NaCl), and dried (Na_2SO_4). Evaporation (Et_2O) and recrystallization (C_6H_6–cyclohexane) yielded 17.5 g (81%) of the amide, mp 76–78°. *Anal.* ($C_9H_9Cl_2NO$).

3-(3,4-Dichlorophenyl)propanol-1. A solution of β-(3,4-dichlorophenyl)propionic acid (0.2 mol, 43.8 g) in 250 ml of THF was added dropwise to a solution of BH_3 (0.6 mol) in 600 ml of THF at 0° under N_2. The mixture was refluxed 3 hr and cooled to 0°, and the excess BH_3 was destroyed with 2 *N* HCl. The THF was removed *in vacuo*; the aqueous layer was neutralized (2 *N* NaOH) and extracted (Et_2O). The extracts were washed (saturated $NaHCO_3$, saturated NaCl solution), dried (Na_2SO_4), and evaporated. The resulting residue was distilled at 121–122° (0.5 mm) to yield 37.6 g (91%). *Anal.* ($C_9H_{10}Cl_2O$).

J. 4-(3,4-Dichlorophenyl)butyronitrile. The propanol (0.1 mol, 20.5 g) was dissolved in 75 ml of dry C_5H_5N and CH_3SO_2Cl (0.11 mol, 8.4 ml) was added dropwise, while maintaining the temperature below 20°. The mixture was stirred at room temperature for 3 hr and then added to 75 ml of concentrated HCl in ice. The aqueous layer was extracted (Et_2O) and the extract washed (10% Na_2CO_3, saturated NaCl solution), dried (Na_2SO_4), and evaporated. The resulting 28 g (99%) of light-yellow liquid sulfonate ester was immediately dissolved in 100 ml of DMF–25 ml of H_2O and cooled to 0°. Solid KCN (0.15 mol, 9.75 g) was added and the mixture stirred at room temperature 72 hr. The mixture was diluted with 100 ml of H_2O and extracted (Et_2O). The Et_2O extracts were washed (H_2O, saturated NaCl) and dried (Na_2SO_4). Evaporation of solvent yielded a red liquid which was distilled at 115–118° (0.1 mm) to yield 18.1 g (84%) of colorless liquid. *Anal.* ($C_{10}H_9Cl_2N$).

Enzyme Assay. The enzyme preparation and the method of enzyme assay have been previously described in detail.[11] An $(NH_4)_2SO_4$ fraction of the high-speed centrifugal supernatant fraction from a homogenate of whole adrenal glands from rabbits was used as the enzyme.[11] L-Norepinephrine (40 μ*M*) was the substrate. Enzyme activity was calculated as picomoles of epinephrine formed per 30 min of incubation. Several concentrations of each inhibitor were tested for enzyme inhibition; the concentrations were spaced on a log basis, *e.g.*, 1, 3, 10, 32, 100, 317 μ*M*, and were selected to achieve a range of inhibition on both sides of 50%. By interpolation, the molar concentration required for 50% inhibition was determined. The negative log of this concentration is defined as the pI_{50} value.

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Inhibition of Phenylethanolamine *N*-Methyltransferase by Benzylamines. 2. *In Vitro* and *In Vivo* Studies with 2,3-Dichloro-α-methylbenzylamine

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2,3-Dichloro-α-methylbenzylamine (DCMB) was a potent inhibitor of phenylethanolamine *N*-methyltransferase *in vitro*. The (+) isomer was more active than the (−) isomer, and the inhibition was reversible and competitive with respect to norepinephrine. The calculated K_i for DCMB was 0.09 μ*M*, compared to the K_m of 12 μ*M* for l-norepinephrine as the substrate. The α-methyl was required for biological stability of benzylamines. DCMB localized in tissues in the following order of concentration when injected intraperitoneally into rats: lung > kidney > liver > brain > adrenal > spleen > heart > blood. The half-life of the compound in the adrenal gland after injection of a dose of 0.2 mmol/kg was 3.4 hr. The concentration of the drug in the adrenal glands bore a nearly linear relation to dose over the dose range of 0.1–0.4 mmol/kg. Treatment of rats with DCMB prior to forced exercise caused a significant reduction of epinephrine, indicative of phenylethanolamine *N*-methyltransferase inhibition *in vivo*.

A specific inhibitor of phenylethanolamine *N*-methyltransferase (PNMT) in the adrenal medulla would suppress the formation of the adrenal medullary hormone, epinephrine, without directly interfering with norepinephrine biosynthesis. Such an inhibitor may find medicinal application, especially if some pathological states are associated with overproduction of epinephrine. Recently we have found that a group of benzylamines are unexpectedly potent inhibitors of PNMT *in vitro*.[1] This paper describes further *in vitro* studies with one of the most active members of that group, 2,3-dichloro-α-methylbenzylamine, and also reports the results of the initial *in vivo* studies with this compound.

In Vitro **Studies.** Figure 1 shows a comparison of the inhibition caused by the stereoisomers of 2,3-dichloro-α-methylbenzylamine. The (+) isomer was more active than the racemic mixture and hence much more active than the (−) isomer. The difference between the isomers was more than tenfold, the pI_{50} value for the (+) isomer being 6.81 and that for the (−) isomer being 5.72.

To determine whether the inhibition by 2,3-dichloro-α-methylbenzylamine was reversible, we measured the ability of dialysis in Visking tubing to remove the inhibitor and thereby restore enzyme activity after the inhibitor and enzyme were mixed *in vitro*. The results in Table I show that dialysis for 24 hr nearly completely restored enzyme activity, indicating that the amount of inhibitor which was sufficient to cause 62–65% inhibition had been removed by dialysis. Thus, the inhibitor appears to form a reversible complex with the enzyme.

Figure 2 shows a Lineweaver-Burk plot. The inhibition by 2,3-dichloro-α-methylbenzylamine was competitive with respect to the methyl-accepting substrate, norepinephrine. From the data in Figure 2, kinetic constants were calculated by the method of Wilkinson.[2] The V_{max} was not altered by the inhibitor, being 57 ± 6 for the control and 58 ± 2 and

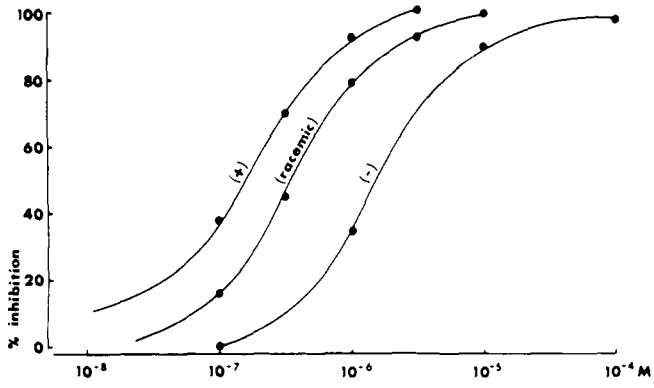


Figure 1. Inhibition by isomers of DCMB.

Table I. Reversal by Dialysis of the Inhibition by DCMB[a]

Conditions	PNMT activity	% inhibition
(a) no inhibitor	40 ± 1	
(b) inhibitor added at end of dialysis	15 ± 1	62
(c) inhibitor added at beginning but not dialyzed	14 ± 1	65
(d) inhibitor added at beginning and dialyzed	35 ± 1	12

[a]Enzyme preparations in 0.5 *M*, pH 7.4 Na phosphate buffer were kept at 4° for 24 hr and then used as the enzyme in the PNMT assay. Mixtures (a), (b), and (d) were dialyzed during this time against frequent changes of the same buffer. Inhibitor was added before (d) or after (b) dialysis. In (c), inhibitor was added at the beginning of the 24-hr period, but the mixture was not dialyzed. Inhibitor concentration was 67 μM in the dialysis mixtures and 1 μM in the final assay mixture. PNMT activity is in picomoles of epinephrine formed per 30 min of incubation.

57 ± 11 pmol/30 min in the presence of the 0.2 and 0.6 μM concentrations of inhibitor, respectively. On the other hand, the apparent K_m was progressively increased by increasing concentrations of inhibitor; the calculated values were 12 ± 3, 40 ± 3, and 110 ± 27 μM for 0, 0.2, and 0.6 μM concentrations of inhibitor, respectively. The increase in apparent K_m characterizes a competitive type of inhibition. The K_i value calculated from these data was 0.08-0.09 μM, which when compared to the K_m value of 12 μM for l-norepinephrine indicates the high affinity of 2,3-dichloro-α-methylbenzylamine for PNMT.

In Vivo **Studies.** Since benzylamines are substrates for monoamine oxidase, we expected that compounds lacking the α-methyl substituent would be rapidly destroyed when administered to animals. Table II shows the levels of several members of the benzylamine series in tissues of the rat, both in the lung (in which amines of this type tend to localize in highest concentration) and in the adrenal glands (the primary target organ in the case of PNMT inhibitors). No tissue levels of the benzylamines without the α-methyl group could be found at 1 hr after injection of the 0.2 mmol/kg dose. Although Zeller[3] has shown that the 2-chloro substituent on benzylamines markedly reduces the activity as a monoamine oxidase substrate *in vitro*, the 2-chloro compounds were still apparently so rapidly oxidized *in vivo* that none remained at 1 hr. In contrast, the compounds with the α-methyl group (both the 2-chloro and the 2,3-dichloro compounds) were present at high concentrations in the lung and at somewhat lower levels in the adrenal glands at 1 hr. Thus, the α-methyl group is required for biological stability of these compounds, and further *in vivo* work was done only with compounds having the α-methyl.

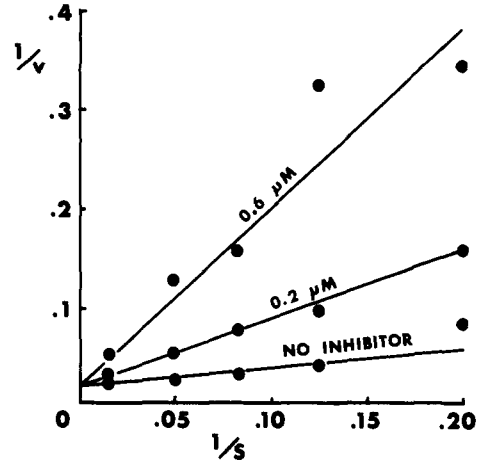
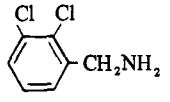
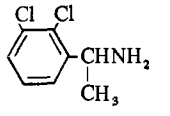
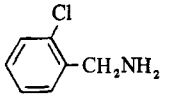
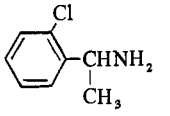
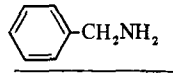


Figure 2. Competitive inhibition by DCMB. The reciprocal of the velocity (picomoles per 30 min) of the enzyme reaction is plotted against the reciprocal of the concentration (micromolar) of l-norepinephrine. Concentrations of racemic DCMB are shown.

Table II. Requirement of an α-Methyl Substituent for Biological Stability of Benzylamines: Tissue Level at 1 Hr[a]

Compound	Drug level in tissue, nmol/g	
	Lung	Adrenal
	0	0
	481 ± 28	240
	0	0
	510 ± 8	189
	0	0

[a]Drugs were injected ip at a dose of 0.2 mmol/kg 1 hr earlier into five rats per group. Mean and s.e.m. are given for lung; adrenals from five rats were combined for analysis.

Figure 3 shows the tissue distribution of 2,3-dichloro-α-methylbenzylamine 1 hr after ip injection. Highest levels of the compound were in the lung. All tissues had the ability to concentrate the drug, since tissue:blood ratios varied from 15 for lung to 3 for heart. The concentration of drug in the adrenal glands was lower than that in lung, kidney, liver, or brain.

Figure 4 shows the amounts of 2,3-dichloro-α-methylbenzylamine in the adrenal glands of treated rats at various times after the 0.2 mmol/kg dose. The compound disappeared from the adrenal glands with a half-life of 3.4 hr as calculated by linear regression analysis of the semilogarithmic plot.

Figure 5 shows the amount of 2,3-dichloro-α-methylbenzylamine present in the adrenal glands at 1 hr after various doses. The drug concentration was directly de-

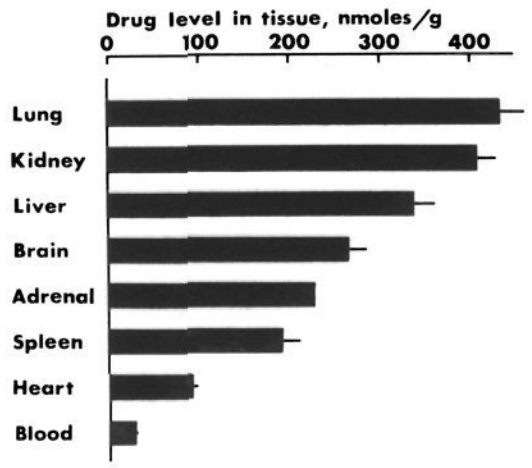


Figure 3. Tissue distribution of DCMB in rats. The compound was injected ip at 0.2 mmol/kg 1 hr earlier. There were five rats per group; mean ± s.e.m. is shown for all tissues but the adrenal glands, in which case the tissues were combined prior to analysis.

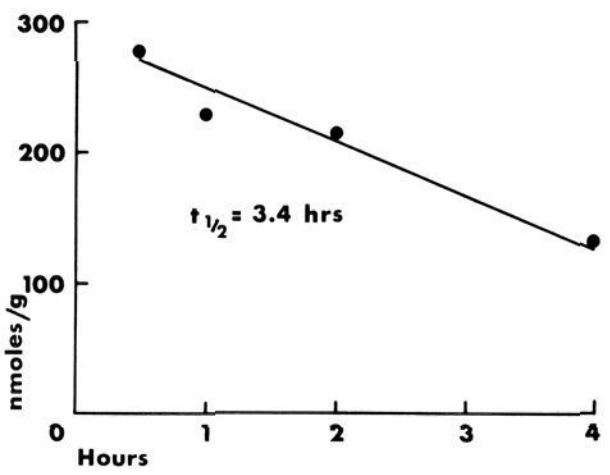


Figure 4. Disappearance of DCMB from the adrenal glands in the rat. The compound was injected ip at zero time at a dose of 0.2 mmol/kg. Adrenal glands from five rats per group were combined for analysis.

pendent upon the dose administered throughout the dose range studied. All of the doses used, from 0.05 to 0.4 mmol/kg, caused signs of CNS stimulation, including exophthalmos, piloerection, and tremors. The incidence and severity of the symptoms were dose-related, being minimal at the lower doses. Some of the animals at the highest dose convulsed. The central effects were observed soon after drug administration (within 10 min) and persisted for a few hours depending on dose; it is doubtful that they were related to PNMT inhibition but rather probably represented a direct action of the drug.

To evaluate PNMT inhibition *in vivo,* we used the experimental system described by Gordon, *et al.*[4] Rats were placed in a wire-mesh drum revolving at 8 rpm and forced to run to remain upright. This forced exercise, as shown by Gordon, *et al.*,[4] accelerates the turnover of catecholamines in the adrenal glands. The animals are thus sensitive to inhibitors of catecholamine synthesis and respond, for example, to inhibitors of tyrosine hydroxylase[4] or of dopamine β-hydroxylase[5] with a decline in catecholamine content. Similar rats treated with the drugs but not forced to exercise show no reduction of catecholamine levels because of the normally slow turnover rate.[4,5] Figure 6 shows the effect of 2,3-dichloro-α-methylbenzylamine. Compared to saline-treated rats, there was a significant increase ($P < 0.01$) in norepinephrine content and a significant decrease ($P < 0.01$) in epinephrine content in the drug-treated rats, indicative of PNMT inhibition. The epinephrine:norepinephrine ratio was decreased from 6.1 in the control rats to 2.0 in the drug-treated rats.

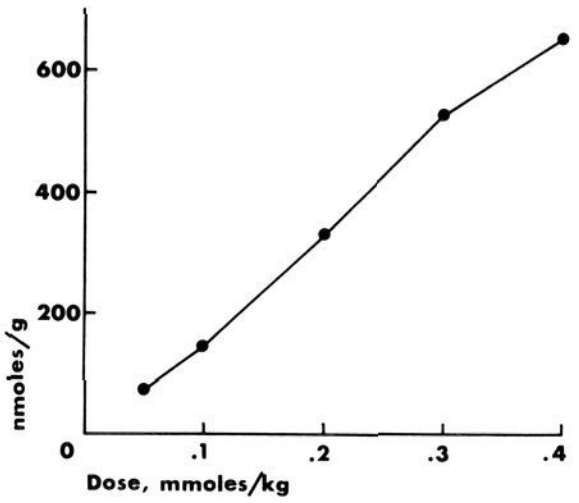


Figure 5. Concentration of DCMB in the adrenal glands of the rat as a function of dose. The compound was injected ip 1 hr earlier. Adrenal glands from five rats per group were combined for analysis.

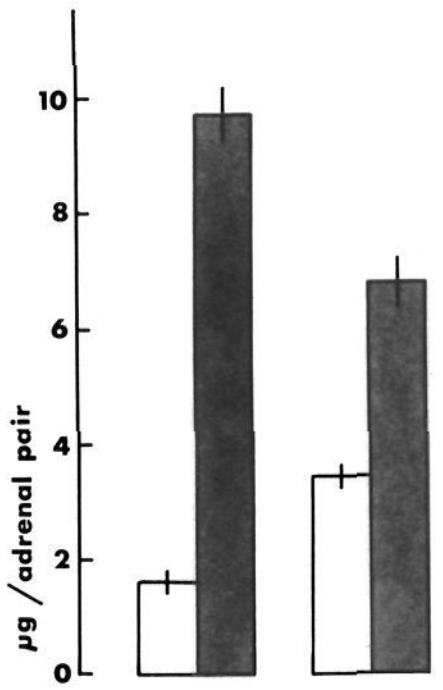


Figure 6. Epinephrine and norepinephrine content in the adrenal glands of exercised rats treated with saline or DCMB. Either saline or DCMB (0.2 mmol/kg) was injected ip 1 hr before the rats were placed in a revolving drum and forced to exercise. After 3 hr of exercise, the rats were killed by decapitation. Mean values ± s.e.m. for norepinephrine (open bars) and epinephrine (shaded bars) are shown for five rats per group.

Experimental Section

Enzyme Assay. Frozen adrenal glands from rabbits were purchased from Pel-Freez Biologicals in Rogers, Ark. PNMT was partially purified through the ammonium sulfate fractionation step as we have previously described.[6] PNMT activity with (*S*)-adenosylmethionine and L-norepinephrine as the substrates was measured by our radioisotopic micromethod.[6]

Animals. Male albino rats from Harlan Industries, Cumberland, Ind., were used for the *in vivo* studies. Drugs were injected ip in aqueous solutions.

Benzylamines. The preparation of the benzylamine inhibitors was described in our earlier paper.[1]

Catecholamine Assay. After rats were killed by decapitation, the adrenal glands were quickly removed and frozen on Dry Ice and then stored frozen prior to analysis. Epinephrine and total catecholamines were determined spectrofluorometrically by the iodine oxidation method of Shore and Olin,[7] and norepinephrine content was calculated as the difference.

Drug Assay. Frozen tissues obtained as above from decapitated rats were homogenized in 4 vol of 0.1 *N* HCl; then 1 vol of 30% $HClO_4$ was added. After centrifugation, the protein-free supernatant fraction was used for colorimetric assay of drug levels by the Methyl Orange method of Axelrod[8] as modified by Dubnick, *et al.*[9]

Resolution of *dl*-2,3-Dichloro-α-methylbenzylamine. *d*-2,3-Dichloro-α-methylbenzylamine Hydrochloride. A 500-ml solution of *dl*-2,3-dichloro-*d*-methylbenzylamine (47.5 g, 0.25 mol) in 95%

EtOH was slowly added to 1500 ml of a hot solution of *l*-dibenzoyltartaric acid (94.0 g, 0.25 mol) in 95% EtOH. Upon cooling overnight the *d*-amine *l*-dibenzoyl tartrate separated (due to its insolubility, specific rotation data were obtained by converting samples to the amine hydrochloride). Four successive recrystallizations from 1000 ml of 95% EtOH were required to obtain optically pure material.

The *d*-amine *l*-dibenzoyl tartrate (8 g, 0.014 mol) was partitioned between 2 *N* HCl and EtOAc. The aqueous layer was evaporated and the residue recrystallized from MeOH-EtOAc to yield 2.55 g of white crystals: mp 225-230°, $[\alpha]^{25}D$ +13.3° (H_2O) (α +0.272, *c* 1.024 g/100 ml, *l* = 2).

l-2,3-Dichloro-α-methylbenzylamine Hydrochloride. The filtrates from the first recrystallization of the *d*-amine *l*-dibenzoyl tartrate were evaporated to dryness, basified with NaOH, and extracted (Et_2O). The Et_2O layer was dried (K_2CO_3) and evaporated. The resultant free amine (18.7 g, 0.098 mol) in 250 ml of 95% EtOH was treated with *d*-dibenzoyltartaric acid (36.95 g, 0.098 mol) in 750 ml of 95% EtOH as for the *d*-amine *l*-dibenzoyl tartrate. The optically pure *l*-amine *d*-dibenzoyl tartrate (6 g, 0.011 mol) thus obtained was converted to the amine hydrochloride which was recrystallized (MeOH-EtOAc) to yield 1.94 g of white crystals: mp 227-231°, $[\alpha]^{25}D$ −12.4° (H_2O) (α −0.250, *c* 1.007 g/100 ml, *l* = 2).

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Syntheses of Cisoid and Transoid Analogs of Phenethylamine

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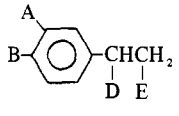
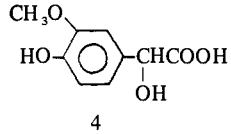
In an attempt to ascertain whether the cisoid or transoid phenethylamine moieties are responsible for the catatonic state produced by bulbocapnine (**5**), *trans*-4-methyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (**6**), *trans*-1-methyl-1,2,3,4,4a,5,10,10a-octahydrobenzo[*g*]quinoline (**7**), aza-1-methyl-1,2,3,7,8,8a-hexahydroacenaphthalene (**8**), and 1-methyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*h*]-quinoline (**9**) were synthesized. Compounds **6** and **7**, both transoid analogs, produced catalepsy in rats. The symptoms produced by **7** in cats were similar to those observed with bulbocapnine (**5**).

Catecholamines along with some of their metabolites and derivatives have been implicated in various neuropsychiatric disorders.[1] Metanephrine (**1**), normetanephrine (**2**), and 3-methoxy-4-hydroxymandelic acid (**4**) have been reported to be excreted in abnormally high concentrations during psychotic episodes in patients with periodic catatonia. Furthermore, 3,4-dimethoxyphenethylamine (3, DMPEA) is capable of producing a catatonic state in animals. It has also been reported to be present in the urine of 92% of the schizophrenic population but was found to be absent from the urine of normal subjects.



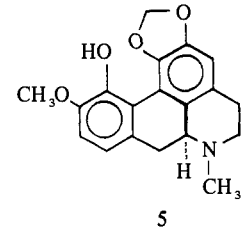


4

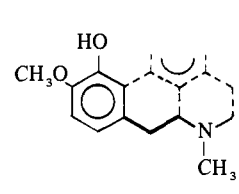
	A	B	D	E
1	CH_3O	OH	OH	$NHCH_3$
2	CH_3O	OH	OH	NH_2
3	CH_3O	CH_3O	H	NH_2

Bulbocapnine (**5**), one of the Corydalis alkaloids, has been widely used to produce experimental catatonia in various animal species.[2] In addition to its ability to produce catatonia, bulbocapnine has been shown to be an α-adrenergic blocking agent.

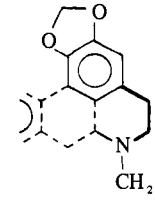
It can be seen that within the bulbocapnine molecule there are two tricyclic components which embody the catecholamine moiety fixed in rigid conformations (**5a** and **5b**). In structure **5a** the catecholamine amine portion is held in a transoid form and in structure **5b** the catecholamine moiety is embodied in a cisoid manner. The possibility exists that one of these rigidly held phenethylamines is responsible for the production of catatonia while the other may be responsible for the α-adrenergic blockade. The purpose of this research was to prepare cisoid and transoid structure of the type shown below (**6-9**) to de-



5



a



b

†Presented in part by S. E-A. before the Midwest Regional Meeting of the American Chemical Society, Lincoln, Neb., Oct 28-30, 1970.

‡Taken in part from the dissertation presented by L. W. H., Oct 1968, to the Graduate School of the University of Kansas in partial fulfillment of the requirements for the Doctor of Philosophy Degree.