Substituted Carbamides: Interrelationship between Anticonvulsant Activity and Inhibition of Nicotinamide Adenine Dinucleotide-Dependent Pyruvic Acid Oxidation

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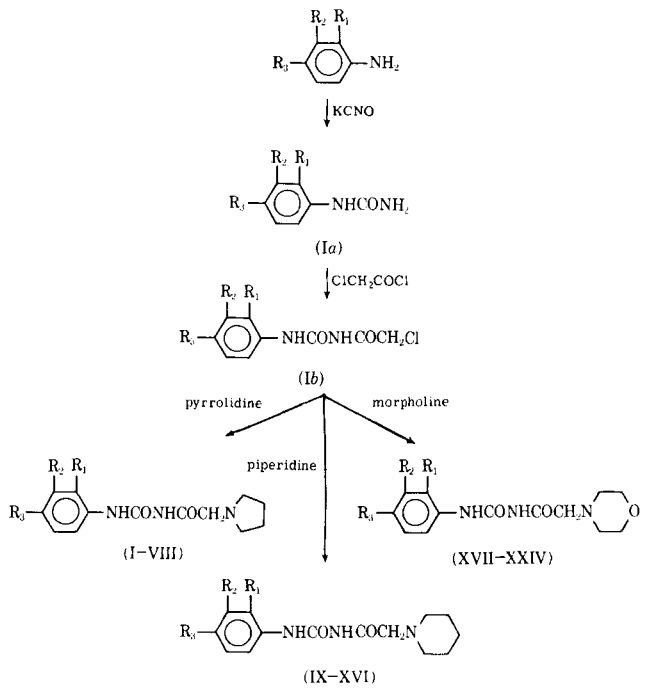
Abstract □ 1-Substituted acetyl-3-aryl carbamides were synthesized and evaluated for their ability to inhibit *in vitro* respiratory activity of mouse and rat brain homogenates. The anticonvulsant activity of these carbamides was determined against pentylenetetrazol-induced seizures to study their structure–activity relationships.

Keyphrases □ Carbamides, 1-substituted acetyl-3-aryl—synthesis, *in vitro* (mouse and rat brain homogenates) inhibition of respiratory activity, *in vivo* anticonvulsant activity □ Inhibition of pyruvic acid oxidation in absence/presence of nicotinamide adenine dinucleotide—1-substituted acetyl-3-aryl carbamides, correlated to anticonvulsant activity □ Anticonvulsant activity—1-substituted acetyl-3-aryl carbamides, correlated to inhibition of nicotinamide adenine dinucleotide-dependent pyruvic acid oxidation

The ability of aryloxy alkyl carbamides to exhibit a profound effect on CNS activity (1) and the ability of 1-carbamoylpyrrolidines and 1-carbamoylpiperidines to possess muscle relaxant and anticonvulsant properties (2) prompted the synthesis of carbamides having pyrrolidine, piperidine, and morpholine substituents. Inhibitory effects of these carbamides have reflected inhibition of nicotinamide adenine dinucleotide-dependent oxidation of pyruvic acid. In the present study, attempts were made to correlate anticonvulsant properties exhibited by these carbamides with their enzyme inhibitory properties. The various substituted carbamides were synthesized following the steps shown in Scheme I.



Scheme I

1-Substituted acetyl-3-aryl carbamides (I–XXIV) were synthesized by the reaction of 1-chloroacetyl-3-aryl carbamides (*Ib*) with appropriate secondary amines. 1-Chloroacetyl-3-aryl carbamides (*Ib*) were prepared by the action of chloracetyl chloride on aryl carbamides (*Ia*).

EXPERIMENTAL

Analyses for carbon, hydrogen, and nitrogen were performed. Melting points were taken in open capillary tubes and were corrected.

Aryl Carbamides (*Ia*)—These carbamides were synthesized according to a known procedure (3) by treating a solution of potassium cyanate with substituted anilines.

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

1-Substituted Acetyl-3-aryl Carbamides (I–XXIV)—A mixture of 1-chloroacetyl-3-aryl carbamide (*Ib*) (0.01 mole) and an appropriate secondary amine (0.02 mole) in 25 ml. of dry benzene was refluxed for 4–5 hr. The reaction mixture was cooled and filtered. The filtrate was concentrated under reduced pressure. The solid which separated out on cooling was collected by filtration, washed several times with water, dried, and recrystallized from ethanol. These carbamides were characterized by their melting points and elemental analyses (Tables I–III).

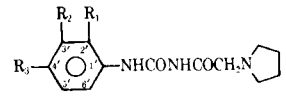
BIOCHEMICAL STUDIES[1]

Assay of Respiratory Activity of Mice and Rat Brain Homogenates—Respiratory activity was determined by measuring oxygen consumption by the conventional Warburg manometric method at 37°, with air as the gas phase. Fresh brain homogenate of healthy mice and albino rats, equivalent to 125 mg. wet weight of brain, was used in each flask. Homogenates were prepared in ice-cold 0.25 *M* sucrose. The reaction mixture in a final volume of 3.0 ml. consisted of 20 m*M* Na_2HPO_4 buffer solution of pH 7.4, 6.7 m*M* $MgSO_4$, 1 m*M* adenosine monophosphate (sodium salt), 33 m*M* KCl, and 500 mcg. cytochrome c. The central well contained 0.2 ml. of 20% KOH solution. The final concentrations of nicotinamide adenine dinucleotide, sodium pyruvate, and carbamides used were 0.5, 10, and 2 m*M*, respectively.

Determination of 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 equal as possible. Carbamides (100 mg./kg.) were injected intraperitoneally in a 5% aqueous suspension of acacia to one group of 10 animals. Pentylenetetrazol (80 mg./kg.) was injected subcutaneously 4 hr. after the administration of carbamides. This dose of pentylenetetrazol has been found to cause convulsions in almost all normal mice. The occurrence of seizures was observed for the

[1] Commercial chemicals were used in the present study. Adenosine monophosphate, cytochrome c, and nicotinamide adenine dinucleotide were obtained from Sigma Chemical Co., St. Louis, Mo.; sodium pyruvate was obtained from E. Merck, Darmstadt, West Germany; and other common chemicals were purchased from British Drug House, Bombay, India.

Table I—1-(*N*-Acetylpyrrolidino)-3-aryl Carbamides



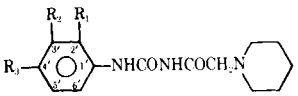
Compound Number	R_1	R_2	R_3	Melting Point	Yield, %	Formula	Analysis, % Calc.	Found
I	H	H	H	100°	60	$C_{13}H_{17}N_3O_2$	C 63.15	63.28
							H 6.88	6.62
							N 17.00	16.83
II	CH_3	H	H	167°	52	$C_{14}H_{19}N_3O_2$	C 64.37	64.18
							H 7.27	7.42
							N 16.09	15.83
III	H	CH_3	H	58°	62	$C_{14}H_{19}N_3O_2$	C 64.37	64.65
							H 7.27	6.98
							N 16.09	15.74
IV	H	H	CH_3	135°	65	$C_{14}H_{19}N_3O_2$	C 64.37	64.42
							H 7.27	7.18
							N 16.09	16.12
V	H	CH_3	CH_3	96°	60	$C_{15}H_{21}N_3O_2$	C 65.45	65.27
							H 7.63	7.82
							N 15.27	15.05
VI	OCH_3	H	H	128°	55	$C_{14}H_{19}N_3O_3$	C 60.65	60.20
							H 6.85	6.62
							N 15.16	14.86
VII	H	H	OCH_3	104°	62	$C_{14}H_{19}N_3O_3$	C 60.65	60.72
							H 6.85	6.73
							N 15.16	14.96
VIII	Cl	H	H	180°	52	$C_{13}H_{16}ClN_3O_2$	C 55.41	55.68
							H 5.68	5.54
							N 14.92	15.06

next 60 min. An episode of clonic spasm that persisted for at least 5 sec. was considered a threshold convulsion. Transient intermittent jerks of tremulousness were not taken into account. Animals not exhibiting even threshold convulsions during a 60 min. were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of these carbamides was represented as percent protection. The mortality of these animals was recorded after 24 hr. to obtain an idea of the ability of these carbamides to protect against pentylenetetrazol-induced death.

RESULTS AND DISCUSSION

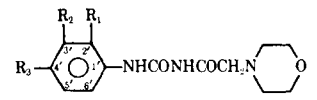
As is evident from Tables IV and V, all carbamides were found to inhibit *in vitro* nicotinamide adenine dinucleotide-dependent oxidation of pyruvic acid, using mice and rat brain homogenates as the source of the enzyme systems. The oxygen uptake in the absence of carbamides was found to be greater with mouse brain as compared with that of rat brain homogenate. Addition of nicotinamide adenine dinucleotide in both these cases was found to increase the respiratory activity of these homogenates during oxidation of pyruvic acid. Addition of nicotinamide adenine dinucleotide caused significant decreases in the degree of inhibition of nicotinamide adenine dinucleotide-dependent oxidation of pyruvic acid, as has been reported earlier with quinazolones (6, 7), β-aminoketones (8), and thiazolidones (9). These studies provided further evidence that inhibition of nicotinamide adenine dinucleotide-dependent oxidations could presumably account for the interference of these carbamides somewhere in the electron transport chain during

Table II—1-(*N*-Acetylpiperidino)-3-aryl Carbamides



Compound Number	R_1	R_2	R_3	Melting Point	Yield, %	Formula	Analysis, % Calc.	Found
IX	H	H	H	88°	64	$C_{14}H_{19}N_3O_2$	C 69.37	69.02
							H 7.27	7.42
							N 16.09	15.92
X	CH_3	H	H	80°	60	$C_{15}H_{21}N_3O_2$	C 65.45	65.28
							H 7.63	7.82
							N 15.27	14.96
XI	H	CH_3	H	54°	68	$C_{15}H_{21}N_3O_2$	C 65.45	65.68
							H 7.63	7.48
							N 15.27	15.02
XII	H	H	CH_3	102°	70	$C_{15}H_{21}N_3O_2$	C 65.45	65.37
							H 7.63	7.82
							N 15.27	14.98
XIII	H	CH_3	CH_3	112°	70	$C_{16}H_{23}N_3O_2$	C 66.43	66.28
							H 7.95	8.21
							N 14.53	14.38
XIV	OCH_3	H	H	114°	62	$C_{15}H_{21}N_3O_3$	C 61.82	62.16
							H 7.21	6.98
							N 14.43	14.24
XV	H	H	OCH_3	90°	69	$C_{15}H_{21}N_3O_3$	C 61.85	61.63
							H 7.21	7.46
							N 14.43	14.35
XVI	Cl	H	H	85°	58	$C_{14}H_{18}ClN_3O_2$	C 56.85	57.13
							H 6.09	5.87
							N 14.21	13.98

Table III—1-(*N*-Acetylmorpholino)-3-aryl Carbamides



Compound Number	R_1	R_2	R_3	Melting Point	Yield, %	Formula	Analysis, % Calc.	Found
XVII	H	H	H	134°	62	$C_{13}H_{17}N_3O_3$	C 59.31	59.17
							H 6.46	6.38
							N 15.96	16.25
XVIII	CH_3	H	H	126°	55	$C_{14}H_{19}N_3O_3$	C 60.65	61.02
							H 6.85	6.63
							N 15.16	14.92
XIX	H	CH_3	H	70°	65	$C_{14}H_{19}N_3O_3$	C 60.65	60.43
							H 6.85	6.72
							N 15.16	15.28
XX	H	H	CH_3	127°	68	$C_{14}H_{19}N_3O_3$	C 60.45	60.30
							H 6.85	7.15
							N 15.16	14.98
XXI	H	CH_3	CH_3	128°	66	$C_{15}H_{21}N_3O_3$	C 61.85	62.02
							H 7.21	7.08
							N 14.43	14.26
XXII	OCH_3	H	H	175°	58	$C_{14}H_{19}N_3O_4$	C 57.33	57.62
							H 6.48	6.26
							N 14.33	14.13
XXIII	H	H	OCH_3	114°	66	$C_{14}H_{19}N_3O_4$	C 57.33	57.18
							H 6.48	6.56
							N 14.33	14.25
XXIV	Cl	H	H	130°	55	$C_{13}H_{16}ClN_3O_3$	C 52.43	52.27
							H 5.37	5.46
							N 14.11	13.95

utilization of nicotinamide adenine dinucleotide as a cofactor in these enzyme systems.

Methyl substitution at various positions of the phenyl nucleus attached to the 3-position of these pyrrolidino, piperidino, and morpholino carbamides was found to play a definite role in influencing their ability to inhibit nicotinamide adenine dinucleotide-dependent oxidations of pyruvic acid. In all these carbamides, the presence of a methyl substituent at the 3′-position of the phenyl moiety (III, XI, and XIX) was found to exhibit maximum inhibition while such a methyl substituent at the 2′-position (II, X, and XVIII) or 4′-position (IV, XII, and XX) decreased the degree of enzyme inhibition; the relative importance of the 3′-position of the phenyl moiety was reflected by an increase in the degree of inhibition of respiratory activity by the introduction of a methyl substituent

Table IV—Inhibition of Pyruvic Acid Oxidation by 1-Substituted Acetyl-3-aryl Carbamides Using Mice Brain Homogenate[a]

Compound Number	Inhibition, % Absence of Nicotinamide Adenine Dinucleotide	Presence of Nicotinamide Adenine Dinucleotide
I	50.83 ± 0.92	31.78 ± 0.78
II	26.64 ± 0.76	15.92 ± 0.63
III	58.77 ± 1.34	42.24 ± 1.63
IV	36.78 ± 0.59	22.90 ± 0.52
V	61.77 ± 1.06	45.65 ± 0.88
VI	35.29 ± 0.54	23.67 ± 0.59
VII	41.07 ± 0.89	29.23 ± 0.63
VIII	72.98 ± 2.20	59.57 ± 1.46
IX	77.38 ± 2.15	51.20 ± 0.76
X	70.49 ± 1.89	56.22 ± 1.38
XI	81.33 ± 2.05	51.21 ± 2.12
XII	41.84 ± 1.76	25.96 ± 0.67
XIII	72.32 ± 0.98	44.19 ± 1.12
XIV	14.27 ± 0.75	10.92 ± 0.49
XV	29.39 ± 0.67	9.35 ± 0.56
XVI	75.53 ± 0.98	62.08 ± 2.25
XVII	62.19 ± 0.79	46.49 ± 0.88
XVIII	56.60 ± 0.98	43.78 ± 0.92
XIX	78.91 ± 1.88	60.92 ± 1.38
XX	59.77 ± 0.58	47.68 ± 0.78
XXI	66.32 ± 2.46	51.07 ± 0.58
XXII	20.94 ± 0.52	14.28 ± 0.60
XXIII	34.82 ± 0.74	21.38 ± 0.55
XXIV	79.17 ± 2.90	68.56 ± 1.87

[a] Each experiment was done in duplicate. The oxygen uptake in control experiments was 189.81 ± 2.56 μl. in the absence of nicotinamide adenine dinucleotide and 209.60 ± 2.87 μl. in the presence of nicotinamide adenine dinucleotide during 60 min. All values represent mean values of percent inhibition, with standard error (*SE*) calculated from two separate experiments. Inhibition was determined by the decrease in the oxygen uptake per 125 mg. wet weight of tissue per hr. Assay conditions are as indicated in the text.

Table V—Inhibition of Pyruvic Acid Oxidation by 1-Substituted Acetyl-3-aryl Carbamides Using Rat Brain Homogenate[a]

Compound Number	Inhibition, % Absence of Nicotinamide Adenine Dinucleotide	Presence of Nicotinamide Adenine Dinucleotide
I	45.36 ± 1.25	25.99 ± 0.98
II	36.34 ± 0.92	24.23 ± 1.75
III	55.96 ± 0.74	39.00 ± 1.82
IV	47.31 ± 2.12	31.90 ± 2.12
V	74.45 ± 1.85	54.17 ± 1.09
VI	39.18 ± 0.90	29.34 ± 0.89
VII	42.04 ± 0.64	36.50 ± 0.87
VIII	79.20 ± 2.00	65.35 ± 1.02
IX	72.67 ± 1.85	50.62 ± 2.50
X	82.06 ± 0.88	71.31 ± 1.85
XI	82.93 ± 2.25	73.87 ± 1.25
XII	45.34 ± 1.45	40.56 ± 1.84
XIII	65.62 ± 1.25	54.12 ± 0.98
XIV	34.96 ± 0.99	16.68 ± 1.09
XV	60.51 ± 0.85	48.14 ± 0.88
XVI	79.88 ± 1.25	75.46 ± 0.74
XVII	61.12 ± 0.85	48.61 ± 0.65
XVIII	54.74 ± 0.55	45.97 ± 0.86
XIX	76.52 ± 0.45	59.43 ± 1.35
XX	62.65 ± 0.92	55.35 ± 1.40
XXI	71.27 ± 1.25	56.28 ± 0.95
XXII	25.61 ± 0.86	21.37 ± 0.45
XXIII	31.23 ± 0.77	24.77 ± 0.25
XXIV	84.30 ± 2.80	83.12 ± 2.85

[a] Each experiment was done in duplicate. The oxygen uptake in control experiments was 134.40 ± 2.06 μl. in the absence of nicotinamide adenine dinucleotide and 144.00 ± 2.28 μl. in the presence of nicotinamide adenine dinucleotide during 60 min. All values represent mean values of percent inhibition, with standard error (*SE*) calculated from two separate experiments. Inhibition was determined by the decrease in the oxygen uptake per 125 mg. wet weight of tissue per hr. Assay conditions are as indicated in the text.

Table VI—Anticonvulsant Activity of 1-Substituted Acetyl-3-aryl Carbamides

Compound Number	Anticonvulsant Activity[a], %	Mortality[b], %
I	10	10
II	40	10
III	70	30
IV	70	10
V	30	10
VI	30	60
VII	10	60
VIII	10	40
IX	30	20
X	30	40
XI	80	20
XII	40	10
XIII	40	40
XIV	30	30
XV	0	50
XVI	20	40
XVII	0	30
XVIII	30	40
XIX	60	30
XX	50	0
XXI	40	20
XXII	30	20
XXIII	20	70
XXIV	0	70

[a] Anticonvulsant activity was determined as described in the *Experimental* section. [b] Represents mortality in each group of animals administered pentylenetetrazol during the 24-hr. period.

at 3′-position of the 4′-methyl-substituted carbamides (V, XIII, and XXI). Substituted carbamides having a methyl substituent at the 4′-position (IV and XX) were found to have greater inhibition as compared to corresponding 2′-methyl-substituted derivatives (II and XVIII), except with the piperidino carbamides where a 2′-methyl-substituted derivative (X) afforded greater inhibition. Unlike methyl-substituted derivatives, introduction of a methoxy substituent at the 4′-position of the phenyl group (VII, XV, and XXIII) resulted in comparatively greater inhibition to that of carbamides possessing a methoxy substituent at the 2′-position (VI, XIV, and XXII). The presence of an electronegative chloro substituent at the 2′-position of the phenyl moiety (VIII, XVI, and XXIV) was found to result in a marked increase in the inhibitory properties of these carbamides. Such a chloro substituent at the 2′-position of the phenyl nucleus exhibited maximum inhibition of pyruvic acid oxidation with both mouse brain and rat brain homogenates in pyrrolidino (VIII) and morpholino carbamides (XXIV).

As is evident from Table VI, all carbamides exhibited anticonvulsant activity, where protection against pentylenetetrazol-induced seizures ranged from 10 to 80%. 3′-Methylphenyl-substituted carbamides were found to exhibit protection that was 70% with pyrrolidino carbamide (III), 80% with piperidino carbamide (XI), and 60% with morpholino carbamide (XIX). Such protection with 3′-methyl-substituted carbamides was found to be comparable with their ability to inhibit nicotinamide adenine dinucleotide-dependent oxidations (Tables IV and V). On the other hand, carbamides possessing a methoxy substituent (VI, VII, XIV, XV, XXII, and XXIII) were able to afford only a low degree of protection. Carbamides having an unsubstituted phenyl group at the 3-position (I, IX, and XVII) had lower, or were almost devoid of, anticonvulsant activity. These results indicated that introduction of electron-pushing methyl or methoxy groups in the phenyl moiety resulted surprisingly in increased anticonvulsant activities. On the other hand, no change in anticonvulsant activity was observed when an electron-pulling chloro substituent was introduced at the 2′-position of the phenyl group of these carbamides (VIII, XVI, and XXIV).

As is evident from Table VI, all substituted carbamides having a methyl substituent in the phenyl nucleus (II–V, X–XIII, and XVIII–XXI) not only elicited a higher degree of protection against pentylenetetrazol-induced seizures but also exhibited lower mortalities. These studies have, however, failed to represent inhibition of nicotinamide adenine dinucleotide-dependent oxidation as a biochemical basis of the anticonvulsant activity of these 1-substituted acetyl-3-aryl carbamides.

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