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ON THE BOND BETWEEN DIKETOPIPERAZINES AND AMINO ACIDS

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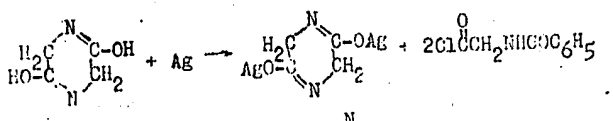
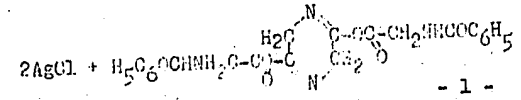
After the natural origin of cyclic forms of bonding was proved by N. D. Zelinskiy, V. S. Sadikov [1], N. I. Gavrilov, [2], and the Abderhalden school [3], the question arose in regard to the structure of more complicated compounds, particularly derivatives of diketopiperazine and peptides or amino acids, the more so since a series of compounds consisting of 3-5 amino acids and possessing a cyclic anhydride [amide] structure were isolated by N. D. Zelinskiy and V. S. Sadikov [4] as well as Abderhalden and his co-workers [5]. The first steps in this direction were taken by Maillard [6].

Abderhalden and Schwab [7] conducted systematic investigations on the synthesis of N-aminoacyl derivatives of diketopiperazines [8-13]. But in not one case did they obtain individual, well defined compounds. The works of M. Bergman [14], N. I. Gavrilov and Kh. N. Lerman [11], and L. N. Akimova [15] showed the fundamental impossibility of the course chosen by Abderhalden for the synthesis of N-aminoacyldiketopiperazines. Thus to this time no one has succeeded in obtaining N-aminoacyl derivatives of diketopiperazines.

The synthesis of acylamine compounds from enols of diketopiperazines also led Karrer [16] to no positive results.





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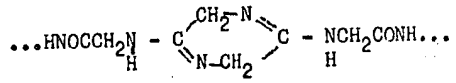
The greatest interest was elicited by Abderhalden's work with chloracyl derivatives of diketopiperizines. In our preceding article [20] were shown the specific reactive properties of N-acyl compounds which led us to methods of obtaining: 1) exo-acyl amino acid (piperazine) amidines, 2) their esters, 3) hydrates of these esters, and 4) free amidines.

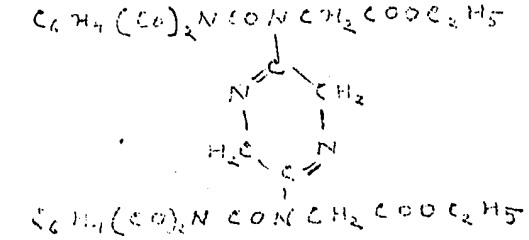
Moreover, we worked out a method for obtaining piperazines and dihydropyrazines which are connected through an amidine bond with amino acids and peptides; in this way the possibility of a fourth form of bond between diketopiperazines and aminoacyls has been demonstrated



In spite of Abderhalden's and our failure to obtain aminoacyl derivatives, we still consider it exceptionally important to synthesize these compounds, which may be found in the structure of a micromolecule of protein. This form of binding has also been accomplished by us.

Proceeding from the ease of acylating diketopiperazines with acid chlorides, we obtained chlorides of glycine, protecting the amino group with phthalyl, toluene sulfonyl and benzylsulfonyl groups.

We combined N, N'-diphathalylglycinediketopiperazine with the ester of glycine by our methods for the synthesis of exo-acylamidines and obtained



two phthalylglycylglycinepeptides joined by means of dihydropiperazine bridge. The proof of the structure of this compound is its hydrolysis to phthalylglycine and glycine, proceeding at 40°, by a 1% hydrochloric acid solution.

I. 1.4-diphthalylglycine-2.5-diketopiperazine. 1.9 g of phthalylglycinchloride [17], carefully ground together with 0.48 g of diketopiperazine, were heated in 2.5 ml of xylene for 20 minutes in a water bath (boiling under), and then for 5 hours in Wood's alloy at 142°. The precipitate was washed with benzene and treated with boiling water to remove diketopiperazine. The residue, insoluble in water, was washed with alcohol and ether. The melting point was 385° under decomposition. The yield was 83%. The product was partially soluble in boiling xylene and methyl acetate, as well as acetone. The picrin and ninhydrin reactions were negative. It was recrystallized from nitrobenzene.

II. 1.4-ditoluenesulfonylglycine-2.5-diketopiperazine. 3.7 g of toluolsulfonylglycinchloride [18], dissolved in 50 ml of nitrobenzene, were heated with 0.85 g of diketopiperazine at 140° for 2 hours. To the hot filtered solution was added absolute ether. The precipitate was filtered off and washed with alcohol and ether. The melting point was 220°. The product gives a positive anhydride reaction. From the analysis it appears to be a monoacylated diketopiperazine.

	Found %:	C 47.85; H 4.51; N 13.21
$C_{13}H_{15}O_5N_5S$.	Calculated %:	C 48.0; H 4.61; N 12.92

III. 1.4-dibenzylsulfonylglycine-2.5-diketopiperazine. 1.3 g of benzylsulfonylglycinechloride [19], dissolved in 30 ml of nitrobenzene, were heated with 0.3 g of diketopiperazine at 140° for 30 minutes. The hot solution, filtered through a glass filter, was precipitated with absolute ether. The precipitate, which settled on standing, was filtered out and washed with alcohol. Then it was recrystallized from nitrobenzene. The boiling point was 226°, accompanied by decomposition. The anhydride reaction was positive, and the ninhydrin reaction negative.

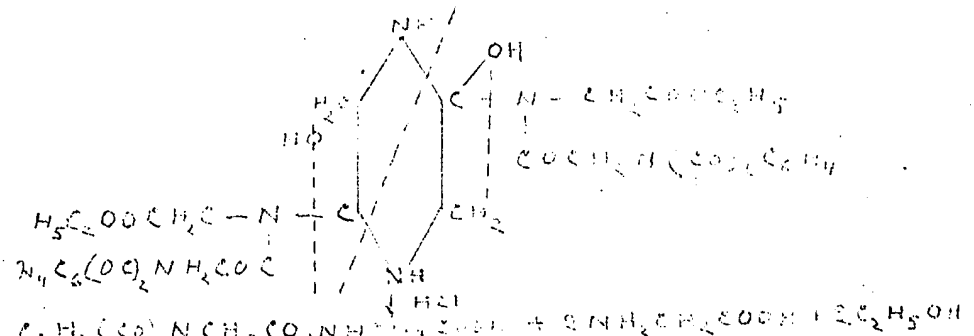
Found %:	C 49.18;	H 4.51; N 10.58
Calculated %:	C 49.25;	H 4.47; N 10.45

IV. Di-exo-N-phthalylglycylglycineethyl ester hydrate-2.5-dihydropyrazineamidine. To 5 g of diphthalylglycine-2.5-diketopiperazine in 25 ml of absolute ether are added 2.1 g (2 moles) of ethyl ester of glycine. The solution was agitated on a rocking device for a week. A stream of dry HCl was passed into the filtrate separated from precipitate 1. The precipitate forming here represented a hydrochloride of the glycine ester. Precipitate 1 was treated with chloroform at a low temperature. The precipitate which formed when the chloroform filtrate was concentrated was treated with methyl acetate. A small portion was dissolved and when concentrated gave a precipitate with a melting point of 183°. The greater portion was not dissolved and represented a substance with a melting point of 145° and with a crystal form resembling leaves. On analysis the substance with a melting point of 145° appeared to be an amidine. Ninhydrin and picrin reactions were negative.

	Found %:	C 55.10; H 4.95; N 11.84
$C_{32}H_{34}O_{12}N_6$.	Calculated %:	C 55.33; H 4.89; N 12.11
	Found (Solution) :	M = 678.7
	Calculated :	M - 694

For evidence as to the regrouping of the phthalylglycine group from the exo-position of nitrogen in the piperazine ring to the exo-position of nitrogen in the side chain amino acid, this compound was hydrolyzed with 1% HCl with and without ferment. After standing for 2 days in a constant temperature chamber a precipitate which seemed to be phthalylglycylglycine formed in both solutions. For analysis this substance was recrystallized from water. The melting point of 229.5°, the crystal form (needles) under the microscope, and the complete absence of an anhydride reaction and a biuret reaction carried out with both $CuCl_2$ and $Co(NO_3)_2$, demonstrate that the substance we isolated is actually phthalylglycylglycine. The synthetic phthalylglycylglycine melts at 230° and gives no depression with the substance obtained above.

Scheme of the Decomposition of Amidine in 1% HCl



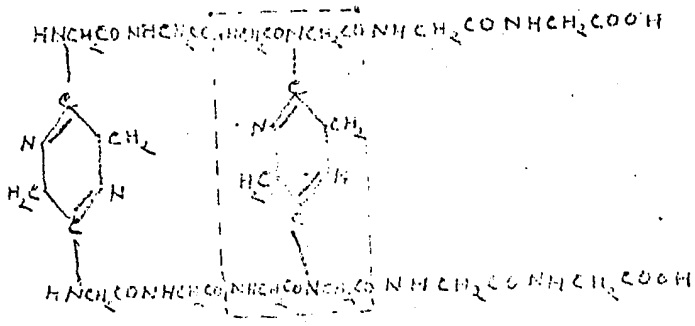
We did not succeed in finding diketopiperazine. Its presence in this compound was proved by the appearance of the ninhydrin reaction in the filtrate after neutralization with HCl. The assumption made earlier as to the location of the phthalylglycine group at the nitrogen of the side chain was verified by this experiment.

CONCLUSIONS

1. The synthesis of doubly N-aminoacylated diketopiperazine was carried out by acylation with chlorides of glycine under protection of the latters' amine group with phthalyl, toluene sulfonyl, and benzylsulfonyl residues.

2. The phthalylglycine group at the nitrogen of diketopiperazine possesses the properties of a mobile acyl group and with glycine ester forms the exo-acyl-(phthalylglycil)-glycine amidine of dihydropiperazine.

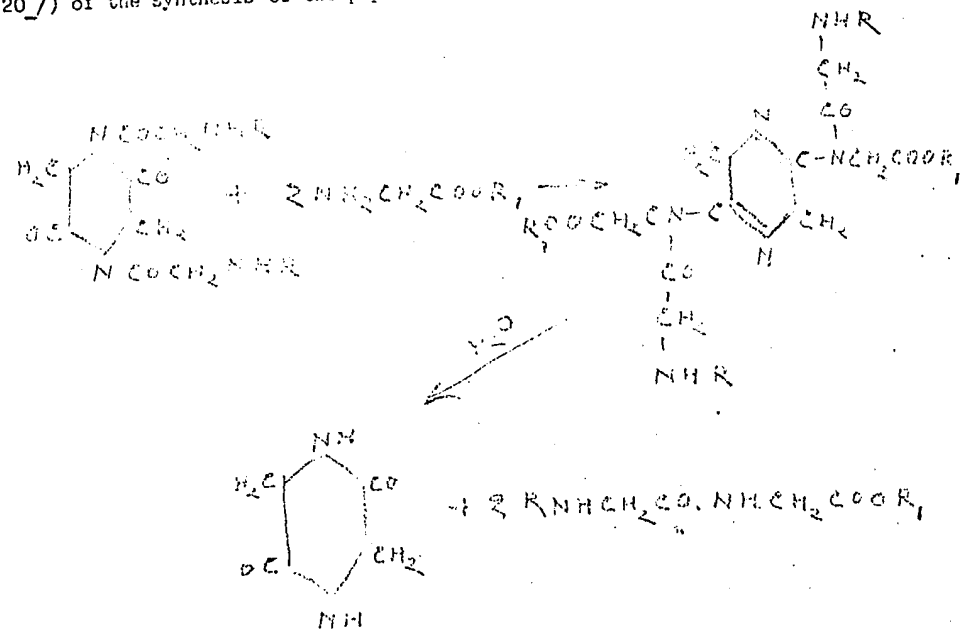
3. By the synthesis of this compound was shown the possibility of the structure put forward by N. I. Gavrilov for the complex (peptone) micromolecule of protein.



The central fragment of this structure has been realized.

4. N-acylamine groups are easily rearranged to the exo-position if the carbonyl is replaced by an aminoacyl residue, and furthermore they can serve

as a model (in agreement with M. Bergmann's reaction, which we have interpreted [20_7) of the synthesis of the peptide link.



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