of the results in Fig. 1 are shown in Table 2. The data clearly show that a single injection of disulfiram (125 mg/kg) strongly inhibits the formation of norepinephrine from its radioactive precursor dopamine at all time intervals tested. The inhibition was at a maximum at approximately 2 hr and was still apparent at the end of the 48-hr period. Disulfiram caused a reduction in norepinephrine and an increase in dopamine tissue levels. In addition, the relative amounts of metabolites 1 and 2 increased appreciably after disulfiram. Little change was observed in the radioactive metabolite 3, corresponding on the paper strip to the position of 3-O-methyl dopamine.

The results obtained in heart (Table 3) show that the degree and duration of inhibition of norepinephrine synthesis with disulfiram was similar to that previously shown in spleen. Higher levels of metabolite 1 were observed in treated animals. The levels of metabolite 2 were quite variable, and metabolite 3 could not be detected in the heart.

Musacchio *et al.*[3] have studied the effect of disulfiram in the heart up to 1 hr after dopamine administration. Goldstein *et al.*[2] reported the inhibition of norepinephrine synthesis with disulfiram in heart and spleen only for a period up to 4 hr. The authors however, injected a total of 400 mg disulfiram/kg 2 hr prior to, and then simultaneously with, the injection of dopamine-^{14}C; thus the degree and duration of disulfiram inhibition cannot be properly evaluated.

The data presented in this investigation have shown that a single injection of disulfiram strongly inhibits the formation of norepinephrine from the radioactive precursor dopamine in spleen and heart over a period of at least 48 hr. The inhibition of norepinephrine is accompanied by increasing levels of dopamine, and in spleen also by increasing levels of some (at present) unidentified metabolites.

The action of disulfiram, through inhibition of dopamine-β-hydroxylase,[2, 3] results in increasing tissue concentration of dopamine which may act as an adrenergic transmitter.[4] If it is assumed that dopamine can act as a less potent transmitter than norepinephrine and that it is released instead of norepinephrine, then it should manifest itself as a reduction in blood pressure in a sensitive test animal such as the DOCA-hypertensive rat. This possibility is now being investigated and will be the subject of a separate report.

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Department of Physiology and Biochemistry,
Schering Corp.,
Bloomfield, N.J., U.S.A.

S. Symchowicz
C. A. Korduba
J. Veals
I. I. A. Tabachnick

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1,2,5-Selenadiazoles: A new class of highly cytotoxic compounds

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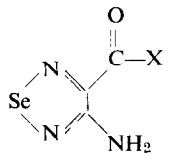
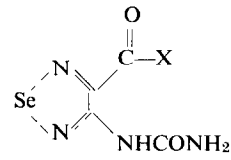
Although the 1,2,5-selenadiazole ring is known as part of fused-ring systems such as the 2,1,3-benzoselenadiazoles[1] and the [1,2,5] selenadiazolo]3,4-*d*] pyrimidines,[2, 3] the only synthesis of monocyclic 1,2,5-selenadiazoles appears to be that reported in the dissertation of Shew.[4] We have synthesized 4-amino-1,2,5-selenadiazole-3-carboxylic acid (NSC 84531) and several of its derivatives and have found that certain of these selenadiazoles were highly cytotoxic.

The method of synthesis is analogous to that previously found to be applicable to the preparation of the corresponding 1,2,5-thiadiazoles.[5, 6] Thus, cleavage of the pyrimidine ring of [1,2,5]selenadiazolo[3,4-*d*]-pyrimidin-7(6*H*)-one[2] (NSC 87430) with aqueous potassium hydroxide and with alcoholic ammonia gave the amino acid (NSC 84531) and 4-amino-1,2,5-selenadiazole-3-carboxamide (NSC 84963), respectively. *N*-Methyl-4-amino-1,2,5-selenadiazole-3-carboxamide (NSC 93169) and *N*-butyl-4-amino-1,2,5-selenadiazole-3-carboxamide (NSC 86047) were formed by treatment of the same selenadiazolopyrimidinone with methylamine and with butylamine, respectively. Both of these reactions also yielded the unsubstituted amide (NSC 84963) as a second product. Ring-cleavage of [1,2,5]selenadiazolo[3,4-*d*]pyrimidin-5,7(4*H*, 6*H*)-dione[3] (NSC 87431) with butylamine or with aqueous potassium hydroxide afforded *N*-butyl-4-ureido-1,2,5-selenadiazole-3-carboxamide (NSC 86048) and the potassium salt of 4-ureido-1,2,5-selenadiazole-3-carboxylic acid (NSC 84532), respectively.





NSC 84531: X = OH NSC 84532: X = OK
NSC 84963: X = NH_2 NSC 86048: X = NHC_4H_9
NSC 93169: X = $NHCH_3$
NSC 86047: X = NHC_4H_9

Studies have been carried out on the biological activity of these compounds in mammalian cell-culture and microbial systems.

METHODS

The cell-culture line used in the cytotoxicity studies was KB (Eagle), derived from a human nasopharyngeal carcinoma.[7] Drug inhibition tests were carried out according to the procedure of Eagle and Foley[8] as modified at the Cancer Chemotherapy National Service Center.[9] Cell growth was measured by the protein determination procedure described by Oyama and Eagle.[10] Initial experiments were carried out in serial one-log dilutions of compound in order to establish a range of cytotoxic activity (Table 1, experiment 1). Subsequent experiments were performed in 0·3 log (twofold) dilutions in order to obtain more exact ED_{50} values. The ED_{50} is defined as that concentration of drug (μg/ml) that will inhibit the growth of cells to 50 per cent of cell-control growth. A brief outline of the procedure follows. Twenty-four-hour tube cultures of the KB cells were fed with Eagle's basal medium[11] supplemented with 10 per cent bovine serum containing selected concentrations of the compound. The cultures were incubated at 37° for 72 hr, at which time the amount of protein contained in all cultures was determined. ED_{50} values were obtained by both IBM 1401 computer calculations and dose–response plots made on semilog paper (e.g. Fig. 1).

Preliminary antimicrobial activity determinations were performed by previously described techniques.[12]

RESULTS AND DISCUSSION

Mammalian cytotoxicity studies of the six selenadiazoles and, for comparison, of two selenadiazolopyrimidines and three inorganic selenium compounds are summarized in Table 1, and a typical dose–response plot is shown in Fig. 1. The inorganic compounds represent three different oxidation states of selenium. The results of these studies show that the four amino selenadiazoles (NSC 84531, 84963, 93169, and 86047) were highly cytotoxic to KB cells in culture; they showed essentially the same degree of cytotoxicity, giving ED_{50} values ranging from 0·2 to 0·5 μg/ml. The two ureido selenadiazoles (NSC 84532 and 86048), the two selenadiazolopyrimidines (NSC 87430 and 87431), and the three inorganic selenium compounds exhibited only minimal cytotoxicity. These studies give no insight into the mechanism of cytotoxic action of the amino selenadiazoles, but it may be noted that the selenadiazole ring, with its carbon and nitrogen atoms situated similarly to corresponding atoms in the imidazole ring, may be regarded as a heterocyclic analog of the imidazole ring. The most

TABLE 1. EFFECT OF 1,2,5-SELENADIAZOLES AND RELATED COMPOUNDS ON THE GROWTH OF KB CELLS IN CULTURE

NSC No.	Compound	ED_{50} (μg/ml)* Experiment 1	2	3	4	5	6	Average
84531	4-Amino-1,2,5-selenadiazole-3-carboxylic acid	<1·0†	0·37	0·26	0·48	0·46		0·39
84963	4-Amino-1,2,5-selenadiazole-3-carboxamide	<1·0†	0·34	0·29	0·20	0·17	0·4	0·28
93169	*N*-Methyl-4-amino-1,2,5- selenadiazole-3-carboxamide	<1·0†	0·23	0·38	0·38	0·3	0·42	0·34
86047	*N*-Butyl-4-amino-1,2,5-selenadiazole-3-carboxamide	<1·0†	0·54	0·34	0·32	0·5		0·43
86048	*N*-Butyl-4-ureido-1,2,5-selenadiazole-3-carboxamide	4·4	7·4	5·3	15·0			8·0
84532	4-Ureido-1,2,5-selenadiazole-3-carboxylic acid potassium salt	15·0	32·0	42·0				30·0
87430	[1,2,5]Selenadiazolo[3,4-*d*]pyrimidin-7(6*H*)-one	15·0	6·9	18·0	23·0			16·0
87431	[1,2,5]Selenadiazolo[3,4-*d*]pyrimidine-5, 7 (4*H*, 6*H*)-dione	15·0	20·0	22·0				19·0
	Sodium selenate	15·0	12·0					13·5
	Sodium selenite	2·7	3·8					3·3
	Sodium selenide‡	7·0	7·0					7·0

* See text.

† Not included in average.

‡ Because of the instability of this compound, its solutions were prepared immediately before addition to the cell medium.

active selenadiazoles (NSC 84531, 84963, 93169, and 86047) have the same positioning of the amino and carboxamide, or amino and carboxyl, groups as the imidazole moieties of AIC ribonucleotide (5-amino-1β-D-ribofuranosylimidazole-4-carboxamide 5′-phosphate) and carboxy-AIR (5-amino-1β-D-ribofuranosylimidazole-4-carboxylic acid 5′-phosphate), both of which are intermediates in the synthesis *de novo* of purine ribonucleotides. Reflections on the mechanism of action of the amino

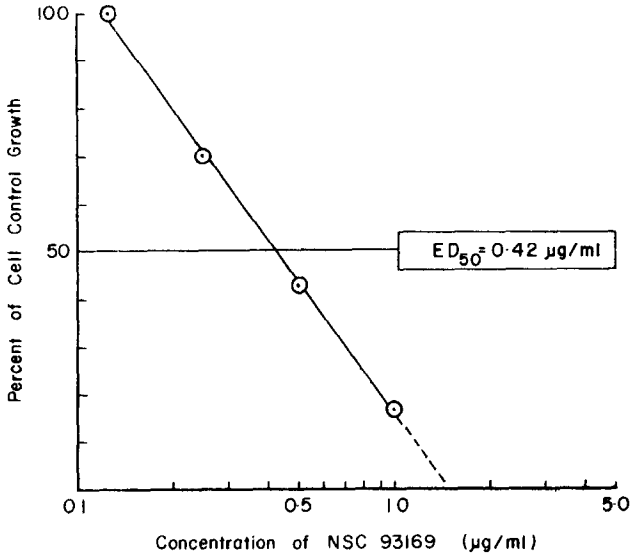


FIG. 1. Response of KB cells to *N*-methyl-4-amino-1,2,5-selenadiazole-3-carboxamide.

selenadiazoles, therefore, should include the possibility that they may function as antagonists at some stage in the biosynthesis of purine nucleotides or in some metabolic area requiring purine nucleotides.

Preliminary antimicrobial tests *in vitro*, illustrated with representative data in Table 2, indicate that the four amino selenadiazoles have marked broad-spectrum antibacterial and antifungal activity.

TABLE 2. ANTIMICROBIAL ACTIVITY OF SELECTED SELENADIAZOLES*

Microorganism	Compound (100 μg/disk)			
	NSC 84531	NSC 84963	NSC 86047	NSC 93169
Escherichia coli ATCC 11303	1·6	3·5	3·2	4·0
E. coli B (Hill)	1·7	3·3	2·9	4·0
Pseudomonas aeruginosa ATCC 10145	N.I.†	1·7	2·0	2·0
Salmonella typhimurium ATCC 7823	N.I.	2·0	1·8	2·3
Streptococcus faecalis ATCC 8043	1·6	2·5	2·8	3·4
S. faecalis ATCC 9790	N.I.	2·4	2·2	3·1
Bacillus subtilis ATCC 6051	trace	2·5	2·7	2·2
B. cereus ATCC 10876	2·7	3·4	2·6	2·0
Staphylococcus aureus ATCC 6538	2·8	3·4	3·3	3·1
Saccharomyces cerevisiae ATCC 4123	trace	4·4	4·4	4·2
Candida albicans Duke 2548	2·1	5·0	5·0	3·4
Aspergillus niger ATCC 1004	3·0	5·5	5·0	4·0
Penicillium roqueforti ATCC 6987	3·4	6·6	6·7	>7·0

* Antimicrobial activity was determined by the paper-disk agar-diffusion method. Numbers in the table give diameters in centimeters of the zones of inhibition surrounding and including the disks. Disk diameter = 1·27 cm.

† N.I. = no inhibition.

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Medicinal Chemistry Section; Y. FULMER SHEALY
JOE D. CLAYTON
Virus Section; GLEN J. DIXON
ELIZABETH A. DULMADGE
Microbiology Section; ROBERT F. PITTILLO
Kettering-Meyer Laboratory, DALE E. HUNT
Southern Research Institute,
Birmingham, Ala., U.S.A.

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Vitamin B_6 and the toxicity of 1,1-dimethylhydrazine*

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IT IS well known that 1,1-dimethylhydrazine (UDMH) inhibits certain enzymatic reactions requiring vitamin B_6 as a cofactor,[1, 2] apparently through the formation of a UDMH-pyridoxal or pyridoxal phosphate hydrazone which depletes the tissues of vitamin B_6. Other workers have demonstrated that intraperitoneally administered pyridoxine (PY) protects rats from UDMH toxicity, while pyridoxal (PAL) and pyridoxal phosphate (PALP) do not.[2, 3] When PAL and PALP were injected immediately after UDMH, convulsions and death occurred much sooner than with UDMH alone. Similar findings with PAL[4] and PALP[2] have been reported for hydrazine and a number of substituted hydrazines.

In the present study, the B_6 vitamers were injected intracerebrally into UDMH-treated rats to circumvent the blood–brain barrier. On the basis of these findings, the protective effects of intraperitoneally injected vitamers then were re-examined.

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