3. The use of 50% sulphuric acid as the solvent for dimethylaminobenzaldehyde and of a Watkins' extractor in making the first ether extraction have been cited as advantages.

4. A better blue color is obtained if the solution of dimethylaminobenzaldehyde and alkaloid is not heated.

The author wishes to express his appreciation to Mr. E. J. Hughes for his friendly criticism.

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LACTUCARIA. I. THE MYDRIATIC ACTIVITY OF LACTUCARIA BY THE MUNCH METHOD.*[1]

BY JAMES C. MUNCH, HARRY J. PRATT AND GEORGE E. BYERS.

Lactucarium is the "dried milk-juice of *lactuca virosa* Linné (Fam. *Compositæ*)" (34). It was official from the First U. S. P. in 1820 to the Ninth of 1916, but was dropped from the recognized drugs in U. S. P. X (24). It has been used in homeopathic medicine in which it is described as the concrete juice of *L. virosa*, *L. sativa*, *L. scariola* and *L. altissima* (1, 20).

If lactucarium were capable of producing the effects attributed to it, it would be very miraculous. Descriptions in Folklore and in early scientific papers affirmed that it was a sedative, resembled opium in its effect, could be used as a substitute for opium, and later still that it contained hyoscyamine or some of the other mydriatic alkaloids (2, 3, 4, 8, 9, 10, 11, 12, 18, 22, 23, 25, 28, 29, 30, 31, 32, 35, 36, 37).

Apparently, the introduction of lactucarium as the dried juice of *L. virosa* was due to some inconclusive experiments by John Redman Coxe, presented at the meeting of the American Philosophical Society in 1797, at Philadelphia. Comparing the effects with those of opium, he decided that the two were similar in action, and called the material "lettuce opium." A careful scrutiny of his report fails to show any basis for this startling deduction (8, 9). Work by Duncan, Sr., in Edinburgh at about the same time appears to be responsible for the introduction of this product into the Dublin and Edinburgh Pharmacopœias (11).

To prepare lactucarium, various species of *lactuca* appear to have been used (*virosa*, *scariola*, *sativa*, *canadensis*, *septiva* and *altissima*, being most frequently reported (1, 2, 6, 7, 8, 12, 14, 16, 19, 20, 21)).

The juice is collected by cutting off the top of the lettuce plant in June, when it is just ready to blossom. The latex is collected daily. As needed, new incisions are made in the stalk. The combined latex is dried, forming irregular brown lumps of a narcotic odor and bitter taste (6, 24, 37).

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[1] Joint communication from the Department of Research, School of Pharmacy, Temple University, and Department of Pharmacology, Sharp and Dohme, Philadelphia, Pa.

A number of active principles have been reported, most of them proving to be impure mixtures on further study. No agreement has been found regarding one specific principle to which the activity may be ascribed. Various extractives have been called lactucin, lactopicrin, lactucic acid, lactucerin, lactucone, lactucerol. In addition, oxalic acid, mannite and other materials have been observed (2, 5, 6, 7, 13, 14, 15, 16, 18, 22, 25, 28, 31, 35, 37, 38).

Adulteration of lactucarium has been reported with bread crumbs (22), gum opium and other materials of similar appearance.

The popular belief has been encountered in various parts of the world that the consumption of lettuce produces a sedative or soporific effect. It is impossible to determine exactly what products were used, but it would appear that many of the earlier reports alleging narcotic activity were conducted on material which was not chemically or pharmacognostically identified. Hirschfeld (19) in 1833 reported observations on sparrows, other birds and rabbits, as well as a few tests on man, concluding that the principal narcotic substance was volatile and recommended the use of lactucarium for various convulsions, coughs and spasms. Many of the older encyclopedias and books refer to the hypnotic or sleep-producing effect and this has been occasionally reprinted without confirmation in recent texts (1, 3, 11, 29, 30, 31, 32, 36, 37).

Cushny states that $^1/_2$ ounce failed to cause any effects on a dog. Wood and Bache were unable to produce any results with doses of 10 to 20 grains or more. Sollmann states that the reputation of lettuce as a hypnotic is probably undeserved as Kelterborn took 12 Gm. of lactucarium without any effect (10, 30, 37).

A commercial mixture has been found containing 0.5 mg. of morphine sulphate with 60 mg. of lactucarium and other constituents: this type of product may be the type which has been reported to be effective (33).

So far as the mydriatic activity is concerned, the earliest reference found was Ludwig, who suggests that "Giftlattick" should be capable of constricting the pupil. This served as the basis of an investigation by Gerber in 1863: applied externally or internally lactucarium did not influence the diameter of pupils of rabbits (17).

Dymond (13) made a chemical examination of an extract of *L. virosa* and obtained a very small amount of a substance which he identified by chemical properties as hyoscyamine. Braithwaite and Stevenson (5) collected some French flowering *L. virosa* in Essex, England, which was crushed, extracted with dilute hydrochloric acid, then the aqueous solution shaken out with ether, made alkaline with ammonia and extracted with ether again. The residue was dissolved in a trace of hydrochloric acid and failed to cause mydriasis in four humans on whom it was tested. Four hundred grams of fresh plant were taken and an extract obtained in about 1 cc.

This work was repeated by Farr and Wright, using 1 kilo of fresh herb obtained from Braithwaite; the herb was dried and extracted with alcohol and acid, the filtrate concentrated, made alkaline with ammonia and extracted with chloroform. About 0.6 mg. of material was obtained, which gave alkaloidal reactions with Mayer's and Thresh's reagents. Applied to the pupils of two humans, powerful mydriatic effects were obtained. In a discussion of this paper (15) Ransom stated that he had obtained 0.015 per cent of a chloroform extract from lactucarium, which was mydriatic. It appeared strange, however, that ether extraction by Braith-

waite and Stevenson had not obtained any mydriatic alkaloid. Wright subsequently (38) repeated this procedure on *lactuca muralis*, finding negative results in a general examination and only minute quantities of alkaloid in a more intensive study. These ranged from 0.06 per thousand in the leaves to 0.15 per thousand in the root.

In order to obtain more definite information regarding the presence of active alkaloids in lactucarium, a series of investigations were initiated. This report deals with the studies made to determine the mydriatic activities of lactucaria.

EXPERIMENTAL PROCEDURE.

A number of adult cats were confined in individual cages. They were handled daily in order to develop their friendship and to facilitate ease in use. The thresholds of each cat were determined for known solutions of atropine, hyoscyamine, hyoscine and in most instances for physostigmine. The technic previously developed by Munch (26), (27) was used: exactly 0.05 cc. of solution was placed on the cornea of a cat, the inner canthus compressed and the lids opened and closed over a period of thirty to sixty seconds, until absorption was apparently complete. Just before this application, and at half-hour intervals over a period of three hours, the pupils were inspected to note any differences in diameter which might develop between treated and untreated eyes. Each cat was placed one foot from a bright light and the degree of pupillary contraction determined. By means of a transparent celluloid scale the pupillary diameters were easily measured. A satisfactory degree of effect was attained when the treated pupil differed by 0.5 mm. from the untreated pupil.

In preparing lactucarium two procedures were used. In one, the method outlined in the Pharmacopœia was followed (grinding with sand, etc.). Similar tests were conducted on the same samples by acidifying and boiling lactucarium with alcohol, under the belief that the mydriatic alkaloids, if any, would dissolve in this solvent. Four samples of lactucarium were studied.

In addition, three different samples of lettuce were purchased on the open market, dried in an incubator at about 37° C., ground and percolated to make a fluidextract. Approximately 70 per cent alcohol was used as the menstruum.

In a few experiments the alcoholic solutions were used as such, although in general the alcohol was removed and the volume made up with distilled water to the original quantity.

Tests were conducted on four samples of lactucarium and three samples of lettuce: no extracts ever showed any mydriatic potency.

CONCLUSION.

1. No evidence of mydriatic action was found in testing four samples of lactucarium and three samples of lettuce.

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THE GRAVIMETRIC AND VOLUMETRIC DETERMINATION OF ANTIPYRINE AS HYDROFERROCYANIDE IN THE PRESENCE OF AMIDOPYRINE.*[1]

BY I. M. KOLTHOFF.

The simple iodometric determination[2] of antipyrine cannot be applied in the presence of amidopyrine, since the latter substance is oxidized by iodine. In the present work it has been found that antipyrine yields a crystalline precipitate with potassium ferrocyanide in acid medium whereas amidopyrine does not react under similar conditions. Use of this precipitation reaction has been made in the quantitative determination of antipyrine; the method can be applied in the presence of amidopyrine.

Composition of the Hydroferrocyanide of Antipyrine.—The crystalline precipitate formed in an acid medium of hydrochloric acid and containing an excess of potassium ferrocyanide was collected, washed with water, alcohol and ether, and air dried. On heating in vacuum at 70° no loss in weight was noticed. It should be mentioned that the air-dry precipitate obtained after washing with alcohol (no ether) contains 0.3 to 0.4% of water. The hydroferrocyanide content of the precipitate was determined by titrating 0.1000-Gm. samples with sodium hydroxide using phenolphthalein as an indicator. The hydroferrocyanic acid behaves as a quadrivalent acid; the antipyrine, being a very weak base, does not affect the titration. It was found that 0.1 Gm. required 12.78, 12.75, 12.72 cc., 0.0529*N* sodium hydroxide, respectively, corresponding to a molecular weight of the precipitate of 592.1. From the above it may be concluded that the crystals consist of a compound of 2 molecules antipyrine and 1 molecule hydroferrocyanic acid:

$$(C_{11}H_{12}N_2O)_2H_4Fe(CN)_6 . M = 592.1$$

The composition is different from that of the hydroferrocyanides of most alkaloids which ordinarily yield precipitates containing water of crystallization and one molecule of alkaloid per one molecule of hydroferrocyanic acid.

Sensitivity of the Precipitation of Antipyrine.—The sensitivity depends upon the concentration of ferrocyanide and the acidity of the mixture. In the following experiments the solution was acidified with hydrochloric acid. It appeared advantageous to have a large excess of potassium ferrocyanide. After some systematic experiments, the following procedure was adopted: 2 cc. 0.5 molar potassium ferrocyanide were added to 5 cc. antipyrine solution, the latter containing the concentration of hydrochloric acid as given in Table I.

The optimum acidity is 0.5 to 0.75*N* of hydrochloric acid. 2 mg. antipyrine can be detected if 2 cc. 0.5-molar potassium ferrocyanide and 0.5 cc. 6*N* hydro-

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* Scientific Section, A. PH. A., Madison meeting, 1933.

[1] Contribution from the School of Chemistry of the University of Minnesota.

[2] Comp. I. M. Kolthoff, "Volumetric Analysis," Vol. 2, page 454, translated by N. H. Furman, John Wiley & Sons, New York, 1929.