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CANCEROLYTIC PEPTIDES HAVING DIRECTED ACTION

COMMUNICATION 5. SOME AMINO ACIDS AND PEPTIDES CONTAINING THE (BIS-2-CHLOROETHYLAMINO)PHOSPHINYLIDENE GROUP*

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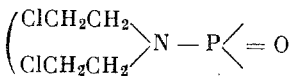
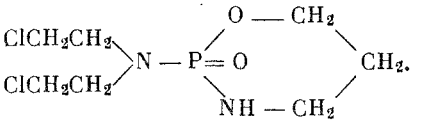
In 1954 Friedman and Seligman [1] synthesized a series of enzymically active antitumor compounds containing the (bis-2-chloroethylamino)phosphinylidene group

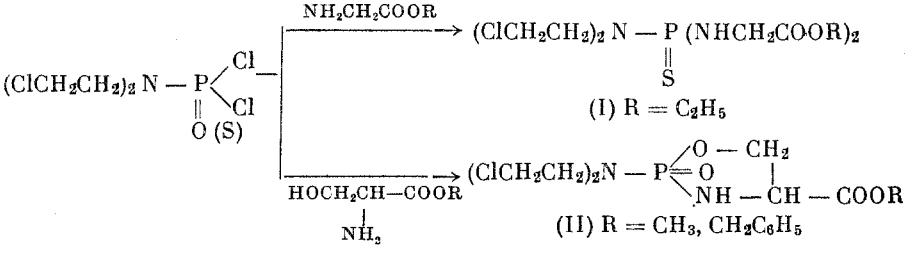


In 1958 Arnold and co-workers [2] published the results of tests on about 300 compounds of this kind. They showed that the most active antitumor compound of this group is the cyclic diamide endoxan



The therapeutic index of endoxan was better than those of previously known antitumor preparations. Also, clinical tests showed that, in comparison with other antitumor preparations, this compound has a number of advantages. However, it was later found that the use of endoxan leads to the development of some side effects (leukopenia, loss of hair).

We have shown previously that the introduction of the bis-2-chloroethylamino group into amino acids and peptides leads to antitumor preparations that are highly effective, but of low toxicity [3]. Hence to increase the selectivity of compounds carrying the (bis-2-chloroethylamino)phosphinylidene group, this group was introduced into amino acids and peptides. By the reaction of glycine ester with N,N-bis-2-chloroethylphosphoramidothioic dichloride we obtained N,N-bis-2-chloroethyl-N',N"-bis(ethoxycarbonylmethyl)phosphorothioic triamide (I).



The reaction was carried out at 20° in dry benzene in presence of triethylamine. The acylation of serine methyl and benzyl esters with bis-2-chloroethylphosphoramidic dichloride under the same conditions led to the formation of cyclic N,O-[(bis-2-chloroethylamino)phosphinylidene] derivatives of serine esters (II, R = CH_3, $CH_2C_6H_5$)

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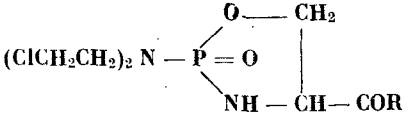
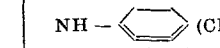
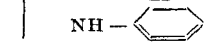
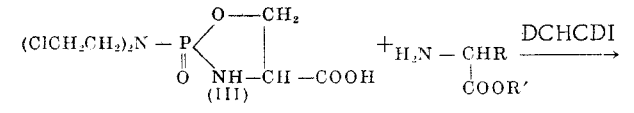
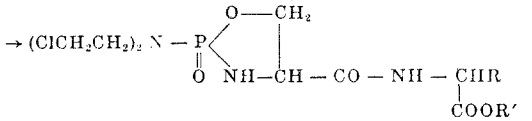
Cyclic N, O-[(Bis-2-chloroethylamino)phosphimylidene] Derivatives of Serine and its Derivatives



Compound	R	M.p., °C (solvent for crystn.)	Yield %	Formula	Found %					Calculated %				
					C	H	N	Cl	P	C	H	N	Cl	P
II	OCH_3	96 (ethanol)	77	$C_6H_{15}O_4N_2Cl_2P$	31,25	4,89	9,31	23,24	10,18	31,48	4,92	9,18	23,28	10,16
III	OH	160 (methanol)	75	$C_5H_{13}O_4N_2Cl_2P$	28,85	4,58	9,69	24,57	10,54	28,86	4,47	9,62	24,40	10,65
IV	$NH-CH(COOCH_2Ph)-CH_2OH$	134 (methanol)	84	$C_{17}H_{20}O_6N_3Cl_2P$	43,59	5,17	9,41	16,32	6,32	43,59	5,13	8,97	15,17	6,62
V	$NH-CH(COOCH_2Ph)-CH(OH)Ph$	147 — 148 (ethyl acetate)	90	$C_{23}H_{24}O_6N_3Cl_2P$	50,78	5,23	7,85	12,73	5,47	50,74	5,15	7,72	13,05	5,70
VI	$NH-CH(COOC_2H_5)-CH_2OH$	130 — 131 (methanol)	95	$C_{12}H_{18}O_6N_3Cl_2P$	35,58	5,48	10,33	17,52	7,51	35,47	5,42	10,34	17,49	7,63
VII	$NH-CH(COOCH_3)-CH(OH)CH_3$	168 (methanol, ethanol)	82	$C_{12}H_{18}O_6N_3Cl_2P$	35,43	5,50	10,68	15,54	7,59	35,47	5,42	10,34	17,49	7,63
VIII		145 (methanol, ethyl acetate)	83	$C_{18}H_{24}O_5N_3Cl_2P$	47,85	5,99	—	15,12	6,47	47,50	5,83	—	14,79	6,46
IX		153 — 154 (methanol)	89	$C_{17}H_{20}O_5N_3Cl_2P$	45,43	5,65	9,72	16,03	6,21	45,13	5,31	9,29	15,71	6,87

(see table). We should mention that we were unable to isolate (II, R = $CH_2C_6H_5$) in the crystalline state; it was converted by catalytic hydrogenation into the cyclic N,O-[(bis-2-chloroethylamino)phosphinylidene] derivative of serine (III). Further investigation showed that in presence of dicyclohexylcarbodiimide (DCHCDI) under the usual conditions (III) readily condenses with amino esters with formation of the corresponding dipeptide derivatives.





IV) R = CH_2OH, R′ = $CH_2C_6H_5$; (V) R = C_6H_5CHOH, R′ = $CH_2C_6H_5$; (VI) R = CH_2OH, R′ = C_2H_5; (VII) R = CH_3CHOH, R′ = CH_3

In all cases of the acylation of hydroxy amino esters the protection of the hydroxy group was not necessary. When ethyl acetate was used as solvent in the acylation, some dipeptides separated together with the dicyclohexylurea formed. These mixtures were separated by treatment with a little boiling ethyl acetate followed by filtration of the hot solution from dicyclohexylurea. The properties of the dipeptides obtained are given in the table. By the acylation of (p-aminophenyl)alkanoic esters with the cyclic compound (III) (also in presence of dicyclohexylcarbodiimide in chloroform solution) we obtained the corresponding derivatives of these compounds [(VIII) and (IX) in table].

EXPERIMENTAL METHOD

Bis-2-chloroethylphosphoramidic dichloride was prepared by the method described by Friedman and Seligman [1]; m.p. 54-56°. The p-toluenesulfonate of serine benzyl ester was prepared by a known method [4]; m.p. 94-95°. Ethyl, methyl, and benzyl esters of amino acids were isolated from the corresponding hydrochlorides and toluenesulfonates by Hillmann's method [5].

Bis-2-chloroethylphosphoramidothioic Dichloride. A mixture of 50 g of 2,2'-dichlorodiethylamine hydrochloride and 140 ml of thiophosphoryl chloride was refluxed for 20 h and then cooled. Unchanged dichlorodiethylamine hydrochloride was filtered off, and excess of thiophosphoryl chloride was distilled off. The residue was vacuum-distilled twice; b.p. 117° (2 mm); m.p. 30-32°; yield 74% (based on the amount of the dichlorodiethylamine hydrochloride that reacted). Found: C 17.48; H 2.96; P 11.42; Cl 51.76; S 11.50%. $C_4H_8NCl_4PS$. Calculated: C 17.49; H 2.91; P 11.27; Cl 51.64; S 11.64%.

N,N-Bis-2-chloroethyl-N′,N″-bis(ethoxycarbonylmethyl)phosphorothioic Triamide (I). A solution of 14 g of bis-2-chloroethylphosphoroamidothioic dichloride in dry benzene was added with stirring and cooling (20°) to a mixture of 10 g of glycine ethyl ester and 14 g of dry triethylamine in dry benzene. The solution was stirred further for two hours and left for one day at room temperature. Triethylamine hydrochloride was filtered off (13.9 g). The filtrate was vacuum-evaporated. The residue (20.7 g) was an oil that crystallized partially when left in a refrigerator. The crystalline precipitate was filtered off and washed with dry ether; the yield of (I) was 10.2 g (50%); m.p. 138-140°, raised to 146-147° by recrystallization from a mixture of ethanol and ethyl acetate. Found: C 35.69; H 5.88, 10.28; Cl 17.63; P 7.66%. $C_{12}H_{24}O_4N_3Cl_2PS$. Calculated: C 35.30; H 5.88; N 10.30; Cl 17.63; P 7.60%.

Cyclic N,O-[(Bis-2-chloroethylamino)phosphinylidene] Derivative of Serine (III). 3.9 g of serine benzyl ester was dissolved in 30 ml of dry benzene, and 5.2 g of bis-2-chloroethylphosphoramidic dichloride was added. Then, with stirring and cooling (20°) 4 g of dry triethylamine in 20 ml of dry benzene was added dropwise. The solution was stirred further for 2 h and then left overnight at room temperature. The precipitate of triethylamine hydrochloride was filtered off and washed with dry ether; weight 5.4 g; m.p. 250° (the literature gives 254°). The benzene solution was vacuum-evaporated at not above 30°; the residue (7.7 g of a colorless oil) was dissolved in 200 ml of dry methanol and hydrogenated in presence of 0.3 g of palladium black. 500 ml of hydrogen was absorbed (theory requires 450 ml). Catalyst was filtered off, and the solution was vacuum evaporated at not above 40°. The weight of the crude crystalline residue was 5.85 g (theory requires 5.82 g); m.p. 138° (decomp.). The substance was treated with hot absolute ethanol. The insoluble residue was filtered off; weight 3 g (52%); m.p. 158-160° (decomp.). After recrystallization: m.p. 160° (decomp.). The alcoholic mother liquor was evaporated to dryness, and we obtained 2.78 g (42%) of a substance of m.p. 138-139° (decomp.). The substance was dissolved in the calculated

amount of 1 N HCl; m.p. 147-148°. After two crystallizations from absolute ethanol: m.p. 160-161° (decomp.); total yield of (III), 75%.

Condensation of Bis-2-chloroethylphosphoramidic Dichloride with Serine Methyl Ester to give (II, R = CH_3). Serine methyl ester (1.2 g) was dissolved in 30 ml of dry ether. 2.6 g of bis-2-chloroethylphosphoramidic dichloride was added to the solution, and then with stirring and cooling (20°) 2 g of dry triethylamine in 20 ml of dry ether was added. The solution was stirred further for 2 h and then left overnight at room temperature. The precipitate of triethylamine hydrochloride was filtered off and washed with dry ether; weight 2.5 g. The solution was vacuum-evaporated, and the colorless oily residue was dissolved in ethyl acetate; petroleum ether was added and the solution was left in a refrigerator. The precipitate formed was filtered off; weight 2.3 g (77%); m.p. 96°. After recrystallization from absolute ethanol (II) had m.p. 96°.

Method of Preparing Dipeptides Containing the (Bis-2-chloroethylamino)phosphinylidene Group (IV, V, VI, VII, VIII, and IX in the Table) Described for the Case of the Acylation of Threonine Methyl Ester with the Cyclic Serine Derivative (III). Threonine methyl ester (0.27 g) was dissolved in ethyl acetate, and 0.58 g of (III) and then 0.45 g of dicyclohexylcarbodiimide were added to the solution. The solution was shaken for 5-10 min and then left for 2 h. The precipitate of dicyclohexylurea was filtered off; weight 0.47 g (theory requires 0.46 g). Petroleum ether was added to the mother liquor until it became turbid, and the mixture was left in a refrigerator. The precipitate formed was filtered off; yield 0.67 g (82.5%); m.p. 152-154°. After successive crystallizations from ethanol, ethyl acetate, and methanol the melting point of the cyclic (bis-2-chloroethylamino)phosphinylidene derivative of serylthreonine (VII) was 168° (decomp.).

SUMMARY

The (bis-2-chloroethylamino)phosphinylidene group was introduced into amino acids, hydroxy amino acids, and dipeptides for the first time.

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