SYNTHESIS AND SOME PHYSICOCHEMICAL PROPERTIES OF ANTAMANIDE, RETROANTAMANIDE, AND PERHYDROANTAMANIDE

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

UDC 547-96

Among natural biologically active peptides, considerable interest has recently been attracted by the cyclic decapeptide antamanide isolated from extracts of Amanita phalloides (deathcup amanita) which in vivo suppresses the action of its main poisons – phallodine, the amanitines, etc. [1, 2]. Together with T. Wieland and his colleagues, we have recently shown that in alcoholic solutions antamanide forms complexes with Na^+ and K^+ ions having stability constants of ~2500 and ~250 liters/mole, respectively [3]. The marked sodium specificity of complex formation is a unique property of antamanide, since other natural complexones of the alkali metals (valinomycin [4], the enniatins [4], macrotetralides [5], etc.) possess potassium specificity, as a rule.

In view of the interesting physiological properties of antamanide and also the possibility of obtaining from it substances capable of selectively inducing the transport of sodium cations through artificial and biological membranes, we undertook an investigation on the relationship between structure, physicochemical properties, and biological activity of antamanide and its analogs. We first performed the synthesis of antamanide (1)* and its retro analog (2), (i.e., the analog differing from the natural compound by the direction of acylation), and also of the perhydro derivative of antamanide (3) in which the L-β-phenylalanine residues were replaced by L-β-cyclohexylalanine (L-Cha) residues.†

$$[\overline{\text{Phe-Phe-Val-Pro-Pro-Ala-Phe-Phe-Pro-Pro}}] \quad (1)$$

$$[\overline{\text{Phe-Phe-Val-Pro-Pro-Ala-Phe-Phe-Pro-Pro}}] \quad (2)$$

$$[\overline{\text{Cha-Cha-Val-Pro-Pro-Ala-Cha-Cha-Pro-Pro}}] \quad (3)$$

In order to obtain antamanide (1) and its retro analog (2), we first synthesized the corresponding linear decapeptides, which were then cyclized under conditions of high dilution. The method of synthesis was selected in such a way that at all stages benzyloxycarbonyl- or tert-butoxycarbonylamino acids or the carboxy groups of peptides with C-terminal proline residues were subjected to activation. In this respect, the synthesis performed differs favorably from the syntheses of antamanide described previously, since, in the latter, carboxy groups of peptides with C-terminal alanine [6] or phenylalanine [1, 7, 8] residues were activated, which greatly increased the risk of racemization. The protected pentapeptides (24, 26, 28, 30) were obtained by the stepwise growth of the peptide chain from the N end. The C-terminal carboxy groups were blocked by p-nitrobenzyl ester groups, which are capable of elimination by hydrogenolysis, and for the protection of the amino groups of the N-terminal residues of the amino acids the tert-butoxycarbonyl and benzyloxycarbonyl groups were used, these being eliminated by the action of HCl or HBr in glacial

*All the amino acids have the L configuration.

†A synthesis of antamanide and a number of its analogs has been described previously by T. Wieland and other workers [1, 6-8].

M. M. Shemyakin Institute of the Chemistry of Natural Compounds, Academy of Sciences of the USSR. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 469-474, July-August, 1971. Original article submitted March 15, 1971.

acetic acid. The methods used to form the peptide bonds were the N-hydroxysuccinimide ester, the mixed anhydride, and the N,N'-dicyclohexylcarbodiimide (DCCDI) methods. The protected di-, tri-, tetra-, and pentapeptides obtained immediately after the reaction consisted of oily or amorphous chromatographically homogeneous products and did not require further purification; the protected decapeptides (34) and (36) were separated from the low-molecular-weight impurities by chromatography on Sephadex LH-20. The cyclization of the free decapeptides was effected by the p-nitrophenyl ester method in pyridine–dioxane solution under conditions of high dilution ($\sim 6 \times 10^{-3}$ M). The cyclization products formed were isolated from the reaction mixture with a yield of about 20% by chromatography successively on Sephadex LH-20, alumina, and silica gel. The antamanide obtained was identical with a natural sample, kindly sent to us by Wieland, with respect to its chromatographic behavior in thin layers of alumina and silica gel, its melting point and its optical rotatory dispersion curve in ethanol (see [3]). The cyclopeptide (3) was readily formed by the hydrogenation of antamanide over a platinum catalyst.

The structures of the cyclopeptides (1)-(3) agreed well with their mass spectra, in which strong peaks of the molecular ions with m/e 1146, 1146, and 1170, respectively, were found. As in the case of antamanide [9], the mass spectrum of retroantamanide (2) has a strong peak corresponding to the elimination of the phenylalanine side chain (M – 91) which is clearly followed by a series of peaks corresponding to the amino-acid type of fragmentation of the two pentapeptide chains (Pro-Pro-Val-Phe-Phe and Pro-Pro-Phe-Phe-Ala), formed as a result of the decomposition of the cyclodecapeptide molecule and each having at the N end the two proline residues playing the role of N-acyl protective groups. A similar type of fragmentation was observed for the hydrogenated analog (3).

The change in the constants of complex-formation of these analogs of antamanide has shown that a reversal of the direction of acylation, equivalent to an interchange of the Val and Ala residues (compound 2) somewhat lowers the stability of the complexes with Na^+ and K^+, while the replacement of phenyl groups by cyclohexyl groups has practically no effect on complex formation. At the same time, the greater solubility of the analog (3) than of antamanide in organic solvents must be mentioned; this may show a fundamental influence on its membrane activity, which we are studying at the present time.

EXPERIMENTAL

All the melting points are uncorrected. The individualities of the compounds obtained were checked by thin-layer chromatography on alumina (activity grade II) or silica gel. For all the compounds the elementary analyses corresponded to the calculated C, H, and N contents. The specific rotations were determined at 20-25° C in 96% ethanol (c 0.3-0.7). In the preparation of the protected amino acids and peptides, the reaction mixtures were washed with water, 10% citric acid solution, saturated $NaHCO_3$ solution, and water again, and were dried with $MgSO_4$.

Benzyloxycarbonylproline p-Nitrobenzyl Ester (5). A solution of 24.9 g (0.1 mole) of benzyloxycarbonylproline (4) in 300 ml of absolute ethyl acetate was treated with 21.6 g (0.1) mole) of p-nitrobenzyl bromide and 14 ml of triethylamine. The solution was boiled for 6 h under reflux, cooled to 20° C, and

TABLE 1. Physicochemical Properties of Compounds (1)-(3)

Compound	Mol. wt.	Mp, °C	$[\alpha]_D^{20}$, deg (in ethanol)	Stability constants of the complexes (K, kcal/mole)		Free energies of complex formation ($-\Delta F = RT \log K$, kcal/mole)	
				Na^+	K^+	Na^+	K^+
Antamanide (1)	1146	172–174	–168 (c 0,5)	2800	270	4,7	3,3
Retroantamanide (2)	1146	176–177	–176 (c 0,08)	400	100	3,6	2,7
Perhydroantamanide (3)	1170	164–166	– 86 (c 0,5)	200	<50	4,5	<2,3

*Measured by the conductometric method [4].

filtered, and the filtrate was washed, dried, and evaporated. This gave a chromatographically homogenous product in the form of a yellow oil. Yield 33.4 g (87%), $[\alpha]_D$ – 20.4°.

Hydrobromide of Proline p-Nitrobenzyl Ester (6). A solution of 19.2 g (0.05 mole) of (5) in 30 ml of glacial acetic acid was mixed with a 35% solution of HBr in glacial acetic acid and after 0.5 h the mixture was evaporated at 35° C and the residue was treated with 200 ml of absolute ether. The precipitate that deposited was filtered off and crystallized from absolute ethanol. Yield 13.4 g (81%), mp 170° C, $[\alpha]_D$ – 28.7°.

Benzyloxycarbonylprolylproline p-Nitrobenzyl Ester (8). A solution of 21.6 g (0.11 mole) of DCCDI in 200 ml of methylene chloride was added at 0° C to a solution of 33.1 g (0.1 mole) of the hydrobromide of (6), 14 ml of triethylamine, and 24.9 g (0.1 mole) of benzyloxycarbonylproline (7) in 400 ml of methylene chloride. After 12 h (20° C), the precipitate of N,N'-dicyclohexylurea was filtered off, the filtrate was evaporated, the residue was dissolved in 300 ml of ethyl acetate, and the solution was washed, dried, and evaporated. The residue was crystallized from a small amount of ethyl acetate. Yield 31.8 g (66%), mp 100-101° C, $[\alpha]_D$ – 83.3°.

tert-Butoxycarbonylphenylalanylprolylproline p-Nitrobenzyl Ester (11). To a solution of 26.5 g (0.1 mole) of tert-butyloxycarboylphenylalanine (9) and 14 ml of triethylamine in 300 ml of absolute tetrahydrofuran at –15° C were added 13.5 ml (0.5 mole) of isobutyl chloroformate and, after 15 min (–10° C), a solution of the hydrobromide of prolylproline p-nitrobenzyl ester (10) obtained from 52.9 g (0.11 mole) of (8) by treatment with a 35% solution of HBr in glacial acetic acid in 100 ml of methylene chloride containing 14 ml (0.1 mole) of triethylamine. The mixture was stirred at 20° C for 12 h and evaporated. The residue was dissolved in 300 ml of ethyl acetate, and the solution was washed, dried, and reevaporated. This gave 49.9 g (84%) of an oil with $[\alpha]_D$ – 109°.

tert-Butyloxycarbonylvalylprolylproline p-nitrobenzyl ester (13) was obtained under the conditions of the preceding experiment from 21.7 g (0.1 mole) of tert-butyloxycarbonylvaline (12) and 42.8 g (0.1 mole) of (10) in the form of a yellow oil, $[\alpha]_D$ – 103°. Yield 44.0 g (81%).

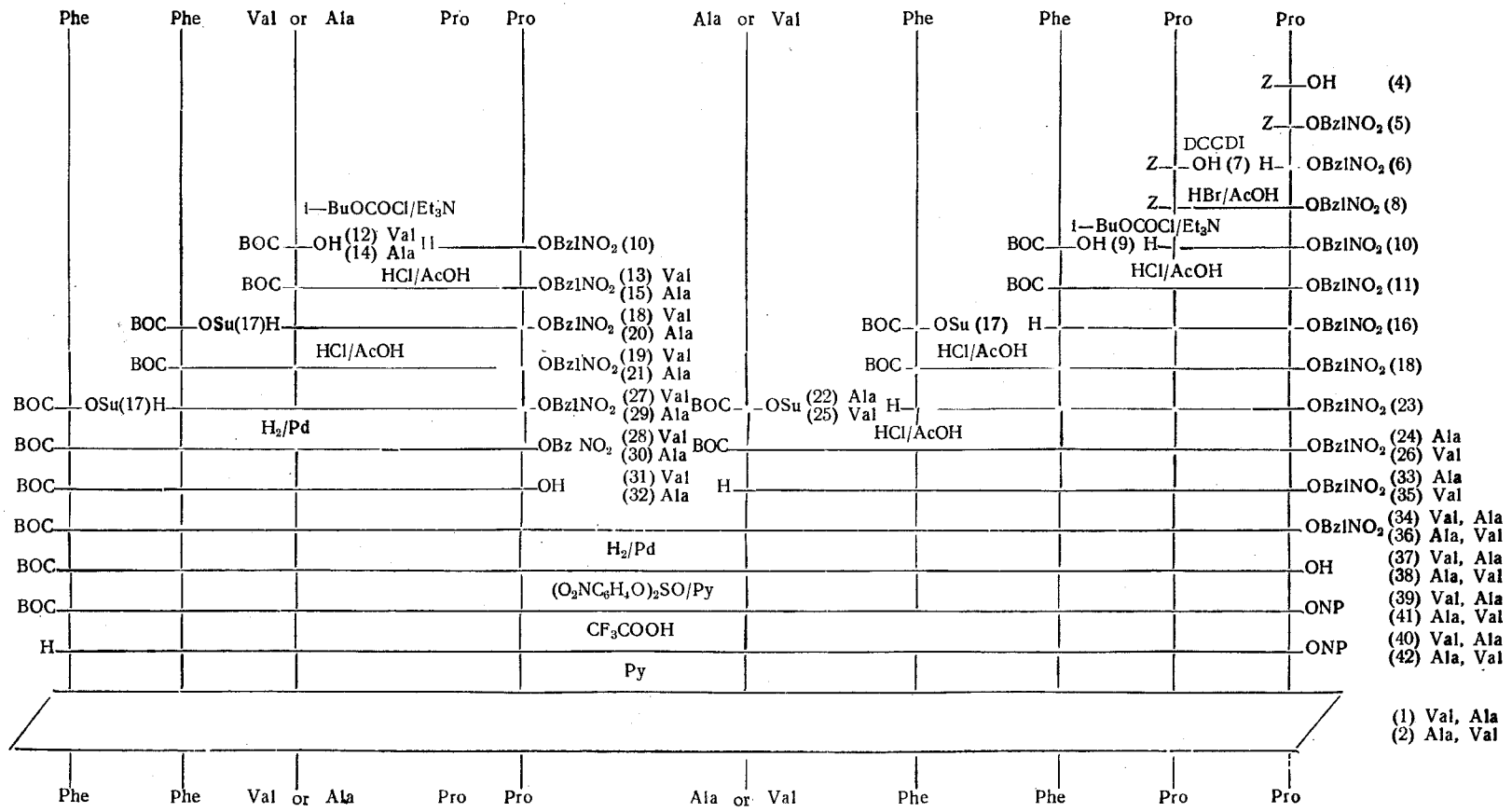
tert-Butoxycarbonylalanylprolylproline p-nitrobenzyl ester (15) was obtained similarly to (11) from 18.9 g (0.1 mole) of tert-butoxycarbonylalanine (14) and 42.8 g (0.1 mole) of (10). Yield 43.0 g (83%). Yellow oil, $[\alpha]_D$ – 125°.

tert-Butyloxycarbonyldiphenylalanylprolylproline p-Nitrobenzyl Ester (18). With ice cooling, a solution of 65.4 g (0.11 mole) of (11) in 130 ml of absolute dioxane was saturated with dry HCl for 30 min. Then it was left for another 40 min and was evaporated. The residue was treated with 300 ml of dry ether, and the hydrochloride of phenylalanylprolylproline p-nitrobenzyl ester (16) was filtered off. After drying in vacuum over P_2O_5 and KOH, the yield of substance was 57.1 g (98%). A mixture of 53.05 g (0.1 mole) of the resulting hydrochloride, 36.2 g (0.1 mole) of the N-hydroxysuccinimide ester of tert-butoxycarbonylphenylalanine (17), 14 ml of triethylamine, and 500 ml of absolute dioxane was stirred at 20° C for 70 h. The solvent was evaporated off, the residue was dissolved in 350 ml of chloroform, and the solution was washed, dried, and evaporated. After treatment with ether, 66 g (89%) of an amorphous powder with $[\alpha]_D$ – 86.5° was obtained.

tert-Butoxycarbonylphenylalanylvalylprolylproline p-nitrobenzyl ester (19) was prepared under the conditions of the preceding experiment from 36.2 g (0.1 mole) of the N-hydroxysuccinimide ester (17) and 48.2 g (0.1 mole) of the hydrochloride of (18) obtained from 60 g (0.11 mole) of the tripeptide (13). Amorphous powder, yield 59 g (85%), $[\alpha]_D$ – 75°.

tert-Butoxycarbonylphenylalanylalanylprolylproline p-nitrobenzyl ester (21) was prepared similarly to (18) from 36.2 g (0.1 mole) of (17) and 45.5 g (0.1 mole) of the hydrochloride of (20) obtained from 57.0 g (0.11 mole) of the tripeptide (15). Yield 59.9 g (90%); $[\alpha]_D$ – 87.3°.

tert-Butoxycarbonylalanyldiphenylalanylprolylproline p-nitrobenzyl ester (24) was synthesized similarly to (18) from 28.6 g (0.1 mole) of the N-hydroxysuccinimide ester of tert-butoxycarbonylalanine (22) and 67.8 g (0.1 mole) of the hydrochloride of (23) obtained from 81.6 g (0.11 mole) of the tetrapeptide (18). The yield of amorphous powder was 73.1 g (90%); $[\alpha]_D$ – 89.5°.



Note. Z= Benzyloxycarbonyl; BOC= t-Butyloxycarbonyl.

tert-Butoxycarbonylvalyldiphenylalanylprolylproline p-nitrobenzyl ester (26) was obtained similarly to (18) from 31.4 g (0.1 mole) of the N-hydroxysuccinimide ester of tert-butoxycarbonylvaline (25) and 67.8 g (0.1 mole) of the hydrochloride of (23), obtained from 74.6 g (0.11 mole) of the tetrapeptide (18). Yield 67.2 g (80%); $[\alpha]_D - 90°$.

tert-Butoxycarbonyldiphenylalanylvalylprolylproline p-nitrobenzyl ester (18) was synthesized like (18) from 36.2 g (0.1 mole) of the N-hydroxysuccinimide ester (17) and 62.8 g (0.1 mole) of the hydrochloride of (27) obtained from 76.1 g (0.11 mole) of the tetrapeptide (19). Yield 72.2 g (86%), $[\alpha]_D - 89.5°$.

tert-Butoxycarbonyldiphenylalanylprolylproline p-nitrobenzyl ester (30) was obtained similarly to (18) from 36.2 g (0.1 mole) of the N-hydroxysuccinimide ester (17) and 60.3 g (0.1 (mole of the hydrochloride of (29) obtained from 73.1 g (0.11 mole) of the tetrapeptide (21). Yield 69.0 g (85%); $[\alpha]_D - 87.5°$.

tert-Butoxycarbonyldiphenylalanylvalylprolylproline (31). A solution of 42.0 g (0.05 mole) of the p-nitrobenzyl ester (28) in 250 ml of absolute dioxane and 2 ml of glacial acetic acid was hydrogenated over 5 g of palladium oxide for 18 h. After the end of hydrogenation (monitored by thin-layer chromatography), the filtrate was evaporated, the residue was dissolved in dioxane–water (3 : 1) and, to eliminate p-toluidine, the solution was passed through a column of Dowex 50 × 2(H^+ form). The eluate was evaporated, the residue was dissolved in a small amount of ethanol, and the product was precipitated with ether in the form of an amorphous powder. Yield 30.3 g (87%), $[\alpha]_D - 92°$.

tert-Butoxycarbonyldiphenylalanylalanylprolylproline (32) was obtained under the conditions of the preceding experiment from 40.6 g (0.05 mole) of the pentapeptide (30) in the form of an amorphous powder with a yield of 27.1 g (80%), $[\alpha]_D - 95°$.

tert-Butoxycarbonyldiphenylalanylvalyldiprolylalanyldiphenylalanylprolylproline p-Nitrobenzyl Ester (34). A solution of 122.7 g (0.028 mole) of (24) in 180 ml of glacial acetic acid was saturated with dry HCl at 20° C for 3 h. Then the solution was evaporated and the residue was treated several times with benzene followed by its evaporation. The hydrochloride of the pentapeptide (33) was washed with dry ether, dried in vacuum over KOH, and dissolved at 0° C in 200 ml of dry methylene chloride containing 3.5 ml (0.025 mole) of triethylamine. The solution was treated with 17.6 g (0.25 mole) of the tert-butoxycarbonyl peptide (31) and 6.2 g (0.03 mole) of DCCDI. The reaction mixture was stirred at 0°C for 1 h and at 20°C for 48 h. The precipitate was filtered off, the filtrate was evaporated, and the residue was dissolved in ethyl acetate and precipitated with petroleum ether. The resulting powder was dissolved in methanol and chromatographed on a column of Sephadex LH-20 (200 × 5 cm). The low-molecular-weight fraction was evaporated and the residue was reprecipitated from ethyl acetate with petroleum ether. This gave the decapeptide (34) in the form of an amorphous powder with a yield of 21.0 g (60%), $[\alpha]_D - 125°$.

The p-nitrobenzyl ester of tert-butoxycarbonyldiphenylalanylalanyldiprolylvalyldiphenylalanylprolyl-proline (36) was isolated in a similar manner to the preceding experiment, starting with 16.9 g (0.025 mole) of the acid (32) and 22.8 g (0.025 mole) of the hydrochloride of the pentapeptide p-nitrobenzyl ester (35), obtained from 23.5 g (0.028 mole) of (26). Amorphous powder, yield 27.2 g (78%), $[\alpha]_D - 106.2°$.

tert-Butoxycarbonyldiphenylalanylvalyldiprolylalanyldiphenylalanylprolylproline (37) was obtained in the same way as (31) from 14.0 g (0.01) mole) of the p-nitrobenzyl ester (34) in the form of an amorphous powder with a yield of 9.9 g (78%), $[\alpha]_D - 122°$.

tert-Butoxycarbonyldiphenylalanylalanyldiprolylvalyldiphenylalanylprolylproline (38) was obtained in a similar manner to (31) from 14 g (0.01 mole) of the p-nitrobenzyl ester (36) in the form of an amorphous powder with a yield of 11.3 g (81%), $[\alpha]_D - 111°$.

cyclo-Diprolylvalyldiphenylalanyldiprolyldiphenylalanylalanine (1) (Antamanide). To a solution of 6.32 g (0.005 mole) of (37) in 25 ml of dry pyridine was added 16.2 g (0.05 mole) of di-p-nitrophenyl sulfite, and after 48 h (20°C), the pyridine was distilled off in vacuum. The residue was washed with ether and dried in vacuum over P_2O_5. The resulting p-nitrophenyl ester of the tert-butoxycarbonyldecapeptide (39) was dissolved in 30 ml of anhydrous trifluoroacetic acid, and after 2 h the solution was evaporated at 35° C. The residue was treated with 200 ml of absolute ether, and the trifluoroacetate of (40) was filtered off and dried over KOH in vacuum. Then it was dissolved in a mixture of 200 ml of absolute dioxane, 10 ml of dry

dimethylformamide, and 0.5 ml of glacial acetic acid and the solution was added by drops over 6 h to 700 ml of absolute pyridine at 65° C. After 72 h (65° C), the solution was evaporated to dryness and the residue was washed with ether, dissolved in methanol, and chromatographed on Sephadex LH-20. The eluate containing the cyclopeptide (monitoring by thin-layer chromatography on silica gel) was evaporated and the residue was dissolved in 10 ml of chloroform–benzene (1 : 1) and deposited on a column of neutral alumina (activity grade II; 20 × 2 cm). The column was eluted with 500 ml of chloroform–benzene (1 : 1) and 600 ml of ethyl acetate. The ethyl acetate solution was evaporated and the residue was dissolved in 10 ml of tetrahydrofuran, deposited on a column of silica (20 × 2 cm), and eluted with tetrahydrofuran. The yield of antamanide after recrystallization from aqueous acetone and drying over P_2O_5 in high vacuum was 0.98 g (17%).

cyclo-Diprolylalanyldiphenylalanyldiprolylphenylalanylvaline (2) (Retroantamanide) was obtained under the conditions of the preceding experiment from 6.32 g (0.005 mole) of the decapeptide (38). After recrystallization from methyl ethyl ketone and drying, the yield of substance was 1.2 g (21%).

cyclo-Diprolylvalyldicyclohexylalanyldiprolyldicyclohexylalanylalanine (3) (Perhydroantamanide). Antamanide (1) (100 mg) was hydrogenated in 10 ml of ethanol–acetic acid (1 : 1) over 0.1 g of platinum oxide for 72 h. After cessation of the absorption of hydrogen, the catalyst was filtered off, the solvent was distilled off, and the residue was dissolved in 2 ml of tetrahydrofuran and chromatographed on 5 g of neutral alumina (activity grade II). The tetrahydrofuran eluate (50 ml) was evaporated and the residue was dissolved in a small amount of ether and precipitated with 50 ml of acetone. The yield after crystallization from cyclohexane was 58 mg (57%).

SUMMARY

The synthesis of antamanide, retroantamanide, and perhydroantamanide has been effected. It has been shown that the inversion of the direction of acylation – the equilibrium interchange of the valine and alanine residues (retroantamanide) – lowers the stability of the complexes with sodium and potassium in ethanolic solutions, while the replacement of the phenyl groups by cyclohexyl groups has practically no effect on complex formation.

LITERATURE CITED

1. T. Wieland, G. Lüben, H. Ottenheym, J. Faesel, J. X. de Vries, W. Konz, A. Prox, and J. Schmigt, Angew. Chem., 80, 209 (1968).
2. T. Wieland, G. Lüben, H. Ottenheym, and H. Schiefer, Ann. Chem., 722, 173 (1969).
3. T. Wieland, H. Faulstich, W. Burgermeister, W. Otting, W. Möhle, M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, and G. G. Malenkov, FEBS Let., 9, 89 (1970).
4. M. M. Shemyakin, Yu. A. Ovchinnikov, V. T. Ivanov, V. K. Antonov, E. I. Vinogradova, A. M. Shkrob, G. G. Malenkov, A. V. Evstratov, I. A. Laine, E. I. Melnik, and I. D. Ryabova, J. Membrane Biol., 1, 402 (1969).
5. L. A. R. Pioda, U. A. Wachter, R. E. Dohner, and W. Simon, Helv. Chim. Acta, 50, 1373 (1967).
6. W. König and R. Geiger, Ann. Chem., 727, 125 (1969).
7. T. Wieland, J. Faesel, and W. Konz, Ann. Chem., 722, 197 (1969).
8. T. Wieland, J. Faesel, and W. Konz, Proc. 9th European Peptide Symposium, Ed. E. Bricas, North-Holland Publishing Company, Amsterdam (1968), p. 243.
9. A. Prox, J. Schmidt, and H. Ottenheym, Ann. Chem., 722, 179 (1969).