Table II—C-3-Hydroxylation of Benzodiazepines by Liver Microsomal Enzymes of Rats and Mice

Substrates	Rat		Mouse	
	K_m, M	V_{max}, moles/min/mg of Protein	K_m, M	V_{max}, moles/min/mg of Protein
III	4.02×10^{-5} [a]	1.14×10^{-10}	4.52×10^{-4}	6.23×10^{-10}
I	3.30×10^{-4}	1.12×10^{-10}	8.02×10^{-4}	4.58×10^{-10}
II	1.52×10^{-3}	2.35×10^{-10}	1.36×10^{-3}	17.15×10^{-10}

[a] Each figure corresponds to the mean value of four different determinations.

Studies on the distribution of I–III and their metabolites after intravenous administration to rats and mice are in progress. The correlation between *in vitro* and *in vivo* metabolism for both animal species should help explain the species variations in metabolism and pharmacological activity.

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Small Animal Model for Myocardial Infarction

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Abstract □ Myocardial infarctions were produced in rats by electrocauterization of the left anterior descending artery, and the extent of myocardial damage was measured by serial serum levels of creatine phosphokinase activity utilizing spectrophotometric analysis. All animals were also evaluated for myocardial damage by electrocardiographic wave alterations. A correlation between myocardial infarct size and serum creatine phosphokinase was demonstrated. Significant arrhythmias and death occurred only in experimental groups where myocardial infarction had been produced. This small animal model offers a quick, inexpensive, and simple method for screening therapeutic agents that alter infarct size.

Keyphrases □ Myocardial infarction—damage correlated with serum creatine phosphokinase levels, rats □ Creatine phosphokinase—serum levels correlated with myocardial damage caused by infarction, rats □ Enzymes—creatine phosphokinase, serum levels correlated with myocardial damage caused by infarction, rats

More than 1,000,000 persons die annually in the United States from cardiovascular disease, the major cause of death in this country. Although a large percentage of deaths resulting from myocardial infarction are associated with cardiac arrhythmias, many patients expire in the coronary care unit due to power failure, especially low output congestive heart failure (1–3). Power failure was suggested to be related to inadequate cardiac output due to necrosis of functional myocardium (4, 5). In power failure, the extent of necrosis, as it is related to the critical mass of functional myocardium, may be used as a determinant of morbidity and mortality in clinical prognosis (6, 7).

BACKGROUND

Several methods have been used to measure the extent of myocardial ischemia, including coronary blood flow, myocardial lactate production, angiography, radioactive scanning, and radioactive potassium tracers (8). Electrocardiographic (ECG) mapping experiments also were used to describe ischemic changes in the myocardium (9, 10). The problem encountered with these methods is that they study the extent of myocardial ischemia without quantitatively ascertaining the infarct size.

The relationship between infarct size and the serum myocardial enzyme levels has been recognized for many years. Myocardial infarct size, measured by excision and weighing of the damaged tissue, correlated closely with the extent of the depletion of myocardial creatine phosphokinase (11). This enzyme offered many advantages over other myocardial enzymes, since it rose first following an infarction and returned to normal levels in approximately 3 days (12). Sequential serum changes of creatine phosphokinase were used to assess damage in the experimentally infarcted dog (13).

A satisfactory quantitative method for the evaluation of myocardial damage would make it possible to determine the effect of therapeutic intervention on limiting the dynamic extension of an infarction. In this study, a simple, inexpensive, small animal model for inducing myocardial infarction, which may prove useful in the preliminary screening of drugs capable of altering infarct size, is presented.

Table I—Experimental Data

Animal Group	Number of Animals	Heart Rate, beats/min ± SE		Incidence of Arrhythmias[a]			Number of Animals with T-Wave Elevations
		Before Surgery	After Surgery	V	VF	VFD	
I	10	350.6 ± 11	328.8 ± 26	0	0	0	0
II	10	353.6 ± 10	335.0 ± 25	0	0	0	0
III	54	354.7 ± 14	322.5 ± 12	13.4[b]	3	14[b]	51

[a] V = ventricular arrhythmias, VF = ventricular fibrillation, and VFD = ventricular fibrillation and death. [b] $p < 0.05$, χ-square analysis.

EXPERIMENTAL

Animals—Seventy-four Sprague–Dawley[1] male rats, 300–400 g, were divided into three groups. Animals in Group I were surgically prepared, but no tissue was infarcted. Group II also served as a control and was handled similarly, except that skeletal muscle was infarcted by cautery. Animals in Group III were prepared surgically, and myocardial tissue was infarcted by cautery. All animals were given food[2] and water *ad libitum*.

Procedure for Inducing Infarction—The surgical preparation was carried out as follows. The animals were anesthesized with pentobarbital sodium[3] (35 mg/kg ip), and the ECG was monitored throughout the surgical procedure. Following depilatation and sterilization with povidone-iodine[4], a 2.54-cm long incision was made through the skin in the left thoracic area to the skeletal muscle. The muscle layers were separated, with minimal damage, using blunt dissection, and a purse string suture was prepared around the incision site.

After the ribs had been spread, the thorax opened, and the heart exposed, a branch of the left anterior descending artery was carefully cauterized[5]. Artificial respiration was not employed, since the procedure involved an open thorax for only 15 sec. The chest was slightly compressed to create a negative intrathoracic pressure, and the purse string suture was closed.

For the measurement of the creatine phosphokinase levels, blood was taken periodically from the tail for 54 hr at intervals of 0, 6, 18, 30, 42, and 54 hr, since creatine phosphokinase activity previously returned to baseline by this time (14–17). After each experiment, the surviving animals were sacrificed, their hearts were removed, and the infarcted tissues were excised carefully. The necrotic tissue was weighed for future correlation with enzyme release data.

Assay of Creatine Phosphokinase—A 0.1-ml volume of serum was used for the assay of creatine phosphokinase levels in a commercial spectrophotometric kit[6].

RESULTS AND DISCUSSION

Significant arrhythmias and deaths occurred in the experimental groups where myocardial infarction had been produced compared to the various control groups (Table I). This finding agrees with previous reports indicating that death associated with an infarct is due to arrhythmias leading to ventricular fibrillation (18). These arrhythmias are believed to be associated with electrically excitable tissue resulting in pacemaker and conduction abnormalities (19).

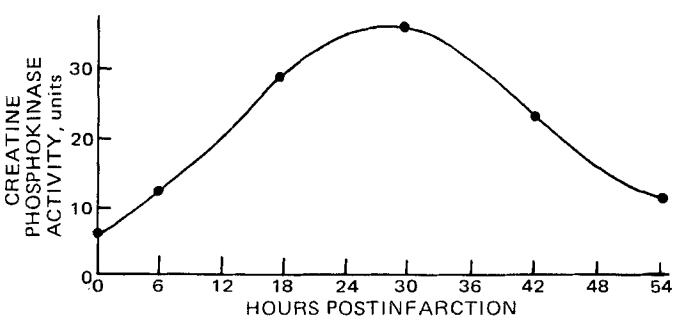

Figure 1—*Variation of serum creatine phosphokinase levels with time in infarcted animals.*

The central zone of an infarct is necrotic tissue that cannot be salvaged and has already released the intracellular enzymes. This zone is believed to be responsible for the early rise in the creatine phosphokinase levels. The intermediate zone consists of ischemic tissue that gradually releases its enzymes. With the incorporation of a proper therapeutic regimen, the conversion of this ischemic zone to necrotic tissue may be precluded (20, 21).

Figure 1 illustrates the mean sequential serum creatine phosphokinase levels[7] for the 54 animals in the experimental group. Since no significant rise in the creatine phosphokinase was observed in control Group II, where the skeletal muscle had been cauterized, it can be concluded that the observed values of creatine phosphokinase in the experimental group are of cardiac origin. The curve is associated with two enzyme pools: a dynamic pool of the enzyme released upon infarction of myocardial tissue and the enzymatic decay associated with the metabolism and excretion. The upslope of the curve (Fig. 1) predominantly represents the release of the enzyme by the myocardium; the apex of the curve represents the

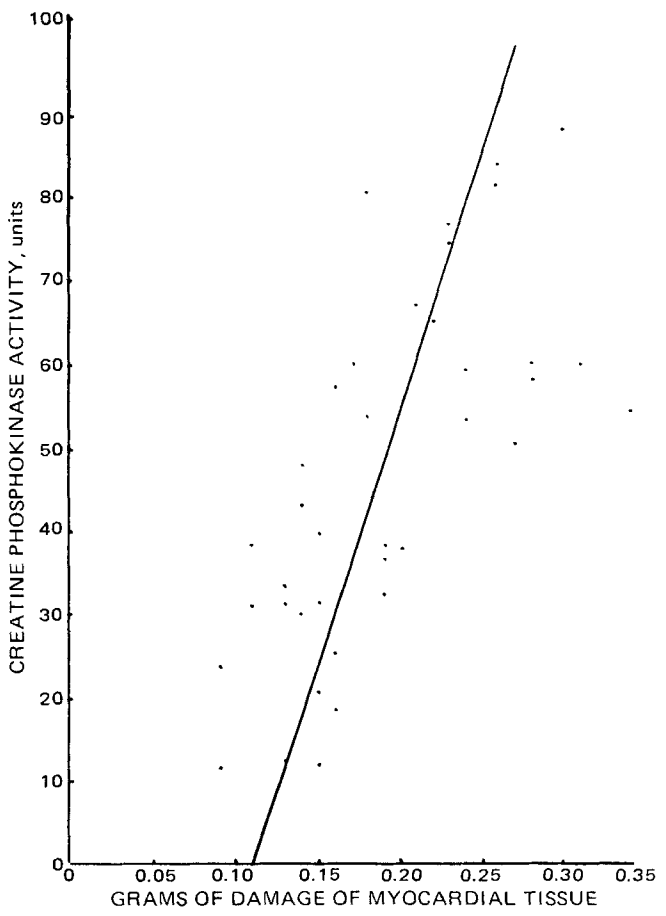

Figure 2—*Creatine phosphokinase activity in terms of the area under the serial creatine phosphokinase curve for each animal in Group III* (n = 54) *plotted* versus *the grams of damage in the excised heart. This linear graph is a result of a standard linear regression analysis. The mean creatine phosphokinase curve area is 50.1975; the mean amount of damage is 0.193 g.*

[1] Taconic Farms, Germantown, N.Y.
[2] Purina Rat Chow, Ralston-Purina, St. Louis, Mo.
[3] Sedasol, Evsco Pharmaceutical Corp., Oceanside, N.Y.
[4] Betadine, Purdue Frederick, Yonkers, N.Y.
[5] Lawton Co., New York, N.Y.
[6] CPK 45-UV, Sigma Chemical Co., St. Louis, Mo.
[7] In Sigma units, Technical Bulletin CPK 45-UV, Sigma Chemical Co., St. Louis, Mo.

equilibrium between myocardial release and decay kinetics. The downslope is predominantly associated with decay phenomena (21).

Animals yielding the upper limits of enzyme release were experiencing serious ventricular arrhythmias and death.

Figure 2 demonstrates the relationship between myocardial damage (in grams) and serum creatine phosphokinase activity. These results agree with a previous conclusion that there is a direct relationship between infarct size and serial enzyme release (22). The pharmacological significance of this model is that it allows study of the dynamic changes of the infarct and also institution of possible therapeutic regimens.

This work also showed that there is a relationship among ECG T-wave elevation, grams of damage, and death in addition to the correlation with enzyme data. Of the 54 experimentally infarcted animals, 51 showed T-wave elevation indicating a hypoxic state. Furthermore, each of the 14 animals experiencing ventricular fibrillation and death was in the group displaying a T-wave elevation. This work demonstrates the sensitivity of enzyme analysis with respect to ECG wave alterations in assessing myocardial infarction.

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Cactus Alkaloids XXXIII: β-Phenethylamines from the Guatemalan Cactus *Pilosocereus maxonii*

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Abstract □ TLC analysis of extracts of *Pilosocereus maxonii* (Rose) Byles and Rowley detected six identifiable alkaloids. Preparative TLC aided in the crystallization of the hydrochlorides of *N*-methyl-3,4-dimethoxyphenethylamine, *N*-methyl-3-methoxytyramine, and *N,N*-dimethyl-3-methoxytyramine. Traces of 3,4-dimethoxyphenethylamine (TLC and mass spectrometry), tyramine (TLC), and *N*-methyltyramine (TLC) were identified. While all of these compounds were isolated and/or detected previously in other cactus species, this study is the first reported crystallization of *N*-methyl- and *N,N*-dimethyl-3-methoxytyramine from a natural source.

Keyphrases □ *Pilosocereus maxonii*—whole plant extract, six alkaloids isolated and identified □ Alkaloids—isolated from whole plant extract of *Pilosocereus maxonii* □ β-Phenethylamines, various—isolated from whole plant extract of *Pilosocereus maxonii*

The cactus genus *Pilosocereus* comprises some 50–60 species, ranging from Florida, the Carribean, and Mexico through Central America into northern South America (1–3). These plants are large, columnar, "giant cacti," and dense wooly hairs or bristles characteristically cover the areoles at the apex of the shoots. Throughout their natural range, species of *Pilosocereus* are used for hedges; a few have edible fruit, but their greatest value in the United States stems from their sale as ornamentals (4, 5). No previous reports have been made regarding the alkaloid content of members of this genus.

In a continuing survey of the Cactaceae for new alkaloid-containing species, extracts of *Pilosocereus maxonii* (Rose) Byles and Rowley were screened. This attractive species is indigenous to Guatemala (3), but propagated plants are available in the United States from commercial cactus dealers. Six β-phenethylamine alkaloids were identified by TLC, and three were successfully crystallized as their hydrochlorides. This paper describes the extraction, isolation, structure elucidation, identification, and synthesis procedures for these alkaloids.

EXPERIMENTAL

Plant Material—Terminal cuttings, both rooted and unrooted, of the plants were received on July 10, 1975, February 5, 1976, and March 5,