SYNTHESIS OF GLUTAMINE AND PYROGLUTAMYLGLUTAMINE DERIVATIVES SUBSTITUTED IN THE CARBOXAMIDE NITROGEN

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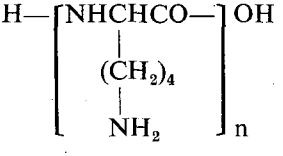
Monomer and dimer type derivatives of polyantin, a substituted polyglutamine of polycationic character, similar to polylysine in structure have been prepared. DL-Glutamic acid γ-diethylamino-ethylamide, tosyl-L-pyroglutamyl-L-glutamic acid bis-diethylamino-ethylamide, and basic N-substituted derivatives of Contergan have also been synthesized. These derivatives are expected to show biological activity and their synthesis may have significance from a peptide-chemical point of view.

As early as 1959, KATSCHALSKI and coworkers [1] synthesized various α-poly-amino acids, starting with basic amino acids. The most characteristic representative of these polymers is poly-L-lysine (I)

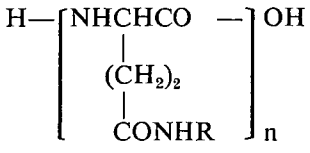


I.

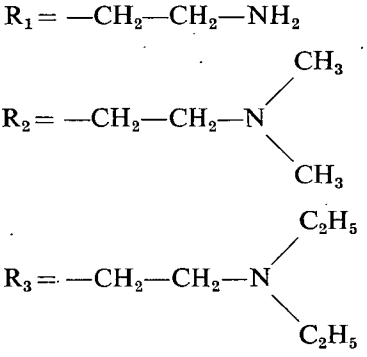
Poly-L-lysine is an interesting model compound, it has significant biological activity and, concerning its structure, it is a polycationic peptide, since the farther primary amino group of the lysine unit is not involved in the peptide bond. The same structural principle was achieved by K. KOVÁCS and A. KÓTAI [2], when neutral α-poly-L-glutamic acid γ-methylester was reacted with ethylenediamine, and furthermore with N,N-dialkylethylenediamines. The polycationic peptide could be prepared also in this way. The structure of the latter differs from that of the polypeptides made up from direct basic amino acids only in that they (II) involve free amino groups originating from ethylene diamine and these are built in with the participation of the γ-carboxylic groups of glutamic acid units, i.e. through a peptide-analogous carboxamide bond.

The R_1 type compound was termed by the authors to „polyanthin" both to refer to the manysided biological activity and for the sake of simplicity. In case of substituents R_2 and R_3 the name is „dimethylpolyanthin" and „diethylpolyanthin",

respectively. In the latter two polybases the basic function is represented by tertiary amino groups and hence these could easily be converted to quaternary derivatives with methyl iodide.



II.

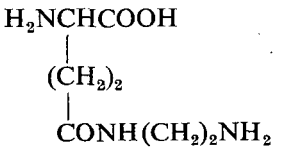


These substances, as compared to KATSCHALSKI's basic polyamino acids (I), are also biologically active, but their activity is much more wide-ranging. Namely, besides the higher microbiological activity they exhibit antiinflammatory and wound healing effects. The latter effect, for instance, was also supported by several hundred clinical observations [3], drawing attention for the possibility of the therapeutical application of these compounds.

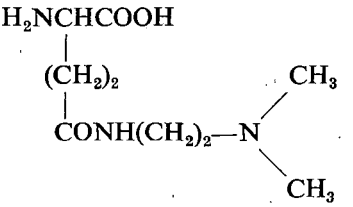
We have found, however, that the structural units for these polybases of interesting properties are unknown in literature. Therefore, in order to compare the chemical and biological properties it seemed reasonable to elaborate the preparation of the monomers. In the course of our preliminary experiments it turned out that the synthesis of the monomeric unit corresponding to polyanthin: glutamic acid γ-2-aminoethylamide (III) is hard to achieve because of the equal reactivity of the ethylenediamine groups. Notwithstanding, FRIEDMANN and coworkers [4] have already described this compound. On the other, since both the synthesis and biological activity of dimethyl- and diethylpolyanthin are more favouring than those of polyanthin it was reasonable to attempt the synthesis of the analogously substituted glutamine derivatives (IV and V).

First of all the racemic modification corresponding to V, DL-glutamic acid $\gamma-2$-diethylaminoethylamide was synthesized, since the polymer of this is the most efficient. SHEEHAN and FRANK [5] applied DL-phthalylglutamic anhydride in the preparation of DL-glutamine. When in dioxan solution instead of ammonia some amine compound is applied, the corresponding derivative of DL-glutamine is formed. The synthesis is made complete by removal of the phthalyl group with hydrazine hydrate in alcohol solution. The selective γ-aminolysis of the phthalylglutamic an-

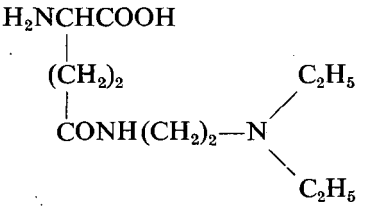
hydride ring is theoretically well explainable. The negative inductive effect of the highly electron-attracting phthalyl group, being propagated through the nearer α-carboxylate makes the carbon atom of the γ-carboxylic group positively polarized, decreasing the negative charge density on it. Thus the nucleophilic ammonia or amine compound decomposes the anhydride ring, suffering, an electron shift directed



III.



IV.

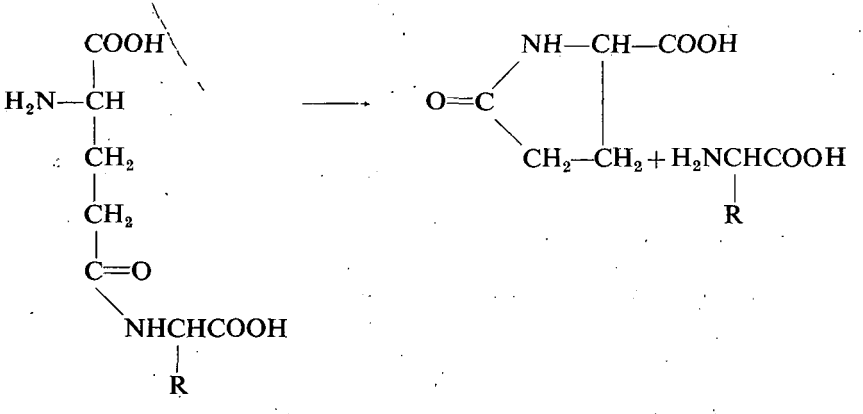


V.

by the phthalyl group, obviously with the formation of a γ-carboxamide bond. Apolar solvent may influence this effect.

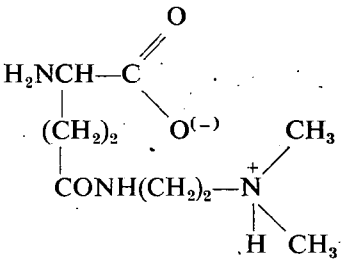
The compounds DL—V and DL—IV can be prepared analogously from phthalylglutamic anhydride with N, N-diethylaminoethylamine and N, N-dimethylaminoethylamine, respectively. The cleavage products obtained in the first step crystallize well. Dephthalylation may be done with hydrazine according to ING and MANSKE [6] as described above. The phthalyl hydrazone by-product is insoluble and hence removable from the reaction mixture by filtration.

Further on the stability of the monomeric, basic derivatives was studied. Namely, QUESNE and YOUNG [7] stated that γ-glutamyl peptides undergo hydrolysis on



heating in aqueous medium and the reaction gives rise to pyroglutamic acid and the amino acid component originally bound with γ-peptide linkage.

The reaction is explained with the nucleophilic attack of the free amino group of the terminal γ-glutamyl group upon the carbonyl carbon atom of the γ-carboxylic group. One of the driving forces of the reaction is the fact that pyroglutamic acid is a stable five-membered cycle. DL-Glutamic acid γ-2-diethylaminoethylamide (V), too, shows a similar tendency for secondary transformations. Therefore the extension of the period of dephthalylation with hydrazine actually favours the transformation, which may be termed also to trans-peptidation, and instead of the expected product also here pyroglutamic acid and the primary reaction component, N, N-diethyl-ethylenediamine can be recovered. The same result is observable on treatment with aqueous alkali. On the contrary, compound V remains intact on heating with dilute aqueous acid even after 8 hours. This fact may be connected with the „zwitterion" structure of compound V. According to infrared spectroscopic analysis the α-carboxylic group of V forms a „zwitterion" not whit the primary, but with the tertiary amino group (Va).



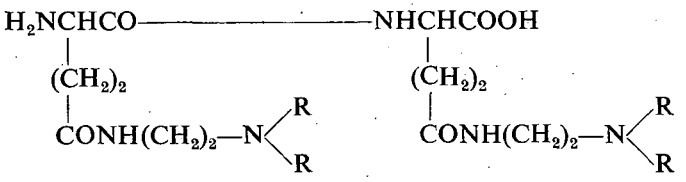
Va.

This „zwitterion" structure is stable, the carboxylate anion and the ammonium cation assume a favouring relative position. Inspection of the Stuart-Briegleb model of the compound supports this statement. Due to steric causes the quasicyclic structure eliminates the possibility for the reverseside nucleophilic replacement of the amide group at the positive polarized carbon atom of the γ-carboxamide group by the α-amine function. On the contrary, in acidic medium the primary amino group is protonized and the participation of the unshared electron pair terminates the nucleophilic character.

In alkaline medium the „zwitterion" structure decomposes and the nucleophilic character of the amino group becomes effective. These statements are obviously valid for compound IV, too, which is accessible analogously with N,N-dimethyl-etylenediamine.

In the second stage of our experiments the possibility for the preparation of model compounds consisting of several units was looked for. Since the synthesis of compounds IV and V had been worked out, our next direct purpose was the pre-

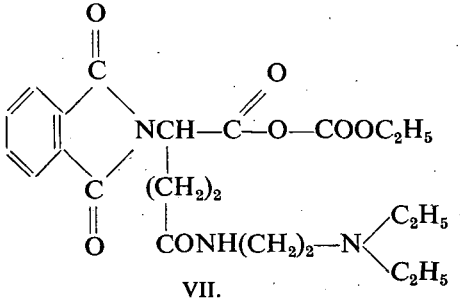
paration of their α-dipeptide (VI)



VI.

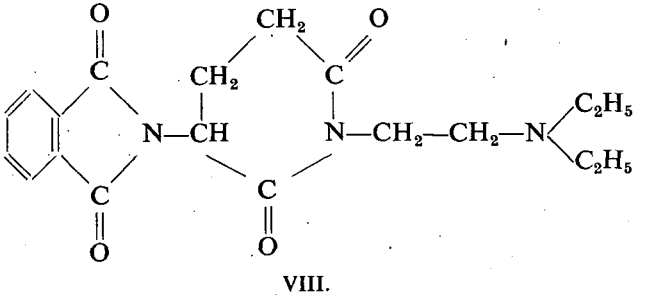
where R = CH_3 or C_2H_5. Though the basic dipeptide of type VI could not be prepared according to our original idea, the reaction path made possible the synthesis of derivatives which are interesting themselves. Namely, the synthesis of N-phthalylglutamic imide N-dialkylaminoethyl derivative (VIII) could be achieved. Its base compound, N-phthalylglutamic imide was reported by KUNCZ and KELLER [8] to have influence upon the nervous system and the compound was applied *pro tempore* as sedative under the name „Contergan". Later on it was withdrawn because of its deleterious by effects.

It has been found by us that N-phthalylglutamic acid γ-2-diethylaminoethylamide can be converted with ethyl chlorocarbonate to a mixed anhydride derivative (VII), when the α-carboxylic group takes part in the reaction.



VII.

However, the mixed anhydride VII is unstable, in practice it can not be isolated, because after its formation an intramolecular acylation takes place to give a cyclic secondary carboxamide derivative (VIII). This compound may be considered as a Contergan derivative substituted in the imide nitrogen, but the substituent may also involve a tertiary amino group.



VIII.

The same compound is formed when N-phthalylglutamic acid γ-2-diethylaminoethylamide is heated with acetic anhydride.

Since in the reaction corresponding to our first idea the otherwise interesting and significant Contergan derivative was obtained and in the same time glutamic acid was built in as the racemic modification, it seemed reasonable to apply tosyl group for the protection of the glutamic acid amino group instead of phthalyl group. The application of tosylglutamic acid and derivatives in peptide synthesis was reported first by RUDINGER [9]. He prepared tosylpyroglutamic chloride from tosylglutamic acid with thionyl chloride. Tosylpyroglutamic chloride was used [9] for the acylation of various amines and amino acids. Moreover, he observed that in tosylpyroglutamyl derivatives the pyroglutamyl ring is opened with amines.

On basis of RUDINGER's observations we reacted tosylpyroglutamic chloride with L-glutamic acid dibenzyl ester and L-glutamic acid γ-methyl ester, respectively, in the presence of sodium hydrocarbonate in aqueous chloroform medium. The amidation reaction of tosyl-L-pyroglutamyl-L-glutamic acid dibenzyl ester proceeds already at room temperature. Thus with N,N-diethylethylenediamine such a derivative is formed in which both carboxylic groups of the C-terminal glutamic acid are amidated and the product involves two basic centre. Actually the compound obtained consists of two amino acids, i.e. two glutamic acid parts and the number of basic groups is also two. Accordingly the compound may be considered a simpler, low-member polyanthin model. The synthesis and biological control of further basic oligoglutamine peptides are in progress.

Experimental

N-Phthalyl-DL-glutamic acid γ-2-diethylaminoethylamide

Phthalyl-DL-glutamic anhydride (20,72 g) was reacted with 2-diethylaminoethylamine (11,3 ml) in abs. dioxan (100 ml). A colourless oil separated which crystallized on standing 2—3 days. The crystalline material was filtered, washed with ether, dried and crystallized from methanol, to yield 18 g product, m. 182—184 °C. Anal.: Calc.: $C_{19}H_{25}O_5N_3$ requires C 60,8 H 6,7; Found: C 61,1 H 7,0.

DL-Glutamic acid γ-2-diethylaminoethylamide dihydrochloride

Anhydrous hydrazine hydrate (4,2 ml) was added to a suspension of the above material (10,5 g) in abs. ethanol (100 ml) and the mixture was refluxed for 10 min. White substance deposited, which was filtered off, washed with ether, dried and treated with 4% H CL solution (60 ml). The insoluble phthalyl hydrazide (3,2 g) was filtered, the filtrate was concentrated *in vacuo*, triturated with abs. ethanol (50 ml) and finally the insoluble hydrazine dihydrochloride was removed. The alcoholic solution was evaporated to dryness, kept over KOH and P_2O_5 for 5 days, and the oily product was treated with abs. ethanol (10 ml) to give 4 g. crystalline material, m. 159—161 °C. Recrystallization from ethanol-methanol mixture afforded 3,5 g substance, M. p.: 164—165 °C. Anal.: Calc.: $C_{11}H_{25}O_3N_3Cl_2$ C 41,5 H 7,9 Cl 22,7; Found: C 41,5 H 8,0 Cl 22,8.

N-Phthalyl-DL-glutamic acid N-diethylaminoethylimide

Phthalyl-DL-glutamic acid γ-2-diethylaminoethylamide (1,18 g) was suspended in chloroform at −10° and treated with ethyl chlorocarbonate (0,26 ml). The mixture was kept at −10° for 30 min. and at room temperature overnight. After removal of the solvent *in vacuo* the residue crystallized on treating with ether, 1 g product, M. p.: 180 °C, on recrystallization 184 °C. Anal.: Calc.: $C_{19}H_{24}O_4N_3Cl$ C 58,0 H 6,1 Cl 9,0; Found: C 58,5 H 6,6 Cl 9,2.

N-Tosyl-L-pyroglutamyl-L-glutamic acid dibenzyl ester

Tosyl-L-pyroglutamic chloride (3 g) was reacted with dibenzyl-L-glutamate (3,6 g) in chloroform solution (50 ml) in the presence of a solution of $NaHCO_3$ (3,4 g) in water (50 ml). After CO_2 formation was over the two phases were separated, the organic one was dried over Na_2CO_3 and concentrated. The oily residue crystallized on adding alcohol, to give 3,6 g product, M. p.: 107 °C, after crystallization from ethanol 107 °C. Anal.: Calc.: $C_{31}H_{32}O_8N_2S$ C 62,8 H 5,4 S 5,4; Found: C 64,3 H 5,5 S 5,5.

Methyl-N-tosyl-L-pyroglutamyl-L-glutamate

The procedure is analogous with that described for the preceding product, M.p.: 100—111°C. Anal.: Calc.: $C_{18}H_{22}O_8N_2S$ C 50,7 H 5,1 N 6,5; Found: 51,3 H 4,93 N 5,3.

N-Tosyl-L-pyroglutamyl-L-glutamic acid bis-2-diethylaminoethylamide

A sample of the above dibenzyl ester (1 g) was shaken with N,N-diethylethylenediamine (1,5 ml) for 4 hours. The colourless, cristalline material obtained was treated with petrol ether to remove excess amine. Subsequent to filtration and drying 0,6 g colourless crystalline substance was yielded, M. p.: 158—160 °C, after crystallization M. p.: 165 °C. Anal.: Calc.: $C_{29}H_{42}O_6N_6S$ C 60,3 H 7,8 N 11,7; Found C 61,4 H 7,7 N 11,8.

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СИНТЕЗ ПРОИЗВОДНЫХ ГЛУТАМИНА И ПИРОГЛУТАМИЛГЛУТАМИНА ЗАМЕЩЕННЫХ НА АЗОТЕ АМИДА КИСЛОТЫ

К. Ковач, Б. Пэнкэ, А. Котаи

Авторами изготовились простые мономерные и димерные производные полиантина, замещенный полиглутамин с поликатионным свойством имеюший структуру подобную к полилизину. Кроме того изготовились γ-диэтиламиноэтиламид DL-глутаминовой кислоты, бис-диэтиламиноэтиламид L-пироглутамил-L-глутаминовой кислоты, и основные производные Контэргана, замещенные на азоте. По всей вероятности они являются биологически активными соединениями, а их синтез имеет важность с точки зрения химии пептидов.