SYNTHESIS AND PROPERTIES OF SYMMETRICAL ANALOGS OF ANTAMANIDE

A. I. Miroshnikov, K. Kh. Khalilulina, N. N. Uvarova, V. T. Ivanov, and Yu. A. Ovchinnikov

UDC 547.964.4+577.17.07

In preceding papers [1, 2] we have described the synthesis and the results of physicochemical investigations of the natural antagonist of deathcup amanita, antamanide (I) [3, 4], and two of its analogs – perhydroantamanide (II) and retroantamanide (III). A unique property of the compounds obtained is their capacity for forming complexes with alkali-metal cations in solution with a well-defined Na/K selectivity in complex formation ([1, 5], Table 1).

1 2 3 4 5 6 7 8 9 10

[Val-Pro-Pro-Ala-Phe-Phe-Pro-Pro-Phe-Phe]

I antamanide

[Val-Pro-Pro-Ala-Cha-Cha-Pro-Pro-Cha-Cha]

II. Cha^5, Cha^6, Cha^9, Cha^{10}-antamanide (perhydroantamanide)*

[Ala-Pro-Pro-Val-Phe-Phe-Pro-Pro-Phe-Phe]

III. Ala^1, Val^4-antamanide (retroantamanide)

Spectral investigations, in combination with a theoretical analysis, have enabled a spatial structure for a Na^+ complex of antamanide to be put forward which explains many features of its behavior [2] and serves as a basis for the interpretation of the relationship between the structure of cyclopeptides of the antamanide group and their capacity for complex formation [6, 7].

In the course of the further development of a study of the relationship between the structure and stability of the complexes, on the one hand, and the biological activity of antamanide and its analogs, on the other hand, it appeared of interest to investigate derivatives of antamanide the molecules of which possess a second-order axis of symmetry (C_2). Analogs of this type would be expected to have an increased tendency to complex formation, since the presence of a pseudo C_2 axis has been shown previously for the Na^+ complex of antamanide [2]. Furthermore, such compounds are more convenient objects for conformational investigation than antamanide itself, since they give considerably simpler NMR spectra. The present paper describes the synthesis of compounds (IV) and (V), and their perhydro derivatives (VI) and (VII); the related cyclodecapeptide Phe^4, Val^6-antamanide has recently been described by Wieland et al. [8].

The cyclopeptides (IV) and (V) were synthesized by the scheme given on p. 208 with the aid of the expedients and methods used previously in the production of antamanide† [1].

*Cha = —NH—CH(CH$_2$—C$_6$H$_{11}$)—CO—

†In contrast to the conditions for the synthesis and cyclization of the ester (XXIV) described in [1], where pyridine was used in the last stage, here the p-nitrophenyl ester (XXIII) was subjected to the cyclization reaction in solution in chloroform containing triethylamine.

M. M. Shemyakin Institute of the Chemistry of Natural Compounds, Academy of Sciences of the USSR.
Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 214-220, March-April, 1973. Original article submitted June 20, 1972.

TABLE 1. Physicochemical Properties of Compounds (I-VII)

Compound	Mol. wt. (mass spectrometer)	mp, °C	$[\alpha]_D^{20}$ (in ethanol), deg	Stability constants of the complexes* (K, liter·mole^{-1})		Free energies of complex formation ($-\Delta F = RT \ln K$, kcal/mole)	
				Na^+	K^+	Na^+	K^+
Antamanide [1, 3] (I)	1146	172—174	—168 (*c* 0,5)	2800	270	4,7	3,3
Perhydroantamanide [1]† (II)	1170	164—166	—86 (*c* 0,5)	2000	<50	4,5	2,3
Retroantamanide [1] (III)	1146	176—177	—176 (*c* 0,08)	400	100	3,6	2,7
Phe^1, Ala^9-Antamanide (IV)	1118	178—179	—107,6 (*c* 1)	6000	500	5,2	3,7
Val^6, Ala^9-Antamanide (V)	1022	320—322	—384 (*c* 1)	25000	1000	6,0	4,1
Perhydro-Phe^1, Ala^9-antamanide (VI)	1142	179—182	—25 (*c* 1)	2800	270	4,7	3,3
Perhydro-Val^6, Ala^9-antamanide (VII)	1034	304—305	—153 (*c* 1)	1700	100	4,4	2,3

* Measured by the conductometric method [10].

†In a preceding paper [1], a value of 200 liters·mole^{-1} was given erroneously for the stability constant of the Na^+ complex of perhydroantamanide (II).

1 2 3 4 5 6 7 8 9 10

[Phe-Pro-Pro-Ala-Phe-Phe-Pro-Pro-Ala-Phe]

IV. Phe^1, Ala^9-antamanide

[Val-Pro-Pro-Ala-Phe-Val-Pro-Pro-Ala-Phe]

V. Val^6, Ala^9-antamanide

[Cha-Pro-Pro-Ala-Cha-Cha-Pro-Pro-Ala-Cha]

VI. Cha^1, Cha^5, Cha^6, Ala^9, Cha^{10}-antamanide

[Val-Pro-Pro-Ala-Cha-Val-Pro-Pro-Ala-Cha]

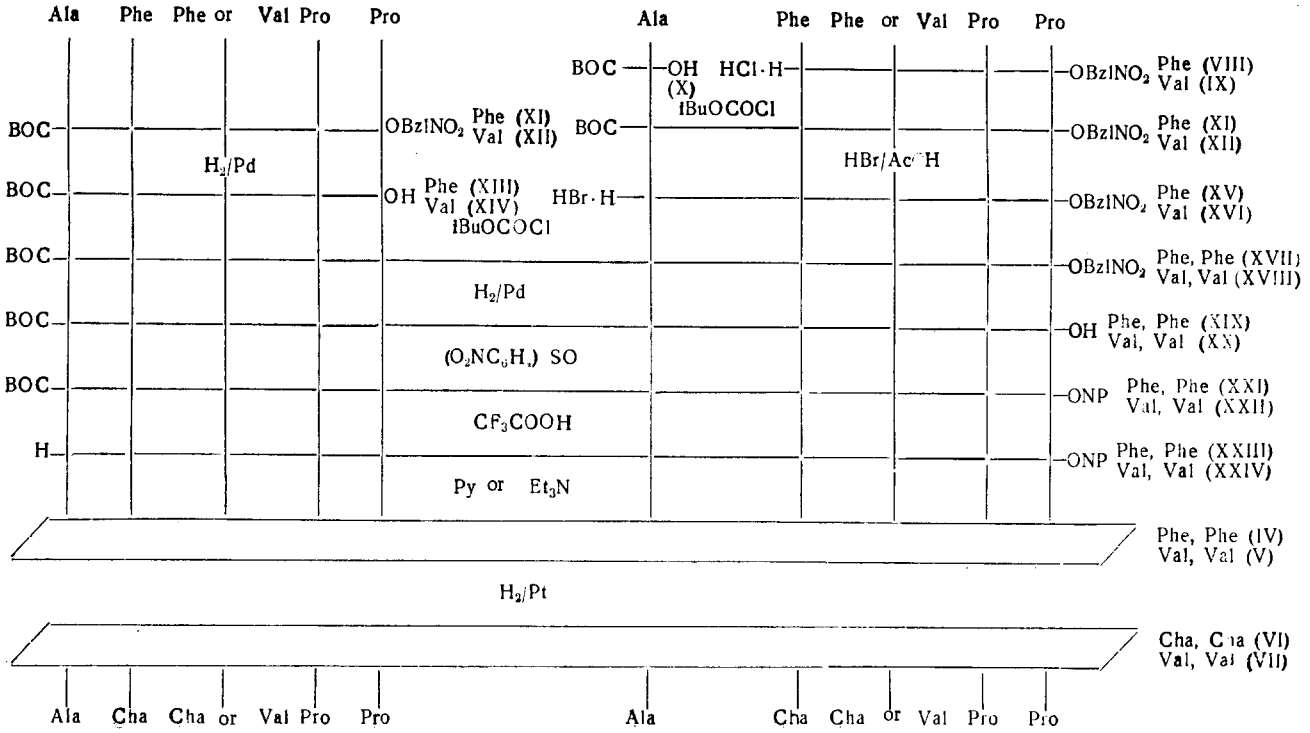
VII. Cha^5, Val^6, Ala^9, Cha^{10}-antamanide

Compounds (VI) and (VII) were obtained by the prolonged hydrogenation of the phenylalanine-containing peptides (IV) and (V) over a platinum catalyst; their comparatively low yields (34 and 61%) are apparently due to the simultaneous occurrence of reductive degradation.

The physicochemical constants and information on complex formation by the cyclopeptides obtained with Na^+ and K^+ ions are given in Table 1. For comparison, it also gives the figures for antamanide (I), perhydroantamanide (II), and retroantamanide (III) taken from the previous paper [1]. The mass spectra of compounds (IV-VII) are extremely similar to the mass spectra of antamanide, giving strong peaks of the molecular ions; the nature of their fragmentation agrees completely with the primary structure of the cyclodecapeptides synthesized.

The symmetrical analogs of antamanide, (IV) and (V), dissolve somewhat more sparingly in water and in the usual organic solvents than antamanide. As can be seen from Table 1, they are distinguished by a higher stability of the sodium and potassium complexes as compared with those of antamanide itself. Furthermore, they differ considerably from the natural cyclopeptide in the selectivity of their complex formation ($\Delta F_{Na^+} - \Delta F_{K^+}$). Measurements of the antitoxic action of compounds (IV) and (V) showed* that the latter are considerably inferior in this respect to antamanide: for (IV), the protective dose against 5 mg/kg of phalloidine ($LD_{50} \approx 2.0$ mg/kg) injected into mice is 10 mg/kg, while at the same concentration (V) showed no protective effect (for antamanide, the protective dose is 0.5 mg/kg). The symmetrical analogs (IV) and (V), like antamanide (I), show no antimicrobial activity at concentrations of up to 50 γ/ml against Sarcina lutea, Bacillus subtilis, Candida albicans, Mycobacterium phlei, Escherichia coli, Staphylococcus aureus 209 P, Staphylococcus aureus UV-3, and Streptococcus faecalis. So far as concerns the hydrogenated analogs (VI) and (VII), as has been reported previously [9], compound (II) suppresses the growth of Staph-

* The testing of biological activity was performed by Prof. T. Wieland (Institute for Medical Research of the Max Planck Society, Heidelberg, GFR), the measurement of the antimicrobial activity by N. D. Ryabova, and the determination of the instability constants of the complexes by G. G. Malenkov and N. A. Skobelev.



ylococcus aureus UV-3 and Streptococcus faecalis in concentrations of 18 and 6-9 γ/ml, respectively. The symmetrical analog (VI) shows no antimicrobial activity against all the strains mentioned even at concentrations of up to 100 γ/ml, and compound (VII) could not be tested because of its low solubility.

Thus, the symmetrical analogs of antamanide (IV)-(VII), while being fairly effective complexones of Na^+ ions, possess an extremely weak biological activity. It is not excluded that this is connected with their low solubility in organic media.

EXPERIMENTAL

The individuality of the compounds obtained was checked by thin-layer chromatography on alumina (activity grade II) or silica gel. For all the compounds, the results of elementary analysis corresponded to the calculated C, H, and N contents. The specific rotations were measured on a Perkin-Elmer 141 polarimeter at 20-25°C in 96% ethanol (c 0.3-0.7). In the preparation of the protected peptides, the reaction mixture was washed with water, 10% citric acid solution, saturated $NaHCO_3$ solution, and water again, and was dried with $MgSO_4$.

p-Nitrobenzyl Ester of tert-Butyloxycarbonylalanyl(phenylalanyl)(phenylalanyl)propylproline (XI). At −15°C, 14 ml of triethylamine and 13.5 ml (0.1 mole) of isobutyl chloroformate were added to a solution of 18.9 g (0.1 mole) of tert-butoxycarbonylalanine (X) in 100 ml of absolute tetrahydrofuran. After 15 min at −15°C, the reaction mixture was treated with 67.8 g (0.1 mole) of the hydrochloride of the tetrapeptide (VIII) [1] and 14 ml of triethylamine in 100 ml of absolute tetrahydrofuran. The mixture was stirred at 0°C for 1 h and at 20°C for 12 h and was then evaporated and the residue was extracted with 300 ml of ethyl acetate. After washing and drying the ethyl acetate solution was evaporated in vacuum. The yield of chromatographically homogeneous amorphous powder ($[\alpha]_D$ −89.5°) was 73.1 g (90%).

p-Nitrobenzyl Ester of tert-Butoxycarbonylalanyl(phenylalanyl)valylprolylproline (XII). This substance was obtained under the conditions of the preceding experiment from 18.9 g (0.1 mole) of (X) and 62.9 g (0.1 mole) of the hydrochloride of the tetrapeptide (IX) [1] with a yield of 68.6 g (90%), $[\alpha]_D$ −261.1°.

tert-Butoxycarbonylalanyl(phenylalanyl)(phenylalanyl)alanylprolylproline (XIII). The hydrogenation of 40.65 g (0.05 mole) of the ester (XI) in 350 ml of absolute dioxane and 2 ml of glacial acetic acid was performed over 5 g of palladium oxide for 20 h. After the end of hydrogenation (monitored by thin-layer chromatography), the filtrate was evaporated, 300 ml of dioxane−water (1 : 1) was added, and the solution was passed through a column of Dowex 50 × 2 (H^+ form). The eluate was evaporated, the residue was dissolved in 50 ml of ethanol, and the product was precipitated with 400 ml of ether−hexane (1 : 1). Yield 30 g (91%), $[\alpha]_D$ −86.5°.

tert-Butoxycarbonylalanyl(phenylalanyl)valylprolylproline (XIV). The substance was obtained under the conditions of the preceding experiment from 38.2 g (0.05 mole) of (XII) in the form of an amorphous powder with a yield of 29 g (92%), $[\alpha]_D$ −80.6°

p-Nitrobenzyl Ester of tert-Butoxycarbonylalanyl(phenylalanyl)(phenylalanyl)prolylprolylalanyl-(phenylalanyl)(phenylalanyl)prolylproline (XVII). A solution of 9.82 g (0.012 mole) of the pentapeptide (XI) in 15 ml of glacial acetic acid was treated with 40 ml of a 35% solution of HBr in glacial acetic acid. After 1 h, the mixture was evaporated at 35°C and the residue was treated with 250 ml of absolute ether. The precipitate of the hydrobromide of (XV) that deposited was filtered off, washed with ether, and dried in vacuum over KOH. Yield 8.85 g (93%). With stirring, 1.4 ml of triethylamine and 1.35 ml (0.01 mole) of isobutyl chloroformate were added to a solution of 6.8 g (0.01 mole) of the pentapeptide (XIII) in 40 ml of absolute tetrahydrofuran cooled to −15°C; after 20 min, another 7.94 g (0.01 mole) of the hydrobromide of (XV) in 50 ml of tetrahydrofuran containing 1.4 ml of triethylamine was added. After 30 h (20°C), the reaction mixture was evaporated, the residue was extracted with ethyl acetate, and the extract was washed and dried. This gave 9.07 g (66%) of the decapeptide (XVII) in the form of an amorphous powder, $[\alpha]_D$ −108.5°.

p-Nitrobenzyl Ester of tert-Butoxycarbonylalanyl(phenylalanyl)valylprolylprolylalanyl(phenylalanyl)-valylprolylproline (XVIII). The substance was obtained under the conditions of the preceding experiment from 6.29 g (0.01 mole) of the tert-butoxycarbonylpentapeptide (XIV) and 7.45 g (0.01 mole) of the hydrobromide of the pentapeptide (XVI) obtained from the protected ester of the pentapeptide (XII). The yield of the decapeptide (XVIII) was 8.91 g (70%), $[\alpha]_D$ −129°.

tert-Butoxycarbonylalanyl(phenylalanyl(phenylalanyl)prolylprolylalanyl(phenylalanyl)(phenylalanyl)-prolylproline (XIX). As in the preparation of (XIII), the hydrogenation of 13.72 g (0.01 mole) of the protected decapeptide (XVII) over 1 g of palladium oxide gave compound (XIX) with a yield of 11.5 g (93%), $[\alpha]_D$ −107.8°.

tert-Butoxycarbonylalanyl(phenylalanyl)valylprolylprolylalanyl(phenylalanyl)valylprolylproline (XX). The substance was obtained in a similar manner to the acid (XIX) from 12.75 g (0.01 mole) of the protected decapeptide (XVIII) in the form of an amorphous powder with a yield of 10.8 g (95%), $[\alpha]_D$ −137°.

Cyclo(phenylalanyl)prolylprolylalanyl(phenylalanyl)(phenylalanyl)prolylprolylalanyl(phenylalanine) (Phe^1, Ala^9-Antamanide) (IV). To a solution of 6.18 g (0.005 mole) of (XIX) in 25 ml of dry pyridine was added 16.2 g (0.05 mole) of di-p-nitrophenyl sulfite. After 48 h (20°C), the pyridine was distilled off in vacuum and the residue was washed with ether and dried in vacuum over P_2O_5. The resulting p-nitrophenyl ester of a tert-butoxycarbonyldecapeptide (XXI) was dissolved in 30 ml of anhydrous trifluoroacetic acid. After 2 h, the solution was evaporated at 35°C. The residue was treated with 200 ml of absolute ether and the trifluoroacetate of (XXIII) was filtered off and dried over KOH in vacuum, after which it was dissolved in 200 ml of dioxane containing 2 ml of acetic acid and, over 6 h, was added at 50°C to 1000 ml of absolute chloroform containing 0.7 ml (0.05 mole) of triethylamine. After 24 h (50°C), the solution was evaporated to dryness, and the residue was triturated under ether and was filtered off. The resulting product was dissolved in 300 ml of methanol–water (9:7) and was passed successively through columns (30 × 2 cm) containing Dowex 50 × 2 (H^+ form) and Dowex 1 × 2 (OH^- form). The eluate was evaporated, and the residue was dried, washed with ether, and then crystallized from acetone–water (50:1). After drying in vacuum over P_2O_5, the yield was 2.56 g (47%), calculated on the acid (XIX).

Cyclovalylprolylprolylalanyl(phenylalanyl)valylprolylprolylalanyl(phenylalanine) (Val^6, Ala^9-Antamanide) (V). The trifluoroacetate of the p-nitrophenyl ester of a decapeptide (XXIV) obtained under the conditions of the preceding experiment from 5.7 g (0.005 mole) of the acid (XX) was dissolved in 200 ml of dioxane–dimethylformamide (20:1) and was added over 6 h at 65°C to 700 ml of absolute pyridine. After 65 h (65°C), the solution was evaporated to dryness and the residue was washed with ether and dried in vacuum and was then dissolved in methanol and chromatographed on Sephadex LH-20 (column 200 × 4 cm). The eluate containing the cyclodecapeptide (monitoring by thin-layer chromatography on silica gel) was evaporated and the residue was crystallized from aqueous acetone. After drying in vacuum over P_2O_5, the yield was 1.53 g (30%), calculated on the acid (XX).

Cyclo-β-cyclohexylalanylprolylprolylalanyl-(β-cyclohexyl)alanyl-(β-cyclohexylalanyl)prolylprolyl-alanyl-(β-cyclohexylalanine) (Perhydro-Phe^1, Ala^9-Antamanide) (VI). The cyclopeptide (IV) (100 mg) was hydrogenated in 10 ml of ethanol–acetic acid (1:1) over a platinum catalyst (from 0.1 g of platinum oxide). After the cessation of the absorption of hydrogen, the catalyst was filtered off, the solution was evaporated, and the residue was recrystallized three times from aqueous acetone. Yield 62 mg (61%).

Cyclovalylprolylprolylalanyl-(β-cyclohexylalanyl)valylprolylprolylalanyl-(β-cyclohexylalanine) (Perhydro-Val^6, Ala^9-Antamanide) (VII). The substance was obtained under the conditions of the preceding experiment from 100 mg of the cyclopeptide (V). After three recrystallizations from aqueous acetone, the yield was 31 mg (34%).

SUMMARY

1. The synthesis of four symmetrical analogs of antamanide has been performed.

2. The capacity of the analogs obtained for complex formation with Na^+ and K^+ ions in ethanolic solution has been studied.

LITERATURE CITED

1. Yu. A. Ovchinnikov, V. T. Ivanov, A. M. Miroshnikov, K. Kh. Khalilulina, and N. N. Uvarova, Khim. Prirodn. Soedin., 469 (1971).
2. V. T. Ivanov, A. I. Miroshnikov, N. D. Abdullaev, L. B. Senyavina, S. E. Arkhipova, N. N. Uvarova, K. Kh. Khalilulina, V. F. Bystrov, and Yu. A. Ovchinnikov, Biochem. Biophys. Res. Communs., 42, 654 (1971).
3. Th. Wieland, G. Lüben, H. Ottenheym, J. Faesel, J. X. de Vries, W. Konz, A. Prox, and J. Schmid, Angew. Chem., 80, 209 (1968).
4. Th. Wieland, G. Lüben, H. Ottenheym, and H. Schiefer, Ann., 722, 173 (1969).
5. Th. Wieland, H. Faulstich, W. Burgermeister, W. Otting, W. Möhle, M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and V. V. Malenkov, FEBS Lett., 9, 89 (1970).
6. Yu. A. Ovchinnikov, V. T. Ivanov, V. F. Bystrov, N. D. Abdullaev, and A. I. Miroshnikov, Proc. 11th European Peptide Symposium, Vienna (1971).
7. Yu. A. Ovchinnikov, V. T. Ivanov, and A. M. Shkrob, Proc. Int. Symposium Molecular Mechanisms of Antibiotic Action on Protein Biosynthesis and Membranes, Elsevier, Amsterdam (1972).
8. Th. Wieland, A. V. Dudgen, and Ch. Birr, FEBS Lett., 14, 299 (1971).
9. Yu. A. Ovchinnikov, V. T. Ivanov, L. I. Barsukov, E. I. Melnik, N. A. Oreshnikova, N. D. Bogolyubova, I. D. Ryabova, A. I. Miroshnikov, and V. A. Rimskaya, Exper., 28, 399 (1972).
10. I. M. Andreev, G. G. Malenkov, A. M. Shkrob, and M. M. Shemyakin, Molekul. Biol., 5, 614 (1971).