Pharmacological Assessment of 3-*tert*-Butylsydnone

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Abstract □ The pharmacological effects of the mesoionic derivative, 3-*tert*-butylsydnone, were investigated. Administration to rats caused clonic convulsions. The CD_{50} of 3-*tert*-butylsydnone was 0.471 ± 0.033 mmole/kg. Trimethadione, but not phenytoin sodium or proadifen hydrochloride, protected the rat from the effects of 3-*tert*-butylsydnone. After administration of this compound, pentobarbital sodium sleeping time was reduced in the rat, but blood pressure and ECG were unchanged in the dog. Pretreatment of the mouse with 3-*tert*-butylsydnone did not influence the LD_{50} of epinephrine hydrochloride. The action of methacholine chloride in the rat was not blocked, and the pupil of the rabbit eye was unaffected. Tests for analgesic and oxytocic activity were negative. Chronic administration of a small dose to the rat for 70 days had no effect on blood glucose, blood urea nitrogen, hemoglobin, or microhematocrit values.

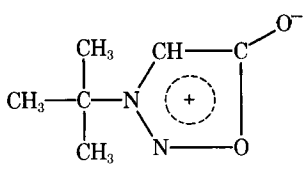
Keyphrases □ 3-*tert*-Butylsydnone—pharmacological and toxicological evaluation □ Sydnones—pharmacological and toxicological evaluation of 3-*tert*-butylsydnone □ Pharmacology—screening of 3-*tert*-butylsydnone

The medicinal chemistry and pharmacology of mesoionic compounds, including the sydnones, have been reviewed (1). Mesoionic compounds generally consist of a five- or six-atom heterocyclic aromatic ring system. The ring has a partial positive charge, which is balanced by a corresponding negative charge on an exocyclic atom or group (2, 3). The sydnones are of interest to the pharmacologist because of their wide spectrum of biological activity, and their unique physical and chemical properties make the compounds potential tools in drug receptor studies. Other than the fact that 3-*tert*-butylsydnone (I) causes convulsions in the mouse, little is known about its actions (4). This study was undertaken to gain additional pharmacological data concerning I.

EXPERIMENTAL[1]

General Data and Criteria—All drugs and 3-*tert*-butylsydnone (I) were administered by the intraperitoneal route, maintaining a constant injection volume if possible. Suspensions of I were made in 0.5% (w/v) acacia. All control animals received equivalent volumes of the suspending agent.

Sprague–Dawley rats (175–200 g), New Zealand white rabbits (3.2–3.8 kg), Swiss Colony mice (16–26 g), and mongrel dogs (10–10.5 kg) were employed (all females). Animals were maintained on commercial laboratory feed[2] and water *ad libitum* but were fasted 12 hr before experimentation. Rats and mice were randomized according to weight.



I

[1] An initial supply of I was generously provided by Dr. L. B. Kier, Massachusetts College of Pharmacy. Supplemental amounts were synthesized in this laboratory.

[2] Purina laboratory chows.

Table I—Effect of Pretreatment with Trimethadione, Phenytoin Sodium, and Proadifen Hydrochloride on 3-*tert*-Butylsydnone-Induced Convulsions and Death in the Rat[a]

Pretreatment	Protected from Convulsions	Deaths
Control (none)	0	4
Trimethadione (2.794 mmoles/kg)	8[b]	0
Phenytoin (0.091 mmole/kg)	0	3
Proadifen hydrochloride (0.077 mmole/kg)	0	7

[a] χ-Square test on each drug in comparison with untreated control. [b] $p < 0.005$ ($n = 8$); all other values are not significant.

Animals demonstrating clonic and/or tonic seizures of at least 5-sec duration within 1 hr of the administration of I or pentylenetetrazol were considered to have convulsed. However, animals demonstrating hyperflexia and tremors or fasciculations were not counted. The poor solubility of I precluded the use of isolated tissue techniques. Exceptions to this protocol are indicated.

CD_{50} Determination—The Reed–Muench (5) method was employed for the determination of the median convulsive dose (CD_{50}) of I for the rat and the median lethal dose (LD_{50}) of epinephrine hydrochloride[3] for the mouse. Five doses of I (0.211, 0.268, 0.345, 0.444, and 0.563 mmole/kg) were administered to 50 rats. Each dose was given to 10 rats. Two groups of 50 mice each were used to determine the effect of I upon the LD_{50} of epinephrine hydrochloride. The test group received 0.246 mmole/kg of I 15 min before epinephrine hydrochloride. The other group served as a control. Each group received five doses of epinephrine hydrochloride (0.009, 0.018, 0.032, 0.050, and 0.091 mmole/kg). The standard errors for the CD_{50} and LD_{50} values were computed by the method of Pizzi (6).

Convulsant Studies—Four groups of eight rats each were employed to determine the effects of two anticonvulsants and an inhibitor of drug metabolism on the convulsant action of I. One group served as a control, and each remaining group was pretreated with one of the following: phenytoin sodium[4], 0.091 mmole/kg; trimethadione[5], 2.794 mmoles/kg; and proadifen hydrochloride[6], 0.077 mmole/kg.

Sixty minutes after pretreatment with anticonvulsants or 30 min after proadifen hydrochloride, the animals were injected with 0.704 mmole/kg of I and the protection or lack of protection from convulsions and the frequency of death were observed. The results (Table I) were analyzed employing a 2×2 χ-square test.

Pentobarbital Sleeping Times—The effect of I on pentobarbital sodium[7] sleeping time in the rat was determined in the following manner. Two groups of 10 rats each were given 0.141 mmole/kg of pentobarbital sodium. Twenty minutes after the loss of the righting reflex, the animals in one group were injected with 0.704 mmole/kg of I. Control rats were sham injected. In place of I, pentylenetetrazol[8], 0.724 mmole/kg, was administered to a third group, and sleeping times were recorded. An animal was judged awake if righting of the anterior limbs could be demonstrated. The Student t test was employed to compare data from control and test animals (Table II).

Action on Anesthetized Dogs—The following procedure was

[3] Adrenalin hydrochloride, Parke-Davis.
[4] Dilantin sodium, Parke-Davis.
[5] Tridione, Abbott.
[6] SK&F 525-A.
[7] Nembutal sodium, Abbott.
[8] Sigma Chemical Co.

Table II—Effect of 3-*tert*-Butylsydnone on the Pentobarbital Sodium Sleeping Time of the Rat[a]

Treatment after Pentobarbital	Mean Sleeping Time, min	
	Test Group	Control
3-*tert*-Butylsydnone (0.704 mmole/kg)	89.40[b]	177.22
Pentylenetetrazol (0.724 mmole/kg)	34.56[b]	146.56

[a] Student *t* test in comparison with control sleeping times. [b] $p < 0.001$ ($n = 10$).

employed to determine the effect of I on the intact dog. One male and two female dogs were anesthetized with 0.141 mmole/kg of pentobarbital sodium, and the tracheas were cannulated. Blood pressure was measured directly off the left carotid artery. ECG recordings were from needle electrodes placed subcutaneously in the midthorax region. Transducers were connected to an amplifier and recorder[9].

Injections were *via* the femoral vein at least 5 min apart and were followed by a 0.9% saline wash. Acacia (0.5%) was injected as a control. One female dog received three doses of 0.070 and two doses of 0.176 mmole/kg of I; the other female received one dose of 0.007, three doses of 0.070, two doses of 0.176, three doses of 0.352, and two doses of 0.704 mmole/kg of I. After the final dose of I, epinephrine hydrochloride (0.046 nmole/kg), levarterenol bitartrate[8] (0.031 nmole/kg), and acetylcholine chloride[8] (0.006 nmole/kg) were injected.

Uterine Studies—Four rabbits, two multiparous and two pregnant, were employed to study the effect of I on the uterus. The animals were anesthetized with 19 mmoles of urethan[10]. A laparotomy was performed, and each uterine horn was attached to a separate myograph connected to a recorder. Throughout the experiment, the uterus and intestines were bathed with 37° isotonic saline. All injections were into the marginal ear vein with a minimum of 10 min between injections and were followed by a saline wash.

One multiparous animal received eight doses of I ranging between 0.070 and 0.352 mmole/kg. The other rabbit received 0.070, 0.141, and 0.211 mmole/kg of I. One pregnant rabbit was given two doses of 0.211 mmole/kg of I, and the second animal received two doses of 0.211 and one dose of 0.282 mmole/kg of I. Ergonovine maleate[11], 4.53×10^{-4} mmole/kg, was administered as a control to each rabbit.

Methacholine Studies—Forty rats were employed to study the effect of I on methacholine chloride[12]-induced salivation and bloody tears. The animals were divided equally into five groups and pretreated with I. Animals within each group received one of the following doses of I: 0.070, 0.092, 0.120, 0.162, and 0.211 mmole/kg 15 min prior to administration of 0.05 mmole/kg of methacholine chloride. Two additional groups were pretreated at 30 and 60 min with 0.162 mmole/kg of I.

Two control groups were employed: methacholine chloride alone and methacholine 15 min after 0.001 mmole/kg of atropine sulfate[10]. The presence or absence of bloody tears or salivation 15 min after methacholine was noted. This method was first described by Winbury *et al.* (7).

Ophthalmic Studies—Six rabbits were equally divided into two groups for the purpose of determining the effect of I on the eye. Into the left eyes of control animals, 0.2 ml of atropine sulfate (1.44×10^{-3} mmole/ml) was instilled. Into the left eyes of test rabbits, 0.2 ml of I (0.018 mmole/ml in a 1% aqueous solution of polysorbate 40)[13] was instilled. Right eyes served as controls.

The diameter of each pupil was measured at time 0, 30, 60, and 120 min and 24 hr after instillation. The pupil's response to light was noted. Constant room light was employed throughout. The procedure was repeated with polysorbate 40 alone (Table III). This method was previously described by Edwards *et al.* (8).

Analgesia—The analgesic screening technique of Marozzi and Malone (9), utilizing the tail flick response to a thermal stimulus from a conduction dolorimeter[14], was employed for the determination of possible analgesic activity. Tail flick responses were measured with an electrical timer equipped with a foot switch. For each rat, three successive reaction times in seconds were determined 1 min apart. The mean of the last two values was recorded.

After determination of control reaction times, the animals were treated with a test substance. Subsequent reaction times were determined at 15, 30, and 60 min after injection. A reaction time greater than 20 sec was considered equivalent to 100% analgesia. Each test group of five rats received one of the following doses of I: 0.070, 0.092, 0.120, 0.162, and 0.211 mmole/kg. There were two control groups; one was sham injected while the other received 0.013 mmole/kg of morphine sulfate[15] (Table IV).

Chronic Effects—To observe the chronic effects of I, 10 rats were injected daily with 0.035 mmole/kg of I for 70 days. Control animals were sham injected. Throughout the treatment period, cage droppings were observed for evidence of diarrhea and other changes. On the final day, 2 ml of blood was withdrawn under ether anesthesia from each animal by cardiac puncture. Blood glucose, blood urea nitrogen, and hemoglobin values were determined[16]. Microhematocrit values were also obtained. The mean values ±*SD* were determined (Table V).

Table III—Effect of 3-*tert*-Butylsydnone and Atropine Sulfate on the Mean Percent Change in the Diameter of the Pupil of the Rabbit Eye[a]

Time after Instillation into Eye	Mean Percent Change	
	0.2 ml 3-*tert*-Butylsydnone (0.018 mmole/ml)	0.2 ml Atropine Sulfate (1.44×10^{-3} mmole/ml)
0 min	0	0
30 min	0	+27
60 min	0	+26
24 hr	0	+9

[a] Percent change = $[(C_p - T_p)/C_p] \times 100$, where C_p = size of control pupil in millimeters, and T_p = size of test pupil in millimeters. Each value represents the mean percent change for three rabbits determined at a given time point.

RESULTS

The CD_{50} of I was 0.417 ± 0.033 mmole/kg. A dose of 0.704 mmole/kg was arbitrarily selected and always produced convulsions in the rat. Within 5 min after injection of I, asymmetric clonic convulsions were apparent in the animals and were indistinguishable from convulsions exhibited by rats poisoned with pentylenetetrazol. Subcutaneous and oral administration of I to rats also caused clonic convulsions.

The results shown in Table I demonstrate that trimethadione, but not phenytoin or proadifen hydrochloride, protected the rat from convulsions due to I. Although the frequency of death was greater in the proadifen hydrochloride group, the results were not significant.

Pentobarbital sodium sleeping time was decreased by convulsive doses of I without causing convulsions. Pentylenetetrazol reduced sleeping time in the control group (Table II).

Blood pressure and the ECG of the dog were unaffected by I. However, supplemental pentobarbital was required to maintain anesthesia after injection of greater than 0.176 mmole/kg of I. No convulsions were observed after the administration of I to the anesthetized dogs. Responses to epinephrine hydrochloride, levarterenol bitartrate, and acetylcholine chloride were all normal in the I-treated animals.

Pretreatment of the mouse with I did not influence the LD_{50} of epinephrine hydrochloride, which was determined to be 0.061 ±

[9] Narco Bio Systems.
[10] Merck Chemical Co.
[11] Ergotrate Maleate, Lilly.
[12] Mecholyl, Merck.
[13] Tween 40, Atlas Chemical Co.
[14] Metro Scientific Co.
[15] Mallinckrodt.
[16] Bio-Dynamics Unitest System.

Table IV—Effect of 3-*tert*-Butylsydnone and Morphine Sulfate on the Tail Flick Response to Thermal Stimulus in the Rat

Treatment	Mean Percent Analgesia[a]		
	15 min	30 min	60 min
3-*tert*-Butylsydnone (0.070, 0.092, 0.120, 0.162, and 0.211 nmole/kg)	0	0	0
Acacia, 0.5%	0	0	0
Morphine sulfate (0.013 mmole/kg)	35.81	52.86	21.35

[a] Percent analgesia = $[(Rx - C_0)/(20 - C_0)] \times 100$, where C_0 = control reaction time in seconds for each rat determined before treatment; Rx = reaction time in seconds for each rat determined 15, 30, or 60 min after treatment; and 20 = 20 sec (100% analgesia). Each value represents the mean percent analgesia of five rats.

0.013 nmole/kg in the control and 0.069 ± 0.010 nmole/kg in the test animals.

The administration of I had no effect on the rabbit uterus. Ergonovine maleate caused uterine contractions.

The production of bloody tears and salivation by methacholine chloride in the rat was not prevented by prior administration of I. However, atropine sulfate blocked the action of methacholine in the control animals.

As indicated in Table III, the instillation of a solution of I into the eye of the rabbit had no mydriatic or other observable effects. When treated with atropine sulfate, the eyes of control animals were dilated and unresponsive to light. Polysorbate 40 alone had no effect on the rabbit eye.

Morphine sulfate, but not I, produced analgesia in the rat (Table IV).

As indicated in Table V, blood glucose, blood urea nitrogen, hemoglobin, and microhematocrit values from control and I-treated rats were within the standard error of the mean of each other. There was no observable difference between the feces of the control and test animals in this study.

DISCUSSION

There is little question as to the convulsant action of 3-*tert*-butylsydnone (I). The physical similarities between the convulsions produced by I and pentylenetetrazol suggest that I may act primarily on the brain stem. Like pentylenetetrazol, convulsant doses of I decrease pentobarbital sleeping time without causing convulsions, indicating that hypnotic doses of pentobarbital sodium and convulsant doses of I antagonize the actions of each other. This finding is supported by the increase in the amount of pentobarbital sodium required to maintain anesthesia after injection of I into the dog.

The protection of the rat by trimethadione, but not by phenytoin, against seizures induced by I suggests that I could be employed in place of pentylenetetrazol as a screening agent for drugs effective against petit mal epilepsy. Furthermore, there may be some benefit to using activity against the convulsant effects of I as a criterion for hypnotic, antianxiety, or antiepileptic activity. However, the poor solubility of I may present a problem.

When compared on a millimolar basis, the doses of I are considerably larger than those of the control drugs. The highest dose of I was 623 times greater than the control dose of ergonovine maleate that caused uterine contractions. The concentration of I instilled into the eye of the rabbit was 12 times the control dose of atropine sulfate. In the methacholine experiment, the highest ineffective dose of I was 211 times in excess of the blocking dose of atropine sulfate. The analgesic dose of morphine sulfate was 16 times smaller than the maximum dose of I. In the experiments with the dog, the dosages of I were usually considerably greater than the doses of epinephrine, levarterenol, and acetylcholine administered as positive controls.

Table V—Blood Analysis of Rats Treated with 0.013 mmole/kg of 3-*tert*-Butylsydnone Daily for 70 Days[a]

	Test	Control
Blood glucose, mg %	115.5	104.45
SE	±5.69	±5.73
Blood urea nitrogen, mg %	12.22	12.05
SE	±0.67	±0.86
Hemoglobin, g %	16.98	16.27
SE	±0.29	±0.44
Microhematocrit, % rbc[b]	46.0	43.09
SE	±0.95	±1.63

[a] n = 10 for each group. [b] % rbc = percent red blood cells.

The comparatively high doses of I resulted in a predominance of negative results with respect to pharmacological activities other than convulsions. This finding suggests that these activities are absent or, if they do exist, that I is considerably weaker than the positive control drugs employed. Another possibility is that an additional pharmacological action of I is masked by the convulsions. However, the uneventful administration of doses of I greater than the CD_{50} (in rats) to anesthetized dogs appears to discount this argument.

It can be concluded from this study that the outstanding pharmacological activity of I was that of a convulsant and that future studies should be of a mechanistic neuropharmacological and neurochemical nature. Compound I is apparently devoid of other pharmacological–toxicological properties and associated problems. This information, coupled with the unique structural properties of sydnones, which lend themselves to the study of drug–receptor interactions, may be of value in determining the structural configuration requirement for convulsant activity and in mapping receptors to convulsant drugs.

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