and ρ_w are the respective densities (2, 3); C_w was estimated from:

$$C_w = \frac{QP_a}{P} \qquad \text{(Eq. 3)}$$

where P_a is the apparent permeability coefficient measured in the presence of surfactant. The decrease in the permeability coefficient from 6.02×10^{-8} cm^2 sec^{-1} (Membrane A) upon addition of surfactant is assumed to be directly attributable to the disappearance of free drug. Plots of Q/C_wW_w against W_m/W_w were constructed from the experimental data (Table IV). The density of the surfactant was taken as unity. When assuming no volume change upon mixing, W_w and W_m were obtained directly. The regression coefficients of these plots are given in Table V, along with the partition coefficient estimated from Eq. 2.

The large partition coefficients found for cetrimonium bromide and sodium lauryl sulfate solutions are due to their ionic nature. At the pH of these experiments, chlordiazepoxide exists in the unionized and cationic forms. In addition to dissolution of unionized drug in the hydrocarbon interior of the micelles, reaction with the charged exterior occurs. Chlordiazepoxide cations react with lauryl sulfate anions to form ion-pairs while unionized drug forms dipole complexes with cetrimonium ions. The strong interaction of chlordiazepoxide with ionic surfactants suggests that the latter should be avoided in preparing formulations of this drug.

CONCLUSIONS

The interaction of chlordiazepoxide with talc and ionic surfactants suggests that care should be exercised in preparing formulations with these substances to avoid conditions under which absorption may be restricted by drug–excipient interaction.

The interaction of magnesium stearate with the polydimethylsiloxane membrane highlights the need for careful membrane selection. The ideal material would be a nonporous, homogeneous, polymeric membrane that contains no filler, crystallites, or other internal microphases which influence the diffusion rate or solubility of the drug in the membrane. Most commercially available, thin elastomeric membranes contain filler to impart physical strength.

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Synthesis of Substituted Piperidino Carbamides: Correlation between CNS Effects and Selective Inhibition of NAD-Dependent Oxidations

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Abstract □ Several 1-(*N*-acetyl-substituted piperidino)-3-aryl carbamides were synthesized. All carbamides possessed anticonvulsant activity, as exhibited by protection against pentylenetetrazol-induced seizures, and potentiated pentobarbital-induced hypnosis. Inhibitory effects of these carbamides on the respiratory activity revealed selective inhibition of NAD-dependent oxidation of the various substrates by the rat brain homogenate. Such a selective inhibition of respiratory activity was in no way related with the pharmacological properties of the compounds that were tested.

Keyphrases □ Carbamides of substituted piperidines—synthesis, relationship between anticonvulsant activity and inhibition of respiratory activity □ Piperidine carbamides—synthesis, relationship between anticonvulsant activity and inhibition of respiratory activity □ Anticonvulsant activity—synthesis and evaluation of 1-(*N*-acetyl-substituted piperidino)-3-aryl carbamides □ Respiratory activity—synthesis and evaluation of carbamides as inhibitors □ CNS activity—synthesis and evaluation of 1-(*N*-acetyl-substituted piperidino)-3-aryl carbamides □ Structure–activity relationships—substituted piperidino carbamides, CNS effects

Continuing interest in the synthesis of substituted carbamides (1–3) and the study of their central nervous system (CNS) effects led to the synthesis of some 1-(*N*-acetyl-substituted piperidino)-3-aryl carbamides. In the present study, the anticonvulsant activity of these compounds against pentylenetetrazol-induced seizures and their ability to potentiate pentobarbital hypnosis were determined. The *in vitro* inhibitory effects of these carbamides on the respiratory activity of the rat brain homogenate were also investigated to elucidate their biochemical mechanism of action. These carbamides were synthesized by following the methods outlined in Scheme I.

Table I—Physical Constants of 1-(*N*-Acetyl-substituted piperidino)-3-aryl Carbamides

Compound	Ar	R	Melting Point[a]	Yield, %	Molecular Formula	Analysis, %	
						Calc	Found
I	2-$CH_3C_6H_4$	2-CH_3	65°	70	$C_{16}H_{23}N_3O_2$	C 66.43	66.18
						H 7.95	8.10
						N 14.53	14.62
II	3-$CH_3C_6H_4$	2-CH_3	67°	76	$C_{16}H_{23}N_3O_2$	C 66.43	66.23
						H 7.95	7.92
						N 14.53	14.68
III	4-$CH_3C_6H_4$	2-CH_3	70°	75	$C_{16}H_{23}N_3O_2$	C 66.43	66.38
						H 7.95	7.88
						N 14.53	14.50
IV	3,4-$(CH_3)_2C_6H_3$	2-CH_3	76°	60	$C_{17}H_{25}N_3O_2$	C 67.32	67.21
						H 8.25	8.13
						N 13.86	13.69
V	2-$OCH_3C_6H_4$	2-CH_3	95°	58	$C_{16}H_{23}N_3O_3$	C 62.95	63.02
						H 7.54	7.50
						N 13.77	13.82
VI	4-$OCH_3C_6H_4$	2-CH_3	68°	70	$C_{16}H_{23}N_3O_3$	C 62.95	62.92
						H 7.54	7.33
						N 13.77	13.69
VII	2-ClC_6H_4	2-CH_3	100°	62	$C_{15}H_{20}ClN_3O_2$	C 58.15	58.38
						H 6.46	6.48
						N 13.57	13.48
VIII	2-$CH_3C_6H_4$	3-CH_3	85°	45	$C_{16}H_{23}N_3O_2$	C 66.43	66.50
						H 7.95	8.00
						N 14.53	14.43
IX	4-$CH_3C_6H_4$	3-CH_3	80°	40	$C_{16}H_{23}N_3O_2$	C 66.43	66.46
						H 7.95	7.92
						N 14.53	14.44
X	3,4-$(CH_3)_2C_6H_3$	3-CH_3	92°	50	$C_{17}H_{25}N_3O_2$	C 67.32	67.52
						H 8.25	8.30
						N 13.86	13.77
XI	2-$OCH_3C_6H_4$	3-CH_3	105°	35	$C_{16}H_{23}N_3O_3$	C 62.95	62.88
						H 7.54	7.48
						N 13.77	13.82
XII	2-ClC_6H_4	3-CH_3	94°	32	$C_{15}H_{20}ClN_3O_2$	C 58.15	58.27
						H 6.46	6.43
						N 13.57	13.44
XIII	2-$CH_3C_6H_4$	4-CH_3	82°	70	$C_{16}H_{23}N_3O_2$	C 66.43	66.48
						H 7.95	7.93
						N 14.53	14.50
XIV	3-$CH_3C_6H_4$	4-CH_3	65°	70	$C_{16}H_{23}N_3O_2$	C 66.43	66.42
						H 7.95	7.93
						N 14.53	14.39
XV	4-$CH_3C_6H_4$	4-CH_3	80°	65	$C_{16}H_{23}N_3O_2$	C 66.43	66.50
						H 7.95	7.87
						N 14.53	14.46
XVI	3,4-$(CH_3)_2C_6H_3$	4-CH_3	73°	75	$C_{17}H_{25}N_3O_2$	C 67.32	67.33
						H 8.25	8.23
						N 13.86	13.88
XVII	2-$OCH_3C_6H_4$	4-CH_3	133°	90	$C_{16}H_{23}N_3O_3$	C 62.95	62.80
						H 7.54	7.50
						N 13.77	13.86
XVIII	4-$OCH_3C_6H_4$	4-CH_3	74°	78	$C_{16}H_{23}N_3O_3$	C 62.95	62.94
						H 7.54	7.52
						N 13.77	13.76
XIX	2-ClC_6H_4	4-CH_3	90°	80	$C_{15}H_{20}ClN_3O_2$	C 58.15	58.42
						H 6.46	6.42
						N 13.57	13.48

[a] Melting points were taken in open capillary tubes and are uncorrected.

EXPERIMENTAL

Synthesis—*Aryl Carbamides*—These compounds were synthesized by treating a solution of potassium cyanate with suitable anilines (4).

1-Chloroacetyl-3-aryl Carbamides—A mixture of aryl carbamide (0.1 mole) and chloroacetyl chloride (0.11 mole) in dry benzene was refluxed for 2–4 hr. On cooling, the solid mass which separated out was filtered, washed with water, dried, and recrystallized from suitable solvents (3,5,6).

1-(*N-Acetylmethyl-substituted piperidino)-3-aryl Carbamides*—To a solution of 1-chloroacetyl-3-aryl carbamide (0.01 mole) in dry benzene was added an appropriate methylpiperidine (0.02 mole), and the reaction mixture was refluxed for 6–8 hr. On cooling, the separated hydrochlorides were removed by filtration and the filtrate was concentrated under reduced pressure. The solid mass which separated out on cooling was filtered, washed with water, dried, and recrystallized from suitable solvents. These carbamides were characterized by their sharp melting points and elemental analyses (Table I).

Pharmacological—*Toxicity*—The approximate 50% lethal dose (LD_{50}) was determined in albino mice by the method of Smith (7). All substituted carbamides were suspended in 5% aqueous gum acacia and were administered intraperitoneally to each group of 10 mice.

Anticonvulsant Activity—Anticonvulsant activity was determined in mice of either sex weighing 25–30 g. The mice were divided in groups of 10, keeping the group weights as near the same

Table II—Pharmacological Properties of 1-(*N*-Acetyl-substituted piperidino)-3-aryl Carbamides

Compound[a]	Approximate LD_{50}, mg/kg	Anticonvulsant Activity[b], % Protection	Pentylenetetrazol Mortality[c], %	Potentiation of Pentobarbital Sleeping Time[d], min	Increase Times Control
I	600	50	20	58.3 ± 5.2	1.62
II	800	30	10	44.5 ± 3.5	1.23
III	1000	50	40	48.5 ± 3.8	1.35
IV	>1000	60	10	62.8 ± 6.5	1.74
V	750	30	10	55.8 ± 5.4	1.55
VI	250	20	80	60.5 ± 2.8	1.67
VII	800	30	20	56.5 ± 6.9	1.57
VIII	>1000	60	10	49.3 ± 5.9	1.37
IX	1000	60	0	39.5 ± 2.8	1.09
X	1000	40	20	39.0 ± 2.3	1.08
XI	>1000	60	10	52.0 ± 5.4	1.44
XII	>1000	10	50	55.5 ± 6.7	1.52
XIII	1000	20	50	59.0 ± 5.3	1.64
XIV	>1000	20	50	64.5 ± 7.0	1.79
XV	>1000	20	50	72.5 ± 8.9	2.01
XVI	>1000	40	50	41.0 ± 3.0	1.14
XVII	>1000	30	30	55.7 ± 7.2	1.54
XVIII	850	50	50	48.5 ± 4.5	1.34
XIX	>1000	20	60	57.0 ± 6.4	1.58

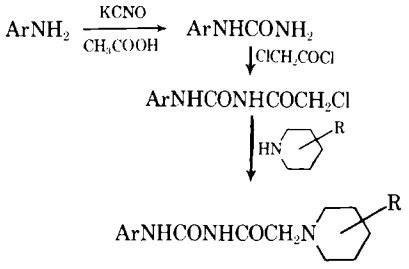
[a] Compound numbers are as reported in Table I. [b] Anticonvulsant activity was determined at a dose of 100 mg/kg ip, as described under *Experimental*. [c] Represents mortality during 24 hr in each group of animals administered pentylenetetrazol. [d] Control values for pentobarbital (40 mg/kg) sleeping time was 36 min, and test compounds were administered at a dose of 100 mg/kg ip, which was taken as 1 for comparison.

as possible. All substituted carbamides were suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). The test compound was injected in a group of 10 animals at a dose of 100 mg/kg ip. Four hours after the administration of the substituted carbamides, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol has been shown to produce convulsions in almost all untreated mice and also to exhibit 100% mortality over 24 hr. However, no mortality was observed during 24 hr in animals treated with 100 mg/kg alone of the test compounds.

The mice were observed for 60 min for the occurrence of seizures. An episode of clonic spasm that persisted for a minimum of 5 sec was considered a threshold convulsion (8). Transient intermittent jerks and tremulousness were not counted. Animals devoid of threshold convulsions during 60 min were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of these substituted carbamides was represented as percent protection. The animals were then observed for 24 hr and their mortality was recorded.

Potentiation of Sodium Pentobarbital Sleeping Time—The method of Winter (8) was followed to investigate the ability of substituted carbamides to potentiate pentobarbital-induced hypnosis. Mice weighing 20–25 g were taken in groups of six animals. One group of six was used for each compound while the other group of six served as the control. Pentobarbital, administered in a dose of 40 mg/kg ip to the control group, produced sleep. All substituted carbamides were suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). The test compounds were injected in a group of six animals at a dose of 100 mg/kg ip 30 min prior to the administration of pentobarbital. The administration time of pentobarbital in both the control and the experimental mice was recorded. The animals were observed regularly for sleep as evidenced by the observance of the loss of the righting reflex until the animal had awakened. The mean average sleeping time in each group was calculated. The degree of potentiation caused by these substituted carbamides was calculated by the total average time of the sleep observed in the experimental animals divided by the total average time of the sleep observed in the control animals.

Biochemical[1]—Male albino rats, kept on an *ad libitum* diet, were used for the assay of respiratory activity of rat brain homogenate. Rats weighing 150–200 g were sacrificed by decapitation. The brains were taken out immediately and homogenized in the



Scheme I

Table III—Effect of 1-(*N*-Acetyl-substituted piperidino)-3-aryl Carbamides on the Oxidation of Pyruvate, α-Ketoglutarate, NADH, and Succinate by Rat Brain Homogenates

Compound[a]	Inhibition of Substrate Oxidation[b], %			
	Pyruvate	α-Ketoglutarate	NADH	Succinate
I	75.9 ± 2.9	65.5 ± 2.0	43.0 ± 1.6	Nil
II	93.6 ± 2.9	83.2 ± 2.4	86.7 ± 2.1	Nil
III	81.7 ± 2.7	68.7 ± 2.8	63.4 ± 1.8	Nil
IV	79.3 ± 2.6	73.4 ± 2.7	78.4 ± 1.9	Nil
V	34.9 ± 1.9	39.9 ± 1.9	30.6 ± 2.0	Nil
VI	53.3 ± 1.4	49.0 ± 2.0	40.3 ± 2.2	Nil
VII	56.5 ± 1.4	40.5 ± 2.0	40.0 ± 1.8	Nil
VIII	46.8 ± 2.7	88.6 ± 2.9	82.3 ± 3.0	Nil
IX	87.4 ± 2.0	85.5 ± 2.6	89.3 ± 2.3	Nil
X	94.3 ± 3.0	95.4 ± 2.9	91.3 ± 3.0	Nil
XI	43.4 ± 1.7	44.1 ± 1.4	63.9 ± 1.9	Nil
XII	62.3 ± 1.9	49.0 ± 1.5	86.8 ± 2.4	Nil
XIII	57.3 ± 1.8	46.7 ± 1.5	55.0 ± 2.1	Nil
XIV	93.1 ± 3.0	94.1 ± 2.9	93.9 ± 2.8	Nil
XV	82.5 ± 2.8	84.4 ± 2.6	81.4 ± 2.4	Nil
XVI	90.0 ± 2.6	88.8 ± 2.4	85.5 ± 2.4	Nil
XVII	32.5 ± 1.9	30.7 ± 1.7	32.5 ± 1.2	Nil
XVIII	78.5 ± 2.1	53.1 ± 2.2	88.4 ± 2.9	Nil
XIX	75.2 ± 2.6	54.9 ± 2.1	80.1 ± 2.5	Nil

[a] Compound numbers are as recorded in Table I. [b] Each experiment was done in duplicate. All values represent mean values of percent inhibition with ± standard error of the mean calculated from three separate experiments. Inhibition was determined by the decrease in the oxygen uptake/125 mg wet tissue weight/hr. Assay conditions are as described in the text. All substituted carbamides were used at a final concentration of 1 m*M*. Different substrates and NADH were used at a final concentration of 10 and 0.5 m*M*, respectively.

[1] Commercial chemicals were used. Adenosine monophosphate, cytochrome c, NADH, α-ketoglutarate, sodium pyruvate, and sodium succinate were purchased from Sigma Chemical Co., St. Louis, Mo.

ratio of 1:9 (w/v) with a Potter–Elvehjem homogenizer in 0.25 *M* cold sucrose. Respiratory activity was determined by measuring the oxygen consumption by the conventional Warburg manometric method at 37° with air as the gas phase. Fresh brain homogenate of healthy albino rats, equivalent to 125 mg wet tissue weight, was used in each flask. The reaction mixture in a final volume of 3.0 ml consisted of 20 m*M* Na_2HPO_4 buffer solution (pH 7.4), 6.7 m*M* $MgSO_4$, 1 m*M* AMP (sodium salt), 33 m*M* KCl, and 500 μg of cytochrome c. The central well contained 0.2 ml of 20% KOH solution. All substituted carbamides were used in the final concentration of 1 m*M* to study their effects on the oxidation of the various substrates and NADH, which were used in a final concentration of 10 and 0.5 m*M*, respectively. The compounds were dissolved in propylene glycol (100%) and an equivalent amount of the solvent was added to the control vessels.

RESULTS AND DISCUSSION

Pharmacological properties exhibited by substituted carbamides are recorded in Table II. The approximate LD_{50} values, ranging from 250 mg/kg, reflect low toxicity for these compounds. Compound VI was the most toxic, with an approximate LD_{50} value of 250 mg/kg. As is evident from Table II, all carbamides possessed anticonvulsant activity and the protection afforded by these compounds against pentylenetetrazol-induced seizures ranged from 10 to 60%. Maximum protection was observed with carbamides having a 2-methyl, 4-methyl, or 2-methoxy substituent at the phenyl moiety at position 3 of 1-(*N*-acetyl-3-methyl)-piperidino carbamide and a 3,4-$(CH_3)_2$ substituent at the phenyl moiety at position 3 of the 1-(*N*-acetyl-2-methyl)piperidino carbamide. In the present study, compounds exhibiting 60% protection afforded greater protection from pentylenetetrazol mortality (0–10%). These results have, however, failed to indicate that greater anticonvulsant activity of these compounds is associated with a greater degree of protection against pentylenetetrazol mortality. All carbamides were found to potentiate sodium pentobarbital hypnosis in mice, where maximum potentiation was observed with the carbamide having a 4-methyl substituent at the phenyl moiety at position 3 of 1-(*N*-acetyl-4-methyl)piperidino carbamide (Compound XV). Their ability to potentiate pentobarbital-induced hypnosis ranged from 1.09 to 2.01 times that observed in normal control rats.

All carbamides were found to inhibit selectively *in vitro* NAD-dependent oxidation of pyruvate and α-ketoglutarate by rat brain homogenate whereas FAD-dependent or NAD-independent oxidation of succinate remained unaltered (Table III). These carbamides, like nitrobenzamides (9), were also found to inhibit the oxidation of NADH, indicating possible inactivation of the electron transfer process in the respiratory chain by acting presumably at the site of electron transfer from NADH to FAD. The variations in the aryl group at position 3 of these carbamides have been found to alter the ability of these carbamides to produce inhibition in the respiratory activity. Maximum inhibition of the respiratory activity was observed with Compound X while V and XVII, possessing a 2-methoxy substituent at the phenyl moiety of these carbamides, exhibited low inhibitory effects.

These results have not only failed to provide any structure–activity relationships with respect to the inhibitory effects or pharmacological properties but have also failed to show any correlation between the inhibition of respiratory activity with the ability to provide protection against pentylenetetrazol-induced seizures and to potentiate pentobarbital-induced hypnosis. Detailed study of the effects of these substituted carbamides on the activity of purified enzyme systems may possibly reflect a biochemical basis for their CNS effects.

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