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SYNTHESIS OF NEW ALKYLANTS WITH ESTERS OF N-(m-AMINOBENZOYL)-L-ASPARAGIC ACID SUPPORT

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ABSTRACT. In order to obtain new antitumoural compounds acting as alkylating agents, some esters were synthesized, to the α- and β-carboxylic group of the N-(m-aminobenzoyl)-L-asparagic acid. These esters were then converted into di-(β-hydroxyethyl)-amino compounds by condensation with ethylene oxide in a diluted acetic acid medium. By substituting the chlorine for the hydroxylic groups the corresponding di-(β-chloroethyl)-amino compounds were obtained.

INTRODUCTION

Among the chemotherapics used for treatment of some malign tumors the N-mustards are particularly important. Their cytostatic activity is exerted by an alkylating effect of blocking some components participating to the anarchical growth of cancerous cells.

The great disadvantage of treating some cancerous forms with N-mustards consists in their high toxicity due to the low selectivity toward the neoplastic cells, the normal cells, especially those with a higher rate of growth, being also affected.

In some extent disadvantage may be overcome by grafting the bis-(β-chloroethyl)-amino group on certain compounds characteristics of the organism. By means of such derivatives the transport of N-mustards till the cell level is facilitated, the toxicity lowered and the cytostatic action improved by their either antagonistic or antimetabolitic effects.

Several investigators [1-4] have synthesized N-mustards of such structure some of that are already used in the cancer chemotherapy.

In previous paper [5-14] a series of new N-mustards containing the active group grafted on derivatives of N-(m-nitrobenzoyl)-DL-asparagine N-(m-aminobenzoyl)-DL-asparagic acid and on N-(m-aminobenzoyl)-DL-asparagine were synthesized.

EXPERIMENTAL

a) N-(m-aminobenzoyl)-L-asparagic acid (II)

10 g (3.035 mole) of N-(m-nitrobenzoyl)-L-asparagic acid Was solved in ammonia. The obtained solution was introduced in a reaction vessel provided with a reflux refrigerator, through which penetrate a glass tube till bottom of the vessel. Through the glass tube was introduced a sulphuretted hydrogen current for 1 hour,

heating the flask for 10 minutes at intervals of 3 hours. In last half of hour, the introduction of sulphuretted hydrogen was made to a continuous heating. After cooling, one part of sulphur obtain from reaction was removed by filtration. The filtrate was shake twice in a separator with 60 ml carbon sulphide for removing the sulphur remained in a reaction mixture. After removing the carbon sulphide, the aqueous level was treated in a flask, under stirring, with a concentrated HCl solution, to pH 2.5-3, when a yellowish precipitate was separated. It was filtered off, dried and recrystallized from boiling water. The solid product melting at 156-158°C was thus obtained in a 70 % yield.

Analyses for $C_{11}H_{12}N_2O_5$:

	C, %	H,%	N,%
Calculated:	52.38	4.76	11.11
Found:	52.99	4.12	11.37

IR (KBr): 2800-3200 cm$^{-1}\nu_{NH\ amidic}$, 1660 cm$^{-1}\nu_{C=O}$, 1540ν cm$^{-1}\nu_{amide\ –II\ band}$ and 860 cm^{-1} disubstituted aromatic ring.

b) *Dimethylic ester of N-(m-aminobenzoyl)-L-asparagic acid (III)*

5 g of m-aminobenzoyl-L-asparagic acid was treated with 40 ml methylic alcohol previously saturated with gaseous hydrogen chloride at 0 °C. The obtained mixture was allowed to stay at the room temperature till the whole quantity of m-aminobenzoyl-L-aspa-ragic acid passed into solution. The hydrogen chloride and the excess of methylic alcohol was removed by evaporation at low temperature of 40-45 °C. The remaining residue was diluted with water till a volume of 250 ml and then neutralized at pH 8 with a concentrated ammonia solution. The dimethylic ester of m-aminobenzoyl-L-asparagic acid was thus precipitated quantitatively. After filtration, drying and recrystallization from ethylic alcohol the pure compound was obtained as needle crystals melting at 124-126 °C.

Analyses for $C_{13}H_{16}N_2O_5$:

	C, %	H,%	N,%
Calculated:	55.71	5.71	10.00
Found:	56.08	5.25	9.77

IR (KBr): 2800-3300 cm$^{-1}\nu_{NH\ amidic}$, 1640 cm$^{-1}\nu_{C=O}$, 1240 cm$^{-1}\nu_{C\text{-}O\text{-}C}$ and 840 cm^{-1} disubstituted aromatic ring.

c) *Diethylic ester of N-(m-aminobenzoyl)-L-asparagic acid (IV)*

It was prepared similarly (*vide supra*) from 5 g (0.02 mole) of I and 40 ml ethylic alcohol 96 % previously saturated with gaseous hydrogen cloride at 0 °C, recrystallized from ethanol. The pure product was obtained as a white powder, yield 62.4%, m.p. 144-146°C.

Analyses for $C_{15}H_{20}N_2O_5$:

	C, %	H,%	N,%
Calculated:	58.44	6.50	9.09
Found:	58.61	7.01	9.57

IR (KBr): 2950-3300 $cm^{-1}\nu_{NH\ amidic}$, 1645 $cm^{-1}\nu_{C=O}$, 1235 $cm^{-1}\nu_{C\text{-}O\text{-}C}$ and 830 cm^{-1} disubstituted aromatic ring.

d) Dimethylic ester of m-[bis-(β-hydroxyethyl)-amino]-benzoyl-L-asparagic acid (V)

To a suspension of 2,27 g III in 50 ml water amd 40 ml glacial acetic acid, 5,5 ml ethylene oxide were added. After maintaining for 24 hours at the room temperature the obtained mixture was neutralized with sodium bicarbonate. An oil product thus separates which was extracted in ethyl acetate and washed with water in a separating funnel. The obtained product was dried on anhydrous sodium sulphate, filtered and the excess of ethyl acetate distilled under vacuum. Cooling separated a light yellow solid product. After repeated washings with anhydrous ethylic ether and drying it was melted at 66-68 °C.

Analyses for $C_{17}H_{27}N_2O_7$:

	C, %	H,%	N,%
Calculated:	54.98	7.28	7.55
Found:	55.22	7.11	7.87

IR (KBr): 3000-3400 $cm^{-1}\nu_{OH}$, 1705 $cm^{-1}\nu_{C=O}$, 1300 $cm^{-1}\nu_{C\text{-}N}$,1230 $cm^{-1}\nu_{C\text{-}O\text{-}C}$, 1050 $cm^{-1}\nu_{C\text{-}OH}$ and 825 cm^{-1} disubstituted aromatic ring.

e) Diethylic ester of m-[bis-(β-hydroxyethyl)-amino]-benzoyl-L-asparagic acid (VI)

It was obtained from 2,5 g IV and 7,5 ml ethylene oxide in 50 ml water and 40 ml glacial acetic acid according to a procedure described upper.

The final product that melts at 72-74 ^{0}C is light yellow. Yield 57.8 %.

Analyses for $C_{19}H_{29}N_2O_7$:

	C, %	H,%	N,%
Calculated:	57.43	7.30	7.05
Found:	57.83	7.02	7.66

IR (KBr): 2800-3400 $cm^{-1}\nu_{OH}$, 1730 $cm^{-1}\nu_{C=O}$, 1335 $cm^{-1}\nu_{C\text{-}N}$,1205 $cm^{-1}\nu_{C\text{-}O\text{-}C}$, 1070 $cm^{-1}\nu_{C\text{-}OH}$ and 835 cm^{-1} disubstituted aromatic ring.

f) Dimethylic ester of m-[bis-(β-chloroethyl)-amino]-benzoyl-L-asparagic acid (VII)

A suspension of 2,3 g compound V in 25 ml anhydrous chloroform was treated with 10 g thionyl chloride. After refluxing the obtained mixture on a bath for 3 hours a homogeneous solution was formed. The solvent as well as the excess of thionyl chloride were removed by vacuum distillation and the remained resinous product was solved in anhydrous benzene. After drying on anhydrous sodium sulphate, filtration and benzene distillation under vacuum a colorless crystalline product melting at 35-37 °C was obtained.

Analyses for $C_{17}H_{25}Cl_2N_2O_5$:

	C, %	H, %	Cl, %	N, %
Calculated:	50.00	6.13	17.40	6.86
Found:	50.23	6.54	17.91	6.32

IR (KBr): 3300 $cm^{-1}\nu_{NH}$, 1720 $cm^{-1}\nu_{C=O}$, 1540 $cm^{-1}\nu_{amide\ –II\ band,}$ 1330 $cm^{-1}\nu_{C\text{-}N}$, 1210 $cm^{-1}\nu_{C\text{-}O\text{-}C}$, 750 $cm^{-1}\nu_{C\text{-}Cl}$ and 830 cm^{-1} disubstituted aromatic ring.

g) Diethylic ester of m-[bis-(β-chloroethyl)-amino]-benzoyl-L-asparagic acid (VIII)

I was prepared similarly started from 2,5 g compound VI and 10 g thionyl chloride in 30 ml anhydrous chloroform. The final product was melted at 37-40 ^{o}C.

Analyses for $C_{19}H_{27}Cl_2N_2O_5$:

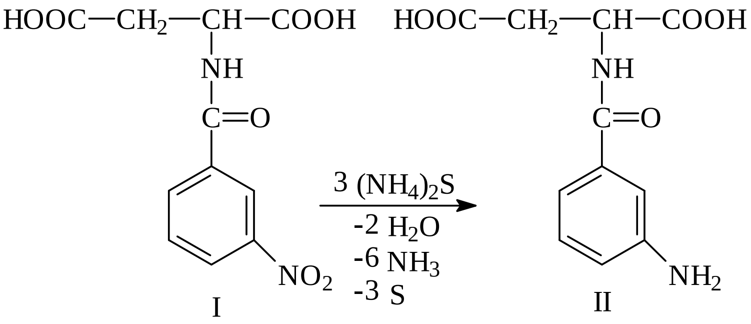
	C, %	H, %	Cl, %	N, %
Calculated:	52.53	6.22	16.85	6.45
Found:	52.05	6.69	17.10	6.22

IR (KBr): 3330 cm$^{-1}\nu_{NH}$, 1735 cm$^{-1}\nu_{C=O}$, 1535 cm$^{-1}\nu_{\text{amide –II band,}}$1340 cm$^{-1}\nu_{C\text{-}N}$, 1205 cm$^{-1}\nu_{C\text{-}O\text{-}C}$, 720 cm$^{-1}\nu_{C\text{-}Cl}$ and 840 cm^{-1} disubstituted aromatic ring.

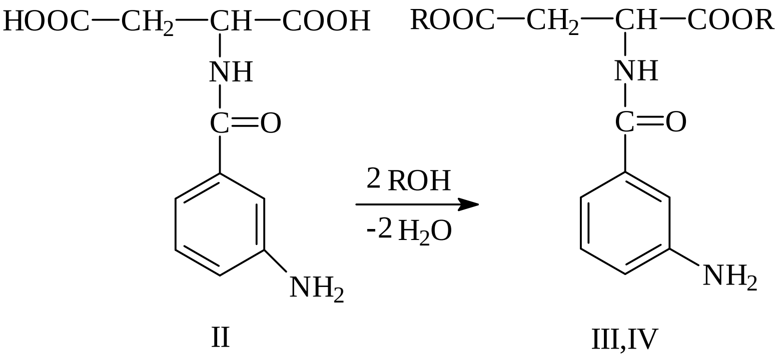
RESULTS AND DISCUSSION

In the present paper two N-mustards with di-(β-chloroethyl)-amino group grafted on a dipeptide, namely on the dimethylic ester of m-aminobenzoyl-L-asparagic acid or diethylic ester of m-aminobenzoyl-L-asparagic acid, respectively, were synthesized.

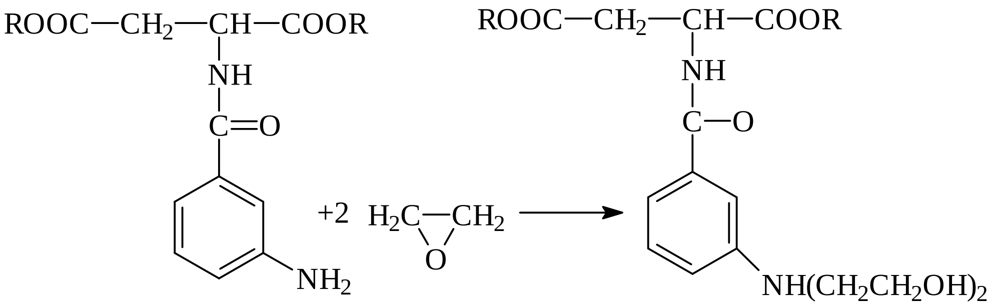
Firstly, N-(m-aminobenzoyl)-L-asparagic acid (II) was synthesized by reducing the N-(m-nitrobenzoyl)-L-asparagic acid (I) with sulphuretted hydrogen current:



The esters (III,IV) were obtained by treating the acid (II) with excess of alcohol saturated previously with gaseous hydrogen chloride:



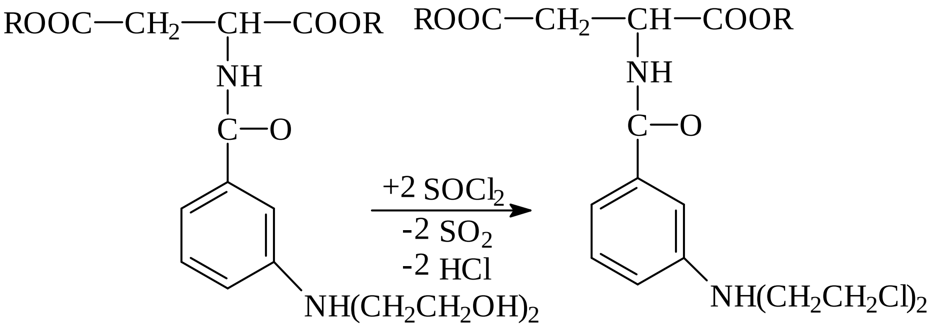
In order to transform the amino into bis-(β-chloroethyl)-amino group, the corresponding bis-(β-hydroxyethyl)-amino intermediates (V,VI) were prepared by treating the amino compounds (III,IV) with ethylene oxide in diluted acetic acid medium:



III,IV V,VI

By substituting the chlorine for hydroxylic group in the bis-(β-hydroxyethylic)-amino compounds the corresponding bis-(β-chloroethylic)-amino compounds were obtained. In this connection, the chlorination of the esters of m-[bis-(β-hydroxyethylic)-amino]-benzoyl-L-asparagic acid (V,VI) was carried out by means of thionyl chloride.

By treating the compounds (V,VI) with an excess of thionyl chloride in chloroformic solution, followed by refluxing on a water bath for 3 hours, solving in anhydrous benzene and removing the excess of the benzene, the final products (VII,VIII) were obtained:



V,VI VII,VIII

CONCLUZII

Two new N-mustards, derivatives of the m-aminobenzoyl-L-asparagic acid were synthesized by chlorinating the m-[bis-(β-hydroxyethyl)-amino]-benzoyl-L-asparagic acid with thionyl chloride.

Most of the bands occurring in the spectra of the synthesized compounds were explained, the molecular structures determined by chemical methods being thus confirmed.

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