ANTICONVULSANT AND SUCCINATE DEHYDROGENASE INHIBITORY ACTIVITY OF SOME NEWER THIOBARBITURATES

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ABSTRACT

Some 1-aryl-3-cyclohexyl thiobarbiturates were synthesized and investigated for their ability to inhibit succinate dehydrogenase (SDH) activity of rat brain homogenate. All compounds exhibited concentration dependent inhibition of enzyme. The degree of enzyme inhibition was also evaluated on the basis of their I_{50} and $I_{50}/(S)$ values. Kinetic study carried out with 1-(2-ethoxyphenyl)-3-cyclohexyl-thiobarbiturate revealed a competitive nature of inhibition. Anticonvulsant activity of the compounds against pentylenetetrazol-induced seizures and the partition coefficients were determined in order to correlate structure-activity relationship.

INTRODUCTION

NUMEROUS CNS depressants and anticonvulsants have been shown to inhibit respiratory activity of brain which ultimately leads to altered energy metabolism and thus impair neuronal functions.[1–3] Furthermore, it has been reported that various barbiturates interfere with the succinate dehydrogenase activity[4–6]. Recently, several 1, 3-disubstituted thiobarbiturates were investigated to show their effects on respiratory enzymes, convulsive seizures and succinate dehydrogenase activity of rat brain homogenate[7,8]. On the basis of these observations, a few 1-aryl-3-cyclohexyl-thiobarbiturates were synthesized and investigated for succinate dehydrogenase activity *in vitro* of rat brain homogenate. The compounds were screened for anticonvulsant activity and the partition coefficients were also determined in order to discover any relationship between structure, biological activity and lipophilicity.

EXPERIMENTAL

1-Aryl-3-cyclohexyl thiocarbamides.—Cyclohexylamine (0·01 mole) and the appropriate aryl isothiocyanate (0·01 mole) were taken in 20 ml of dry benzene and refluxed on a steam bath for 2 hr. The reaction mixture was then concentrated under reduced pressure. The solid mass which separated out on cooling was filtered, washed (Et_2O, dil. HCl), dried and recrystallized from EtOH. All thiocarbamides have been reported earlier[9] except 1-(2-ethoxyphenyl)-3-cyclohexyl thiocarbamide m.p. 104° C, yield 82%. Analyses for $C_{15}H_{22}N_2OS$

Calcd. C, 64·74%; H, 8·27%; N, 10·07%.

and

Found C, 64·46%; H, 8·4%; N, 10·32%

1-(4-ethoxyphenyl)-3-cyclohexyl thiocarbamide, m.p. 126° C, yield 80%. Analyses for $C_{15}H_{22}N_2OS$

Calcd. C, 64·74%; H, 8·27%; N, 10·07%

Found C, 64·86%; H, 8·15%; N, 9·80%.

1-Aryl-3-cyclohexyl thiobarbiturates

Appropriate thiocarbamide (0·001 mole) and malonic acid (0·015 mole) were heated slowly on a water bath with acetyl chloride (7 ml) at 60–80° for 1 hr. After cooling, the pasty mass was triturated with water and the brown solid was separated and recrystallized from ethanol. All thiobarbiturates were characterized by their sharp melting points, elemental analyses (Table I) and infrared spectra.

Succinate dehydrogenase activity.—Succinate dehydrogenase activity of rat brain homogenate was determined by the spectrophotometric method described by Slater and Bonner[10].

Determination of I_{50} value.—The concentration of the compound required to inhibit 50% of the enzyme activity was obtained by plotting a graph of log molar concentration of compounds against per cent inhibition.

Anticonvulsant activity.—Anticonvulsant activity of 1-aryl-3-cyclohexyl thiobarbiturates was determined at a dose of 100 mg/kg i.p. against pentylenetetrazol-induced seizures[11].

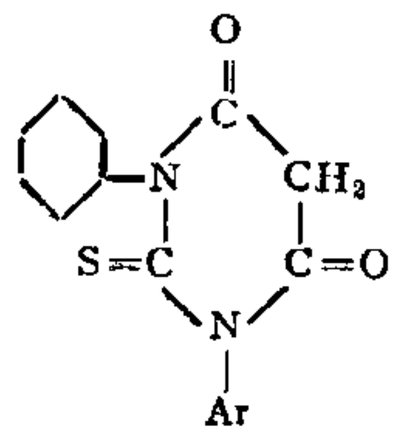
Determination of partition coefficients.—The partition coefficients were determined by dissolving the compounds in chloroform, shaking the solution with water and assaying the concentration of the compound in two phases spectrophotometrically.

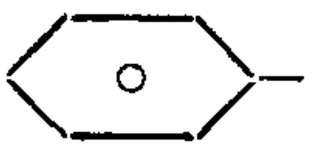
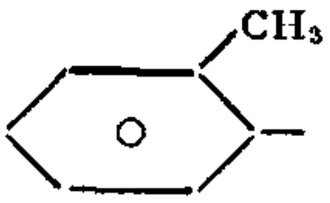
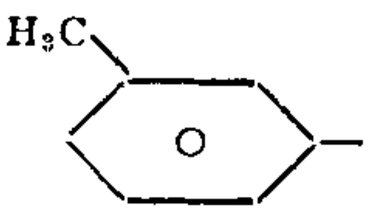
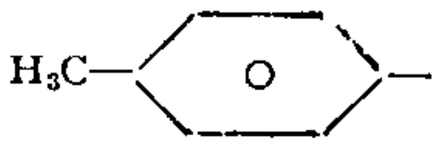
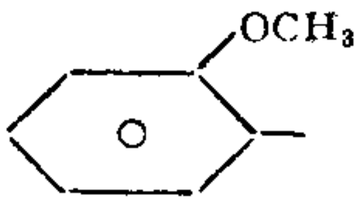
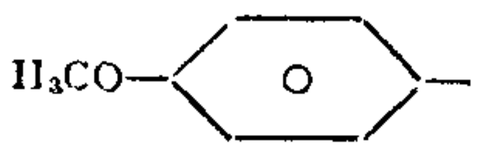
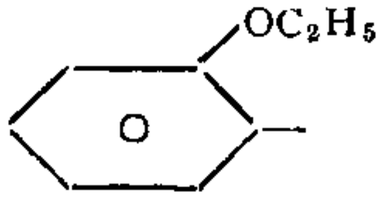
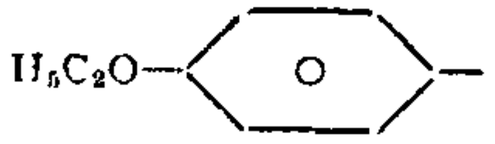
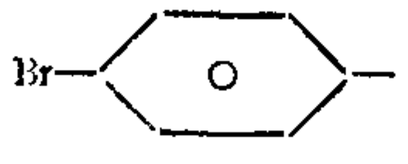
RESULTS AND DISCUSSION

All 1-aryl-3-cyclohexyl thiobarbiturates were found to inhibit rat brain succinate dehydrogenase *in vitro* at final concentrations of $1 \times 10^{-3}\,M$, $5{\cdot}0 \times 10^{-4}\,M$, $2{\cdot}5 \times 10^{-4}\,M$, $1 \times 10^{-4}\,M$ and $5 \times 10^{-5}\,M$ (Table II). The degree of inhibition of succinate dehydrogenase was found to be concentration dependent. This necessitated the determination of I_{50} values of the thiobarbiturates and the ratio of I_{50} values to substrate concentration (Table III) for a better comparative evaluation of their inhibitory activity. 1-(2-Ethoxyphenyl)-3-cyclohexyl thiobarbiturate (Compound VII) exhibited maximum inhibition.

TABLE I

Physical constants of 1-aryl-3-cyclonexyl thiobarbiturates

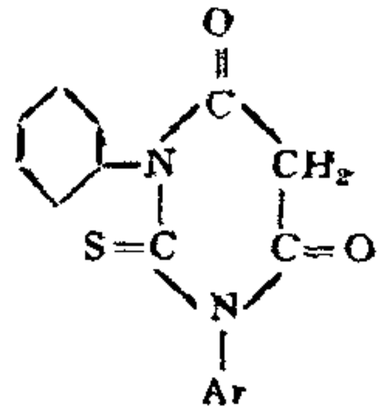


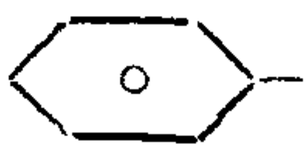
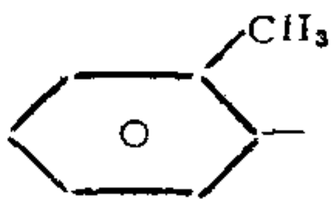
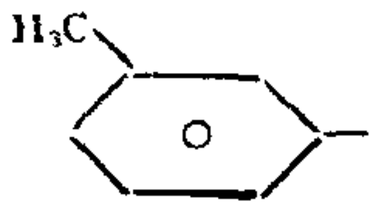
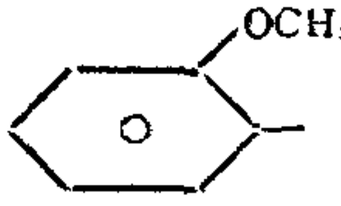
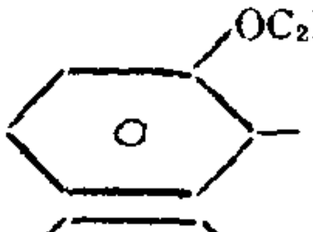
Compound No.	Ar.	Melting point	Yield %	Molecular formula	Analysis %	Calcd.	Found
I		124° G	72	$C_{16}H_{18}N_2O_2S$	C H N	63·57 5·96 9·27	63·78 6·01 9·15
II		132° C	68	$C_{17}H_{20}N_2O_2S$	C H N	64·56 6·33 8·86	63·36 6·29 9·06
III		182° C	70	$C_{17}H_{20}N_2O_2S$	C H N	64·56 6·33 8·86	64·85 6·47 8·65
IV		90° C	76	$C_{17}H_{20}N_2O_2S$	C H N	64·56 6·33 8·86	64·68 6·42 8·99
V		142–144° C	64	$C_{17}H_{20}N_2O_3S$	C H N	61·44 6·02 8·43	61·73 5·96 8·68
VI		152° C	68	$C_{17}H_{20}N_2O_3S$	C H N	61·44 6·02 8·43	61·27 6·23 8·36
VII		138° C	71	$C_{18}H_{22}N_2O_3S$	C H N	62·43 6·36 8·09	62·57 6·42 8·27
VIII		158° C	63	$C_{18}H_{22}N_2O_3S$	C H N	62·43 6·46 8·09	62·20 6·48 8·15
IX		148° C	64	$C_{16}H_{17}N_2O_2SBr$	C H N	53·54 4·46 7·34	53·79 4·28 7·55

Substitution of various groups at pheryl ring attached to position 1 of the thiobarbiturate nucleus was found to elicit a varied degree of inhibition of SDH activity. The shifting of methoxy/ethoxy group from position 2 to 4 (Compounds, V, VI, VII, VIII) resulted in the decrease in the enzyme inhibition. Moreover, the compounds possessing ethoxy groups were found to shew greater activity as compared to those having methoxy groups at the respective positions in the phenyl ring. The introduction of an

TABLE II

Effect of 1-aryl-3-cyclohexyl-thiobarbiturates on rat brain succinate dehydrogenase



Compound No.	Ar	$1 \times 10^{-3} M^a$	Per cent inhibition*			
			$5{\cdot}0 \times 10^{-4} M^b$	$2{\cdot}5 \times 10^{-4} M^c$	$1 \times 10^{-4} M^d$	$5 \times 10^{-5} M^e$
I		58·0±0·3	33·0±0·8	26·9±0·7	20·8±0·9	14·4±0·6
II		70·0±0·08	41·4±0·9	32·1±1·0	22·3±0·4	5·8±0·7
III		72·0±0·1	62·9±0·7	33·8±0·5	31·3±0·6	12·7±0·9
IV		76·0±1·0	51·4±0·6	35·7±0·7	28·3±0·8	2·90±0·4
V		92·3±1·0	61·4±0·2	42·8±0·8	38·8±0·6	17·4±0·8
VI		70·7±0·2	51·4±0·8	33·8±0·4	23·8±0·9	12·7±1·0
VII		98·2±0·4	66·1±0·8	58·5±0·8	47·7±0·7	21·2±0·6
VIII		94·8±0·3	51·4±0·5	54·6±0·3	29·8±0·5	12·6±0·8
IX		92·1±0·5	47·1±0·3	44·6±0·8	25·3±1·3	12·6±0·9

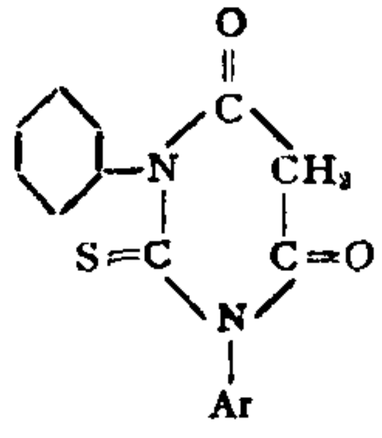
*Assay procedures and contents of the reaction mixture were as described in the text. Each experiment was done in triplicate and the values are the mean values of three separate experiments with ± standard error of the mean.

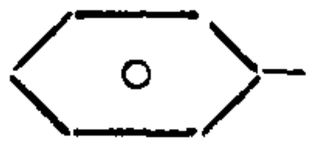
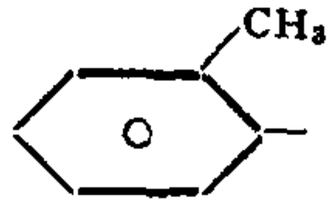
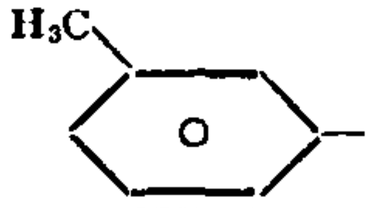
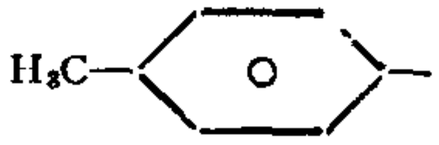
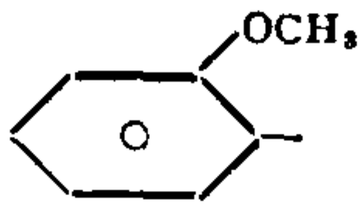
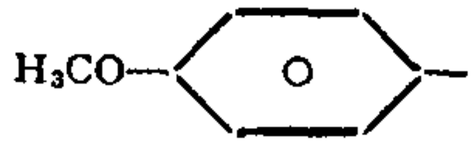
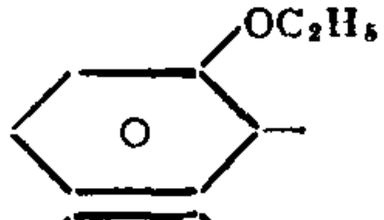
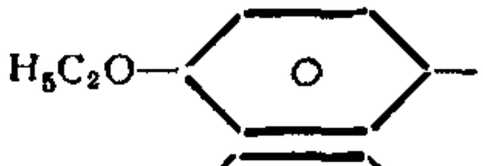
a, b, c, d, e — Indicate the final concentration of the test compound used in the reaction mixture.

electronegative Br atom in the phenyl ring (Compound IX) exhibited a similar degree of inhibition as compared to the electron-donating methoxy and ethoxy groups. In the case of methyl substituted barbiturates (II, III and IV), the degree of inhibition was found to be in order of $-p > -m > -o$ at the final concentrations of $1 \times 10^{-3} M$ and $2{\cdot}5 \times 10^{-4} M$. The order $-m > -p > -o$ was observed at the final concentration of $5 \times 10^{-4} M$ and $1 \times 10^{-4} M$ while the order was $-m > -o > p$ at $5 \times 10^{-5} M$. However, the compound possessing an unsubstituted phenyl ring exhibited minimum inhibition at various con-

TABLE III

I_{50} values of 1-aryl-3-cyclohexyl thiobarbiturates



Compound No.	Ar.	I_{50}*	$\frac{I_{50}}{(S)}$**
I		$8{\cdot}4 \times 10^{-4}$ *M*	0·168
II		$6{\cdot}6 \times 10^{-4}$ *M*	0·132
III		$3{\cdot}8 \times 10^{-4}$ *M*	0·076
IV		$4{\cdot}8 \times 10^{-4}$ *M*	0·096
V		$3{\cdot}4 \times 10^{-4}$ *M*	0·068
VI		$4{\cdot}9 \times 10^{-4}$ *M*	0·098
VII		$1{\cdot}2 \times 10^{-4}$ *M*	0·024
VIII		$4{\cdot}5 \times 10^{-4}$ *M*	0·090
IX		$5{\cdot}4 \times 10^{-4}$ *M*	0·108

* Represents the molar concentration of inhibitor causing 50% inhibition of enzyme under the experimental conditions described in the text.

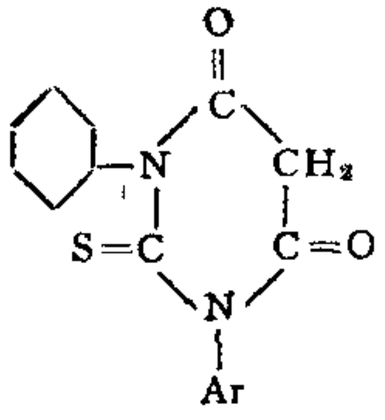
** Indicates the ratio of molar concentration of compounds to substrate giving 50% inhibition.

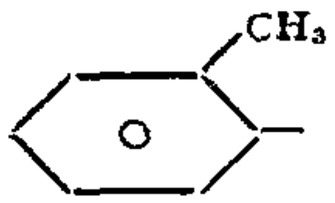
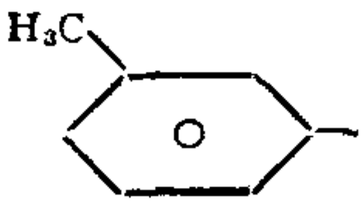
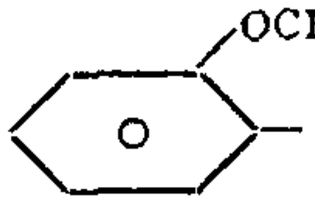
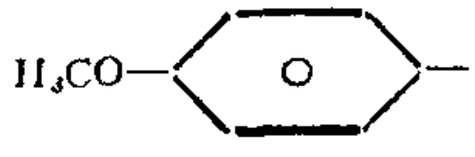
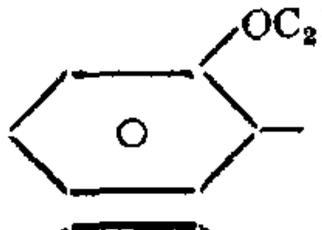
concentrations. Preincubation of these compounds with the enzyme system for varying lengths of time prior to the addition of substrate in no way altered their degree of inhibition (Table IV). Kinetic study of 1-(2-ethoxyphenyl)-3-cyclohexyl thiobarbiturate using Lineweaver and Burk[12] plots as modified by Dixon[13] has been demonstrated in Fig. 1. Ki value obtained graphically, was found to be $0{\cdot}125 \times 10^{-4}$ *M*.

All thiobarbiturates afforded protection (10%–60%) aganist pentylenetetrazol-induced seizures (Table V). Maximum protection was shown by compound IV while the minimum was exhibited by compound I. In general,

TABLE IV

Preincubation studies with 1-*aryl*-3-*cyclohexyl thiobarbiturates*



Compound No.	Ar.	Per cent inhibition* (Preincubation time in min.)			
		0 min.	10 min.	20 min.	30 min.
I		58·1±0·2	58·0±0·2	58·0±0·4	58·1±0·1
II		70·1±0·8	70·0±0·5	70·1±0·3	70·2±1·4
III		72·0±0·5	72·0±0·4	72·2±0·3	72·1±0·9
IV		76·0±1·5	76·1±0·7	76·1±0·6	76·0±0·9
V		92·3±0·5	92·2±0·4	92·1±0·4	92·2±0·7
VI		70·8±1·5	70·5±0·9	70·6±0·3	70·7±0·6
VII		98·1±1·0	98·2±0·8	98·2±0·5	98·3±0·7
VIII		94·7±0·9	94·8±1·3	94·7±0·3	94·8±0·6
IX		92·3±0·4	92·1±0·8	92·0±1·0	92·2±1·2

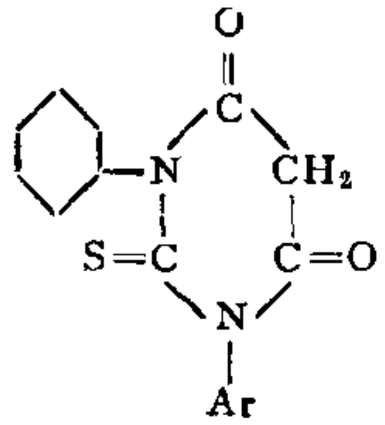
* Assay procedures and contents of the reaction mixture were as described in the text. Each experiment was done in triplicate and the values are the mean of three separate experiments with ± standard error of the mean. The compounds were used at a final concentration of 1×10^{-3} *M*.

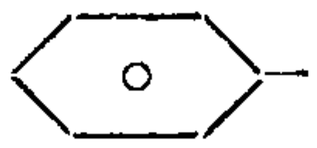
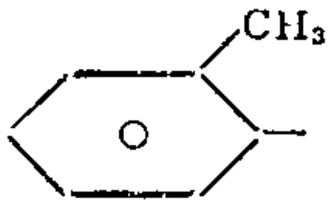
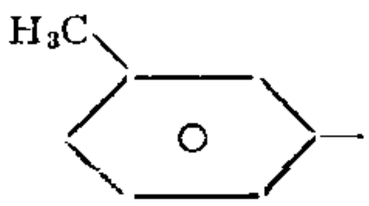
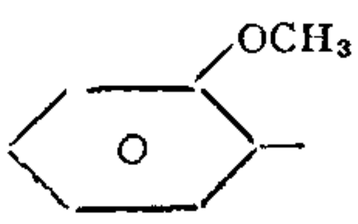
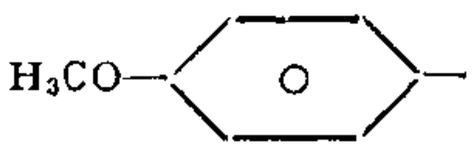
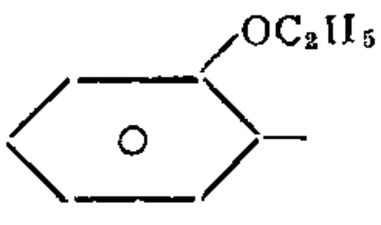
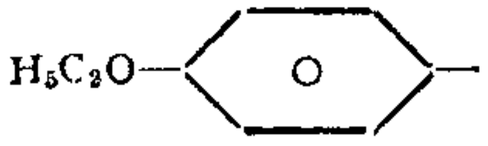
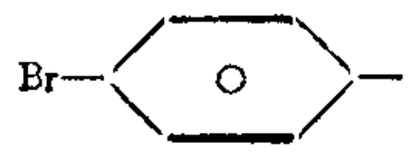
shifting of electron-donating groups from position 2 to 4 of the phenyl nucleus produced greater degree of protection, while the inhibition of succinate dehydrogenase activity was lowered. The partition coefficients of these compounds are also shown in Table V. These values could not be correlated to either the anticonvulsant activity or SDH inhibitory activity.

Some 1, 3-disubstituted thiobarbiturates have been reported to affect the succinoxidase activity of rat brain homogenate and the site of action of these

TABLE V

Anticonvulsant activity and partition coefficients of 1-aryl-3-cyclohexyl thiobarbiturates



Compound No.	Ar	Anti-convulsant activity[a] %	Mortality[b] %	Partition coefficients
I		10	70	7·40
II		40	30	6·14
III		40	60	7·33
IV		60	20	6·73
V		30	40	25·56
VI		50	30	19·75
VII		20	60	15·20
VIII		30	40	12·15
IX		20	70	28·41

[a] Anticonvulsant activity was determined as described in the experimental section.

[b] Represents mortality in each group of animals administered pentylenetetrazol during 24 hr period.

compounds has been demonstrated to be presumably at the complex I and complex II of the electron transport chain[7]. The present investigation was made in an attempt to investigate the effect of 1-aryl-3-cyclohexyl-thiobarbiturates on complex II of electron transport chain. In the present study the inhibitory action of these thiobarbiturates on complex II of E.T.C. can be ascribed to be due to the action of these compounds on succinate dehydrogenase, a constituent of complex II. However, these studies do not show a definite

correlation between the anticonvulsant activity of these 1-aryl-3-cyclohexyl thiobarbiturates and their ability to inhibit succinate dehydrogenase of rat brain homogenate as a biochemical basis of their anticonvulsant activity.

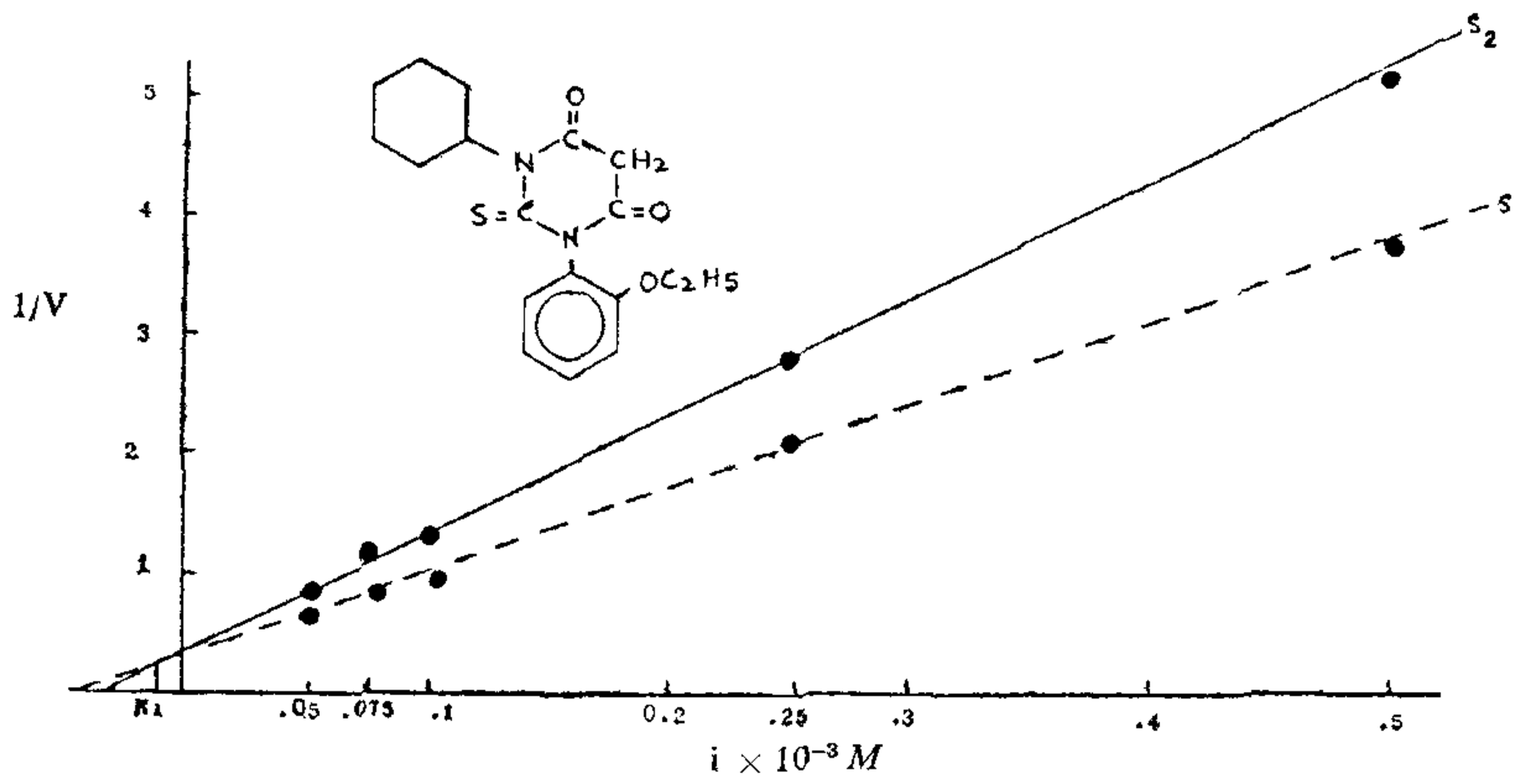


FIG 1. Kinetic study showing competitive inhibition of rat brain succinate dehydrogenase by 1-(2-ethoxyphenyl)-3-cyclohexyl-thiobarbiturate, 1/V represents reciprocal of the enzyme activity while (i) represents inhibitor concentrations. Final concentrations of the substrate were $S_1 = \cdot 005\ M$, $S_2 = \cdot 0025\ M$.

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