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The Pharmacognosy of *Chionanthus**

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The purposes of this investigation were to endeavor to improve the National Formulary VI monograph on *Chionanthus*, to verify the presence of a saponin in this drug, and to develop a method of assay for *Chionanthus* based upon its saponin content.



Fig. 1.—*Chionanthus virginicus* L. Leaf and Flowering Branches.

Description of Plant.—*Chionanthus virginicus* Linné, the Fringe Tree or Old Man's Beard, is a

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dioecious shrub or small tree occurring along the banks of woodland streams from southern New Jersey and Pennsylvania to the shores of Tampa Bay, Florida, and through the Gulf States to southern Arkansas and Texas. It is characterized by its large opposite, petiolate, narrowly elliptic or ovate to obovate-oblong leaves and drooping panicles of white, unisexual flowers. The pistillate plant bears ellipsoidal, dark blue drupes up to 2 cm. in length.

Materials.—Fresh materials consisting of roots, stems, root bark, stem bark and leafy branches of *Chionanthus virginicus* Linné were collected by H. W. Youngken in the Arnold Arboretum, Jamaica Plain, Mass., in the fall of 1939. Another sample of authentic root bark was later collected in North Carolina and four commercial samples of the drug were obtained. Some of the fresh material was pressed and preserved on herbarium sheets as reference basic material. Portions of the stem and root barks were dried and portions preserved in diluted alcohol. Sections of the root and stem barks of varying ages were examined in water, in chloral hydrate, and in phloroglucin-hydrochloric acid mounts and as permanent, stained balsam mounts. The four lots of commercial drug have been compared with the dried barks gathered by Youngken and, save for some few pieces of stem bark found in two of them, represented *Chionanthus* N. F. Root Bark. Some of the authentic root bark was ground and studied microscopically. Drawings have been prepared, and measurements of the length and thickness of the wall of the stone cells and of the diameter of the starch grains found in the root bark have been made and the results recorded.

External Appearance.—A comparison of the

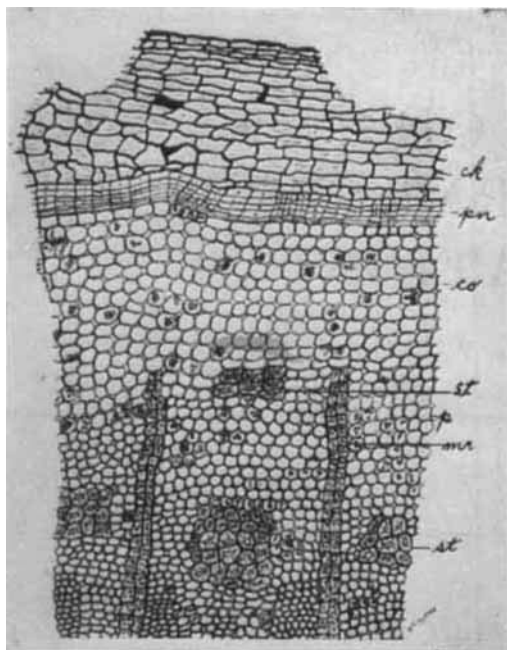


Fig. 2.—Chionanthus Root Bark. Transverse Section.

ck, Cork; *pn*, phellogen; *co*, cortex; *p*, phloem; *st*, stone cells; *mr*, medullary ray.

authentic root bark specimens with the paragraph on Unground Chionanthus in the N. F. VI showed the following differences:

Circular, raised lenticels were present on some of the pieces of the freshly gathered barks and on the commercial root barks. These should be mentioned in the N. F. monograph.

The outer surface of the root bark was not found to be usually reddish brown in the samples examined, but most of the pieces were grayish brown and fewer were reddish brown. It is suggested that a range in color of the outer surface be cited, following the ISCC-NBS system, *e. g.*, dusky to light yellowish brown ("dusky" brown is a "reddish" brown).

As a result of our studies we would suggest the following revised paragraph on Unground Chionanthus for the N. F. monograph:

In transversely curved and flattened pieces, occasionally in single quills up to 15 cm. long; bark from 2 to 10 mm. thick, heavy, some pieces sinking in water; outer surface dusky to light yellowish brown with few transverse wrinkles, more or less scaly, roughened by shallow pits, ridges and depressions, and with occasional circular or elliptical rootlet scars; inner surface moderate to weak yellowish orange, frequently with a purplish tinge, irregularly striate and undulate, some pieces exhibiting circular depressions; fracture short, hard and coarsely granular, the broken surface yellowish white to light brown.

Structure.—After examining numerous transverse,

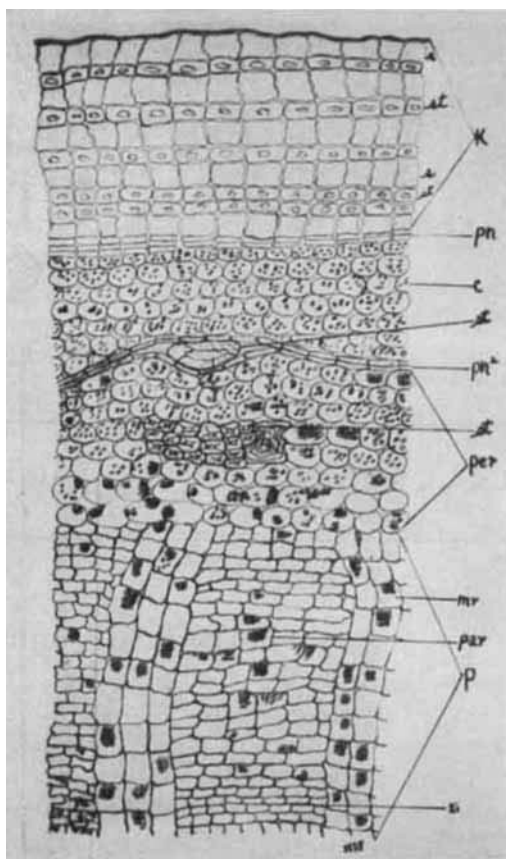


Fig. 3.—Chionanthus Stem Bark. Transverse Section.

k, Cork; *s*, suberized tissue; *st*, stone cells; *pn*, primary phellogen; *c*, cortex; *pn*², secondary phellogen; *per*, pericycle; *p*, phloem; *mr*, medullary ray; *par*, parenchyma; *st*, sieve tissue.

radial-longitudinal and tangential sections, we find the middle bark is thin, instead of very thick as stated in National Formulary VI, page 79, and that the inner bark or phloem is very thick. We have not seen the occasional bundles of fibers purported to be in this region of the root bark (1). We have, however, found numerous bundles of fibers in the pericycle of the stem bark. No groups of fibers were found in the phloem of most pieces of the root bark. Occasional groups of fibers did occur, however, in pieces of bark taken from the part of the root adjacent to the base of the stem (representing stem bark covered by soil), which no doubt accounts for their presence in some of the mounts of the powdered drug. There were also a number of elongated stone cells with a fiber-like appearance present in the sclerenchyma groups of both cortex and phloem of the root bark.

We suggest the following revised paragraph on Structure for the National Formulary monograph:

A thin zone of polygonal to irregular-shaped cork cells with suberized and lignified walls but without stone cells (distinction from stem bark); a

secondary cortex of varying thickness consisting of starch-bearing parenchyma with few or no cells possessing lignified walls; a broad phloem traversed by nearly straight medullary rays from 1 to 3 cells in width, and with numerous groups of stone cells; in old, thick bark two or more periderms may extend as far inward as the phloem, causing cortex exfoliation.

Powdered Chionanthus.—The color of the powders prepared from each of the authentic root barks was weak yellowish orange.

The diameters of the individual starch grains in a measured area in a mount of the powdered root bark from the specimen collected in North Carolina were as follows:

2 grains were	3.4 μ^1
9 grains from	3.5 μ to 6.8 μ
40 grains from	6.9 μ to 13.6 μ
10 grains from	13.7 μ to 17 μ
16 grains from	17.1 μ to 20.4 μ
9 grains above	20.5 μ
Maximum size	27.2 μ

The root bark collected in the Arnold Arboretum contained, in a measured area, starch grains of the following diameters:

18 grains up to	5.68 μ
27 grains from	5.69 μ to 11.36 μ
5 grains above	11.37 μ
Maximum size	17.04 μ

It is probable that a maximum diameter of 20 μ would cover the starch grains in the average mount, with occasional grains exceeding this diameter.

The shape of the grains varied from simple spheroidal and irregularly spheroidal to ovate; some were 2- to 4-compound.

The stone cells in both powders presented a large range of forms, many being irregularly lobed. They occurred isolated and in groups; some of them were elongated with tapered or blunt ends, and some were fiber-like, but with distinct pore canals. Fifty-one stone cells were measured, the longest being 295.36 μ . The thickness of the cell wall was determined, the thickest wall being 68.16 μ .

Fibers were relatively few in the powdered root bark as compared with the numerous fibers observed in the powder of authentic stem bark. They were lignified, with thin walls, and with rounded or truncate ends, the walls thicker than the lumen. The cork cells occurred mostly in groups and were polygonal to rectangular or irregular in shape. Their walls were brown and thin, and varied from suberized to lignified in character. Most of the parenchyma contained starch grains. A number of small, irregular, brownish, amorphous masses were detected which stained blue with 1% aqueous solution of cyanin followed by irrigation with glycerin, but no prismatic crystals other than silica or

calcium sulfate could be found in the material examined.

Our studies to date would suggest the following revised paragraph on Powdered *Chionanthus*:

Weak yellowish orange in color; odor characteristic; taste bitter, gritty upon mastication; starch grains simple or 2- to 4-compound, the individual grains variable in shape from spheroidal to angular convex, generally with 20 μ as a maximum diameter, rarely up to 27 μ in diameter; stone cells numerous, isolated and in groups, the individual cells variable in shape, some elongated, up to 300 μ in length, mostly of 125 μ as maximum diameter, with lamellated and strongly lignified walls showing branching pore canals, the walls usually not exceeding 50 μ , rarely up to 68 μ in thickness; numerous fragments of parenchyma tissue, many of the cells containing starch grains; occasional brown resin masses; fibers very few, mostly with lignified walls, simple pores and rounded or truncated ends; numerous fragments consisting of light brown cork cells with walls suberized to lignified.

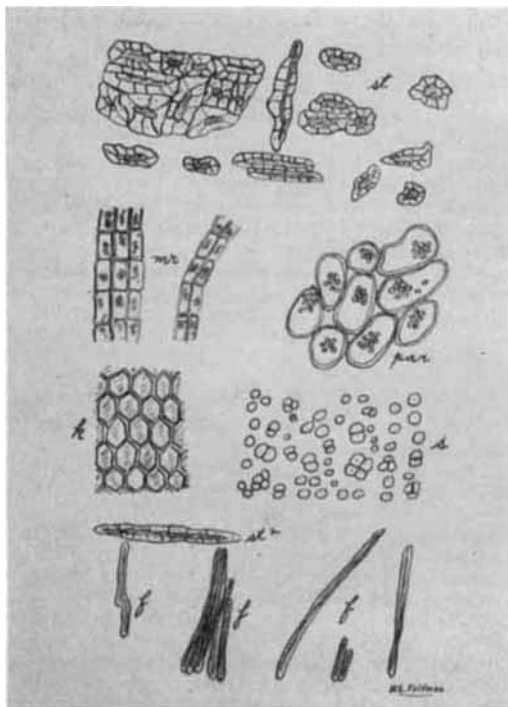


Fig. 4.—Powdered *Chionanthus*.
st, Stone cells; *mr*, medullary ray fragments; *s*, starch grains; *par*, parenchyma; *k*, cork; *st*², elongated, fiber-like stone cell; *f*, fibers from bark taken from basal portion of stem which was covered with soil and which has been marketed as a part of commercial root bark.

Alcohol-Soluble Extractive by U. S. P. XI Method.—The alcohol-soluble extractive was determined by the U. S. Pharmacopœial method (U. S. P. XI, pp. 473, 475), and using U. S. P. alcohol, with the following results:

¹ Many starch grains were smaller, but their measurements were not recorded, as they were considered to be non-diagnostic.

ROOT BARK

	Sample 1	Sample 2
Weight of hydrous sample	2.5019 Gm.	1.9075 Gm.
Weight of anhydrous sample ¹ (moisture 5.62%)	2.3613 Gm.	1.8003 Gm.
Weight of anhydrous marc	1.8880 Gm.	1.4127 Gm.
Weight of alcohol-soluble extractive (by difference)	0.4733 Gm.	0.3866 Gm.
Percentage of alcohol-soluble extractive	20%	21.5%

STEM BARK

	Sample 1
Weight of hydrous sample	1.6840 Gm.
Weight of anhydrous sample (moisture 6.64%)	1.5722 Gm.
Weight of anhydrous marc	1.1228 Gm.
Weight of alcohol-soluble extractive (by difference)	0.4494 Gm.
Percentage of alcohol-soluble extractive	28%

Alcohol-Soluble Extractive by the N. F. VI Method.

—The alcohol-soluble extractive was determined by the National Formulary method (N. F. VI, p. 79) on lots of the root and stem bark obtained from a reliable collector in North Carolina and using 73% alcohol for the extraction. The following results were obtained:

ROOT BARK

	Sample 1	Sample 2	Sample 3
Weight of hydrous sample	2.0457 Gm.	2.2404 Gm.	2.5085 Gm.
Weight of anhydrous residue (from the 50% aliquot)	0.4240 Gm.	0.5200 Gm.	0.5346 Gm.
Yield of residue from sample	0.8480 Gm.	1.0400 Gm.	1.0680 Gm.
Extractive soluble in 73% alcohol	41.5%	43.2%	47.2%

Average, 44%

STEM BARK

	Sample 1	Sample 2	Sample 3
Weight of hydrous sample	2.0121 Gm.	2.0425 Gm.	2.0530 Gm.
Weight of anhydrous residue (from the 50% aliquot)	0.3786 Gm.	0.4454 Gm.	0.3881 Gm.
Yield of residue from sample	0.7572 Gm.	0.8908 Gm.	0.7762 Gm.
Extractive soluble in 73% alcohol	37.6%	43.6%	37.8%

Average, 39.7%

Several points of interest are to be noted; *viz.*: (a) The U. S. P. method requires 95% alcohol for the extraction of the drug; the N. F. method requires but 73% alcohol; the difference in the yield of extractive is very marked, more than 100%. What is the significance of the 73% alcohol? Would not 95% alcohol be more appropriate for such a test, if not more significant? (b) The root barks yield extractive by the N. F. method far in excess

¹ The percentage of moisture in the drug was determined by the toluene distillation method, the weight of moisture in the sample was calculated and this weight was subtracted from the original weight of the drug taken for the test.

of the N. F. requirement of 25%. If the purpose of the test is to insure a good quality of root bark, should not the 25% minimum be considerably increased, at least to 35%? (c) The stem bark meets the test very easily; in fact, the yield of extractive with either 95% alcohol or 73% alcohol from the stem bark is greater than or almost as great as from the root bark; if the purpose of the test is to exclude stem bark, it must fail of its purpose.

The Hemolytic Saponin of Chionanthus.—The occurrence of a saponin in *Chionanthus*, according to Justice (2), was verified by us. The presence of a saponin in the crude drug is based upon the ability of the extractive material to hemolyze blood cells in normal saline suspensions, as well as to produce a froth in aqueous solution.

Preparation of Crude Saponin: The powdered root bark (127 Gm. of a commercial lot) was extracted with 95% alcohol, yielding a clear reddish percolate. This percolate was evaporated to a glassy, dark reddish brown solid extract, the yield being 13.8%, and forming a light brown powder having a saponaceous odor. The extract dissolved in water with frothing. To this solution was added a hot saturated solution of barium hydroxide until no further precipitation occurred. This precipitate was collected on a filter paper, washed with more of the barium hydroxide solution, dried and redissolved in water. Carbon dioxide gas was passed through this solution to precipitate the excess barium. The filtrate was evaporated to a semi-solid extract which was spread on glass and dried at room temperature. A brown powder (1.524 Gm. of 1.12% of the crude drug) was obtained, which responded to the following chemical tests: no coloration with concentrated sulfuric acid; reddish brown upon the addition of concentrated nitric acid; green with ferric chloride T. S. (indicating presence of tannin); red with potassium hydroxide T. S. Reactions identical to these were reported by Justice (2).

Von Schulz (3) claimed to have isolated a glucoside from *Chionanthus* which he called chionanthin, having the chemical formula $C_{22}H_{36}O_{10} \cdot 2H_2O$. He also stated in a preliminary examination, the bark gave indications of alkaloids with Mayer's reagent and potassium triiodide. The crude saponin obtained by us appeared to differ in physical and chemical properties from Von Schulz's glucoside, chionanthin. We contemplate work on the separation of the pure saponin and its comparison with the material called "chionanthin" by Von Schulz.

Qualitative Determination of the Crude Saponin: To prove that the substance obtained contained a hemolytic saponin, a test for saponins according to Leach (4) was applied as follows: Dissolve 0.1 Gm. of the crude substance in 25 cc. of normal saline solution and filter; agitate 1-, 2- and 3-cc. portions of this solution in small test tubes with 1-cc. portions of 1% suspension in normal saline of red blood cells from defibrinated rabbit's blood; run controls using 1-cc. quantities of the red blood cell suspension; incubate for 12 hrs. at 36° C., and note that in the

saponin tubes an opaque red to clear brownish solution appears, while the blood suspensions in the controls show no alteration. Microscopic examination of the material from both the saponin and control tubes proved that hemolysis had taken place. It was noted in mounts of the blood-saponin material that many of the red corpuscles had undergone solution, while several had swollen and become strongly refractive. A mount of the control material presented red blood cells in a normal condition.

Quantitative Evaluation of the Crude Saponin: The smallest amount of crude saponin from *Chionanthus* that would hemolyze blood cells by the method devised by Fantus and Dyniewicz (5) was determined as follows: Prepare seven series of three tubes each, each tube containing 1 cc. of the 1% red blood cell suspension in normal saline; add to the tubes in each series, 1 cc., 2 cc. and 3 cc., respectively, of a solution of the crude saponin in varied concentrations. The following table demonstrates the concentrations used and the results obtained:

Series	Test Tube Number	Crude Saponin, 0.1 Gm., in Normal Saline, Cc.	Result
1	1-3	25	Hemolysis
2	4-6	50	Hemolysis
3	7-9	100	Hemolysis.
4	10-12	200	Hemolysis
5	13-15	400	No hemolysis in tube 13
6	16-18	800	No hemolysis
7	19-21	1600	No hemolysis

Apparently 1 cc. of normal saline solution, containing 0.5 mg. of crude saponin of *Chionanthus*, will cause hemolysis of the red blood corpuscles of the rabbit.

Assay of Chionanthus: The hemolytic index of its constituent saponin (Fantus and Dyniewicz (5)) has been used as a basis for the assay of *Chionanthus*. The smallest amount of crude saponin causing hemolysis within a given period of time is compared with that amount of standard pure saponin producing a similar reaction within an equivalent period.

The pure saponin called standard or reference saponin was obtained from the LaMotte Chemical Products Company, and was stated to be pure *Quillaja saponin*.

In computing a quantitative percentage of the pure *Chionanthus* saponin, we base our calculations on the crude saponin which contains the pure material and on the standard hemolytic index.

Two samples of crude *Chionanthus* saponin were used in this investigation. Sample No. 1 was obtained by a modification of Justice's method (2), as already presented. The yield was 1.12% of crude saponin.

Sample No. 2 was obtained by the Rochleder process cited by Allen (6): Extract 100 Gm. of powdered *Chionanthus* root bark completely with hot water; add a saturated solution (aqueous) of barium hy-

droxide to this aqueous extract and collect the resulting precipitate; dry the precipitate, dissolve it in water, bubble carbon dioxide gas through the solution to precipitate the barium, filter and add to the filtrate a mixture of equal parts of ether and alcohol to precipitate the saponin; collect, dry and weigh the precipitate. Our yield was 2.003% of the crude saponin.

Determination of the Hemolytic Index: The following method employed by Fantus and Dyniewicz (5) was used to determine the hemolytic index of the pure saponin contained in the crude substance: Defibrinate freshly drawn rabbit's blood by shaking it with glass beads; wash the corpuscles from 2 cc. of this blood several times with normal saline solution; place 5 cc. of the red blood cell suspension in each test tube and add the saponin in normal saline solution in progressively increasing amounts (0.1 cc., 0.2 cc., 0.3 cc., etc.); make up the mixtures in each tube to 10 cc. with normal saline solution; keep at body temperature for 12 hrs., then read the results.

The saponin acts directly on the cells according to E. Ponder (7) and belongs to the simple hemolytic system, the saponin uniting with the cholesterol bodies. The criterion of complete lysis is the disappearance of all opacity and cloudiness in the test tube. The tube containing the smallest amount of saponin hemolyzing completely within 12 hrs. at body temperature is taken as the reading, and the hemolytic index calculated. As an example, using a 1:1000 solution of saponin:

- 0.1 cc., not complete in 12 hrs.
- 0.2 cc., not complete in 12 hrs.
- 0.3 cc., not complete in 12 hrs.
- 0.4 cc., complete in 12 hrs.
- 0.5 cc., complete in 12 hrs.

Hence the hemolytic index is 0.0004 Gm. of saponin in 10 cc. of blood suspension or 1:25,000.

TABLE I.—HEMOLYTIC INDEX OF CRUDE CHIONANTHUS SAPONIN No. 1

Crude Saponin Solution	Cc. Added to Red Blood Cell Suspension	Results
1:1000	0.1	Hemolysis not complete in 12 hrs.
	0.2	
	0.3	
	0.4	
	0.5	
	0.6	
	0.7	
	0.8	
	0.9	
	0.95	
1.00		
1:100	0.1	Hemolysis not complete in 12 hrs.
	0.2	
	0.3	
	0.4	
	0.5	
	0.6	
	0.7	
	0.8	
	0.9	
	1.0	

(Table I continued on p. 134)

Crude Saponin Solution	Cc. Added to Red Blood Cell Suspension	Results
1:50	0.1	Hemolysis not complete in 12 hrs.
	0.2	
	0.3	
	0.4	
	0.5	
	0.6	
	0.7	
	0.8	
	0.9	
1:25	0.1	Hemolysis complete in 12 hrs.
	0.2	
	0.3	
	0.4	
	0.5	
	0.6	
	0.7	
	0.8	
	0.9	
1.0		

Hemolytic index of sample No. 1 is 1:1250.

TABLE II.—HEMOLYTIC INDEX OF CRUDE CHIONANTHUS SAPONIN NO. 2

Crude Saponin Solution	Cc. Added to Red Blood Cell Suspension	Results
1:1000	0.1	Hemolysis not complete in 12 hrs.
	0.2	
	0.3	
	0.4	
	0.5	
	0.6	
	0.7	
	0.8	
	0.9	
	0.95	
1.00		
1:100	0.1	Hemolysis not complete in 12 hrs.
	0.2	
	0.3	
	0.4	
	0.5	
	0.6	
	0.7	
	0.8	
	0.9	
1.0		
		Hemolysis complete in 12 hrs.

Hemolytic index of sample No. 2 is 1:1110.

TABLE III.—HEMOLYTIC INDEX OF PURE QUILLAJA SAPONIN

Standard Saponin Solution	Cc. Added to Red Blood Cell Suspension	Results
1:10,000	1.00	Hemolysis not complete in 12 hrs.
	1.10	
	1.20	
	1.30	
	1.40	
	1.50	
	1.60	
	1.70	
	1.80	
	1.90	
2.00		
		Hemolysis complete in 12 hrs.

Hemolytic index of standard saponin is 1:55,000.

The standard hemolytic index was obtained by using pure Quillaja saponin as the standard or reference saponin. A 1:1000 solution of this saponin was first tested by the hemolytic index determination method. An index of 1:25,000 was obtained within 5 min. but this index could not be compared with the crude saponin indexes because of the time difference. A 1:10,000 standard saponin solution was then tested and a hemolytic index was obtained at 12 hrs.

As 1.80 cc. of the standard saponin solution, containing 0.18 mg. of pure saponin, causes hemolysis, then 0.2 cc. of the 4% solution of crude saponin No. 1 and 0.9 cc. of the 1% solution of crude saponin No. 2 each contains 0.18 mg. of standard pure saponin; hence 1 Gm. of crude saponin No. 1 contains 22.5 mg. of pure saponin (0.2 cc./0.18 mg. = 25 cc./x mg.; x = 22.5 mg.); and 1 Gm. of crude saponin No. 2 contains 20 mg. of pure saponin (0.9 cc./0.18 mg. = 100 cc./x mg.; x = 20.0 mg.).

As 100 Gm. of powdered Chionanthus yielded 1.12 Gm. of crude saponin No. 1, the Chionanthus is calculated to contain 25.2 mg., equivalent to 0.0252% of pure saponin; however as 100 Gm. of the same lot of powdered Chionanthus yielded 2.003 Gm. of crude saponin No. 2, the Chionanthus could be calculated to contain 40.06 mg., equivalent to 0.04% of pure saponin.

Evidently an evaluation of the drug based on its saponin content depends upon the ability to extract the saponin and get it into solution; our work would indicate that the Rochleder method of extraction is the better one.

The following tentative assay has been developed for Chionanthus:

Extract 100 Gm. of Chionanthus in moderately fine powder in a suitable percolator to exhaustion with hot distilled water, and add to the percolate a hot, saturated aqueous solution of barium hydroxide until no further precipitation occurs. Collect the precipitate, dry it and dissolve it in 150 cc. of distilled water; bubble carbon dioxide gas through this solution until precipitation ceases, and then filter; to the filtrate add a mixture of equal parts of ether and alcohol until no further precipitation occurs; collect, dry and carefully weigh the precipitate. Prepare a 1% solution of this crude saponin in normal saline solution and add progressively increasing amounts from 0.1 cc. to 1.0 cc. of this solution to ten test tubes each containing 5 cc. of a 2% suspension in normal saline solution of red blood cells (animal); add sufficient normal saline solution to each test tube to make 10 cc. Keep the tubes at body temperature for 12 hrs., then note the test tube containing the least amount of the crude saponin that shows complete hemolysis (a clear brownish solution).

Each cubic centimeter of the 1% crude saponin solution is equivalent to 0.00018 Gm. of standard pure saponin from the bark of *Quillaja saponaria*.

It appears from tests made by us that the Chionanthus saponin is a slow-acting saponin which

accounts for the amount of time required in obtaining an end-point.

The Uses of Chionanthus.—The root bark of the Fringe Tree, *Chionanthus virginicus* L. (Fam. *Oleaceae*), has long been used in the form of its fluidextract in the treatment of hepatic disorders. Its purported efficiency in jaundice was discovered by Prof. I. J. M. Goss (8) of Georgia, who, in 1843, while suffering from an attack of this disease, tested it upon himself and reported its positive results. Dr. Goss considered it the best remedy for all cases of jaundice not dependent on gallstones. Later Prof. Scudder (9) considered it efficient even when calculi were present. He recommended an eclectic preparation of it during the paroxysm. "King's American Dispensatory" (8) states that "hypertrophy of the liver, chronic hepatic inflammation and portal congestion are speedily relieved by *Chionanthus*." The same work also states that "it acts principally upon the abdominal glandular organs, and to some extent upon the venous system, relieving congestion."

Other uses for this drug as reported in the literature are tonic, diuretic, and astringent vulnerary. Rafinesque (10) is stated to have reported its use in a cataplasm for the healing of wounds. Dr. F. S. Smith (10) judged the fluidextract of *Chionanthus* as one of the best remedies in certain forms of bilious sick headache.

Chionanthus first became official in National Formulary IV (1916) and the crude drug and its fluidextract have been official in all later editions. It is also recognized in the "Homeopathic Pharmacopoeia."

SUMMARY AND CONCLUSIONS

1. A brief description is given of *Chionanthus virginicus* and its distribution.

2. Comparisons between the authentic root bark of *Chionanthus virginicus* and the N. F. VI description of *Chionanthus* have been made and discrepancies have been found in the paragraphs pertaining to the description of the unground drug, its histology and the powdered drug. Suggestions are offered for their improvement.

3. No groups of fibers were found in the phloem of authentic root bark. Occasional groups of fibers did occur in pieces of the commercial bark which had been gathered from the basal portion of the stem adjacent to the root and which represent stem bark covered with soil.

4. A number of stone cells were present in the sclerenchyma groups of both cortex and phloem of the root bark.

5. The maximum size of the individual starch grains found in root bark was 27.2 μ .

6. *Chionanthus* root bark yields extractive by the N. F. VI method far in excess of the N. F. requirement of 25%. It is suggested that the minimum extractive standard be considerably increased.

7. The difference between the yield of alcohol-soluble extractive by the N. F. and U. S. P. methods, when applied to *Chionanthus* root bark, was very marked.

8. The yield of alcohol-soluble extractive from the stem bark with either 95% or 73% alcohol is greater than or almost as great as from the root bark. It is found that the N. F. test fails of its purpose if it is intended to exclude the *Chionanthus* stem bark.

9. The occurrence of a saponin in *Chionanthus* as first reported by Justice has been verified.

10. The smallest amount of *Chionanthus* crude saponin which will hemolyze red blood cells was determined by the method of Fantus and Dyniewicz to be 1 Gm. in 200 cc.

11. The hemolytic index of crude *Chionanthus* saponin ranged from 1:1110 to 1:1250, whereas that of standard saponin from *Quillaja* bark was found to be 1:55,000.

12. A method for the assay of *Chionanthus* has been developed.

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