SYNTHESIS AND NEUROTROPIC ACTIVITY OF A NUMBER OF N-[Di(*n*-PROPYL)ACETYL]LACTAMS

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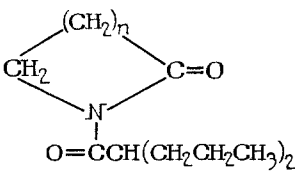
Di(*n*-propyl)acetic (valproic) acid and its derivatives are known to have anticonvulsive [11] and antihypoxic [2] activities. The sodium and calcium salts of valproic acid are among the most active anticonvulsives presently available [12]. The great variety of states seen in clinical practice, especially those involving disturbances of cognitive and memory functions in epilepsy, requires the spectrum of the neuropharmacological properties of anticonvulsive agents to be widened. Many of the standard anticonvulsants (benzodiazepines, barbiturates) worsen intellectual and memory functions. The standard nootropic agents (piracetam, aniracetam) lack anticonvulsive activity. This gives a basis for searching for anticonvulsive compounds which do not have adverse effects on memory and, even better, which might improve memory.

We report here the synthesis and pharmacological investigation of a number of N-(valproyl)lactams [1, 3], which are of interest because, as new derivatives of valproic acid, they are also acyllactams. We might therefore expect these compounds to have new pharmacological properties which may be useful in the treatment of nervous diseases.

N-di(*n*-propyl)acyllactams (I-III) were synthesized by the interaction of pyrrolidone, piperidone, or hexahydroazepinone with di(*n*-propyl)acetyl chloride.



I-III

$n = 2$ (I), 3(II), 4(III)

Acylation was carried out in benzene or toluene, and preferably in the presence of HCl acceptors, i.e., potassium carbonate of Et_3N. Acylation of lactams in the absence of acceptors required more stringent conditions, i.e., prolonged boiling in toluene. Lactams I-III were colorless oily liquids, soluble in organic solvents and oils, and insoluble in water. The structures of these compounds were confirmed by elemental analysis and IR spectroscopy. The IR spectra lacked absorption peaks due to the presence of a secondary amide group, and contained absorption peaks due to the carbonyl groups of the N-di(*n*-propyl)acetyl and lactam fragments. It should be noted that in the case of lactams II and III, the absorption peaks of the carbonyl fragments of the two groups coincided, and were in the ranges 1690-1969 and 1990-1704 cm^{-1}, respectively, while the absorption peaks of the carbonyl groups of the chain (1691 cm^{-1}) and the pyrrolidone ring (1738 cm^{-1}) were separated.

The neurotropic activity of N-[di(*n*-propyl)acetyl]lactams was studied by comparing them with the known anticonvulsant calcium valproate and the standard nootropic agent piracetam.

The results presented in Table 1 show that all agents investigated had anticonvulsive properties, and that the N-(valproyl)lactams had greater activity than calcium valproate. The most active in the maximum electric shock text (MEST) were

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TABLE 1. Comparison of the Neurotropic Activities of Piracetam, Calcium Valproate, and N-Acyllactams

Compound	Spontaneous movement activity*	Rotating bar	MEST (ED_{50})	No effect at up to 8000 mg/kg	Acetaldehyde-induced convulsions**	One-dayto-xicity, 50%	GABA content	
							μmoles/g of tissue	% of control
Isotonic saline (control)				Convulsions 98%; lethal outcome 58%	1,5 points		1,97±0,10	100±5
Piracetam	No effect up to 1000 mg/kg	2840 (2469—3266)	No effect up to 8000 mg/kg	No protective effect up to 1000 mg/kg	1,1 points (500 mg/kg)	8000 mg/kg	1,62±0,18 (700 mg/kg)	82 %
Calcium valproate	100 mg/kg	390 (336—452)	310 (294—350)	Convulsions 87%; lethal outcome 20% (120 mg/kg)	0,5 points (100 kg/mg)	860 mg/kg	2,25±0,24 (200 mg/kg)***	114±12
I	150 mg/kg	210 (189—233)	175 (154—197)	Convulsions 50% lethan outcome 0% (120 mg/kg)	0,5 points (75 kg/mg)	750 mg/kg	2,43±0,29 (250 mg/kg), $p \leq 0,05$	123±12
II	100 mg/kg	180 (152—214)	275 (241—313)	Convulsions 50%; lethal outcome 0% (160 mg/kg)	0,8points (200mg/kg	550 mg/kg	2,54±0,12 (262 mg/kg), $p \leq 0,01$	129±6
III	100 mg/kg	205 (183—229)	158 (133—186)	Convulsions 60%; lethal outcome 0% (140 mg/kg)	0,3 points (200 mg/kg	520 mg/kg	2,40±0,18 (284 mg/kg), $p \leq 0,05$	122±9

*Dose giving a doubling (in comparison with initial) in the reduction in spontaneous movement activity in mice placed as a group of 10 animals in an Opto-Varimex multichannel detector.

**Acetaldehyde was administered i.p. to mice at a dose of 250 mg/kg, and the intensity of convulsions was measured on a 3-point scale; compounds were given 15 min before acetaldehyde, and doses are shown in brackets.

***Acyllactams were given in doses equimolar to the content of the valproate radical in the threshold dose of calcium valproate, i.e., 200 mg/kg.

compounds I and III; the ratios of the effective doses of N-acyllactams I and III to that of calcium valproate were 175:310 and 158:310 (doses in mg/kg). The higher activity of N-(valproyl)lactams as compared to that of calcium valproate is even more evident when the results are recalculated for doses equimolar to those of calcium valproate. Compounds I-III protected from bicuculline-induced convulsions at doses lower than those required for calcium valproate. Piracetam lacked anticonvulsive effects. The compounds studied here were also active against acetaldehyde-induced convulsions.

The data shown in Table 1 also show that the compounds studied here significantly weakened the convulsive effects of acetaldehyde, and were more active than piracetam. This type of result is known to be a prognostic sign for possible therapeutic effects in patients with symptoms arising from alcohol withdrawal.

Unlike piracetam, the N-acyllactams and calcium valproate gave moderate reductions in orientational-investigative behavior, at doses of 100-150 mg/kg, as shown by decreases in movement activity (see Table 1). N-(valproyl)lactams had more profound depressive effects at doses exceeding 200 mg/kg. A comparison of the doses at which these agents had protective effects in the MEST and, especially, against bicuculline-induced convulsions, and the doses inducing depriming and toxic effects, showed that the N-(valproyl)lactams, especially compound I, had some selectivity as compared to the myorelaxant. The difference between the doses at which calcium valproate induced anticonvulsant and myorelaxant effects, i.e., the therapeutic index, was significantly smaller than the differences found for the compounds of interest.

The compounds studied here increased the duration of life of animals placed in a sealed chamber with a low initial oxygen level; they reduced lethality in conditions of decreased atmospheric pressure, and they also prevented the increase of lactate and its predominance over pyruvate, which are characteristic of hypoxia ("the lactate excess") (Table 2). A comparison of the equieffective threshold doses of these compounds showed that the N-acyllactams had antihypoxic activity in the sealed chamber when used at lower doses than required for calcium valproate and piracetam. The N-acyllactams also differed qualitatively from piracetam in that they increased resistance to oxygen deficiency in hypobaric hypoxia, where piracetam was not effective. Compound I had a narrow therapeutic ratio for its antihypoxic effect.

Valproic acid derivatives, such as decapine, are known to increase brain GABA levels. The results shown in Table 1 demonstrate that when the minimal effective dose of sodium valproate is compared with equimolar (in terms of the valrpoyl moiety) doses of compounds I-III, the latter had greater activity on GABA levels. The compounds differed from calcium valproate in that when they were used at threshold doses, they produced statistically significant increases in brain GABA levels. There were no significant changes in glutamic and aspartic acid levels in the cerebral cortex.

TABLE 2. Comparison of the Activity of Valproyl Derivatives, Piracetam, and Calcium Valproate in Models of Normobaric (Sealed Chamber) and Hypobaric (Barometric Chamber) Hypoxia, and Prevention of the Accumulation of a Lactate Excess in the Brain

Compound	Mean duration of life in sealed chamber*	Content in brain (%)**		Animals surviving in barometric chamber (%)**
		lactate	pyruvate	
Isotomic saline in hypoxic conditions (control)	20 min	33	330	148
	28 min	35	228	113
Piracetam	(660 mg/kg)	(500 mg/kg)		(300 mg/kg)
Calcium valproate	32	66	123	130
	(140 mg/kg)	(320 mg/kg)		(150 mg/kg)
I	30	65	118	88
	(84 mg/kg)	(110 mg/kg)		(200 mg/kg)
II	32	64	167	114
	(100 mg/kg)	(105 mg/kg)		(210 mg/kg)
III	31	67	181	108
	(110 mg/kg)	(105 mg/kg)		(220 mg/kg)

*The chamber volume was 250 ml, and the initial oxygen content was 8% (v/v), and mice weighed 18-20 g.

**Animals were elevated to 10,500 m.

***Lactate and pyruvate levels in mice in the sealed chamber, % in relation to control (dosage with 0.9% sodium chloride, respiring normal air).

Table 3 shows the protective effects of these compounds against the amnestic actions of electric shocks and alcohol, as measured in a conditioned active escape reflex (CAER). These results show that compound II, like piracetam and, to a lesser extent calcium valproate (at low doses), gave antiamnestic effects, increasing the number of conditioned reflex actions which had been reduced by amnestic treatments. Compounds I and III had no activity in this test.

N-acyllactams and calcium valproate did not reduce post-convulsive retrograde amnesia in a conditional passive escape reaction test.

Of the N-acyllactams, only compound II had activating effects in a forced swimming test, reducing the duration of immobilization by 13.4% ($p \leq 0.05$).

It is of interest to compare this result with data published by Schmidt [10], who showed that both antidepressants and substances with nootropic activity were effective in the forced swimming test developed by Porsolt [9].

Thus, these compounds had anticonvulsive activity greater than that of calcium valproate. The intensity of this effect was significantly greater than that of piracetam, as was the intensity of the antihypoxic effect. None of the compounds of interest increased the damaging effect of electric shock and ethanol on learning and memory, and compound II actually weakened their amnestic effects, and was thus comparable to piracetam. This leads to the conclusion that compound II may have nootropic activity. This combination of properties is of interest in relation to the requirement, mentioned above, for developing substances which normalize cognitive functions in patients with convulsive syndromes.

The presence of qualitatively new pharmacological properties in N-(valproyl)lactams may make them interesting as subjects of further study.

EXPERIMENTAL (CHEMICAL)

N-[di(*n*-propyl)acetyl]pyrrolidone (I). Compound I was prepared by mixing a solution of 17 g (0.2 mole) of pyrrolidone-2 in 200 ml of anhydrous benzene and a solution of 32.5 g (0.2 mole) of the chloroanhydride of di(*n*-propyl)acetic acid in 50 ml of benzene. The mixture was stirred with cooling, and a solution of 20.2 g (0.2 mole) of Et_3N in 50 ml of benzene was added dropwise over 15 min. The reaction mixture was kept at room temperature for 1 h with stirring, and then the benzene was boiled off for 2 h. After cooling, 100 ml of water was added, the organic layer was separated, washed with water (2 × 50 ml), washed with 50 ml of 5% aqueous potash, and again with water (2 × 50 ml). The benzene solution was dried with $MgSO_4$, the drying agent was removed by filtration, and the benzene was evaporated. The oily residue was distilled in vacuo and a fraction with a boiling point of 111-112°C/2 mm Hg was collected. The yield of compound I was 34 g (80.5%),

TABLE 3. Effect of N-Valproyl Derivatives of Lactams, Piracetam, and Calcium Valproate on Retention of Learned Active Escape in Amnesia-Induced Conditions

Compound	Number of conditioned reflex responses							
	compound + electric shock				compound + alcohol			
	day 1	day 2	day 3	day 4	day 1	day 2	day 3	day 4
Isotonic saline (control)	23±2,1	31±6,2	40±4,2	43±4,0	14±2,2	41±8,0	33±4,6	44±4,8
Piracetam, 100 mg/kg	23±2,1	50±5,2*	62±7,0**	73±4,3**	25±4,8*	56±7,6	71±5,7**	73±4,2**
Isotonic saline (control)	30±2,6	46±4,6	50±3,3	56±3,1	17±3,3	35±3,7	48±3,3	56±2,2
Calcium Valproate, 30 mg	30±2,6	51±4,3	57±6,0	64±3,1*	20±2,1	43±2,6*	56±4,3	66±3,7*
Isotonic saline (control)	27±7,9	51±16,0	61±13,4	59±11,0	13±1,5	35±3,4	47±4,2	61±4,3
I, 63 mg/kg	28±8,2	51±5,2	57±9,9	63±9,5	16±1,6	42±3,9	50±2,6	68±3,3
Isotonic saline (control)	30±1,5	49±2,3	53±4,2	60±2,1	13±1,5	35±3,4	47±4,2	61±4,3
II, 70 mg/kg	31±2,3	55±4,5	67±4,0*	75±4,3**	23±2,1**	47±2,1**	64±3,1**	73±4,3*
Isotonic saline (control)	30±1,5	49±2,3	53±4,2	60±2,1				
III, 237 mg/kg	30±2,1	50±3,9	60±3,0	56±6,2				

*$p \leq 0.05$.

**$p \leq 0.01$.

the elemental formula was $C_{12}H_{21}NO_2$, and n_D^{20} was 1.4701. The elemental analysis agreed with the calculated composition in this and all other cases.

N-[di(*n*-propyl)acetyl]piperidone-2 (II). Compound II was prepared by the same method used for compound I, using 19.8 g (0.2 mole) of piperidone-2 and 32.5 g (0.2 mole) of the chloroanhydride of di(*n*-propyl)acetic acid in 250 ml of benzene. The yield was 37 g (82.2%), the elemental formula was $C_{13}H_{23}NO_2$, the boiling point was 119-120°C/2 mm Hg, and n_D^{20} was 1.4764.

N-[di(*n*-propyl)acetyl]hexahydroazepinone-2 (III). A solution of 11.3 g (0.1 mole) of hexahydroazepinone-2 (caprolactam) and 16.3 g (0.1 mole) of the chloroanhydride of di(*n*-propyl)acetic acid in 25 ml of toluene was boiled under reflux for 12 h. After cooling, the solution phase was decanted from the dark brown oil. The toluene solution was washed with 5% aqueous potash, and then with water. Toluene was evaporated and the residue was distilled in vacuo, and a fraction with a boiling point of 133-134°C/2 mm Hg was collected. The yield was 17 g (70%), the elemental formula was $C_{14}H_{25}NO_2$, and n_D^{20} was 1.4770.

EXPERIMENTAL (BIOLOGICAL)

Anticonvulsive activity was measured using models of maximal electric shock and convulsions induced by bicuculline and acetaldehyde. Electric shocks of 40 V lasting 200 msec were applied transcorneally. Anticonvulsive effects were evaluated as the number of mice in which the substance of interest prevented the tonic extension phase. ED_{50} values were calculated as described by Mitchell and Wilcoxon. Mice received s.c. bicuculline at a dose of 2.5-3 mg/kg, and the effect was evaluated as the number of animals in which clonic convulsions and death occurred. Acetaldehyde convulsions were induced by i.p. doses of 250 mg/kg.

Depriming effects were studied using the effects on spontaneous movement activity of mice. Measurements were made over 15 min in an Opto-Varimex multichannel recorder (USA). Substances were given i.p. 30 min before measurements, starting with doses which were one tenth of the toxic dose and increasing until clear depriming effects were observed (except for piracetam, where there were no depriming effects even at high doses). Values for control animals were taken as 100%.

The criterion used for determining alterations in movement coordination consisted of the ability of mice to stay on a bar rotating at 6 rpm for 60 sec.

One-day toxicity was determined using doses at which 50% of experimental mice died within 24 h.

Effects on GABA content were studied by measuring GABA in the frontal cortex of mice, using paper electrophoresis [7].

Antihypoxic properties of compounds were measured in mice subjected to normobaric hypoxia (measurement of duration of life in the sealed chamber) and hypobaric hypoxic hypoxia (per cent survival at an altitude of 10,500 m), as

described in [2]. The sealed chamber experiment was also used to determine the effects of agents on the basic biochemical parameter used as a measure of hypoxia, i.e., the lactate and pyruvate levels in the brain. Lactate was measured as decrease by Barker and Sommerson [4], and pyruvate as described by Friedman et al. [6].

The protective effects of compounds in relation to amnestic factors such as alcohol were studied in rats using a CAER test [8], and also in relation to electric shock. Animals received p.o. alcohol (10% solution, 4 ml/day for 13 days). Animals were trained to CAER responses on days 15-18 after the start of alcoholization. Single doses of agents were given i.p. on days 11 and 14 after the start of alcoholization; 2 doses were given on day 12 and 13, and daily doses were given on days 15-18, 60 min before training.

Animals were trained to conditioned passive escape reactions with a single reinforcement by a modification of the method of Bureš and Burešova [5]. Electric shocks were applied immediately after training. Substances were given immediately after electric shocks.

The forced swimming test ("desperate behavior") was carried out using mice, as described by Porsolt et al. [9]. Agents and isotonic sodium chloride were given i.p. 1 h before the experiment. The total duration of episodes of immobility was measured over a period of 6 min.

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