(3) Exler, *Pharm. Weekblad*, 65 (1928), 1152.
(4) Reindollar, JOUR. A. PH. A., 14 (1925), 789.
(5) Stikarofsky, *Ibid.*, 16 (1927), 30.

NOTE: The authors take this opportunity to thank Prof. E. D. Davy, School of Pharmacy of Western Reserve University, for his suggestions, criticisms and advice regarding this investigation.

THE PHARMACOGNOSY, CHEMISTRY AND PHARMACOLOGY OF VIBURNUM. III. HISTORY, BOTANY AND PHARMACOGNOSY OF VIBURNUM OPULUS L. VAR. AMERICANUM (MILLER) AIT.*

BY HEBER W. YOUNGKEN.

INTRODUCTION.

The American variety of *Viburnum Opulus* has been the theme of many contradictory discussions and reports within the past half century alike by botanists, pharmacognosists and pharmacologists. The purpose of this investigation, at least so far as the botanical and pharmacognic phases are concerned, is an attempt to straighten out this tangle first through historical studies and then by new research work upon authentic plants, representative specimens of which, including separated barks, have been permanently deposited in the herbarium of the Massachusetts College of Pharmacy.

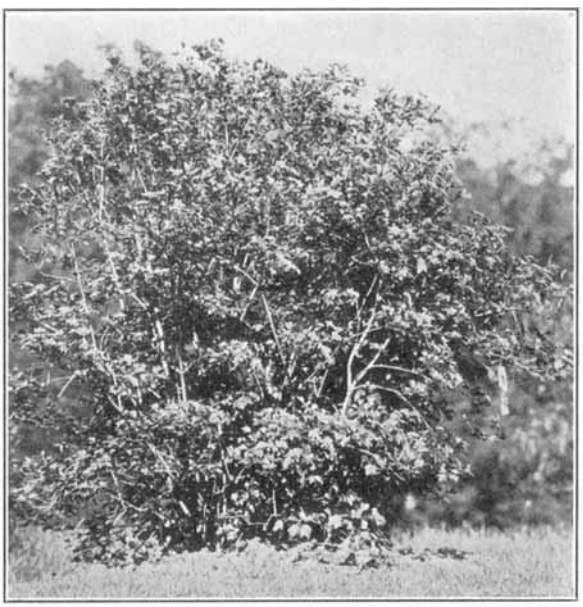

Fig. 1.—*Viburnum Opulus* Linné var. *americanum* (Miller) Aiton. Shrub growing in Arnold Arboretum.

Detailed reports upon the chemistry and pharmacodynamics of the botanically standardized bark of this species will be published later by the collaborators on these phases of the investigation who are Florin J. Armhein (on chemistry) and James C. Munch (on pharmacodynamics).

HISTORY.

The taxonomic history of this plant takes us back to less than a decade before the American Revolution.

As early as 1768 Philip Miller in his Gardener's Dictionary (1) briefly described it as a distinct species which he named *Viburnum Americanum* or American Guelder Rose. He took

* This investigation was aided by a grant from the AMERICAN PHARMACEUTICAL ASSOCIATION Research Fund. Presented before Scientific Section, A. PH. A., Miami meeting.

cognizance of the European *Viburnum Opulus* or cranberry bush which he described separately on the same page referring to the description previously given it by Linnaeus.

In 1785, Humphrey Marshall in "Arbustrum Americanum" (2) named this plant *Viburnum trilobum* or Mountain Viburnum and described it as follows:

"This plant grows naturally upon mountains in the interior parts of Pennsylvania rising with slender stems to the height of 8–10 ft. The leaves are somewhat like those of the Guelder Rose or Snowball tree; they are narrowed at the base, but spreading and divided into three sharp pointed lobes, the middle ones longest and sometimes slightly toothed. The flowers are produced in the form of the others and are succeeded by berries of the same shape, of a pretty large size and a red color when ripe."

In 1789, Aiton in Hortus Kewensis (3) divided *Viburnum Opulus* into *V. opulus europea* α *ramulis viridibus opacis*, the Marsh Viburnum or Guelder Rose, β *Viburnum Opulus americana, ramulis rubicundis lucidis* or Red Twiged Viburnum or Guelder Rose and γ *V. opulus rosea*, the Snowball Viburnum or Guelder Rose. He thus took up Miller's species, *V. americanum* and reduced it to a variety of *V. Opulus* L.

In 1803, Michaux in his "Flora Boreali-Americana" (4) described *Viburnum Opulus* as *V. foliis lobatis; petiolis glandulosis, cymis floribus sterilibus radiatis.* He recognized three varieties which he named **var.** α *europeanum*, β *Pimina* and γ *edule*. His Pimina variety was described as follows: "β *Pimina: foliis tricuspidatis; lobis sursum angustatis, promisee acuminatis.*" It corresponds to the common wild American plant.

Fig. 2.—*Viburnum Opulus* Linné **var.** *americanum* (Miller) Aiton. Leaf and flowering branch. Collected by H. W. Youngken at Framingham, Mass., May 20, 1929.

In 1814, Pursh in his "Flora Americana Septentrionalis" (5) describes Aiton's *V. Opulus americanum* and Michaux's *V. Opulus Pimina* and Marshall's *V. trilobum* under the name of *Viburnum Oxycoccos*. He states that the berries are red and have an agreeable acid taste resembling that of cranberries, *Vaccinium macrocarpum*, for which he claims they are a very good substitute. In the 2nd edition of this same work (6) he states this variety is found in swamps, shady woods of Canada and on the mountains of New York and New Jersey.

Rafinesque (7) in Medical Flora (1828–30) points out that the bark of this *Viburnum* was used by the North American Indians as a diuretic usually in the form of a decoction, that some of the western tribes smoked the bark instead of tobacco and that the leaves of this and other species of *Viburnum* were employed as a tea by Indians and the early settlers.

In 1838, the same writer in his "Alsographia Americana" (8) readopted Michaux's *V. Opulus Pimina* for this plant, gave its distribution as from Ohio to Canada and described a form of this variety with subcordate leaves which he found in the Alleghanies of western Pennsylvania under the name of *Vib. Op. Pimina* **var.** *subcordatum* Raf.

In their "Illustrated Flora of the Northeastern U. S. and Canada" of 1913, Britton and Brown (9) describe the plant which Aiton called *V. Opulus americanum* and Marshall as *V. trilobum*

under the title of *V. Opulus* L., thus recognizing no distinction between the European and American species.

"Gray's Manual of Botany," 7th ed., revised by Robinson and Fernald (10), lists the American Cranberry-Tree under the section *Opulus* (Tourn.) DC. of the genus Viburnum as *Viburnum Opulus* L. **var.** *americanum* (Mill.) Ait. and calls it by the names or synonyms of Cranberry-Tree, High Bush Cranberry and Pimina. This work describes it in part as follows: "Nearly smooth, upright, 1–4 m. high; leaves 3–5 ribbed, strongly 3-lobed, broadly wedge-shaped or truncate at the base, the spreading lobes pointed, mostly toothed on the sides, entire in the sinuses; petioles bearing two glands at the apex; cyme broad, the marginal flowers neutral, with greatly enlarged flat corollas; stamens elongate." Its distribution is given as in woods and along streams from Newfoundland and Eastern Quebec to British Columbia, south to New Jersey, Pennsylvania, Michigan, Wisconsin and northeastern Iowa.

In 1927, Bush (11), discussing the taxonomy of some species of Viburnum calls attention to the earlier work of Blake and agrees with the latter that Miller's *Viburnum Americanum* rests on *Hydrangea arborescens*. He states in part: "It is quite apparent that Aiton intended to take up Miller's species americanum and reduce it to a variety under the European *V. Opulus* L. Even if he did not mention Miller as the author of the name americanum, and this being the case, the next available name for this species would be the *V. trilobum* Marshall, 1785."

In the same year in his "Manual of Cultivated Trees and Shrubs," Rehder (12) readopted the name of *Viburnum trilobum* Marsh. for the American variety of *Viburnum Opulus* thus giving it full species rank. In this same work he separately describes as a separate species the *V. Opulus* L. or European Cranberry Bark. A comparison of his two descriptions shows that this author bases his concepts for the elevation of the American variety to species rank mainly upon the longer, less toothed lobes of the leaves, shallow instead of narrow, grooved petiole and the small usually stalked glands instead of the large disk-shaped glands of the European species.

The more important events dealing with the morphologic and pharmacognic history has been largely discussed by the writer in the first article of this series (13). To this the following may be added: The bark of *Viburnum Opulus* was official in the seventh and eighth editions of the United States Pharmacopœia. The U. S. P. VIII (1905) gave a description which applied to Mountain Maple (*Acer spicatum*) bark.

Under the name of *Viburnum Opulus* or High Bush Cranberry Bark the bark of *Viburnum Opulus* L. **var.** *americanum* (Miller) Aiton became official in the National Formulary IV (1916). Here material which is apparently authentic is described as to physical characteristics, inner structure and powder.

It was continued as official in the National Formulary V (1926), this addition adding the synonym of Cramp Bark and describing the unground drug, structure and powdered drug and giving the list of official preparations into which it entered and the average dose.

The Homeopathic Pharmacopœia of the United States 3rd ed., revised 1914, page 581, recognizes as official the fresh bark of *Viburnum Opulus* including the bark of the root.

In his "Physiological Plant Anatomy," Haberlandt (14) calls attention to the fact that certain roots of Viburnum have cortical layers immediately outside of the endodermis furnished with thickening ridges forming a close to continuous mesh-work. He also states that in *V. Opulus* the inner walls of the cork cells are thicker than the others (15) and that the wood fibres very frequently are arranged in perfectly regular, radial rows (16).

Viehoever, Ewing and Clevenger (17) state that sclerenchyma fibres are usually absent in the secondary bark and are few and scattered in the primary cortex of young bark of *V. Opulus*, that the root bark of this species differs from the stem bark by showing almost an entire absence of stone cells and sclerenchyma fibres.

The earliest record of chemical studies upon *Viburnum Opulus* is that of Chevreul (18) who, in 1817, found valerianic acid in the European plant.

In 1844, Krämer (19) reported the presence in the European species of tannic acid, a bitter principle (viburnin), brown resin acid, chlorophyll, pectin, gum, wax, potassium malate, calcium malate, calcium sulphate, iron oxide and magnesia.

In 1900, Gibson (20) subjected the American *Viburnum Opulus* stem bark to proximate examination and found the air-dried drug contained 6.92% of moisture, 5.52% of ash, 87.56% organic constituents, 5.271% resin, 6.342% of waxy matter, 9.431% organic acids, 36.62% NaOH

extractive, 15.80% cellulose, 4.378% of colored extractive together with glucose and earthy phosphates and carbonates. He further stated that he believed the active principle to be a glucoside which he described of resinous character, greenish or greenish yellow in color, slightly soluble in water and completely soluble in alcohol.

In 1920, Clevenger and Ewing (21) analyzed the tree bark of American *Viburnum Opulus* and found the powdered drug to contain 10.7% total ash, 0.3% acid-insoluble ash and 11.04% of olive-green ether extract of which 1.14% was volatile with a characteristic valerianic acid odor.

By far the most extensive chemical examination upon the stem bark of the American Viburnum Opulus up to the present was that of Heyl (22) who in 1922 analyzed bark obtained through regular channels from northern Minnesota which conformed to the U. S. P. standards. He found the following substances: valerianic, acetic, caporic, caprylic, formic, oleic, linoleic, cerotic and palmitic acids, traces of paraffin, myricyl alcohol, phytosterolin ($C_{33}H_{56}O_4$), a phytosterol, an acidic resin readily yielding acetic and valerianic acids on hydrolysis and indications of the presence of a glucoside (by the Bourquelot method) which was not isolated. No alkaloid was found.

The same year Bemis (23) analyzed the fruit of this plant and found 2.92% of glucoside (viburnin), valeric acid, 0.19% oxalates, 0.16% citric acid, 0.81% malic acid, 6.65% sugar, 0.4% tannic acid and 3.45% of ash representing calcium phosphate and traces of potassium. He claimed that the valerianic acid and the glucoside exist independently of each other in that organ and that the acid did not form from the breaking down of the glucoside.

The history of the medicinal use of this plant dates back to the American aborigines of the time of Rafinesque (7) who reported them employing the bark as a diuretic.

The first account of its use in the practice of medicine was reported by Wooster Beach (24) in 1833.

King's "American Dispensatory" 18th ed. (25) states "Like *Viburnum Prunifolium* it is a remedy for the prevention of abortion and to prepare the way for the process of parturition. It allays uterine irritation with a tendency to terminate in hysteria, while in neuralgia and spasmodic forms of dysmenorrhoea it is a favorite remedy with many physicians."

According to W. Boericke (26), it is a general remedy for cramps, it often prevents miscarriage and is useful in the treatment of false labor pains and spasmodic and congestion affections of ovarian or uterine origin.

J. V. Shoemaker (27) in his "Materia Medica and Therapeutics" states the bark is used in the form of the fluidextract and is given to prevent cramps of all kinds resulting from hysterics, dysmenorrhoea or pregnancy.

In 1916 Pilcher (28) immersed longitudinal strips of the uterus (usually pregnant) in a bath of well-oxygenated Tyrode's fluid and tested the extract and infusion in strength of 1 : 1000 of the bark of *Viburnum Opulus* and *V. Prunifolium* upon these strips and recorded the contractions. He reported that *V. Opulus* produced slight depression and *V. Prunifolium* inconstant stimulation which he interpreted as practically negative results. Nothing is stated regarding the authenticity of the crude drugs used in the fluidextracts and infusions employed in his tests and the results he obtained are therefore of questionable scientific value.

Sollman (29) states that *Viburnum Prunifolium* and *Viburnum Opulus* enjoy the widest reputation among the uterine sedatives although their usefulness is no more demonstrated. He apparently bases his opinion upon the earlier work of Pilcher which he cites as evidence.

Wood (30) states that the berries of *V. Opulus* are antiscorbutic but that there is no sufficient reason to believe the bark has any medicinal properties of any kind.

In their "Essentials of Physiology and Pharmacodynamics," Bachman and Bliss (31) include *Viburnum Opulus* and *Viburnum Prunifolium* in the group of uterine sedatives and hemostatics and state that any action of these drugs on the uterus is questionable and the mechanism of action, if there be any, unknown.

Cohen and Githens in their Pharmacotherapeutics (32) claim that both *Viburnum Opulus* and *Viburnum Prunifolium* are much praised and also repeatedly stated to be without effect. That the fluidextract is given with apparent good effect in threatened abortion, that it may relieve pain and uterine contraction during pregnancy, that in dysmenorrhoea it acts by relieving spasm and congestion of the pelvic organs and that it is also given in subinvolution of the uterus

following pregnancy, as well as to quiet tearing after pains and to give tone to the uterus after the removal of one or more small fibroids. They express uncertainty of opinion as to all of these actions on account of the alcohol in the liquid preparations. They also state that there seems to be little difference in the action of the two forms of *Viburnum* and that the best preparations of either drug are the fluidextract and elixir.

It is quite evident from the foregoing citations that therapeutic opinion has been chaotic regarding the action of the bark of *Viburnum Opulus*. Search of the literature has failed to reveal any evidence that the particular bark used in preparations for the animal tests was botanically standardized. Moreover, it is clear that this drug as well as *V. Prunifolium* have been employed empirically with apparently good results.

MATERIALS AND METHODS.

The materials for this investigation consisted of entire shrubs of *Viburnum Opulus* L. **var.** *americanum* (Miller) Aiton and *Viburnum Opulus* L. growing in the

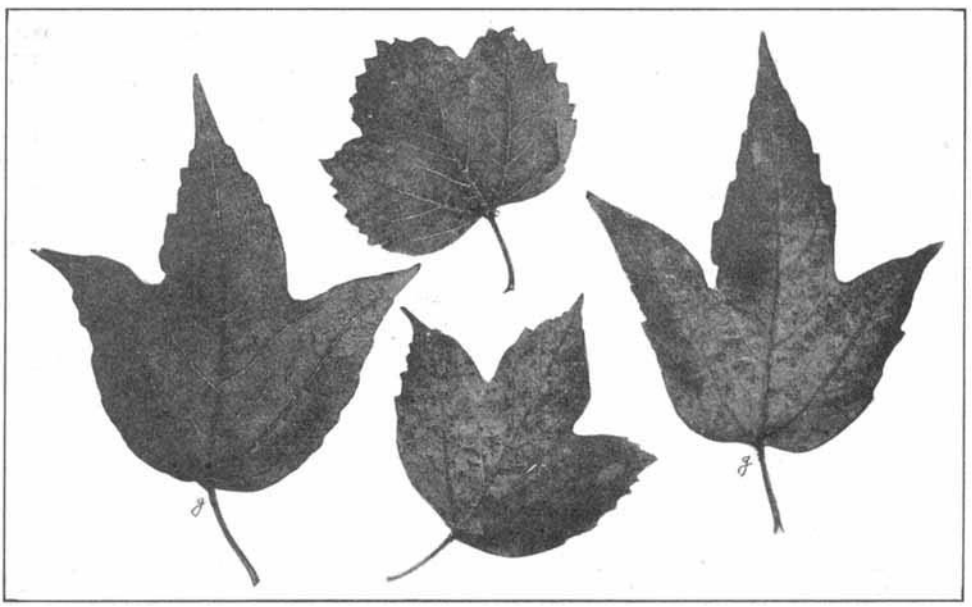

Fig. 3.—Types of leaves found on *Viburnum Opulus* Linné **var.** *americanum* (Miller) Aiton. Note petiole glands (g).

Arnold Arboretum and studied at various times during their growth and dormant periods by the writer, specimens of roots, stems, root and stem barks, leaf and flowering and leaf and fruiting branches gathered from these shrubs at various times during the spring, summer and autumn of 1927, 1929, 1930 and in the late spring of 1931, also of entire shrubs of *V. Opulus americanum* obtained from a nursery at East Boxford, Mass., and at Framingham, Mass., in May 1929, and compared with the Arnold Arboretum material and found to be authentic by the writer, also of 5 entire shrubs, 3 root systems and representative parts of aerial portion of the same plants and 50 lbs. of stem bark of *V. Opulus americanum* collected from authentic plants growing near Falmouth, Michigan, in late September and early October of 1930.

The methods employed by the writer in the investigation of the American variety of *Viburnum Opulus* were similar to those described under "Materials and Methods" in my earlier investigation of *Viburnum Lentago* (See JOUR. A. PH. A. 20; (1931) 315–317.

DESCRIPTION OF VIBURNUM OPULUS L. VAR. AMERICANUM (MILLER) AITON.

This plant commonly known as Cranberry Bush, Cranberry Tree, High Cranberry, High Bush Cranberry, American Cranberry Bush, American Guelder Rose and Pimina is an erect, nearly smooth, handsome shrub of open habit found in the woodlands and along streams from Newfoundland to British Columbia, south to New Jersey and Oregon and extending to Pennsylvania, and into Michigan, Wisconsin and Iowa, in many places of which it attains a height of 4 meters. Its stems are repeatedly dichotomously branched toward the summit and bear scaly winter buds. The trunk divides near the ground into two primary branches and these branch at successive levels typically in bifurcate fashion.

Its leaves are simple, opposite, petiolate, broadly ovate, palmately-3-lobed, broadly cuneate, rounded or truncate at base, up to 13 cm. long and 9.5 cm. in width, with lobes coarsely and irregularly toothed or nearly entire along the margin and acuminate at apex, the middle lobe being frequently elongated. The upper surface is dark green and glabrous, the lower surface pale green and pilose along veins. The venation is palmate-reticulate, the veins of the first order alternate. The petiole is up to 3 cm. long, grooved on upper surface with stalked to sessile glands at its apex and with a pair of elongated slender glandular stipules at its base.

The inflorescence is a stalked, broad, compound cyme bearing neutral marginal flowers and numerous minute, inconspicuous greenish fertile florets.

The fruit is a subglobular, scarlet drupe with a sour, succulent sarcocarp and a flat stony endocarp enclosing a single seed.

DESCRIPTION OF THE ROOT BARK.

The root bark occurs in short pieces or chips, externally dark brown to purplish brown, irregularly longitudinally wrinkled, reddish brown where cork is abraded, scaly on older roots and nearly smooth on younger roots. The fracture is short, very brittle and uneven, the fractured surface exhibiting a brown outer bark and a whitish middle and inner bark. The odor is indistinct in bark which has been stored for some time but becomes strongly valeric-acid like when the bark is triturated with phosphoric or hydrochloric acids. Odor of freshly gathered drug is very strongly valeric-acid like. The taste is bitter and slightly astringent.

When 0.5 Gm. of ground root bark was boiled with 20 cc. of distilled water and filtered, a very pale brown colored clear filtrate resulted. When a portion of this filtrate was treated with 4 drops of a 2% $FeCl_3$ solution, a dark green mixture resulted. When this was filtered, the filtrate was a clear green and the precipitate dark green to greenish-black. When a second portion of the first filtrate was treated with ammonium molybdate T. S. (U. S. P.), a yellowish color resulted with the separation of a dirty yellow precipitate.

When the inner surface of the root bark was treated with a 2% solution of $FeCl_3$ a blue-green color resulted.

HISTOLOGY OF THE ROOT.

Piliferous Region.

Cross sections through the root a very short distance above the cap exhibit the following structures:

1. Epidermis of large polygonal to slightly radially elongated cells with thick outer cuticle and clear contents, a number of the cells being extended into short papillæ and root hairs.

2. Primary Cortex of 4 to 5 layers of rounded to polygonal parenchyma cells and small angular air spaces.

3. Endodermis, of ellipsoidal outline, the poles of the ellipse being marked by cells with thick, brownish walls.

4. Pericambium of a layer of small, thin-walled cells.

5. Radial fibrovascular bundle consisting of an oblong strand of xylem flanked on either side by an elongated strand of phloem.

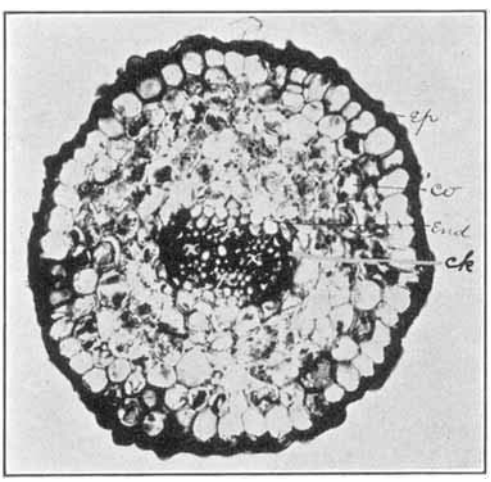

Fig. 4.—Photomicrograph of a cross section of the root of primary growth of *Viburnum Opulus* var. *americanum* showing a diarch bundle in the center of the illustration. × 52. *ep*, epidermis; *co*, primary cortex; *end*, endodermis; *ck*, early appearance of cork; *x*, xylem strands and *pr*, phloem strands of a diarch radial fibrovascular bundle.

Six Inches from Tip.

1. Epidermis with radially-elongated epidermal cells having a thick cuticle.

2. Primary cortex of 5 to 6 layers of rounded to tangentially elongated cortical parenchyma cells and small angular air spaces.

3. Endodermis of tangentially elongated endodermal cells, the radial walls of which are not averagely thicker than the outer or inner walls.

4. Pericambium which has divided into outer and inner layers and begun to lay down cork in the interrupted patches, of a single layer in thickness.

5. Protophloem of soft bast. The secondary phloem has begun to be laid down. An interfascicular cambium has formed which has already tagged on to the intrafascicular cambium and formed a complete cambium ring. Four primary xylem strands are evident. These have been somewhat deepened by a secondary xylem having been laid down on their outer faces.

6. Pith, beginning to be crushed out.

Eleven Inches from Tip. (*Early Secondary Growth.*)

Section cut through this region show: (*1*) an epidermis which has almost completely sloughed off, having been replaced by 6 or 7 layers of cork, (*2*) a phellogen in the state of division, (*3*) secondary cortex of about 4 layers of parenchyma cells and

air spaces, (*4*) a circle of open collateral bundles separated by narrow medullary rays which are 1 cell in width. Each bundle shows about 5 layers of phloem, a distinct cambium and a broad xylem wedge which in radial longitudinal section exhibits tracheæ with scalariform and oval bordered pore markings together with elongated xylem cells and wood fibres with attenuated ends.

Root of Medium Thickness.

Sections through a portion of the root of 2 years of age exhibited further secondary increase in thickness. The cortex and phloem were somewhat broader. Rifts had begun to appear in cortex and small sclerenchyma patches were evident in protophloem and outer cortex. Rosette aggregates of calcium oxalate were observed in a number of cells of the cortex and phloem. The pith had become completely crushed out.

In surface view the cork cells were polygonal to somewhat tangentially elongate up to 106.20μ × 56.64μ. In cross sections the cork cells measured were up to 70.8μ × 17.7μ.

Sections through portions of a root 3 years of age showed tabular cork cells which in cross sections were occasionally up to 85.53μ × 28.32μ and frequently to 70.8μ × 17.7μ. Many of the cork cells slightly lignified and showed evidence of vertical thickenings in their walls. In surface sections the cork cells appeared irregularly polygonal and were up to 106.20μ × 63.72μ.

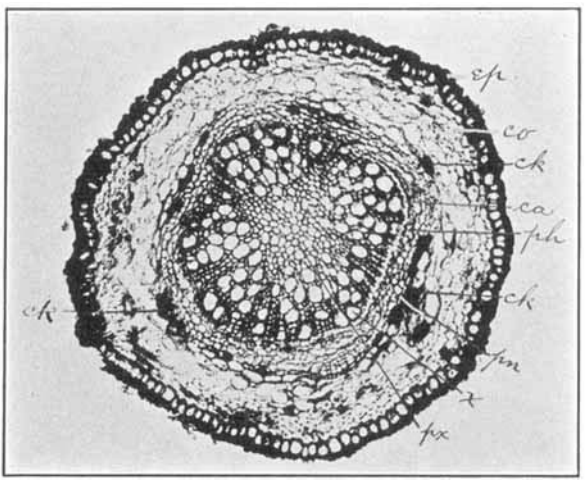

Fig. 5.—Photomicrograph of a transverse section of the root of early secondary growth of *Viburnum Opulus* var. *americanum*, cut 6 in. from tip, × 52, *ep*, epidermis; *co*, primary cortex; *ck*, cork cut off by a phellogen originating from division of pericambium; *pn*, cork cambium; *ph*, phloem; *ca*, cambium; *x*, secondary xylem; *px*, primary xylem.

The medullary rays were 1 to 2 cells in width. Occasional groups of stone cells were evident in the phloem.

The xylem wedges were deeper than in the younger sections studied and showed numerous scalariform tracheæ, fewer pitted tracheæ and many wood fibres, the latter arranged in radial rows.

Calcium oxalate crystals were numerous in the cortex and phloem occurring as rosette aggregates usually from 15μ to 25μ but occasionally up to 35μ in diameter.

Old Thick Root.

Sections cut through older portions of the root showed the following structural peculiarities:

1. Cork of numerous layers of tangentially-elongated cells with lignified walls.

Many of the cork cells were up to 70.8μ long $\times$ 21.24μ in breadth, a few up to 120.75μ $\times$ 41.4μ when examined in surface view.

2. Phellogen of tangentially-elongated meristematic cells.

3. Secondary cortex of several layers of tangentially elongated parenchyma cells most of which have starch or rosette aggregate-crystal contents, a few having brownish contents.

4. Phloem, a broad zone traversed by numerous phloem portions of medullary rays which are mostly 1 cell wide but up to 2 cells in width and vary in their path from straight to curved, forming convergent groups in places.

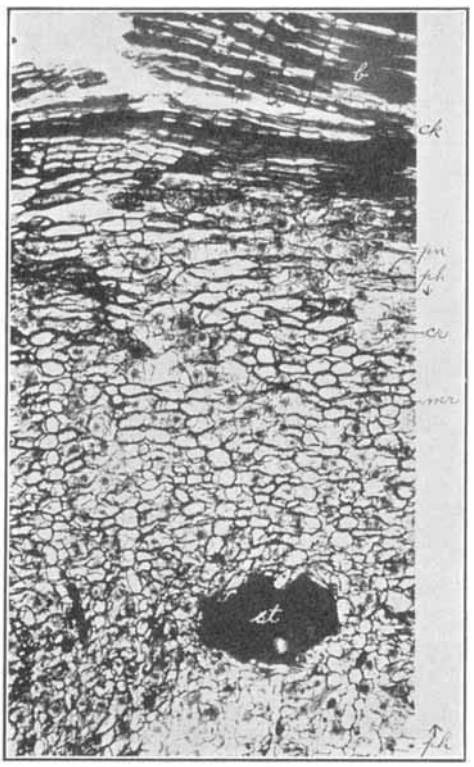

Fig. 6.—Photomicrograph of a cross section of the root bark of *Viburnum Opulus* var. *americanum* obtained from the oldest portion of a root, $\times$ 77, *b*, borke; *ck*, cork tissue; *pn*, a secondary phellogen; *ph*, phloem; *cr*, rosette aggregate of calcium oxalate; *mr*, phloem medullary ray; *st*, group of stone cells in phloem.

Scattered groups of stone cells and small islets or individual stone cells occur in the phloem, some intercepting medullary rays. The largest groups observed were up to 340μ $\times$ 170μ.

5. Cambium, wavy in outline, or meristematic cells.

6. Xylem, a broad porous, radiate zone traversed by xylem portions of medullary rays which separate numerous elongated wood wedges that are chiefly composed of straight rows of wood fibres and tracheæ of scalariform and pitted types. The medullary rays of the xylem are 1 to 2 cells in width.

The starch grains occurring in the parenchyma cells were simple, spheroidal, subspheroidal to occasionally ovate or 2-3 compound, more rarely up to 4-5 compound, the simple grains up to 10.62μ in diameter, some with distinct central, rounded to cleft hilum. The rosette aggregate crystals of calcium oxalate were up to 38μ in diameter but mostly up to 25μ.

POWDERED ROOT BARK.

The bark powdered was removed by the writer from a root system of an entire shrub growing near Falmouth, Michigan. It was passed through a No. 60 sieve. Both mill and sieve were thoroughly cleansed before the operation.

The powdered drug was grayish brown and exhibited the following histological elements: Numerous rosette aggregates of calcium oxalate from 10.62μ to 38μ in diameter together with angular to deltoid fragments of these crystals; numerous starch grains which were simple, spheroidal to ovoid and 2- to 3-compound, rarely up to 4- to 5-compound, the individual grains often showing a central spheroidal, angular or curved hilum and up to 10.62μ in diameter or length; numerous fragments of starch and crystal parenchyma, some cells of which contained

brownish amorphous contents; fragments of brownish cork with irregularly polygonal to rectangular shaped cells whose walls were more or less suberized to lignified, many with unevenly thickened walls, frequently up to 70.8μ x 28.32μ, occasionally up to 120.75μ x 41.4μ; stone cells of various shapes with lignified walls and prominent pore canals, up to 217.4μ x 53μ; few fragments of sclerenchyma fibres with walls thicker than lumen and giving a pink color with phoroglucin and HCl.

DESCRIPTION OF STEM BARK.

The bark of the stem occurs in strips, quills or chip-like fragments usually up to 1.5 mm. in thickness but occasionally up to 3 mm. As examined on the entire shrub allowed to dry in the laboratory, it was purple on young thin twigs, greenish to greenish brown or greenish yellow on young portions of main shoots and irregularly longitudinally wrinkled. Passing downward for a distance the next older bark was grayish brown with areas of silvery (light gray) lustre. Still older bark was grayish black to blackish and greenish to light brown to brown where abraded.

The lenticels were slightly raised, ovoid, circular to elliptic in shape and arranged both transversely and longitudinally. They were numerous in middle aged and older stem portions. The old bark toward the base of the stem displayed irregular brownish areas, many circular stem scars and cork exfoliating in this area.

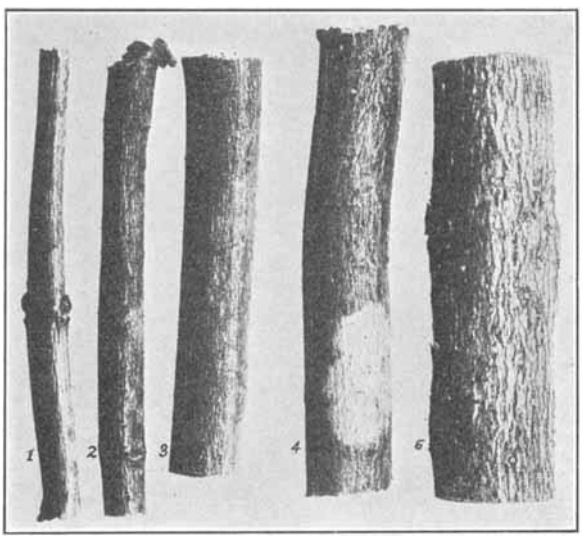

Fig. 7.—Stem bark of *Viburnum Opulus* Linné var. *americanum* (Miller) Aiton obtained from an entire shrub growing near Falmouth, Michigan. The numbers refer to successive year's growth of the stem from which the bark was taken.

The inner surface varied from greenish yellow to yellowish to rusty brown and was irregularly transversely to obliquely or longitudinally striated. The transverse and oblique striae were undulate. The fracture was short and weak, the fractured surface showing a brown to rusty brown outer bark, a greenish phelloderm and pale brown to yellowish inner bark. The odor of the dried bark was slight and characteristic but became strongly valeric-acid like upon trituration in a mortar with phosphoric or hydrochloric acid. The taste was bitter and moderately astringent.

When the inner surface of the stem back was treated with a 2% solution of $FeCl_3$, a dark green color resulted changing to black on drying.

When 0.5 Gm. of ground stem bark was boiled with 20 cc. of distilled water and filtered, a very pale yellow, clear filtrate resulted. When a portion of this filtrate was treated with 4 drops of a 2% solution of $FeCl_3$, a precipitate resulted which, when separated by passing the mixture through filter paper, was dark green to greenish

black while the filtrate was green. When to the second fraction of the first filtrate 2 drops of ammonium molybdate T. S. (U. S. P.) was added, a deep yellow color resulted. Both young and old stem barks were tested as above with similar results.

HISTOLOGY OF THE STEM.

A series of sections were studied which were cut through successive levels of the

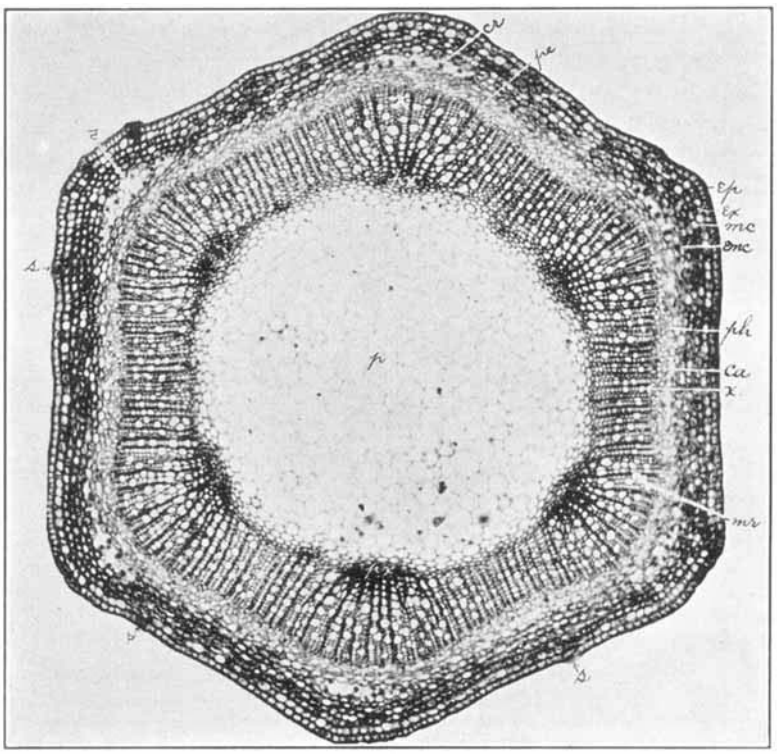

Fig. 8.—Photomicrograph of transverse section of stem of *Viburnum Opulus* var. *americanum* cut directly beneath terminal bud, × 52. *ep*, epidermis; *s*, stomata; *ex*, exocortex; *mc*, mediocortex; *enc*, endocortex; *pe*, pericycle; *ph*, phloem; *ca*, cambium; *x*, secondary xylem; *px*, primary xylem; *r*, right; *cr*, rosette aggregate of calcium oxalate; *p*, pith; *mr*, medullary ray in xylem.

stem starting just beneath the terminal bud and proceeding downward toward the base.

A. Just below Terminal Bud. (Early First Year Growth.)

The cross section was 6-angled with rounded angles. The following structures were noted:

1. Epidermis of rounded to slightly tangentially elongated cells with cutinized outer walls and scattered stomates. In regions of stem angles the epidermal cells tend to become radially elongate with convex outer walls.

2. Primary Cortex of 5 to 6 layers of largely chloroplastid containing cells consisting of an outer hypodermis or exocortex of more or less collenchymatous cells

which tend to be larger than most of the subjacent layers of the cortex, the latter being mostly parenchymatous. Rifts occur in the inner part of the mediocortex and are always seen in the angle regions where they harbor a number of rosette aggregates of calcium oxalate. Smaller rifts occur elsewhere in the cortex and pericycle. In the angle regions the cells of both exocortex and mediocortex (beyond the rifts) are collenchymatous. The endocortex (endodermis) is composed of a layer of parenchyma cells of averagely large size. Some of the outermost layer of cells of the cortex (hypodermis) show division through formation of a tangential wall through center (early formation of phellogen).

3. Pericycle of parenchyma cells resembling those of the endocortex. Here and there are to be noted the beginning of pericyclic fibers as isolated modified cells with empty lumen and clear glistening walls which show thickening.

4. Primary Phloem, a narrow zone of sieve tubes and phloem cells, traversed by narrow phloem medullary rays.

5. Cambium, 6-angled and composed of meristematic cells. The intrafascicular cambium had already been laid down.

6. Xylem. Broadest in regions of stem angles and showing numerous, narrow wood wedges composed of tracheids, wood fibres, scalariform and pitted tracheæ separated by narrow medullary rays.

7. Pith, a broad zone of thin-walled, pitted parenchyma cells and angular air spaces. Some of the pith cells contain rosette aggregates of calcium oxalate, a few monoclinic prisms, others spheroidal starch grains up to 10.62μ in diameter. In 50% alcohol mounts a number of the pith cells contain brownish sphæro-crystals, a few colorless rhombohedral crystals. Some of the cells of the conjunctive tissue surrounding the pith also show this last type.

The maximum thickness of the bark was 49.56μ, of the xylem 63.72μ.

Two Inches below Terminal Bud. (First Year's Growth.)

The outermost layer of cells of the cortex (hypodermis) has already divided to form a phellogen which by this time has cut off 3 to 4 layers of young cork cells on its outer face beneath the epidermis. Some of the young cork cells show crystalline contents when examined in alcohol mounts. The cells throughout the mediocortex have become collenchymatous. Rosette aggregates of calcium oxalate have made appearance in the phloem. Single sclerenchyma fibers and small groups of 2 or 3 usually occur at wide intervals in the pericycle. The contour of the margin of the stem and of the cambium is less angular. The xylem has been deepened through deposition of secondary xylem on the inner face of the cambium ring. The maximum thickness of the bark was 70.8μ, that of the xylem 106.2μ.

Five Inches from the Terminal Bud. (First Year's Growth.)

Cross section are practically circular in outline. The bark region had a maximum width of 416.5μ, the xylem 500μ.

The epidermis was still present but many of its cells contained air and were nearly ready to sluff off.

The subepidermal cork zone had been augmented by an additional layer or two of cork.

The phloem, pericycle and inner cortex regions showed an increased number of rosette aggregate crystals of calcium oxalate. The pericycle showed a few additional pericyclic fibres. The protophloem showed an occasional bast fibre with thick walls and narrow lumen. The pericyclic fibres were up to 31.86μ in width, the rosette aggregates up to 26.5μ. The cambium line was wavy in outline. The xylem showed considerably more secondary wood than in the previous section.

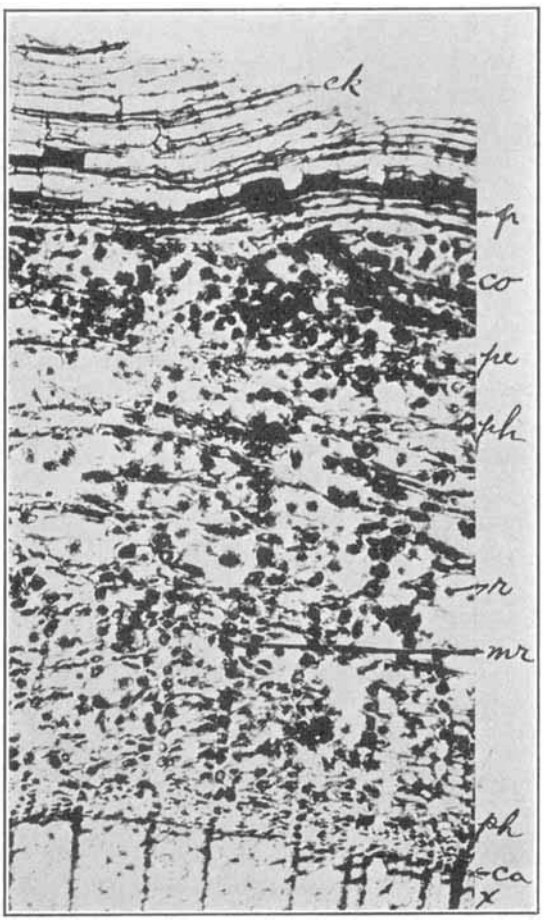

Fig. 9.—Photomicrograph of a cross section of the five-year stem bark of *Viburnum Opulus* **var.** *americanum*, × 80. *ck*, cork; *p*, phellogen; *co*, cortex; *pe*, pericycle; *ph*, phloem; *mr*, medullary ray; *r*, rosette aggregates of calcium oxalate.

Eight Inches from Terminal Bud. (Second Year's Growth.)

Cross sections were circular in outline. Maximum width of bark was 450.5μ, of xylem 1983μ.

The epidermis was in the process of exfoliation. The bark showed but slight increase in thickness over previous sections (34μ), but the xylem had increased materially in diameter. It showed wood fibres with bordered pores and scalariform and pitted tracheæ when examined in radial longitudinal section.

The pericyclic fibres and bast fibres showed increase in thickness of their walls, some fibres being up to 45μ in diameter.

Twelve Inches below Terminal Bud. (Three Year's Growth.)

Maximum thickness of bark 749.7μ, of xylem 2918μ. The epidermis has completely disappeared together with some of the outer cork layers. The walls of the cork cells have become suberized and to some extent lignified. Many of the cork cells show the greater thickening on their inner walls. They varied from 17.7μ to 71.8μ in length × 10.62μ to 24.78μ in width in cross sections and longitudinal radial sections.

The xylem elements showed increased lignification. The medullary rays were 1 to 2 cells wide in tangential longitudinal sections. Radial longitudinal sections showed occasional non-lignified pericyclic fibres but these were not seen in every section. A number of crystal fibres with rosette aggregates as well as isolated rosette aggregates occurred in the phloem and pericycle.

The tracheæ in cross sections were mostly arranged singly and in groups of 2, more rarely in groups of 3. The wood fibres in such sections occurred in radial rows. Some of the conjunctive tissue cells showed considerably lignification of their walls as observed in radial longitudinal sections.

The sections through the several successive regions of the stem so far discussed will serve to illustrate progressive steps in the development of the stems of this plant. All of them showed a bark which is similar to authentic commercial samples designated as "Young Cramp Bark."

Seven Feet below Terminal Bud. (Five Year's Growth.)

Maximum thickness of bark 1322μ; width of xylem 6.5 to 8.5 mm. Transverse sections show an outer zone of fissured cork of up to 8 or 9 layers of tangentially elongated cork cells whose inner walls are considerably thickened and lignified, the thickenings being irregularly wavy in character. These cells measured up to 95μ $\times$ 39μ. A phellogen, a cortex and pericycle of about 20 layers of tangentially elongated to rounded parenchyma cells and spaces in which regions are to be found many rifts. The parenchyma cells contain either rosette aggregates of calcium oxalate, minute starch grains or amorphous yellowish brown contents. Pericyclic fibres, as in the previous growth of the stem were only occasional and not seen in the majority of sections studied.

The phloem was broad and traversed by numerous medullary rays 1–2 cells wide which ran straight in some regions but tended to curve and converge in groups in their outer path. No bast fibres were observed in the phloem patches. Rosette aggregates of calcium oxalate were abundant in the phloem cells. The medullary ray cells had pitted walls and prominent large nuclei. The calcium oxalate crystals were up to 38.9μ in diameter being mostly under 35μ.

As in the previous sections the xylem showed the wood fibres largely arranged in radial rows. Radial longitudinal sections showed occasional non-lignified pericyclic fibres, numerous crystal fibres containing rosette aggregates in pericycle and phloem. The xylem in these sections showed scalariform and pitted tracheæ and tracheids as well as wood fibres with simple oblique pits and with bordered pits. Tangential sections showed the medullary rays to range from 1–2 rows of cells in width. The starch grains found in the phloem cells were spheroidal, ovate or ellipsoidal with central hilum and up to 14μ in diameter.

Stem of Six Year's Growth.

The microscopical features of these sections resembled those of the 5-year stem except that we note here the first occurrence of stone cells in the phloem. These are of a variety of shapes and sizes with lamellated walls showing varying degrees of lignification. Many are more or less isodiametric, others elongated and a number quite fibre-like but showing pore canals.

Cross sections cut at further levels downward show secondary phellogens arising in cortex, pericycle and outer phloem causing a sluffing off of the primary bark tissues. This accounts for the presence of pericyclic fibres and a moderate number of bast fibres in the young bark of commerce and their almost complete absence in commercial older barks.

HISTOLOGY OF OLD STEM BARK (10 YRS. OLD).

Sections were cut through the basal portion of the stem adjoining the root. The xylem of these showed 10 annual rings. The bark portion had a thickness of 1500μ.

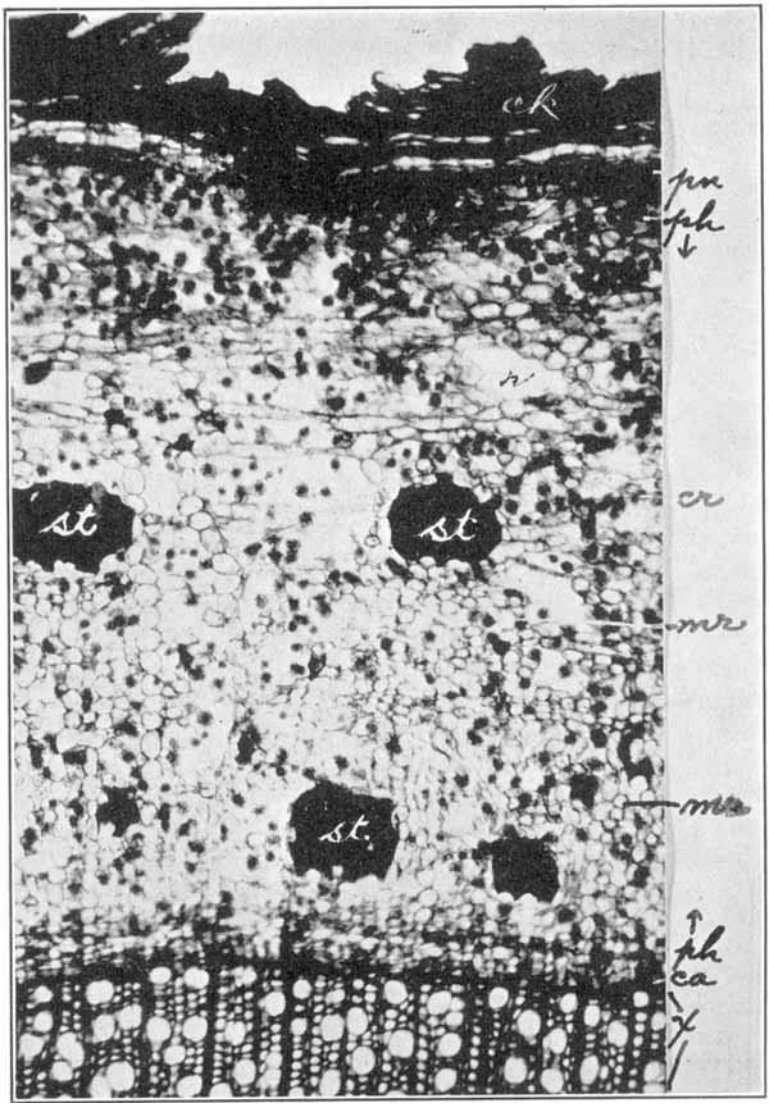

Fig. 10.—Photomicrograph of old stem bark of *Viburnum Opulus* **var.** *americanum* with adherent wood, as seen in cross section, × 81. *ck*, cork; *pn*, secondary phellogen; *ph*, phloem; *cr*, rosette crystal of calcium oxalate; *mr*, medullary ray; *ca*, cambium line; *x*, xylem; *r*, rift between parenchyma cells of phloem; *st*, groups of stone cells.

These sections showed a complete absence of cortex and pericycle. The following bark structures were noted passing from periphery to center:

1. Outer phloem region of dead cells or completely exfoliated in some sections.

2. Several layers of tabular cork cells whose tangential walls are for the greater part both suberized and lignified. While the radial walls of most of the cells observed were suberized, a few show lignification. Many of the cork cells showed ridge-like thickenings in their walls. The cork cells varied from 17.7μ to 95.5μ in length and from 10.62μ to 35.4μ in breadth.

3. A secondary phellogen of clear-looking meristematic cells in the process of division.

4. A broad secondary phloem separated into a number of irregularly oblong phloem patches by numerous medullary rays which run nearly straight in the inner region but which for the greater part pursue curved courses in the outer portion of the phloem. Most of the phloem patches consist of starch- and crystal-containing phloem parenchyma and sieve tubes but scattered here and there and intercepting medullary rays were observed some groups of sclerenchyma consisting of stone cells of variable size and shape some of which are elongated and fibre like.

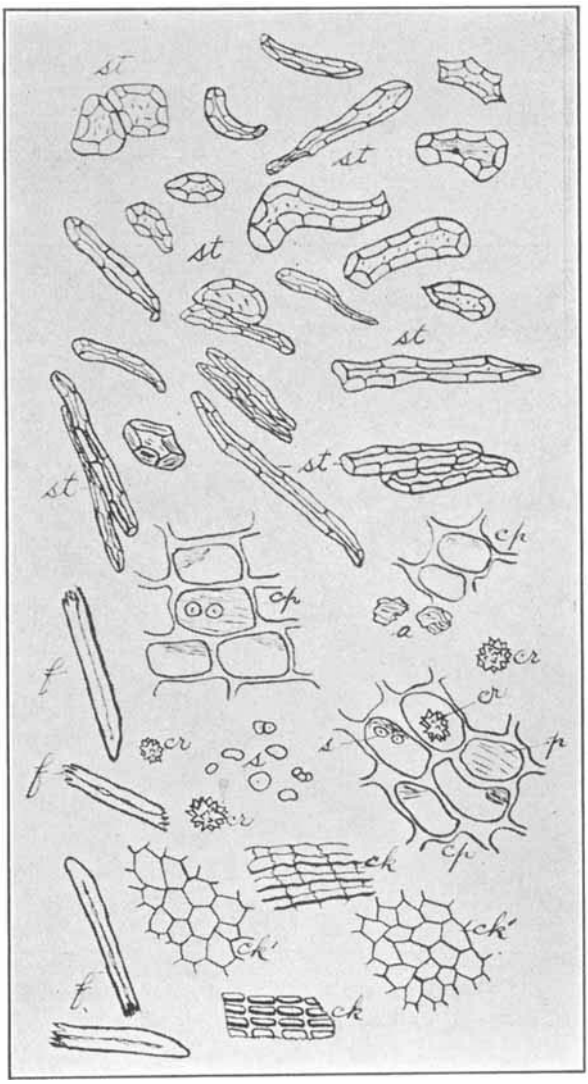

Fig. 11.—Powdered *Viburnum Opulus* var. *americanum* stem bark. *st*, stone cells; *cp*, cortical parenchyma; *a*, resinous material occurring in parenchyma of bark; *s*, starch grains; *cr*, rosette aggregates of calcium oxalate; *f*, pericyclcic fibers from young bark; *ck*, cork tissue in transverse section; *ck*, cork tissue in surface view.

The sclerenchyma groups are for the greater part widely scattered but occasionally are found fairly close, never however as numerous as in the older *Acer spicatum* stem bark. A few isolated stone cells are also present. In some sections the groups assumed a tier-like arrangement but, unlike the sclerenchyma fibres of *Acer spicatum* bark, had broad intervening spaces of soft bast between the series of groups.

A striking feature of these sections was the great abundance of rosette aggregates of calcium oxalate.

Radial-longitudinal sections showed many cells in the outer phloem with brownish to reddish contents and thick lamellated walls. These in some places were disposed in groups, the groups appearing collenchymatous.

The cork cells were tangentially elongated and for the greater part were lignified on their tangential walls. Here and there a pericyclic fibre was imbedded in the borke mass. Calcium oxalate crystals in the form of rosette aggregates were very numerous, the larger crystals measuring up to 42.48μ in diameter. Most were up to 35.4μ. Stone cells of a large variety of shapes and thickenings occurred, some with hooked and crenated margins. Some of the stone cells were elongated and appeared like fibres but usually showed distinct pore canals. The stone cell groups measured were up to 1499μ in length and up to 416.5μ in width.

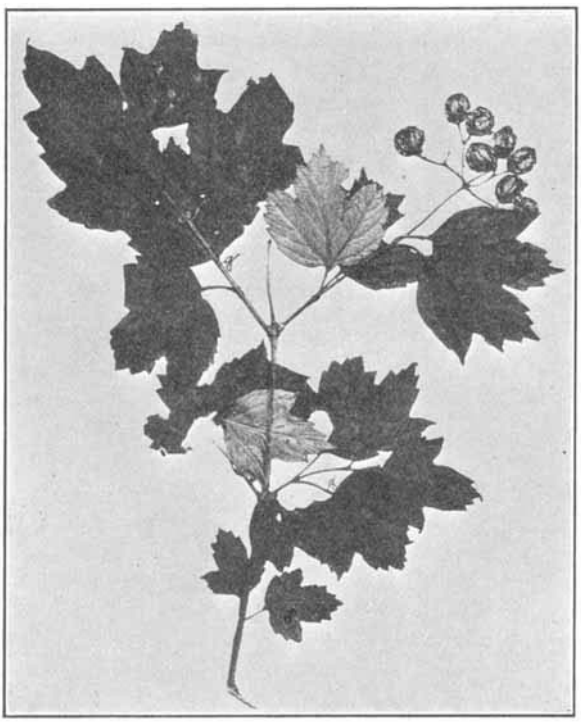

Fig. 12.—*Viburnum Opulus* L. Leaf and fruiting branch of European Cranberry bush × 1/2; g. petiolar glands.

The medullary rays traversed the other elements at right angles, their cells showing thick porous walls and starch, tannin or rosette crystals, occasionally reddish brown contents.

Tangential-longitudinal sections exhibited numerous medullary rays 1 cell in width and a scattering of others 1–2 cells in width, more rarely in some sections a medullary ray is seen 1–3 cells wide. In this type of section the medullary ray cells appear rounded ovate to ellipsoidal. The stone cell groups were up to 1450μ in length and up to 416.5μ in width. Some isolated stone cells of variable outline were also seen. The cork cells appear tangentially elongated and many show lignification on their tangential walls. Numerous crystal fibres containing rosette aggregates of calcium oxalate are present as well as isolated rosette aggregates, the latter occurring in many of the parenchyma and medullary ray cells.

POWDERED STEM BARK.

The powders studied were those of barks picked from young and older portions of stems of shrubs collected at Falmouth, Michigan, and from the same lot of plant material used in the description of the foregoing sections. The bark was carefully deprived of adherent wood and ground in a previously cleansed mill and passed through a 60-mesh sieve.

They were light grayish brown in color and showed the following elements: Numerous fragments of starch- and crystal-parenchyma and medullary ray cells, the latter with beaded

walls; starch grains mostly small, somewhat less numerous than in the root bark and frequently adherent to walls and dead protoplasm of parenchyma cells, the grains spheroidal to ovoid, very small and usually not exceeding 6μ; a few thick walled pericyclic fibres; numerous fragments of brownish cork tissue with cells of polygonal or tabular outline, the latter usually in layers and showing thickened tangential and occasionally radial walls, walls of cork cells suberized to lignified; the polygonal cork cells usually up to 70.8μ × 35.4μ, the tabular up to 106.20μ in length and up to 35.4μ in width; stone cells of a variety of shapes and degrees of lignification up to 124μ long and 35.4μ in breadth; numerous rosette aggregates of calcium oxalate up to 42.48μ in diameter; a few fragments of adherent wood elements (practically unavoidable in preparation) consisting of lignified wood fibres with oblique and bordered pits and scalariform and pitted tracheæ.

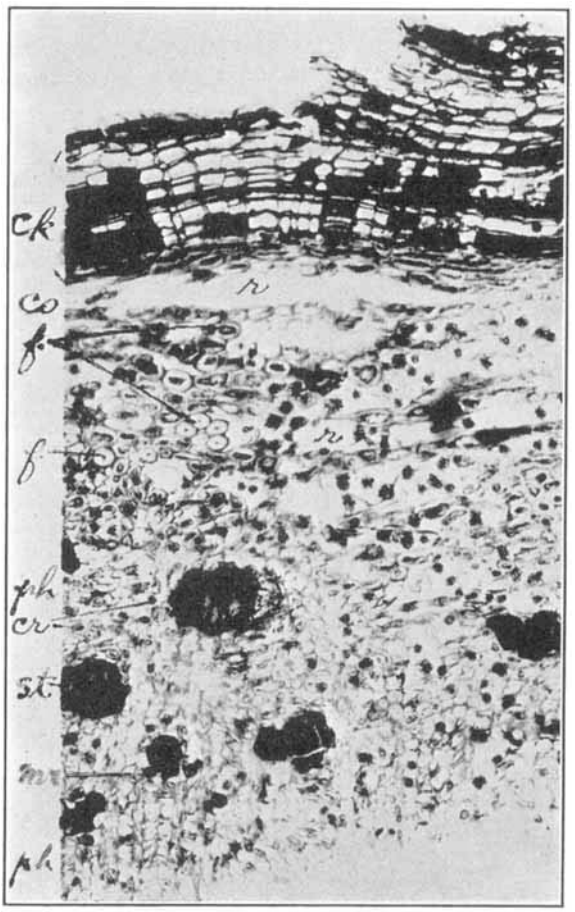

Fig. 13.—Photomicrograph of a cross section of 5-year stem bark of *Viburnum Opulus* (European species). × 80. *ck*, cork; *co*, cortex; *f*, sclerenchyma fibres in pericycle; *ph*, phloem; *st*, groups of stone cells; *mr*, medullary ray in phloem; *r*, rifts between parenchyma cells; *cr*, rosette aggregates of calcium oxalate.

DISTINCTIONS BETWEEN VIBURNUM OPULUS VAR. AMERICANUM AND VIBURNUM OPULUS (EUROPEAN).

For this purpose the writer compared shrubs of both the American and European *V. Opulus* plants growing in adjacent plots in the Arnold Arboretum, herbarium sheets of both kinds of plants grown in Europe and America and sections of stems of the same age collected from American and European varieties with the following results:

The petiolar groove of *V. Opulus* var. *americanum* is more flattened and open whereas that of *V. Opulus* is narrow and often closed to the very top. When the petiole of *V. Opulus americanum* dries up and shrivels, the groove becomes narrower, hence this difference is best noted on fresh material.

The petiolar glands (at apex of petiole) of *V. Opulus* var. *americanum* are smaller and more numerous whereas those of *V. Opulus* are fewer (usually only 2) and larger.

The fruits of *V. Opulus* var. *americanum* are short ellipsoidal and averagely slightly larger whereas the fruits of *V. Opulus* are subglobose, averagely somewhat

smaller. The former are edible and devoid of the bitter taste which characterizes the fruits of the latter.

The stem of the 5th year's growth of *V. Opulus americanum* usually possesses very few non-lignified sclerenchyma fibres in the pericycle and few or no stone cells in the phloem whereas that of *V. Opulus* shows many small groups of non-lignified sclerenchyma fibres in the pericycle and numerous groups of stone cells and some isolated ones in the phloem.

The cork of this year's stem of *V. Opulus americanum* possesses more layers showing lignified thickening of the inner tangential walls than that of European *V. Opulus*. The cork of the latter tends to have alternate zones of non-lignified cells between zones showing lignification.

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