T H E S I S

on

SOME PHARMACOLOGICAL ASPECTS OF OIL OF CALIFORNIA LAUREL

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SOME PHARMACOLOGICAL ASPECTS OF OIL OF CALIFORNIA LAUREL

INTRODUCTION

The oil of California laurel, or Oregon myrtle, has had very little pharmacological investigation. The pharmacognosy of the tree from whose leaves the oil is obtained has been thoroughly studied, and the chemistry of the constituents of the oil has been thoroughly recorded.

It is said by the old settlers in the regions where the tree is found that the Indians bruised the leaves which they placed on the head or face to relieve headache or conditions of neuralgia. Heaney,[1] in 1875, stated that the oil could be used as a remedy for nervous headache, but no remarks were made as to dosage or method of administration, nor was mention made of any likely actions or reactions the oil might produce.

During the year 1925, a large quantity of myrtle leaves were distilled to obtain oil for chemical research. During the process of distillation one of the workers was overcome by the escaping vapors and remained unconscious for a period of over six hours. Another helper shortly afterward experienced similar results. Though it was realized that there was some

principle in the oil responsible for the above results, no effort was made to study the case.

The chemical investigation of the oil has shown it to contain a ketone principle, which has been called umbellulone, and which seems to possess the characteristic pungency with which the oil is endowed. This constituent is probably responsible for the peculiar properties which the oil possesses.

BOTANICAL DESCRIPTION OF CALIFORNIA LAUREL

The California laurel, or myrtle tree, is the only representative of the Genus Umbellularia, of the Laurel family, on the Pacific coast. The tree is described by Sudworth[2] as follows:

California Laurel: Oregon Myrtle Tree.

Umbellularia californica (Hook. & Arn.) Nutt.

"An evergreen tree, distinguished from all others on its range by the strong camphoric-pungent odor of its crushed leaves and stems. Under favorable conditions of growth it attains a height of sixty to seventy feet, and a diameter of two and a half to three feet. In dense forests it has a clean straight trunk thirty to forty feet high and a narrow crown of close, small, upright branches. Elsewhere it has a short trunk, surmounted by long limbs which turn upward and form an exceedingly wide dense crown. In moist shady mountain canyons it appears as a many-stemmed shrubby form in clumps of thickets ten to fiteen feet high.

"The bark of the tree is smooth and dull gray in color. New leaves are produced throughout the summer on the stems which grow consistently in height. This results in the branches being densely foliaged. As a rule the season's growth persists about two years.

When mature the leaves are shiny, deep yellow-green in color, from three to six inches in length and one-third to five-eighths inches in width. The yellowish green fruit resembling the olive has a thin outer skin, leathery in appearance, which contains a large thin-shelled seed. The fruits mature in one season and are ripe usually in October when they fall. They germinate shortly after. They are frequently washed along the banks of mountain streams and in this way extend along many narrow gulches.

"The wood is very heavy when green, moderately heavy when dry, and very hard and firm, fine grained, and of a rich yellow-brown.

"The range of the tree is from Oregon, the Coos River district, through California, along the Coast Range and western slopes of the Sierra Nevadas to the southern slopes of the San Bernardino Mountains.

"Names also used in reference to Umbellularia californica are:

Mountain Laurel	Pepperwood
California Bay Laurel	Oreodaphne
Cajeput	Yellow Myrtle
California Olive	Black Myrtle
Myrtle	White Myrtle
Spice Tree	Bay

California Spice Tree Bay Laurel

Oregon Myrtle."

The oil is described as being clear and colorless when first distilled but almost immediately assuming a light yellow color. The odor is pungently aromatic and reminds one of the odor of nutmeg and cardamon. The taste is warm and camphorlike.

WORK OF PREVIOUS INVESTIGATORS ON OIL OF MYRTLE

The earliest records[3] obtainable on oil of myrtle give only an indefinite summarization of its physical character and chemistry. Some time later Heaney[1] reported the oil to contain 35 per cent of a hydrocarbon and 65 per cent of an oxygenated body. Stillman[4] obtained from 60-70 pounds of leaves a yield of 820 grams of oil having a specific gravity of 0.940 at 11° C. Reference is also made here of its remedial value, it being used in nervous disorders, atonic dyspepsia, and as an antispetic.

The work of Power and Lees[5] is perhaps the most extensive investigation made on the oil. This work, however, is solely from a chemical standpoint. Investigation carried out on 1200 grams of oil yielded the results shown below:

The oil was obtained by steam distillation and had a specific gravity of 0.983; optical rotation -22°; soluble in 1 to 5 parts of 70 per cent alcohol. The oil formed no solid compound with sodium hydrogen sulphite.

The oil was separated by distillation at atmospheric pressure into fifteen fractions, boiling between 165-250° C.

Fraction	Specific Gravity	Rotation	Identification
1.	0.8659	-20°	L-pinene
2.	0.8770	-18° 20'	Pinene odor camphoraceous
3.	0.8903	-13° 12'	More camphoraceous
4.			Fraction very small
5.	0.9092	-30° 30'	Camphoraceous with odor of cineol
6.			Very small
7.			Relatively small Odor of cineol
8.	0.9308	-22° 48'	Odor of cineol with increased pungency
9.	0.9640	-30° 3'	Fraction small and pungent
10.	0.9546	-34° 35'	Increased pungency
11.	0.9614	-36° 33'	Largest fraction
	Having a mint-like odor, pungent. The greatest amount came over at 218° C., and was identified as the ketone umbellulone.		
12.	0.9842	-29° 17'	Small fraction, with odor of safrol
13.	0.9976	-20° 27'	Odor of safrol strong
14.	1.013	8° 40'	Small fraction, odor of safrol
15.	1.021	2° 22'	Yellow in color, consists of eugenol-methyl ester

Summary:

The following compounds were identified in the percentages shown:

Eugenol	1.6%		
L-Pinene	6.0%	Eugenol Methyl Ester	10.0%
Cineol	20.0%	Mixture of fatty acids,	trace
Umbellulone	60.0%	Formic, acid	trace
Safrol	(Trace)		

NOTE: The above constants were determined at 15° C.

According to the results of Power and Lees, umbellulone, when purified by regeneration from its semi-carbazide semi-carbazone, has the following constants:

Boiling point 219°-220° C.

Specific gravity 0.950

Optical rotation 36° 30'

Reference index 1.18325.

Gildemeister and Hoffman[6] report the following results:

The oil obtained from the leaves varied in yield from 2.45 per cent to 4 per cent; specific gravity 0.963; contains a hydrocarbon boiling at 175°; and another boiling at 210° C., which has a specific gravity of 0.960. The fraction boiling between 167°-168° C., contains a terpineol substance, and the fraction boiling between 215°-216° C., contains the

ketone umbellulone.

Sawyer[7] states the oil is recommended for nervous headache. In regard to physical constants he records the following results:

The pure dry oil has a solubility of 1:1000, and a specific gravity of 0.936. On fractional distillation that fraction obtained at 175° C. was found to be highly inflammable. It dissolves iodine assuming a deep red color. With nitric acid the fraction produces a violent reaction. The specific gravity of the fraction was 0.894 at 15° C.

According to Parry[8] the yield of the oil varies from 2.5 per cent to 5.5 per cent; specific gravity from 0.935 to 0.950; optical rotation -22°.

FOREWORD TO PRELIMINARY WORK ON OIL

It was not the purpose of the investigator to undertake any further chemical analysis of the oil. The reason for the following analysis was one of personal interest only. Owing to the fact that no immediate supply of the pure oil was available, it was deemed advisable to undertake a preliminary investigation of the oil obtained from the leaves, in order to insure a pure sample of the oil upon which to work.

PRELIMINARY WORK ON THE OIL

Extraction and Determination of Physical Constants

Three samples of leaves were distilled to obtain oil for the following investigation. These samples were collected in February, March, and September of 1928. Percentage yield was calculated only on the September sample.

The February and March samples of leaves were distilled in a semi-dry condition. The September sample was thoroughly dry.

The still used in the operation consisted of a galvanized iron can capable of holding five pounds of dry leaves. To this was connected two Liebig condensers in series.

The oil obtained from the February and March sample of leaves was combined. Each sample of oil after being dried over anhydrous sodium sulphate gave the following constants.

Sample	Specific Gravity	Rotation	Index
February	0.9134	----	----
March	0.9242	-23.1°	1.47382
September	0.9218	-22.6°	1.47334

The February and March samples after being combined were fractionated under vacuum. Three hundred and twenty grams of oil were used and four fractions were collected.

Fraction	Weight Gm.	Per cent of Total	Range Deg. C.	Pressure
1.	139.25	43.3	66-87	7 mm
2.	93.26	28.6	87-100	7 mm
3.	47.86	14.8	100-103	8 mm
4.	23.03	7.1	remaining in flask	8 mm

The September sample of leaves was thoroughly dried by spreading on the floor in a room with a temperature practically constant at 25° C. The sample distilled weighed 11,697.94 Gm. The total yield of oil was calculated at 5.16 per cent, based on dry weight. Three hundred and twenty-eight grams of this sample of oil was then fractionated under vacuum and fractions collected as follows:

Fraction	Weight Gm.	Per cent of Total	Range Deg. C.	Pressure
1.	115.65	35.6	40-66	6-7 mm
2.	48.56	14.8	67-86	5 mm
3.	129.26	39.4	86-102	5 mm
4.	24.82	7.5	fraction left in flask	5 mm

The fractions collected were then combined in the manner of number one with one, etc., and refractionated under vacuum with fractions collected as follows:

Fraction	Weight Gm.	Per cent of Total	Range Deg. C.	Pressure
A	79.14	12.7	47-52	5 mm
B	84.24	13.5	52-58	4.5
C	50.10	8.4	55-58	4.5
D	22.58	5.2	45-58	3.5 - 3
E	111.45	17.8	74-91.5	4.5 - 5
F	28.64	6.3	66-87	4.0 - 4.5
G	126.63	21.22	87-88	4
H	26.92	4.3	83-99	3.5 - 4
I	17.42	2.8	103-110	4
J	24.61	3.9	above 110	4

Physical constants were determined on the preceding fractions with the following results:

Fraction	Optical rotation at 20° C.	Specific Gr. at 25° C.	Index at 25°C.
A	-21.40	0.8764	1.46269
B	-13.80	0.8892	1.46244
C	- 4.52	0.9015	1.46103
D	- 5.95	0.9054	1.46326
E	-35.84	0.9431	1.48052
F	-36.66	0.9411	1.48060

Fraction	Optical rotation at 20° C.	Specific Gr. at 25° C.	Index at 25° C.
G	-39.90	0.9549	1.48049
H	-44.51	0.9437	1.48169
I	-27.10	0.9608	1.49861
J		1.0011	

During the process of fractionation, as fraction "G" was approached, the pungency increased and became most pronounced with fraction "G". As the fractions from "H" to "J" were gathered the pungent odor disappeared and was replaced with one which became more empyreumatic until fraction "J," or the last fraction was obtained.

Though the distillation of the leaves was carried out in a well-ventilated room, the small amount of vapor which escaped from the still made the atmosphere very disagreeable to breathe. Even this very high dilution of oil vapor produced an intense burning in the mucous membranes of the nose and throat, and produced a flow of tears. Several times it was necessary for the operator to leave the room to relieve the action produced by the escaping vapors.

In handling a sample of the oil, a small bit was spilled on the wrist. This was immediately wiped off, but the area was not washed. Shortly afterward an

intense burning was set up, which became decidedly painful. The skin showed signs of slight blistering and the surrounding surface was reddened. The sleeve of the laboratory coat which was being worn at the time absorbed a portion of the oil which was spilled. One week later the same coat was worn again, and the oil remaining in the coat over that period of time produced the same reaction in a slightly milder form.

Fraction "G," which we shall now call umbellulone, produces all the burning sensations to the mucous membranes, increases the flow of tears, and produces the rubefacient action when placed on the skin. Since umbellulone is probably the only important constituent of the oil that has not been subjected to a thorough pharmacological investigation, it is probably responsible for the peculiar action demonstrated by the oil.

ESTIMATION OF THE PHENOL COEFFICIENT

The estimation of the phenol coefficient of the oil was carried out according to the procedure of the United States Hygienic laboratory method[9] briefly outlined as follows:

The culture used is B. Typhosus maintained on nutrient agar as specified by the method. Three days before the tests are run, the test culture is transplanted at 24 hour intervals to successive tubes of meat extract broth. The transfers are made with a platinum loop having a diameter of four millimeters.

The phenol standard used must meet the requirements of the U.S.P. IX. The standard solution is made to a five per cent strength and standardized against a deci-normal bromine solution.

Oil solutions were made up arbitrarily as indicated on the chart. Since the United States Hygienic method provides only for those disinfectants which are soluble in water, a modification at this point was necessary. To bring the oil into as nearly a perfect suspension as possible, sufficient powdered acacia was added to form an emulsion with considerable shaking.

The proportion of the culture added to the disinfectant was 0.5 cc. to 5 cc. of oil emulsion.

Seeding tubes were incubated for 48 hours at $37\frac{1}{2}^{o}$ C.

Only four dilutions were carried out at a time. Innoculations were made every thirty seconds, and at intervals of 5, $7\frac{1}{2}$, 10, $12\frac{1}{2}$, and 15 minutes transfers were made from each dilution of oil to the nutrient broth.

Calculation of the coefficient was made by dividing the greatest dilution of the oil capable of killing the organism in less than ten, but more than five minutes, with the corresponding phenol dilution.

In the absence of organic matter the coefficient calculated was 1.1 as compared to the phenol standard. The results are as follows:

Dilution oil	5'	$7\frac{1}{2}$'	10'	$12\frac{1}{2}$'	15'
1: 50	--	--	--	--	--
1:100	**	**	--	--	--
1:133	**	**	--	--	--
1:200	**	**	**	**	**
Phenol					
1: 90	**	--	--	--	--
1: 95	**	**	**	**	**
1:100	**	**	**	**	**
1:110	**	**	**	**	**

Dilution oil	5'	7½'	10'	12½'	15'
1: 50	**	--	--	--	--
1:100	**	**	--	--	--
1:133	**	**	**	--	--
1:200	**	**	**	**	--
Phenol					
1: 90	**	**	--	--	--
1: 95	**	**	**	**	**
1:100	**	**	**	**	**
1:110	**	**	**	**	**

** indicating positive reaction

-- indicating negative reaction

Summary:

These results show a coefficient somewhat lower than that which might have been expected form an oil having the characteristics of oil of myrtle. There is a possibility that the method involved in the determination may be in a way responsible for the results. Since the oil was not very soluble in water it was necessary to emulsify it by means of acacia, thereby preventing it, perhaps, from coming into sufficient contact with the organism.

THE ACTION OF UMBELLULONE ON BLOOD PRESSURE

The following experiments were carried out entirely on cats, the first three cats being injected intravenously through the femoral vein. The remainder of the cats received ketone by intraperitoneal injection. Tracings were made on a single drum kymograph, using a Becker mercury manometer. All the pressure recordings were obtained from the right carotid artery. A 25 per cent aquerous magnesium sulphate was used as the anti-clot solution.[11,12,13]

The following is a typical protocol of the three cats receiving the intravenous injection of umbellulone.

Cat Number Three

Weight 2100 gm.

The animal was anesthesized with ether, which was immediately followed by an intraperitoneal injection of chloretone in alcoholic solution. The ether was removed after the injection of chloretone.

11:07 Cat placed on the table and normal reading taken. (Fig. 1.)

11:10 One-tenth cubic centimeter of the ketone was introduced into the femoral vein undiluted. The pressure reading continued normal, no increase in pulse. Respiration showing

slight increase.

11:12 Injection of 0.2 cc. of ketone. This injection was followed by a gradual progressive decrease in pressure. (Fig. 2.)

11:16 Cessation of respiration followed immediately by arrest of heart action.

Post Mortem: On opening the lung cavity the odor of umbellulone was very strongly present. The lungs were in a severe state of congestion. The entire surface of the lungs was spotted with blood clots.

The animals in the following experiments were treated in the same manner as the cats in the foregoing experiments, except that all injections, with one exception, were made in the area just below the abdomen. In none of the experiments was artificial respiration given.

The following is a protocol of the cats treated by intraperitoneal injection of the ketone.

Cat Number Four

9:39 Cat placed on table and normal reading observed. (Fig. 3.)

9:42 Injection of 0.1 cc. of ketone made well toward the diaphram. No noticeable reaction.

9:44 At this point there was a gradual decline of pressure until the line upon the kymograph was

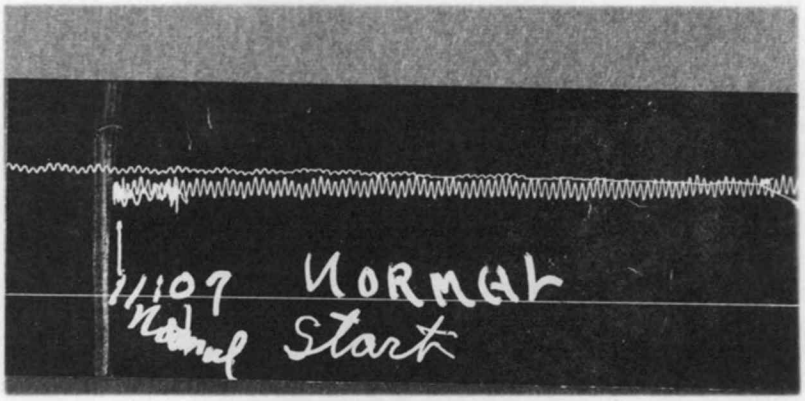

Figure 1

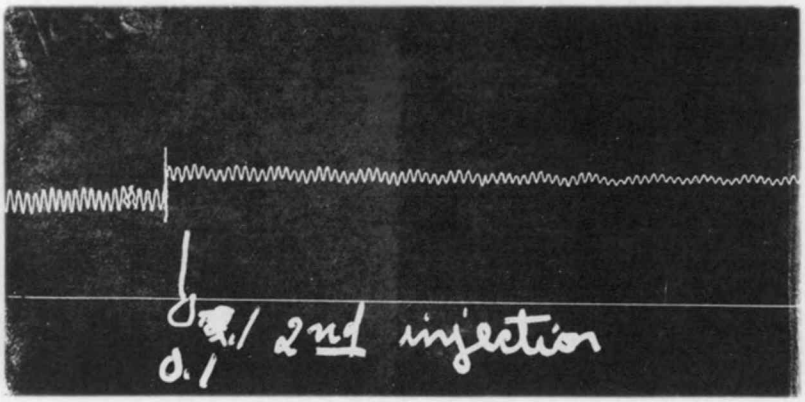

Figure 2

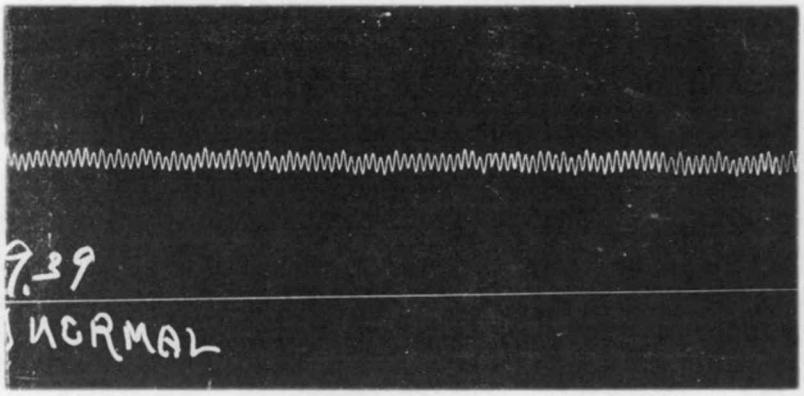

Figure 3

slightly more than a straight impression. (Fig. 4.)

9:47 The pressure at this point took a sudden rise which became progressive but gradually reverted regaining its normal beat. (Fig. 5.)

9:50 Pressure again at normal, with perhaps a slight increase in the systolic contraction.

9:51 Injection of 0.2 cc. of ketone in a similar manner. Pressure the same. Rate of respiration slightly increased, depth decreasing.

9:55 A sudden increase in pressure, with respiration tending to become spasmodic, of the Cheyne-Stokes type.

9:56 Respiration ceased followed immediately by stoppage of heart action.

Cat Number Five

3:05 Cat placed on the table and normal reading observed. (Fig. 6.)

3:07 Injection of 0.3 cc. of umbellulone just below right side of the abdomen. Pressure normal, no signs of distress.

3:10 Pressure rise, very sudden with a slight pause at the termination of the systolic contraction. (Fig. 7.)

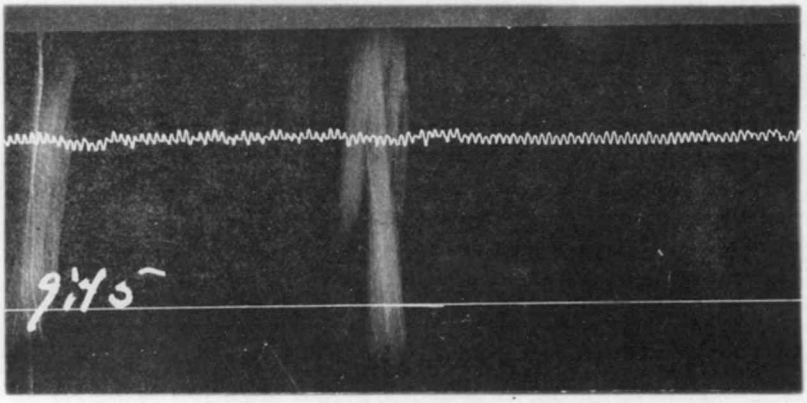

Figure 4

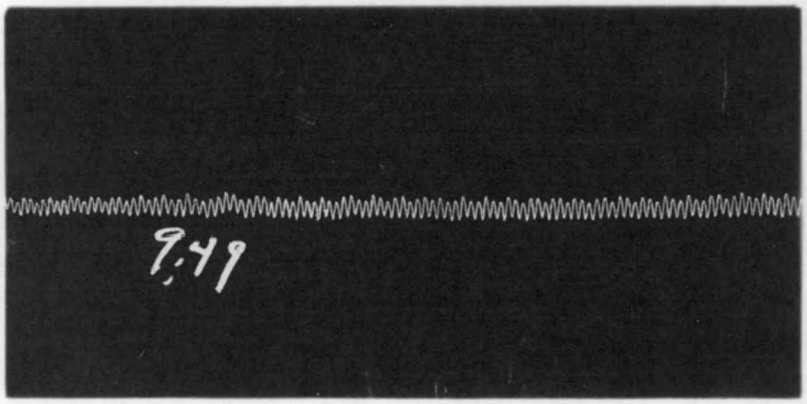

Figure 5

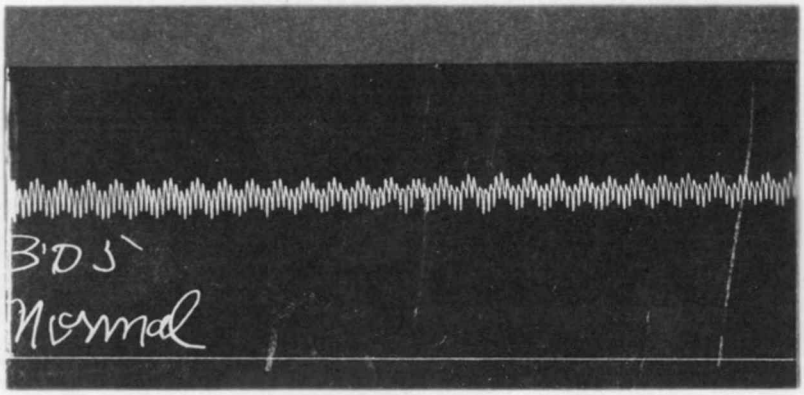

Figure 6

3:12 Pressure showing a slight rise over the preceding rise.

3:13 Injection of 0.3 cc. of ketone.

3:15 Abrupt stoppage of both heart action and respiration. (Fig. 8.)

Cat Number Six

2:13 Cat placed on board and reading taken of normal pressure. (Fig. 9.)

2:18 Injection of 0.2 cc. of ketone. Slight increase in pulse rate.

2:21 Injection of 0.4 cc. of ketone. Reading same as before.

2:23 Pressure constant: rate somewhat slower.

2:25 Injection of 0.3 cc. of ketone into the lung cavity. Pressure remaining constant, rate slightly less.

2:27 There was a slight rise in pressure for a period of ten seconds, then a return to normal.

2:31 Injection of 0.3 cc. of ketone: rate and pressure constant.

2:37 Pressure still constant, breathing quite shallow with an intermediate spasmodic expansion of the lungs.

2:44 Pressure remaining constant, but a quickened pulse beat. (Fig. 10.)

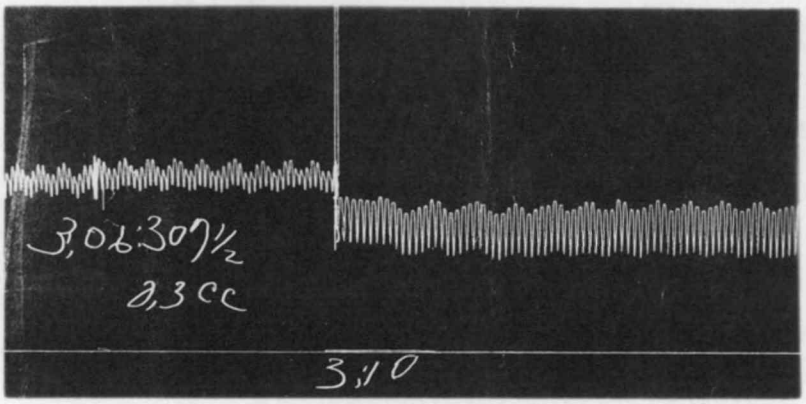

Figure 7

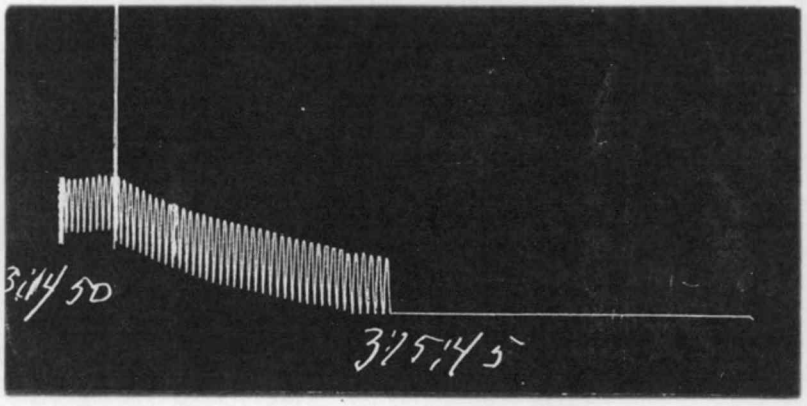

Figure 8

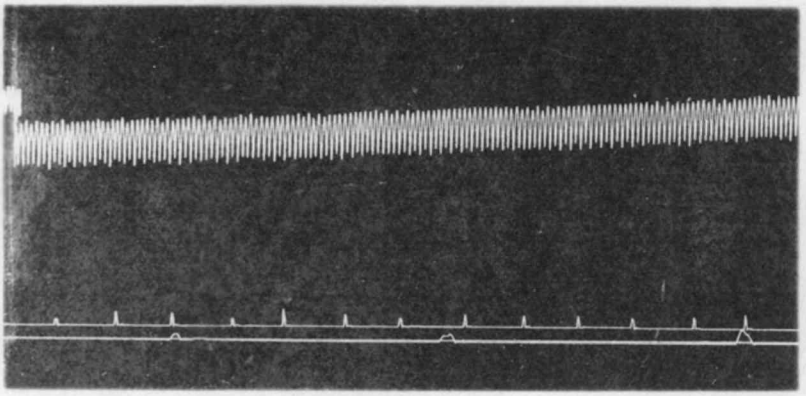

Figure 9

3:07 Pressure still constant with a marked increase in rate of beat. (Fig. 11.)

3:11 Respiration and heart action ceased almost simultaneously.

Post Mortem:

In all cats where injection of the ketone was made only in the peritoneal cavity, the odor of the ketone was found to be only in that cavity. The tissue in the cavity and the portion surrounding the spot where the injection was made showed no signs of rubefaction. The thoracic cavity contained no odor of the ketone. The lungs were in a perfectly normal state.

In cat number six, where injection was made into the lung cavity, there was a decided irritation of the surface of the lungs. Their appearance was similar to that in the animals which had received the ketone through intravenous injection. The appearance was one of extreme redness without the blood clots.

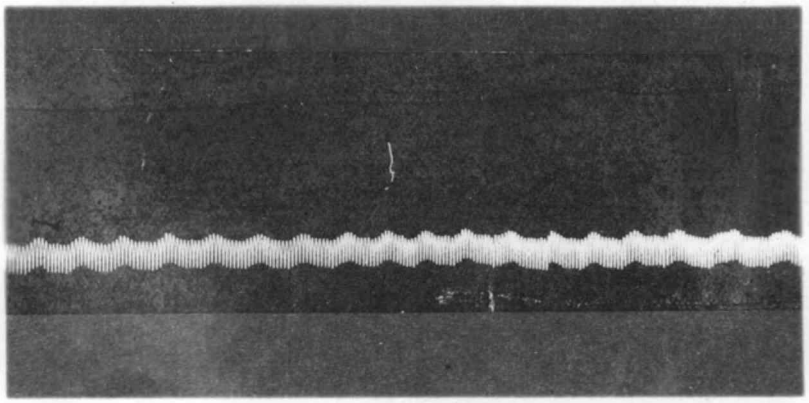

Figure 10

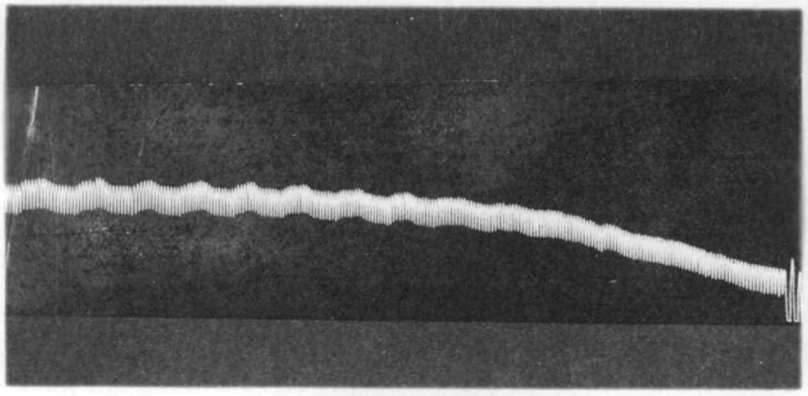

Figure 11

Conclusions

The results obtained from the foregoing experiments do not give sufficiently consistent data to warrant any definite conclusions on the action of the ketone upon the blood pressure. At best, it may be said that the ketone produces a slight increase in pressure followed by a relative decrease, and accompanied by an increase in pulse rate. Further experimentation could not be undertaken due principally to the lack of animals. The fact that artificial respiration was not given, no doubt enters into the results obtained. Without further experimentation a more concise conclusion cannot be drawn.

ACTION OF UMBELLULONE ON RESPIRATION

The following experiment was carried out on only one cat. Injection of the ketone was made intravenously in the manner similar to the experiments on blood pressure. Records were made on a single drum kymograph using a Becker Plethsymograph.

The animal was first anesthesized with ether and a normal reading obtained, a normal reading signifying, of course, the respiratory movements under the influence of ether. (Fig. 12.)

One and three-tenths (1.3) cc. of the ketone was injected over a period of twenty-five minutes. Shortly after the first injection of ketone (approximately ten minutes) the ether mask was removed and no more ether was given throughout the remainder of the experiment.

Shortly after the first injection the respiration became considerably more even than it had previously been. (Fig. 13.)

There was an increase in the rate of movement accompanied by a decrease in depth. The above reaction was continuous over a period of one and one-quarter hours, that is, the reaction was one of progression, a gradual increase in rate with a proportional decrease in depth of breathing, up to the time of respiratory

collapse. Figure 14 shows the resulting progression just before collapse of respiration occurred.

On opening the thoracic cavity, the lungs were found in a severe state of congestion with the odor of the ketone strongly present. Collapse was probably due to disruption of the capillaries of the lungs due to the mechanical effect on the insoluble ketone.

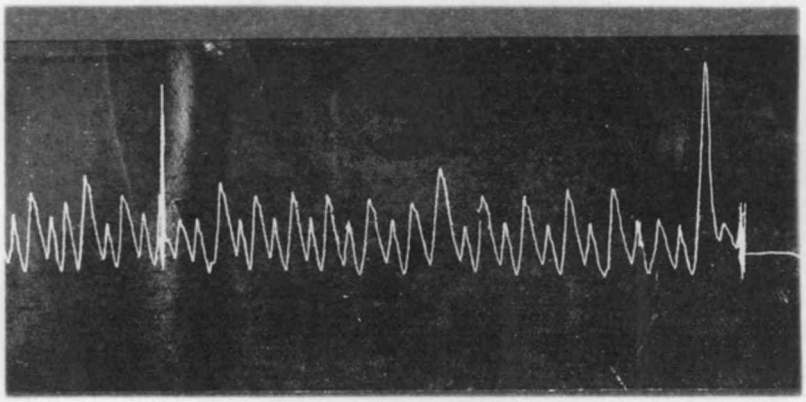

Figure 12

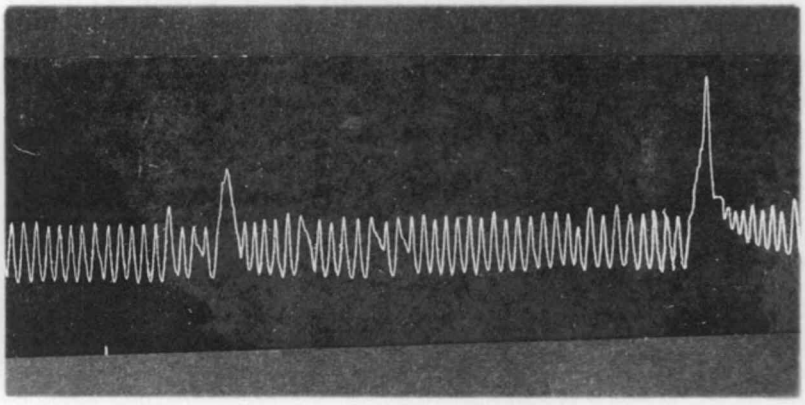

Figure 13

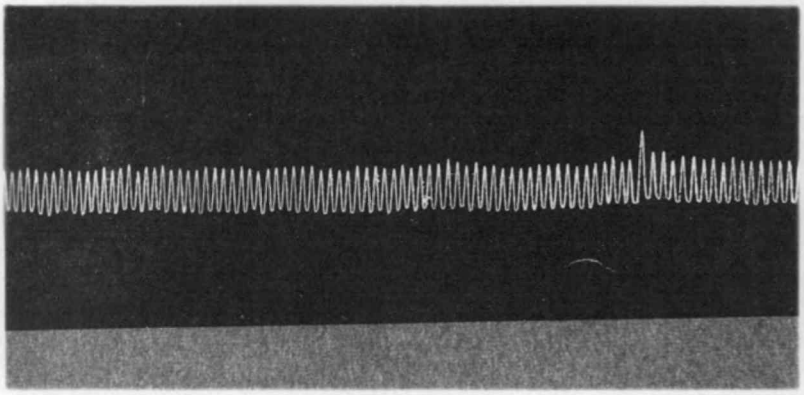

Figure 14

THE ACTION OF UMBELLULONE ON THE ISOLATED TISSUE OF THE RABBIT AND GUINEA PIG

The following experiments were carried out according to the procedure of Salant and Mitchell.[14] The rabbits used in the experiments were of the Chinchilla variety, and were taken from pedigreed stock.

The segments of the intestine were suspended in 200 cc. of Locke-Ringer's solution through which a stream of oxygen was continuously bubbled. Recordings were made on a single drum kymograph, using a Becker universal lever. Temperature regulations were maintained between 38° C. and 40° C. The umbellulone was prepared in the form of an emulsion, using 1 per cent powdered acacia as the emulsifying agent. A stock solution of 1:100 was first prepared and dilutions of 1:1000, 1:10,000 and 1:100,000 made from it.

The rabbits were killed by the neck stroke. The small intestine was immediately removed and placed in cold Ringer's solution, to which was added the defibrinated blood of the animal. The tissue was used within six hours after being removed from the animal.

The segments of tissue were fixed to the lever and counterpoised. The following is a protocol of the

various dilutions.

I. Dilution of 1:100,000

Figure 15 represents a normal reaction of tissue suspended in Locke-Ringer solution.

Ten minutes after normal reading was begun, 2 cc. of the 1:100,000 dilution was added to the bath. The reaction at first was very slight for the first five minutes. At this time, however, there was a slight increase in rhythm. At this point 1 cc. of the dilution was added to the bath. This time the reaction became more pronounced, the contractions becoming more spasmodic, accompanied by an increase in tonus. (Fig. 16.)

Ten minutes later, 1 cc. of dilution was again added. The spasmodic contractions which had occurred up to this time became lessened in depth, accompanied by an increase in rate of contraction, and decrease in tone. (Fig. 17.)

II. Dilution of 1:10,000

A fresh piece of tissue was adjusted to the lever, and the above dilution added according to the following protocol.

10:48 Normal reading. (Fig. 18.)

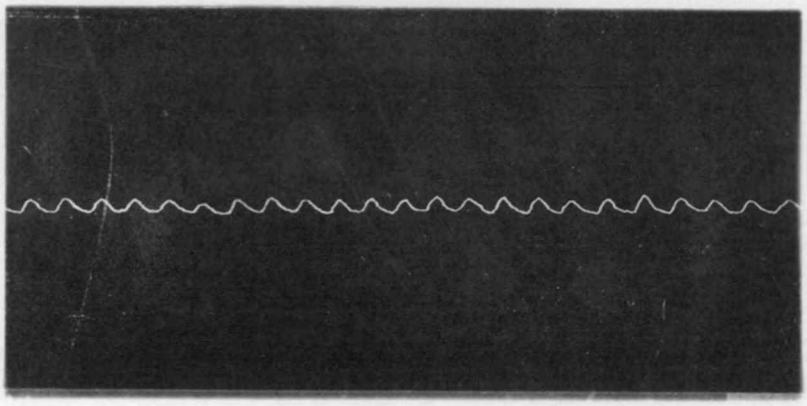

Figure 15

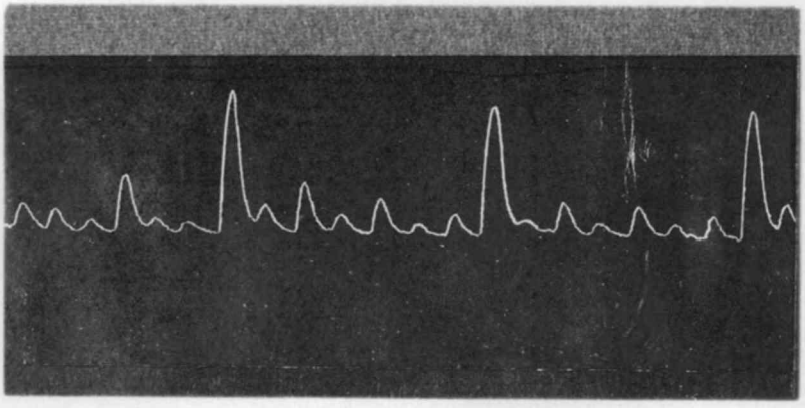

Figure 16

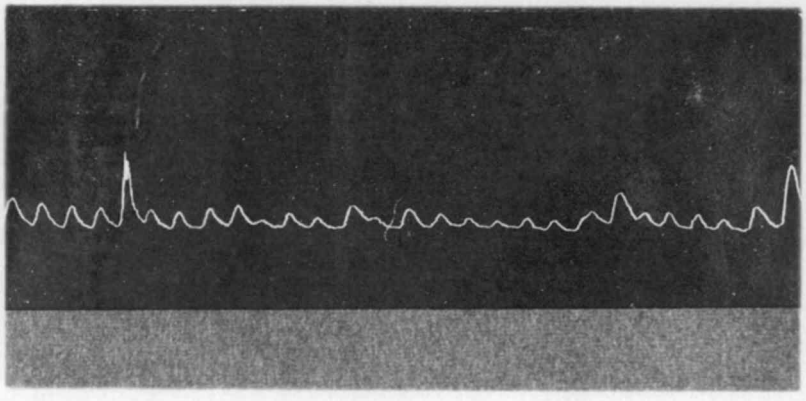

Figure 17

10:58 Introduction of 1 cc. of ketone emulsion to the bath. This produced a slight increase in contraction and rate. The tone remained the same.

11:08 One cubic centimeter of ketone solution added to the bath. The contractions became more marked with a relative increase in rate.

11:18 One cubic centimeter added to the bath. There followed a slowing in the rate of contractions, accompanied with a marked increase in tonus. (Fig. 19.)

11:28 A further increase in rate of contraction and rate. At this point the experiment was stopped. No attempt was made to determine whether the tissue would revive if placed in a fresh Ringer solution.

The segments of guinea pig intestine were prepared in the same manner as those from the rabbit. The following is a protocol of a segment exposed to a dilution of 1:10,000.

8:45 Normal reading.

8:51 Introduction of 1 cc. of ketone dilution. The contractions which were slightly spasmodic in the normal reading now became even more pronounced. The contractions were followed by a depression which lasted over a period of as long as twelve seconds. (Fig. 20.)

8:56 One cubic centimeter of ketone solution added to the bath. The preceding reactions were even more slowed with an increasing tone.

9:07 The Ringer solution was removed from the tissue and substituted for a fresh solution. The tissue immediately came back to its normal contraction and rhythm. (Fig. 21.)

At this point the experiment was terminated, and a fresh piece of tissue substituted. The following is the protocl using a 1:1000 dilution.

10:20 Normal reading.

10:27 Introduction of 1 cc. of 1:1000 dilution of ketone to the bath. Immediately after this

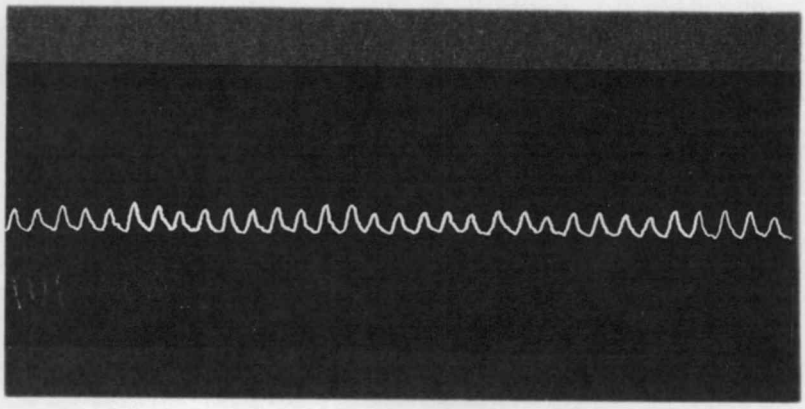

Figure 18

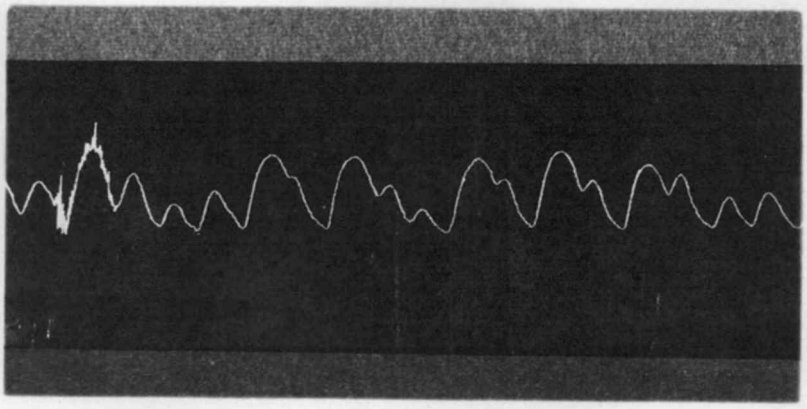

Figure 19

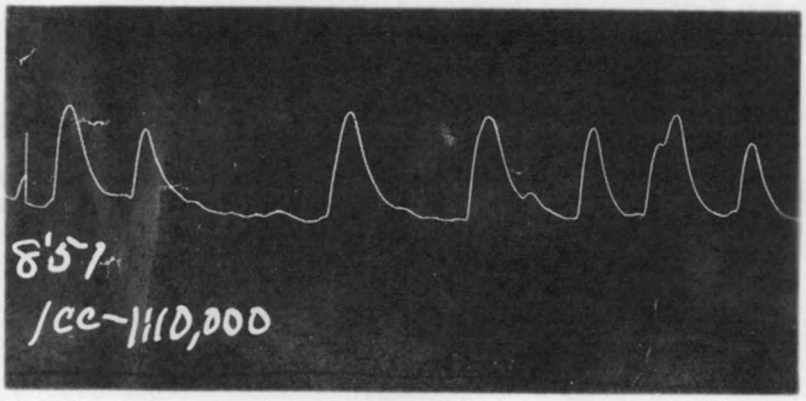

Figure 20

introduction the contractions of the tissue became very weak. Practically all tonus was lost, and the contractions were indicated by little more than a slight raise of the lever.

10:30 One cubic centimeter of the ketone dilution was again added to the bath, this time producing more severe sedative effect on the tissue. The recording line has now become little more than a straight line. (Fig. 22.)

10:37 The original Locke-Ringer solution at this point was removed and the tissue washed and a fresh solution added. There was no return to normal action of the tissue. The loss of contractibility was not recovered although the tissue was observed over a period of an hour before being discarded.

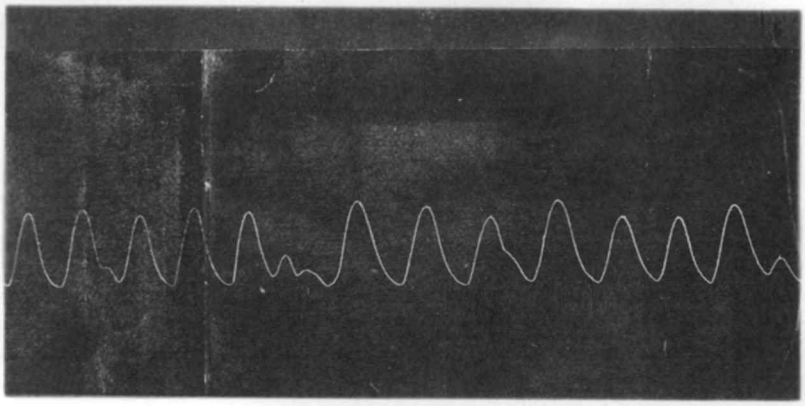

Figure 21

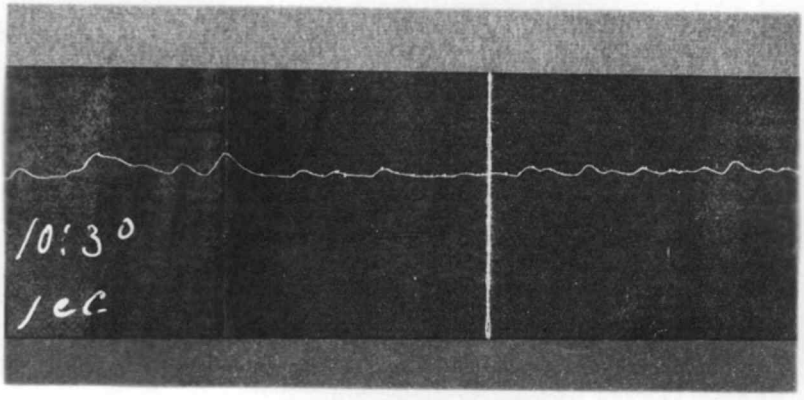

Figure 22

Conclusions

The bath in which the tissue was suspended in each case throughout the following experiments was 200 cc. The dilutions of the ketone were from 1:1000 to 1:100,000. The dilution to which the tissue was actually subjected was, therefore, from 1:200,000 to 1:200,000,000. These dilutions are unusually high, which show an equally unusual reaction. The tissue subjected to the dilution of 1:100,000 showed the most marked increase in contractibility, while the dilution of 1:1000 entirely prevented the tissue from performing any reaction. In high dilutions it is to be noted there exists the ability of the tissue to regain its normal tonus and contractibility when subjected to a fresh Locke-Ringer solution. This reaction is entirely prevented when the same tissue is treated with a ketone dilution of 1:1000.

According to the above results, umbellulone may be said to produce, in high dilutions, a decreased frequency accompanied with an increase in amplitude. This increase in amplitude, is not constant, it taking, after a period of twenty or thirty minutes, a less progressive increase. With low dilutions there results an almost immediate disappearance of contractibility which

cannot be recovered by the addition of fresh Ringer solution.

SUMMARY

To insure a pure sample of oil on which to conduct the foregoing experiments, it was deemed advisable to extract a sample of oil directly from fresh leaves. Three samples of leaves were collected at different intervals of the year, distilled separately, fractionated, and the fractions combined for distillation and separation of the ketone principle, umbellulone. All fractions were carried out under reduced pressure to decrease the amount of decomposition of the oil by heat. A determination of the physical constants of the fractions agreed quite closely with those obtained by previous investigators in the field.

The estimation of the phenol coefficient was carried out according to the latest methods approved by the United States Hygienic Laboratory. The results obtained are more or less in accordance with values accorded to most volatile oils, a low coefficient.

The blood pressure and respiration experiments were carried out entirely on cats, but were not sufficiently extensive to warrant a conclusive statement to be drawn on the results in this work. However, the ketone seems to induce a slight rise of pressure which in turn is followed by a gradual progressive decrease

of pressure, accompanied by a rise of pulse rate.

Isolated tissue experiments were carried out on both rabbits and guinea pigs. In all procedures only segments of the small intestines were used. The results in this work show umbellulone to cause a marked contractibility of the intestinal muscles, accompanied with an increase in tonus and a slowing of rhythm. These results are obtained only with high dilutions, while the lower dilutions produce an inactivity of the tissue from which it could not be revived by the addition of a fresh Locke-Ringer solution.

The present investigation has not exhausted any phase of the work undertaken. The work on changes of blood pressure and respiration should be covered more completely, using a longer period of time and more animal experimentation than was possible at present. The work on isolated tissue should be extended to cover other tissue. The one disadvantage of working out a problem in a new field is that so much must be left untouched.

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