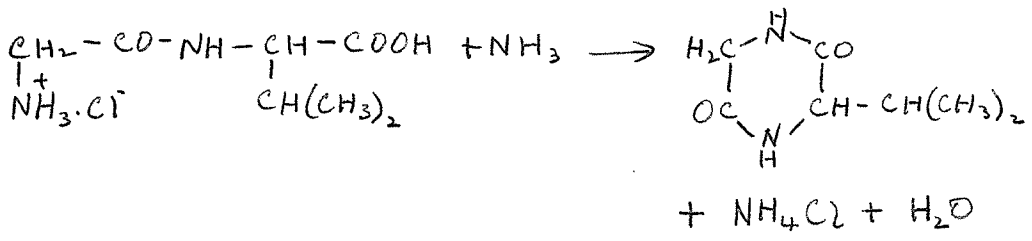


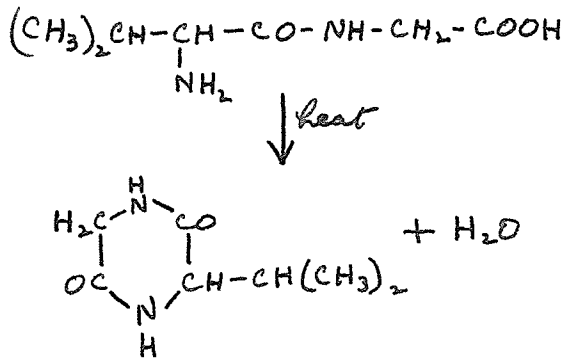
Bergell attempted to synthesise alanylglycinamide by the action of aqueous ammonia solution on α -bromopropionylglycine. The amide, if formed, was not sufficiently stable to be isolated, and the only product of the reaction was a small yield of 3-methyl-2,5-diketopiperazine (5).

Levene and co-workers reported its formation as a result of the catalytic effect of erepsin on the hydrolysis of alanylglycine and of glycylalanine (7).

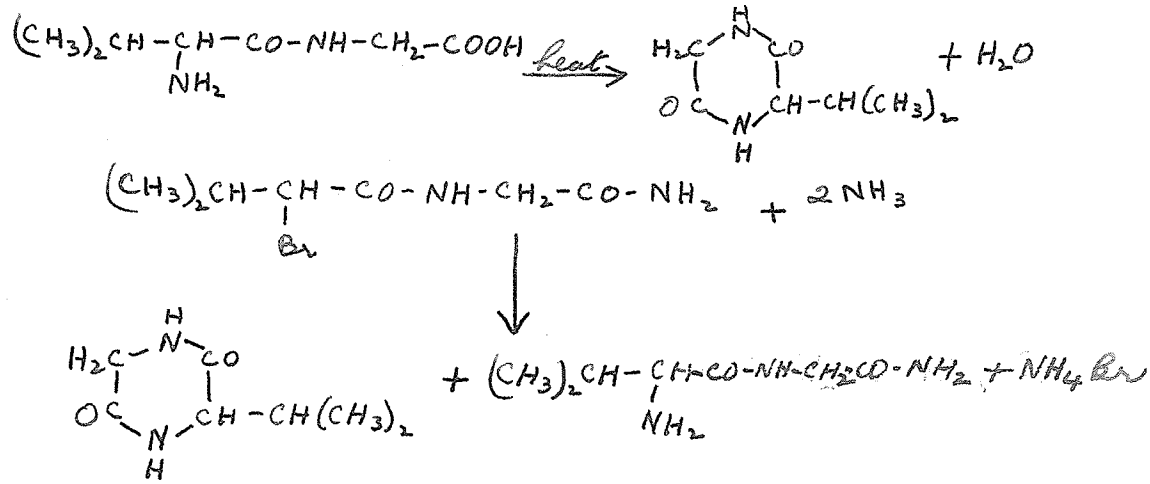
3-Isopropyl-2,5-diketopiperazine, or glycylvaline anhydride.

Preparations of this compound have been made by Fischer and his associates, from appropriatedipeptides and on protein hydrolysis, by Bergell and by Lichtenstein. Fischer's preparations were carried out by treating glycyl-D-valine hydrochloride with saturated alcoholic ammonia at low temperature (17), by heating DL-valylglycine to its melting point, (18), and by hydrolysing elastin with strong hydrochloric acid, and isolating the compound from other products in the hydrolysate (19).



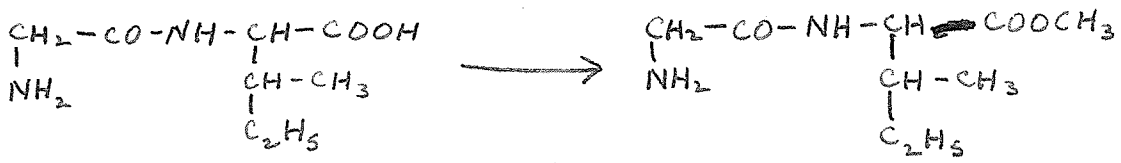


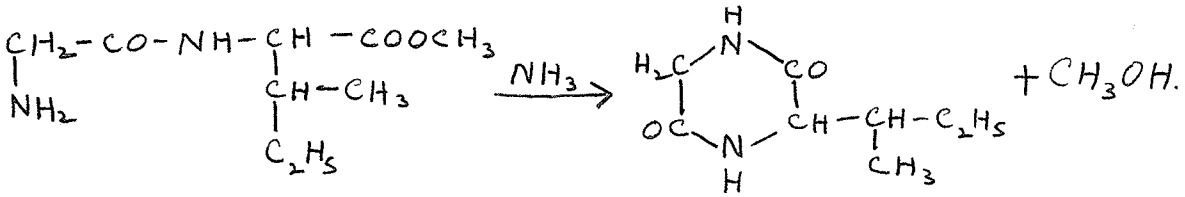
Lichtenstein prepared it by heating DL-valylglycine to 135-140° in ∝-naphthol (8). Bergell was studying the action of ammonia on (∝-bromovaleryl) glycine amide (5). Aqueous ammonia had hardly any action, but on heating to 120° in a sealed tube with alcoholic ammonia the diketopiperazine was formed along with the dipeptide amide.



3-Sec. butyl-2,5-diketopiperazine, or glycylisoleucine anhydride.

Abderhalden, Hirsch and Schuler effected its synthesis from glycyl-D-isoleucine, by first converting it to the methyl ester, and treating the latter with concentrated ammonia solution (20).

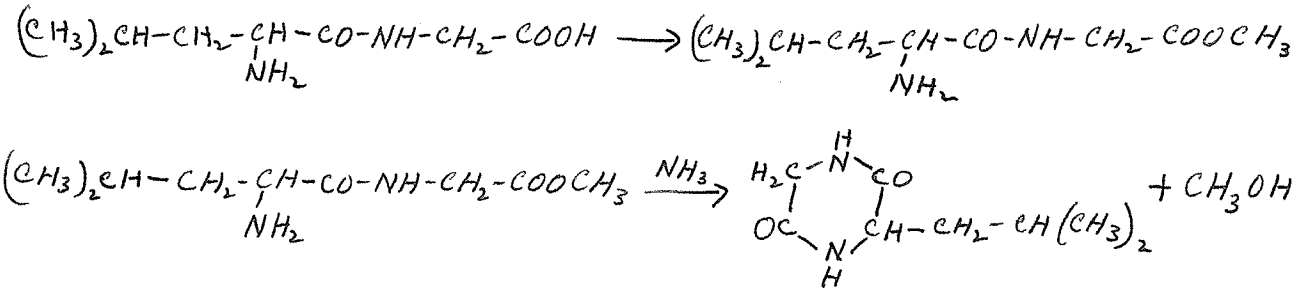




3-Isobutyl-2,5-diketopiperazine or glycylleucine anhydride.

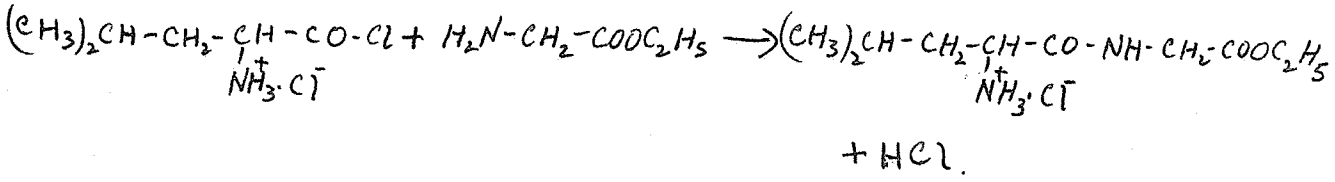
Numerous preparations of this compound have been carried out in the laboratories of Fischer, Abderhalden, and Lichtenstein. Fischer's methods were:-

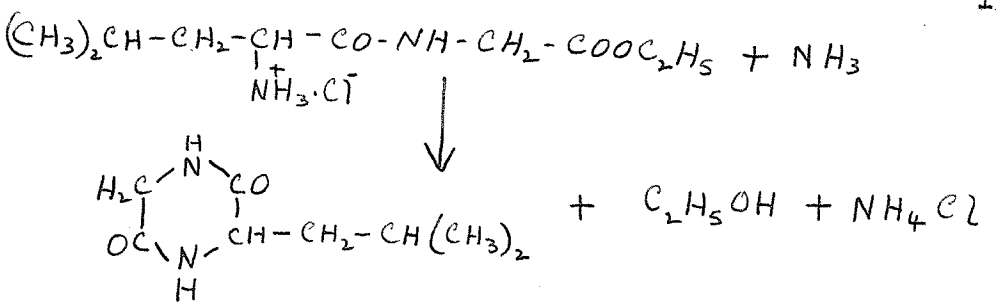
(i) Conversion of L-leucylglycine to its methyl ester and treating the latter with concentrated methyl alcoholic ammonia solution (21).



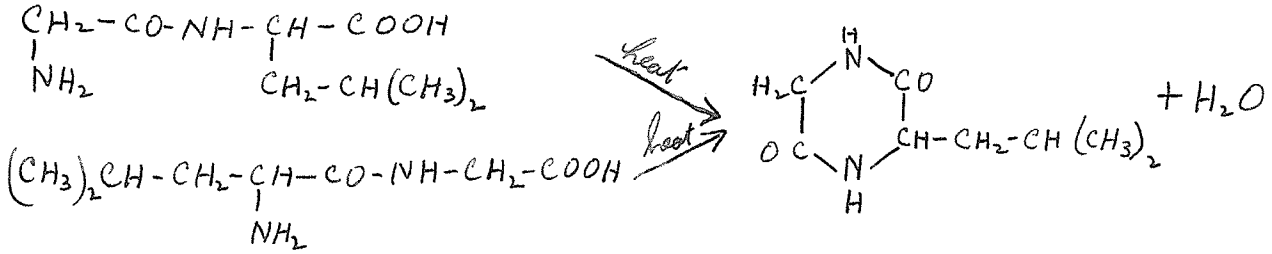
(ii) Hydrolysis of elastin with 70% sulphuric acid, esterifying the hydrolysate with ethyl alcohol in presence of hydrogen chloride, and treating the ester with ammonia in alcohol (22).

(iii) Shaking DL-leucyl chloride hydrochloride with glycine ethyl ester, and evaporating the solution with ammonia (23).





(iv) Heating glycyl-DL-leucine or DL-leucylglycine to the melting point (24, 25).

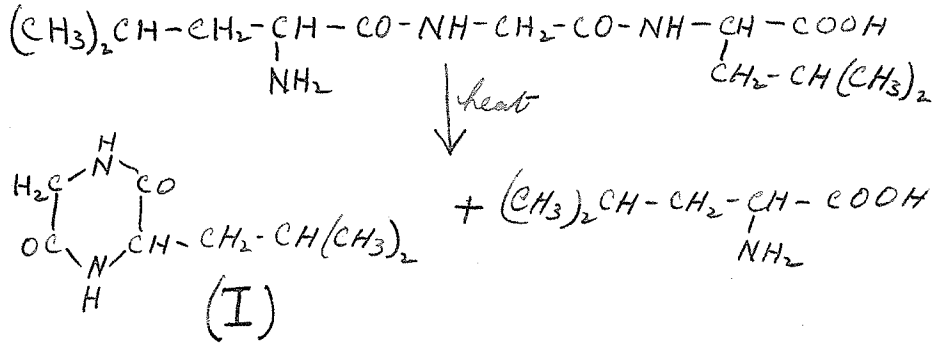


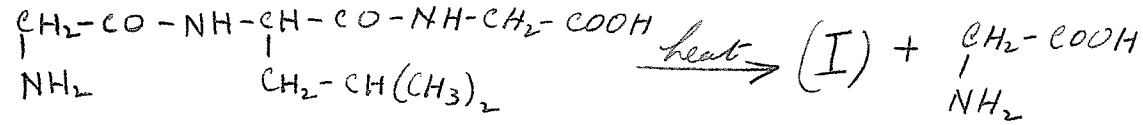
(v) Refluxing L-leucylglycine with quinoline (the DL-compound was formed) (24).

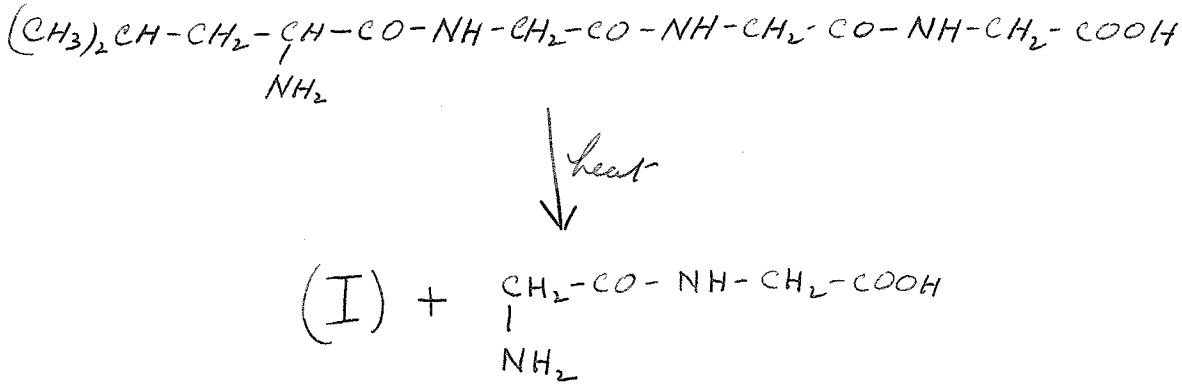
Abderhalden and Komm also obtained the compound by heating various dipeptides, polypeptides, and blood protein in an autoclave, thus:-

(i) Glycylleucine and leucylglycine each formed the substance when heated with water to 150-160° in a sealed tube (6).

(ii) L-leucylglycyl-L-leucine, glycyl-DL-leucylglycine and DL-leucylglycylglycylglycine were each heated in an autoclave (26).







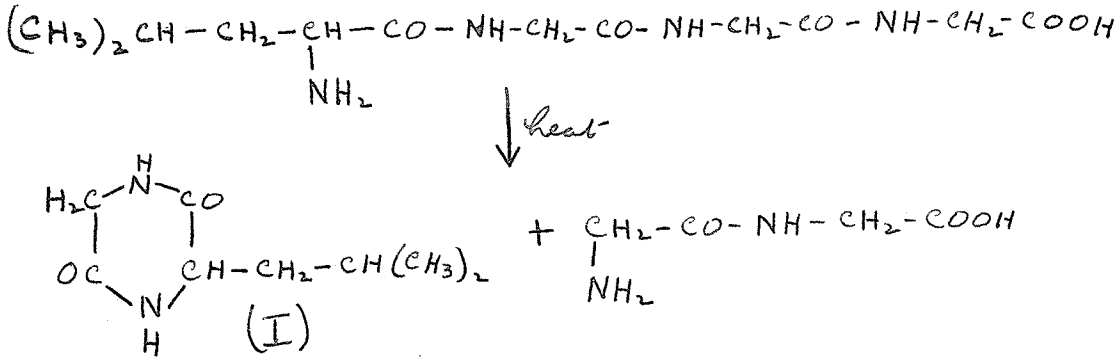
(iii) Isolation from the hydrolysate after blood protein had been heated with water at 180° for seven hours (26).

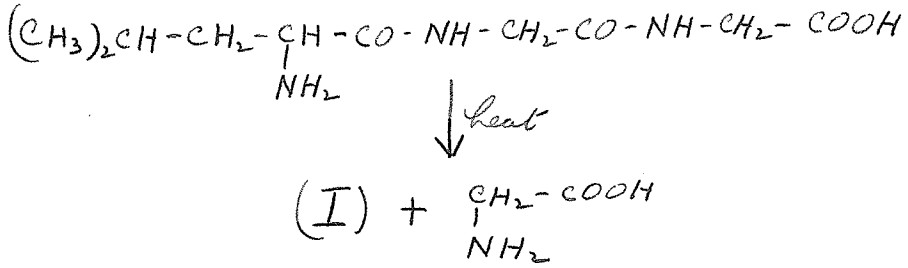
Lichtenstein converted each of the following peptides (8) to the anhydride by heating to 135-140° in α-naphthol:-

DL-leucylglycine; DL-leucylglycylglycylglycine;

glycyl-DL-leucine; DL-leucylglycylglycine;

The overall equations for the conversion of the two dipeptides are the same as with Fischer's method no. (iv) above. The conversion of the other peptides is as shown below:-





3,6-Dimethyl-2,5-diketopiperazine or alanine anhydride, was prepared by Fischer, Preu, Pellizzari, Bergmann, Lichtenstein, and Sannié. Fischer's preparations were carried out by the following reactions:-

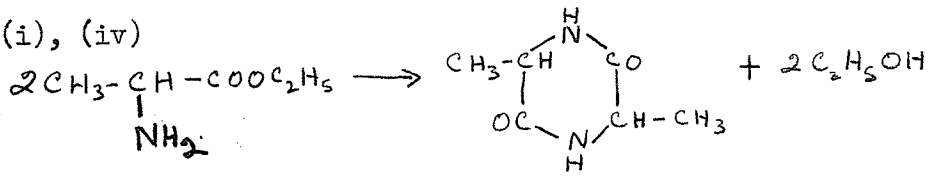
(i) Heating D-alanine ethyl (or methyl) ester for 12 hours or several days at 100° (27).

(ii) Condensing D-alanyl chloride hydrochloride with D-alanine ethyl ester, neutralising with sodium ethoxide and saturating the free ester with ammonia at 0° (27).

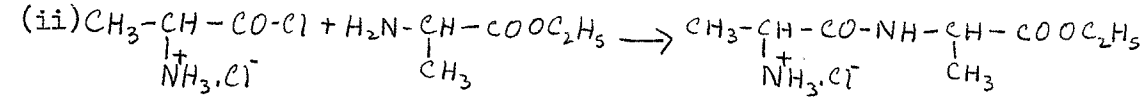
(iii) Esterifying alanylalanine and treating with alcoholic ammonia in the cold (27) (28).

(iv) Allowing DL-alanine ethyl ester to stand for several weeks, or, better, by heating to 180° in a sealed tube for 24 hours. (29). The reactions for those procedures are as represented by the equations below.

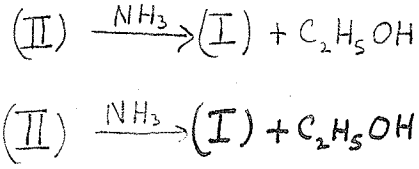
(i), (iv)



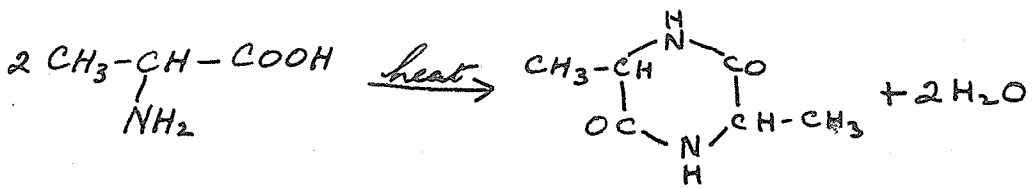
(ii)



(iii)

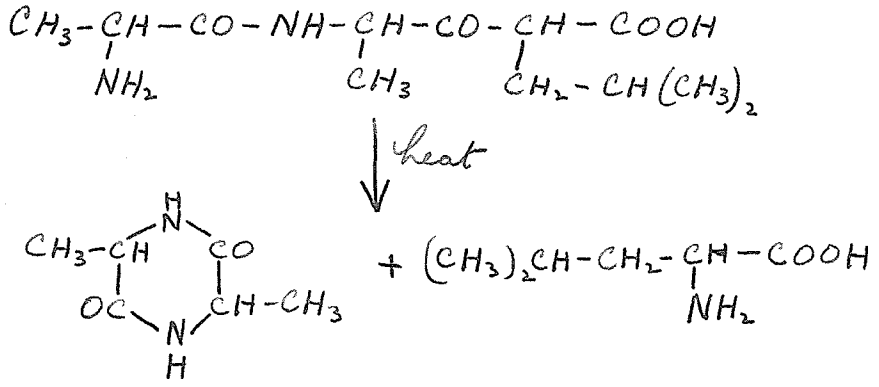


Preu's method was to heat DL-alanine to 180° in a stream of hydrogen chloride (30), while Pellizzari effected the synthesis by heating DL-alanine hydrochloride with ethyl benzoate (34).

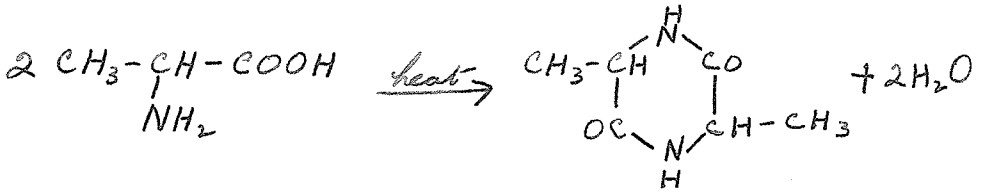


Bergmann carried out an indirect synthesis of the compound from alanine and serine. These two amino acids were condensed to form a compound $C_6H_{11}O_3N_2Cl.HCl$; the product was converted to 3-methylene-2,5-diketopiperazine by ammonia. Reduction of this unsaturated material by catalytic hydrogenation gave alanine anhydride (16).

When Lichtenstein heated DL-alanyl-DL-alanyl-DL-leucine to 135°-140° with ∝-naphthol (8), DL-alanine anhydride and DL-leucine were formed.

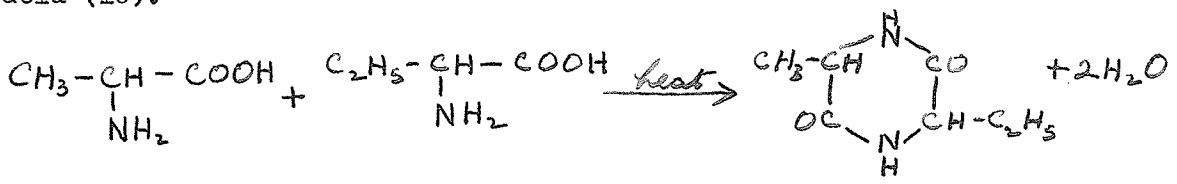


DL-Alanine was one of the amino acids whose behaviour Sannié studied; on heating with glycol DL-alanine anhydride was formed (10).



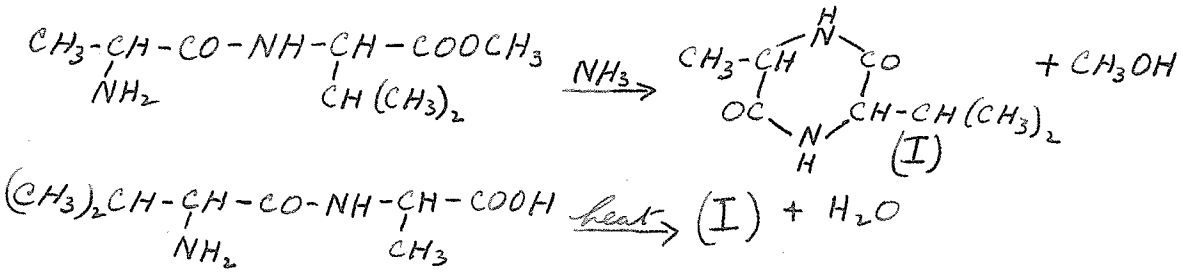
3-Methyl-6-ethyl-2,5-diketopiperazine or alanylbutyric anhydride.

Sannié describes the preparation of this compound in his paper on the heating of amino acids in glycol, by carrying out the reaction on a mixture of DL-alanine and DL-∝-aminobutyric acid (10).

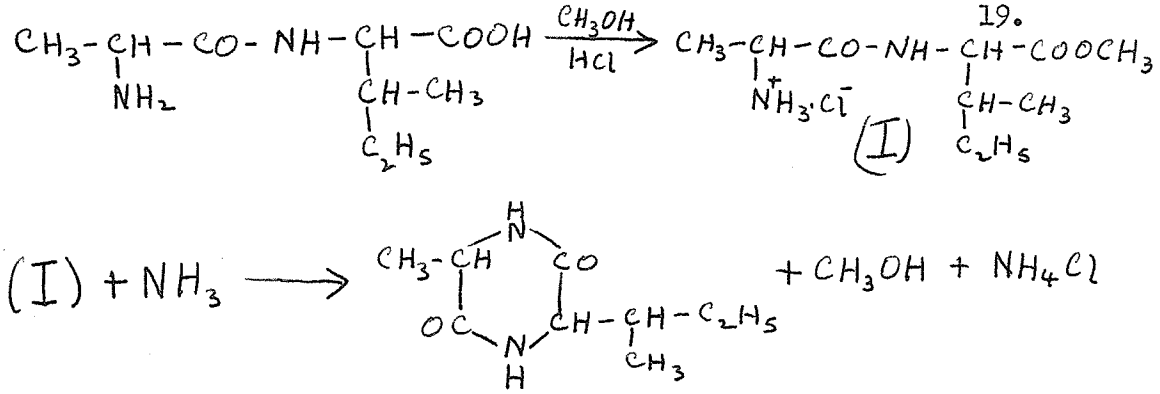


3-Methyl-6-isopropyl-2,5-diketopiperazine or alanylvaline anhydride.

By converting D-alanyl-D-valine to its methyl ester and subsequently treating the ester with ammonia, or by heating DL-valyl-DL-alanine to its melting point, Fischer and Scheibler obtained this diketopiperazine (17).



3-Methyl-6-sec.butyl-2,5-diketopiperazine or alanylisoleucine anhydride, was prepared by Fischer, Hirsch and Schuler. Hydrogen chloride was passed into a mixture of D-alanyl-D-isoleucine and methyl alcohol, followed by the action of methyl alcoholic ammonia at low temperature (20).



3-Methyl-6-isobutyl-2,5-diketopiperazine or alanylleucine anhydride.

Abderhalden obtained it from the hydrolysis of hog bristles by 1% hydrochloric acid.

Fischer obtained it in low yield by the prolonged action of concentrated ammonia solution at 25° on D-bromoisocaproyl-D-alanine, and by warming L-leucyl-D-alanine to 100°. A better yield was obtained by esterifying the latter dipeptide, followed by the action of methyl alcoholic ammonia (21). He also effected its preparation by heating DL-leucyl-DL-alanine to about 250° (25).

Lichtenstein's conversion of peptides to diketopiperazines by heating in ∝ -naphthol was effective when applied to DL-alanyl-DL-leucine and DL-alanyl-DL-leucylglycine (8). The method of Sannié when applied to a mixture of alanine and leucine also produced the anhydride (10).