matographic conditions described, baseline resolution between suprofen and the three potential metabolite reference standards was achieved. Retention times and the relative separation of these potential metabolites from suprofen are reported in Table I.

Linear regression analysis of the curve, described by plotting microvolt seconds *versus* micrograms injected, indicated a virtual linear fit of the data ($r^2 = 0.99992$).

The response factor, F_i, generated by the computer is described by $F_i = W_i/A_i$, where W_i is the amount injected and A_i is the area in microvolt seconds. It was shown to be linear over the concentration range evaluated (0.05–0.2 μg/injection). Over a 2-month period of analysis, this response factor demonstrated little change, with a coefficient of variation equal to 2.4% ($n = 23$).

The results from the extracted control plasma to which ^{3}H-suprofen had been added are shown in Table II. The average extraction efficiency based on total recovered radioactivity was 81.2 ± 4.8% *SD*. The average recovery of suprofen from plasma samples to which unlabeled suprofen had been added was 80.0 ± 8.5% *SD* (Table II). Representative chromatograms of extracts from plasma containing 0.2 μg/ml (20 μl from a 100-μl dilution with tetrahydrofuran) and 20.0 μg/ml (5 μl from a 500-μl dilution with tetrahydrofuran) are shown in Fig. 2. A multicomparison analysis of the percent recovery data shown in Table II was performed using the two-tailed Dunnett (2) and Scheffé (3) test. No statistically significant differences were found in extraction efficiencies among the four groups of plasma samples with added suprofen.

These data suggest that there is no concentration dependence in extraction efficiencies over the range of expected plasma drug[12] levels following oral administration of 200 mg of suprofen. The results of this study will be the subject of a separate report.

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[12] Preliminary studies, conducted by Janssen Pharmaceutica and Ortho Pharmaceutical Corp., indicated that peak plasma levels of suprofen, equal to approximately 10–20 μg/ml, would be achieved following the oral administration of 200 mg of suprofen to normal human subjects.

Anticonvulsant and Antiproteolytic Properties of 2,5-Disubstituted Oxadiazoles and Their Inhibition of Respiration in Rat Brain Homogenates

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Received September 12, 1977, from the **Jawahar Lal Nehru Laboratory of Molecular Biology, Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow 226003, India,* and the ‡*Department of Physiology, School of Medicine, University of North Dakota, Grand Forks, ND 58202.* Accepted for publication November 7, 1977. §Present address: National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Abstract □ Eight 2-(3,4-methylenedioxyphenyl)-5-arylamino-1,3,4-oxadiazoles were synthesized, characterized by their sharp melting points, elemental analyses, and IR spectra, and evaluated for anticonvulsant activity. The protection afforded by oxadiazoles (100 mg/kg ip) against pentylenetetrazol (90 mg/kg sc)-induced convulsions ranged from 50 to 80%. All oxadiazoles inhibited the respiratory activity of rat brain homogenates during oxidation of pyruvate, α-ketoglutarate, and succinate. The presence of added nicotinamide adenine dinucleotide (NAD) to the reaction mixture during oxidation of pyruvate decreased the degree of inhibition. All oxadiazoles possessed antiproteolytic activity that was reflected by their ability to decrease trypsin-induced hydrolysis of bovine serum albumin. Such an inhibition was concentration dependent and ranged from 10.2 to 47.5 and from 15.7 to 71.8% by 0.5 and 1 m*M* oxadiazoles, respectively. All oxadiazoles competitively inhibited *in vitro* succinate dehydrogenase activity of rat brain homogenates.

Keyphrases □ Oxadiazoles, various—synthesized, evaluated for effect on enzyme activity in rat brain homogenates, anticonvulsant activity in mice, and antiproteolytic activity *in vitro* □ Enzyme activity—effect of various oxadiazoles in rat brain homogenates □ Anticonvulsant activity—various oxadiazoles evaluated in mice □ Antiproteolytic activity—various oxadiazoles evaluated *in vitro* □ Structure–activity relationships—various oxadiazoles evaluated for effect on enzyme activity in rat brain homogenates, anticonvulsant activity in mice, and antiproteolytic activity *in vitro*

Earlier studies indicated central nervous system depressant (1, 2), analgesic (3), muscle relaxant (4), and tranquilizing (5, 6) properties of substituted oxadiazoles. The inhibitory effects of 2,5-disubstituted 1,3,4-oxadiazoles on the respiratory activity of rat brain homogenates were reported (7–9). These observations led to the synthesis of 2,5-disubstituted oxadiazoles, which were evaluated for anticonvulsant activity.

In the present study, the ability of these oxadiazoles to inhibit respiratory activity of rat brain homogenates and trypsin activity during hydrolysis of bovine serum albumin was determined to investigate the biochemical mechanism of action for the anticonvulsant activity of these newer substituted oxadiazoles.

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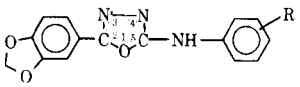
Table I—Physical Constants of 2-(3,4-Methylenedioxyphenyl)-5-arylamino-1,3,4-oxadiazoles



Compound	R	Melting Point[a]	Yield, %	Molecular Formula		Analysis, % Calc.	Found
I	H	224°	60	$C_{15}H_{11}N_3O_3$	C	64.05	64.21
					H	3.91	3.92
					N	14.94	14.68
II	2-CH_3	174°	55	$C_{16}H_{13}N_3O_3$	C	65.08	65.20
					H	4.40	4.36
					N	14.23	14.20
III	4-CH_3	210°	56	$C_{16}H_{13}N_3O_3$	C	65.08	65.00
					H	4.40	4.48
					N	14.23	14.22
IV	2,4-$(CH_3)_2$	170°	48	$C_{17}H_{15}N_3O_3$	C	66.01	65.94
					H	4.85	4.62
					N	13.59	13.67
V	2,6-$(CH_3)_2$	184°	50	$C_{17}H_{15}N_3O_3$	C	66.01	66.20
					H	4.85	4.94
					N	13.59	13.70
VI	2-OCH_3	162°	52	$C_{16}H_{13}N_3O_4$	C	61.73	61.50
					H	4.18	4.20
					N	13.50	13.47
VII	4-OCH_3	214°	54	$C_{16}H_{13}N_3O_4$	C	61.73	61.55
					H	4.18	4.23
					N	13.50	13.36
VIII	4-Cl	274°	57	$C_{15}H_{10}ClN_3O_3$	C	57.05	57.00
					H	3.15	3.26
					N	13.31	13.54

[a] Melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected.

EXPERIMENTAL[1]

1-(3,4-Methylenedioxybenzoyl)-4-substituted Thiosemicarbazides—The various substituted thiosemicarbazides were synthesized and reported previously (10).

2-(3,4-Methylenedioxyphenyl)-5-arylamino - 1,3,4-oxadiazoles (I–VIII)—The various 1-(3,4-methylenedioxybenzoyl)-4-substituted thiosemicarbazides were cyclized to the corresponding 2,5-disubstituted 1,3,4-oxadiazoles following the method of Silberg and Cosma (11). To an ethanolic solution (300 ml) of the appropriate thiosemicarbazide (0.01 mole) was added 5 ml of 4 *N* NaOH, with cooling and shaking, to obtain a clear solution. To this solution was added iodine in 5% KI, gradually with stirring, until the color of the iodine persisted at room temperature.

The mixture was refluxed on a steam bath, and more iodine solution was added carefully until a permanent color of excess iodine was obtained. The reaction mixture was poured over crushed ice (500 g), and the solid mass that separated was filtered, washed first with water and then with hot carbon disulfide, dried, and recrystallized from ethanol in the presence of activated charcoal. Compounds I–VIII (Table I) were characterized by their sharp melting points, elemental analyses, and IR spectra.

The presence of characteristic bands of C=N (1640 cm^{-1}), NH–Ar (1299 cm^{-1}), NH (3300 cm^{-1}), and pentaatomic ring (1389 cm^{-1}) in the IR spectra of 2,5-disubstituted oxadiazoles provided further support for their structures.

Assay of Respiratory Activity of Rat Brain Homogenate[2]—Swiss albino rats, 100–150 g, were kept on an *ad libitum* diet. Rat brains isolated from decapitated animals were homogenized[3] immediately in ice-cold 0.25 *M* sucrose in a ratio of 1:9 (w/v). All incubations were carried out at 37°, and the oxygen uptake was measured by conventional Warburg manometric techniques with air as the gas phase (12).

Fresh brain homogenates, equivalent to 100 mg wet weight, were added to chilled Warburg vessels containing 6.7 m*M* magnesium sulfate, 2 m*M* sodium hydrogen phosphate buffer (pH 7.4), 1 m*M* adenosine monophosphate (sodium salt), 33 m*M* potassium chloride, and 500 μg of cytochrome c in a final volume of 3 ml unless otherwise stated. The central well contained 0.2 ml of 20% KOH. Pyruvate, α-ketoglutarate, and succinate were used at a final concentration of 10 m*M*, while the concentration of nicotinamide adenine dinucleotide (NAD) was 0.5 m*M*.

Compounds I–VIII were dissolved in propylene glycol (100%) and incubated with rat brain homogenates at 37° for 10 min prior to the addition of various substrates. An equal volume of the solvent was added to the control vessels. The oxygen uptake was measured every 10 min for a total of 60 min.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in Swiss albino mice, 25–30 g, of either sex. The mice were divided into groups of 10, keeping the group weights as near the same as possible. Each 2-(3,4-methylenedioxyphenyl)-5-arylamino-1,3,4-oxadizole was suspended in 5% aqueous gum acacia to give a concentration of 1% (w/v). The test compounds were injected intraperitoneally in a group of 10 mice at a dose of 100 mg/kg.

Four hours after the administration of the test compounds, the mice were injected subcutaneously with 90 mg of pentylenetetrazol/kg. This dose of pentylenetetrazol produced convulsions in almost all untreated mice and exhibited 100% mortality during 24 hr. However, no mortality was observed during the 24-hr period in animals treated with 100-mg/kg doses of the test compounds alone.

The mice were then observed for 60 min for seizures. An episode of clonic seizure that persisted for at least 5 sec was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not counted. Animals devoid of the threshold convulsion during 60 min were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of I–VIII was represented as percent protection. In the present study, no anticonvulsant activity was observed in animals treated with 5% aqueous gum acacia solution alone. The animals were then observed for 24 hr, and their mortality due to the administration of pentylenetetrazol was recorded.

Assay of Proteolytic Activity of Trypsin—The inhibitory effects of substituted oxadiazoles on the hydrolysis of bovine serum albumin by trypsin were determined by the method reported earlier (13). The reaction mixture consisted of 0.05 *M* tromethamine buffer (pH 8.2), 0.075 mg of crystalline trypsin (1 g is sufficient to hydrolyze 250 g of casein), 3.3×10^{-5} *M* bovine serum albumin, and water in a total volume of 1 ml. All oxadiazoles were dissolved in dimethylformamide and were used at 1×10^{-3} and 5×10^{-4} *M*. An equivalent amount of dimethylformamide, added to the control tubes, had no effect on the activity of trypsin during hydrolysis of bovine serum albumin.

All oxadiazoles were preincubated with trypsin for 10 min prior to the addition of bovine serum albumin, unless otherwise stated, and the reaction mixture was further incubated for 5 min after the addition of bovine serum albumin. The reaction was stopped by the addition of 0.5 ml of 15% (w/v) trichloroacetic acid. The acid-soluble products of protein breakdown obtained after centrifugation were determined by the method of Lowry *et al.* (14). A decrease in the formation of the products of protein breakdown in the presence of the test compounds was used to determine their antiproteolytic activity (14).

Assay of Succinate Dehydrogenase Activity of Rat Brain Homogenate—The method of Kun and Abood (15) was followed for the determination of succinate dehydrogenase activity of rat brain homogenates, using succinate as the substrate and triphenyltetrazolium as a proton acceptor. Tissue homogenates in the presence of succinate in a buffered (pH 7.4) medium reduce the colorless tetrazolium salt to a red-colored water-insoluble formazan. The formazan can be dissolved easily in acetone, which, by precipitating tissue proteins, leaves a colored supernate ready for colorimetric determination.

The reaction mixture in a total volume of 3 ml consisted of 0.2 ml of 0.25 *M* sodium phosphate buffer (pH 7.4), 0.5 ml of 0.2 *M* sodium succinate, 1.0 ml of rat brain homogenates equivalent of 100 mg wet weight of tissue, and 1.0 ml of 0.1% triphenyltetrazolium chloride solution (freshly prepared). After shaking, the tubes were incubated for 30 min at 38°. The various oxadiazoles were dissolved in propylene glycol (100%) and were used at final concentrations of 1 and 2 m*M*.

All oxadiazoles were preincubated for 10 min with rat brain homogenates prior to the addition of succinate, and the reaction was continued for 30 min. The tubes were removed from the constant-temperature bath, and 7 ml of extra pure acetone was added immediately to arrest the enzyme reaction. The precipitate was then centrifuged, and the absorbance of the clear supernate was determined. The color intensity was measured[4]

[1] All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected. IR spectra were taken with a Perkin-Elmer Infracord spectrophotometer model 137, equipped with sodium chloride optics, in potassium bromide films in the 700–3500-cm^{-1} range.

[2] Commercial chemicals were used. Adenosine monophosphate (sodium salt), monosodium α-ketoglutarate, sodium pyruvate, NAD, sodium succinate, and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo.

[3] Potter-Elvehjem homogenizer.

[4] Perkin-Elmer spectrophotometer.

Table II—Anticonvulsant Activity of 2-(3,4-Methylenedioxyphenyl)-5-arylamino-1,3,4-oxadiazoles and Their Effect on the Respiratory Activity of Rat Brain Homogenates

Compound	Anticonvulsant Activity[a], % Protection	Pentylenetetrazol Mortality[b], %	Inhibition of Respiratory Activity[c], %			
			Pyruvate		α-Ketoglutarate	Succinate
			−NAD	+NAD		
I	50	50	67.8 ± 1.5	59.3 ± 1.4	37.2 ± 1.2	76.2 ± 1.3
II	50	30	69.5 ± 1.7	60.4 ± 1.6	31.1 ± 1.3	72.0 ± 1.3
III	80	0.0	70.8 ± 1.4	61.5 ± 1.5	49.3 ± 1.3	66.1 ± 1.0
IV	70	0.0	63.8 ± 1.3	50.3 ± 1.5	55.6 ± 0.8	70.6 ± 1.0
V	50	0.0	49.2 ± 1.5	41.6 ± 1.8	37.3 ± 1.3	33.4 ± 1.3
VI	70	0.0	84.5 ± 1.9	75.4 ± 1.8	30.0 ± 1.0	80.7 ± 1.5
VII	60	30	56.6 ± 0.9	50.3 ± 1.3	41.6 ± 1.5	71.1 ± 1.5
VIII	50	50	46.5 ± 1.3	40.6 ± 1.1	28.2 ± 1.3	40.3 ± 1.4

[a] Screening procedures were as described in the text. All substituted oxadiazoles were administered (100 mg/kg ip) 4 hr before the administration of pentylenetetrazol (90 mg/kg sc). [b] Represents pentylenetetrazol-induced mortality during 24 hr in each group of 10 animals. [c] Each experiment was done in duplicate. All values represent the mean of six values of percent inhibition, with the standard error of the mean calculated from three separate experiments. Inhibition was determined by the decrease in oxygen uptake by 100 mg of rat brain weight/hr. The final concentration of pyruvate, α-ketoglutarate, and succinate was 10 m*M*; NAD was used at 0.5 m*M*. All oxadiazoles were used at a final concentration of 2 m*M*. Assay procedures and the contents of the reaction mixture were as indicated in the text.

at 420 nm. An increase in the percent transmission provided a direct measurement of reduced triphenyltetrazolium chloride, which was taken as an index of the enzyme activity. The percent inhibition was calculated from the decrease in the absorbance with 100 mg of fresh tissue weight/30 min.

In addition, kinetic studies were carried out, and the enzyme inhibition by substituted oxadiazoles was determined by using the graphic method of Lineweaver and Burk (16) as modified by Dixon (17). In preincubation studies, rat brain homogenates in the incubation mixture were incubated with or without oxadiazoles at 38° for 10, 20, and 30 min prior to the addition of succinate. The zero-time experiments represent those in which the oxadiazoles and the substrate were added simultaneously to the reaction mixture containing appropriate brain homogenate preparations.

RESULTS

The anticonvulsant activity of the substituted 2-(3,4-methylenedioxyphenyl)-5-arylamino-1,3,4-oxadiazoles is recorded in Table II. The ability of oxadiazoles to provide protection at a dose of 100 mg/kg ip against pentylenetetrazol (90 mg/kg sc)-induced convulsions in mice ranged from 50 to 80%; maximum protection was observed with 2-(3,4-methylenedioxyphenyl)-5-(4-methylphenylamino)-1,3,4-oxadiazole (III). Administration of substituted oxadiazoles also provided significant protection against pentylenetetrazol-induced mortality during 24 hr since no mortality was observed with animals treated with III–VI.

The effects of substituted oxadiazoles on the *in vitro* respiratory activity of rat brain homogenates are shown in Table II. All substituted oxadiazoles inhibited oxidation of both NAD-dependent oxidation of pyruvate and α-ketoglutarate and NAD-independent oxidation of succinate. The maximum inhibition of pyruvate and sodium succinate was observed with 2-(3,4-methylenedioxyphenyl)-5-(2-methoxyphenylamino)-1,3,4-oxadiazole (VI), while 2-(3,4-methylenedioxyphenyl)-5-(2,4-dimethylphenylamino)-1,3,4-oxadiazole (IV) caused maximum inhibition of the oxidation of α-ketoglutarate.

The degree of inhibition by substituted oxadiazoles at a final concentration of 2 m*M* ranged from 46.5 to 84.5, from 28.2 to 55.6, and from 33.4 to 80.7% during oxidation of pyruvate, α-ketoglutarate, and succinate, respectively. It was presumed that endogenous NAD in rat brain homogenates was sufficient for the oxidation of pyruvate and α-ketoglutarate. On the other hand, the presence of added NAD to the reaction mixture not only increased the respiratory activity of rat brain homogenates during pyruvate oxidation but also decreased the inhibitory effectiveness of substituted oxadiazoles (40.6–75.6%).

All substituted oxadiazoles exhibited antiproteolytic activity (Table III). The degree of inhibition of trypsin activity during hydrolysis of bovine serum albumin increased with the increase in the concentration of these substituted oxadiazoles and ranged from 10.2 to 47.5 and from 15.8 to 71.8% at final concentrations of 0.5 and 1 m*M*, respectively. Maximum antiproteolytic activity was observed with 2-(3,4-methylenedioxyphenyl)-5-(2,6-dimethylphenylamino)-1,3,4-oxadiazole (V), while 2-(3,4-methylenedioxyphenyl)-5-(2-methylphenylamino)-1,3,4-oxadiazole (II) provided the least protection against trypsin-induced hydrolysis of bovine serum albumin.

The ability of substituted oxadiazoles to inhibit *in vitro* activity of succinate dehydrogenase activity of rat brain homogenates is recorded in Table IV. All compounds inhibited succinate dehydrogenase activity, and the degree of inhibition increased with the increase in their concentration. The inhibition ranged from 20.0 to 48.6 and from 55.0 to 87.5% at final concentrations of 1 and 2 m*M*, respectively.

The *in vitro* inhibition of succinate dehydrogenase of rat brain homogenates by substituted oxadiazoles was investigated by preincubation studies. Preincubation of these substituted oxadiazoles for varying times prior to the addition of succinate in *in vitro* studies in no way altered the degree of succinate dehydrogenase activity (Table V). These results indicated a rapidly reversible and possibly competitive inhibition by substituted oxadiazoles. This finding was further supported by kinetic studies with 2-(3,4-methylenedioxyphenyl)-5-(4-methoxyphenylamino)-1,3,4-oxadiazole (VII), which revealed the competitive nature of inhibition of rat brain succinate dehydrogenase (Fig. 1). The inhibitor constant, K_i, for VII was 0.015 m*M*.

DISCUSSION

These results indicating the equal effectiveness of substituted oxadiazoles in providing protection against pentylenetetrazol-induced convulsions and pentylenetetrazol-induced 24-hr mortality have failed to provide structure–activity relationships with respect to anticonvulsant

Table III—Effect of 2-(3,4-Methylenedioxyphenyl)-5-arylamino-1,3,4-oxadiazoles on the Inhibition of Trypsin-Induced Hydrolysis of Bovine Serum Albumin

Compound	Inhibition[a], %	
	1×10^{-3} *M*	5×10^{-4} *M*
I	36.3 ± 1.3	31.1 ± 0.8
II	15.8 ± 0.8	10.2 ± 1.2
III	31.4 ± 1.2	20.2 ± 1.4
IV	35.7 ± 1.2	19.7 ± 1.5
V	71.8 ± 1.5	47.5 ± 1.4
VI	50.0 ± 1.2	44.3 ± 1.2
VII	35.0 ± 1.3	18.8 ± 0.8
VIII	25.2 ± 1.2	21.3 ± 1.2

[a] Assay procedures were as indicated in the text. Each experiment was done in duplicate. All values represent the mean of six values of percent inhibition obtained from three separate experiments with the standard error of the mean. Concentrations given were the final concentrations of the oxadiazoles in the reaction mixture.

Table IV—Effect of 2-(3,4-Methylenedioxyphenyl)-5-arylamino-1,3,4-oxadiazoles on the Succinate Dehydrogenase Activity of Rat Brain Homogenates

Compound	Inhibition[a], %	
	2×10^{-3} *M*	1×10^{-3} *M*
I	57.5 ± 0.8	36.0 ± 0.9
II	55.0 ± 1.0	20.0 ± 0.6
III	75.0 ± 1.1	48.6 ± 0.7
IV	65.0 ± 0.6	46.3 ± 0.3
V	50.0 ± 0.5	37.1 ± 0.8
VI	80.0 ± 0.7	38.3 ± 0.4
VII	87.5 ± 0.9	36.5 ± 0.7
VIII	60.0 ± 1.0	40.0 ± 0.6

[a] Contents of the reaction mixture and assay procedures were as described in the text. Each experiment was done in triplicate. All values are the mean of nine values obtained from three separate experiments with the standard error of the mean. Concentrations given were the final concentrations of the oxadiazoles in the reaction mixture.

Table V—Preincubation Studies with 2-(3,4-Methylenedioxyphenyl)-5-arylamino-1,3,4-oxadiazoles during Inhibition of Rat Brain Succinate Dehydrogenase

Compound	Inhibition of Succinate Dehydrogenase[a], %			
	0 min	10 min	20 min	30 min
I	57.2 ± 0.1	57.3 ± 0.8	56.9 ± 0.3	57.4 ± 0.8
II	55.2 ± 0.6	55.6 ± 1.0	54.9 ± 0.6	55.1 ± 0.5
III	75.2 ± 0.7	75.3 ± 0.7	75.8 ± 0.4	75.2 ± 0.4
IV	65.0 ± 0.5	65.9 ± 1.0	65.3 ± 0.4	65.6 ± 0.3
V	50.0 ± 0.8	50.5 ± 0.9	50.0 ± 0.3	50.0 ± 0.8
VI	81.2 ± 0.4	81.7 ± 1.1	81.0 ± 0.2	81.5 ± 0.9
VII	87.5 ± 0.3	87.4 ± 0.6	87.4 ± 0.1	87.2 ± 0.7
VIII	60.3 ± 0.1	60.5 ± 0.1	60.5 ± 0.9	60.4 ± 0.3

[a] Contents of the reaction mixture and the assay procedures were as indicated in the text. The enzyme preparations were incubated with oxadiazoles for 10, 20, and 30 min before the addition of succinate. Zero-time experiments indicate that both succinate and oxadiazoles were added to the reaction mixture containing enzyme preparations simultaneously. All oxadiazoles were used at a final concentration of 2×10^{-3} *M*.

activity (Table I). The data indicate, in general, a trend of more protection from convulsions with less mortality. The unspecific inhibition of both the NAD-dependent and NAD-independent oxidations (Table II) and reversible and competitive inhibition of succinate dehydrogenase in rat brain homogenates (Tables III and IV and Fig. 1) indicated the possible sensitivity of substituted oxadiazoles toward both NADH–CoQ (oxido) reductase (Complex I) and succinate–CoQ (oxido) reductase (Complex II) of the electron transport chain. It is also possible that hydro CoQ–cytochrome c–O_2 (oxido) reductase (Complex III) and/or cytochrome c–O_2 (oxido) reductase (Complex IV) are also susceptible to these substituted oxadiazoles as reported earlier (9).

These substituted oxadiazoles inhibit flavine adenine dinucleotide-dependent oxidation of succinate (NAD-independent) and NAD-dependent oxidations of pyruvate and α-ketoglutarate by rat brain homogenates and thus differ from other anticonvulsants belonging to the group of quinazolones (18), thiazolidones (19), and nitrobenzamides (20) in their ability to inhibit both NAD-dependent and NAD-independent oxidations by rat brain homogenates, as observed with phenothiazines (21) and salicylic acid derivatives (22). The concentration-dependent antiproteolytic activity of the substituted oxadiazoles (Table III) was unrelated to their anticonvulsant activity.

These results failed to provide any relationship between *in vitro* antiproteolytic activity and *in vitro* inhibition of respiratory activity and succinate dehydrogenase activity of rat brain homogenates with anticonvulsant activity. Detailed investigations of the effects of these substituted oxadiazoles on the activity of purified enzyme systems possibly may reflect a biochemical basis for their anticonvulsant activity.

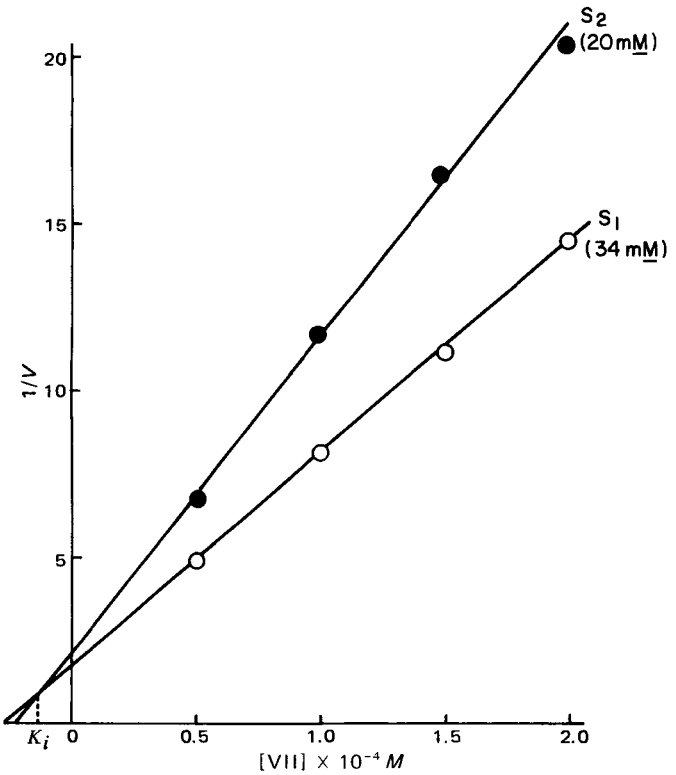


Figure 1—*Kinetic study showing competitive inhibition of rat brain succinate dehydrogenase by VII. The 1/V represents the reciprocal of enzyme activity as described in the text. The concentrations of succinate were 20 and 34 mM. The* K_i *value for VII was 0.015 mM.*

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