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β-Carbobenzoxylation of DL-αβ-Diaminopropionic Acid and some Acylated Derivatives

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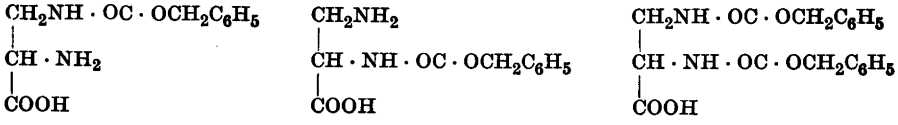
In connection with a synthetic problem in this laboratory, it became of importance to prepare DL-α-amino-β-carbobenzoxyaminopropionic acid (I). A search of the literature revealed that only a few partially acylated derivatives of the diaminopropionic acids are known. Schneider [1] prepared methyl L-α-amino-β-carbobenzoxyaminopropionate by an application of the elegant Bergmann procedure to L-dicarbobenzoxyaminopropionic acid *via* the acid chloride and the corresponding N-carboxy (Leuchs') anhydride. Miyanoki [2], in a study of enzyme substrate models, made a mono-chloroacetyldiaminopropionic acid by direct acylation of the diamino-acid and showed that the chloroacetyl-group had entered the amino-group in the β-position.

Although the Bergmann method leads to the β-isomer in an unambiguous way, the procedure is long and time-consuming. Therefore it became desirable to investigate whether or not it would be possible, in a fair yield, to prepare the β-mono-carbobenzoxylated diaminopropionic acid by a selective one-step acylation of the DL-αβ-diaminopropionic acid.

Greenstein [3] has determined the apparent dissociation constants for αβ-diaminopropionic acid and gives the following pK' values: 1.33 (COOH), 6.80 (α-NH_3^+) and 9.60 (β-NH_3^+). From these figures it is apparent that the ion needed for exclusive β-acylation, *viz.* $CH_2NH_2 \cdot CHNH_3^+ \cdot COO^-$, does not exist at any pH, and that the only possibility of obtaining the β-isomer is by performing the carbobenzoxylation in strongly alkaline solution where the ion $CH_2NH_2 \cdot CHNH_2 \cdot COO^-$ prevails, thereby hoping to obtain chiefly the β-isomer in a competitive reaction. By conducting the acylation in a slightly alkaline solution, however, it should be possible to obtain, in a high yield, the pure α-isomeride (II), because in this pH-region the predominant ionic form is $CH_2NH_3^+ \cdot CHNH_2 \cdot COO^-$. Experimentally this was verified, and the prepa-

ration of DL-α-carbobenzoxyamino-β-aminopropionic acid and some derivatives will be the subject of a future publication.

It was found that when DL-$\alpha\beta$-diaminopropionic acid was treated in strongly alkaline solution (pH>11) with one mole of carbobenzoxy chloride, the main product formed was $\alpha\beta$-dicarbobenzoxyaminopropionic acid which was isolated in a yield of 65-70 %. In addition, there was obtained in 20 % yield a mono-carbobenzoxyderivative which appeared to be quite homogenous and proved on further investigation to be the desired β-isomeride. Careful examination of the mother liquors did not reveal the presence of any of the α-isomeride, some unreacted diaminopropionic acid being the only material that could be isolated. The simultaneous occurrence of only the β-isomeride (I) and the $\alpha\beta$-diacylated acid (III) may be interpreted as a primary attack of the acyl halide on the β-amino group, which according to the titration data possesses the highest basicity, followed by further reaction of the α-amino group in (I) with a second molecule of carbobenzoxy chloride.



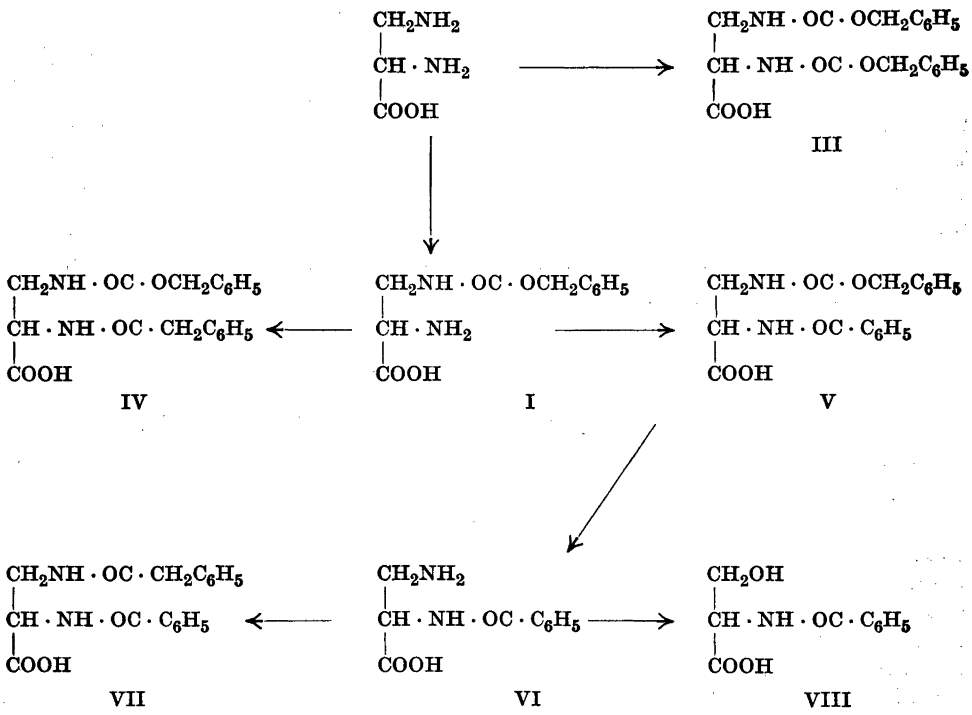
I II III

This is considered to be the most likely explanation, although a simultaneous formation of (I) and (II) followed by a secondary acylation, which in the case of (II) takes place with a velocity considerably higher than for (I), is a mechanism which also satisfies the experimental findings.

This method of preparing the β-isomeride (I) has certain advantages over the Bergmann procedure, which in our hands worked less smoothly in the DL-series than in the L-series, apparently because of the lower solubility of the racemic derivatives. The main product (III) of the carbobenzoxylation can readily be recovered as $\alpha\beta$-diaminopropionic acid by hydrogenolysis with palladized charcoal, and resubjected to carbobenzoxylation.

In order to prove the structure of the mono-carbobenzoxylated $\alpha\beta$-diaminopropionic acid the series of reactions shown in the following flow sheet were carried out.

As mentioned above, carbobenzoxylation in strongly alkaline solution of DL-$\alpha\beta$ diaminopropionic acid yields a mixture of one mono- and the diacylated products. The former (I) was treated with phenacetyl chloride to give the compound (IV) which proved to be identical with DL-α-phenacetylamino-



β-carbobenzoxyaminopropionic acid which the May & Baker group[4] prepared by a completely different route during the penicillin programme*.

Further confirmatory evidence for the correctness in allocating the carbobenzoxy radical to the β-amino group was provided by benzoylation of the mono-carbobenzoxy derivative to give a reaction product (V) which, in turn, was hydrogenolyzed to a mono-benzoyl derivative (VI). By deamination of this substance with barium nitrite in slightly acid solution, a compound was isolated which by comparison with an authentic sample, was proved identical with DL-N-benzoyl-serine (VIII).

Finally DL-α-benzoylamino-β-phenacetylaminopropionic acid (VII) was obtained by phenacetylation of (VI) as a nicely crystallizing substance melting at 165°. In the Penicillin Monograph, the May & Baker group (*l. c.*) describe the isomeric DL-α-phenacetylamino-β-benzoylaminopropionic acid* (m.p. 160—161°) and a mixture of the two compounds melted at 142—145°. Thus by three different sets of reactions, it has been proved that the mono-carbobenzoxy-diaminopropionic acid in question is the β-acylated product.

* A sample for comparison was kindly supplied by Dr. K. N. Langford, May & Baker Ltd.

During the work reported here, the 'ninhydrin' reaction was examined on the different derivatives and it was noticed that under the conditions used — heating aqueous solutions of equimolar amounts in a boiling water bath with a constant amount of the reagent — the results were not very conclusive as to the presence of a free α-amino group. Although the compounds containing such a group gave a more rapid color formation, the substances containing a free β-amino group gave on prolonged heating the same color with about the same intensity. Negative results were, however, observed on the $\alpha\beta$-diacylated derivatives.

EXPERIMENTAL *

DL-$\alpha\beta$-Dicarbobenzoxyaminopropionic acid

To a solution of 7.0 g (37.8 mM) of diaminopropionic acid mono-hydrobromide in 37 ml of 2.07 *N* NaOH (78.2 mM), 75 ml of 2.07 *N* NaOH and 22.8 ml (114 mM) of a solution of carbobenzoxy chloride in toluene (0.85 g/ml) were simultaneously added from two burettes under cooling in ice and vigorous stirring in the course of one hour. During the addition, a crystalline precipitate separated (the sodium salt of the dicarbobenzoxy-aminopropionic acid) which was not completely brought into solution by dilution with 125 ml of water. After all had been added, the reaction mixture was stirred for 30 min in the ice bath and for another half an hour at room temperature. With cooling, 7 ml of conc. hydrochloric acid were cautiously added dropwise to a distinctly acidic reaction. A colorless syrup separated which rapidly crystallized in the refrigerator. The product (m.p. 114—5°) was dried *in vacuo* and weighed 13.4 g (95 % yield). The compound was purified by dissolving in chloroform and adding petroleum ether (b. p. 40—55°). After chilling, a colorless product was obtained. 11.3 g (80 %). M. p. 124°.

The corresponding L-dicarbobenzoxyaminopropionic acid, prepared according to Schneider (*l. c.*), melted at 99—100° and was found in contrast to the DL-compound reported here to be a poorly crystallizing substance. While the optically active compound is readily soluble in carbon tetrachloride and ether, the solubility of the racemic form in these solvents is considerably smaller.

$C_{19}H_{20}O_6N_2$ (372.4)	Calc.	C	61.31	H	5.41	N	7.52
	Found	»	61.35	»	5.30	»	7.31

Carbobenzoxylation of DL-diaminopropionic acid

During 45 min there was added simultaneously to a well stirred and cooled solution of 4.45 g (24.1 mM) of DL-diaminopropionic acid hydrobromide in 50 ml of 0.990 *N* sodium hydroxide, 12.5 ml of an ethereal solution containing 4.25 g (24.9 mM) of carbobenzoxy chloride and 49.0 ml of 0.990 *N* sodium hydroxide. The pH was thereby kept

* All the melting points here reported are uncorrected. Analyses were carried out in this laboratory by Mr. A. Grossmann.

above 11 during the reaction. The precipitate formed was removed by filtration and washed with ice-cold water. It was suspended in water and, with cooling, acidified with concentrated hydrochloric acid. The colorless syrup was readily transformed into a crystalline product by cooling and seeding. After drying *in vacuo*, the compound weighed 2.45 g (m. p. 122—3°) and consisted of practically pure dicarbobenzoxyaminopropionic acid. The filtrate and washings were made very strongly acidic and extracted with three 25 ml portions of chloroform. After drying over sodium sulfate, the chloroform was removed *in vacuo* leaving 1.00 g of an oil which crystallized on standing to almost pure dicarbobenzoxyaminopropionic acid, thereby increasing the total amount of this compound to 3.45 g or 68 % of the theoretical, provided all the acid chloride had been used in the formation of the diacylated compound.

The aqueous phase was freed of chloroform *in vacuo* and brought to pH 7 by careful addition of 1 *N* sodium hydroxide. The separation of crystals started within a few minutes and after cooling overnight in the refrigerator, 764 mg of a colorless crystalline material was collected. M. p. 239—41° with vigorous gas evolution. By concentration of the filtrate, there was obtained a second crop, (252 mg), m. p. 230—2°. The total yield of DL-monocarbobenzoxydiaminopropionic acid was thus 1.016 g or 18.5 % of the theoretically possible. An analytical sample was prepared by recrystallization from hot water. M. p. 239—41° with decomp.

$C_{11}H_{14}O_4N_2$ (238.2)	Calc.	C	55.45	H	5.92	N	11.76
	Found	»	55.33	»	5.87	»	11.75

From the mother liquor a small amount of unreacted diaminopropionic acid in form of the hydrochloride was isolated by way of the mercuric acetate complex.

DL-α-Benzoylamino-β-carbobenzoxyaminopropionic acid

To a solution of 749 mg (3.15 mM) of the monocarbobenzoxyderivative described above in 3.5 ml of 0.990 *N* (3.46 mM) sodium hydroxide, was added gradually 0.40 ml (3.48 mM) of freshly distilled benzoyl chloride and 3.7 ml of 0.990 *N* (3.66 mM) sodium hydroxide with cooling and vigorous shaking. The precipitate formed was transformed into a colorless syrup on acidification. On cooling and scratching, the syrup crystallized to a product which, after drying and repeated extractions with hot petroleum ether, weighed 1.030 g. M. p. 150—1°. Recrystallization from 8 ml of 50 % acetone yielded 959 mg of analytically pure substance, melting at 151°. By concentration of the mother liquor, was obtained an additional 44 mg, thereby increasing the yield to 93 %.

$C_{18}H_{18}O_5N_2$ (342.3)	Calc.	C	63.13	H	5.30	N	8.19
	Found	»	63.01	»	5.23	»	8.33

The compound was readily soluble in ethanol and acetone and slightly soluble in cold water.

DL-α-Benzoylamino-β-aminopropionic acid

A solution of 894 mg of α-benzoylamino-β-carbobenzoxyaminopropionic acid in 20 ml of 50 % methanol to which 0.5 ml of glacial acetic acid and 200 mg of palladized charcoal (5 % Pd) were added, was treated with a fairly rapid stream of hydrogen, while

the reaction mixture was kept at 50°. In the course of the reaction, the methanol was gradually displaced by water in order to keep the reactants in solution. After six hours the carbon dioxide evolution ceased and the catalyst was removed by filtration through celite. By concentration and chilling of the filtrate, the separation of small colorless plates began. The crystals weighed 296 mg and melted with evolution of gaseous products at 227°.

$C_{10}H_{12}O_3N_2$ (208.2)	Calc.	C	57.68	H	5.81	N	13.46
	Found	»	57.65	»	5.80	»	13.46

From the mother liquor there was obtained, by the addition of ethanol, an additional 117 mg of the same grade of purity, the yield thereby reaching 413 mg or 76 %.

The successful decarbobenzoxylation reported here is in marked contrast to the failure noted by the May & Baker group (*l. c.*) in hydrogenolyzing the corresponding α-phenacetylamino-β-carbobenzoxyaminopropionic acid.

DL-α-Benzoylamino-β-phenacetylaminopropionic acid

One hundred and sixteen mg (0.56 mM) of α-benzoyl-diaminopropionic acid dissolved in 0.60 ml of 0.990 *N* (0.59 mM) sodium hydroxide was treated with 0.09 ml (0.67 mM) of freshly distilled phenacetyl chloride and 0.60 ml of 0.990 *N* (0.59 mM) sodium hydroxide with cooling and shaking. The crystalline precipitate formed was dissolved by the addition of a few ml of water, and the solution was decanted from a small amount of sticky material and acidified, with cooling, with hydrochloric acid. The precipitate crystallized in the ice box in contact with a little highly diluted methanol. After washing and drying it weighed 148 mg (81 %) and melted at 163—5°.

Recrystallized from very dilute ethanol it weighed 116 mg, m. p. 165°. The melting point of the isomeric DL-α-phenacetylamino-β-benzoylaminopropionic acid was determined under the same conditions and found to be 160—1°. The mixed melting point of the two compounds was 142—5°.

$C_{18}H_{19}O_4N_2$ (326.3)	Calc.	C	66.27	H	5.56	N	8.59
	Found	»	66.34	»	5.46	»	8.67

DL-α-Phenacetylamino-β-carbobenzoxyaminopropionic acid

A solution of 100 mg (0.42 mM) of α-carbobenzoxy-diaminopropionic acid in 0.5 ml of 0.990 *N* (0.495 mM) sodium hydroxide was treated in the usual way with 0.065 ml (0.49 mM) of pure phenacetyl chloride and 0.5 ml of 0.990 *N* (0.495 mM) sodium hydroxide. After acidification, there was obtained 151 mg of a yellowish oil which tenaciously resisted all attempts of crystallization. Only after a crystalline sample from the laboratories of May & Baker Ltd. became available, and the solution in ethylene chloride was seeded did a deposite of crystalline material result. Several recrystallizations from ethylene chloride-petroleum ether were necessary in order to obtain a well crystallizing sample with the correct m. p. 129—30°. This was not depressed on admixture with an authentic sample from the British laboratory.

DL-*N*-Benzoyl-serine

To a solution of 380 mg of barium nitrite monohydrate (1.54 mM) in 3 ml of water was added 250 mg of α-benzoylamino-β-aminopropionic acid. The suspension was acidified with 1.0 ml of 0.1 *N* hydrochloric acid, and kept very slightly acidic by intermittent addition of 0.1 ml portions of 0.1 *N* hydrochloric acid. The mixture was shaken occasionally and, after having been kept for 3 days at room temperature, the solution was made strongly acidic and extracted thoroughly with ethyl acetate. The organic phase was dried and the solvent removed *in vacuo*. The residue wat recrystallized several times from water, thereby giving 80 mg of a colorless product, m. p. 151°, undepressed on admixture with an authentic sample of DL-N-benzoyl-serine, prepared by benzoylation of DL-serine.

SUMMARY

The preparation of pure DL-β-carbobenzoxyamino-α-aminopropionic acid by selective acylation of DL-αβ-diaminopropionic acid is described.

The structural proof of the position of the carbobenzoxy-radical is given by three different sets of reactions.

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