Anticonvulsant Activity and Succinate Dehydrogenase Inhibitory Property of New Substituted Thiobarbiturates

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Eight 1-aryl-3-(2-pyridyl)thiobarbiturates were synthesized and evaluated for their anticonvulsant property and their ability to inhibit succinate dehydrogenase activity of rat brain homogenates. These substituted thiobarbiturates (100 mg./kg., i.p.) provided 20-60% protection against pentylenetetrazol-induced convulsions in albino mice. Low toxicity of these compounds was reflected by their high approximate LD_{50} values which were found to range from 500-1000 mg./kg. All substituted thiobarbiturates (1m*M*) inhibited *in vitro* succinate dehydrogenase activity and the degree of inhibition ranged from 10-72%.

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Various barbiturates are known to interfere with succinate dehydrogenase activity (1). Recent studies have indicated the anticonvulsant property of 1,3-disubstituted thiobarbiturates and their ability to inhibit the activity of succinate dehydrogenase (2,3). These observations prompted the synthesis of 1-aryl-3-(2-pyridyl)thiobarbiturates which were evaluated for their anticonvulsant and succinate dehydrogenase inhibitory properties.

In the present study, eight 1-aryl-3-(2-pyridyl)thiobarbiturates were synthesized from 2-aminopyridine. The cyclization of 1-aryl-3-(2-pyridyl)thiocarbamides, which were obtained by the reaction of 2-aminopyridine and the appropriate aryl isothiocyanate, was carried out in the presence of acetyl chloride and malonic acid at 60-80°; and this resulted in the formation of 1-aryl-3-(2-pyridyl)-thiobarbiturates (**1-8**).

All substituted thiobarbiturates on intraperitoneal administration at a dose of 100 mg./kg. provided protection of 20-60% against pentylenetetrazol-induced convulsions in mice (Table II). Among halogen substituted thiobarbiturates, a decrease in the anticonvulsant activity was observed by the movement of chlorine and fluorine substituents from position 3 (**5,7**) to position 4 (**6,8**) in the phenyl ring. It was also observed that the greater anticonvulsant activity of these substituted thiosemicarbazides was not reflected by their greater ability to provide protection against 24 hour mortality due to pentylenetetrazol. These substituted thiobarbiturates were found to possess low toxicity, which was reflected by their higher approximate LD_{50} values; these were found to range from 500-1000 mg./kg. on intraperitoneal administration in mice (Table II). As is evident from Table II, all substituted thiobarbiturates at a final concentration of $1 \times 10^{-3}M$ inhibited *in vitro* succinate dehydrogenase activity of rat brain homogenates. Maximum inhibition of 72% was observed with 1-(4-methylphenyl)-3-(2-pyridyl)thiobarbiturates (**4**), while 1-(4-methoxyphenyl)-3-(2-pyridyl)thiobarbiturate (**3**) showed minimum inhibition of 10%. The presence of a substituent in the phenyl moiety at position 1 of the thiobarbiturate nucleus has not been shown to play a significant role in their ability to inhibit the activity of rat brain succinate dehydrogenase. These results have failed to provide any definite correlation between the anticonvulsant activity of substituted thiobarbiturates and their ability to inhibit succinate dehydrogenase activity. Thus, the enzyme inhibitory effectiveness cannot provide a biochemical basis for the anticonvulsant activity of 1-aryl-3-(2-pyridyl)thiobarbiturates.

EXPERIMENTAL

All melting points were analyzed for their carbon, hydrogen, and nitrogen contents. Melting points were taken in open capillary tubes with a partial immersion thermometer and are uncorrected.

1-Aryl-3-(2-pyridyl)thiocarbamides.

A mixture of 2-aminopyridine (0.01 mole) and the appropriate arylisothiocyanate (0.01 mole) in 20 ml. of dry benzene was refluxed on a steam bath for 2 hours. The reaction mixture was then concentrated under reduced pressure. The solid mass which separated on cooling was filtered, washed (ether and diluted hydrochloric acid), dried and recrystallized from ethanol (4).

1-Aryl-3-(2-pyridyl)thiobarbiturates.

The appropriate thiocarbamide (0.01 mole) and malonic acid (0.015 mole) were heated slowly with acetyl chloride (7 ml.) on a water bath at 60-80° for 1 hour. After cooling, the pasty-mass was triturated with water and the brown solid which separated was recrystallized from benzene (Table I).

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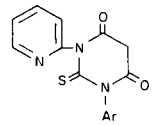
Table I

Physical Constants of 1-Aryl-3-(2-pyridyl)thiobarbiturates



Compound No.	Ar	Melting Point	Yield %	Formula	Analysis % Calculated C	H	N	Found C	H	N
1	C_6H_5	190°	72	$C_{15}H_{11}N_3O_2S$	60.60	3.70	14.14	60.45	3.56	14.32
2	$2\text{-}OCH_3C_6H_4$	200°	70	$C_{16}H_{13}N_3O_3S$	58.71	3.97	12.84	58.60	3.82	12.66
3	$4\text{-}OCH_3C_6H_4$	170°	70	$C_{16}H_{13}N_3O_3S$	58.71	3.97	12.84	58.82	3.86	13.78
4	$4\text{-}CH_3C_6H_4$	224°	64	$C_{16}H_{13}N_3O_2S$	61.73	4.18	13.50	61.56	4.25	13.78
5	$3\text{-}ClC_6H_4$	150°	72	$C_{15}H_{10}ClN_3O_2S$	54.29	3.01	12.66	54.15	2.88	12.57
6	$4\text{-}ClC_6H_4$	156°	71	$C_{15}H_{10}ClN_3O_2S$	54.29	3.01	12.66	54.18	2.90	12.52
7	$3\text{-}FC_6H_4$	216°	68	$C_{15}H_{10}FN_3O_2S$	57.14	3.17	13.33	57.26	3.02	13.10
8	$4\text{-}FC_6H_4$	185°	70	$C_{15}H_{10}FN_3O_2S$	57.14	3.17	13.33	57.02	3.04	13.12

Table II

Anticonvulsant Activity and Succinate Dehydrogenase Inhibitory Property of 1-Aryl-3-(2-pyridyl)thiobarbiturates

Compound No.	Anticonvulsant Activity, Protection % (a)	Pentylenetetrazol Mortality % (b)	Approximate LD_{50} (mg./kg., i.p.)	Inhibition of Succinate Dehydrogenase Activity, % (c)
1	40	20	500	70.1 ± 0.3
2	60	40	500	68.8 ± 0.3
3	60	30	750	10.0 ± 0.2
4	20	40	500	72.1 ± 0.4
5	40	30	500	70.4 ± 0.3
6	20	100	500	60.1 ± 0.5
7	60	60	500	58.3 ± 0.6
8	40	100	1000	42.0 ± 0.2

(a) Compounds were administered at a dose of 100 mg./kg., i.p., 4 hours before the administration of pentylenetetrazol (90 mg./kg., s.c.). (b) Represents mortality in each group of animals administered pentylenetetrazol during the 24 hour period. (c) Each experiment was done in duplicate and the values are the mean values of three separate experiments with ± standard error of the mean. All compounds were used at a final concentration of 1 x $10^{-3}M$.

Anticonvulsant Activity.

Anticonvulsant activity was determined against pentylenetetrazol-induced seizures in albino mice of either sex weighing 25-30 g. (5). The mice were divided into groups of ten, keeping the group weights as equal as possible. All thiobarbiturates were suspended in 5% aqueous gum acacia to give a concentration of 0.25% (weight volume). Each test compound was injected intraperitoneally into a group of ten mice at a dose of 100 mg./kg. Four hours after the administration of the test compounds, the mice were injected with pentylenetetrazol (90 mg./kg., s.c.). This dose of pentylenetetrazol was shown to produce convulsions in almost all untreated mice and the mice exhibited 100% mortality during the period of 24 hours. The mice were then observed for 60 minutes for the occurrence of seizures. An episode of clonic spasm persisting for at least 5 seconds was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not counted. Animals devoid of threshold convulsions during 60 minutes were considered protected. The number of animals protected in each group was recorded and the anticonvulsant activity of these thiobarbiturates was represented as the percent protection. The mice were then observed for 24 hours and their mortality was recorded.

Toxicity Studies.

The approximate LD_{50} values were determined by the intraperitoneal administration of these substituted thiobarbiturates in mice following the method of Smith (6).

Assay of Succinate Dehydrogenase.

Succinate dehydrogenase activity of rat brain homogenates was determined by spectrophotometric method described by Slater and Bonner (8). The rate of reduction of potassium ferrocyanide ($0.01M$) was measured in the presence of sufficient sodium cyanide ($0.01M$) to inhibit cytochrome oxidase following the rate of decrease of optimal density at 400 nm. The rat brain homogenates in the presence of the substrate (succinate) in the buffered (pH 7.2) medium reduces the yellow colored potassium ferrocyanide.

Albino rats weighing 100-150 g. kept on *ad libitum* diet were used in

the present study. Rat brains isolated from decapitated animals were immediately homogenized in ice cold 0.25*M* sucrose in a Potter-Elvehjem homogenizer in a ratio of 1:9 (w/v). The reaction mixture in a total volume of 2 ml. consisted of 0.4 ml. of phosphate buffer (*p*H 7.2, 0.5*M*), 0.1 ml. of 0.1*M* sodium succinate, 0.4 ml. of 0.01*M* potassium ferrocyanide, 0.2 ml. of 0.1*M* sodium cyanide and 0.2 ml. of fresh rat brain homogenates equivalent to 20 mg. wet weight of the tissue. All compounds were dissolved in propylene glycol (100%) and used at a final concentration of $1 \times 10^{-3}M$. The compounds were incubated with rat brain homogenates at 37° for 10 minutes prior to the addition of the substrate. The reaction mixture was further incubated for 30 minutes at 37° after the addition of the substrate. The enzyme reaction was found to be linear for 30 minutes. Enzyme reaction was stopped by the addition of 2 ml. of 10% trichloroacetic acid (w/v) and the precipitated proteins were removed by centrifugation. The clear supernatant was drawn off for the determination of the optical density. The color intensity was measured at 400 nm in Perkin-Elmer Spectrophotometer. An increase in percent transmission provided a direct measurement of reduced potassium ferrocyanide which was taken as an index of the enzyme activity. The percent inhibition was calculated from the decrease in optical density/20 mg. fresh tissue weight/30 minutes. Proper controls were also run under similar experimental conditions.

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