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BIS(N-ADAMANTYLPIPERAZIDES) OF ALIPHATIC DICARBOXYLIC ACIDS AND THEIR PHARMACOLOGIC ACTIVITY

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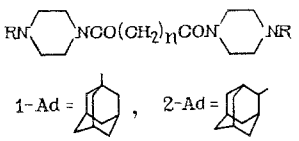
In recent years interest has arisen in adamantane derivatives as biologically active substances. The introduction of adamantyl substituents into the molecule of a physiologically active compound in several cases markedly alters the distribution and absorption of the preparation, the conditions of its interaction with receptors, and its ability to pass through biological membranes [1-3].

We have continued our work on the correlation between the biological activity and chemical structure of adamantane derivatives with the synthesis of bis(N-adamantylpiperazides) of succinic and sebacic acids and an examination of their immunotropic activity (compounds I-IV); we also evaluated the curariform activity of their ammonium salts.



I: $n = 2$, R = 1-Ad;
II: $n = 2$, R = 2-Ad;
III: $n = 8$, R = 1-Ad;
IV: $n = 8$, R = 2-Ad;
V = I·2CH_3I;
VI = III·2CH_3I;
VII = II·2$CH_3C_6H_4SO_4CH_3$;
VIII = IV·2$CH_3C_6H_4SO_3CH_3$.

We prepared the adamantylpiperazides by refluxing 1- or 2-adamantylpiperazine hydrochloride in an inert solvent with the appropriate dicarboxylic acid dichlorides. We also synthesized these compounds by another method, interaction of 1- or 2-adamantylpiperazine with the dicarboxylic acid in the presence of dicyclohexylcarbodiimide, carrying out the reaction with an equimolar ratio of the components and a slight excess of dicyclohexylcarbodiimide. We prepared the dimethiodides V and VI by refluxing the base with excess methyl iodide in methanol. We were unable to prepare the methiodides of the compounds containing the 2-adamantyl radical; the starting compounds were recovered unchanged when the bases were heated in methanol with excess methyl iodide. We converted amides II and IV to the bisquaternary ammonium salts VII and VIII by heating with methyl p-toluenesulfonate.

EXPERIMENTAL PHARMACOLOGY

We evaluated the immunotropic activity of the 1- and 2-adamantylpiperazides of succinic and sebacic acids from the antibody titers (hemolysins and hemagglutinins) in the serum of mice immunized with ram erythrocytes, the number of antigen-binding or plaque-forming cells (PFC) in the spleens of mice immunized with ram erythrocytes, and also from the time of rejection of skin allotransplants in mice.

We used male mice tetrahybrids A/He (A)×Balb/c(c)×C_{57}Bl/6(B_6)×CC_{57}W(W) weighing 18-20 g. The animals were immunized by intraperitoneal administration of a standard dose of washed ram erythrocytes (0.5 ml of a 5% suspension). Water-insoluble test compounds were

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TABLE 1. Effect of Succinic and Sebacic Acid bis[N-adamantylpiperazides] on Antibody Titers in Mice Immunized with Ram Erythrocytes

Compound	Dose mg/kg	Protocol, days of administration	Antibody titer, ID		Death of the mice
			hemolysins	hemagglutinins	
I	0,5	−3, −2, −1	0,9	0,8	0/5
	5,0	—	0,7	0,8	0/5
	50,0	—	0,8	0,8	0/5
	0,5	−1,0,+1	0,9	0,8	0/5
	5,0	—	1,0	0,9	0/5
	50,0	—	0,9	0,8	0/5
	0,5	+1, +2, +3, +4	1,0	0,9	0/5
	5,0	—	1,0	1,0	0/5
	50,0	—	1,0	0,9	0/5
II·2HCl	0,5	−1,0,+1	0,3	0,3	0/5
	5,0	—	0,6	0,7	0/5
	50,0	—	1,0	1,0	1/5
III	0,5	−1,0, +1	0,3	0,3	0/5
	5,0	—	0,3	0,3	0/5
	50,0	—	0,3	0,3	0/5
	1,5	−1	0,3	0,2	0/5
	1,5	0	0,3	0,5	0/5
	1,5	+1	0,6	0,7	0/5
	1,5	+2	1,0	0,9	0/5
IV	0,5	−1,0, +1	1,0	0,9	0/5
	5,0	—	0,9	0,8	0/5
	50,0	—	0,8	0,8	0/5
	0,5	+1, +2, +3, +4	0,8	0,7	0/5
	5,0	—	0,8	0,7	0/5
	50,0	—	0,7	0,8	0/5

dissolved in a Tween—alcohol—water mixture and water-soluble compounds in 0.95% sodium chloride solution. Compounds were administered intraperitoneally in different doses according to the protocol shown in Table 1. The control animals received the appropriate volume of solvent in the same way. On the fifth day after immunization the animals were killed and blood was collected from each mouse in a separate tube. The antibody titers (hemolysins and hemagglutinins were determined the serum with a Takachi microtitrator. We expressed the results of the titration as $\log_2$ of the reciprocal antibody titers and calculated the index of effectiveness equal to the average value of $-\log_2$ of the antibody titers in the test (the average value of $-\log_2$ of the antibody titers in the control). An index of effectiveness of 0.7 and lower demonstrated the existence of a significant immunosuppressant effect [4]. In addition to observation of the immunological status of the mice we also monitored the toxicity of the compounds (Table 1). The day of immunization was symbolically designated as day 0 and the days of administration of the compounds before and after antigen challenge were indicated with the sign (−) or (+), respectively. Compounds that displayed marked immunotropic activity were tested further. Here in analogous tests on mice immunized with ram erythrocytes we determined the dependence of the activity of the compounds not only on dose but also on the time of administration with respect to the time of antigen challenge, since this factor is important in the evaluation of immunotropic compounds. We also ascertained the effect of the test compounds on the number of PFC's in the spleens of mice immunized with ram erythrocytes by Zaalberg's method [5]. We evaluated the state of cell immunity from the reaction of rejection of skin allotransplants in mice of lines CBA and $C_{57}Bl$, which differ at the strong (H-2) locus of histocompatibility. We evaluated the results statistically using Student's t test.

These experiments revealed that two of the test compounds (II and III) have marked immunosuppressant activity. Compound III considerably reduced the circulating antibody titers over a wide dose range (from 0.5 to 50 mg/kg) (Table 1). We examined the dependence of its effect on the relation of the time of administration to the time of immunization. We found that in an extremely low dose (1.5 mg/kg) this compound caused strong immunosuppression (Fig. 1). It was much less effective when used after immunization. Compound II had the ability to reduce not only the antibody titers but also the number of PFC's in the spleens of mice immunized with ram erythrocytes (Fig. 2). Thus, if on the fifth day after immunization the number of PFC's per 10^3 nucleus-containing cells in the control was 22 (27-17), after threefold administration (−1, 0, +1 days) of compound II in a dose of 0.5

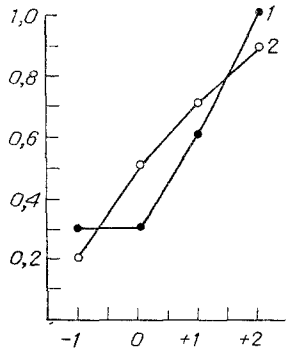

Fig. 1

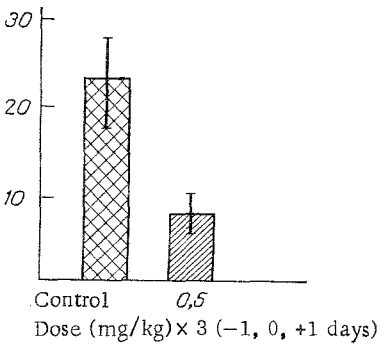

Fig. 2

Fig. 1. Dependence of the immunosuppressant effect of compound II (1.5 mg/kg) on the time of administration (in days) in relation to the time of immunization with ram erythrocytes ($5 \cdot 10^8$ cells); 1) hemolysins; 2) hemagglutinins.

Fig. 2. Effect of compound II on the number of PFC's in spleens of mice immunized with ram erythrocytes ($5 \cdot 10^8$ cells); abscissa axis is the dose (mg/kg) ×3 (−1, 0, +1); ordinate axis is the number of PFC/10^3.

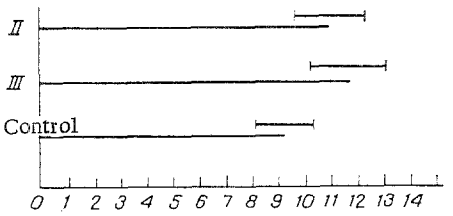

Fig. 3. Effect of compounds II and III on the viability of skin allotransplants in mice; abscissa axis is the time after grafting (in days). Preparations were administered daily in a dose of 0.5 mg/kg, starting from the time of grafting and continuing until rejection of the skin graft.

mg/kg the number of PFC's was 8 (10-6) and the activity of the PFC population was strongly suppressed. Compound III was capable of slightly prolonging the time before rejection of skin grafts on daily administration in a dose of 0.5 mg/kg starting from the time of grafting and continuing until rejection of the allotransplant (Fig. 3).

Thus we have shown that of the four test adamantane derivatives containing the piperazine ring, two (II and III) had immunosuppressant activity in doses that did not cause death of the animals.

These compounds differ markedly from known cytostat immunosuppressants (cyclophosphane, 6-mercaptopurine, etc.), which are typified by a proportional dependence of immunosuppressant activity on dose and a correlation of this activity with toxicity. The immunosuppressant agents described here, which contain the adamantyl radical, do not follow this dependence. Their immunosuppressant activity develops in doses that do not cause toxic effects.

We also examined the curariform activity of the bisquaternary ammonium salts V and VI. The tests were carried out in cats anesthetized by chloralose (60 mg/kg) with urethane (0.4 g/kg). The peripheral section of the sciatic nerve was stimulated by supramaximal square pulses (0.5 msec). We recorded the contractions of the gastrocnemius muscle in the semiisometric regime. All compounds were administered intravenously.

The blocking activity of the compounds depends to a considerable extent on the interion separation. Thus, when n = 2, i.e. with 10 atoms separating the quaternary nitrogen atoms, compound V blocks neuromuscular transmission in a dose of 60-70 μg/kg. On increase in n to 8 (compound VI), i.e.,with 16 atoms between the cation centers, the myoparalytic activity is roughly 15 times lower. The compound blocked neuromuscular transmission in a dose of the order of 1 mg/kg.

In terms of their mechanism of action the test preparations are antidepolarizing myorelaxants.

EXPERIMENTAL CHEMISTRY

The preparation of N-(1- or 2-adamantyl)piperazines has been described earlier [6].

Succinic Acid Bis[N-(1-adamantyl)piperazide] (I). To a mixture of N-(1-adamantyl)-piperazine (1.7 g) and succinic acid (0.5 g) in methylene chloride (40 ml) was added dropwise with stirring a solution of dicyclohexylcarbodiimide (2.3 g) in dichloroethane (20 ml). The reaction mixture was stirred for 5 h and then filtered. The filtrate was evaporated under vacuum to dryness and the residue was triturated with diethyl ether and filtered. The precipitate was dissolved in water acidified with hydrochloric acid until weakly acidic. The acidic solution was saturated with sodium bicarbonate and the amine was extracted with benzene. The benzene extract was evaporated to dryness to give compound I (1.2 g, 60.3%), mp 252-254°C (from methanol). Found, %: C 73.46; H 9.60; N 10.47. $C_{32}H_{50}N_4O_2$. Calculated, %: C 73.52; H 9.63; N 10.72. Dihydrochloride, mp 308-310°C. Found, %: Cl 12.05. $C_{32}H_{52}Cl_2N_4O_2$. Calculated, %: Cl 11.84.

Dimethiodide (V). To a solution of I (0.5 g) in methyl alcohol (10 ml) was added methyl iodide (2 ml). The reaction mixture was refluxed for 20 h and then poured into dry ether (50 ml). The precipitate was filtered off and recrystallized from absolute ethanol to give compound V (0.4 g, 52%), mp 230-231°C. Found, %: I 31.69. $C_{34}H_{56}I_2N_4O_2$. Calculated, %: I 31.46.

Succinic Acid Bis[N-(2-adamantyl)piperazide] (II). A mixture of N-(2-adamantyl)-piperazine hydrochloride (2 g), succinyl dichloride (0.55 g), and triethylamine (7 ml) in chloroform (20 ml) was kept at 20°C for 12 h and then refluxed for 7 h. Ether saturated with hydrogen bromide was added to the cooled mixture, which was then extracted with water. The aqueous layer was saturated with sodium bicarbonate. The precipitate was filtered off, washed with water, and dissolved in benzene; after drying over magnesium sulfate the solvent was stripped off to give the diamide (3.3 g, 70%), mp 215-216°C (from methanol). Found, %: C 73.16; H 9.56; N 10.88. $C_{32}H_{50}N_4O_2$. Calculated, %: C 73.52; H 9.65; N 10.72. Dihydrochloride (II), mp 295-298.5°C (from ethanol). Found, %: Cl 7.98. $C_{32}H_{52}Cl_2N_4O_2$. Calculated, %: Cl 8.41.

Sebacic Acid Bis[N-(2-adamantyl)piperazide] (IV). By the same method as the preceding synthesis a mixture of N-(2-adamantyl)piperazine hydrochloride (2 g), sebacoyl dichloride (0.85 g), chloroform (20 ml), and triethylamine (7 ml) after standing for 16 h gave base IV (1.2 g, 58.4%), mp 127.5-129°C. Found, %: C 74.90; H 10.31; N 9.33. $C_{38}H_{62}N_4O_2$. Calculated, %: C 75.20; H 10.29; N 9.23. Dihydrochloride of (IV), mp 267-270°C. Found, %: Cl 10.41. $C_{38}H_{64}Cl_2N_4O_2$. Calculated, %: Cl 10.43.

Sebacic acid bis[N-(1-adamantyl)piperazide] (III) was prepared in the same way, yield 57%, mp 141-143°C. Found, %: C 75.41; H 10.23; N 9.62. $C_{38}H_{62}N_4O_2$. Calculated, %: C 75.20; H 10.29; N 9.23. Dihydrochloride of III, mp 293-297°C (from ethanol). Found, %: Cl 10.51. $C_{38}H_{64}Cl_2N_4O_2$. Calculated, %: Cl 10.43.

Dimethiodide (VI). A solution of the base of bis-piperazide III (1 g) in methanol (10 ml) was refluxed with methyl iodide (2 ml) for 20 h. The precipitate was filtered off and recrystallized from methanol to give dimethiodide VI (1.2 g, 81.5%), mp 224-226°C. Found, %: I 28.43. $C_{40}H_{68}I_2N_4O_2$. Calculated, %: I 28.49.

Sebacic Acid [N-(2-adamantyl)piperazide] Dimethyl-p-toluenesulfonate (VIII). A mixture of sebacic acid bis[N-(2-adamantyl)piperazide] (0.2 g) and methyl p-toluenesulfonate (0.18 g) was heated at 150°C for 1.5 h. The cooled mass was triturated with dry ether and the precipitate was filtered off and recrystallized from aqueous acetone to give salt VIII (0.2 g, 62%), mp 222-225°C. Found, %: S 6.91. $C_{54}H_{82}N_4O_8S_2$. Calculated, %: S 6.59.

Succinic acid bis[N-(2-adamantyl)piperazide] dimethyl-p-toluenesulfonate VII was prepared in the same way, yield 45%, mp 240-242°C (with decomposition). Found %: N 6.14; S 6.87. $C_{48}H_{70}N_4O_8S_2$. Calculated, %: N 6.25; S 7.16.

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SYNTHESIS AND PHARMACOLOGIC ACTIVITY OF AMIDES OF γ-AMINOBUTYRIC ACID

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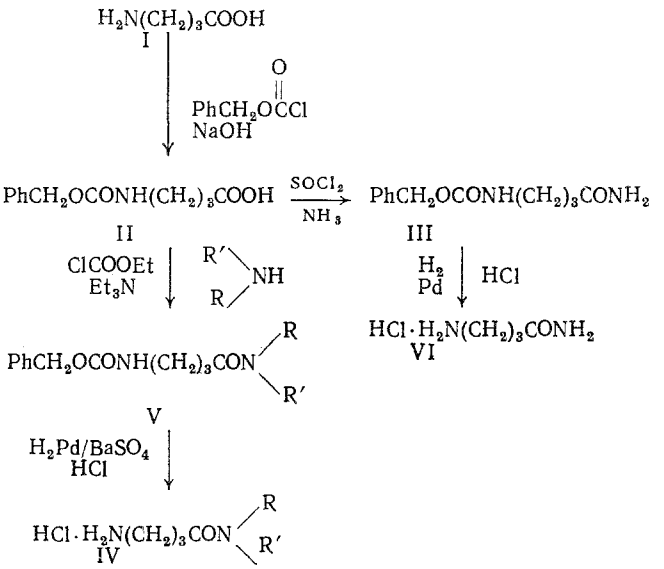
Earlier we had prepared a series of esters of γ-aminobutyric acid (I) and examined their neurotropic activity [1]. We showed that esterification of I with alcohols containing lipophilic radicals forms esters with marked neurotropic activity and the ability to increase the life span of animals under conditions of hypoxia; the most active of these compounds was the cetyl ester of I. We set out to make a further study of the dependence of the neurotropic activity of derivatives of I on structure with the synthesis and pharmacologic study of a series of amides of I, including some with lipophilic radicals on the nitrogen.

Synthesis of the amides by the mixed anhydride method required firstly blocking of the amino group of I, which we did with the N-carbobenzoxy protecting group [2]



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