CICUTOXIN: THE POISONOUS PRINCIPLE IN WATER HEMLOCK (CICUTA).

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Received December 31, 1914.

Introductory.

Water hemlock is a very widely distributed poisonous plant, whose botanical character varies with its geographical location. Several different species are described as occurring, not only in the same country, but in restricted areas of the same country. Two or perhaps three distinct varieties may be recognized in this country: the eastern *Cicuta maculata* and *Cicuta bulbifera* and the western *Cicuta occidentalis*. The *maculata* appears to be identical with the tall variety of the French Ciguë Vireuse and the German Wasserschierling (*Cicuta virosa*). These varieties are, however, to be clearly distinguished from the spotted hemlock (*Conium maculatum*) of Socrates fame, which is a different genus.

A very complete bibliography of water hemlock literature was published by Marsh and Clawson,[1] but among the dozens of publications on the subject there is but one, namely, Pohl's,[2] purporting to give a chemical discussion of the poisonous principle of the plant.

The effect of the poison on frogs has been investigated by Wikszemski,[3] and the effect on dogs and cats by Boehm.[4] A great deal of experimentation on the plant has been carried out in several of our agricultural experiment stations, but with conflicting and indefinite results. The reason for this may be found in the fact that the chemical character of the poison itself has not been understood. Extractions of the plant have been made and fed to stock, and in some cases found to be poisonous, while in others not. One man states that swine are immune from the poison, another describes the death of two sows after eating the roots. In one case an experimenter fed as high as 41 pounds of the water hemlock roots to a sheep weighing 91 pounds without pronounced effects, but when he fed 2.5 pounds of the roots to another weighing 93 pounds, the sheep died. Nothing was said about the character of the roots nor when they were dug.

These are instances of the mass of conflicting data found on the subject, and the problem of undertaking to get more reliable and definite results seemed well worth while. Hendrick[5] estimates that over 100 cattle were lost in Oregon in one year from this source, and Chestnut and Wilcox[6] record 150 deaths among sheep and cattle in Montana in one year. Several cases of cattle poisoning and two suicides among Indians,

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[1] U. S. Dept. Agric., *Bull.* 69.

[2] *Archiv. Exp. Pathol.*, 34, 262 (1894)..

[3] Dissertation, Dorpat (1875).

[4] *Archiv. Exp. Pathol.*, 5, 279 (1876).

[5] Oregon Agr. Exp. Station, *Bull.* 46 (1897).

[6] U. S. Dept. of Agric., *Bull.* 26.

caused by eating the roots of this plant, have come to the author's attention during the progress of this work.

The main efforts in the present investigation have been directed along purely chemical lines, although the work had to be supplemented by toxicological experiments in order to trace the poisonous constituent in the various chemical transformations. For these experiments, rabbits, cats and guinea pigs were used.

Botanical.

Water hemlock belongs to the Umbelliferae, or Parsley family. It is a bulbiferous annual plant, two to five feet tall, growing along the edges of streams, ditches and lakes, and in marshy ground. Nine or ten species of cicuta have been recorded as occurring in this country. About thirty-five foreign varieties are found in the literature, a number of which are already known to be duplications.

The following experimental work was carried out with the *Cicuta vagans*, Greene, which in the opinion of the author is identical with *Cicuta occidentalis*, Greene, and whose essential parts are shown in the accompanying cut. The small greenish white to white flowers are dispersed in clusters of clusters. The leaves are compound, lanceolate and medium serrate. The stem is smooth and hollow with nodes four to eight inches

Water hemlock (*cicuta vagans*).

apart. The seed is smooth, compound and markedly ribbed, and when ripe separates easily into two carpellary parts. The rootstalk from which the stem or stems grow, varies in size from that of a hazel nut to that of a man's fist. Its shape is not uniform. It has one to four or five tapering roots and these are covered with fine, white root hairs. The rootstalk is transversely chambered and next to the layer of brown cells is a fibro vascular region containing ducts from which the poisonous liquid exudes.

Chemical.

When the water hemlock rhizome, described in the foregoing section, is cut in two, drops of a yellowish balsam-like liquid ooze out upon the cut surface. A penetrating aromatic odor is observed, which is characteristic of this substance. In about a minute the light yellowish color of the droplets changes to light red, then to ruby red and reddish brown. The color change is not wholly produced by light, nor is it an oxidation process.

This yellowish viscid liquid is the poisonous principle of the plant. It is also found in the stem, particularly in the lower portions, but in relatively small amount. A Frenchman by the name of M. Brandes, recorded by Trumel,[1] named this poison cicutoxine. At least thirty-eight years later, Prof. Boehm,[2] of Dorpat, Russia, apparently not aware of the Frenchman's name for the substance, renamed it with the same name. The name cicutoxin has since been woven into the literature so extensively that it would hardly seem advisable to rename it, although this could now be done upon a rational chemical basis.

Methods of Preparation.—Boehm[2] describes three methods for the preparation of cicutoxin, but expresses doubt as to the purity of the substance obtained by all of them. In the first method he extracts the *dried* and finely ground tubers with ether, the ethereal residue is then treated with 70% alcohol and finally extracted with petrolic ether. The petrolic ether insoluble portion he calls cicutoxin. His second method consists in the extraction of the *dried* and ground roots with boiling water and subsequent treatment of the residue with 95% alcohol and petrolic ether, while his third method employs 70% alcohol containing 10% strong ammonium hydroxide as the solvent, subsequently precipitating the poison with lead acetate.

The method employed by Pohl[2] differs from all three of Boehm's methods in that warm 95% alcohol is used for extracting the sliced and *air-dried* tubers. The dissolved cicutoxin is precipitated from the alcohol with lead acetate, the lead removed from the filtrate with sodium sulfate and the final residue taken up in dilute alcohol and shaken out 20–30 times with petrolic ether.

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[1] *Thèse-Faculté de Medecine de Paris,* No. 239, p. 6 (1838).

[2] *Loc. cit.*

From the following chemical discussion it will be seen that this substance polymerizes and decomposes easily at temperatures above 50°, and especially when in contact with alcohol or hot water. Experiments have also shown that when the cicuta tubers are extracted with 95% alcohol a sugar or glucoside is extracted simultaneously with the toxic substance, which has a sweet taste, and when hydrolyzed gives a strong reduction of Fehling's solution. In fact, a yellow osazone was prepared from the water solution of one of these hydrolyses, which formed in 5–6 minutes' heating and melted with decomposition at 203–204°. It was unquestionably glucosazone.

In view of the above facts the following method of preparation was adopted for obtaining the purest possible product: The fresh water hemlock tubers were ground to a fine pulp in a meat chopper and extracted with ether at room temperature, pouring the ethereal solution from flask to flask, so as to obtain it as concentrated as possible. No attempt was made to get a complete extraction, except for one quantitative determination. The ethereal solution was dried by shaking with anhydrous copper sulfate, filtered and allowed to evaporate in the air or in a stream of dry air. The residue, after all the ether had apparently passed off, continued to lose weight for several weeks. When the loss in weight amounted to 0.05% or less for a period of 24 hours the substance was considered sufficiently dry for analytical purposes. It was discovered that the substance could be precipitated as an amorphous solid from petrolic ether (b. p. 25–45°) having a temperature of 6–10°; consequently some of the ethereal residue obtained above was dissolved in a small quantity of absolute alcohol and poured, drop by drop, with constant stirring into the cold petrolic ether. The precipitate was washed by decantation with more cold petrolic ether, the solvent allowed to drain off and the residue dried in a stream of dry air.

Combustions were run on two different lots of the poisonous product, with and without precipitating from petrolic ether, yielding the following results:

	Grams cicutoxin.	CO_2.	H_2O.	% C.	% H.
	(Without precipitating from petrolic ether.)				
Lot 1 (*a*)	0.2804	0.7818	0.2180	76.04	8.70
Lot 1 (*b*)	0.2341	0.6535	0.1830	76.13	8.75
Lot 2 (*c*)	0.2219	0.6194	0.1701	75.38	8.34
Lot 2 (*d*)	0.2261	0.6287	0.1699	75.10	8.17
			Mean,	75.66	8.49
	(Precipitated from petrolic ether.)				
Lot 1 (*e*)	0.2013	0.5618	0.1587	76.11	8.82
Lot 2 (*f*)	0.2075	0.5724	0.1500	75.23	8.07
			Mean,	75.67	8.44
Calculated composition for $C_{19}H_{26}O_3$:				75.44%	8.67%

The above results go to show that the ether does not dissolve any other substance from the tubers to an appreciable extent; and that the precipitation from petrolic ether does not influence the composition of the product. The less handling, and the fewer transformations to which the substance is subjected, the purer it will be. When cicutoxin is allowed to stand in contact with alcohol for some time, especially warm alcohol, or is heated with water, it decomposes and yields a petrolic ether soluble constituent having, in alcohol, a greenish fluorescence and non-toxic properties. This substance, Boehm calls umbelliferon. It is in all probability a decomposition product of cicutoxin.

Yield.—The yield of cicutoxin obtained by the ether extraction was found to be between 0.3% and 0.4% of the weight of the green tubers.

Molecular Weight.—Molecular weight determinations were made on the purified cicutoxin, resulting as follows:

Cryoscopically, with glacial acetic acid as solvent, 268.5, 265.4, 288.2; ebullioscopically, with alcohol as solvent, 926, 418, 798; with chloroform as solvent, 601.2. The molecular weight of cicutoxin calculated from analytical results is 302.21.

Empirical Formula.—$C_{19}H_{26}O_3$.

The low values obtained for the molecular weight by the freezing-point method are in all probability due to impurities, while the high and erratic results by the boiling point are due to polymerization. In the first experiment three molecules polymerized into one, and in the last one with chloroform, two molecules. This polymerization process is continuous after the cicutoxin has been removed from the plant, but proceeds very much slower at ordinary temperatures. Complete polymerization with the accompanying decomposition destroys the toxicity of the substance. The polymerization of cicutoxin diminishes its volume without perceptibly affecting its weight.

Properties of Cicutoxin.—It is a yellowish liquid resin, viscous like ordinary cane sirup, polymerizing spontaneously into a semisolid body of a ruby-red to reddish brown color. It has an odor like that described for the rhizome, a very bitter taste which persists for several minutes. The polymerization is apparently accompanied by a disengagement of a volatile or gaseous substance, for when cicutoxin is kept in a glass-stoppered bottle the polymerization is very slow compared to what it is when exposed to the air. When the substance is kept in an evacuated desiccator over concentrated sulfuric acid, the process is the most rapid and after some hours visible decomposition sets in, shown by the liberation of gas. The substance darkens and carbonizes, the acid in the desiccator assuming a ruby-red color. The visible decomposition may be retarded or entirely inhibited by lowering the temperature, increasing the pressure, or both. It cannot be dried over sulfuric acid.

Cicutoxin is very sensitive to an increase in temperature above that normal to its formation in the plant, and differences have been observed in the products obtained in April and October from those obtained in July and August. The former is the yellower product and the more toxic, gram for gram, but the midsummer tubers contain the larger quantity of material. When cicutoxin is heated quite rapidly in a melting-point tube, it begins to decompose at 100°, the decomposition becoming pronounced at 115° and strong at 125–130°. At 150° the substance chars in a few minutes. When a larger quantity of the substance is heated in a test tube connected with a delivery tube, gases come off between 120° and 148°, and at the latter temperature there seems to be a pause in the decomposition process.

Cicutoxin is readily soluble in ethyl and methyl alcohol, ether, acetone, chloroform, phenol and glacial acetic acid, but is very slightly soluble in carbon tetrachloride, benzene and petrolic ether. It is insoluble in water, glycerol and aniline.

Cicutoxin has never been obtained in crystalline form, but is precipitated as an amorphous solid when a concentrated alcoholic solution is poured, drop by drop, into a large volume of cold, low-boiling petrolic ether. When the temperature rises to about 20°, the precipitated cicutoxin melts into the original sirupy mass. It is not volatile with steam nor can it be distilled in any manner without decomposition, and consequent loss of toxicity. When pure it is neutral to litmus, phenolphthalein, congo red and sodium alizarinesulfonate, but if dissolved in alcohol and the solution allowed to stand at room temperature for a few hours, sufficient decomposition will take place to render the solution slightly acid. Cicutoxin is not an aldehyde, acid, ester, carbohydrate, anhydride, ketone or hydrocarbon, but by acetylation with acetic anhydride it shows weak alcoholic properties. When ignited it burns with a smoky flame leaving a carbonaceous residue. It unites with free bromine very energetically, with the liberation of gas, the reaction being strongly exothermic. When in solution it also takes up some bromine and iodine. It ignites spontaneously when a few drops of concentrated nitric acid are poured upon it. In contact with concentrated sulfuric acid and phosphorus trichloride it chars immediately.

Cicutoxin does not haemolize bovine, sheep or rabbit blood. It has a specific gravity of 0.9659 at 22°, and an index of refraction of 1.5885 at 25°. Its solutions exhibit no absorption bands in the visible spectrum but a general absorption is noticed in the violet. The ultraviolet was not examined.

Tests for Cicutoxin.—No satisfactory chemical test for the detection of this poison has heretofore been submitted. Takayama,[1] however, gives the following color test: a concentrated solution of cicutoxin in

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[1] *Mitteil. Med. Ges. Tokyo,* 17, Heft 6 (1903).

phenol, or a mixture of phenol and ether (3 : 1), exhibits a beautiful green color when a drop of concentrated sulfuric acid is brought in contact with it on a watch glass, changing to blue and violet by reflected light and finally to brown and reddish brown.

The author finds that this test is not entirely satisfactory because the given color changes are not only produced by the pure cicutoxin but by the polymerized and decomposed product which is no longer poisonous.

The following chemical test may be given as one not open to the above objection and, as far as the author's experience goes, is entirely characteristic for the pure cicutoxin: Add a 2% aqueous barium hydroxide solution, in small quantities, to a 5% alcoholic solution of the poison, until a voluminous precipitate appears and continue the addition till the color changes to a light green, then a few drops more, after which the solution is set aside. In from one to ten minutes the precipitate will have assumed a pea-green to olive-green color, which changes to a reddish brown (O—Shade I, Mulliken Color Standard, Sheet A), if an excess of barium hydrate has been added. On the addition of a dilute mineral acid in slight excess, the color changes to a darker red (OR—Shade I, Mulliken Color Standard, Sheet A).

For detection in stomach contents, an ethereal extraction is made and the residue tested by the above method. If the quantity allows, a subcutaneous injection of 0.05 g. of substance in dilute alcohol is given to a rabbit or guinea pig and observations made of the resulting symptoms. One-tenth this amount may be used for the production of characteristic symptoms in frogs.

Decomposition Products of Cicutoxin.—The dry distillation of cicutoxin yields a gas, between 125° and 148°, burning with a blue flame, which is not absorbed by solutions of bromine, potassium hydroxide or ammoniacal cuprous chloride. 4.7 g. material yielded 11.6 cc. of the gas, measured at 20° and 650 mm. pressure. Unsuccessful attempts were made to explode this gas with three and four volumes of oxygen. The material at hand could not be sacrificed for further attempts along this line. A previous distillation of cicutoxin (36.6 g.) had been carried out at diminished pressure, collecting the distillate in the following fractions:

Fraction.	Temp.	Pressure.	Grams distillate.	Sp. gr.	*n*.
1	45.5–52°	150–100 mm.	0.27	$0.8316_{24°}$	$1.363_{24°}$
2	52–85°	135–100 mm.	1.78	$0.8874_{20°}$	$1.370_{24°}$
3	110–168°	70–64 mm.	3.13	$0.9360_{20°}$	$1.4845_{12°}$ $1.4812_{21.5°}$ $1.4766_{30°}$ $1.4726_{40°}$
4	120–150°	160 mm.	3.82	$0.9608_{20°}$	.....
5	150–176°	180 mm.	0.80	....	.....

The charred residue remaining in the flask weighed 19.37 g. Great volumes of white and yellow fumes passed off during the distillation. These had hydrocarbon odors. Analyses made of the oil obtained in fraction 3, gave the following results:

(*a*) 0.3829 g. oil gave 1.0455 g. CO_2 and 0.3465 g. H_2O or C 74.47 and H 10.13;
(*b*) 0.4090 g. oil gave 1.1133 g. CO_2 and 0.3781 g. H_2O or C 74.24 and H 10.34.
Calc. for $C_{13}H_{21}O_2$: C, 74.58%; H, 10.12%.

The steam distillation of cicutoxin yields a colorless oil. It is only partially soluble in water, the major portion being found as a globule floating on the water in the receiver. This product has a pleasant essential-oil odor, and polymerizes in the course of a few weeks into a clear transparent semisolid resin. A combustion of this oil gave the following values:

0.2545 g. oil gave 0.7592 g. CO_2 and 0.2412 g. H_2O.
Calc. for $C_{14}H_{22}O$: C, 81.44%; H, 10.75%; found: C, 81.36; H 10.61.

Not enough material was at hand for molecular weight determinations.

When cicutoxin is treated with a fairly mild oxidizing agent such as potassium permanganate or nitric acid (1 : 3), it breaks up into various well-known substances. Pohl[1] detected oxalic acid as a decomposition product of cicutoxin, by using potassium permanganate. The correctness of his observation was confirmed by the work in this laboratory, where oxalic acid has been obtained with both permanganate and dilute nitric acid oxidations. Carbon dioxide is another product of this treatment.

In order to determine the amount of oxalic acid and carbon dioxide resulting from the oxidation, the following experiment was carried out: 0.7681 g. cicutoxin was heated in a flask on a water bath together with 50 cc. of water solution of nitric acid (1 : 2) for 24 hours, water being added from time to time, to maintain the approximate concentration of the acid. The vapors from the flask were aspirated through water, concentrated sulfuric acid and potassium hydroxide, respectively. At the end of the experiment the tubes containing the alkali gained 0.5639 g. in weight, which represented carbon dioxide. This amount, reduced to carbon, was 20.23% of the weight of the oxidized cicutoxin. There remained in the flask an unoxidized residue weighing 7.7 mg. This residue can be broken up by prolonging the oxidation. The oxalic acid was obtained from the nitric acid solution by neutralizing the latter with KOH, acidifying with acetic acid, treating with calcium chloride and titrating the oxalic acid liberated from its calcium salt, with standard potassium permanganate, giving 0.19297 g. $(COOH)_2$. Assuming that the carbon atoms and one oxygen atom of the oxalic acid came from cicutoxin, it would mean that 11.27% of the cicutoxin went to oxalic acid. The ratio of 2C + O to $C_{19}H_{26}O_3$ is 13.23%.

Three additional products resulting from the oxidation were identified,

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[1] *Loc. cit.*

namely, hydrocyanic acid, isobutyric acid and acetyl-2-cyclopentanone. The first of these was found in the wash water through which the carbon dioxide was aspirated. This acid, precipitated with silver nitrate, gave a white salt, not sensitive to light, and upon burning showed the cyanide composition. The nitrogen had been supplied by the nitric acid. When the nitric acid solution resulting from the cicutoxin oxidation was shaken with ether, the ethereal solution treated with metallic zinc to remove the dissolved nitric acid, and the filtrate from the zinc treatment allowed to evaporate at room temperature, a liquid remained, having acid properties and a decided isobutyric acid odor. This liquid was distilled, the distillate shaken out with ether and, after drying, the substance was recovered from the ether and burned, with results indicating the composition $C_4H_8O_2$.

In a subsequent oxidation of cicutoxin with nitric acid, the major portion of oxalic acid was removed by crystallization and the larger portion of nitric acid by evaporation over caustic soda. The resulting paste was extracted with ether, the ethereal solution treated with zinc as above and the resulting liquid distilled under a pressure of about 15 mm., giving a constant and sharp boiling point of 73.5°. It had a rather pleasant ethereal odor and yielded combustion values indicating the composition $C_7H_{10}O_2$. Blaise and Koehler[1] synthesized a substance with this composition, whose boiling point was 75° at 8 mm. and showed it to be acetyl-2-cyclopentanone.

Attempts at hydrolyzing the cicutoxin with dilute sulfuric and hydrochloric acids were unsuccessful, but as a result there was obtained a very inert polymerization product, which was labelled "K." The same product was evidently formed by both processes. Cicutoxin in contact with 50 times its weight of 20–25% sulfuric acid was at first heated on the water bath for a couple of hours and then subjected to steam distillation for three hours. The substance swelled, darkened and became porous like a sponge. After removal from the flask it hardened and became brittle. It was powdered and washed free of acid with boiling water, dried to constant weight and burned.

(*a*) 0.2021 g. "K" by H_2SO_4 gave 0.5497 g. CO_2 and 0.1414 g. H_2O.
(*b*) 0.1809 g. "K" by H_2SO_4 gave 0.4932 g. CO_2 and 0.1295 g. H_2O.
Mean composition from (*a*) and (*b*) C 74.27%, H 7.91%.

The same procedure was carried out with cicutoxin and 20% hydrochloric acid, yielding apparently the same "K."

0.2363 g. "K" by HCl gave 0.6474 g. CO_2 and 0.1734 g. H_2O.
Composition of "K" by HCl, C 74.75%, H 8.21%.
Calculated for $C_{17}H_{22}O_3$: C 74.40%, H 8.09%.

A similar treatment of cicutoxin with nitric acid yields a different substance, which indicates the introduction of oxygen into the polym-

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[1] *Bull. Soc. Chim. de France*, **4**, 7, 710 (1910).

erized product. Combustions of two different lots rendered the following values:

	Grams "K" HNO_3.	Grams CO_2.	Grams H_2O.	% C.	% H.
Lot 1 (*a*)	0.1998	0.4028	0.0971	54.98	5.44
Lot 1 (*b*)	0.2036	0.4106	0.0964	55.00	5.30
Lot 2 (*a*)	0.2728	0.5585	0.1291	55.85	5.29
			Average,	55.28	5.34
Calc. for $C_{15}H_{17}O_8$				55.35	5.29

"K" obtained by the first two processes, as well as that obtained by heating cicutoxin with water in a sealed bomb tube to 200°, is one of the most stable and chemically inert substances known to the author. It is very sparingly soluble in hot ethyl and amyl alcohol, amyl acetate, naphthalene and phenol, but wholly insoluble in water, chloroform, pyridine, methyl alcohol, turpentine, carbon disulfide, carbon tetrachloride, potassium hydroxide solution, formic and acetic acids. No reagent was found to dissolve it to any appreciable extent except concentrated nitric and sulfuric acids, which destroy its identity.

The decomposition of cicutoxin with hydriodic acid (sp. gr. 1.7) was attempted in the hope of determining the number of methoxyl groups present in the compound. Various alkyl determinations were made, both with the free cicutoxin and when dissolved in glacial acetic acid, rendering values between 1.50% and 1.75% CH_3. Judging from the molecular weight determinations and subsequent work, these values do not indicate the presence of a CH_3O- group in the molecule.

Combinations of Cicutoxin.—Besides being very unstable, cicutoxin is relatively inert and only a few combinations of definite character have been successfully made. After working with the substance for three years, Boehm[1] says he is unable to arrive at an understanding of its chemical nature. This was doubtless due to the fact that he did not recognize the great instability of the substance at temperatures above 50°, and also when dissolved in alcohol.

Cicutoxin is best precipitated from its alcoholic solution as a compound of lead or barium. The former is the more stable and definite. The barium salt seems to be a combination of the partly decomposed cicutoxin molecule, and is itself unstable. For precipitating the lead compound, a clear water or alcoholic solution of basic lead acetate is prepared, of about 10% strength, and added slowly to an alcoholic solution of cicutoxin at room temperature, producing immediately a light yellow precipitate. The reagent is added until no further precipitation results. The precipitate is first washed by decantation and then on a filter paper with 50% alcohol until the filtrate is free from lead. The compound

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[1] *Loc. cit.*

does not melt, but begins to decompose and char at about 230°. It ignites spontaneously when concentrated nitric acid is added to it.

	Gram Pb salt.	Gram $PbSO_4$.	Gram CO_2.	Gram H_2O.	% Pb.	% C.	% H.
(*a*)............	0.2062	0.1136	....	....	37.63	...	....
(*b*)............	0.2040	0.1131	....	....	37.87	...	....
(*c*)............	0.2103	....	0.3289	0.0825	...	42.65	4.39
(*d*)............	0.2238	....	0.3550	0.0927	...	43.26	4.61
Calc. for $C_{19}H_{27}O_5Pb$............					38.19	42.04	5.01

The barium salt is made by slowly adding a 5% aqueous barium hydroxide solution to a dilute solution of cicutoxin in alcohol, at room temperature, with constant stirring. A yellowish precipitate at first appears, which turns greenish to dark green after the point of saturation has been reached and the solution becomes slightly alkaline. This precipitate settles slowly and is stable towards alcohol and water so that the compound may be washed as in the case of the lead salt, but if the barium hydrate is added to excess the color of the precipitate changes to nearly that of Shade I, Yellow-Orange, in Mulliken's Color Standards. If an excess of acid is added to the barium salt, a reddish brown sirupy substance resembling the original material separates out. The above observations would indicate that the barium salt thus obtained is unstable and that great importance cannot be attached to the values obtained for its composition. With a little experience one may be able to interrupt the precipitation at the right moment, so that a compound of uniform composition can be obtained. When this barium salt is kept for some months in a desiccator, or in the open air, the green color gradually fades into a sandy brown.

The washed and dried barium salt yielded the following analytical values:

	Gram Ba salt.	Gram CO_2.	Gram H_2O.	Gram $BaSO_4$.	% C.	% H.	% Ba.
(*a*)............	0.2100	0.4305	0.1053	....	55.91	5.61	...
(*b*)............	0.2036	0.4182	0.0998	....	56.02	5.49	...
(*c*)............	0.3762	....	....	0.1488	...	....	23.29
(*d*)............	0.3926	....	....	0.1566	...	....	23.47
Calc. for $(C_{14}H_{17}O_3)_2Ba$............					55.66	5.68	22.75

The barium salt behaves like the lead salt in the melting-point tube, charring above 200°.

According to preliminary tests, cicutoxin possesses alcoholic properties, therefore attempts were made to produce a pure acetyl product of the poison. Both acetic anhydride and acetyl chloride were used but it was found that the latter reagent was the more adaptable. A dilute solution of cicutoxin in glacial acetic acid was made and acetyl chloride added in excess. The solution turned from a ruby-red to a dark brown, with a faint tint of green, but yielded no precipitate. The reaction mixture was evaporated to dryness at a low temperature. The brown residue was dissolved in a small amount of hot alcohol and precipitated from low-boiling petrolic ether. This was repeated and then the brown powder dried in a desiccator to constant weight and burned.

(*a*) 0.2016 and 0.2019 g. acetyl prod. gave 0.5012 and 0.5216 g. CO_2; 0.1266 and 0.1300 g. H_2O.

Calc. for $C_{14}H_{16}O_3(OCCH_3)_2$: C 67.89%, H 6.97%; found: C 67.80, 69.09 and H 7.03, 7.07.

The acetyl product also precipitates from the alcoholic solution upon cooling, and this might have been a better method for the purification of the substance.

The iodine value of cicutoxin was found according to Hübl, the mean of two determinations being 125.5. Assuming the molecular weight of the substance to be 302.21, three atoms of iodine entered the molecule. The number obtained by calculation, using the above iodine value, was 2.99.

The bromine and iodine derivatives of cicutoxin have been made but they are difficult to purify, and a good deal of variation in the halogen content of different lots have been found, so that too much weight should not be attached to these determinations. The iodine compound, which is the more satisfactory to work with, is made by adding a dilute solution of iodine in glacial acetic acid to a solution of cicutoxin in the same solvent. Almost immediately a brown precipitate settles out, which is washed free of iodine with carbon tetrachloride. The substance is further purified by extracting with ether, until the latter solvent remains colorless. The ether removes a small amount of a substance that contains iodine. The ether insoluble residue is then dried and subjected to analysis. The compound ignites spontaneously when heated to 100°.

	Gram I comp.	Gram AgI.	% I.		Gram I comp.	Gram CO_2.	Gram H_2O.	% C.	% H.	% I.
(*a*)	0.1997	0.1394	37.76	(*d*)	0.2043	0.3522	0.0813	47.02	4.46	....
(*b*)	0.2000	0.1311	35.43	(*e*)	0.2028	0.3493	0.0910	46.97	5.02	....
(*c*)	0.2215	0.1450	35.38	(*f*)	0.2079	0.3566	0.0851	46.78	4.58	....
							Mean,	46.94	4.69	
Calc. for $C_{14}H_{17}O_3I$								46.66	4.76	35.25

The bromine compound was made similar to the iodine, but in this case a dark brown to black liquid separated. This was purified in a similar way to the one above, but it was difficult to wash free of bromine and almost impossible to dry. Carius' determinations on this product yielded 22.15% and 23.53% bromine. The percentage of bromine calculated for $C_{14}H_{17}O_3Br$ is 25.54, showing that the composition is not far remote from that suggested by the iodine product.

It was found that the halogen acids form addition products with cicutoxin, or, more correctly, with a modified form of the cicutoxin molecule. The hydrochloride of cicutoxin was made by passing dry hydrochloric acid gas into a solution of the substance in absolute alcohol until the solution became saturated at the temperature of an ice-salt mixture. After saturation the flask was kept in the freezing mixture for 24 hours, with the hope of getting a crystalline product to separate, but without success. The dark brown solution resulting was evaporated to dryness at about 50°, the residue taken up in ether and shaken free of hydrochloric acid with water. The ethereal solution was then dried with calcium chloride and evaporated to dryness, leaving a brown amorphous powder which was subjected to analysis.

	Gram salt.	Gram AgCl.	%Cl.	Gram. CO_2.	Gram. H_2O.	% C.	% H.	Cl.
Lot 1 (*a*)....	0.2254	0.1225	13.44	...	....	...	....	....
Lot 1 (*b*)....	0.2355	0.1292	13.54	...	....	...	....	....
Lot 2 (*c*)....	0.2248	0.1212	13.31	...	....	...	....	....
Lot 1 (*d*)....	0.1847	....	...	0.4705	0.1246	69.47	(7.55)	....
Lot 2 (*e*)....	0.2269	....	...	0.5682	0.1688	(68.30)	8.33	....
Lot 3 (*f*)....	0.2346	....	...	0.5986	0.1691	69.59	8.07	....
Lot 3 (*g*)....	0.2534	....	...	0.6474	0.1917	69.68	8.55	....
					Mean,	69.51	8.29	
Calc. for $C_{31}H_{42}O_3.2HCl$..........						69.50	8.29	13.25

The amine hydrochloride of cicutoxin was prepared by saturating the alcoholic solution with dry ammonia gas and heating to 165° in a sealed bomb for two hours. The resulting solution was treated with an excess of hydrochloric acid, evaporated to dryness and the residue taken up in ether, washed with water and the substance precipitated from a mixture of low-boiling petrolic ether and carbon tetrachloride (three to one, by volume). The resulting brown product was washed, dried and subjected to analysis with the following results:

0.1985 g. subs. gave 0.1061 g. AgCl which is 13.22% Cl.

0.4487 g. subs. gave by Dumas' method, 19.6 cc. N at 26° and 646.2 mm. pressure, equivalent to 4.07% N.

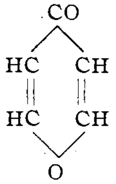
(*a*) 0.1642 g. subs. gave 0.3952 g. CO_2 and 0.1000 g. H_2O equals C 65.64 and H 6.82.

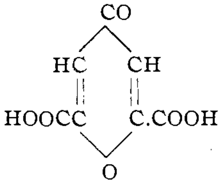
(*b*) 0.1871 g. subs. gave 0.4519 g. CO_2 and 0.1134 g. H_2O equals C 65.87 and H 6.78.

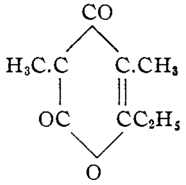
Calc. for $C_{59}H_{73}O_7N_3Cl_4$: C 65.71%, H 6.83%, N 3.90%, Cl 13.16%.

Structure of the Molecule.—Recognizing that no constitutional formula can be ascribed with certainty to a complex molecule upon purely analytical data, yet when a certain familiarity with a substance is gained, combined with a knowledge of a large number of transformations, it would seem justifiable to incorporate that knowledge into an expression best explaining the facts known. Besides the facts recorded in this paper the author has made countless observations upon cicutoxin and its chemical behavior. Four-fifths or more of the experiments undertaken with the substance have been negative or inconclusive and therefore omitted from this discussion. Each observation, however, whether positive or negative, has contributed to its knowledge.

More and more as the facts accumulated the author was led to believe that in the molecule of cicutoxin he had a pyrone nucleus to deal with. γ-Pyrone has the constitution shown in (I), in which one or more hydrogen atoms may be substituted by an element or group, retaining the double linkage between the two pairs of carbon atoms as in chelidonic acid (II). One side of the molecule may be saturated by another pair of elements or groups as in α-ethyl-β,β′-dimethylpyronone (III), which was synthesized by Wedekind.[1]


(I).


(II).


(III).

According to the records of Brühl,[2] Willstätter and Pummerer,[3] Hantzsch and Denstorff,[4] Feist and Baum,[5] Staudinger,[6] Ruhemann[7] and others, concerning pyrone and its derivatives, a marked similarity in properties exists between this class of substances and cicutoxin. Both classes polymerize and change color in the air and especially when heated, both oxidize and yield oxalic acid quite readily, both are unsaturated and have indefinite basicity, both have carbonyl oxygen without yielding an oxime or phenylhydrazone, both add halogen acids and salts, but not free halogens, both substitute halogens for hydrogen, their iodine substitution products being very stable, with high melting points. Both classes form colored salts with certain basic elements and both substitute oxygen for the nitrogen in ammonia. These, together with some other minor similarities, have led the author to believe that cicutoxin is a derivative of γ-pyrone.

The reason for not being able to isolate and determine this constituent may be found in the fact that unstable groups are attached to the pyrone nucleus, which break up and effect immediate polymerization when the molecule is subjected to higher temperatures or chemical treatment. This explanation becomes more plausible when one considers the character

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[1] *Ann.*, 378, 261 (1911).

[2] *Ber.*, 24, 2450 (1891).

[3] *Ibid.*, 37, 3740 (1904).

[4] *Ibid.*, 40, 241 (1907).

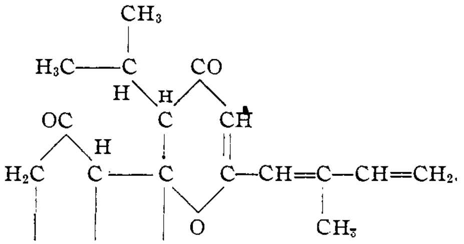
[5] *Ibid.*, 38, 3562 (1905).

[6] *Ann.*, 380, 288 (1911); 384, 62 (1911).

[7] *J. Chem. Soc.*, 75, 415 (1899).

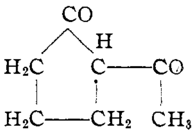
of these groups and the extraordinary stability of the polymerization product "K."

We then have the following building material out of which to construct the graphic molecule: a pyrone nucleus, an acetyl-2-cyclopentanone group, an isobutyl group, which when oxidized away would yield either isobutyric aldehyde or isobutyric acid. In case the former resulted it would, according to Taipale,[1] be oxidized to isobutyric acid by the hydrocyanic acid shown to be present. Besides these, an unsaturated and unstable group must be introduced on purely empirical grounds, which is chosen only after careful consideration of its properties and the transformations it is likely to occasion. In view, therefore, of the facts adduced, the author feels justified to submit the following structural formula for the cicutoxin molecule (IV),



(IV).

This expression corresponds to the empirical formula $C_{19}H_{26}O_3$ obtained by analysis. The lower left hand radical is cyclopentanone containing a labile hydrogen atom, making it tautomeric with cyclopentanol, in which condition the formation of the acetyl product and barium salt are rendered possible. The same condition of tautomerism is presumed for the β'-hydrogen atom of the pyrone ring, shifting to the γ-position under certain conditions. The decomposition product obtained from the nitric acid oxidation was acetyl-2-cyclopentanone, shown in (V), instead of the straight pentanone, shown as a radical in (IV).



(V).

Since the pyrone molecule is far less stable than the five-membered carbon ring it is obvious that, when the molecule is split up by the nitric acid, the break should occur at the most vulnerable place. We therefore have the isobutyl group liberated and oxidized to isobutyric aldehyde or acid, the pentanone group split off, taking with it the oxygen and α'-carbon atoms of the pyrone together with the attached methyl. The

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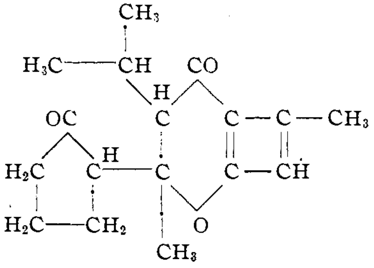
[1] *J. Russ. Phys. Chem. Ges.*, 41, 815 (1909).

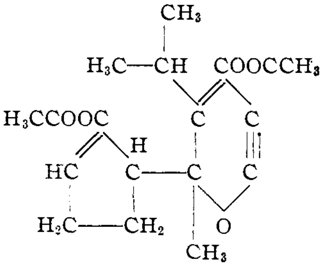
rest of the molecule is oxidized to oxalic acid and carbon dioxide, with the exception of a carbon-hydrogen group which forms hydrocyanic acid with the nitrogen from nitric acid, and one or more hydrocarbons that are undoubtedly liberated in the polymerization immediately preceding the oxidation.

The right hand isoprene group is the most unstable part of the entire molecule. Isoprene decomposes readily under certain circumstances and polymerizes under others, and these properties especially fit the conditions of the cicutoxin molecule. Suppose the isoprene radical should be split in two, liberating C_2H_4, and the free bond of the isoprene carbon unite with the β-carbon of the pyrone ring, we should get the structure represented in (VI).



(VI).

If this molecule should polymerize, which is not beyond the range of possibility, we would have a product whose composition would be that of "K," or $(C_{17}H_{22}O_3)_x$. On the other hand, if under certain conditions, particularly an acid medium, the entire isoprene group were to be removed and acetyl groups substituted for the two labile hydrogens, we should get the form represented in (VII), which has the same composition as the acetyl product isolated from cicutoxin.[1]



(VII).

The iodine product obtained may be represented by the same formula,

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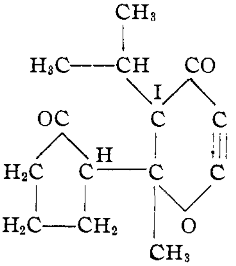
[1] NOTE.—An objection to this formula may be made on the ground that the introduction of a triple bond in the pyrone nucleus is unprecedented, but it is nevertheless within the range of possibility. It has already been noted that, although pyrone contains double bonds, it does not add halogen, and this property is apparently not changed by the introduction of a triple bond.

one iodine being substituted for the β′-hydrogen in the pyrone ring and the isoprene group eliminated as shown in (VIII).



(VIII).

The lead salt of cicutoxin may be considered as having been formed like the neutral salt combinations with pyrone itself in which oxonium compounds have resulted. In this case the PbO and OH groups from the basic lead acetate are in the same manner supposed to satisfy the quadrivalent oxygen of the pyrone nucleus making the formula for the lead salt $C_{19}H_{26}O_8$.PbO.OH. Descriptions of somewhat similar organic lead oxide combinations may be found in the literature,[1] but too much credence should not be placed on either the lead or the barium salt for the reason that their purification could not be accomplished satisfactorily. The barium salt is not supposed to be an oxonium compound, like the lead salt and the hydrochloride, but a cyclopentanolate, obtained by the substitution of the cyclopentanole hydrogen in two molecules of cicutoxin. No doubt other alkali and alkaline earth salts of the same composition are formed although their isolation proved unsuccessful.

It has already been pointed out that in a mineral acid medium cicutoxin easily decomposes, with a recombination or polymerization of certain parts resulting from the decomposition. Consequently, it was not to be expected that a straight hydrochloride or amine hydrochloride of the substance could be made. However, the hydrochloride obtained bears a simple relation to certain decomposition products obtained from cicutoxin. The product "K," obtained from a mineral acid medium, is considered some multiple of $C_{17}H_{22}O_3$. If this group were to combine with the oil obtained by steam distillation ($C_{14}H_{22}O$), with the elimination of H_2O, and if this compound molecule should add two molecules of hydrochloric acid, we would get a compound corresponding to the analytical values obtained for the hydrochloride made from cicutoxin.

Not so simple a relation as this is disclosed by the composition of the amine hydrochloride, but the analytical values for this compound have been included without attempting to show what the relation is. It is

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[1] *Jahresber. Chem.*, **16**, 566 (1863); *Ann.*, **255**, 195 (1890); *Deutsche Zucker Industrie*, **12**, 1367.

not improbable that this product is the hydrochloride of a modified imino cicutoxin corresponding to pyridone which is obtained from pyrone with ammonia, or oxypicolinic acid obtained from comanic acid.

It is interesting to note that chelidonic acid, a simple derivative of pyrone, has been isolated from the roots of the poisonous veratrum or white hellebore belonging to the lily family, a plant also growing in swamps and marshy ground.

Toxicological.[1]

A fairly concordant literature is found on the physiological effects of this poison. With the exception of the frog, the symptoms are very similar in all the lower animals, as well as man. Cicutoxin may be classed as a narcotic cramp poison which attacks certain brain centers.

Three well-defined stages of symptoms are recognized in cicuta poisoning, namely, a prodromal, a paroxysmal and a paralytic stage. The first represents the time prior to the beginning of visible symptoms, and is usually not of more than forty minutes' duration.

When the poison is administered intravenously the animal at once passes into the second or paroxysmal stage, beginning with a strong salivation and tremors. The animal is then seized by convulsions at irregular intervals, characterized by violent chewing and frothing at the mouth. The tonic and clonic spasms, into which the animal is thrown, render it unconscious and irresponsive to stimulation. There is also dilation of the pupils, a more rapid and shallow respiration, as well as an increase in body temperature. This stage usually lasts from twenty minutes to six or eight hours.

The paralytic or final stage represents the time when the animal has relapsed into total paralysis and is no further responsive to stimulation. This stage is only of a few minutes' duration.

Wikszemski[2] has shown that cicutoxin attacks the cramp center of the brain, located at the base of the fourth ventricle. It also affects the pneumogastric centre so that a small dose stimulates and a larger one paralyzes the action of the lungs and heart. The animal dies from asphyxiation and exhaustion.

In regard to the toxicity of the poison it has been found that a root the size of a walnut is sufficient to kill a cow. 150–200 mg. cicutoxin kills the average rabbit and 200 mg. the average cat. Boehm[2] found that, per kilo body weight of dogs, 110 mg. per mouth and 21 mg. intravenously, was sufficient to kill; and that 50 mg. per kilo body weight of cats, when administered per mouth, was found sufficient to kill. From other in-

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[1] A detailed account of the botanical and toxicological investigations of water hemlock will be published in *Bulletin* 81 of The Nevada Agricultural Experiment Station.

[2] *Loc. cit.*

vestigations, however, it would appear that the lethal dose of this poison cannot be correctly given in terms of milligrams per kilo body weight.

No abnormalities whatsoever are noticed after death, with the exception of a pronounced venous hyperemia of the brain and lungs, and in certain cases, a reddening of the inside lining of the stomach.

No antidote for cicutoxin is known. The most successful treatment, in human cases of poisoning, consists in producing vomiting and moderating the effects of the convulsions by means of a narcotic, such as morphine or opium.

The author wishes to acknowledge the assistance of Mr. August Holmes during the major portion of this work.

Conclusion.

Water hemlock is an umbelliferous, poisonous plant, growing along the banks of streams and in marshy ground. It is recognized under at least three distinct species in this country, all three containing the same poisonous principle, cicutoxin, which is located primarily in the rhizone or rootstalk of the plant.

Cicutoxin is an unstable resin-like substance of the formula $C_{19}H_{26}O_3$, and is a complex derivative of pyrone. A tentative structural formula for the cicutoxin molecule has been proposed. It decomposes and polymerizes readily, especially at temperatures above 50°. It is extracted from the tubers by means of ether and enters violently into combination with free bromine.

It forms combinations with lead, barium, hydrochloric acid, ammonia, and yields the double acetyl derivative.

A reliable chemical test for its presence has been found.

Cicutoxin is a spasmotoxin, producing symptoms that may be separated into a prodromal, a paroxysmal and a paralytic stage. Death ordinarily results in from thirty minutes to eight hours. The lethal dose of cicutoxin for the average rabbit is 175 mg., and "50 mg. per kilo body weight" for cats, when administered per mouth. Cicutoxin attacks a nerve center in the calamus scriptorius and kills by asphyxiation and exhaustion.

It is not a constitutional poison and the lethal dose cannot properly be given in terms of milligrams per kilo body weight. No antidote is known for this poison, and the most reliable treatment at present consists in producing vomiting and allaying the convulsions by means of a narcotic.

RENO, NEV.

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[1] NOTE.—In order to make the knowledge of this plant as complete as possible, any information regarding its character, distribution and harmfulness will be gratefully received by the author and duly acknowledged in a subsequent paper or bulletin.