top of the chain, the isolated fragment soon loses part of its charge by hydrolysis and is pulled back by gravity to the surface of the bed or to another chain. With an anion-exchange resin the only difference is that the chains are attracted to the cathode.

The corresponding electro-osmotic flow of the water in the opposite direction was studied, but was not reproducible.

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Selective Rat Toxicant

Abstract. *The maleimide adduct of an unusual fulvene is highly toxic to rats when administered orally or parenterally. It does not induce significant toxic or other biological changes in common house pets and commercially useful mammals and birds when given at dose levels 200 times greater than the dose which is lethal to 50 percent of the rats it is administered to.*

One member of a series of norbornenedicarboximides has been found to be selectively toxic to rats. This compound, 5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide, which we have called McN-1025, does not affect mice or other species thus far tested even at high doses.

The material was administered orally to female rats (*1*), and the median lethal dose (LD_{50}) was determined, by the method of Litchfield and Wilcoxon (*2*), to be 5.3 (4.4 to 6.5) mg/kg (*3*). This compound is almost nontoxic in the mouse, for the LD_{50} is 2250 (1760 to 2800) mg/kg. It was also administered to a variety of other species (Table 1) and had no effect on the common varieties of mammals and birds. The animals were observed closely after initial administration and periodically for at least 5 days. Except in guinea pigs and rabbits, high doses (1000 mg/kg, orally) did not induce any significant behavioral changes.

The dicarboximide was given orally to two groups of dogs (eight animals each). One group received a daily amount equal to 0.1 percent of the daily food intake for 60 days without exhibiting deleterious effects. The second group received 10 times this amount. This treatment was terminated in two animals after 15 days and in two more after 30 days, but was continued in the other four animals for 60 days. All the animals in this group survived the designated treatment period, but they became somewhat ill and did not consume their normal amount of food.

The compound was also administered orally to a group of 50 wild Norway rats (*Rattus norvegicus*) captured at a local waste disposal plant. These animals were maintained in captivity for periods ranging from 2 to 30 days. The LD_{50} in this group of wild males and females was similar to that found in albino rats (Table 1). Likewise, a group of wild Roof rats (*Rattus rattus*) were obtained and given the dicarboximide by stomach tube. The data (Table 1) indicate that the LD_{50} in this species is approximately 30 to 40 mg/kg of body weight.

Table 1. Relative toxicity of a dicarboximide (McN-1025) to mammals and birds. In each instance the compound was administered orally by stomach tube as a solution in dilute acid or in dry powder tablet or capsule form.

Common name and species	Sex	No. of animals	Dose (mg/kg)	Remarks
Rat, albino (*Rattus norvegicus*)	F	30	5.3 (4.4–6.5)	LD_{50}
Rat, wild Norway (*R. norvegicus*)	M, F	50	12 (10–13)	LD_{50}
Rat, roof (*R. rattus*)	M, F	7	60	7/7 dead
		8	55	8/8 dead
		8	50	5/8 dead
		8	45	5/8 dead
Cattle (*Bos taurus*)	M, F	2	100	No effect
Cat (*Felis catus*)	M, F	6	100–1000	No effect
Dog (*Canis familiaris*)	M, F	11	100–1000	No effect
Guinea pig (*Cavia porcellus*)	M, F	12	30–1000	Deaths at 300 & 1000
Horse (*Equus caballus*)	M	1	100	No effect
Monkey, Rhesus (*Macaca mulatta*)	F	2	1000	No effect
Rabbit (*Oryctolagus cuniculus*)	M, F	31	10–1000	1/9 deaths at 1000
Sheep (*Ovis aries*)	M, F	2	1000	No effect
Chicken (*Gallus domesticus*)	M, F	20	10–1000	No effect
Turkey (*Meleagris gallopavo*)	M, F	4	1000	No effect

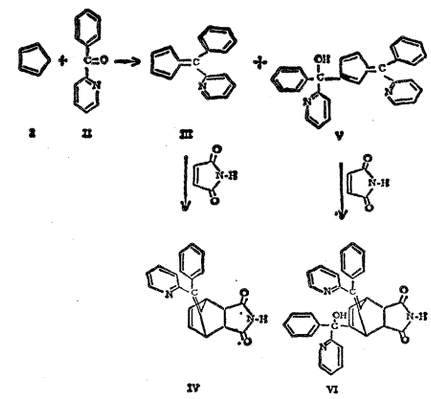

Fig. 1. Synthesis of the toxic carboximide.

After oral administration of an overdose of this material to rats, death occurred rapidly, that is, within 15 minutes to 4 hours. In the primary stages of illness, the animals initially assumed a huddled position and later there was locomotor impairment due to a weakening but not paralysis of the hind extremities. Struggling, labored breathing, and, in some instances, a mild convulsion preceded death. The peripheral extremities were blanched but not cyanotic. Gross and histopathological examination of selected vital organs and tissues revealed no characteristic abnormalities. These findings suggest that this carboximide induces some type of circulatory impairment which may, in turn, cause death.

The synthesis of this toxic carboximide (VI) is shown in Fig. 1. The base-catalyzed reaction of cyclopentadiene (I) and 2-benzoylpyridine (II) leads to a mixture of several products. The maleimide adduct IV of the expected 6-phenyl-6-(2-pyridyl) fulvene (III) has already been described (*4*).

In addition to this fulvene, the condensation of cyclopentadiene and 2-benzoylpyridine produces 2-(α-hydroxy-α-2-pyridylbenzyl)-6-phenyl-6-(2-pyridyl) fulvene (V), which can be obtained in high yield by varying the reaction conditions. Except for the multiple condensation of acetone with cyclopentadiene to give a complex mixture of products (*5*), there is no precedent in the literature for the formation of a trisubstituted fulvene such as this one.

A solution of 5.02 g of sodium in 1420 ml of absolute ethanol was pre-

pared, and 400 g of 2-benzoylpyridine was added and dissolved. The solution was cooled to 10°C, and 252 g of freshly distilled cyclopentadiene was added dropwise over a 30-minute period. After stirring under nitrogen for 16 hours at 10° to 13°C, the orange-red crystalline product was separated by filtration, washed with cold 1 : 1 alcohol-ether solution, and dried; 322 g (72-percent yield), mp 138° to 160° C; $\lambda_{max}^{CH_3OH}$, 324 mμ (ε, 23,400). The infrared spectrum, combustion analyses (C, H, N, and O), and molecular weight determinations were all consistent with structure V. Fractional recrystallization from ethyl acetate served to separate compound V into geometric isomers which melted at 175°–76°C and 181°–82°C, respectively.

Fulvene V reacts readily with maleimide to give 5-(α-hydroxy-α-2-pyridylbenzyl)-7-(α-2-pyridylbenzylidene)-5-norbornene-2,3-dicarboximide (McN-1025, VI). Thus 5.4 g of V (mixed isomers) and 1.26 g of maleimide were dissolved in 25 ml of benzene and heated under reflux for 4½ hours. After cooling in an ice bath, filtration, concentration of the filtrate, cooling, and filtration, a stable, white crystalline solid, mp 190°–98°C; $\lambda_{max}^{CH_3OH}$, 250 mμ (ε, 17,500), identified as VI (5.9 g, 90-percent yield), was obtained. Combustion analyses (C, H, and N), the infrared spectrum, and nuclear magnetic resonance spectrum give support to the structure designated.

Adduct IV and several analogous compounds (*4*) were much less toxic to rats. Results obtained so far have shown that relatively minor changes in the structure of VI give compounds which are much less toxic to rats. For example, the mixture of the four possible stereoisomers of compound VI (two *endo* and two *exo*isomers) have been separated. Two of the isomers (60 to 70 percent of the total mixture) appear to be far more toxic to rats than the other two.

The carboximide is a substance which is selectively toxic to the genus *Rattus*. It does not produce significant changes in species other than the rat, even at extremely high doses. This high specificity indicates that even closely related species manage this chemical in different ways and suggests an underlying physiological difference between rats and other species.

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Growth-Inhibiting Agents from Mercenaria Extracts: Chemical and Biological Properties

Abstract. *Preliminary studies of the chemical nature of an antitumor agent from the common quahog,* Mercenaria mercenaria, *are reported. The agent has a regressive and an inhibiting effect on sarcoma 180 and on Krebs 2 carcinoma in Swiss albino mice.*

The effect of constitutents of normal cells on malignant growths has recently received renewed interest in the work presented by Szent-Györgyi *et al.* (*1*). These workers have suggested that the term "autobiotics" be used for such tumor-inhibiting substances (*2*). The fact that cellular extracts of many origins have shown both a stimulating and inhibiting effect on cancer cells has been studied for a number of years in these laboratories (*3*), while the action of normal tissue extracts from spleen, brain, and liver has been reported from the Institutum Divi Thomae Research Laboratories over the past 20 years (*4*). Preliminary studies have been made of extracts from several different kinds of marine invertebrates as possible sources of antitumor activity (*5*). The most promising extracts, as demonstrated by their effect on sarcoma 180 in female Swiss albino mice, were those obtained from the common edible quahog, *Mercenaria mercenaria.* In this report I present some of the results of an investigation of the chemical and biological nature of the active principle believed to be responsible for the antitumor activity of the extracts.

The active *Mercenaria* agent, which can be extracted with water (*6*), is not precipitated by 20 to 25 percent saturation with $(NH_4)_2(SO_4)$, but greater concentrations of this salt decrease the yield of active agent in the supernatant. Seventy percent of activity can be extracted in the supernatant when the extract is treated with four volumes of methyl alcohol chilled to −20°C and the extract chilled to +2°C. At room temperature, 15 to 20 percent of the activity can be found in the supernatant. The agent is destroyed by boiling at 100°C for 25 minutes, its activity is decreased by heating at various temperatures above 50°C for 25 minutes, but 100 percent of the activity is retained in extracts heated to 37°C. The substance is nondialyzable, and lyophilyzation at −20°C does not destroy the antitumor activity. Partial purification of the active material has been achieved by chromatography on Sephadex-gel columns. Lipids do not appear to be responsible for the antitumor activity, as shown by testing the cellulose acetate strips obtained after electrophoresis with Nigrosine. Extracts of *Mercenaria* in previous experiments inhibited the growth of sarcoma 180 and had no toxic effects in the dilutions used. While untreated mice (controls) died within 10 days after implantation of tumor—by the trocar method in the axillary region—animals treated with extract were still healthy and normal after 6 months. These animals, which were kept so that the long-range effects of the extract could be determined, showed no evidence of the recurrence of tumors and produced normal litters (*6*).

Mercenaria extracts also inhibited the Krebs-2 ascites tumor in female Swiss mice, 3 to 4 weeks old. Fifteen control animals and groups of 10 experimental mice were each injected with 0.25 ml of ascites fluid in the right axillary region. Four days later, animals without tumors were rejected, and the remaining mice in the experimental groups were injected subcutaneously in the left axillary region, once daily for 7 days, with *Mercenaria* extract partially purified on Sephadex-gel columns. One unit of extract (*7*) was injected per day in a total volume of 0.25 ml. Control mice received 0.25 ml of normal saline daily. Extracts in concentrations higher than those used were toxic. This toxicity, which was probably due to the potassium present, could be