crystallized twice from aetone and then twice from 95% ethanol to yield white needles, mp 244–246° [lit. (5) mp 260–262° for 29-norlupan-20-one-3β-yl acetate [lit. (5) mp 249–251° for 29-nor-19α-H-lupan-20-one-3β-yl acetate]. The product (0.57 g, 0.0012 mole) was obtained in a 27.0% yield.

The IR spectrum indicated loss of the peaks corresponding to the vinylidene group at 3100, 1650, and 880 cm^{-1}. Carbonyl absorption at 1700 cm^{-1} was observed.

Anal.—Calc. for $C_{31}H_{50}O_3$: C, 79.15; H, 10.64. Found: C, 79.01; H, 10.50.

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Synthesis of Substituted Benzylidinohydrazines and Their Monoamine Oxidase Inhibitory and Anticonvulsant Properties

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Abstract □ Some N^1-(4-acetamidobenzoyl)-N^2-(substituted phenyl carboxylate)benzylidinohydrazines were synthesized, characterized, and evaluated for their ability to inhibit monoamine oxidase *in vitro*. All substituted benzylidinohydrazines inhibited monoamine oxidase activity of rat brain homogenates. These compounds possessed anticonvulsant activity, which was reflected by the protection afforded against pentylenetetrazol-induced convulsions; they also potentiated pentobarbital-induced hypnosis in mice. Monoamine oxidase inhibitory effectiveness of these substituted benzylidinohydrazines was unrelated to their anticonvulsant activity and their ability to potentiate pentobarbital-induced hypnosis.

Keyphrases □ Benzylidinohydrazines—synthesis, characterization, and *in vitro* inhibition of monoamine oxidase □ Structure–activity relationships—benzylidinohydrazines, anticonvulsant activity and inhibition of monoamine oxidase activity of rat brain homogenates □ Monoamine oxidase inhibitors—synthesis of benzylidinohydrazines □ Anticonvulsant activity—benzylidinohydrazines

Many hydrazine derivatives are monoamine oxidase inhibitors (1). Such enzyme inhibitors have been shown to possess anticonvulsant properties (2). Furthermore, psychotropic (3) and anticonvulsant (4) properties exhibited by benzylidine derivatives led to the synthesis of N^1-(4-acetamidobenzoyl)-N^2-(substituted phenyl carboxylate)benzylidinohydrazines. The ability of these benzylidinohydrazines to inhibit monoamine oxidase activity of rat brain homogenates was investigated in an attempt to correlate enzyme inhibitory effectiveness with anticonvulsant activity and ability to potentiate pentobarbital-induced hypnosis.

EXPERIMENTAL

Chemistry—*Ethyl 4-Aminobenzoate*—Ethyl 4-aminobenzoate was prepared by the esterification of 4-aminobenzoic acid by the method reported earlier, mp 91° (5).

Ethyl 4-Acetamidobenzoate—In a conical flask containing water, 18.3 ml of concentrated hydrochloric acid and 35 g of ethyl 4-aminobenzoate (0.22 mole) were introduced with stirring. To this solution, 256 ml of distilled acetic anhydride was added. The reaction mixture was added to a solution of 33 g of sodium acetate in 100 ml of water and stirred vigorously. On cooling, the solid that separated was filtered, washed, dried, and recrystallized from ethanol, mp 117°.

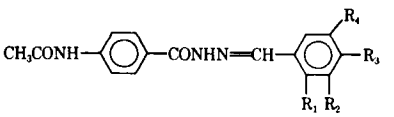
(4-Acetamidobenzoyl)hydrazine—A mixture of ethyl 4-acetamidobenzoate (0.4 mole) and 0.4 mole of hydrazine hydrate (99–100%) in absolute ethanol was refluxed on a steam bath for 15 hr. Excess ethanol was distilled, and the hydrazine that separated on cooling was filtered and recrystallized from ethanol, mp 277°.

N^1-*(4-Acetamidobenzoyl)*-N^2-*substituted Benzylidinohydrazines*—A mixture of 4-acetamidobenzoylhydrazine (0.1 mole) and a suitable salicylaldehyde (0.1 mole) in ethanol with a few drops of acetic acid was refluxed on a steam bath for 4–5 hr. Excess ethanol was removed by distillation. The solid mass that separated was collected by filtration, washed with water, dried, and recrystallized from ethanol. These compounds were characterized by their sharp melting points and elemental analyses (Table I).

N^1-*(4-Acetamidobenzoyl)*-N^2-*(aryl-substituted Phenyl Carboxylate)benzylidinohydrazines*—N^1-(4-Acetamidobenzoyl)-N^2-substituted benzylidinohydrazine (0.025 mole) in dry benzene (20 ml) was mixed with the appropriate benzoyl chloride, and the resulting mixture was refluxed on a steam bath for 5–6 hr. Excess benzene was removed by distillation, and the crude product that separated on cooling was filtered, washed first with sodium bicarbonate and then with water, and recrystallized from ethanol. These substituted benzylidinohydrazines, characterized by their sharp melting points and elemental analyses, are recorded in Table II.

Determination of Monoamine Oxidase Activity—Male rats weighing 150–200 g were killed by decapitation. Brains were quick-

Table I—Physical Constants of N^1-(4-Acetamidobenzoyl)-N^2-substituted Benzylidinohydrazines



Compound	R_1	R_2	R_3	R_4	Melting Point[a]	Yield, %	Molecular Formula[b]	Analysis, % Calc.	Found
I	H	H	OH	OCH_3	255°	80	$C_{17}H_{17}N_3O_4$	C 62.38	62.36
								H 5.20	5.22
								N 12.84	12.88
II	OH	H	H	H	275°	65	$C_{16}H_{15}N_3O_3$	C 64.64	64.53
								H 5.05	5.00
								N 14.14	14.20
III	H	OH	H	H	285°	70	$C_{16}H_{15}N_3O_3$	C 64.64	64.49
								H 5.05	5.20
								N 14.14	14.18
IV	H	H	OH	H	300°	85	$C_{16}H_{15}N_3O_3$	C 64.64	64.70
								H 5.05	5.10
								N 14.14	14.10

[a] Melting points were taken in open capillary tubes and are uncorrected. [b] All compounds were recrystallized from ethanol.

ly removed and homogenized[1] in ice-cold 0.25 *M* sucrose (2%, w/v). The reaction mixture (final concentration) consisted of 0.5 ml of phosphate buffer (0.2 *M*, pH 7.5), 1×10^{-4} *M* kynuramine, 0.5 ml of brain homogenate (equivalent to 10 mg of wet weight of the tissue), and water to a total volume of 3 ml. The monoamine oxidase activity of brain homogenates was determined after incubation at 37° in air for 30 min (6).

The various substituted benzylidinohydrazines were added to the brain homogenate to produce a final concentration of 5×10^{-5} and 1×10^{-4} *M* and incubated for 10 min before adding kynuramine. The mixture was then incubated for an additional 30 min. The reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid (w/v), and the precipitated proteins were removed by centrifugation.

Suitable 1-ml aliquots of the supernatant solution were taken in 2 ml of 1 *N* NaOH and were assayed spectrophotofluorometrically[2] for 4-hydroxyquinoline content. An increase in absorbance provided a direct measurement of 4-hydroxyquinoline formation, which was taken as an index of the enzyme activity. The percent inhibition was calculated from the decrease observed in absorbance, and this value provided an index of the inhibitory property of these substituted benzylidinohydrazines.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in mice of either sex weighing 25–30 g. The mice were divided into groups of 10, keeping the group weights as nearly the same as possible. All benzylidinohydrazines 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 10 animals at a dose of 100 mg/kg ip. Four hours later, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol has been shown to produce convulsions in most untreated mice and was also found to produce 100% mortality during a 24-hr period.

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 (7). Transient intermittent jerks and tremulousness were not counted. Animals devoid of threshold convulsions during the 60-min period were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of the benzylidinohydrazines was represented as percent protection. The mice were then observed for 24 hr and mortality was recorded.

Potentiation of Pentobarbital (Sodium) Sleeping Time—The method of Winter (8) was followed to investigate the ability of benzylidinohydrazines to potentiate pentobarbital-induced hypnosis. Mice weighing 20–25 g were divided into groups of six animals. One group of six animals was used for each compound, while another group of six mice served as the control. Pentobarbital, when administered in a dose of 40 mg/kg ip to the control group, was found to produce sleep. All benzylidinohydrazines were suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v) and were injected at a dose of 100 mg/kg ip 30 min prior to the administration of pentobarbital.

The animals were observed for sleep as evidenced by loss of the righting reflex. The degree of potentiation produced by these benzylidinohydrazines was calculated by the total average time of sleep observed in experimental animals divided by the total average time of sleep observed in control animals.

Toxicity—The approximate LD_{50} value for each benzylidinohydrazine was determined in albino mice by the method reported by Smith (9). The test compounds, suspended in 5% aqueous gum acacia, were administered intraperitoneally to groups of 10 mice.

RESULTS AND DISCUSSION

Monoamine oxidase inhibitory effectiveness of benzylidinohydrazines at the final concentrations of 1×10^{-4} and 5×10^{-5} *M*, using kynuramine as the substrate and rat brain homogenates as the source of the enzyme, are recorded in Table III. All evaluated compounds possessed monoamine oxidase inhibitory activity. Maximum inhibition was observed with the compound possessing a 3-nitrophenyl carboxylate group at position R_1 of the benzylidine moiety (Compound X). The presence of a 4-acetamidophenyl carboxylate group at R_1, R_2, or R_3 of the benzylidine moiety was found to result in a relatively lower degree of monoamine oxidase inhibition when compared to other substituted phenyl carboxylates.

As is evident from Table III, benzylidinohydrazines at a dose of 100 mg/kg possessed anticonvulsant activity against pentylenetetrazol-induced convulsions; the degree of protection ranged from 10 to 50%. Maximum protection was observed with the compounds having a 3-nitrophenyl carboxylate group (Compund VI) or an acetyl salicylate group (Compound XVIII) at R_3 of the benzylidine moiety of these hydrazines. Benzylidinohydrazines having a 4-acetamidophenyl carboxylate group at R_2 or R_3 (Compounds XV and XIX) possessed weak anticonvulsant activity.

The degree of protection afforded by these compounds against pentylenetetrazol-induced death during 24 hr in mice was unrelated to their anticonvulsant activity (Table III). The low toxicity of these compounds in albino mice was reflected by their high approximate LD_{50} values, which were 1000 mg/kg or greater on intraperitoneal administration.

All evaluated benzylidinohydrazines potentiated sleeping time induced by pentobarbital in mice at a dose of 100 mg/kg (Table III). Benzylidinohydrazines having a phenylcarboxyl, 4-acetamidophenyl carboxylate, or acetyl salicylate group at R_3 with a methoxy substituent at R_4 and a 3-nitrophenyl carboxylate group at R_3 of the benzylidine moiety prolonged the sleeping time twice that observed with the administration of pentobarbital alone.

[1] Potter–Elvehjem.
[2] Aminco-Bowman spectrophotofluorometer.

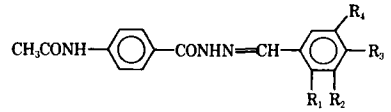


Table II—Physical Constants of N^2-(4-Acetamidobenzoyl)-N^2-(aryl-substituted Phenyl Carboxylate)benzylidinohydrazines

Compound	R_1	R_2	R_3	R_4	Melting Point[a]	Yield, %	Molecular Formula[b]	Analysis, %	
								Calc.	Found
V	H	H	$OOCC_6H_5$	OCH_3	240°	80	$C_{24}H_{21}N_3O_5$	C 66.82	66.78
								H 4.87	4.82
								N 9.74	9.62
VI	H	H	3-$(NO_2)C_6H_4OCO$	OCH_3	243°	60	$C_{24}H_{20}N_4O_7$	C 60.50	60.47
								H 4.20	4.18
								N 11.76	11.82
VII	H	H	4-$(NHCOCH_3)C_6H_4OCO$	OCH_3	125°	85	$C_{26}H_{24}N_4O_6$	C 63.93	63.78
								H 4.92	4.88
								N 11.47	11.52
VIII	H	H	2-$(OCOCH_3)C_6H_4OCO$	OCH_3	215°	60	$C_{26}H_{23}N_3O_7$	C 63.80	63.77
								H 4.70	4.73
								N 8.59	8.61
IX	$OOCC_6H_5$	H	H	H	263°	65	$C_{23}H_{19}N_3O_4$	C 68.83	68.82
								H 4.74	4.73
								N 10.47	10.51
X	3-$(NO_2)C_6H_4OCO$	H	H	H	218°	68	$C_{23}H_{18}N_4O_6$	C 61.88	61.92
								H 4.04	4.21
								N 12.56	12.40
XI	4-$(NHCOCH_3)C_6H_4OCO$	H	H	H	260°	72	$C_{25}H_{22}N_4O_5$	C 65.50	65.70
								H 4.80	4.77
								N 12.23	12.20
XII	2-$(OCOCH_3)C_6H_4OCO$	H	H	H	250°	68	$C_{25}H_{21}N_3O_6$	C 65.36	65.32
								H 4.58	4.47
								N 9.15	9.10
XIII	H	$OOCC_6H_5$	H	H	275°	75	$C_{23}H_{19}N_3O_4$	C 68.83	68.88
								H 4.74	4.80
								N 10.47	10.52
XIV	H	3-$(NO_2)C_6H_4OCO$	H	H	268°	59	$C_{23}H_{18}N_4O_6$	C 61.88	61.73
								H 4.04	4.00
								N 12.56	12.62
XV	H	4-$(NHCOCH_3)C_6H_4OCO$	H	H	265°	78	$C_{25}H_{22}N_4O_5$	C 65.50	65.30
								H 4.80	4.72
								N 12.23	12.42
XVI	H	2-$(OCOCH_3)C_6H_4OCO$	H	H	266°	65	$C_{25}H_{21}N_3O_6$	C 65.36	65.33
								H 4.58	4.56
								N 9.15	9.20
XVII	H	H	$OOCC_6H_5$	H	275°	75	$C_{23}H_{19}N_3O_4$	C 68.83	68.66
								H 4.74	4.71
								N 10.47	10.58
XVIII	H	H	3-$(NO_2)C_6H_4OCO$	H	280°	70	$C_{23}H_{18}N_4O_6$	C 61.88	61.90
								H 4.04	3.98
								N 12.56	12.50
XIX	H	H	4-$(NHCOCH_3)C_6H_4OCO$	H	270°	80	$C_{25}H_{22}N_4O_5$	C 65.50	65.38
								H 4.80	4.76
								N 12.23	12.30
XX	H	H	2-$(OCOCH_3)C_6H_4OCO$	H	276°	63	$C_{25}H_{21}N_3O_6$	C 65.36	65.25
								H 4.58	4.62
								N 9.16	9.12

[a] Melting points were taken in open capillary tubes and are uncorrected. [b] All compounds were recrystallized from ethanol.

Table III—Monoamine Oxidase Inhibitory and Pharmacological Properties of N^1-(4-Acetylaminobenzoyl)-N^2-(substituted Phenyl Carboxylate)benzylidinohydrazines

Compound	Monoamine Oxidase Inhibition[a], %		Approximate LD_{50}, mg/kg	Anticonvulsant Activity[b], % Protection	Pentylenetetrazol Mortality[c], %	Potentiation of Pentobarbital Sleeping Time, × Control[d]
	5×10^{-5} *M*	1×10^{-4} *M*				
V	16.7 ± 0.8	36.7 ± 0.5	>1000	20	50	2.2
VI	31.7 ± 0.9	57.9 ± 0.8	>1000	50	30	1.7
VII	15.4 ± 0.7	36.7 ± 0.6	>1000	30	40	2.1
VIII	19.2 ± 0.6	52.9 ± 1.1	>1000	30	30	2.1
IX	67.9 ± 1.2	81.7 ± 2.1	>1000	30	50	1.5
X	81.7 ± 1.1	92.9 ± 2.0	1000	30	50	2.3
XI	45.4 ± 1.0	65.4 ± 1.5	1000	30	50	1.6
XII	77.9 ± 1.0	84.2 ± 2.2	1000	30	50	1.9
XIII	19.2 ± 0.7	55.4 ± 2.4	>1000	40	40	1.7
XIV	30.4 ± 0.7	52.9 ± 1.9	>1000	40	50	2.0
XV	22.9 ± 0.6	26.7 ± 0.7	>1000	10	60	1.6
XVI	20.4 ± 0.8	30.4 ± 0.5	>1000	30	50	1.8
XVII	32.9 ± 0.9	50.4 ± 1.1	>1000	30	50	1.7
XVIII	33.7 ± 0.7	57.9 ± 1.3	>1000	50	20	2.1
XIX	27.9 ± 0.6	50.2 ± 0.9	>1000	10	90	2.0
XX	54.2 ± 0.8	71.7 ± 2.0	>1000	50	50	1.4

[a] 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. Assay procedure and the contents of the reaction mixture are as described in the text. All compounds were dissolved in propylene glycol (100%), and an equivalent amount of propylene glycol was added to the control tubes containing kynuramine alone in the absence of the test compounds. [b] Anticonvulsant activity was determined at a dose of 100 mg/kg as described in the *Experimental* section. [c] Represents mortality during 24 hr in each group of animals administered pentylenetetrazol. [d] Control value for pentobarbital (40 mg/kg) sleeping time was 29 min, which was taken as 1 for evaluation of the ability of substituted benzylidinohydrazines to potentiate pentobarbital sleeping time.

These observations did not provide a correlation between the monoamine oxidase inhibitory effectiveness of these benzylidinohydrazines and their anticonvulsant activity or their ability to potentiate pentobarbital-induced hypnosis. Detailed pharmacological studies of the effects of these benzylidinohydrazines may possibly reflect a basis for their ability to inhibit monoamine oxidase.

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