

A CHEMICAL EXAMINATION  
OF  
THE ROOTS OF PLANTAGO MAJOR

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## TABLE OF CONTENTS

- I Introduction
  - A. Material Used
    - 1. Botanical Description
    - 2. Collection and Preparation
- II Preliminary Extraction with Selective Solvents
  - A. Petroleum Ether
    - 1. Extract Obtained
    - 2. Description
    - 3. Quantity and Percentage
  - B. Ether
    - 1. Same as A.
  - C. Alcohol
    - 1. Same as A.
    - 2. Water Soluble Portion
    - 3. Sugar Content
  - D. Tabulation of Extraction
- III Extraction of Material with Alcohol
  - A. Extract Obtained
    - 1. Weight and Percentage
    - 2. Acid Value
    - 3. Saponification Value
  - B. Extraction of Alcoholic Extract with Petroleum Ether
    - 1. Petroleum Ether Extract
      - a) Acid Value
      - b) Saponification Value

## TABLE OF CONTENTS(Cont)

- 2. Residue After Extraction
    - a) Same as B-1
  - C. Extraction of Alcoholic Extract with Acetone
    - 1. Acetone Extract
      - a) Same as B-1
      - b) Solubility and Melting Point
    - 2. Residue
      - a) Acid Value
      - b) Saponification Value
      - c) Unsaponifiable Matter
  - D. Tabulation of Extractions
- IV Bibliography

A CHEMICAL EXAMINATION OF THE ROOTS  
OF PLANTAGO MAJOR

Material Used:-

The material used in this investigation was the roots of *Plantago major* Linné gathered within the city of Madison.

The following botanical description of this plant is quoted from Britton and Brown - Illustrated Flora of the Northern States and Canada.

Plantago major Linné (Family Plantaginaceae)

Perennial, glabrous or somewhat pubescent; rootstock short, thick, erect, scapes longer than the leaves. Leaves spreading or ascending long-petioled, mostly ovate, obtuse or acutish, narrowed, rounded or cordate at the base, entire, or coarsely dentate, 1' to 10' long, 3 to 11 ribbed; scapes 2' to 3' high; spike linear-cylindric, usually very dense, commonly blunt at the summit, 2' to 10' long, 3" to 4" thick; flowers perfect, proterogynous; sepals broadly ovate or obovate, scarious on margins, one-half to two-thirds as long as the ovoid obtuse or subacute, 5 to 16 seeded pyris, which is circumscissile at about the middle; corolla-lobes spreading or reflexed at the summit of the pyris; stamens 4.

Another description is quoted from Gray's Lesson's and Manual of Botany - 6th Edition, page 423.

Plantago major, Linné (Common Plantain)

Smooth or rather hairy, rarely roughish; leaves ovate,

oblong, oval, or slightly heart-shaped, often toothed abruptly narrowed into a channelled petiole; spike dense, obtuse; sepals round-ovate or obovate; capsule ovoid, circumscissile near the middle, 8 to 18 seeded; seed angled, reticulated.

According to the above authors:

The ecological habitat of *Plantago major* Linné is in waste places, waysides near dwellings, nearly everywhere throughout North America.

Naturalized from Europe, or more likely indigenous in the far north and on salt meadows; native from Lake Superior and North Minnesota, northward. Small leaves are occasionally borne near the bases of the spikes, and the spikes are rarely branched. The salt meadow plant may be distinct from May to September.

Old Names and Synonyms - Bird seed, Broad-leaf, Hen plant, Lamb's-foot, Waybread, Healing blade, Door-yard Plantain, Common Plantain, and Greater Plantain.

#### Collection and Preparation:-

The roots for this investigation were gathered in the summer and fall of 1935 by Royal Beaudry, who was engaged in a study of the leaves of *Plantago major* Linné. The material was the common door-yard plantain with leaves not more than 12 inches long all growing at the base of the spike. After drying for several months in dry air the roots were placed in a mill and ground to a very fine powder. This powder

3.

was grayish-brown in color and was quite soft to the touch.  
The total weight of the powder obtained upon grinding was  
around 700 Gms.

### Preliminary Extraction with Selective Solvents

After the material had been ground to the proper fineness preliminary extractions of the soluble constituents of the roots were made by means of selective solvents using 50 Gms. of the powder. This material was placed in an extraction apparatus of the Soxhlet type set up for continuous percolation and, using 150 cc of petroleum ether, was extracted for 12 hours after which another 50 cc of petroleum ether was added and the extraction continued for another 12 hours. The petroleum ether was then allowed to evaporate at room temperature and a pasty, black extract was obtained. On drying in an oven at 100°C for 12 hours the extract was somewhat more firm although still possessing its pasty properties. This extract was insoluble in cold water and very slightly soluble in alcohol.

The weight of this extract on drying in air was 0.3280 Gm or 0.656% of the total material used. After drying in an oven at 100°C for 12 hours the weight of the extract was reduced to 0.2194 Gms or 0.439% of the total material used.

Next the powder in the percolator was extracted with ether following the same procedure as mentioned under the petroleum ether extraction. The extract obtained was more firm in consistency than the petroleum ether extract but was still dark in color. It was somewhat soluble in alcohol.

The weight of this extract after drying in an oven was 0.1013 Gm. or 0.203% of the total material.

On further extraction alcohol was used as the solvent following the same procedure as mentioned before. This extract was of a very pasty consistency, black in color and slightly soluble in cold water. The weight of the extract on drying in an oven was 6.1932 Gms. or 12.386% of the total material.

Of the three solvents used in these preliminary extractions it was observed that alcohol removed a much greater amount of the constituents present in the roots than either petroleum ether or ether.

The alcoholic extract was then heated on a water bath with 165 cc of water for 30 minutes. The mixture was filtered and the insoluble residue weighed.

Wt. of Alcoholic Extract.....6.1932 Gms.

Wt. of Insoluble Residue.....4.3914 Gms.

Wt. of Portion Soluble in Water...1.8018 Gms.

Therefore 1.8018 Gms. of extract were soluble in water, 29.09% of the total alcoholic extract.

Test with Fehling's Solution:

The aqueous solution obtained above was then tested for its reducing sugar content. To reduce 5 cc of Fehling's Solution 91.8 cc of this aqueous solution were required.

Calculation:-

5 cc Fehling's Solution ~~=====~~ 91.8 cc of Aqueous Solution  
 1 cc of Aqueous Solution equals  
 $1.8018 \div 165$  equals 0.01092 Gms. of Alcoholic Extract

5 cc Fehling's Solution        0.025 Gm. Reducing  
Sugar

Then:

$0.025 \div 91.8$  equals 0.000272 Amount of Reducing  
Sugar present in  
each cc of solution

And:

$0.000272 \div 0.01092$  equals .0249 or 2.49% Redu-  
cing Sugar in Alco-  
holic Extract

Tabulation of Extraction with Selective Solvents

	<u>WEIGHT</u>	<u>PERCENT</u>
Material.....	50 Gms.	
Solvents:		
1. Petroleum Ether....(Air)	0.3280 Gm.	0.656%
	(100 C)0.2194 Gm.	0.439%
2. Ether.....(100 C)	0.1013 Gm.	0.203%
3. Alcohol.....(100 C)	6.1932 Gms.	12.386%
a) Solubility in water	1.8018 Gms.	29.090%
b) Reducing Sugar.....		2.490%

### Extraction of Material with Alcohol

After having made the preliminary extractions with selective solvents the remaining portion of the material was extracted to exhaustion with alcohol, using an apparatus arranged for continuous percolation. The weight of the material used was 618 Gms. which was evenly packed in the percolator. This was extracted with 500 cc of alcohol for 24 hours at the end of which time another 300 cc of alcohol were added and the percolation was continued for another 12 hours. The extraction was not complete at the end of this time so another 200 cc of alcohol were added and the percolation was continued for another 12 hours. The extract was then poured into a 6 inch evaporating dish and allowed to dry in the air. This extract weighed 152.7 Gms. but was reduced to 142.8 Gms. on drying in an oven at 100°C. The appearance of the extract was much like that obtained in the preliminary extractions although much less firm in consistency and probably slightly darker in color. The extract obtained by this operation was 21.49% of the crude material.

An acid number of this material was made according to the methods of the U.S.P.X using 2.8816 Gms. of the extract. To neutralize the free acid, 7 cc of 0.466 N/I KOH were required.

1 cc NKOH equals 56.1 mg. KOH  
 1 cc 0.466 KOH equals 26.1717 mg. KOH  
 26.1717 x 7 equals 183.2019  
 183.2019 ÷ 2.8816 equals 63.5764 (Acid Number)

Another small accurately weighed portion of the alcoholic extract was used to make a saponification value determination according to the U.S.P.X method. The weight of the material used was 1.5830 Gms. which was placed in a 500 cc flask and mixed with exactly 25 cc of 0.466 N/l.KOH. This mixture was then heated on a steam bath, using a reflux condenser, for one-half hour, during which time a blank test was also being made. The excess KOH was titrated with N/2 HCL. The difference in the number of cc's of N/2 HCL consumed in the actual test and in the blank test was determined.

1.5830 Gms. Extract required 13.8 cc 0.532 N/l HCL  
to neutralize  
Blank Test - Required 21.1 cc 0.532 N/l HCL

Then: 21.1 x .53196 equals 11.324 cc NHCL  
13.8 x .53196 equals 7.341 cc NHCL  
11.324 - 7.341 equals 3.983 cc NHCL

Therefore:  
3.983 x .0561 x 1000 equals 223.446  
223.446 ÷ 1.583 equals 141.154  
(saponification  
value)

#### Extraction of Alcoholic Extract with Petroleum Ether

The next step was to extract the alcoholic extract with petroleum ether. This was done by adding four 100 cc portions of the solvent to the extract, mixing and pressing the extract thoroughly with the petroleum ether. The weight of the alcoholic extract to begin with was 135.48 Gms. and upon extraction 4.89 Gms. of the substance were removed by the petroleum ether.

## Percentage Calculations:

4.89  $\div$  135.48 equals 0.0361 or 3.61% (of Alcoholic  
Extract Soluble in  
Petroleum Ether)

Then:

142.83 x .0361 equals 5.156 Gms. would have been ex-  
tracted by petroleum ether  
if the total weight of al-  
coholic extract had been  
used.

And:

5.156  $\div$  618 equals 0.00834 or 0.834% Petroleum ether  
Extract in crude  
drug.

This result is somewhat higher than that obtained by  
the method of selective solvents.

An acid number of the petroleum ether extract was de-  
termined using the same method as given before. The follow-  
ing results were obtained:

1.7474 Gms. of material required 1.7 cc 0.466 N/1 KOH  
to neutralize the free acid.  
1 cc NKOH equals 56.1 mg. KOH  
1 cc 0.466 N/1 KOH equals 26.1717 mg. KOH

Then:

26.1717 x 1.7 equals 44.49089

44.49089  $\div$  1.7474 equals 25.4612 (Acid Number)

Upon another portion of the petroleum ether extract  
the saponification value was determined using the same  
method as for the alcoholic extract. The following results  
were obtained:

1.3626 Gms. of material plus 25 cc KOH required 14.9  
cc of 0.532 N/1 HCL to neutralize the free  
fatty acid and saponify the ester.  
Blank Test (25 cc KOH) equals 21.1 cc 0.532 N/1 HCL

Then:

21.1 x 0.532 equals 11.324 cc NHCL

14.9 x 0.532 equals 7.926 cc NHCL

The difference is 3.398 cc NHCL

Then:

3.398 cc NHCL ~~=====~~ 3.398 N KOH

And:  $3.398 \times 0.0561 \times 1000$  equals 196.6278  
 $196.6278 \div 1.3626$  equals 139.8266 (Saponification Value)

Upon the residue left after extracting the alcoholic extract with petroleum ether similar acid and saponification values were determined with the following results:

#### Acid Number

2.1320 Gms. of material required 3.2 cc of 0.466 N/1 KOH to neutralize free acid.

1 cc N KOH equals 56.1 mg. KOH

1 cc 0.466 N/1 KOH equals 26.1717 mg. KOH

Then:

$26.1717 \times 3.2$  equals 89.74944

$89.75 \div 2.132$  equals 39.2821 (Acid Number)

#### Saponification Value

1.8848 Gms. of material plus 25 cc KOH required 10.1 cc 0.532 N/1 HCL to neutralize free fatty acid and saponify the ester.

Blank Test (25 cc KOH) equals 21.1 cc 0.532 N/1 HCL

Then:

$21.1 \times 0.532$  equals 11.324 cc NHCL

$10.1 \text{ cc} \times 0.532$  equals 5.373 cc NHCL

The difference is 5.951 cc NHCL

5.951 cc NHCL ~~=====~~ 5.951 cc N KOH

And:

$5.951 \times 0.0561 \times 1000$  equals 333.8511

$333.8511 \div 1.8848$  equals 177.1281 (Saponification Value)

The residue which remained after extraction with petroleum ether was then extracted with acetone. This was done by adding 100 cc portions of acetone to the material and by using a spatula, mixing the two thoroughly. To completely extract all the soluble material 600 cc of acetone, in several small portions, were required. The weight of the residue left after extraction with petroleum ether was 114.8637 Gms. Upon extraction with acetone 15.1766 Gms. of substance were found to be soluble in this solvent. The acetone extract was greenish-black in color and was very pasty in consistency. The residue became much more firm but still possessed its dark color.

Percentage:

$15.1766 \div 114.8637$  equals 0.1321 or 13.21% of Petroleum ether residue soluble in acetone.

Original weight of alcoholic extract - 142.8256 Gms.

Then:

$142.8256 \times 0.1321$  equals 18.8672 Gms. of total alcoholic extract would have been soluble in acetone.

And:

$18.8672 \div 618$  equals .0305 or 3.05% of acetone soluble constituents in total crude material.

An acid number and saponification value determination were made upon the acetone extract. The following results were obtained:

Acid Number

1.9837 Gms. of extract required 2.9 cc of 0.466  
N/1 KOH to neutralize the free acid.

1 cc NKOH equals 56.1 mg. KOH

1 cc 0.466 N/1 KOH equals 26.1717 mg. KOH

Then:

26.1717 x 2.9 equals 75.89793

75.89793  $\div$  1.9837 equals 38.2708 (Acid Number)

Saponification Value

1.894 Gms. of material plus 75 cc KOH required  
59.7 cc of 0.532 N/1 HCL to neutralize the free fatty acid and saponify the ester.

Blank Test (75 cc KOH) equals 63.3 cc 0.532 N/1 HCL

Then:

63.3 x 0.532 equals 33.673 cc NHCL

59.7 x 0.532 equals 31.758 cc NHCL

The difference is 1.915 cc NHCL

And:

1.915 cc NHCL ~~=====~~ 1.915 cc NKOH

1.915 x 0.0561 x 1000 equals 107.4315

107.4315  $\div$  1.894 equals 56.7222 (Saponification  
Value)

Small portions of the acetone extract were then tested for its solubility placing it in test tubes and treating with various solvents. The following results were observed:

<u>Solvent</u>	<u>Solubility</u>
Alcohol.....	Partly Soluble
Methyl Alcohol.....	More Soluble
Chloroform.....	Insoluble
Ether.....	Slightly Soluble

The coloring matter, largely chlorophyll, which was present in the acetone extract was more completely removed by methyl alcohol than any of the other solvents used. The remaining acetone extract was then treated with 50 cc of methyl alcohol removing the greenish colored matter present in the material. The precipitate collected was light grayish-brown in color. After drying, this precipitate still contained slight traces of oil as was detected from its appearance. The melting point of this material was 79°C and when recrystallized from acetone, more chlorophyll being removed, the melting point was raised to 82°C. The residue which was insoluble in acetone had a melting point of 260°C.

The residue left from the alcoholic extract after extraction with petroleum ether and acetone was much more firm than it was before either extraction. It was still slightly soluble in cold water and possessed a dark color. An acid number and saponification value determination were made as before upon this material with the following results:

Acid Number

2.0966 of material required 0.2 cc of 0.466 N/l KOH to neutralize free acid.

1 cc NKOH equals 56.1 mg. KOH

1 cc 0.466 N/l KOH equals 26.1717 mg. KOH

Then:

26.1717 x 0.2 equals 5.23434

5.23434 ÷ 2.0966 equals 2.4965 (Acid Number)

Saponification Value

2.6926 Gms. of material plus 25 cc KOH required 10.3 cc of 0.532 N/l HCL to neutralize the free fatty acid and saponify the ester.

Blank Test (25 cc KOH) equals 21.1 cc 0.532 N/l HCL

Then:

21.1 x 0.532 equals 11.324 cc NHCL

10.3 x 0.532 equals 5.479 cc NHCL

The difference is 5.845 cc NHCL

And:

5.845 cc NHCL ~~=====~~ 5.845 cc NKOH

5.845 x 0.0561 x 1000 equals 327.9045

327.9045  $\div$  2.6926 equals 121.78 (Saponification Value)

A determination of the unsaponifiable matter present in this material was also made. This determination was made according to the U.S.P.X using 11.9404 Gms. of the extract.

Material.....11.9404 Gms.

Residue..... 0.0498 Gm.

Then:

0.0498      11.9404 equals 0.00417 or 0.417% unsaponifiable matter present in the residue after extracting with petroleum ether and acetone.

TABLUATION OF THE ACID NUMBER, SAPONIFICATION VALUE  
AND UNSAPONIFIABLE MATTER DETERMINATIONS

Also Percentages Of Extract Compared  
With Crude Material

Extracts and Residues	Acid Number	Saponifica- tion Value	Unsaponifi- able Matter	Percentage Of Crude Material
Alcoholic Extract	63.5764	141.1535	Not Determined	21.49%
Petroleum Ether Extract	25.4612	139.8266	Not Determined	.834%
Residue, After Ex- traction With P.E.	39.2821	177.1281	Not Determined	----
Acetone Extract	38.2708	56.7222	Not Determined	3.05%
Residue, After Ex- traction With P.E. & Acetone	2.4965	121.7800	.417%	----

BIBLIOGRAPHY

Mauil, D. W.

1858

Plantago major

Med. and Surgical Reporter, 10,--(Proc. A. Ph. A.,  
7, p. 73).

The expressed juice of the fresh leaves of plantago major, or yard plantain, administered in doses of 3-4 ounces is said to give immediate relief in cases of bite inflicted by the venomous spider.

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1864

Plantago major

Buffalo Med. and Surg. Journ.,--,--;(A.D. Circ.,  
8, p. 105; Proc. A. Ph. A., 121, p. 107.)

The juice of the plant, given freely internally, also applied externally found useful in treating a dog bitten by a rattle snake.

Watson, R.

1878

Plantago major

Proc.A. Ph. A., 26, p. 167.

The author makes note the drug Plantago major, local name, Baibula, was used in Malta; this plant is to be found in cultivated parts during November. The lower superstitious class attribute imaginary virtues to it.

Brown, R. J.

1881

Plantago major

Proc. A. Ph. A., 29, 449

Brown in his catalogue of the Medicinal Flora of the State of Kansas gives Common plantain as a synonym of Plantago major.

Colcord, J. W.

1883

Plantago major

Proc. A. Ph. A., 31, p. 358.

The author in his experiments on the fresh plant of Plantago major expressed the juice from 3 lbs. of the leaves yielding 16 ounces of a light green colored, very turbid juice which was evidently caused by a large amount of chlorophyll.

A fluid-extract, which represents virtually an equal weight, of the dried plant, was founded by exhausting the residue with dilute alcohol, evaporating on a water bath to 2 ounces, mixing with 12 ounces expressed juice and adding 2 ounces alcohol. This preparation, dark-brown in color, showed no signs of precipitation after standing several months.

Another preparation of equal quantities of expressed juice with glycerine also showed no sign of precipitation. The author believed this to be the most efficient preparation and would prove valuable as an injection in treating of leucorrhœa, hemorrhages, and similar disorders.

Two other experiments included mixing (1) equal parts expressed juice with alcohol, and (2) 3 parts juice with 1 part alcohol, but both formed precipitates on standing. After filtering no further precipitation took place.

The author found that the leaves possessed styptic properties which were believed to be due to the tannic acid present.

Watson, F.

1884

Plantago major

Pharm. Journ. and Trans., --, p. 101; (Proc. A. Ph. A., 33, p. 121; Am. Jour. Pharm., 57, p. 107.)

In his "Index to Native and Scientific Names", the author identifies bartung with various species of Plantago, viz: Pl. lanciolata, Lin. (Irvine), Pl. major, Lin. (Honigberger), and Pl. psyllium, Lin. (Birdwood).

The seed of bartung is much thought of in Persia as a remedy for dysentery.

Koller, T.

1886

*Plantago major*

N. Jahrb. d. Pharm., 30, 139; (Amer. Jour. Pharm., 58, p. 418; Proc. A. Ph. A., 35, p. 113).

In 1868 Koller obtained from the leaves of *Plantago major*, *P. lanceolata* and *P. media*, chlorophyll, resin, wax, albumin, pectin, citric acid, and oxalic acid.

Rosenbaum, D.

1886

*Plantago major*

Amer. Jour. Pharm., 58, 418; (Proc. A. Ph. A., 35, p. 113).

Leaves of *Plantago major* were examined with the following results: Petroleum benzin extract 4% of wax and chlorophyll, the extract fusing at 83°C. Ether dissolve 4.4% resin and chlorophyll. Alcohol extract weighed 10%, 6% being soluble in water this portion containing considerable sugar, the remaining 4 parts dissolved by ammonia.

Soluble matter taken up by water weighed 13%, 7.2% of which was insoluble in 66% alcohol. Soda solution dissolved 6%, and dil. acid 10%, the latter containing a notable quantity of calcium oxalate. The bleached lignin weighed 35.5%.

Powdered leaves contained 8% of moisture and yield 12.85% ash, 2.85 soluble in water and 9.5 soluble in hydrochloric acid.

Tannin, Saponin, Alkaloids, etc. were not found.

Sonnenburg, C. E.

1893

*Plantago major*

Drug. Circ., 37, 51; (Proc. A. Ph. A., 41, p. 688).

An account of the constituents, properties and preparations of this plant.

Strawinski, J. F.

1898

*Plantago major*

Amer. Jour. Pharm., 70, p. 189-191; (Proc. A. Ph. A., 46, p. 793).

Strawinski made a proximate analysis of the rhizome and rootlets using relative proportion 30.5 and 19.5 respectively.

Air-dried drug contained 6.92% moisture and yielded 24.7% of a reddish-brown ash, of which only a small proportion was soluble.

Petroleum ether dissolved 0.57% consisting of caoutchouc, wax and fatty matter.

Ether 0.41% chiefly wax - neither alkaloid nor glucoside being present.

Absolute Alcohol, 8.60%, phlobaphene or traces protocatechuic acid, glucose (1.61%) and saccharose (0.87%).

Tannic possible slight traces present.

The drug appeared to be free from albuminous matter, but contained mucilaginous matter and starch.

Bourdier, L. L.

1908

*Plantago major*

Arch. d. Pharm., 2, 81-88; )Proc. A. Ph. A., 56, p. 192).

The author found that an infusion of the leaves of Plantago major when subjected to action of emulsin assumed a nearly black color after several hours' exposure, which was characteristic of the presence of Aucubin. He succeeded in isolating a glucoside in a pure crystallized condition which proved to be identical with the Aucubin ( $C_{13}H_{18}O_8$  plus  $H_2O$ ) of Aucuba Japonica, L.

The author also succeeded in demonstrating the

presence of invertin and of emulsin. These ferments being observed in all of the organs - in leaves, roots, flowers and seeds.

Zizka, - -

1918

*Plantago major*

Chem. and Drug.; through Merck's Rep., 27, 73 (Year Book A. Ph. A., 7, p. 473).

The fixed oil expressed from the seeds is recommended as a better substitute for olive oil than any that has yet been found. It has a greenish yellow color; sp. gr., 0.9315; acid value, 0.9; ester value, 182.0; saponification value, 182.9; and iodine value, 138.5. The seeds yield 10.5 percent of the oil.

Ogata, A and Nishioju, R.

1925

*Plantago major*

Jour. Pharm. soc. Jap., 514, 1040, through Pharm. Zentralh., 66, 299 (Year book A. Ph. A., 14, p. 95).

The seeds of the Japanese plantain are used in Japan and China in the treatment of whooping cough. O. and N. treated the aqueous extract of the seeds of *Pl. major* var. *asiatica* with alcohol which was treated with lead acetate after filtering. The precipitate was a mixture of lead salts from which there was separated succinic acid (M.P. 182-183°C) and a syrupy acid ( $C_5H_8O_3$ ) plantenolic acid by acidifying and shaking out with ethyl acetate. The latter was not obtained in the crystalline condition. Its salts are amorphous. The Ba, Cu, and Pb salts were analyzed. The acid reduces Fehling's solution and gives Lieber's iodoform reaction very markedly. In the aqueous extract choline and adenine were identified.

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APPROVED

Nellie Wakeman

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July 26, 1936