10 times (once every 30 s) to determine whether or not they were mobile. Antidepressant drugs reduce the score in this test in a dose-dependent fashion.

Rat Foot Edema Test. The procedure of Winter[18] was modified as follows: Adult male, fasted, unanesthetized, Charles River CD Sprague-Dawley rats of 170-205 g body weight were numbered and weighed, and an ink mark was placed on the right lateral malleolus. Each paw was immersed in mercury exactly to the ink mark. The mercury was contained in a plastic cylinder connected to an Omega pressure transducer. The output from the transducer was fed through a Buxco organ volume monitor. The volume of mercury displaced by the immersed paw was read, then printed on a Texas Instruments Silent 700. Drugs were given by gavage in a 25 mL/kg volume. One hour after drug administration, edema was induced by injection of 0.05 mL of a 2-week-old 1% solution of carrageenan in distilled water into the plantar tissue of the marked paws. Immediately thereafter, the volume of the injected paw was measured. The increase in foot volume 3 h after the injection of carrageenan constitutes the individual response. Compounds were considered active if they inhibited the edema response by 30% or more in a group of six rats.

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Registry No. **1a**, 27693-67-0; **1b**, 114918-39-7; **2a**, 114918-37-5; **2b**, 114918-35-3; **3a**, 130985-15-8; **3b**, 130985-16-9; **3c**, 114934-47-3; **3d**, 130985-17-0; **3e**, 114918-24-0; **3f**, 114918-31-9; **3g**, 114918-32-0; **4a**, 114934-48-4; **4b**, 114934-49-5; CalPDE, 9036-21-9; $PhCH_2NCO$, 3173-56-6; 2-chlorobenzoic acid, 118-91-2; 2-chloronicotinic acid, 2942-59-8; ethyl isocyanate, 109-90-0; 2-norbornylacetic acid, 1007-01-8; cyclopentylacetic acid, 1123-00-8; 3-aminobenzoic acid, 99-05-8.

Synthesis and Anticonvulsant Activity of 1-Acyl-2-pyrrolidinone Derivatives

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Several 1-acyl-2-pyrrolidinone derivatives were synthesized as derivatives of γ-aminobutyric acid (GABA), and their pharmacological activities and stabilities were investigated. The derivatives showed anticonvulsant effect on picrotoxin-induced seizure at a dose of 200 mg/kg. In particular, 1-decanoyl-2-pyrrolidinone (**7**) and 1-dodecanoyl-2-pyrrolidinone (**8**) had a high activity. The anticonvulsant activity showed a dose dependency. Some of 1-acyl-2-pyrrolidinone derivatives prolonged sleeping time which was induced by sodium pentobarbital and showed a recovery from disruption of the memory of passive avoidance response, which was induced by an electroconvulsive shock. As shown by the results of the stability study of 1-acetyl-2-pyrrolidinone (**1**), it was degraded in an acidic buffer and an alkaline buffer although 2-pyrrolidinone was stable. 1-Acyl-2-pyrrolidinone derivatives were degraded in liver and brain homogenates of mouse and rat. They showed a degradation rate in rat plasma. Conversion of **8** to GABA in mouse liver homogenate was demonstrated. These results suggested that the pharmacological activity of 1-acyl-2-pyrrolidinone is probably due to the release of GABA by hydrolysis of derivatives although further work is necessary.

Several neurological and neuropsychiatric disorders such as epilepsy and Huntington's disease have been reported to be associated with a decrease in γ-aminobutyric acid (GABA) levels in the central nervous system (CNS).[1-3] These observations suggest that increased levels of GABA in the CNS may be useful in the treatment of such neuropsychiatric disorders. However, attempts to use GABA in clinical trials failed due to the extremely high doses required to force GABA across the blood-brain barrier.[4,5]

Numerous derivatives of GABA, including alkyl ester of GABA,[6,7] γ-acetylenic GABA,[8] aliphatic and steroid esters of GABA,[9-11] Schiff bases of GABA,[12] soft drug of GABA,[13] and isonicotinoyl GABA,[14] have been developed in the hope of facilitating the uptake of GABA into the brain.

Callery et al.[15,16] suggested another possibility, use of 2-pyrrolidinone as a GABA prodrug. 2-Pyrrolidinone, the lactam of GABA, is more lipophilic than GABA and penetrates readily into the CNS. However, attempts to increase whole-brain GABA concentrations with single large doses of 2-pyrrolidinone have been unsuccessful.[4] The failure may be explained by a slow hydrolysis of 2-pyrrolidinone to GABA.[16]

The tight amide bond of 2-pyrrolidinone may be weakened by introduction of an acyl function to the 1-position. Also the lipophilicity of drug may be controlled by the chain length of the acyl function to be introduced. Therefore, several 1-acyl-2-pyrrolidinone derivatives having various alkyl chains were synthesized and their GABA-like pharmacological activities such as anticonvulsant activity and sedative activity were evaluated. Antiamnesic activity

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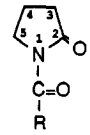
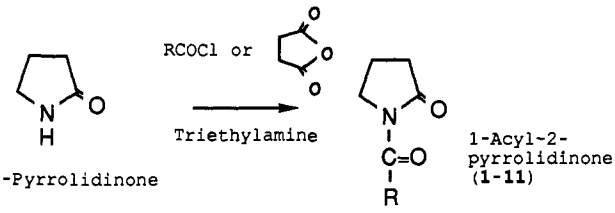
Table I. Structures and Physicochemical Properties of 1-Acyl-2-pyrrolidinone Derivatives



	compd or R	MW[a]	mp, °C	R_f[b]	log k'_0[c]	log P_c[d]	S_{aq}[e]
GABA		103.1	202			-3.33	$>10^4$
2-pyrrolidinone		85.1	23–25	0.51	0.074	-0.85	*g*
1	CH_3	127.1	<4[f]	0.72	0.75	-0.05	*g*
2	C_2H_5	141.2	<4[f]	0.73			
3	C_3H_7	155.2	<4[f]	0.79	1.5	1.18	280
4	C_4H_9	169.2	<4[f]	0.80			
5	$C(CH_3)_3$	169.2	<4[f]	0.81			
6	C_6H_{13}	197.3	<4[f]	0.83	3.0	3.04	24.4
7	C_9H_{19}	239.4	16–17	0.86	4.4	4.33	0.347
8	$C_{11}H_{23}$	267.4	25–28	0.87	5.5	5.05	0.048
9	(C_6H_5)	189.2	89–90	0.81			
10	$CH_2(C_6H_5)$	203.2	40–41	0.82			
11	$(CH_2)_2COOH$	185.2	106–108	0.56			
12	(*N*-dodecanoyl-GABA)	285.4	96–98	0.54			

[a] Molecular weight. [b] R_f values in TLC. [c] Lipophilic indices determined by HPLC. [d] Logarithmic value of apparent partition coefficient between 1-octanol and pH 5.0 phosphate buffer (0.1 M) at 37 °C. [e] Solubility in pH 5.0 phosphate buffer (0.1 M) at 37 °C. [f] The compound was liquid when stored at 4 °C. [g] Miscible.

Scheme I. Synthesis of 1-Acyl-2-pyrrolidinone Derivatives



of the derivatives was also examined. Their fundamental stability and conversion to GABA in biological media were demonstrated.

Results and Discussion

Chemistry. 1-Acyl-2-pyrrolidinone derivatives (**1–8**) were prepared in good yield by a direct acylation of 2-pyrrolidinone with acyl chloride at room temperature in the presence of a proton capturer, triethylamine, as shown in Scheme I. The oily products were purified with distillation under reduced pressure. The derivatives having longer acyl chains showed higher boiling point for distillation. 1-Benzoyl-2-pyrrolidinone (**9**) and 1-(phenylacetyl)-2-pyrrolidinone (**10**) were purified by recrystallization. 1-Succinyl-2-pyrrolidinone (**11**) was prepared by acylation of 2-pyrrolidinone with succinic anhydride and purified by recrystallization. *N*-Dodecanoyl-GABA (**12**) was prepared by acylation of GABA with dodecanoyl chloride in 2 M NaOH for comparing its conversion and anticonvulsant activity with 1-dodecanoyl-2-pyrrolidinone (**8**). The NMR spectra and elemental analysis of the compounds were consistent with their proposed structures.

Structure and Physicochemical Property of Derivatives. Table I summarizes the structures and physicochemical properties of the derivatives. The derivatives showed various R_f values in TLC due to the introduction of the functional group. Lipophilicity and solubility of 2-pyrrolidinone and its derivatives, **1**, **3**, and **6–8**, were examined in detail. The elongation of the alkyl side chain increased lipophilicity and decreased solubility in pH 5.0 phosphate buffer (0.1 M). The relationship between carbon number of the alkyl side chain (R) and physicochemical properties is represented in Figure 1. They showed good linear relationships. Controlling the lipophilicity of a compound is important for its brain uptake.[9-11]

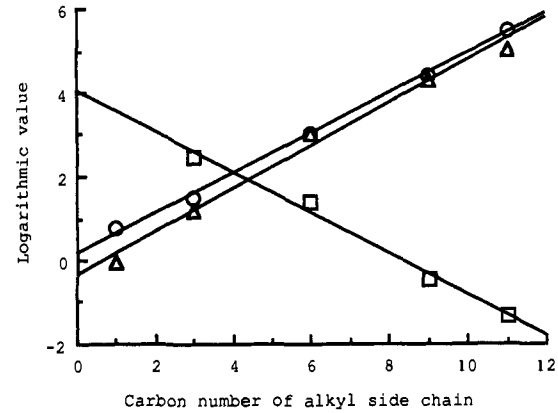


Figure 1. Relationship between physicochemical properties and carbon number of the alkyl side chain of derivatives **1**, **3**, and **6–8**. ○, log k'_0; △, log P_c; □, S_{aq}. The lines through the data were obtained from a linear regression fit: log k'_0, $Y = 0.477X + 0.167$, $r = 0.999$; log P_c, $Y = 0.514X - 0.374$, $r = 0.994$; S_{aq}, $Y = -0.487X + 4.047$, $r = 0.994$.

Pharmacological Properties. GABA is the major inhibitory neurotransmitter in the mammalian CNS. The GABA receptor is an oligometric complex composed of a GABA-binding site, a chloride channel, binding sites for benzodiazepines and β-carbolines, and sites coupled to the chloride channel, which bind picrotoxin and barbiturates.[13,17,18] GABA receptor–ionophore complex mediates the flux of chloride ions across nerve membranes and may be relative to anticonvulsant and sedative actions of GABA-mimetic drugs.[17,19-21]

The anticonvulsant effect of test compounds on picrotoxin-induced seizure in mice is shown in Table II. Test compounds were suspended in 1% (carboxymethyl)cellu-

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Table II. Anticonvulsant Effect of 1-Acyl-2-pyrrolidinone Derivatives on Picrotoxin-Induced Seizure in Mice

compd[a]	N[b]	onset of clonic[c] seizure, % of control	survival[c] time, % of control	survival ratio
control	20	100	100	0
GABA	5	115 ± 13	112 ± 8	0
2-pyrrolidinone	9	107 ± 10	102 ± 10	0
1	9	97 ± 10	144 ± 13[g]	0
2	4	104 ± 9	112 ± 7	0
3	8	92 ± 3	142 ± 20[f]	0
4	4	78 ± 7	107 ± 20	0
5	8	101 ± 7	120 ± 11[d]	0
6	10	124 ± 15	116 ± 13	0
7	8	302 ± 118[e]	364 ± 82[g]	50
8	10	129 ± 13[d]	494 ± 55[g]	80
9	5	114 ± 5	93 ± 6	0
10	4	106 ± 9	281 ± 154[f]	33
11	6	113 ± 5	93 ± 7	0
12	10	493 ± 108[e]	388 ± 54[f]	60
valproic acid	8	171 ± 14[g]	472 ± 64[g]	63
ethosuximide	6	792 ± 174[g]	577[g]	100

[a] The compounds were administered intraperitoneally (200 mg/kg, a final volume of 0.1 mL/10 g of body weight) in the form of pH 7.4 isotonic phosphate buffer suspension containing 1% CMC-Na 15 min prior to subcutaneous administration of picrotoxin (5 mg/kg). [b] Number of animals. [c] They represent the means ± SEM. [d-g] Statistical significance (Student's t test) from control: $^dp < 0.05$, $^ep < 0.02$, $^fp < 0.01$, $^gp < 0.001$.

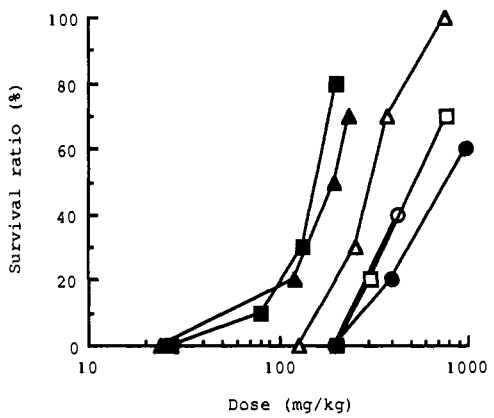

Figure 2. Dose dependency of anticonvulsant activity of 2-pyrrolidinone and its derivatives, 1, 3, and 6–8: ○, 2-pyrrolidinone; △, 1; □, 3; ●, 6; ▲, 7; ■, 8.

lose sodium salt (CMC-Na) and administered intraperitoneally (200 mg/kg). The effect was represented as a ratio of time for the treated group to time for the control group. GABA and 2-pyrrolidinone did not show significant activity, which agreed with the results reported by Maynert and Kaji.[4] However, some 1-acyl-2-pyrrolidinone derivatives showed a significant anticonvulsant activity. In particular, lipophilic **7** and **8** showed a high activity equivalent to that of valproic acid, which has been used clinically as an anticonvulsant agent.[22-24] Compound **12** also showed high activity. It is well-known that N-alkylation and N-acylation of GABA leads to an almost complete loss of affinity for the GABA receptor site. However, some *N*-acyl-GABA derivatives exhibit anticonvulsant activity in rats because presumably they act as GABA via prodrug.[25] Ethosuximide showed further

Table III. Effect of 1-Acyl-2-pyrrolidinone Derivatives on Sleeping Time Induced by Sodium Pentobarbital in Mice

compd[a]	N[b]	onset for[c] sleep, min	sleeping time,[c] min
control	9	7.7 ± 0.9	34.9 ± 6.6
GABA	4	10.1 ± 0.8	25.7 ± 9.0
2-pyrrolidinone	5	10.2 ± 1.2	24.8 ± 11.5
1	4	6.9 ± 0.7	38.2 ± 15.2
3	5	7.2 ± 0.8	92.0 ± 11.3[e]
6	10	7.9 ± 0.4	61.8 ± 9.8[d]
8	10	9.6 ± 1.4	47.4 ± 8.0
valproic acid	4	10.0 ± 0.3[d]	36.4 ± 8.5

[a] The compounds were administered intraperitoneally (50 mg/kg, a final volume of 0.1 mL/10 g of body weight) in the form of pH 7.4 isotonic phosphate buffer suspension containing 1% CMC-Na 30 min prior to intraperitoneal administration of sodium pentobarbital (45 mg/kg). [b] Number of animals. [c] They represent the means ± SEM. [d,e] Statistical significance (Student's t test) from control: $^dp < 0.05$, $^ep < 0.001$.

Table IV. Effect of Derivatives 1, 6, and 8 on Electroconvulsive-Shock-Induced Disruption (ECS) of the Memory of Passive Avoidance Response in Mice

compd[a]	N[b]	learning[c] time, s	step-through[c] latency, s
control	10	17.0 ± 3.2	222.5 ± 26.9[e]
ECS	7	13.7 ± 1.5	20.1 ± 3.8
1	9	15.3 ± 1.8	101.7 ± 36.9[d]
6	8	20.4 ± 3.7	240.6 ± 21.5[e]
8	6	19.4 ± 3.5	278.8 ± 20.0[e]

[a] The compounds were administered intraperitoneally (200 mg/kg, a final volume of 0.1 mL/10 g of body weight) in the form of a pH 7.4 isotonic phosphate buffer suspension containing 1% CMC-Na 60 min before training of the memory of passive avoidance. [b] Number of animals. [c] They represent the means ± SEM. [d,e] Statistical significance (Student's t test) from ECS: $^dp < 0.1$, $^ep < 0.001$.

superior effect. Figure 2 shows dose–response curves of 2-pyrrolidinone and 1-acyl-2-pyrrolidinone derivatives (**1**, **3**, and **6–8**). Their anticonvulsant activities showed dose dependency. Slight anticonvulsant activity of 2-pyrrolidinone was found at a dose of 426 mg/kg. They showed various ED_{50} (311 mg/kg for **1**, 551 mg/kg for **3**, 799 mg/kg for **6**, 200 mg/kg for **7**, and 150 mg/kg for **8**).

Effect of 2-pyrrolidinone and derivatives (**1**, **3**, **6**, and **8**) on sleeping time induced by sodium pentobarbital in mice was examined to determine their sedative action. The results are shown in Table III. Compounds **3** and **6** significantly prolonged the sleeping time. The difference between anticonvulsant and sedative potencies suggests different pharmacological mechanisms.

Some cyclic analogues of GABA including 1-*p*-anisoyl-2-pyrrolidinone (aniracetam) have been developed as antiamnesic agents which are expected to be used for treatment of disruption of memory.[26-29] Therefore, the effect of compounds **1**, **6**, and **8** on electroconvulsive-shock-induced disruption (ECS) of the memory of passive avoidance response in mice was examined. The mice, which have an instinct to escape from a lighted compartment into a darkened compartment, received an electrical foot shock in the darkened compartment. Learning time is the time in which the mouse entered the

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Table V. Degradation Rates of 2-Pyrrolidinone and 1 in Various pH Buffer Solutions at 37 °C

compd	pH	degradation rate constant, h^{-1}	compd	pH	degradation rate constant, h^{-1}
2-pyrrolidinone	2.0	0.0003	1	6.0	0.0018
	7.4	<0.0001		7.4	0.0152
	10.0	0.0003		8.0	0.0609
1	2.0	0.0090		9.0	0.2455
	4.5	0.0007		10.0	3.316
	5.0	0.0006			

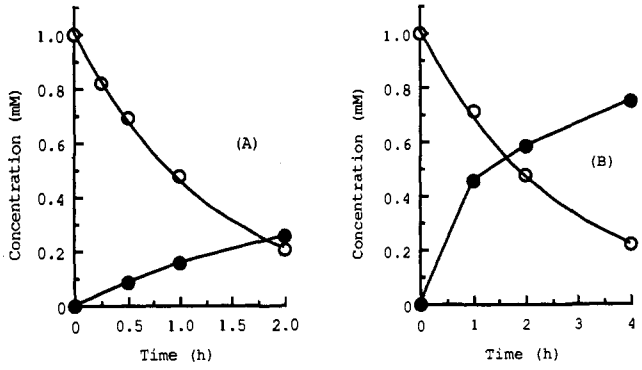


Figure 3. Conversion of 8 (A) and 12 (B) to GABA in mouse liver homogenate: ○, derivative; ●, GABA.

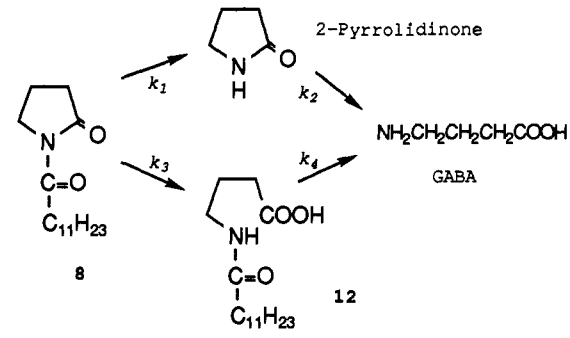
darkened compartment and came out of it by the electrical shock. The memory is described as the length of step-through latency from the nonelectrical, lighted compartment to the electrical darkened compartment. The results are summarized in Table IV. The derivatives showed little influence on learning time. ECS actually reduced the time for step-through latency, suggesting that mice forgot the memory of the electrical foot shock. Compounds 1, 6, and 8 significantly recovered the disruption of the memory.

Stability Study. The degradation of 2-pyrrolidinone and its derivative 1 was determined in various pH buffers. Their degradations showed pseudo-first-order kinetics and their apparent degradation rates are listed in Table V. Derivative 1 was degraded under acid and alkaline conditions. The maximum stabilized pH is about 5.0 although 2-pyrrolidinone was very stable.

Table VI summarizes the degradation rates of 1-acyl-2-pyrrolidinone derivatives in pH 7.4 buffer and biological media at 37 °C. The derivatives were more stable in pH 7.4 buffer than in biological media. They degraded in liver and brain more than in plasma. The plot of degradation rate on lipophilicity of compounds showed a parabolic pattern and the maximum-degraded derivative was 6 in liver homogenate and 7 in brain homogenate. There was no big species difference between rat and mouse. 2-Pyrrolidinone was more stable than its derivatives in biological media.

Figure 3 (A, B) shows the degradation of 8 and 12 and the regeneration of GABA in mouse liver homogenate. They rapidly converted to GABA. Regeneration of GABA from 8 was lower than that of 12. Scheme II shows the simplified regeneration route of GABA from 8 via 2-pyrrolidinone and via 12. In the scheme, k_1–k_4 are first-order rate constants. The rate constants calculated from data in Figure 3 using a nonlinear least-squares program[30] were $k_1 = 0.0297$, $k_2 = 0.0342$, $k_3 = 0.727$, and $k_4 = 0.396$. These results suggest that derivative 8 predominantly converts to GABA via *N*-acyl-GABA. However, the identity and properties of the actual intermediates and the potential for reversibility of some of the reactions are necessary for confirming this simple regeneration model.

Scheme II. Conversion of 8 to GABA



Conclusion

Thus, 1-acyl-2-pyrrolidinone derivatives have GABA-like depressant effects on the CNS such as anticonvulsant and sedative actions. They also showed antiamnesic activity. Derivative 8 showed a conversion to GABA in biological media. These results suggested that the pharmacological activity of 1-acyl-2-pyrrolidinone is probably due to the release of GABA by hydrolysis of derivatives as a prodrug. However, the derivatives may also act intact on various targets such as the GABA receptor, the uptake systems, and the catabolic enzyme.[17] Perlman and Goldstein[31] also reported that some fatty acids have sedative and anticonvulsant actions in mice by disruption of membrane structure. Skondia[32] demonstrated that an antiamnesic agent having a 2-pyrrolidinone group shows in its chemical structure a kinship to GABA, but it has no significant effect upon neurotransmitter levels. Further work is necessary to elicit their mechanisms.

Experimental Section

Chemistry. GABA, 2-pyrrolidinone, valproic acid, and ethosuximide were obtained from Nacalai Tesque Inc. (Kyoto, Japan) and used without further purification. NMR spectra were taken on a Hitachi R-600 FT-NMR spectrometer (Hitachi Co., Ltd., Tokyo, Japan) and elemental analysis were performed by the Center for Organic Elemental Micro-analysis, Nagasaki University. The analytical results obtained were within ±0.4% of the theoretical values. All other reagents were of reagent grade. TLC analysis of derivatives was performed on silica gel plates (60 F_{254}, E. Merck, Darmstadt, Federal Republic of Germany) with the following solvent system: chloroform/ethyl acetate/acetic acid/methanol 90:7:3:10, v/v/v/v. The products on TLC plate were detected by staining with bromocresol green and iodine after removing the solvent completely.

Physicochemical Property. The lipophilic indices (log k') were determined by HPLC employing the equation $\log k' = \log [(t_R - t_0)/t_0]$, where t_R is the retention time and t_0 is the elution time of a solvent. The log k' values were extrapolated to 0% methanol concentration to obtain the lipophilic indices (log k'_0).[33]

Apparent partition coefficients of derivatives were determined in 1-octanol (2 mL) and pH 5.0 phosphate buffer (0.1 M, 8 mL) system at 37 °C. Final drug concentration was 2 mM.

The solubilities of compounds in pH 5.0 phosphate buffer were determined at 37 °C. Excess amounts of compounds (200 mg/mL) were suspended in the solvent, shaken for 30 min, sonicated for 30 min, and kept for 2 days in a thermostated chamber at 37 °C. The supernatant was filtered with an HV filter (pore size 0.45 μm, Nihon Millipore Kogyo K.K., Yonezawa, Japan) and supplied for analysis. The supernatant for oily compounds was supplied for analysis without filtration.

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Table VI. Degradation Rates of 1-Acyl-2-pyrrolidinone Derivatives in pH 7.4 Buffer and Biological Media of Rat and Mouse

compd	degradation rate constant, h^{-1}					
	pH 7.4 buffer	rat[a]			mouse[a]	
		plasma	liver	brain	liver	brain
2-pyrrolidinone	<0.0001	0.018	0.030	0.016	0.034	0.002
1	0.015	0.028	0.097	0.014	0.221	0.010
3	0.006	0.054	3.11	0.016	1.35	0.016
6	0.012	0.056	14.6	0.075	11.9	0.086
7	0.008	0.025	2.87	0.381	2.53	0.326
8	0.007	0.016	1.23	0.233	0.789	0.155

[a] Apparent degradation rate constant in 40% homogenate.

Synthesis. 1-Acetyl-2-pyrrolidinone (1). Acetyl chloride (0.4 mol) was added gradually to triethylamine (0.6 mol) and 2-pyrrolidinone (0.6 mol) in 500 mL of tetrahydrofuran at 0 °C and was stirred for 30 min at room temperature. A white solid, triethylamine chloride, was filtered and the filtrate was evaporated. The residual oily product was distilled under a reduced pressure to give a colorless fluid, 1-acetyl-2-pyrrolidinone. The yield was about 60% based on pyrrolidinone: bp 90–93 °C (0.5 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.80 (2 H, t, J = 7.0 Hz, 5-H), 2.59 (2 H, t, J = 7.4 Hz, 3-H), 2.48 (3 H, s, $COCH_3$), 1.80–2.40 (2 H, m, 4-H). Anal. ($C_6H_9NO_2$) C, H, N.

The following 1-acyl-2-pyrrolidinone derivatives were prepared in the same manner.

1-Propionyl-2-pyrrolidinone (2): bp 96–99 °C (0.2 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.85 (2 H, t, J = 7.0 Hz, 5-H), 2.94 (2 H, q, J = 7.0 Hz, $COCH_2CH_3$), 2.62 (2 H, t, J = 7.0 Hz, 3-H), 1.80–2.40 (2 H, m, 4-H), 1.17 (3 H, t, J = 7.5 Hz, $COCH_2CH_3$). Anal. ($C_7H_{11}NO_2$) C, H, N.

1-Butyryl-2-pyrrolidinone (3): bp 86–87 °C (0.5 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.80 (2 H, t, J = 7.0 Hz, 5-H), 2.88 (2 H, t, J = 7.4 Hz, $COCH_2CH_2CH_3$), 2.59 (2 H, t, J = 7.0 Hz, 3-H), 1.80–2.40 (2 H, m, 4-H), 1.30–1.90 (2 H, m, $COCH_2CH_2CH_3$), 0.96 (3 H, t, J = 7.0 Hz, $COCH_2CH_2CH_3$). Anal. ($C_7H_{11}NO_2$) C, H, N.

1-Valeryl-2-pyrrolidinone (4): bp 95–100 °C (0.2 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.83 (2 H, t, J = 7.0 Hz, 5-H), 2.92 (2 H, t, J = 7.4 Hz, $COCH_2(CH_2)_2CH_3$), 2.62 (2 H, t, J = 7.0 Hz, 3-H), 1.80–2.40 (2 H, m, 4-H), 1.20–1.80 (4 H, m, $COCH_2(CH_2)_2CH_3$), 0.94 (3 H, t, J = 6.0 Hz, $COCH_2(CH_2)_2CH_3$). Anal. ($C_9H_{15}NO_2$) C, H, N.

1-Pivaloyl-2-pyrrolidinone (5): bp 85–87 °C (0.2 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.82 (2 H, t, J = 7.0 Hz, 5-H), 2.59 (2 H, t, J = 7.4 Hz, 3-H), 1.80–2.40 (2 H, m, 4-H), 1.29 (9 H, s, $COC(CH_3)_3$). Anal. ($C_9H_{15}NO_2$) C, H, N.

1-Heptanoyl-2-pyrrolidinone (6): bp 120–122 °C (0.2 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.78 (2 H, t, J = 7.0 Hz, 5-H), 2.85 (2 H, t, J = 7.4 Hz, $COCH_2(CH_2)_4CH_3$), 2.56 (2 H, t, J = 7.0 Hz, 3-H), 1.80–2.40 (2 H, m, 4-H), 1.00–2.00 (8 H, m, $COCH_2(CH_2)_4CH_3$), 0.84 (3 H, t, J = 5.0 Hz, $COCH_2(CH_2)_4CH_3$). Anal. ($C_{11}H_{19}NO_2$) C, H, N.

1-Decanoyl-2-pyrrolidinone (7): bp 146–148 °C (0.2 mmHg); mp 16–17 °C; ^{1}H NMR ($CDCl_3$) δ 3.78 (2 H, t, J = 7.0 Hz, 5-H), 2.85 (2 H, t, J = 7.0 Hz, $COCH_2(CH_2)_7CH_3$), 2.56 (2 H, t, J = 7.4 Hz, 3-H), 1.80–2.40 (2 H, m, 4-H), 1.23 (14 H, s, $COCH_2(CH_2)_7CH_3$), 0.84 (3 H, t, J = 5.0 Hz, $COCH_2(CH_2)_7CH_3$). Anal. ($C_{14}H_{25}NO_2$) C, H, N.

1-Dodecanoyl-2-pyrrolidinone (8): bp 212–215 °C (1.0 mmHg); mp 25–28 °C; ^{1}H NMR ($CDCl_3$) δ 3.78 (2 H, t, J = 7.0 Hz, 5-H), 2.89 (2 H, t, J = 7.4 Hz, $COCH_2(CH_2)_9CH_3$), 2.59 (2 H, t, J = 7.4 Hz, 3-H), 1.80–2.40 (2 H, m, 4-H), 1.25 (18 H, s, $COCH_2(CH_2)_9CH_3$), 0.87 (3 H, t, J = 4.7 Hz, $COCH_2(CH_2)_9CH_3$). Anal. ($C_{16}H_{29}NO_2$) C, H, N.

1-Benzoyl-2-pyrrolidinone (9). This derivative was recrystallized with acetone to give a white solid. The yield was about 80% based on 2-pyrrolidinone: mp 89–90 °C; ^{1}H NMR ($CDCl_3$) δ 7.40–7.80 (5 H, m, COC_6H_5), 3.93 (2 H, t, J = 7.0 Hz, 5-H), 2.56 (2 H, t, J = 7.0 Hz, 3-H), 1.80–2.40 (2 H, m, 4-H). Anal. ($C_{11}H_{11}NO_2$) C, H, N.

1-(Phenylacetyl)-2-pyrrolidinone (10). This derivative was purified with a silica gel column (Art 7734, 70–230 mesh, E. Merck, 300 × 20 mm i.d.), eluted with chloroform. The derivative was recrystallized with acetone and the yield was about 60% based on 2-pyrrolidinone: mp 40–41 °C; ^{1}H NMR ($CDCl_3$) δ 7.28 (5 H, s, $COCH_2C_6H_5$), 4.25 (2 H, s, $COCH_2C_6H_5$), 3.80 (2 H, t, J = 7.0 Hz, 5-H), 2.58 (2 H, t, J = 7.0 Hz, 3-H), 1.80–2.40 (2 H, m, 4-H). Anal. ($C_{12}H_{13}NO_2$) C, H, N.

1-Succinyl-2-pyrrolidinone (11). This derivative was synthesized by acylation of 2-pyrrolidinone by succinic anhydride instead of acyl chloride and recrystallized with chloroform and hexane to give a white solid of 1-succinyl-2-pyrrolidinone. The yield was about 70% based on 2-pyrrolidinone: mp 106–108 °C; ^{1}H NMR ($CDCl_3$) δ 3.80 (2 H, t, J = 7.0 Hz, 5-H), 3.22 (2 H, t, J = 6.6 Hz, $COCH_2CH_2COOH$), 2.69 (2 H, t, J = 6.2 Hz, $COCH_2CH_2COOH$), 2.61 (2 H, t, J = 6.6 Hz, 3-H), 1.80–2.40 (2 H, m, 4-H). Anal. ($C_8H_{11}NO_4$) C, H, N.

***N*-Dodecanoyl-GABA (12).** Dodecanoyl chloride (0.4 mol) was added gradually to GABA (0.6 mol) in 2 M NaOH and was stirred vigorously for 2 h at room temperature. The pH of solution was maintained at 10–12 with 4 M NaOH during the reaction. The derivative was precipitated by addition of 6 M HCl (adjusted to pH 1–2) and was extracted with ethyl acetate. The organic solution was washed with water 10 times and dried with an anhydrous sulfate overnight. The solution was evaporated and the residual was recrystallized with isopropyl ether and methanol to give a white solid of *N*-dodecanoyl-GABA. The yield was about 80% based on pyrrolidinone: mp 96–98 °C; ^{1}H NMR ($CDCl_3$) δ 5.80 (1 H, br, $-NHCO-$), 3.30 (2 H, q, J = 7.1 Hz, γ-H), 2.42 (2 H, t, J = 7.0 Hz, $COCH_2(CH_2)_9CH_3$), 2.19 (2 H, t, J = 7.0 Hz, α-H), 1.60–2.20 (2 H, m, β-H), 1.21 (18 H, s, $COCH_2(CH_2)_9CH_3$), 0.84 (3 H, t, J = 5.0 Hz, $COCH_2(CH_2)_9CH_3$). Anal. ($C_{16}H_{31}NO_3$) C, H, N.

Anticonvulsant Activity. Male ddY mice weighing 20–30 g were used in the experiment. Test compounds were administered intraperitoneally (200 mg/kg) in the form of pH 7.4 isotonic phosphate buffer suspension containing 1% CMC-Na 15 min prior to subcutaneous administration of picrotoxin (5 mg/kg). The final volume of administration for all test compounds and picrotoxin was 0.1 mL/10 g of body weight. The behavioral changes of the mice were observed for 2 h. The latency to the onset of generalized clonic epileptic seizures and the protection against lethality by picrotoxin were measured and evaluated as a percent of the control. The control mice received the vehicle. Dosage of test compound was changed and the survival ratio was calculated for determination of dose dependency.

Sedative Activity. Male ddY mice weighing 20–30 g were used in the experiment. The test compounds were administered intraperitoneally (50 mg/kg) in the form of pH 7.4 isotonic phosphate buffer suspension containing 1% CMC-Na 30 min prior to intraperitoneal administration of sodium pentobarbital (45 mg/kg). The final volume of administration for all test compounds was 0.1 mL/10 g of body weight. Sleeping time and the time of loss of righting reflex was determined for each mouse as sedative activity.

Antiamnesic Activity. Male ddY mice weighing 20–25 g were used in the experiment. The apparatus consisted of two compartments with an opening between the compartments. Both compartments had grid floors. An electrical foot shock of 30 V could be applied in one darkened compartment. One hour after intraperitoneal administration of each compound (200 mg/kg in 1% CMC-Na), each mouse was placed into a nonelectrical, lighted compartment. Immediately the mouse escaped from the lighted compartment through a small hole into the darkened compartment. After 3 s, the mouse received a foot shock and was removed from the darkened compartment. After this training, immediately

each mouse received a single electroconvulsive shock, 100 V for 0.4 s, delivered through clips attached to the ears. The mice were individually returned to the lighted compartment 24 h after ECS treatment to measure the retention of the inhibitory avoidance response. The period the mice remained in the nonelectrical lighted compartment was measured for at least 5 min.

Stability Study. Stock solution of all compounds was prepared in acetonitrile at a concentration of 0.1 M and an appropriate volume was mixed with an aqueous buffer or biological media for stability study.

All stability experiments were carried out in aqueous buffer solutions at 37 ± 0.2 °C. The pH of the solution was maintained at the desired value by using appropriate buffer systems (sodium citrate/HCl and NaH_2PO_4/Na_2HPO_4). The total buffer concentration was 0.1 M. The ionic strength of each buffer solution was adjusted to 0.3 with sodium chloride. Degradation was initiated by the addition of the stock solution to a preheated, buffered solution to give a concentration of 1 mM. Aliquots of the solution were withdrawn at appropriate time intervals.

Male ddY mice weighing 20–30 g and male Wistar albino rats weighing 200–250 g were used to obtain plasma, brain, and liver homogenates. The liver and brain were homogenized at 0–5 °C in a glass–Teflon homogenizer to prepare 40% homogenate with a pH 7.4 isotonic phosphate buffer, then centrifuged at 2500*g* for 15 min, and the supernatant was used for the experiments. Degradation experiments were performed at 37 ± 0.2 °C and initiated by adding the stock solution to give a final concentration of 1 mM. At appropriate time intervals aliquots of the solution were withdrawn and acetonitrile was added to them to precipitate the protein. The supernatant was subjected to HPLC analysis.

Analysis. The derivatives were determined by the use of an HPLC system (LC-6A, Shimadzu Co., Kyoto, Japan) equipped with a variable-wavelength UV detector (UVIDEC 100-II, Japan Spectroscopic Co., Ltd., Tokyo, Japan) in a reverse-phase mode. Derivatives were monitored at 205 nm. The stationary phase used was a Cosmosil $5C_{18}$-P packed column (150 × 4.6 mm i.d., Nacalai Tesque Inc.). Mixtures of methanol/water (15:85 for 1, 40:60 for 3, 65:35 for 6, 80:20 for 7 85:15 for 8, v/v) were used as the mobile phase with a flow rate of 1.0 mL/min.

GABA regenerated from derivatives was determined by the use of an HPLC system equipped with a fluorescence spectromonitor (RF-530, Shimadzu Co.) after derivatization with dansyl chloride according to the method of Tapuhi et al.[34] Twenty microliters of 5-amino-*n*-valeric acid (internal standard, 2 mM) in a pH 9.5 lithium carbonate buffer (0.04 M) and 20 μL of dansyl chloride (5.56 mM) in acetonitrile were added to the sample (2 mL). After 30 min, the derivatization was terminated by adding 2% methylamine solution (2 mL). In all reactions, vials were wrapped with aluminum foil to exclude light. The stationary phase used was a Cosmosil $5C_{18}$-P packed column (150 × 4.6 mm i.d., Nacalai Tesque Inc.). Mixtures of methanol/0.01 M Tris HCl buffer, pH 7.4 (36:64, v/v), were used as the mobile phase with a flow rate of 1.0 mL/min. Excitation wavelength was 250 nm and emission wavelength was monitored at 470 nm.

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Renin Inhibitory Peptides. Incorporation of Polar, Hydrophilic End Groups into an Active Renin Inhibitory Peptide Template and Their Evaluation in a Human Renin Infused Rat Model and in Conscious Sodium-Depleted Monkeys

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We previously reported that Boc-Pro-Phe-N-MeHis-Leuψ[$CHOHCH_2$]-Ile-Amp (1) is a potent and specific inhibitor of human renin in vitro. It was shown to resist degradation by selected proteases and a rat liver homogenate. It was shown to inhibit plasma renin activity and to reduce blood pressure in renin-dependent-animal models both by the intravenous and by the oral routes using dilute citric acid as vehicle. In an effort to discover compounds with improved pharmacological efficacy, we set out to modify the physical characteristics of this highly lipophilic renin inhibitor by incorporation of hydrophilic end groups. We report here a variety of water-solubilizing groups and the resulting structure–activity relationship of these compounds. They all maintain an extremely high level of enzyme inhibitory activity in vitro. Evaluation of these potent renin inhibitors in a human renin infused rat model suggests that some of these compounds exhibit improved pharmacological efficacy in vivo. This observation was further confirmed in the conscious sodium-depleted cynomolgus monkey. Importantly, the oral efficacy was demonstrated in a water vehicle in the absence of citric acid.

The renin–angiotensin system has been implicated in several forms of hypertension.[1] Renin catalyzes the first and rate-limiting step of the cascade by cleaving the substrate angiotensinogen to form the decapeptide angiotensin I. This intermediate is further hydrolyzed by angiotensin converting enzyme to yield the biologically active octapeptide angiotensin II. This octapeptide is one of the most potent vasoconstrictors known, and it also stimulates secretion of aldosterone and catecholamines, all of which lead to elevation of blood pressure. The uniquely high enzyme specificity of renin offers the potential of selective pharmacological intervention by compounds designed to be specific inhibitors.[2] Interest in the blockade of renin has led to rapid development of potent inhibitors by the design of substrate analogues. The most successful approach has been based on the concept of transition-state analogue of the catalytic mechanism of the aspartyl protease.[3]

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