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Toxic Effects of a New Boron Containing Heterocycle: 4,4,8,8-Tetraethyl-3,3a,4,8-Tetrahydro 3a,4a,4-Diazabora-S-Indacene (39051)

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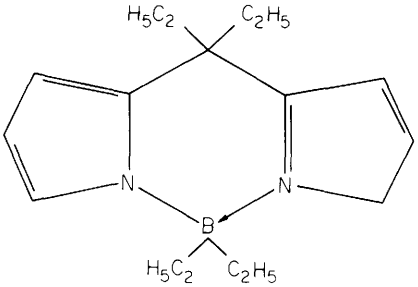
A great deal of research has been done on the toxicity of boron hydrides and the common boron compounds such as boric acid and borax. However, the toxic properties of organoboron compounds has been relatively sparse (1). In particular, the boron containing heterocycles have been rather neglected owing to their general lack of hydrolytic stability (2–4). If the boron containing heterocycles could be made more stable to hydrolysis, they could serve as vital tools in exploring the area of the neutron capture therapy of cancer. (1).

Dewar and his associates have described a class of compounds which may permit the synthesis of boron containing purines and pyrimidines (5–11). Also, Yale *et al.* have reported the synthesis of very stable boron heterocycles (12).

More recently, however, Bellut *et al.* (13) have prepared some new boron heterocycles as intermediates to other compounds. These intermediates have shown unusually high stability towards acids, alkalies, and water. In this paper is presented the basic toxicity of one of these hydrolytically stable heterocycles.

Material and methods. Animals. Both male and female I. C. R. strain albino mice from 25 to 35 g were used in each series of experiments. These animals were reared in our laboratory from stock purchased from Ward's Natural Science Establishment, Inc., Rochester, New York, and were maintained on Purina Laboratory Chow and tap water, *ad libitum*. The mice were caged in polypropylene closed bottom cages with galvanized mesh wire tops. A clay base absorbent was used to cover the bottoms of these cages. The ambient conditions were such that the temperature was approximately 72°F and diffused fluorescent lighting was maintained for 9–10 hr/day.

Test compound. Crude 4,4,8,8 tetraethyl 3,3a,4,8 tetrahydro-3a,4a,4-diazabora-S-indacene (Myborin) was obtained from Dr. Clifford D. Miller, by whom the compound was originally prepared (13). The structure of this compound is as shown in Scheme 1.



The crude Myborin was recrystallized from methanol under aspirator vacuum. The crystals, which have a very pale yellow color when pure, were then desiccated for 18 hr under mechanical vacuum to remove traces of methanol. The crystalline form was stored at room temperature in an amber bottle and in the dark.

For injection, Myborin was prepared as a solution in Mazola corn oil at 10 mg/ml and as a suspension at 20 mg/ml. During the course of experimentation, Myborin was found to undergo an apparent photolytic decomposition when stored at ambient temperature in a lighted room. As a consequence, the solutions used in the following investigations were stored at 4 ± 1° in the dark and were used within 1 month. This procedure seemed to effectively retard decomposition. It is noteworthy that in experiments conducted with solutions which had been purposefully degraded using ultraviolet light, the gross toxicology did not change within the limits of experimental error.

Treatment of animals. Acute intraperitoneal toxicity. Several trials at various

dosage levels following the method of Deichmann and LeBlanc (14) indicated that the LD_{50} was in the range of 56–104 mg/kg (80 ± 30%). Consequently, three equivalent groups of seven mice each were injected ip with a Myborin-corn oil solution (10 mg/ml) at 70, 80, and 90 mg/kg. These animals were observed for a 24-hr period after which the LD_{50} value was determined by the method described by Litchfield and Wilcoxon (15). Survivors were then observed for at least 6 more days and weights recorded daily. Control animals received 0.5–1.0 ml of corn oil.

Acute oral toxicity. Mice were fasted for 4 hrs prior to administration of the test solution *via gavage*. Myborin was given at levels of 10, 16, 24, 37, 55, 80, 120, 180, 280, and 420 mg/kg. LD_{50} values were determined after 24 hrs, taking the lowest dose at which death occurred consistently to be the median lethal dose ± 30% (14). The concentration of the solution was varied in order to keep the amount less than 1.0 ml. Body weights were recorded for survivors for 7 days. Controls received 1.0 ml of pure Mazola corn oil.

Acute subcutaneous toxicity. Myborin was administered sc in the dorsal thoracic region using 23 gauge 1.5 in. needles. Also, several animals were given injections under the skin of the abdomen to determine if the dermatologic effects, to be discussed later, were of a local nature. Animals were dosed at 80, 120, 180, 280, 420, and 620 mg/kg after the method of Deichmann and LeBlanc (14). Animals receiving dosages from 280 mg/kg and up, were injected with a suspension of Myborin in corn oil at a concentration of 20 mg/kg. Below this range, mice were administered Myborin in corn oil at 10 mg/ml. Several series of experiments were attempted using equal numbers of males and females at each dosage level. Mice were observed for a minimum of 7 days and the LD_{50} determined after this time. Body weights were recorded daily. Controls received 0.5 or 1.0 ml of Mazola corn oil.

Establishment of hepatomegaly. Ten mice (five males and five females) were each injected with a Myborin-corn oil solution sc at a level of 180 mg/kg. Also, five male and five female mice were given 1.0 ml of Mazola corn oil sc. Each group was maintained under the ambient conditions described previously for 7 days. On day 7, the mice were weighed to the nearest 0.1 g and sacrificed by a blow to the skull, decapitated and exsanguinated. Livers were excised, trimmed of excess tissue, and gall bladders removed. The livers were then washed with a 0.9% (w/v) NaCl solution, blotted, and weighed to the nearest 0.01 g on a quadruple beam balance. Liver weight to body weight ratios were then calculated, and the mean of each group compared by Student's *t* test.

Boron levels in liver. Five male and five female mice were given ip injections of Myborin in corn oil at 50 mg/kg. After 24 hr the livers were removed as described above and prepared for analysis by the technique described by Hatcher and Wilcox (16). The analytical method described is for plant tissue, but was found to be readily applicable to mammalian tissue. Concentrations of boron were determined colorimetrically based on a carmine-boron complexing reaction.

Ashing of samples was carried out for 5–5.5 hr.

The results were compared with boron concentrations found in an untreated group of five male and five female mice.

Establishment of photosensitivity. (Results discussed w/acute sc toxicity.) Five male and five female mice were given dosages of 180 mg/kg sc and maintained in total darkness for a period of 7 days. These animals were observed daily using a red 25 W incandescent light source. At the end of 7 days, the animals were placed into the main lab where the lights were of a diffused fluorescent type. The mice were then observed daily for an additional 7 days.

Results. Acute ip toxicity. The ip LD_{50} value as calculated by the method of Litchfield and Wilcoxon (15) was 69.5 mg/kg with the upper and lower confidence limits at 77.8 and 62.1 mg/kg, respectively. The initial toxic symptoms were generally noticed within 3 hr; however, occasionally they did not appear for as long as 6 hr. The first noticeable effect was an increase in muscle tone. This tonic state, over a period of 1–3 hr, grew more extreme and was followed

by a state of severe hyperreflexia. The affected animals were observed to give convulsive response to visual, auditory, and physical stimuli.

The convulsions take the form of a generalized seizure of a tonic-clonic nature, characterized by running motion and intermittent tonic extension of the limbs. Also, dyspnea and cyanosis were observed. These convulsive states were often transient and animals may undergo several such episodes before expiration. Convulsions were also observed to occur spontaneously.

Death was usually immediately preceded by convulsions, with tetanic convulsions as an end result. Opisthotonus and *risus sardonicus* were frequently encountered but were less severe than those observed in strychnine poisoning. Death was apparently a result of hypoxia, and generally occurred within 8 hr, but may be extended to 24 hr occasionally, especially at lower doses.

After 24 hr, moderate to severe erythema was noticeable in the ears and other exposed skin. Dilation of the ear vessels was also quite noticeable. These animals were usually marasmic and attendant wt losses of 4–8% were not uncommon. Frequently there was a reddish exudate from one or both eyes. Edema of the face and head region was usually observed within 24 hr.

On day 2 (48 hr after injection) there was virtually always jaundice apparent in the ears of these survivors. Accompanying the jaundice were wt losses up to 14% (Fig. 1).

By 72 hr most survivors exhibited beginning necrosis of the tips of the ears and hair losses from the eyelids and dorsal thoracic region (Fig. 2). Necrosis of the ears continued until there was eventual loss of 0.25–0.5 of both ears.

Oral toxicity. The lethal effects by the po route were found to be highly variable at lower doses. The results of many trials, however, established the best approximation of the LD_{50} to be 180 mg/kg (14). Doses at this level consistently produced death. Considering the 30% confidence limits, the true LD_{50} should lie in the range of 126–234 mg/kg.

The toxic manifestations by the po route paralleled those by ip administration as described above. However, the onset of

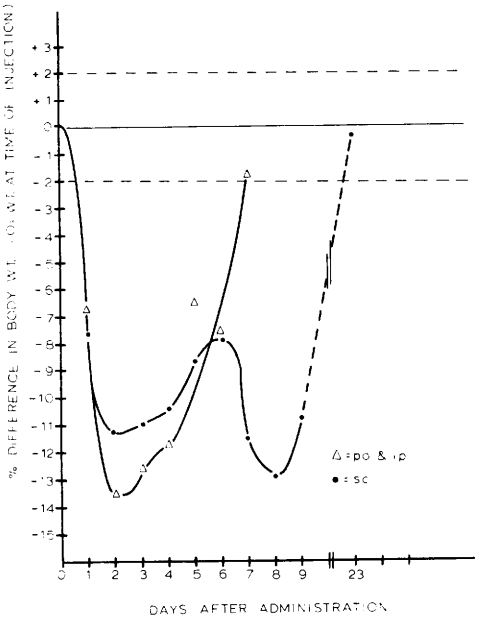


FIG. 1. Average daily weight losses, expressed as % of original body weight, are shown for mice receiving sc (●), ip, and po (△) injections of Myborin at all dosage levels combined. Also, the daily fluctuations of control mice are shown as the dashed lines. The somewhat bi-modal variation for sc injected mice seems to correspond with the peak of hepatic dysfunction on day 5 or 6. The general tendency of ip and po dosed mice is to regain their original weight by day 7.

symptoms generally occurred within 2 hr and were seldom delayed more than 4 hr.

By day 3, some animals developed minor necrosis of the ear tips and some loss of hair. Neither of these symptoms were as severe as the same symptoms attending ip and sc administration. All mice were observed to recover hair after 7 days and necrosis did not generally lead to separation of the ear tips.

Subcutaneous toxicity. The median lethal dose by this route was found to be 420 mg/kg. The upper and lower confidence limits (420 ± 30%) are 546 and 294 mg/kg (14).

Death as a result of sc administration did not follow the sequence described for ip and po routes. Death is apparently a result of acute induced porphyria.

After 24 hr, erythema and vasodilation of the ears was apparent. Weight losses were extreme (Fig. 1) and most animals were marasmic and lethargic. Forty-eight hours

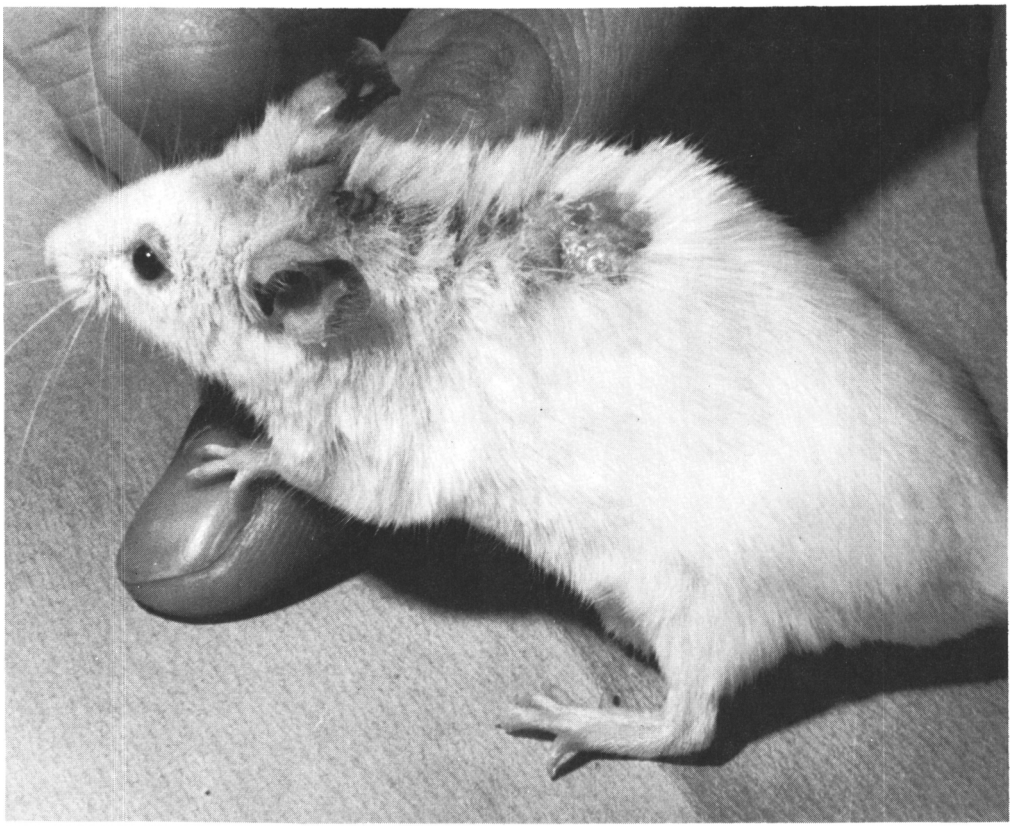


FIG. 2. This picture is representative of the necrosis and dermatological symptoms after administration of Myborin by all routes. Oral administration, however, does not produce these symptoms as severely. The picture was taken 7 days after sc administration of 100 mg/kg of Myborin in Mazola Corn Oil. The injection was given under the skin of the abdomen.

after first exposure, jaundice had begun to appear and continued to worsen until necrosis of the ears and skin appeared on day 3. The urine became dark yellow and had a fluorescentlike character.

After the onset of dermal symptoms, the necrosis of the ears and skin loss from the back and head increased in severity. Edema was usually present about the head and face. The skin loss usually extended from the nose to upper lumbar region and left permanent scar tissue. Necrosis of the ears led to complete loss of $\frac{1}{3}$ to $\frac{3}{4}$ of each ear. The toes and tip of tail were frequently found to undergo necrosis and gave the appearance of dry gangrene.

Severe scratching was found to accompany the onset of dermal symptoms and may be either a direct or indirect result of Myborin.

Animals injected under the abdominal skin underwent the same hair loss patterns. Skin was not sloughed from the site of injection and was, therefore, apparently not a result of direct contact with the compound.

The symptoms described almost invariably appeared within 7 days of administration and mice surviving this long continued to live until sacrificed on day 31. All survivors began showing signs of hair recovery and nearly regained their normal body weights. Upon autopsy all treated animals showed evidence of hepatomegaly.

Mice kept in total darkness for 7 days after receiving a dosage of 180 mg/kg of Myborin in corn oil developed mild to moderate jaundice but showed no erythema, necrosis, edema, or hair loss at the end of this time. When these same mice were again exposed to normal room lighting they began showing signs of necrosis, edema, increased jaundice,

and erythema within 24 hr. After 2 days of this exposure, three animals (two females and one male) had lost ½ to ⅔ of each ear from necrosis. Also, one female showed injury to the retina of the right eye and was blind in this eye. From this point on, the animals continued to undergo the same pattern of affliction as previously described for sc administration. However, the females suffered the greatest losses, in that all were adversely affected but only ⅖ of the males were affected. Even hair losses were not as severe in males; the latter showing only patchy losses from the back and head, but complete loss from eyelids.

Hepatomegaly. The observed mean liver wts for control mice and mice injected sc with Myborin in corn oil at a dosage of 180 mg/kg were 1.71g ± 0.13 (± SE) and 3.11g ± 0.19, respectively. The ratios of liver wt to body wt for those groups of mice were: $0.054 \pm 1.73 \cdot 10^{-3}$ (± SE) and $0.093 \pm 4.03 \cdot 10^{-3}$. These ratio values give a Student's t value of 8.90, corresponding to $P < 0.01$.

The livers of experimental mice were much darker and less friable than those of the controls. The gallbladders of the experimental mice were very much more distended than those of control mice. All Myborin dosed mice exhibited jaundice and erythema of the ears and very yellowed skins. All jaundiced mice showed evidence of oxidation of bilirubin to biliverdin in various places on the underside of the skin.

Boron levels in liver. The colorimetric analysis for boron in groups of control and experimental mice gave values of 8.11 ± 0.58 μg/g and 15.46 ± 0.37 μg/g (wet wt), respectively (± SE). Analysis of these values gives a Student's t of 15.53 corresponding to $0.01 > P$.

The increase in boron concentration is 91% and the average quantity of boron found in these livers corresponds to 23% of the injected amount.

Discussion. The toxicity of boron hydrides and organoboron compounds has generally been found to be quite high. In addition, these compounds have a very high tendency to produce CNS effects including convulsions, dyspnea, and opisthotonus. However, the toxicity of these compounds does not seem to be directly related to either the amount of boron in a compound or to the boron containing by-products, (e.g., hydrolysis to boric acid) (1, 17, 18).

In these respects, Myborin fits the general scheme of heterocyclic boron compounds (1). Weir *et al.* (19) report po LD_{50} values of 0.51 gB/kg and 0.6 gB/kg for borax and boric acid respectively. The value obtained in our experiments with Myborin by the po route corresponded to 5.04 mgB/kg–9.36 mgB/kg. As these values show, there is obviously no correlation with the amount of boron injected. Most assuredly, this would hold true for ip and sc administration. These facts do not, however, preclude the possibility that the toxic effects of the compound are due to the innate toxicity of Myborin per se or some catabolic intermediate such as that obtained by the loss of the boron diethyl moiety. In fact, the observed increase in the boron concentration of the liver corresponds to a level of ~182.8 mg Myborin/kg liver which indicates a high propensity for Myborin to concentrate in liver tissue.

Svirbly (18) reports very similar toxic reactions in mice after ip administration of decaborane. Among these are dark exudates from the eyes, convulsive tremors upon handling, lethargy, and loss of appetite.

The exact chemical mechanism of decomposition and elimination of Myborin from the body is not known at this time. However, the convulsive state invoked and resultant tetany after ip and po administration resemble closely those of hypocalcemia and/or hypomagnesemia. It can be reasonably speculated that Myborin may lose the boron diethyl moiety and in this state possibly function as a bidentate ligand, which could then tie up available magnesium and calcium.

On the other hand, neurologic attack is frequently associated with the inherited porphyrias which are symptomatically not distinguishable from the acquired forms. It has been speculated that the severity of this symptom may be related to the degree of hyponatremia. Additionally, hypocalcemia and hypomagnesemia have been observed apparently without relation to chelates (20). Also, Soloway reports that organic boron containing chemicals with high liposolubility

invariably penetrate the brain readily and produce toxic symptoms to the central nervous system (1).

It is the propensity to induce porphyrialike complications that is one of the most significant effects of Myborin, especially when such effects are not usually associated with organo-boron compounds. Reference to Table I shows that in nearly every respect Myborin induces symptoms paralleling hepatic porphyrias. While these similarities are most noticeable and severe in mice injected sc, the same effects were also noticed via the ip and po routes.

While porphyrias are generally described as caused by the accumulation of either the normal precursors of heme or of its by-products, it has certainly been shown that certain chemicals readily induce hepatic porphrias (20–22).

Careful scrutiny of the structure of Myborin reveals that it is very similar to half of a porphyrin ring system. While conclusive proof is not yet available, it is reasonable to assume that the Myborin molecule, in either its original state or after having lost the boron diethyl moiety, is structurally similar enough to the normal porphyrinogens to stimulate the liver into increased activity.

TABLE I. COMPARISON OF THE CLINICAL SYMPTOMS OF PORPHYRIAS AND MYBORIN TOXICITY.

Porphyrias	Myborin[a]
Jaundice	+
Hepatomegaly	+
Erythema	+
Edema	+
Vesiculation of exp'sd skin	+
Eczematoid lesions	+
Darkened urine	+
Neurologic attacks	+[b]
Visceral pain	?
Photosensitivity	+
Urticaria	+[c]
Increased excretion of uro- and protoporphyrinogens	—[d]

[a] Observed by all routes of administration.

[b] Only by ip and po routes.

[c] Scratching was often observed to be severe; wheals not demonstrated.

[d] Not determined in this study.

Note: + = observed.

The available weight loss data from the experiments reported yields some possibly relevant correlations (Fig. 1). On day 5 or 6 after sc dosing, mice began to lose wt after recovering from the initial wt losses. It would appear that this coincides with the acme of the hepatic syndrome. In ip and po administration, the initial losses are of the same magnitude, but recovery is more consistent after day 3. This may be due to faster elimination in the ip and po routes and the more chronic nature of sc dosages.

In conclusion, while Myborin remains primarily a laboratory curiosity and an intermediate to other compounds, it may well prove to be valuable in the study of acquired porphyrias. Also, as stated in the introduction, boron containing heterocycles in general, remain a relatively unstudied class of compounds having potential use in the neutron-capture therapy of cancer (1).

Summary. LD_{50} values in mice for 4,4,8,8-tetraethyl-3,3a,4,8-tetrahydro-3a,4a,4-diazabora-S-indacene (Myborin) were determined by the ip, po, and sc routes. The LD_{50} value for ip was 69.5 mg/kg found by the method of Litchfield and Wilcoxon, with upper and lower confidence limits of 77.8 and 62.1 mg/kg. Oral and sc LD_{50}'s were approximated after the method of Deichmann and LeBlanc and were found to be 180 mg/kg (po) and 420 mg/kg (sc). Each of these values has a confidence range of $\pm 30\%$.

Myborin induced convulsions, hyperreflexia, and death accompanied by tetany when given by either the ip or oral routes. Moreover, Myborin induced porphyria in animals surviving for 24 hr after these routes of administration and in virtually all animals dosed sc. Death by the sc route is probably a result of acute porphyria.

Hepatomegaly and skin photosensitivity were demonstrated to be profound.

Boron levels in the livers of mice were determined colorimetrically 24 hr after ip injections of Myborin and in untreated control mice. The quantity of boron found in the experimental group was 15.46 μg/g wet liver as compared to 8.11 μg/g wet liver for con-

trols ($P < 0.01$). The difference corresponds to 23% of the injected quantity of boron.

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