added dropwise, at 0° and under nitrogen, a solution of IV in methylene chloride (5 ml). The addition took 10 min. The reaction was allowed to reach room temperature and then refluxed for 24 hr. At that time, a 30% excess of isopropylidene-*sn*-glycerol was added and the reaction was refluxed for an additional 16 hr or until TLC indicated maximum conversion.

The reaction mixture was diluted with ether and washed successively with 3 *N* hydrochloric acid, 5% sodium bicarbonate, and water. The bicarbonate extract was acidified with concentrated hydrochloric acid to give 1.92 mCi of 1-^{14}C-adamantanecarboxylic acid. The organic layer was dried over magnesium sulfate, filtered, and concentrated to dryness under aspirator vacuum. The residual oil was used directly in the next step. The radiochromatographic purity of the product was 95% as determined by TLC.

In a typical cold run, the reaction was stopped after 24 hr of refluxing in methylene chloride. After workup in the usual manner, the residual clear oil was stored in a stoppered glass vial at 4°. After a few days, a white solid crystallized out of the oil. The solid was triturated with cold acetonitrile and vacuum filtered to give 0.060 g, mp 204–205°; IR (KBr): 1810 and 1730 cm^{-1}; NMR ($CDCl_3$): 1.9 (m, 18H) and 1.7 (m, 12H) ppm; mass spectrum *m/e* 168, 135; identical to an authentic sample of adamantanecarboxylic anhydride prepared by the method of Stetter and Rauscher (7).

3-(α-^{14}C-1-Adamantoyl)-*sn*-glycerol (VII)—A solution of V in ether (2.5 ml), methanol (1 ml), and 3 *N* hydrochloric acid (0.4 ml) was stirred magnetically at room temperature for 16 hr. An additional 0.2 ml of 3 *N* hydrochloric acid was added, and the mixture was stirred for 15 hr or until TLC indicated complete conversion. The reaction mixture was diluted with ether and washed successively with water, 5% sodium bicarbonate, and saturated sodium chloride solution. The organic layer was dried over sodium sulfate, filtered, and concentrated to dryness under aspirator vacuum. The residual oil was used directly in the next step. The radiochromatographic purity of the product was 98.5% as determined by TLC.

1,2-Dioleoyl-3-(α-^{14}C-1-adamantoyl)-*sn*-glycerol (I)—To a solution of VII in methylene chloride (2 ml) was added pyridine (0.48 g, 6 mmoles) in methylene chloride (1 ml). The solution was cooled to 0–5° in an ice–water bath. To this magnetically stirred solution under nitrogen atmosphere was added dropwise oleoyl chloride (1.8 g, 6 mmoles) in methylene chloride (3 ml) over 5 min. The reaction was allowed to reach room temperature, stirred magnetically for 15 hr, diluted with ether, and washed successively with water, 3 *N* hydrochloric acid, 5% sodium bicarbonate, and saturated sodium chloride solution.

The ether solution was dried over sodium sulfate, filtered, and concentrated under aspirator vacuum to an oil. The oil was dissolved in 2 ml of petroleum ether–ether (1:1 v/v) and applied to a glass column packed with 50 g of magnesium silicate. The column was eluted with five 30-ml volumes of petroleum ether–ether (1:1 v/v). Fractions 4 and 5, which contained the desired product, were combined and evaporated to an oil under a vacuum of 5 μm. The residual oil weighed 1.35 g (13.8 mCi). The radiochemical yield was 63.8% (based on III), and the radiochromatographic purity was 94.3% as determined by TLC (Fig. 2); the specific activity was 8 mCi/mmole.

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Anticonvulsant Activity of Enzyme Inhibitors in Rats

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Abstract □ Three liver microsomal enzyme inhibitors, proadifen, 2,4-dichloro-6-phenylphenoxyethyldiethylamine, and 2,4-dichloro-6-phenylphenoxyethylamine, and a hepatotoxic agent, carbon tetrachloride, were tested for anticonvulsant activity in adult male albino rats using the maximal electroshock seizure technique. All four substances exhibited significant anticonvulsant activity 1 hr after intraperitoneal administration. This protection was absent when tested 24 hr later.

Keyphrases □ Enzyme inhibitors, liver microsomal—proadifen and two substituted ethylamines screened for anticonvulsant activity, rats □ Hepatotoxic agents—carbon tetrachloride, screened for anticonvulsant activity, rats □ Anticonvulsant activity—evaluation of liver microsomal enzyme inhibitors proadifen and substituted ethylamines, rats

Several reports indicated that enzyme inhibitors, when combined with antiepileptic drugs, increased the therapeutic effects of antiepileptic drugs (1–3). These results suggested the need to study the effect of enzyme inhibitors *per se* for anticonvulsant activity. Preliminary results are reported now.

EXPERIMENTAL

Adult male albino rats, 100–200 g, were allowed free access to food and water prior to testing. Anticonvulsant potency was determined by the maximal electroshock seizure test (150 mamp for 0.2 sec through ear clip electrodes) (4). Abolition of the hindlimb tonic extensor phase of the maximal electroshock seizure test was taken as the end-point for measuring anticonvulsant activity (5).

Five groups of animals (20–37/group) were used, one group each for proadifen[1] (β-diethylaminoethyl diphenylpropylacetate) (I), 2,4-dichloro-6-phenylphenoxyethyldiethylamine[2] (II), 2,4-di-

[1] Also designated by the code number SKF-525A; courtesy of Smith Kline and French Laboratories, Welwyn Garden City, Herts., England.

[2] Also designated by the code number Lilly 18947; courtesy of Eli Lilly.

Table I—Effects of Enzyme Inhibitors on Maximal Electroshock Seizure

Group	Drug	Dose, per 100 g	Number of Animals Protected / Number of Animals Used: After 1 hr	After 24 hr	Percentage of Animals Showing Abolition of Extensor Phase: After 1 hr	After 24 hr	Statistical Significance[a]: After 1 hr	After 24 hr
1	Sodium chloride (0.9%)	0.1 ml	2/20	0/20	10	0	N.S.[b]	—
2	Carbon tetrachloride (95%)	0.1 ml	27/37	1/37	73	3	<0.001	N.S.
3	I	5 mg	24/35	2/35	69	6	<0.001	N.S.
4	II	1 mg	20/20	6/20	100	30	<0.001	N.S.
5	III	1 mg	15/20	1/20	75	5	<0.001	N.S.

[a] Determined by the χ-square test. [b] N.S. = not significant.

chloro-6-phenylphenoxyethylamine[3] (III), and carbon tetrachloride. One group served as a control.

Rats previously shown to produce a positive hindlimb extensor phase for maximal electroshock when tested 1 hr before the actual study were injected intraperitoneally with the drug. For the control group, 0.9% sodium chloride was injected intraperitoneally. All animals were subjected to maximal electroshock 1 and 24 hr after injection, and the number of animals showing absence of the extensor phase was noted.

RESULTS

A summary of the results obtained is given in Table I. The control group did not show any apparent change.

All three enzyme inhibitors and the hepatotoxic agent showed significant anticonvulsant activity 1 hr after administration. The protection afforded was gone when tested 24 hr later.

DISCUSSION

Of the agents used, anticonvulsant activity was reported previously only for I (1). In that study, 129–141 mg/kg administered orally to mice produced anticonvulsant activity within 1–2 hr, reached a peak effect after 3 hr, and entirely disappeared within 10 hr. It also was reported that 50 mg/kg had no observable effect on the pattern of maximal electroshock seizures. In the present study, 50 mg/kg ip of I in rats produced significant anticonvulsant activity. Absence of such activity 24 hr later is in agreement with previous results (1).

A comparative time course study of the duration of anticonvulsant activity in rats for I, II, III, and carbon tetrachloride is currently underway. Compound I was reported (6) to have a membrane-stabilizing activity, which may be one possible mechanism for its anticonvulsant action.

To date, except for liver enzyme-inhibiting activity (7–9), no pharmacological data are available for II and III[4]. Hence, a detailed study of these agents, particularly for their membrane-stabilizing activity, is in progress.

Peak enzyme inhibition after the intraperitoneal injection of I, reported to be 1–2 hr (10), correlates with the time of its peak anticonvulsant effect. Since both enzyme inhibitors and the hepatotoxic agent have been shown to have anticonvulsant activity, the possible role of biochemical changes produced by these agents (including alteration in functional enzymic level) in affording protection against maximal electroshock warrants further study.

[3] Also designated by the code name DPEA; courtesy of Eli Lilly.

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