CANCEROLYTIC PEPTIDES

COMMUNICATION 12. DERIVATIVES OF L-ORNITHINE, BEARING THE p-DI-(2-CHLOROETHYL)AMINO GROUP

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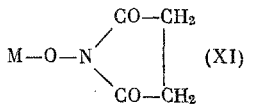
As an expansion of our studies on the synthesis of antitumor compounds employing the principal amino acids [1-3], we inserted p-di-(2-chloroethyl)aminophenylacetic acid* into α,δ-diaminovaleric acid or ornithine. It is known that ornithine does not enter into the composition of proteins, but it is an important intermediate metabolism product and, being an arginine antagonist, it possesses a quite strong cancerostatic action [4].

The following ornithine derivatives were obtained in the present study:

N^{α}, N^{δ}-bis-M-*L*-Orn — OR... (I) R = Bzl, (II) R = H
N^{α}-M, N^{δ}-Cbzo-*L*-Orn — OR... (III) R = CH_3, (IV) R = Bzl
N^{α}-M-*L*-Orn — OR ... (V) R = CH_3, (VI) R = H
N^{α}-M, N^{δ}-Phth-*L*-Orn — OR ... (VII) R = Bzl, (VIII) R = H

For the synthesis of compounds (I)-(VIII) we prepared the following key compounds: ornithine benzyl ester p-toluenesulfonate (IX) and N^{δ}-Phth-L-ornithine benzyl ester p-toluenesulfonate (X). The condensation of M – OH with N-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide (DCC) gave


(XI)

The N^{α},N^{δ}-bis-M-acyl derivative of L-ornithine benzyl ester (I) was obtained by the condensation of the L-ornithine benzyl ester: a) with M – OH in the presence of DCC, b) with (IX), and c) with the p-nitrophenyl ester of M – OH. For comparison, the results of these experiments are given in Table 1.

Benzyl ester (I) was converted to N^{α},N^{δ}-bis-M-L-ornithine (II) by catalytic hydrogenolysis. The methyl ester of N^{α}-M, N^{δ}-Cbzo-L-ornithine (III) was obtained by the condensation of the methyl ester of N^{δ}-Cbzo-L-ornithine with M – OH in the presence of DCC. The condensation of the benzyl ester of N^{δ}-Cbzo-L-ornithine with the p-nitrophenyl ester of M – OH gave N^{α}-M, N^{δ}-Cbzo-L-ornithine (IV). Removal of the carbobenzoxy group from the methyl ester of N^{α}-M, N^{δ}-Cbzo-L-ornithine was effected by catalytic hydrogenolysis over palladium black in the presence of concentrated hydrochloric acid. Here the methyl ester of N^{α}-M-L-ornithine was obtained as the hygroscopic hydrochloride. Removal of the carbobenzoxy group with a solution of hydrogen bromide in glacial acetic acid gave the hydrobromide of the methyl ester of N^{α}-M-L-ornithine, also as an oil. In both cases the methyl ester of the N^{α}-acylated ornithine (V) was identified by conversion of the corresponding hydrohalides to the more stable solid picrate. The simultaneous removal by catalytic hydrolysis of the protective carbobenzoxy and benzyl groups from N^{α}-M, N^{δ}-Cbzo-L-Orn-OBzl (IV) led to N^{α}-p-di-(2-chloroethyl)aminophenacetyl-L-ornithine (VI), with a free amino group

*Here and subsequently this acid will be designated by the letters M – OH, while its acyl moiety $(ClCH_2CH_2)_2N-C_6H_4-CH_2CO$ will be designated by the letter M.

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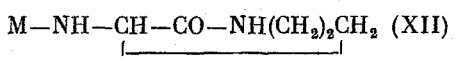
TABLE 1

Method of preparation of compound (I)	Medium	Yield,%	Mp of crude product, °C
a	$CHCl_3$	32,0	102—110
b	$CHCl_3$	20,0	95—102
c	$CHCl_3$	43,0	113—115
	CH_2Cl_2	60,0	113—115

in the δ-position. The condensation of the benzyl ester of N^{δ}-Phth-L-ornithine (X) with the p-nitrophenyl ester of M—OH gave the benzyl ester of N^{α}-M, N^{δ}-Phth-L-ornithine (VII), which was converted by catalytic hydrogenolysis of N^{α}-M, N^{δ}-Phth-L-ornithine (VII). Usually $(C_2H_5)_3N$ is used to remove the p-toluenesulfonic acid when the benzyl ester of ornithine is isolated as the free base. However, an attempt to use a solution of NH_3 in $CHCl_3$ for this resulted in cyclization of the benzyl ester of ornithine to the known 3-L-aminopiperidone. The acylation of the latter with M—OH in the presence of DCC led to 3-N-[p-di-(2-chloroethyl)aminophenacetyl]aminopiperidone



M—NH—CH—CO—NH$(CH_2)_2CH_2$ (XII)

The constants of all of the synthesized ornithine derivatives and the analysis results are given in Table 2.

EXPERIMENTAL

L-Ornithine Benzyl Ester Di-p-toluenesulfonate (IX). Obtained in the same manner as L-histidine benzyl ester di-p-toluenesulfonate [3]. The reaction was practically ended in 5-6 h.

Benzyl Ester of N^{α},N^{δ}-Bis-[p-di-(2-chloroethyl)aminophenacetyl]-L-ornithine (I). Under cooling, a chloroform solution of 1.3 g of (IX) was mixed with chloroform solutions of 0.55 ml of $(C_2H_5)_3N$, 1.1 g of M—OH and 0.8 g of DCC. The obtained solution was allowed to stand at room temperature for 2 days. The precipitate of 1,3-dicyclohexylurea was filtered (0.38 g), the filtrate was evaporated in vacuo, and the oily residue was dissolved in ethyl acetate and washed with water. The organic layer was dried over $MgSO_4$ and then evaporated. The residual oil was recrystallized from ethyl acetate—ether.

N-Hydroxysuccinimide of p-Di-(2-chloroethyl)aminophenylacetic Acid (XI). Equimolar amounts of M—OH, DCC and N-hydroxysuccinimide [5] were dissolved in the minimum amounts of either dioxane or THF. The obtained solutions were cooled, mixed, and allowed to stand for a day in the refrigerator. After removal of the 1,3-dicyclohexylurea, the filtrate was evaporated in vacuo. The residue was dissolved in $CH_3CO_2C_2H_5$ (150 ml) and washed in succession with 5% $NaHCO_3$ solution, 5% HCl solution and water. The ethyl acetate layer was dried over $MgSO_4$ and then evaporated in vacuo. The residual syrupy ester (XI) is readily soluble in THF and $CHCl_3$, and is insoluble in water and ether. Yield 75%. Found: C 51.70; H 5.04; Cl 18.60%. $C_{16}H_{18}O_4N_2Cl_2$. Calculated: C 51.50; H 4.86; Cl 19.00%.

A solution of 1.13 g of (IX), 0.55 ml of $(C_2H_5)_3N$ and 1.4 g of (XI) in $CHCl_3$ was allowed to stand at room temperature for 2 days. Then 0.5 ml of $(CH_3)_2NH$ was added to destroy the unreacted (XI). After 30 min the solution was washed with 1 N HCl solution and then with water until neutral. The ethyl acetate layer was dried, evaporated in vacuo, and the residue was recrystallized from either ethyl acetate—ether or absolute ethanol.

To a solution of 1.13 g of (IX) and 0.55 ml of $(C_2H_5)_3N$ in CH_2Cl_2 was added 1.48 g of the p-nitrophenyl ester of M—OH [6], and the mixture was allowed to stand at room temperature for 2 days. Then the mixture was diluted with CH_2Cl_2, washed in succession with 1 N HCl solution, water, 5-7 times with 5% $NaHCO_3$ solution, and again with water until neutral. After drying over $MgSO_4$ the solvent was vacuum-distilled, while the residue was recrystallized from ethyl acetate—ether. The yield was lower when the reaction was run in $CHCl_3$ (see Table 1).

The diacylated benzyl ester (I) is a white powder that darkens in the light. It is readily soluble in acetone or $CHCl_3$, and insoluble in ether or water.

N^{α},N^{δ}-Bis-[p-di-(2-chloroethyl)aminophenacetyl]-L-ornithine (II). A solution of 1 g of benzyl ester (I) in 30 ml of an acetone—absolute ethanol mixture (2 : 1) was hydrogenated over Pd black. The product was recrystallized from ethyl acetate—ether. The free acid (II) is readily soluble in acetone, THF and dioxane, and in hot $CH_3CO_2C_2H_5$ and $CHCl_3$, and is insoluble in ether, water, and aqueous $NaHCO_3$ solution. For biological testing we prepared the water-soluble salt of acid (II) with diethanolamine, for which 4 g of acid (II) was dissolved in THF and the solution was mixed with 0.6 g of $(OHC_2H_4)_2NH$. The clear mixture was evaporated in vacuo, while the residue was rubbed with anhydrous ether. The obtained salt is not very hygroscopic, is readily soluble in ethanol, and is soluble in water.

TABLE 2 R—HN—$CH_2CH_2CH_2CH$(NH—$COCH_2$—C$_6$H$_4$—$N(CH_2CH_2Cl)_2$)—$COOR'$

Compound	R	R'	Yield, %	Mp, °C (recrystallization solvent)	Found, %				Formula	Calculated, %			
					C	H	N	Cl		C	H	N	Cl
I	M	Bzl	60	114,5-116 (from ethylacetate – absolute ether)	58,35	6,30	7,67	—	$C_{36}H_{44}O_4N_4Cl_4$	58,55	6,14	7,58	19,20
II	M	H[a]	98	127-129 (from ethyl acetate)	53,99	5,88	—	22,00	$C_{29}H_{38}O_4N_4Cl_4$	53,71	5,90	8,64	21,83
III	Cbzo	Me	73	92-94 (from ethyl acetate)	59,00	5,00	7,87	13,40	$C_{2c}H_{25}O_5N_3Cl_2$	58,90	4,72	7,93	13,40
IV	Cbzo	Bzl	63	94-96 (from CCl_4)	62,45	6,00	—	11,32	$C_{32}H_{37}O_5N_3Cl_2$	62,53	6,06	6,84	11,53
V	H	Me[b]	50	57-60 (from methanol – water)	45,81	4,80	—	10,12	$C_{28}H_{30}O_{10}N_6Cl_2$	45,51	4,76	—	11,19
VI	H	H	63	153-155 (from methanol – absolute ether)	52,20	6,50	—	18,20	$C_{17}H_{25}O_3N_3Cl_2$	52,31	6,46	10,76	18,17
VII	Phthc	Bzl	65	119-122 (from methanol)	62,91	5,43	7,19	—	$C_{32}H_{34}O_5N_3Cl_2$	62,85	5,60	6,87	11,60
VIII	Phthc	H	92	78-82 (from absolute methanol)	57,34	5,20	—	13,50	$C_{25}H_{28}O_5N_3Cl_2$	57,59	5,41	8,06	13,60
IX	H[d]	Bzl	97	177-179 (from methanol – absolute ether)	55,32	5,86	—	—	$C_{26}H_{34}O_8N_2S_2$	55,30	6,01	4,94	—
X	Phth[d, e]	Bzl	77	173-176 (from methanol – absolute ether)	62,00	5,41	—	—	$C_{27}H_{29}O_7N_2S$	61,70	5,56	5,44	—
XI	—	—	65	130-133 (from benzene)	55,11	6,46	19,14	—	$C_{17}H_{23}O_2N_3Cl_2$	54,87	6,23	11,29	19,05

Note: a) The ethanolamine salt was analyzed; b) isolated as the picrate; c) phthalyl protection instead of RH; d) hydrogen instead of M on N^{α}, isolated as the salt with two molecules of p-toluenesulfonic acid; e) isolated as the salt with p-toluenesulfonic acid.

Methyl Ester of N^{α}-[p-Di-(2-chloroethyl)aminophenacetyl]-N^{δ}-carbobenzoxy-L-ornithine (III). To a solution of 1.2 g N^{δ}-carbobenzoxy-L-ornithine methyl ester hydrochloride [7] in $CHCl_3$ (15 ml) was added 40-50 ml of anhydrous ether, containing 0.55 ml of $(C_2H_5)_3N$. The mixture was stirred well and, after cooling, the $(C_2H_5)_3N \cdot HCl$ was filtered. The filtrate was evaporated in vacuo to a volume of 10 ml, and then mixed in the cold with chloroform solutions of 0.8 g of DCC and 1.1 g of M – OH. The solution was allowed to stand at room temperature. The next day the 1,3-dicyclohexylurea was filtered, and the filtrate was evaporated in vacuo. The residue was dissolved in 2-3 ml of $CH_3CO_2C_2H_5$ and filtered again. The filtrate was placed in the refrigerator overnight. Product (III) was obtained as white crystals, which are soluble in $CHCl_3$, methanol and ethanol, and insoluble in water or ether.

Methyl Ester of N^{α}-[p-Di-(2-chloroethyl)aminophenacetyl]-L-ornithine (V). a) To a solution of 1.06 g of methyl ester (III) in methanol was added 0.17 ml of conc. HCl solution and the mixture was hydrogenated over Pd black. The catalyst was filtered, and the filtrate was evaporated in vacuo. The residual oil was rubbed with anhydrous ether to give the hygroscopic hydrochloride of compound (V), which was identified as the picrate.

b) A mixture of 1.06 g of (III) and 2 ml of a saturated solution of HBr in glacial acetic acid was shaken until all of (III) had dissolved (20-30 min). Then 20 ml of anhydrous ether was added to give the oily hydrobromide of compound (V).

Preparation of Picrate. The oil, obtained by either method a) or b), was dissolved in the minimum amount of methanol and poured into a saturated aqueous solution of 0.6 g of picric acid. The obtained red oil was washed well with water and then dried in a vacuum-desiccator. The picrate of compound (V) was obtained as a brownish-red powder.

N^{δ}-Carbobenzoxyornithine benzyl ester p-toluenesulfonate was obtained by the procedure described in [8].

N^{δ}-Phthalyl-L-ornithine Benzyl Ester p-Toluenesulfonate (X). A mixture of 6.25 g of N^{δ}-phthalyl-L-ornithine hydrochloride [9], 3.97 g of p-toluenesulfonic acid monohydrate, 9 ml of $C_6H_5CH_2OH$, and 4.5 ml of benzene was refluxed in an apparatus equipped with a Dean – Stark water separator. To the cooled reaction mixture was added 1.5 ml of anhydrous ether and the mixture was placed in the refrigerator. The crystals of the p-toluenesulfonate (X) were filtered and washed with anhydrous ether. Compound (X) is soluble in $CH_3CO_2C_2H_5$ and water.

Benzyl Esters of N^{α}-[p-Di-(2-chloroethyl)aminophenylacetyl]-N^{δ}-carbobenzoxy-L-ornithine (IV) and N^{α}-[p-Di-(2-chloroethyl)aminophenacetyl]-N^{α}-phthalyl-L-ornithine (VII). To a solution of 2 mmoles of the p-toluenesulfonate of the corresponding benzyl ester and 0.27 ml of $(C_2H_5)_3N$ in the minimum amount of CH_2Cl_2 was added 0.79 g of the p-nitrophenyl ester of M – OH [6]. The mixture was let stand at room temperature for 2 days. Then the solution was diluted with CH_2Cl_2 and washed in succession with 1 N HCl solution, water, 5-7 times with 5% $NaHCO_3$ solution, and again with water until neutral. The organic layer was dried and the solvent was vacuum-distilled, while the residue was recrystallized from CCl_4 in the case of (IV), and from $CH_3CO_2C_2H_5$ – anhydrous ether in the case of (VII).

N^{α}-[p-Di](2-chloroethyl)aminophenacetyl]-L-ornithine (VI). Benzyl ester (IV) in an acetone – ethanol mixture (1 : 1) was hydrogenated over Pd black. The hydrogenation product deposited in the precipitate. The mixture of catalyst and substance was filtered and then recrystallized from methanol – ether. Compound (VI) at room temperature is insoluble in water and in the common inorganic solvents. It is soluble in hot water, methanol and $HCON(CH_3)_2$, and is readily soluble in acidulated water. The test with ninhydrin is negative.

N^{α}-[p-Di-(2-chloroethyl)aminophenacetyl]-N^{α}-phthalyl-L-ornithine (VIII). A solution of 3 g of benzyl ester (VII) in an acetone – absolute ethanol mixture (3 : 1) was hydrogenated over Pd black, and the product was recrystallized from absolute ethanol.

3-L-Aminopiperidone. A suspension of 11.3 g of (IX) in 30 ml of a 2% solution of NH_3 in $CHCl_3$ was stirred at room temperature for 30 min. The ammonium p-toluenesulfonate was filtered and the filtrate was evaporated in vacuo. The oily residue was recrystallized from anhydrous ether. The compound was obtained as white hygroscopic crystals that darken rapidly in the light, mp 40-42°. The constants and properties of the compound coincided with those given in [10].

3-[p-Di-(2-chloroethyl)aminophenacetyl]-L-aminopiperidone (XII). The oil, obtained from 11.3 g of (IX) by the above described method, was dissolved in $CHCl_3$ and mixed in the cold with chloroform solutions of 5.52 g of M–OH and 4.12 g of DCC, and the whole was allowed to stand at room temperature until the next day. The 1,3-dicyclohexylurea was filtered, the filtrate was evaporated in vacuo, and the residue was dissolved in a small amount of ethyl acetate. Compound (XII) was obtained. It is readily soluble in methanol, dioxane, $CHCl_3$ and acetone, and is insoluble in ether and water.

CONCLUSIONS

We synthesized N^{α}-p-di-(2-chloroethyl)aminophenacetyl-L-ornithine, N^{α},N^{δ}-bis-p-di-(2-chloroethyl)aminophenacetyl-L-ornithine, and some of their derivatives.

LITERATURE CITED

1. S. É. Zarubyan, L. P. Rasteikene, O. V. Kil'disheva, and I. L. Knunyants, Izv. Akad. Nauk SSSR, Ser. Khim., 1899 (1964).
2. M. I. Dagene, L. P. Rasteikene, O. V. Kil'disheva, and I. L. Knunyants, Izv. Akad. Nauk SSSR, Ser. Khim., 917 (1965).
3. M. I. Dagene, L. P. Rasteikene, O. V. Kil'disheva, and I. L. Knunyants, Izv. Akad. Nauk SSSR, Ser. Khim., 1822 (1969).
4. Kh. Kotobuki, M. Fumio, and O. Saburo, Osaka Ika Daigaku Zasshi, 20, 366, 591 (1960); Ref. Zh., Khim., 12 C 1490.
5. G. Anderson, J. Zimmerman, and F. Callahan, J. Am. Chem. Soc., 86, 1839 (1964).
6. D. A. Dzhyuvene and Yu. A. Degutis, Scientific-Research Institute of Oncology of the LithSSR, Data of Tenth Scientific Session [in Russian], Vilnius (1967), p. 27.
7. R. L. M. Synge, Biochem. J., 42, 99 (1948).
8. G. Losse, H. Jeschkeit, and H. Zaschke, Ann., 676, 234 (1964).
9. M. Bodanszky, M. Ondetti, C. Birkhimer, and P. Thomas, J. Am. Chem. Soc., 86, 4452 (1964).
10. K. Golankiewicz and M. Wiewiorowski, Acta Biochim. Polon., 10, 443 (1963).