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A Pharmacological Investigation of *Valeriana Officinalis* L.

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A PHARMACOLOGICAL INVESTIGATION OF

VALERIANA OFFICINALIS L.

BY

JOHN A. ROSECRANS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

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1960

MASTER OF SCIENCE THESIS

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ABSTRACT

Valeriana officinalis, a perennial herb indigenous to both Europe and Asia, was investigated for its pharmacological properties. An attempt was also made collaterally with the Department of Pharmacognosy of the University of Rhode Island College of Pharmacy to isolate the most active fraction of valerian. Valerian was extracted by solvent extraction procedures developed by the Department of Pharmacognosy and each fraction was tested and compared for its biological activity. The bioassay procedures were used to compare each fraction prepared. These were: (1) The effects of various fractions on the pentobarbital sleeping time in rats, and (2) The effects of various fractions on the blood pressure of rats.

The results obtained revealed that valerian had no significant effect on the pentobarbital sleeping time in rats, but that two fractions, V103 and V115, did show significant hypotensive activity.

V115 which proved to be the most potent hypotensive fraction was reextracted utilizing counter-current extraction procedures. This procedure yielded two antagonistic fractions. One produced a hypertensive effect and the other a hypotensive effect in rats.

Modification of the extraction procedure during attempts to duplicate the results obtained from the first counter-current extraction of V115 resulted in the loss of activity in the new V115 fraction, and thus, the V103 fraction (alcohol extract) of valerian was further investigated for its pharmacological properties.

The V103 fraction of valerian depressed the general activity of rats without producing sleep, and also exhibited respiratory depressant properties.

V103 produced hypotensive effects in rats, rabbits, cats and dogs. The hypotension may be due to several principles in the fraction tested. There appears to be a parasympathetic principle present which produces its effects at least in part through vagal stimulation. V103 was found to block the carotid sinus reflex and was also found to potentiate epinephrine activity in cats and dogs. The effects of V103 on the blood pressure may be due to principles in common with the V115 fraction.

V103 relaxes isolated intestinal smooth muscle at low doses and produces a secondary contraction at higher doses. Atropine blocked the secondary smooth muscle excitant effects.

V103 is relatively non-toxic as evidenced by its lack of effect on the weight, blood and urine of rats and fairly high LD50 dose in male and female mice.

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ABSTRACT

Valeriana officinalis, a perennial herb indigenous to both Europe and Asia, was investigated for its pharmacological properties. An attempt was also made collaterally with the Department of Pharmacognosy of the University of Rhode Island College of Pharmacy to isolate the most active fraction of valerian. Valerian was extracted by solvent extraction procedures developed by the Department of Pharmacognosy and each fraction was tested and compared for its biological activity. The bioassay procedures were used to compare each fraction prepared. These were: (1) The effects of various fractions on the pentobarbital sleeping time in rats, and (2) The effects of various fractions on the blood pressure of rats.

The results obtained revealed that valerian had no significant effect on the pentobarbital sleeping time in rats, but that two fractions, VI03 and VII5, did show significant hypotensive activity.

VII5 which proved to be the most potent hypotensive fraction was reextracted utilizing counter-current extraction procedures. This procedure yielded two antagonistic fractions. One produced a hypertensive effect and the other a hypotensive effect in rats.

Modification of the extraction procedure during attempts to duplicate the results obtained from the first counter-current extraction of VII5 resulted in the loss of activity in the new VII5 fraction, and thus, the VI03 fraction (alcohol extract) of valerian was further investigated for its pharmacological properties.

The VI03 fraction of valerian depressed the general activity of rats without producing sleep, and also exhibited respiratory depressant properties.

V103 produced hypotensive effects in rats, rabbits, cats and dogs. The hypotension may be due to several principles in the fraction tested. There appears to be a parasympathomimetic principle present which produces its effects at least in part through vagal stimulation. V103 was found to block the carotid sinus reflex and was also found to potentiate epinephrine activity in cats and dogs. The effects of V103 on the blood pressure may be due to principles in common with the V115 fraction.

V103 relaxes isolated intestinal smooth muscle at low doses and produces a secondary contraction at higher doses. Atropine blocked the secondary smooth muscle excitant effects.

V103 is relatively non-toxic as evidenced by its lack of effect on the weight, blood and urine of rats and fairly high LD50 dose in male and female mice.

I. INTRODUCTION

Valeriana officinalis is a perennial herb indigenous to Europe and Asia. In 253 A.D. Valerianus, Emperor of Rome, used valerian in medicine. In ancient times valerian was used in epilepsy, and in recent times it has been used as a universal cure-all in India.

During the past fifty years valerian has been described in pharmaceutical and medical literature, and has been an official drug in United States Pharmacopoeia (1930-1940) and National Formulary (1942-1950).

As an official drug it was used as a calative, sedative and anti-spasmodic, but with the advent of more reliable drugs, it was deleted from the official compendia in 1950. Recently, a new class of compounds, the tranquilizers, has come into prominence. Reserpine, another drug of plant origin, Rauwolfia serpentina, was found to be a very potent tranquilizing and hypotensive agent (Rein, 1955).

Similarities in the historical usage of valeriana to rauwolfia led to a study of valerian's sedative properties at the University of Washington. Bajrablaya (1957) reported that valerian potentiated pentobarbital sleeping time, as do most tranquilizing drugs (Barger, 1956). It was therefore suggested that valerian might also possess tranquilizing properties.

It may be added that several researchers in the nineteenth century had also mentioned that valerian was used to bring about "tranquility of the mind". Their definition of tranquility may have been different from ours, but if one continues to search the literature, it will be seen that valerian has been used frequently in diseases affecting man's mental activity.

The present pharmacological investigation was undertaken in order to assess the possible therapeutic value of this drug by modern experimental methods.

II. REVIEW OF THE LITERATURE

A. HISTORY

The history of Valeriana officinalis can be traced back to Pliny and Dioscorides, 100 A.D. (Lloyd, 1911). Pliny, a Roman physician, whose avocation was natural history, compiled the first medical encyclopedia (Castiglioni, 1947). The work on valerian appears in his Historical Naturalis. (Lloyd, 1911).

Dioscorides and Galen mentioned valerian as an aromatic and diuretic. At that time it was noted that valerian roots were highly delightful to cats and rats, and that "rat-catchers", employed them to draw the rats together, (Woodville, 1810).

Valerian was named around the ninth century after Valerianus, Emperor of Rome from 253 to 260 A.D., who was one of the first to employ the plant medicinally, (Gathercoal and Wirth, 1948).

Riverius (1584-1655), Sylvius (1614-1672), Ghmel (1723-1858) and Tissot (1723-1787) reported great success in treating epilepsy with valerian, (Woodville, 1810).

Lloyd (1911) also mentioned that valerian was not so named prior to the ninth and tenth centuries, and that Dioscorides called it "wild hard".

Stille and Haiesch (1879) mentioned that another ancient name for valerian was "setnull". They also verified the strange passion that cats possess for valerian.

Gerarde in 1567 mentioned that the poorer classes in northern England esteemed, "no broths, potage, or physical meats," to be worth anything without valerian (Lloyd, 1911). Gathercoal and Wirth (1948) also note that it is presently employed as a perfume in the Orient.

Wood and Eschke (1847) stated that valerian was a gentle stimulant, not a narcotic and that it was useful in conditions of central nervous system irregularity not connected with inflammation and/or an excited condition of the central nervous system.

Wood (1866) described valerian as an excitant, antispasmodic and useful in females affected with hypochondriases and hysteria. He stated further that it acted on the cerebrospinal axis with large doses causing headache, mental excitement, and illusions.

In 1879 valerian was said to improve the appetite and digestion without producing constipation and to cause a sense of heat and weight in the abdomen, acceleration of pulse rate, general hyperaemia, and either perspiration or diuresis. In small doses it rendered the mind tranquil and produced a feeling of good humor and activity (Bentley and Trimen, 1880).

Dastour² gave a summary of the actions of valerian according to its use by Pakistanians and Indians. It was used as a cardiac and respiratory stimulant, nerve tonic, antispasmodic and diuretic. It was further used in menopause, delirium tremens, mental derangement, nervous conditions, and in creating mental tranquility.

D. PHARMACOLOGY

Chevalier (1907) showed that valerian produced depressant and paralyzing effects on the central nervous system and concluded that this action was due to a volatile alkaloidal constituent.

The activities of various derivatives of the valeric acid group were tested in an attempt to isolate the active principle of valerian

²No date is presented with the volume published by Dastour.

(Sokhout, 1908). It was found that most of the derivatives were either feebly active or inactive compared to bromal.

Gynoval, one of the first commercial preparations of valerian was described by Froehlich (1909). It was allegedly useful in neurasthenia without affecting the gastrointestinal tract.

Pouchet felt that valerian, which possessed an odor, should be considered inert. It was reported to act solely in a propulsive and psychic manner (Diehl, 1912).

Valerian's uterine depressant property was demonstrated by Pilcher (1916), who reported that the infusion of valerian and sodium valerate (1:1000) were inactive.

Army (1918) described a French preparation of valerian known as valerobromine, a sedative combination of sodium valerate and sodium bromide.

The sedative properties of valerian were demonstrated by Bortoluzzi (1918). It intensified sodium diethylbarbituric acid induced sleep in man, but produced no such effect in animals.

The work of Halimowski in 1893 was confirmed by Goris and Vischniac (1921), who found two alkaloids (chatinine and valerine) in valerian. They believed that the effects of the drug were not due to the alkaloids because of the small amounts of each in the crude drug.

Hart and Ting (1921) attempted to prove that the odor of valerian was responsible for its activity. They could only show that the odor had the ability to slow down rats trained to find the center of a maze.

Valerian's antispasmodic properties were described by Snodlark (1921) who demonstrated that the tincture was the most reliable of all preparations.

Dalles (1929) reported that valerian was useful in hysteria, in insomnia and as an analgesic. Large amounts of the drug could be administered without toxic side effects (Grier, 1929). Weger reported that the infusion and the alcohol-free preparations produced an increase in the body temperature of guinea pigs (1932). Nevertheless, these preparations increased the antipyretic property of antipyrine. The activity was described as a synergistic paralyzing effect on the central nervous system.

The possible local anesthetic properties of valerian were investigated in 1933. Marsh et al (1933) developed a quantitative test for comparing such anesthetics and found valerian to possess one-fiftieth the potency of cocaine.

Rylander (1935) demonstrated by a biological assay on frogs that the tincture was the most active of the valerian preparations tested. The fluid extract was the next most active and the infusion least active.

Redman and Run (1935) developed a method of bioassay for valerian based on its ability to paralyze the cerebral portions of frog brains. One hundred units of valerian were designated as that amount of drug which would completely suppress the stimulating effects resulting from a subcutaneous injection of eighteen milligrams of caffeine per kilogram. It was also mentioned that the drug produced a narcotic effect in mice approaching the toxic level.

Hauser (1937) recommended valerian for certain mental states, and noted that it possessed a considerably delayed onset of action.

Using a decalcified mouse preparation, Lukrey and Kohler (1937) reported that one-twentieth of valerian's lethal dose was found to possess sedative properties.

Blackie and Ritchie (1936) found that valerian lowered blood pressure and that atropine blocked this effect by fifty percent.

Supniewski and Taschner (1938) prepared an alkaloid, alpha-methipyrrol ketone, which corresponded in properties to extractions of valerian root. The compound administered subcutaneously to mice in low doses elicited analgesic and hypnotic effects. As the dose was increased, respiratory depression, narcosis and finally, death occurred. Death of the animal was preceded by clonic-tonic convulsions. Valerian when administered to rabbits produced respiratory depression, then stimulation. It was also noted that valerian produced a hypotensive effect said to be due to its peripheral action.

Romanowski-Majcherowicz (1939) determined the magnitude of the sedative action of fresh and dried valerian by finding the smallest amount of drug that would just neutralize the motor activity produced by one milligram of cocaine hydrochloride administered to mice. Fresh valerian was three times as active as the dried plant.

Rusiecki (1937) found that the alcohol extract was the most active of the valerian preparations tested. It was also mentioned that the effects produced by the oil of valerian were not immediate, but prolonged and recurred at about the same time each day for several days.

The hypnotic effects of valerian in mice were discussed by Schaffhauser (1943) who reported that the duration of action lasted from five to ten hours.

The sedative effect of valerian in mice was observed by Lemert-Paterson (1946) who utilized metabolism cages. He measured the activity of mice by determining the oxygen uptake in a constant temperature metabolism chamber.

According to Green (1944), many neurologists believe valerian possesses psychic activity. On the other hand, Allport (1944) felt that the activity of valerian was derived solely from its strong odor and taste which ultimately produces a mental impression.

Using gold fish (Carassius auratus) Fauconnet (1947) found four different principles which produced sedative effects.

Guidetti (1953) stated that two milligrams of valerian is able to reduce significantly strychnine induced convulsions.

Ordynskie (1953) showed that although valerian stimulated the respiration of cats, no effect was noted against chloral hydrate induced respiratory depression.

The ability of valerian to potentiate barbiturates was mentioned by Pfeifer (1956). It was demonstrated that in a two to one ratio, it was synergistic with sodium phenobarbital.

There appears to be evidence in the literature just cited to justify a further study of the actions of valerian.

III. THE INVESTIGATION

A. OBJECTIVES

Valeriana officinalis, as presented in the review of the literature, possesses the ability to produce many different pharmacological effects. Even though this drug once had a significant role in medicine, its pharmacology and chemistry have not been completely analyzed.

An investigation was undertaken in an attempt to determine what principle or principles might be responsible for the biological activity of valerian.

The objectives of this project are as follows:

1. To screen pharmacologically the various fractions obtained from valerian with special emphasis upon the water soluble portions.
2. To elaborate on the pharmacology of the active fraction obtained in the first objective.

B. PREPARATION OF FRACTIONS

The fractions of valerian employed were prepared from Belgian Valeriana officinalis powder. The powder was subjected to various solvent extraction procedures; one fraction was extracted further by a counter-current distribution apparatus. All fractions were prepared by the Department of Pharmacognosy of the University of Rhode Island College of Pharmacy. The extraction procedures are outlined in Flow charts 1, 2, and 3.

Samples of the various fractions for animal testing were prepared by either of the following procedures:

1. A weighed quantity of extract was dissolved in a minimal amount of distilled water and the solution was filtered through a tared filter.

The filter paper was then placed in a desiccator and after a twenty-four hour period was again weighed. The weight of the filtered dried portion was then calculated by subtracting the weight of the undissolved extract from the original weight of the extract.

2. A quantity of drug was mixed with a minimum amount of distilled water. The supernatant liquid was collected and filtered, the insoluble portion being discarded. One millimeter of filtrate was then added to each of three previously tared weighing bottles. These were placed in an oven over night, and then allowed to cool in a desiccator. The bottles were weighed and the weight of drug in mg/ml was determined. All solutions were aqueous.

C. ANIMALS USED

Rats - All were Albino rats of the Wistar (181-2) strain.

Mice - The C57 strain was used except in the studies of the effects of V103 on the general activity of mice (Section 3a.). These latter animals were of a heterogeneous group.

Rabbits - All were Albino.

Dogs and Cats - Mongrel dogs and cats obtained from a laboratory supply house.

D. EXPERIMENTAL PROCEDURES AND RESULTS

This investigation has been divided into three major sections:

- 1- Effects of Various Fractions of Valerian on the Pentobarbital Sleeping Time in Rats
- 2- Effects of Various Fractions of Valerian on the Blood Pressure of Rats
- 3- Investigation of the Pharmacology of the Alcohol Extract (V103) of Valerian

FLOW CHART 1 The first extraction procedure of *Valeriana officinalis*

*These fractions were tested for activity utilizing potentiation of pentobarbital sleeping time as an index of activity.

Extraction procedure developed by the Department of Pharmacology
of the University of Rhode Island College of Pharmacy

FLOW CHART 2 The second extraction procedure of Valeriana officinalis

*Tested for activity

Extraction procedure developed by the Department of Pharmacognosy of
the University of Rhode Island College of Pharmacy

FLOW CHART 3 The third extraction procedure of Valeriana officinalis

*Fractions tested for hypotensive effects.

Fraction V115 was also subjected to counter current extraction.

Extraction procedure developed by the Department of Pharmacognosy
of the University of Rhode Island College of Pharmacy

1. Effects of Various Fractions of Valerian on the Pentobarbital
Sleeping Time in Rats

a. Experimental Procedure

Male albino rats were starved twenty four hours prior to each test; the animals were allowed access to water ad libitum. Two groups of ten rats each were used for each test, ten as controls, and ten as experimental. Following a period of not less than one week, the two groups were crossed over so that each group was used twice, except where indicated.

The dose of pentobarbital sodium was 35 mg/Kg. As the active dose of valerian was unknown, 100 mg/Kg was used initially. This was increased in each experiment by increments of approximately 100 mg until a maximal dose was found.

The procedure used for determining the potentiation of pentobarbital sleeping time is as follows:

(1) The experimental animals were administered doses of valerian intraperitoneally, while the control animals received an equal volume of 0.9% sodium chloride solution.

(2) Thirty minutes after the administration of valerian and sodium chloride solution, both the control and experimental animals were injected with pentobarbital sodium.

(3) The duration of sleeping time was recorded from the time the animals lost their righting reflex until the time the reflex was regained.

The fractions subjected to this bioassay appear in FLOW CHARTS 1 and 2.

The results were subjected to a statistical analysis according to the method of Burn (1952).

b. Results

The results include the following:

- (1) The effects of various fractions of Valeriana officinalis on the pentobarbital sleeping time in rats (TABLES 1,2 and 3)

TABLE 1 Effects of the alcohol extract (V103) of valerian on the pentobarbital sleeping time in rats

GROUP NO.	NO. OF ANIMALS		DOSE (mg/kg)	SLEEPING TIME MINUTES $\pm$ S.D.		% CHANGE OF MEAN SLEEPING TIME
	CONTROL	EXPT'L.		CONTROL	EXPT'L.	
1	6	9	114	85.6 $\pm$ 27.9	102.1 $\pm$ 41.1	19.3
2	9	8	228	50.3 $\pm$ 31.1	85.4 $\pm$ 55.7	69.8
3	8	8	300	85.4 $\pm$ 36.1	109.2 $\pm$ 34.6	27.4
4*	9	9	300	77.3 $\pm$ 12.8	84.6 $\pm$ 14.7	10.0
5	10	8	396	91.8 $\pm$ 31.6	126.3 $\pm$ 40.2	37.1
6*	7	6	400	83.4 $\pm$ 10.2	85.5 $\pm$ 10.8	2.5
7*	7	10	400	91.8 $\pm$ 20.2	99.3 $\pm$ 16.2	8.1
8*	7	8	500	86.5 $\pm$ 12.5	104.5 $\pm$ 16.3	21.1**
9	7	10	528	123.1 $\pm$ 40.1	84.8 $\pm$ 24.1	-31.1**
10	8	6	536	71.2 $\pm$ 21.3	77.1 $\pm$ 16.3	7.6
11	9	10	528	140.0 $\pm$ 23.9	105.0 $\pm$ 48.6	-25.0
12	10	6	528	83.9 $\pm$ 21.6	127.0 $\pm$ 35.7	51.9**

*Alcohol Extraction from Flow Chart #2

**significant at .05 level

TABLE 2 Effects of various fractions of valerian on the pentobarbital* sleeping time in rats

VALERIAN FRACTION	NO. OF ANIMALS		DOSE** VALERIAN FRACTION mg/kg	SLEEPING TIME HOURS $\pm$ S.D.		% CHANGE OF MEAN SLEEPING TIME***
	CONTROL	EXPT'L.		CONTROL	EXPT'L.	
BDE-20	9	10	124	67.3 $\pm$ 14.5	75.1 $\pm$ 16.1	10.1
BDE-20	7	10	247	66.4 $\pm$ 14.3	69.1 $\pm$ 17.3	4.1
BDE-2	10	9	210	69.5 $\pm$ 20.5	69.5 $\pm$ 18.6	0.0
BaCl Ext.	5	9	95	85.6 $\pm$ 11.3	85.0 $\pm$ 20.1	- .7
MeOH Ext.	7	10	96	83.7 $\pm$ 15.8	84.0 $\pm$ 14.0	.3
AcOEt Ext.	10	10	22	83.8 $\pm$ 15.7	83.4 $\pm$ 21.9	- .4

*35 mg/kg I.P.

**Calculated on basis of equivalence to alcohol extract maximal dose of approximately 400 mg/kg.

***None of the results recorded were significant.

TABLE 3 Effects of the BMS-3 fraction of valerian on the pentobarbital* sleeping time in rats

GROUP NO.	NO. OF ANIMALS		DOSE (mg/kg)	SLEEPING TIME (MINUTES $\pm$ S.D.)		% CHANGE OF MEAN SLEEPING TIME
	CONTROL	EXPT'L.		CONTROL	EXPT'L.	
1	9	9	200	71.2 $\pm$ 22.4	102.2 $\pm$ 21.9	43.1**
2	10	10	200	67.2 $\pm$ 24.1	77.4 $\pm$ 26.3	15.2
3	8	8	200	91.3 $\pm$ 17.1	107.0 $\pm$ 20.5	16.5
4	8	10	200	83.6 $\pm$ 25.6	92.6 $\pm$ 33.8	10.8
5	9	9	400	60.0 $\pm$ 15.8	80.8 $\pm$ 21.7	33.0**
6	9	9	400	88.4 $\pm$ 21.5	80.0 $\pm$ 26.7	-10.0
7	10	9	500	76.3 $\pm$ 18.8	82.2 $\pm$ 7.2	4.9

*35 mg/kg intraperitoneally

**were significant at the .05 level

2. Effects of Various Fractions of Valerian on the Blood Pressure of Rats

a. Experimental Procedure

The effects on blood pressure were recorded by direct cannulation of the right carotid artery of albino rats weighing between 150 and 250 g.

The system consisted of a mercury manometer connected to the cannula by a fluid bridge of 7.5% sodium citrate solution. The blood pressure fluctuations were recorded on a slowly moving, smoked kymograph. To prevent clotting, the rats received an intravenous injection of 0.01 ml of a 1:5000 solution of heparin sodium prior to cannulation and 0.01 ml of heparin sodium was added to the cannula. The animals were anesthetized either with a combination of pentobarbital sodium 30 mg/kg and urethane 200 mg/kg, or urethane 1.2 g/kg. Injections were made intravenously usually via the femoral vein. Male animals were employed except where indicated.

The indices of activity used to compare different fractions possessing a hypotensive effect are as follows:

- 1- The maximum percentage drop of the blood pressure caused by active fractions. (The minimal effect recorded was 40-50%)
- 2- The duration of hypotensive effect.

The fractions subjected to this test appear in **FLOW CHART 3**. Fraction VII5, because of its greater activity was also subjected to counter current distribution extraction. Each fraction obtained was subjected to this bioassay involving the hypotensive effects in rats.

b. Results

The results include the following:

- (1) Effects of the VII5 fraction of Valeriana officinalis, and the effects of fractions obtained from the counter current extraction of the VII5 fraction on the blood pressure of rats (TABLES 4-8 and Figs. 1-5)
- (2) Effects of a second VII5 fraction on the blood pressure of rats, and a comparison of the VII5 fractions and other fractions prepared (TABLES 9-13)

TABLE 4 Effects of the VII5 fraction of valerian on the blood pressure of rats

DOSE (mg/kg)	% DROP IN BLOOD PRESSURE (%)	DURATION OF HYPOTENSIVE EFFECT (min.)
50	17	10
100	54	45
200	36	105

Fig.1 Effects of the alcohol extract of valerian (V103) on the blood pressure of a rat

Fig. 2 Effects of the V115 fraction of valerian on the blood pressure of a rat

Fig. 3 A comparison of the hypotensive effects produced by the V103 and V115 fractions of Valeriana officinalis in rats

TABLE 5 Effects of fractions obtained from the counter current
extraction of the V115 fraction of valerian on the blood
pressure of rats: extraction number one

FRACTION NO.	DOSE (mg/kg)	EFFECT ON B.P.*	DURATION OF EFFECT (Min.)
0**	610	L	26
1	99	L	5
2	30	L	212***
3	33	N.E.	—
4	28	R (10% RISE)	60
5	115	L	10
6-11	632	L	250***
12-17	260	N.E.	—
18-23	360	N.E.	—
24-29	188	N.E.	—
30-35	71	N.E.	—
36-44	165	N.E.	—
46-50	94	L	65

*Abbreviations:

L - Lowered

R - Raised

N.E. - No effect

**Q-Fraction - Residual portion of V-115

***Animals that died at that time

TABLE 6 Effects of fractions obtained from the counter current extraction of the V115 fraction of valerian on the blood pressure of rats: extraction number two

FRACTION NO.	DOSE (mg/kg)	EFFECT ON B.P.*	DURATION OF EFFECT (Min.)
0**	600	L	44
1	585	L	12
2	112	L	112***
2	1000	L	10
3	254	N.S.	—
4	132	R (14% RISE)	77
5	66	L	6
6-8	205	L	2
9-11	542	L	120***
39-50	155	L	60

*Abbreviations:

L - Lowered

R - Raised

N.S. - No effect

**0-Fraction - Residual portion of V-115

***Animals that died at that time

TABLE 7 Effects of fractions obtained from the counter current extraction of the VII5 fraction of valerian on the blood pressure of rats: extraction number three

FRACTION NO.	DOSE (mg/kg)	EFFECT ON B.P.*	DURATION OF EFFECT (Min.)
0	200	L	8
0	300	L	26
0	300	L	59
0	360	L	45
0	600	L	12**
2 (TABLE 12)			
4	183	L	46***
4	200	L	2
4	300	L-R (24% RISE)	2-25
9-11	100	L	67
9-11	175	R	42
9-11	200	N.E.	
9-11	300	R	51
6-8	100	R	38

*Abbreviations:

L - Lowered
 R - Raised
 L-R - Lowered, then raised

**Animal died

***Lowering may have been due to hemorrhage.

Fig. 4 Effects of fraction 2 on the blood pressure of a rat (the first counter current extraction of V115)

Fig. 5 Effects of fraction 4 on the blood pressure of a rat (the third counter current extraction of V115)

TABLE 8 Effects of fractions obtained from the counter current extraction of fraction 2 (third counter current extraction of the V115 fraction of valerian) on the blood pressure of rats

FRACTION NO.	DOSE (mg/kg)	EFFECTS ON B.P.*	DURATION OF EFFECT (Min.)
0	64	L	3
0	200	L	40
0	200	L	4
0	305	L	11
1	32	L	3
1	64	L	9
1	64	L	3
1	200	L	3
2	16	L	5
2	24	L	8
2	48	L	14
2	64	L	17
2	64	L	13

*Abbreviations:

L - Lowered

Table 9 Relative potency of the residual fraction of each counter current extraction of the V115 fraction of valerian

C.C.E.*	DOSE OF FRACTION 0 (mg/kg)	DURATION OF EFFECT (Min.)	RELATIVE POTENCY**
1	600	26	1/12
2	600	44	1/6
3	300	42	1/3

*Counter Current Extraction

**Relative potency refers to the relationship of the contents of fraction 0 to the V115 fraction, 100 mg/kg being equivalent to 1.

TABLE 10 Effects of the **second V115** fraction on the blood pressure of rats

DOSE (mg/kg)	% DROP IN BLOOD PRESSURE	DURATION OF EFFECT (Min.)
100	27	5
200	34	12
300	55	28
400	55	60

TABLE 11 Effects of the V115 and V106 fractions of valerian on the blood pressure of rats

FRACTION	DOSE (mg/kg)	% DROP IN BLOOD PRESSURE	DURATION OF EFFECT (Min.)
V115 Dialysed	100	55	4
V106	50	9	2
V106	100	14	4
V106	900	59	48

TABLE 12 A comparison of the hypotensive potency between the first and second preparations of the V115 fractions of valerian on the blood pressure of rats

AGE OF SOLUTION	DOSE (mg/kg)	DURATION OF EFFECT (Min.)
5 Months	200	2
	300	20
3 Months	200	3
	300	43

3. Investigation of the Pharmacology of the Alcohol Extract (V103) of Valerian

a. Experimental Procedures

- (1) The effects of V103 on the general activity of rats and mice.

The purpose of this series of experiments was to determine the following:

- (a) The effects of the drug on the general motor activity, with special reference to respiratory rate and righting reflex.
- (b) An approximate effective sedative dose.
- (c) An approximate intraperitoneal lethal dose in each species of animal.

Mice and rats of both sexes were utilized in this experiment. All animals were fasted twenty-four hours prior to each experiment with water being provided ad libitum. The alcohol extract was administered via intraperitoneal injection in doses ranging from 0.3 g to 6.0 g/kg.

Effects were recorded as a change from the normal activity of the animal.

- (2) The effects of (V103) on the general activity of mice, utilizing activity cages.

Two circular cages suspended from a ring stand by two springs were used in this investigation. Spontaneous movements of the animals in each cage were recorded on a rapidly moving kymograph by a lever attached to the cages via a pulley system sensitive to both vertical and horizontal movement.

Two groups of six mice each were used; one group served as control, the other group as experimental. The animals were deprived of food twenty-four hours prior to each test with water provided ad libitum. A crossover technique was employed in subsequent tests.

V103 was administered intraperitoneally to the experimental animals thirty minutes prior to being placed in their respective cages. The control animals were injected with an equivalent volume of 0.9% sodium chloride solution thirty minutes prior to beginning of the experiment. Activity was recorded for one hour. The activity was calculated as the total number of movements per minute every ten minutes for an hour.

(3) The effects of V103 on the blood pressure of rats and rabbits.

Blood pressures of albino rabbits of both sexes and rats were determined as previously described. The rabbits were anesthetized with 35 mg/kg pentobarbital sodium intraperitoneally. All drugs were administered via the femoral vein.

(4) The effects of V103 on the carotid sinus reflex, nictitating membrane, and various drugs.

Cats, dogs and rats were used in these experiments. The cats and dogs were anesthetized with pentobarbital sodium intraperitoneally in doses ranging from 30 to 35 mg/kg. Blood pressure was recorded directly from the femoral artery except where indicated.

The carotid sinuses were isolated in all experiments except in those with rats. The left superior cervical sympathetic ganglion was isolated in two cats. In the later experiments, the nictitating

membrane was prepared for recording its activity.

In experiments where indicated, epinephrine (0.025 ml of a 1:1000 solution) was administered prior to and during the hypotensive effect produced by V103.

The procedure for testing the effects of V103 on the carotid sinus reflex was as follows:

Bilateral carotid ~~sinus~~ occlusion was carried out before and during the hypotensive effects produced by V103 by applying a small clamp to both carotid arteries at a point distal to the carotid sinus for 30 seconds.

The procedure for determining the effects of V103 on the superior cervical ganglion is outlined as follows:

- (a) The minimal electrical stimulating threshold was determined for contraction of the nictitating membrane.
- (b) Responses from stimulation of the pre- and postganglionic fibers were recorded on a smoked kymograph prior to the administration of V103.
- (c) Procedure (b) was repeated several times during the period of hypotension produced by V103.

After demonstrating the effects of V103 on the carotid sinus reflex and on the nictitating membrane, atropine (1 mg/kg) was administered prior to a second dose of V103.

The effect of atropine on V103 induced hypotension was also demonstrated in rats.

Total vagotomy was performed in two rats prior to a dose of V103.

- (5) The effects of V103 on the isolated rabbit ileum.

A male rabbit was sacrificed by a sharp blow on the back of the head. The ileum was resected and cut into several segments, one cm in length. A segment of intestine was then placed in a muscle bath containing 70 ml of Locke Ringer's solution and contractions were recorded on a slowly moving smoked kymograph. A constant flow of oxygen was maintained at 37°C. V103 was added to the muscle bath with a syringe and allowed to act for a period of no longer than five minutes. A segment of tissue was used until it became refractory. A least three different pieces of tissue were utilized during this experiment.

(4) A chronic toxicity study of V103

Two groups of rats designated as Group A and Group B were used in this study.

V103 was administered intraperitoneally daily for forty-three days to Group A and for forty-one days to Group B.

Group A:

This group consisted of nine experimental and two control male rats.

The dosage schedule was as follows:

1 to 21 days (400 mg/kg)

22 to 33 days (500 mg/kg)

34 to 43 days (600 mg/kg)

Besides administering V103 daily, a blood and urine analysis was conducted at approximately weekly intervals, before and during the experimental periods.

(a) Blood analysis:

Erythrocyte and leukocyte counts were determined by a standard hemacytometer (Levy-Hausser H₂-Life Counting Chamber, Arthur H.

Thomas Co., Phila., Pa.). Clotting times were determined using the standard capillary tube method (Todd and Sanford, 1953). Blood was obtained from each animal by snipping the end of the tail with a pair of scissors. The tail was cauterized at the end of the procedure.

(b) Urine analysis

- 1- Specific gravity was determined with a 25 g pycnometer.
- 2- pH was determined with a Beckman pH meter (model GS).
- 3- Total albumin and dextrose were determined using Kristin Reagent Strips (Ames Company, Inc., Elkhart, Indiana).

The animals were housed in cages specially constructed for daily urine collection. Experimental animals were housed three rats per cage while the controls were in one cage.

The urine was collected for three days prior to testing.

At the end of the twenty-second day of the experiment there was some evidence of an antidiuretic effect in the experimental rats. In order to facilitate the calculation of daily percentage of urine excretion, the daily water intake was also determined. At this time the experimental animals were rearranged two rats per cage to enable greater accuracy in calculating the percent urine excretion.

$$\% \text{ Urine Excretion} = \frac{\text{Daily excretion (ml/g)}}{\text{Daily water intake (ml/g)}} \times 100$$

The results were based on pooled urine from each cage.

Group B

This group of animals was utilized to determine the effects of V103 on the weights of rats of both sexes.

All animals were fed daily an equal quantity of standard Purina Rat Chow which was given in proportion to the weight of the animals

(two pellets of chow per 50 g of animal). Water was provided ad libitum. The rats weighed 50 g at the start.

The dosage schedule was as follows:

1 to 19 days (400 mg/Kg)

20 to 31 days (500 mg/Kg)

32 to 41 days (600 mg/Kg)

(7) The determination of the LD50 dose of V103 in mice.

Fifty mice of the C57 strain were utilized for this work. The mice were fasted for twenty-four hours, and divided into five groups of ten mice each. Each group was composed of six females and four males. The doses ranged from 0.9 to 4 g/Kg, and only one dosage level administered to each group. The doses were administered intraperitoneally and mortalities were recorded within twenty-four hours following injection.

b. Results

- (1) Effects of V103 on the general activity of rats and mice
(TABLES 13, 14, 15 and 16)
- (2) Effects of V103 on the blood pressure of rats, rabbits, cats
and dogs (TABLES 17, 18, 19 and 21)
- (3) Effects of V103 on the carotid sinus reflex of cats and dogs
(TABLES 20 and 22; Fig. 8)
- (4) Effects of V103 on epinephrine activity of cats and dogs
(TABLES 23 and 24; Fig. 9)
- (5) Effects of atropine and total vagotomy on the hypotensive
effects of V103 (TABLES 25 and 26; Fig. 10)
- (6) Effects of V103 on the nictitating membrane in cats (TABLE 27)
- (7) Effects of V103 on the isolated intestines of a rabbit
- (8) Effects of V103 on the specific gravity and % excretion of
urine of rats (TABLES 28 and 29)
- (9) Effects of V103 on the constituents of rat urine
- (10) Effects of V103 on the blood of rats
- (11) A determination of the LD50 dose of V103 in mice (TABLE 30)
- (12) A comparison of the percent gain of weights between experimental
and control groups of rats

TABLE 13 Effects of VIO3 on the general activity of rats (female)

RAT NO. ^a	DOSE (g/kg)	GENERAL ACTIVITY	RESPIRATION RATE	COLOR ^a	RIGHTING REFLEX	MORTALITY 24 HOURS
1	0.300	N.C.	N.C.	N.C.	N.C.	—
2	0.600	Slightly Sedated	N.C.	Red	N.C.	—
3	0.900	D	I	Slight Cyanosis	N.C.	—
4	1.2	D	Labored	Slight Cyanosis	N.C.	—
5	1.8	D	Slow	Cyanotic	N.C.	—
6	2.4	D	Slow	Cyanotic	N.C.	Med
7	3.00	D	Labored	Cyanotic	N.C.	—
8	3.6	D	Labored	Cyanotic	N.C.	Med
9	4.8	D	Labored	Cyanotic	None	Med
10	6.0	Med (Clonic Convulsions)	Labored	Cyanotic	None	Med (with in 10 min.)

Abbreviations: N.C. = No Change

D = Depressed

I = Increased

^aRats weighted from 112 to 174 g.^aColor = Refers to the rat's ears and paws

TABLE 14. Effects of V103 on the general activity of male and female mice

MIKE NO.	DOSE (g/kg)	GENERAL ACTIVITY CHANGES	RESPIRATION RATE	RIGHTING REFLEX	MORTALITY 24 HOURS
1	0.30	H.C.	H.C.	H.C.	—
2	0.60	Slight Depression	H.C.	H.C.	—
3ms	0.90	Slight Depression	H.C.	H.C.	—
4	1.00	Depressed	Increased	H.C.	—
5	1.50	Depressed	Depressed	H.C.	—
6ms	1.50	Depressed	Increased	H.C.	—
7	2.40	Very Depressed	Labored	Sluggish	—
8ms	3.00	Very Depressed	Labored	Sluggish	Med
9ms	3.60	Very Depressed	Labored	Sluggish	Med
10	4.80	Very Depressed	Labored	Sluggish	Med
11	4.80	Very Depressed (Clonic Contractions)	Labored	Negative	Med
12	6.00	Extremely Depressed	Labored	Negative	Med (with in 10 min.)

Mice weighed from 21 to 25 g.

H.C. = No Change

Med = Med

TABLE 15 Effects of V103 on the general activity of rats (male)*

RAT NO.**	DOSE (g/kg)	GENERAL ACTIVITY	RESPIRATION RATE	COLOR***	RIGHTING REFLEX	MORTALITY 24 HOURS
1	0.30	N.C.	N.C.	N.C.	N.C.	—
2	0.60	N.C.	N.C.	Red	N.C.	—
3	0.90	Slightly Depressed	N.C.	Red	N.C.	—
4	1.2	N.C.	I	Red	N.C.	Med
5	1.8	D	Labored	Slightly Cyanotic	N.C.	Med
6	2.4	D	I	N.C.	Sluggish	Med
7	3.00	D	Labored	Slightly Cyanotic	Sluggish	Med
8	3.6	D	Labored	Cyanotic	None	Med
9	4.8	D	Labored	Red	N.C.	Med
10	6.0	Med (Clonic Convulsions)	Labored	Cyanotic	None	Med (with in 10 min.)

*Abbreviations: N.C. -- No Change

D -- Depressed

I -- Increased

**The rats weighed from 65 to 82 g.

***Color -- Refers to the rat's ears and paws

TABLE 16 Effects of V103 on the general activity of mice, utilising activity cages

DOSE (mg/kg)	MOVEMENT OF CONTROLS	MOVEMENT OF EXPERIMENTALS	% INHIBITION OF MOVEMENTS
0.300	311	287	7.8
0.450	867	572	34.1
0.600	551	220	60.1
0.750	1057	273	74.3
0.900	805	301	62.7

Fig. 6 The effects of V103 on the general activity of mice
(The curve was calculated according to Burm (1952))

TABLE 17 The hypotensive effects of V103 in rats*

DOSE (mg/kg)	% DROP IN BLOOD PRESSURE	DURATION OF EFFECT (Min.)
200	42	10
200	55	20
200	66	47
250	63	75
250	52	20
250	66	20
275	49	10
275	50	31
275	43	25
300	40	30
325	73	60
350	66	95
375	50	35
375	73	60
400	60	15
400	80	105
450	43	35
475	67	105
500	63	260
500	75	120

*Effects of doses approaching the lethal dose, 600 mg/kg, were not included because of their irregularity.

Fig. 7 Effects of the alcohol extract (V103) of valerian on the blood pressure of a rat

TABLE 21 Effects of V103 on the blood pressure of male and female dogs

DOSE (mg/kg)	% DROP IN BLOOD PRESSURE	DURATION OF EFFECT (min.)
1. 84	69	98
2. 100*	64	158**
3. 100	22	92

*Female dog

**Blood pressure did not return to normal

TABLE 22 Effects of V103 on the carotid sinus reflex of dogs

DOSE (mg/kg)	% BLOCKAGE OF THE CAROTID SINUS REFLEX													
	TIME IN MINUTES													
	5	10	15	20	25	30	35	40	45	50	60	70	80	90
84			100					80	60	40	20	20		0
100*	92				75		50							50
100		100		60		40					20		20	

*Female dog

Fig. 8 The effects of V103 (84 mg/kg) on the carotid sinus reflex
in the dog
(BCO - Bilateral Carotid Occlusion)

TABLE 19 The effects of V103 on the blood pressure of female cats*

DOSE (mg/kg)	% DROP IN BLOOD PRESSURE	DURATION OF EFFECT (min.)
1. 100	50	78
2. 189	67	80
3. 200	61	35
4. 250	67	180**

*The blood pressure was recorded via the right carotid artery in cats 2 and 4.

**The animal almost died due to respiratory failure. At 180 minutes the blood pressure had not returned to normal.

TABLE 20 Effects of V103 on the carotid sinus reflex of cats

DOSE (mg/kg)	% BLOCKAGE OF THE CAROTID SINUS REFLEX							
	TIME IN MINUTES							
	4	10	32	39	44	60	80	82
100	50			17		0		
189		75			17		0	
200			50					
250		83				83		83

TABLE 18 Effects of V103 on the blood pressure of rabbits

DOSE (mg/kg)	% DROP IN BLOOD PRESSURE	DURATION OF EFFECT (Min.)
190	44	120
324	55	210
324	69	30
457	63	300

TABLE 23 Effects of V103 on epinephrine activity in cats

DOSE (mg/kg)	% INCREASE IN EPINEPHRINE RISE	% INCREASE IN DURATION OF EPINEPHRINE ACTIVITY
250	100	200
100*	-8	150
100	50	250

*Both 100 mg/kg doses were administered to the same animal in the same order as presented here.

TABLE 24 Effects of V103 on epinephrine activity in dogs

DOSE (mg/kg)	% INCREASE IN EPINEPHRINE RISE	% INCREASE IN DURATION OF EPINEPHRINE ACTIVITY
84	100	200
100*	50	200

*Female dog

Fig. 9 The effects of V103 on epinephrine activity in the dog (V103 - 84 mg/kg)

1-Normal epinephrine pressor activity

11-During the peak hypotensive activity of V103

111-Following the recovery from the hypotensive activity of V103

(A - epinephrine - 0.025 ml (1:1000))

TABLE 25 Effects of atropine on the hypotensive effects of V103

ANIMAL	DOSE (mg/kg)	% INHIBITION OF THE HYPOTENSIVE DROP	DECREASE OF THE DURATION OF HYPOTENSION
Cat 3.	200	36	57
Dog 1.	84	43	43
Rat 1.	200	38	79

TABLE 26 Effects of total vagotomy on the hypotensive effects of V103 in the rat

DOSE (mg/kg)	% INHIBITION OF THE HYPOTENSIVE DROP	DECREASE OF THE DURATION OF HYPOTENSION
200	44	67
315	59	91

TABLE 27 Effects of V103 on the eliciting response in cats

ANIMAL NO.	RESPONSE TO STIMULATION OF THE PREGANGLIONIC FIBER*	RESPONSE TO STIMULATION OF THE POSTGANGLIONIC FIBER*
2	+	+
4	+	+

* Positive response

Fig. 10 The effects of atropine on the hypotensive activity of V103 in the dog

At - Atropine (1 mg/Kg)

V103 - 84 mg/Kg

BCO - Bilateral Carotid Occlusion

- (7) Effects of VIO3 on the isolated intestines of a rabbit
- (a) 0.0071% VIO3 relaxes smooth muscle. The duration of relaxation being proportional to the dose.
 - (b) At a dose of 0.43% the usual relaxation period is followed by a period of increased contraction.
 - (c) This secondary period of increased contraction can be totally blocked by atropine (108).

Fig. 11 A comparison of the percent gain of weights between experimental and control groups of rats

TABLE 28 Effects of V103 on the specific gravity of the urine of rats

GROUP NO.	DAYS OF THE EXPERIMENT		
	0	10	18
Control I.	1.009	1.003	1.003
Expt'l I.	1.021	1.031	1.031
Expt'l II.	1.019	1.026	1.031
Expt'l III.	1.017	1.033	1.031

TABLE 29 A comparison of the effects of V103 on the specific gravity and % urine excretion

GROUP NO.	TESTS*	DAYS OF THE EXPERIMENT**				
		27-33	37-43	44	46-52	55-59
Control I.	Sp.G.	1.027	1.024		1.022	1.023
	%U.E.	26%	30%	49%	40%	29%
Expt'l I.	Sp.G.	1.032	1.030		1.022	1.032
	%U.E.	13%	10%	33%	19%	15%
Expt'l II.	Sp.G.	1.033	1.029		1.027	1.022
	%U.E.	11%	16%	44%	27%	14%
Expt'l III.	Sp.G.	1.032	1.031		1.028	1.018
	%U.E.	11%	19%	30%	37%	32%
Expt'l IV.	Sp.G.	1.029	1.027		1.026	1.022
	%U.E.	16%	19%	43%	20%	30%

*Abbreviations:

Sp.G. - Specific Gravity

%U.E. - Urinary excretion

**The experimental period ended at the forty third day.

(9) Effects of V103 on the constituents of rat urine

V103 had no observable effects on either the dextrose or albumin content of the urine of rats. The only noticeable effect was the development of a slight alkalinity of the urine of rats given V103.

(10) Effects of V103 on the blood of rats

V103 produced a slight rise in both the leukocyte and erythrocyte counts as compared to controls. V103 had no effect on the clotting time of the blood of rats.

TABLE 30 Intraperitoneal LD50 dose of V103 in mice (C-57)

DOSE (g/kg)	MORTALITY (Dead/Tested)	SURVIVED (Alive/Tested)	% MORTALITY (Dead/Tested x 100)
0.9	0/10	10/10	0
2.0	2/10	8/10	20
3.0	5/10	5/10	50
4.0	6/10	4/10	60

The results were analysed according to the method of Litchfield and Wilcoxon (1949).

The LD50 dose was determined to be 3.3 g ranging from 2.53 g to 4.29 g.

Fig. A comparison of the percent gain of weights between experimental and control groups of rats

IV. Discussion

1. Effects of Various Fractions of Valeriana Officinalis on the Pentobarbital Sleeping Time in Rats

Since Dejrakhaya (1957) has previously shown that various fractions of valerian potentiate the pentobarbital sleeping time in rabbits, it was thought that this action might be useful in attempting to isolate the most active fraction of valerian.

On gross observation, the results obtained from these experiments indicate that the alcohol extract of valerian has a marked potentiating effect on the pentobarbital sleeping time in rats. However, upon statistical analysis of the results, only five of the twenty-five experiments produced results of any significance (TABLES 1, 2 and 3). In groups 9 and 11 (TABLE 1) there appeared an antagonistic effect on the pentobarbital sleeping time in rats rather than potentiation as exhibited by other animal groups. However, an average of the control sleeping times of the rats in all twenty-five experiments revealed that the sleeping times of control groups 9 and 11 were at least 39 minutes greater. Since this difference was so great, it was felt that the results from these groups might not be significant.

Even though these experiments did not help in isolation of the most active fraction of valerian, there was some indication that the alcohol extract and the HSW-3 fractions of valerian did potentiate the pentobarbital sleeping time in rats. This may indicate that these fractions possess some central nervous system activity.

2. Effects of Various Fractions of Valeriana Officinalis on the Blood Pressure of Rats

Since the first method of bioassay was found to be inadequate

in attempting to isolate the most active fraction of Valeriana officinalis, a new method was used.

Through several preliminary experiments, it was demonstrated that valerian (V103; FLOW CHART 3) produced hypotensive effects in rats. Because of the definite end point presented by this hypotensive effect, this was used as a bioassay procedure in attempting to isolate the most active fraction of valerian.

Ikami (1958) has shown that the V115 fraction of valerian (FLOW CHART 3) also produced a hypotensive effect in rats. Therefore, several experiments involving the effects of V103 and V115 on the blood pressure of rats were conducted (TABLES 4 and 17; Figs. 1, 2 and 7).

It was shown that the V115 fraction possessed a greater hypotensive effect in rats than did V103 (Fig. 3). In order to concentrate the active hypotensive principle, V115 was subjected to counter-current distribution extraction.

The contents of each counter-current fraction obtained were tested for their effects on the blood pressure of rats. The results of these experiments show that through counter-current distribution extraction it was possible to separate the V115 fraction into three differently acting fractions.

These fractions are (TABLE 5):

- (a) Fraction 2 exhibited an immediate hypotensive effect in rats with death ensuing within 212 minutes following administration (Fig. 4).
- (b) Fraction 4 produced a hypertensive effect in rats which lasted for one hour. The magnitude of the rise in blood

pressure was 10% above normal (Fig. 5).

- (c) Fractions 6-11, which were combined because of the small quantities of material obtained, also produced a hypotensive effect in rats, but differed from fraction 2 in that the onset of the hypotensive effect was delayed.

A second counter-current extraction of VII5 was prepared in order to duplicate the effects produced by the previous extraction. Fractions 2 and 9-11 (fractions 6-8 possessed no hypotensive activity) again produced a hypotensive effect in rats, but the hypotension was not as great as was previously observed (TABLE 6). Only fraction 4 exhibited an effect equal to that observed with the first counter-current extraction.

A third counter-current extraction of VII5 was made in a final attempt to isolate the most active fraction of Valeriana officinalis. Bioassay of the fractions prepared revealed that these fractions were not as potent as those previously prepared (TABLE 7). Only fraction 4 exhibited a potency relatively equal to previous preparations of this fraction (Fig. 5).

The results indicated that the hypotensive and hypertensive fractions were spread out over other fractions rather than being concentrated into individual fractions. Even fraction 4 exhibited a hypotensive effect at one dose level. Fractions 9-11 also exhibited a hypertensive effect at one dose level, and at a lower dose level a hypotensive effect was observed.

Fraction 2 which was itself subjected to counter-current extraction, also displayed this spreading effect of the hypotensive principle (TABLE 8).

The three counter-current extractions were then compared to explain why the two attempts to duplicate the first extraction had failed (TABLE 9). Using the residue of each extraction as the basis of the comparison, it was observed that with each succeeding extraction, less of the hypotensive principle of the whole Vll5 fraction was removed as evidenced by the increase in the hypotensive potency of the residual fraction of each fraction. Therefore, it seems that some technical difficulty was encountered in extracting these fractions which ultimately prevented duplication of the first counter-current extraction.

In order to continue the work on Vll5, a new Vll5 fraction of Valerian officinalis was prepared and subjected to bioassay on the blood pressure of rats. The results showed that this new Vll5 fraction lacked the hypotensive potency displayed by the first Vll5 fraction (Fig. 3; TABLE 10). To explain why this newly prepared Vll5 fraction (FLOW CHART 3) possessed no hypotensive potency, the Vlo6 fraction (TABLE 11) was also tested since its yield was greater in the second preparation of Vll5 than in the first (Evans, 1959). The results failed to show any evidence that the Vlo6 fraction had been the source of error because it possessed no comparative hypotensive activity. A dialyzed preparation of Vll5 was prepared and tested on the blood pressure of rats. It too was found to be devoid of activity (TABLE 11).

Because of the lack of potency displayed by the second Vll5 fraction, samples of the first Vll5 fraction were reinvestigated for their hypotensive potency in rats (TABLE 12). The results indicate that the hypotensive principle of Vll5 may be unstable in aqueous solution.

It is difficult to explain why or how these fractions prepared from either solvent extraction procedures or from counter-current

extractions not so differently. One plausible explanation lies in the fact that there may be differently acting principles in these fractions which are antagonistic. A particular effect will be observed as a sum total of their effects depending on their relative concentrations.

The failure to produce an active VII5 fraction or an active fraction through counter-current extraction left the VI03 fraction of Valeriana officinalis as the most active fraction thus far prepared.

3. Investigation of the Pharmacology of the Alcohol Extract (VI03) of Valerian

The first two sections of this investigation dealt primarily with an attempt to isolate the most active fraction of valerian. This third and last section was arranged so that the most active fraction isolated in either section 1 or 2 would be subjected to a general pharmacological investigation. The last stage of this research dealt with the pharmacology of the VI03 fraction of valerian.

VI03 appears to depress the general activity of unanesthetized rats and mice (TABLES 13-15) without producing sleep. It also appears to be a respiratory depressant as evidenced by the development of cyanosis in animals receiving a large dose. An effective depressant dose lies in a range from 0.6 to 1.2 g/Kg. At these dosage levels activity was depressed with little or no interference with respiration. However, even at these dose levels some of the animals developed a red color in their tissues indicating either a peripheral vasodilation, or cyanosis. The minimal lethal intraperitoneal dose was shown to be approximately 3.0 g/Kg in mice. As the lethal dose was approached, the animals began to lose their righting reflex and

appeared to be in a state of complete paralysis since they could be turned over onto their backs without their being able to right themselves.

Death ensued at a dose of 6 g/Kg in about 10 minutes in both rats and mice. The progressive gross effects leading to death were as follows:

- (a) Development of redness of the ears and paws.
- (b) Depression of the general activity.
- (c) Depression of respiration with cyanosis.
- (d) Loss of the righting reflex (A state of complete paralysis).
- (e) Death, sometimes preceded by clonic-tonic convulsions.

Employing activity cages it was found that 620 mg/Kg of V103 when administered intraperitoneally to mice inhibited their general activity by fifty percent (TABLE 16; Fig. 6). These results again substantiate previous experimental results pointing out the depressant effect of V103 on the central nervous system.

The relationship between the dose of V103 and duration of hypotension produced in rats is proportional only up to a dose of 500 mg/Kg. Above this dosage level the relationship takes on an inverse character (TABLE 17; Figs. 7 and 8). Death apparently due to respiratory depression appears at 600 mg/Kg. Several animals died at 400 mg/Kg and 475 mg/Kg, also apparently due to respiratory failure as was evidenced by the development of cyanosis prior to death. The effect on respiration is one possible explanation for the lack of a proportional dose response relationship in rats. The vasomotor centers of the brain are very sensitive to a high carbon dioxide tension or to a low oxygen tension causing an increase in vasomotor tone and an

increase in blood pressure (Best and Taylor, 1955). If either the carbon dioxide tension is increased or the oxygen tension decreased under the influence of V103, then the reflexes compensating for these effects will antagonize any hypotension simultaneously produced by V103.

There was also some indication of a dose response relationship of the hypotensive effects produced by V103 in rabbits (TABLE 18). However, the apparent respiratory depression tends to antagonize the hypotensive effects produced by V103 at higher doses (324 mg/kg). V103 produces similar hypotensive effects in cats and dogs (TABLES 19 and 21).

An attempt to demonstrate V103's mechanism of action was also carried out. The carotid sinus reflex which aids in controlling the normal blood pressure of an animal (Best and Taylor, 1955) was found to be blocked by V103 (TABLES 20 and 22; Fig. 8). The results obtained from testing this reflex before, during and following the hypotensive effects of V103 indicated that some principle present in V103 possesses the ability to block the carotid sinus reflex either completely or partially (50-100%). The hypotensive principle within V103 is thus able to exert its effects for a longer period of time when this reflex is blocked.

When epinephrine was administered before, during and following the hypotensive effect of V103, it was observed that V103 potentiated both the magnitude and duration of the hypertensive response produced (TABLES 23 and 24; Fig. 9).

V103 could potentiate epinephrine activity by either of two mechanisms:

- (a) Autonomic ganglionic blockade

- (b) Direct potentiation of epinephrine or by a sympathomimetic activity

In attempting to prove either 1 or 2, V103 was tested for its ability to block the action of the autonomic ganglia using the nicotinating medium preparation. The results indicate that V103 does not block the superior cervical ganglion, and thus it must potentiate epinephrine activity by a direct or sympathomimetic effect (TABLE 27).

To explain the mechanism whereby V103 produces its hypotensive effects, atropine, a parasympatholytic drug, was administered prior to a dose of V103. It was observed that atropine blocked both the percent drop of blood pressure (36%-43%), and the duration of the hypotensive effect (43%-79%) (TABLE 25; Fig. 10). Total vagotomy in two rats produced a similar inhibition of the hypotensive effects produced by V103 (TABLE 26). Thus it is possible that the parasympathomimetic principle present in V103 may produce hypotension through vagal stimulation.

The possible mechanisms of action of V103 may be summed up as follows:

- (a) V103 produces its hypotensive effects in part by a parasympathomimetic action, which may act primarily by vagal stimulation. As atropine did not block these effects completely, V103 may also act through another mechanism which may be mediated through the vagus nerve centers or on the peripheral circulation.
- (b) V103 blocks the carotid sinus reflex permitting the major hypotensive principles to act for a longer period.
- (c) V103 because of its effect on epinephrine may also possess

a principle which is sympathomimetic in character.

- (d) V103 produces sedation. The principle producing this effect has not been isolated and may be a different principle from those already mentioned.

Counter-current extraction of V115 revealed three differently acting fractions (TABLE 5; Figs. 4 and 5) which may be responsible for the actions described for the V103 fraction.

V103 also has the ability to relax smooth muscle at a minimal dose of 0.0073%, but at a dose of 0.43% the period of relaxation is followed by a period of stimulation which can be blocked by atropine. It is plausible, therefore, that the principle producing the excitatory state is parasympathomimetic in nature.

V103 is relatively non-toxic when administered over a relatively long period of time as evidenced by its failure to produce any significant changes in the weight, blood or urine of rats (Results (9) and (10); Fig. 11).

There was also some evidence that V103 produced an antidiuretic effect as evidenced by the decreased urinary excretion (TABLE 29) of the experimental animals during the test period as compared to the control animals. Experimental groups III and IV showed the greatest antidiuretic effect. These two groups exhibited normal % urinary excretion at the termination of the experiment (46-59 days).

The high specific gravity of the urine of the experimental animals during the testing period as compared to the control animals (TABLE 28) may also indicate an antidiuretic effect of V103.

The LD50 dose determined on a group of male and female mice (ST57) was 3.3 g/kg (4.29 - 2.53 g/kg) (TABLE 30). This high LD50 dose again indicates the relative non-toxic nature of V103.

V. SUMMARY

Fractions of Valeriana officinalis were prepared by solvent extraction and counter-current distribution extraction procedures. These fractions were assayed biologically in order to isolate the most active fraction.

The two bioassay procedures used were:

- 1- Effects of various fractions on pentobarbital sleeping time in rats
- 2- Effects of various fractions on the blood pressure of rats

The first bioassay procedure proved to be statistically inadequate to identify the most active fraction. However, the alcohol extract and the HNS-3 fractions appeared somewhat active and may possess some central nervous system activity.

The second bioassay procedure indicated that both the alcohol extract and the V115 fractions are potent hypotensive agents. It was also observed that the V115 fraction was more active, and because of its potency was subjected to counter-current distribution extraction in order to concentrate further the hypotensive principle.

By testing fractions prepared through counter-current distribution extraction of V115, three differently acting fractions were identified. Two of these fractions produced hypotensive effects in rats, whereas the third produced a hypertensive effect.

Two attempts to reproduce the active fractions obtained by the first counter-current extraction failed. Only the fraction demonstrating a hypertensive effect was obtained. Failure to reproduce a second V115 fraction brought about a reinvestigation of the first

V115 fraction which was shown to be somewhat unstable in aqueous solution.

Failure to reproduce these active fractions was explained on the basis of the order of extraction procedures employed, and on the observation that two antagonistic principles were present in valerian.

Since the only fraction which could be reproduced was the alcohol extract (V103), the emphasis of the continued research was on this particular extract.

V103 demonstrated central nervous system depressant properties without producing sleep. Through gross observation of conscious rats and mice that received V103, the animals demonstrated depression of their general activity at 600 mg/kg, and at higher doses, 2.4 - 6 g/kg, complete paralysis with loss of the righting reflex without producing sleep.

V103 was shown to depress the general activity of mice at an intraperitoneal ED50 dose of 620 mg/kg using activity cages. V103 was also shown to possess respiratory depressant properties (above 600 mg/kg, intraperitoneally) as evidenced by the development of cyanosis. It was also observed that when administered a toxic dose of V103, rats and mice sometimes developed clonic-tonic convulsions prior to death.

V103 produced a hypotensive effect in rats, rabbits, cats and dogs. In rats, V103 produced a somewhat proportional dose-effect (hypotension) relationship up to 500 mg/kg, but then due to apparent respiratory depression, the response varied inversely with the dose. A similar antagonism was noticed in rabbits, cats and dogs.

V103 appears to produce its hypotensive effects by a parasympathetic

principle since this effect was blocked by atropine. This principle may act primarily through the vagal nerves since total vagotomy also blocks the hypotensive effect of V103.

The carotid sinus reflex was also blocked by V103 either partially or completely. This effect would enhance the above mentioned hypotensive effect of V103.

The hypertensive principle identified within V115 may also be present in V103 as evidenced by V103's ability to potentiate epinephrine activity. V103 potentiated epinephrine's pressor effect by at least 50%, and its duration of activity by at least 150%. It was also demonstrated that V103 is not a ganglionic blocking agent, and appears to potentiate epinephrine directly.

It may also be that the parasympathetic principle identified in V103 represents the hypotensive principle in V115.

V103 has the ability to relax smooth muscle, but at higher doses also produced a secondary excitatory state which is blocked by atropine. The parasympathetic principle in V103 may produce this secondary excitatory state on smooth muscle.

V103 is a relatively non-toxic drug when administered to rats over a considerably long period of time (43 days) as it had no effect on the weight, blood or urine of rats.

A slight antidiuretic effect was observed as evidenced by a decrease in percent urinary excretion and an increase in urinary specific gravity of the experimental rats during the experiment.

The intraperitoneal LD50 dose was 3.3 g/Kg (4.29 - 4.59 g/Kg) in male and female mice (ST97).

VI. CONCLUSIONS

1. The alcohol extract (V103) of Valeriana officinalis produces depression of the central nervous system without producing sleep.
2. Valerian (V103) possesses respiratory depressant properties.
3. There may be two antagonistic principles within valerian (V103); one, a hypertensive principle, and the other, hypotensive.
4. The principles present in V115 are probably present in V103 and may produce their effects through three mechanisms:
 - a. sympathomimetic
 - b. parasympathomimetic
 - c. inhibitions of the carotid sinus reflex
5. V103 is relatively non-toxic when administered either chronically or acutely.
6. Valerian (V103) relaxes and excites smooth muscle.

BIBLIOGRAPHY

- Allport, H.L.: Chemistry and Pharmacy of Vegetable Drugs, Chasie Pub. Co. Inc., Brooklyn, N.Y., 1944
- Army, H.V.: Yearbook Amer. Pharm. Ass. 7:185, 1918
- Bein, H.J.: Pharmacol. Revs. 8:435, 1956
- Bojrablaya, M.S.: Sedative Actions of Valerian Officinalis L., University of Washington (1957), M.S. Thesis, University of Washington, 1957
- Bentley, R. and Trimen, H.: Medical Plants, Vol. 2, J. & A. Churchill, London, 1880
- Bergar, F.H.: Bull. N.Y. Acad. Sci. 67:685, 1957
- Best, C.H. and Taylor, H.B.: The Physiological Basis of Medical Practice, 6th Ed., p. 146, The Williams and Wilkins Co., Baltimore, 1955
- Blackie, J.J. and Ritchie, P.: Pharm. J. 142:249, 1933
- Bortultan, H.: Dtsch. med. Wochr. 44:1109, 1918; CA. 14:3464, 1916
- Bunkley, J.P., DeFoe, J.J. and Reif, H.G.: A Manual of Experimental Pharmacology, 2nd Ed., p. 64, University of Pittsburg Bookstore, Pittsburg, 1957
- Burn, J.H., Finney, D.J. and Cookwin, L.G.: Biological Standardization, 2nd Ed., p. 411, Oxford University Press, London, 1952
- Chevalier, J.: C.R. Soc. Biol., Paris 144:154, 1907
- Dastour, J.F.: Medicinal Plants of India and Pakistan, p. 166, D.B. Tarapurivala Sons & Co., Ltd., Bombay
- Diehl, L.: Yearbook Amer. Pharm. Ass. 1:178, 1912
- Dukrey, H. and Kohler, B.: Arch. exp. Path. Pharmac., 183:106, 1936; Pharm. Abs. 3:369, 1937
- Dulles, A.G.: Yearbook Amer. Pharm. Ass., 18:100, 1929
- Eckhout, A.V.D.: Arch. exp. Path. Pharmac. 57:339, 1938; CA. 2: 668, 1938
- Evans, H.: Unpublished results, 1959
- Fausonnet, L.: Pharm. Acta. Helv. 22:265, 1947

- Fruchlich, E.: *Dtsch. med. Wochr.* 35:157, 1909
- Gastiglioni, A.: *A History of Medicine*, 2nd Ed., P. 215, Alfred A. Knopf, New York, 1947
- Gathercole, E.H. and Wirth, E.H.: *Pharmacognosy*, 2nd Ed., P. 615, Lea Febiger, Philadelphia, 1948
- Goris, A. and Vischniac, G.: *O.J. Soc. Biol.*, Paris 172:1059, 1921; *CA.* 15:2525, 1921
- Green, H.W.: *J. Amer. Pharm. Ass., Pract. Pharm. Ed.* 5:168, 1944
- Grier, J.: *Pharm. J.* 122:302-4, 1929
- Guidetti, E.: *Pitoterapia* 24:421, 1953; *CA.* 47:9574, 1953
- Kochman, H. and Hum, H.: *Arch. exp. Path. Pharmac.* 181:421, 1936; *Pharm. Abs.* 3:273, 1937
- Lennert-Petersen, O.: *Dansk. Tids. Pharm.* 20:251, 1946; *Pharm. Abs.* 13:106, 1946
- Litchfield, J.T. and Wilcoxon, F.: *J. Pharmacol. Exptl. Therap.* 96:99, 1949
- Lloyd, J.V.: *Bulletin of the Lloyd Library, Pharmacy and Materia Medica* No. 19, 1921, p. 19
- Mact, D.I. and Ting, G.C.: *J. Pharmacol.* 18:373, 1921
- Masui, P.: Unpublished results, 1958
- Murch, J.G.: *J. Amer. Pharm. Ass., Sci. Ed.* 22:1073, 1933
- Nylander, A.: *Farm. Recog.* 34:531, 1935; *Pharm. Abs.* 24:342, 1935
- Ordynskie, S.I.: *Urachie* 8:143, 1953; *CA.* 48:27458, 1954
- Pfeifer, E.: *Scientia Pharm.* 24:92, 1956; *CA.* 50:14124, 1956
- Pilcher, J.P.: *J. Pharmacol.* 8:110, 1906
- Rennier, A.: *Dtsch. med. Wochr.* 63:816, 1937; *Pharm. Abs.* 8:54, 1937
- Romanowski-Majcherowik, J.: *Acta. Polon. Pharm.* 3:57, 1939; *CA.* 35:3716, 1941
- Rusiecki, W.: *Farm. Wapoleosena.* 6:3, 1937; *Pharm. Abs.* 5:148, 1939
- Schaffhausen, H.: *Arch. exp. Path. Pharmac.* 301:391, 1943; *Pharm. Abs.* 9:308, 1944

- Smolkin, H.: Pharm. J. 5:402, 1921; CA. 16:1126, 1922
- Stille, A. and Haisch, J.M.: The National Dispensatory, p. 1470,
Henry C. Lea, Phil., 1879
- Supniewski, J.V. and Taschner, E.: Ber. ges. Physiol. exp. Pharmak.
95:380, 1938; CA. 32:9279, 1938
- Todd, J.C. and Sanford, A.H.: Clinical Diagnosis by Laboratory
Methods, 12th Ed., p. 174, W.B. Saunders Co., Phil. and London, 1953
- Weger, P.: Arch. exp. Path. Pharmak. 165:625, 1932; CA. 32:4864, 1932
- Wood, G.B. and Bachs, F.: The Dispensatory of the United States, 7th Ed.,
p. 730, Gregg, Elliot and Co., Phil., 1847
- Wood, H.C.: Pereira's Materia Medica and Therapeutics, H.C. Lea Co.,
p. 605, Phil., 1866
- Woodville, W.: Medical Botany, Vol. 1, p. 77, W. Phillips, G. Yard
Leonard Street, London 1810

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A PHARMACOLOGICAL INVESTIGATION OF CERTAIN VALERIANA
OFFICINALIS L. EXTRACTS*

By J. A. Rosecrans, J. J. DeFoe and R. W. Youngken, Jr.**

The dried roots of Valeriana officinalis L., a perennial herb indigenous to Europe and Asia, have been employed for sedative purposes in various forms since early times (1, 2, 3). The drug has been described in the United States Pharmacopeia (1920 - 1940) and National Formulary (1940 - 1950), and various investigators have reported the alcoholic tinctures of the root drug to be useful in the treatment of hysteria, some types of insomnia and as analgesics.

Chevalier (4) showed that Valerian root produced depressant and paralyzing effects on the central nervous system and concluded that this action was due to a volatile alkaloidal constituent. Valerian's sedative properties were further investigated by Boruttau (5) who showed that it potentiated barbiturate induced sleep in man, but not in lower animals. The sedative properties were discussed by others who concluded that Valerian was a useful sedative, especially in cases of hysteria (6, 7, 8).

Several other pharmacological actions have been described for this plant drug. Pilcher (9) demonstrated that the fluid extract of Valerian depressed uterine activity. It was also shown that the infusion of Valerian and sodium valerate were inactive. Valerian was also shown to possess one fiftieth the potency of cocaine as an anesthetic (10). Blackie and Ritchie (11) also found that Valerian possessed hypotensive properties. They demonstrated that atropine blocked this effect by fifty percent. Bajrablaya (12) reinvestigated Valerian's sedative properties. The results

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indicated that alcoholic extracts of the dried roots had some ability to potentiate barbiturate sleeping time in animals, a property common to several tranquilizers (13).

In the course of a phytochemical examination of the Valerian root by our Department of Pharmacognosy several **extractives** and their isolates were prepared by conventional solvent extraction procedures, including the use of counter current distribution methods. This report describes certain pharmacological properties observed from the study of the most active extract, a 90% ethyl alcohol extract of the crude valerian root and of two fractions prepared from this extract.

METHODS

Two kilograms of dried and powdered number 40 mesh Valerian root previously obtained from the S. B. Penick Co.* were extracted in a continuous extraction chamber with petroleum ether (b. p. 38°-43°) until completely exhausted. Approximately 2 kg. of the insoluble residue were then further extracted by 90 percent ethyl alcohol to completion in a soxhlet apparatus. The ethyl alcohol soluble extract (211 g.) was designated V103 and then set aside for testing.

Aliquots of the alcohol extract (V103) were then refluxed for six hours with a mixture (4:1) of methyl alcohol and conc. HCl. The soluble portion was filtered and the solution shaken with distilled water. Insoluble materials were filtered off and the aqueous extract subjected to further fractional extraction using U.S.P. ether. The aqueous phase was then concentrated and extracted with n-butyl alcohol whereupon the water soluble portion was separated, evaporated to dryness and re-extracted with methyl alcohol. The methyl alcohol extract was refluxed with activated charcoal for 2 hours, filtered and set aside for further testing. This extract was designated V115 and approximately 4 g. were obtained in each case.

*S. B. Penick Co., 50 Church Street, New York City, N. Y.

Two to 5 g. aliquots of V115 were mixed in a Post Counter-Current Distribution Apparatus with ethyl acetate as the moving phase and water as the stationary phase. Following each cycle of extraction, tubes number 1-50 were tested. Of these, tubes number 2, 4 and 6-11 showed activity during preliminary pharmacological testing. These were therefore set aside for more thorough evaluation.

Alcoholic extracts of Valerian were first screened for their effects on the pentobarbital sleeping time in rats (14) and for their hypotensive effects in rats. The alcohol extract (V103) was further evaluated as to its general pharmacological properties and its mechanism of action on blood pressure.

PENTOBARBITAL SLEEPING TIME IN RATS - Each experiment utilized twenty male albino rats (Wistar strain) which were starved twenty-four hours prior to the experiment. Ten animals were used in each control and experimental group. The Valerian extracts in each case were concentrated to dryness, dissolved in distilled water and administered intraperitoneally thirty minutes prior to an anesthetic dose of pentobarbital sodium. The duration of sleeping time was recorded from the time the animals lost their righting reflex until the time of recovery.

HYPOTENSIVE STUDIES - The experiments were conducted in rats (150-225 g.), cats (2.3-3.0 Kg.), dogs (14-19 Kg.) and rabbits (2.75-3.2 Kg.) of both sexes. All animals except the rats were anesthetized with an intraperitoneal injection of 25-35 mg./Kg. pentobarbital sodium. The rats were anesthetized with an intraperitoneal injection of 1.2 g./Kg. urethane because of its lack of effect on blood pressure. The effects of the extracts on blood pressure were recorded by direct cannulation of either the carotid or femoral artery with a system consisting of mercury manometer connected to the cannula by a fluid bridge of 7.5% sodium citrate solution. The effects on the blood pressure were recorded on a slowly moving smoked kymograph. Heparin sodium, 0.01 mg./Kg. was injected intravenously, and an equal amount added to the cannula to prevent clotting. Extracts in a dry form were dissolved in distilled water and administered via the femoral vein.

The indices of activity used to compare different fractions possessing a hypotensive effect are as follows:

- (1) The maximum percentage drop of the blood pressure caused by an active fraction (40-50%)
- (2) The duration of hypotensive effect.

In attempting to determine the mechanism of hypotension of the alcoholic extract (V103) several experiments were conducted in dogs and cats. The effects of the drug on the carotid sinus reflex were tested by bilateral carotid occlusion for thirty seconds prior to, during, and following the hypotensive effects of the alcohol extract of Valerian. Epinephrine (0.025 ml. of a 1:1000) was administered prior to and during the hypotensive effects of the drug. The pre- and post ganglionic fibers of the superior cervical ganglia innervating the nictitating membrane were also isolated, and stimulated electrically prior to and during the hypotensive effects of the drug. In three animals atropine (1 mg./Kg.) was administered prior to a second dose of Valerian. Total vagotomy was performed before a second injection of Valerian in two rats.

INTESTINAL MOTILITY - One cm. segment of rabbit ileum was resected and placed in a muscle bath containing 70 ml. of oxygenated Locke Ringers solution maintained at 37°C. Rhythmical contractions of the muscle were recorded on a slowly moving smoked kymograph via a pulley system. The alcohol extract of Valerian was added to the bath with a 1 ml. syringe and allowed to remain in the bath for a period no longer than five minutes.

A SHORT TERM TOXICITY STUDY - Two groups of albino rats were employed in these studies. The first group consisted of eleven male rats. This experiment terminated at the end of 45 days during which the alcohol extract was administered intraperitoneally daily in doses ranging from 400-600 mg./Kg. At weekly intervals blood and urine analyses were conducted. Erythrocyte and leukocyte counts and clotting times were determined by the usual methods (15).

Urine albumin and dextrose, urine pH and specific gravity were likewise also determined.

A second group of twelve albino male and female rats weighing 40 to 60 g. also received Valerian intraperitoneally daily in doses ranging from 400-600 mg./Kg. This experiment was performed to determine the effect of Valerian on the weights of growing rats.

LD50 DETERMINATION - Fifty male and female mice of the C57 strain were utilized. The mice were starved twenty-four hours prior to the experiment and were divided into five groups of ten each. Each group consisting of six female and four male mice was administered the same dosage ranging from 0.9-4.0 g./Kg. The doses were administered intraperitoneally and mortality was recorded within twenty-four hours following administration of the drug. The results were analyzed by the Litchfield and Wilcoxon (16) method.

GENERAL ACTIVITY - In a preliminary experiment twelve mice and twenty rats of both sexes were administered the alcohol extract intraperitoneally and observed for a period of several hours. The drug was administered in doses ranging from 0.3 to 6.0 g./Kg. Effects were recorded as a change from the normal activity of the animal.

A second experiment utilizing activity cages was conducted. Spontaneous movements of male mice (Strain C56) were recorded on a slowly moving smoked kymograph via a pulley system attached to a cage which was sensitive to vertical and horizontal movements. The activity of two groups of six mice each were recorded simultaneously in two different cages, with one group acting as a control. The alcohol extract of Valerian was administered intraperitoneally in doses ranging from 300 to 900 mg./Kg. thirty minutes prior to beginning the experiment. The control mice received an equal volume of normal saline. The activity of the control and experimental groups was calculated as the total number of movements per minute every ten minutes for one hour.

RESULTS

Bajrablaya (12) reported that several fractions prepared from Valerian potentiated the pentobarbital sleeping time in rabbits. Besides the implication of a tranquilizing or sedative action of Valerian, it was felt that this procedure could yield an excellent bioassay procedure in attempting to isolate an active fraction of Valerian. However, in twenty-five experiments utilizing this procedure, only five experiments indicated any statistical significance, and it could only be concluded that the VI03 and VII5 fractions had effects on the sleeping time of anesthetized rats (Fig. 1).

The failure of this method led to the use of a hypotensive test in rats. Two fractions, VI03 and VII5, proved to have hypotensive properties. The VII5 fraction, because of its greater potency, was then extracted by a counter-current distribution method as previously described. This procedure yielded three fractions which acted differently on the blood pressure of rats. Two fractions (Tube 2 and the combined 6-11 tubes) produced hypotensive effects, one with a delayed onset of action, (the combined tubes 6-11) and the third (Tube 4) produced a hypertensive effect (Fig. 2). In attempting to duplicate these results two other counter-current extractions were performed, but only the hypertensive fraction was obtained. By comparing the residue of the three counter-current extractions to that of VII5 it was apparent that with each succeeding extraction less of the hypotensive principle was extracted.

Following this difficulty in producing a second series of active VII5 fractions and because of the greater potency of the alcohol extract (VI03) (Fig. 3) it was subjected to further study with the following results:

The VI03 fraction of Valerian was found to depress the general activity of unanesthetized mice and rats. It depressed respiration as evidenced by the development of cyanosis in animals receiving large doses (3 g./Kg.) of the drug. An apparent sedative dose lies in a range from 0.6 to 1.2 g./Kg. as observed

from the preliminary experiments on general activity. This sedative dose range was further verified utilizing activity cages where it was found that 620 mg./Kg. of VLO3 inhibited the activity of mice by fifty percent. At this dose, 500 mg./Kg., there was some evidence of a peripheral dilation and a moderate cyanosis as evidenced by the development of red color in the paws and ears of rats and mice.

The minimal lethal intraperitoneal dose was calculated to be about 3.0 g./Kg. At this dose the animals appeared to be in a state of complete paralysis since they were unable to right themselves when placed on their backs. It should also be mentioned that even though these animals could not right themselves, they did respond to external stimuli and did not appear to be asleep. Death in rats and mice ensued at a dose of 6 g./Kg. in about ten minutes with death sometimes being preceded by clonic-tonic convulsions.

The VLO3 extract produced hypotensive effects in rats, rabbits, cats, and dogs, but it was difficult to demonstrate a proportional dose-response relationship due to respiratory depression.

In cats and dogs, VLO3 blocked the carotid sinus reflex in all experiments (Fig. 4). The drug had no blocking effect on epinephrine, and even appeared to potentiate its activity. Atropine, however, did block Valerian's hypotensive effects by 50% as was also pointed out by Blackie and Ritchie (11). In two experiments with rats, total vagotomy blocked the effects of Valerian equal to the same degree produced by atropine. Valerian did not block the superior cervical ganglia as evidenced by the lack of an effect on the nictitating membrane preparation of the cat.

It thus appears that the Valerian root extract does not produce its hypotensive action by either ganglionic or sympathetic blockade. However, there is evidence that Valerian possesses a parasympathetic action mediated through vagal stimulation which can explain part of its hypotensive activity. It can further be added that since Valerian extract produced hypotension only by partial parasympathetic action, its central nervous system depressant activity could add to the hypotension. Further

evidence that Valerian could produce its hypotensive effects through central nervous system depression was reported by Dukrey and Kohler (17). They showed that Valerian still possessed sedative activity after decerebration thus placing the site of central nervous system depression at some area lower than the cortex, possibly the medulla, which is also the site of the vasoconstrictor center. It is possible that this central effect could in part explain the effects on the carotid sinus reflex.

Valerian (V103) also has the ability to relax smooth muscle in very low concentrations (0.0071%), but as the dose is increased to 0.43% a secondary excitant effect which can be blocked by atropine was observed. It is possible that this excitant effect is also related to the parasympathetic action referred to previously.

V103 is relatively non toxic as evidenced by its failure to produce any significant change in the weight, blood and urine of rats. There was some evidence that Valerian produced some antidiuretic effects as shown by the decreased urinary excretion as compared with the percent urinary excretion following the termination of the experiment. The high specific gravity of the urine during the testing period as compared to the control animals may also indicate an antidiuretic effect in rats.

The relatively high LD50 dose, in mice, 3.3 g./kg. (4.29-2.53 g./kg.) indicates V103 has a low toxicity.

SUMMARY

A study was made of certain pharmacological actions produced by extracts of Valeriana officinalis L roots. An alcoholic extract, V103, and a fraction separated from it, V115, were found to potentiate the pentobarbital sleeping time in rats. Both also produced hypotensive effects in rats. The V115 fraction showed greater hypotensive activity.

Three fractions which were extracted from V115 through counter current extraction exhibited different effects on the blood pressure of rats. Two fractions exhibited hypotensive properties, and the third exhibited hypertensive properties.

The alcoholic extract, V103, also produced hypotensive effects in rabbits, cats and dogs. Its hypotensive effects appeared to be due to a combination of parasympathetic activity, carotid sinus blockade, and probably through central nervous system depression action. The latter action was also implied by the work of Dulzrey and Kohler (17). V103 was further shown to lack any effect on the autonomic ganglia and on sympathetic fiber activity. It produced a depression of the central nervous system without producing sleep and showed evidence of causing respiratory depression in higher dosage.

The alcoholic extract relaxed smooth muscle in low concentrations, but at higher concentrations produced secondary excitant effects which can be blocked by atropine. Some antidiuretic effects were also observed. The LD50 dose (3.3 g./kg.) of the extract in rats was considered rather high, thus indicating a low order of toxicity.

REFERENCES

- (1) Lloyd, J.V., Botany, Pharmacy and Materia Medica, Bulletin of the Lloyd Library, No. 18, 1911, p. 89
- (2) Woodville, W., Medical Botany, W. Philips, London, 1810, p. 77
- (3) Dastour, J.F., Medicinal Plants of India and Pakistan, D.B. Taraporivala Sons and Co., Ltd., Bombay, p. 168
- (4) Chevalier, J., Compt. Rend. Soc. Biol., Paris 144, 154 (1907)
- (5) Boruttau, H., Dtsch. med. Wochr. 44, 1109 (1918)
- (6) DuRoi, A.G., Yearbook Amer. Pharm. Ass. 18, 100 (1929).
- (7) Renner, A., Dtsch. med. Wochr. 63, 816 (1937); Pharm. Abstr. 3, 54 (1937)
- (8) Schaffbaueser, H., Arch. exp. Path. Pharmac. 201, 391, (1943); Pharm. Abstr. 2, 303, (1944)
- (9) Pilcher, J. P., J. Pharmacol and Exptl. Therap. 8:110 (1916)
- (10) Munch, J. G., J. Amer. Pharm. Assoc., Sci. Ed. 22, 1078, (1933)
- (11) Blackie, J. J. and Ritchie, P., Pharm. J. 142, 299, (1939)
- (12) Bejrablava, D., Sedative Actions of Valeriana Officinalis L., M.S. Thesis, University of Washington, Seattle, Washington, 1957
- (13) Berger, F.H., Ann. N.Y. Acad. Sci. 67, 685 (1957)
- (14) Winter, C.A., J. Pharmacol. Exptl. Therap. 94, 7-11, (1948)
- (15) Todd, J.C. & Sanford, A.H., Clinical Diagnosis by Laboratory Methods, 12 Ed. W. B. Saunders, Phila., 1953, p. 174
- (16) Litchfield, J.T. & Wilcoxon, F., J. Pharmacol. Exptl. Therap. 90, 99 (1949)
- (17) Dulcroy, H. & Kohler, B. Arch. Exp. Path. Pharmac. 183, 106, (1936); Pharm. Abstr. 3, 369 (1937)

Fig. 1a. Effects of the V103 fraction of Valerian on the average pentobarbital sleeping time in rats

Fig. 1b. Effects of the V115 fraction of Valerian on the average pentobarbital sleeping time in rats

Fig. 2b. The hypertensive effect of tube 4 in a rat
(Tube 4 was obtained from the third counter-
current extraction of V115)

Fig. 2a. The hypotensive effect of tube 2 in a rat
(Tube 2 was obtained from the first counter-
current extraction of V115)

Fig. 3 The hypotensive effect of the V103 fraction of Valerian in a rat
(Dose of V103- 500 mg/Kg.)

Fig. 4 The hypotensive effect of the V103 fraction of Valerian (84 mg./Kg.) in the dog with special reference to the carotid sinus reflex (BCO - Bilateral Carotid Occlusion)