A CHEMICAL EXAMINATION

OF THE

WOOD OF THE ROOT OF CELASTRUS SCANDENS

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Description of the Plant:

Scientific name:

Celastrus scandens Linne (Natural order Celastraceae).

Sex, Syst. Pentandria Monogynia.

Derivation of the scientific name:

Celastrus: from "kelastras", an ancient Greek name for an evergreen tree.[1]

Scandens: from the Latin "scandens" meaning climbing, which refers to the twining, climbing habit of the plant.[1]

Synonyms:

American bittersweet,[2] false bittersweet,[2] climbing bittersweet,[2] shrubby bittersweet,[2] fevertwig,[2] fever-twitch,[2] staff tree,[2] climbing staff tree,[2] staff vine,[2] waxwork,[2] Roxbury waxwork,[2] yellow root,[2] climbing orange-root,[2] Jacob's ladder,[2] Celastre, F.;[3] Celaster, G..[3]

Habitat:[4]

This shrub is indigenous to North America. It is found in woods and thickets along streams or in damp rich soil. Its range is from Ontario west to Manitoba in southeastern Canada and south to North Carolina and New Mexico.

1) Merck's Index, 1907, p. 129;
2) Misc. Publication 77, U.S. Dept. of Agriculture, p.4;
3) The National Dispens., 1, p. 370;
4) Habitat and description:
   Misc. Publication 77, U.S. Dept. of Agriculture, p.4;
   Eclectic Dispens., 1, p. 113;
   Am. Dispens., 6, p.209;
   National Dispens., 3, p.407;
   King's Am. Dispens., 18, p. 476;

Description:[4]

American bittersweet is a twining shrub which winds itself about hedges, rocks, and trees and ascends to great heights. Its woody twining stem is without thorns or prickles.

The leaves of this shrub are alternate, thin, smooth, oblong, acuminate, serrate, stipulate and petiolate. The greenish-white or yellowish-white flowers exist in small axillary racemes. These fragrant, dioecious flowers have a flat, five-lobed calyx and a corolla of five sessile petals. The stamens stand around a five-toothed, glandular disk. The style is thick, and the stigma is three-cleft. The fruit, a roundish orange-yellow, three-valved capsule, contains from three to six seeds which are covered with a scarlet red arillus. The fruit opens in the autumn and discloses the scarlet-red seeds. The capsules remain on the plant even during part of the winter.

The root bark, the portion of the plant which has been used medicinally, occurs in irregular thin quills which are nearly smooth. Externally it brown or dark orange brown. This layer is rather tough and separates concentrically showing another bright orange colored layer which when removed discloses another concentric layer. This layer is white and fragile, and the internal surface is finely striate. The stem bark resembles the root bark but is of an "ash-gray" or brown-gray color. The root bark is practically odorless but possess a bitter-sweet taste. The wood is tough and fibrous.

The bark of the root has been used medicinally. it has never been a popular remedy and at no time have any preparations containing it been official. Several remedial actions have been at-

tributed to the drug.

It is said to be alterative, diaphoretic, and diuretic with some narcotic powers. It and its preparations have been used in "scrofula, secondary syphilis, chronic hepatic affections, leucorrhea, rheumatism and obstructed menstruation; an ointment has successfully used in inflamed and indurated breasts of nurses, in pruregо of the vulva, burns, excoriations, etc.."[5]

In domestic practice it is said to be used as an emetic, discutient, and anti-syphilitic.[6]

According to Ugolino Mosso (Revista Clinica 1891) a white crystalline substance isolated from the bark and called Celastrine arrests a frog's heart in systole. It has no marked effect on the blood pressure in mammals, and only through its action on the vagus does it affect respiration. Its stimulant actiom is not followed by a secondary depression and manifests itself on the brain. When used, it is followed by a marked rise in temperature.[7]

Three preparations containing the drug have been employed. The first is "Decoctum Celastri" which was given in doses 2-4 fluidounces, three times a day. The extract was administered in 5-10 grain doses.[8] The third is Syrupus Rumecis Compositus often known as Scrofulous Syrup. It contains yellow dock root, bark of American Ivy, figwort, and the root bark of false bittersweet.[9]

5) Am. Dispens., 6, p. 209;
6) Nat. Dispens., 3, p. 407;
7) Dispens. of the U.S.A., 19, p.1438;
8) Am. Dispens., 6, p.209;
9) Ibid., 10, p. 1208;

Few references are found in the literature concerning the constituents of Celastrus scandens. These are listed here.

E.S. Wayne on Cincinnati claimed the discovery of a neutral principle which occurs in masses on minute white crystals resembling chloral hydrate. He called this substance Celastrine. (1872).[10]

C.H. Bernhard examined the bark in 1882. He found an acid, neutral resin, starch, glucose, gum, a caoutchouc-like body, coloring matter and volatile oil. He reported the ash content as 7.52% and found the ash to contain sulphuric, hydrochloric, phosphoric and silicic acids, potassium, sodium, magnesium, calcium and iron. When dried for four hours at 200° F. the bark lost 11.62% of water.[11]

In 1891 J. Hoch reported the moisture content as 12.2% and the ash as 7.15%. He found no alkaloids or glucosides, but several resins, tannin, a vegetable acid, glucose, starch, and an orange-red coloring matter.[12]

In 1896 J.A. Keller reported his studies of the solubility and the reactions of the coloring matter of the aril of the seed of Celastrus scandens.[13]

In 1907 A.A. Wells and G.S. Reeder reported the following constituents in the fruit of Celastrus scandens: Fructose, tar-

10) Am. Jr. of Pharm., 44, p.133;
11) Ibid, 54, p.1;(Proc. A. Ph. A., 30, p.248;
12) Ibid, 63. p. 523;
13) Ibid, 68, p. 183;(Proc. A.Ph.A., 44, p. 639;

taric, gallic and oxalic acids, a coloring matter, yellow, which they called fisetin, and a chocolate-brown resinous substance. In the seed they found fixed oils, one portion of which was soluble and the other portion insoluble in alcohol. Both contain olein and palmitin but in different proportions.[14]

In 1934 H. Kimbel[15] worked upon the bark of the stem. He reported the presence of galactose, identified by its phenyl hydrazone, and a white crystalline substance, non sugar in character, which melted at 184° C.. In a similar study of the wood of the stem carried on at the same time, A. Ruzeck[16] isolated the same white crystalline product which melted at 183°-186° C. This substance was supposed by both Kimbel and Ruzeck to be dulcitol. It seems not improbable that the white crystalline material isolated by Wayne in 1772, and which he and others called Celastrine, is in reality identical with that found by Kimbel and Ruzeck, namely dulcitol.

14) Chem. News. 95, p.199;(Proc. A.Ph.A., 56, p.247;
15) Jr. A.Ph.A., 23, p. 873;
16) Ibid, 23, p. 873;

Experimental.

The entire plant of Celastrus scandens was obtained from the estate of Dr. Edward Kremers, at the Highlands west of Madison, Wisconsin. The plants were dug up in early October while the leaves were still green. The parts of the plant, i.e. the roots, leaves, stems and fruits, were distributed, each to a different student, for investigation. The wood of the root was allotted to the writer.

The roots were air-dried before further examination. The bark was then stripped from the wood. It was found that this could be done most efficiently by scraping the root with a pocket knife, the blade of which should be held almost perpendicular to the root. By exerting a slight pressure when scraping the bark chipped off quite readily. The wood thus prepared was again allowed to dry in the air to insure a minimum moisture content. The wood was tough and fibrous. Its color ranged from nearly white to very pale yellow. The roots ranged from 1/8 of an inch to 3/8 of an inch in diameter. These root sections were cut into pieces about an inch and a half long and powdered in a high speed rotary mill.

Moisture determination:

1. Drying in an oven:

Two samples of the air-dried powdered drug were each accurately weighed and dried to constant weight in an electric oven at a temperature of 80° C.. The results of these determinations were:

	sample 1	sample 11
Weight of crucible and sample	11.2210 Gm.	11.6700 Gm.
Weight of crucible	8.9772 Gm.	9.5680 Gm.
Weight of air-dried sample	2.2438 Gm.	2.1020 Gm.
Weight of crucible and sample after oven drying	11.1240 Gm.	11.5772 Gm.
Loss in weight due to drying	0.0970 Gm.	0.0928 Gm.
Percent of moisture	4.32%	4.41%

11. Xylene method:

The accurately weighed, air-dried, powdered wood was placed into a 500 cc. round bottom flask and covered with 100 cc. of xylene saturated with water. The flask was connected with a Dean-Stark apparatus and the mixture was refluxed until no more water was collected. The results of this determination were:

Weight of the sample	10Gms.
Amount of water collected	0.44 cc. equivalent to 0.44 Gm.
Percent of moisture	4.4%

Ash Determination:

Total ash:

Two samples of the powdered air-dried wood were placed into tared crucibles and accurately weighed. The wood was first charred and then burned until a white ash was obtained. The crucibles and their contents were cooled in a desiccator and then reweighed. The amount of total ash was determined. The results of these determinations were:

A. Determinations made during a period of dry weather.

	sample 1	sample 2
Weight of crucible and sample	9.5446 Gm.	9.4432 Gm.
Weight of crucible	8.9214	8.8394
Weight of sample	0.6232	0.6038
Weight of crucible and ash	8.9350	8.8524
Weight of ash	0.0136	0.0130
Percentage of ash	2.19%	2.15%

B. Determinations made during several days of rain.

	sample 1	sample 2
Weight of crucible and sample	19.3332 Gm.	25.0240 Gm.
Weight of crucible	16.7860	23.2200
Weight of sample	2.5472	1.8040
Weight of crucible and ash	16.8323	23.2525
Weight of ash	0.0463	0.0325
Percentage of ash	1.82%	1.80%

Acid insoluble ash:

Samples A-1 and A-2 used in determining total ash were each boiled with 25 cc. of diluted hydrochloric acid for five minutes. The solutions were filtered through ashless filter paper. The filters were ignited in a manner similar to that used in the total ash determination. When the ignition was completed, the crucibles and their contents were cooled in a desiccator and weighed. The acid insoluble ash was determined. The results were as follows:

	sample 1	sample 2
Weight of crucible and sample	19.5446 Gm.	9.4432 Gm.
Weight of crucible	8.9214	8.8394
weight of sample	0.6232	0.6038
weight of crucible and acid insoluble ash	8.9236	8.8416
Weight of acid insoluble ash	0.0022	0.0022
Percentage of acid insoluble ash	0.349%	0.361%

Extraction With Selective Solvents.

Petroleum Ether Extract.

328 grams of the powdered, air-dried root were placed in a percolator arranged for continuous percolation. The sample was completely extracted with light petroleum ether(Skelly Sol B., B.P. 60°-70° C.). The solution was evaporated to dryness in a previously tared beaker. The resulting extract was syrupy in consistency and greenish-brown color. Its odor was agreeable.

Weight of beaker	28.2234 Gm.
Weight of beaker and petroleum ether extract	30.8344 Gm.
Weight of petroleum ether extract	2.6110 Gm.

Saponification Value of the Petroleum Ether Extract.

The saponification value of the petroleum ether extract was determined by heating an accurately weighed portion of the sample on a water bath with a known amount of standard alcoholic potassium hydroxide. The residual potassium hydroxide was titrated with standard acid and the saponification value was determined as directed in the U.S.P.X, page 457. The results of this determination were:

Weight of the sample	0.4730 Gm.
cc. of N/2 alcoholic KOH added	36.54
cc. of N/2 HCl used	34.95
cc. of N/2 alcoholic KOH actually used	1.59
Saponification value	94.29

Non Saponifiable Residue of the Petroleum Ether Extract.

A sample of the petroleum ether extract weighing 2.1380 grams was saponified as above. The volume of the resulting

solution was approximately doubled by the addition of an equal volume of distilled water. The mixture was placed into a separatory funnel and extracted with three successive 50 cc. portions of diethyl ether. The ether solution was placed into a tared beaker and allowed to evaporate spontaneously. The percentage of non saponifiable material was determined.

Weight of the sample	2.1380 Gm.
Weight of beaker and non saponifiable residue	57.1745 Gm.
Weight of the beaker	56.1245 Gm.
Weight of nonsaponifiable residue	1.05 Gm.
Percentage of non saponifiable residue	49.11%

Tests for sterols:

A portion of the nonsaponfiable residue was dissolved in alcohol. A solution of 1% digitonin was added to the solution of non saponifiable matter. A white precipitate resulted indicating the presence of sterols.

A small amount of the non saponifiable matter was dissolved in chloroform. The chloroformic solution was gently agitated with concentrated sulphuric acid. The chloroform layer became red whereas the sulphuric acid assumed a green fluorescence when held against a black background.

A sample of the nonsaponifiable matter was dissolved in a mixture of 2-3 drops of chloroform and 10 drops of acetic anhydride. Upon the addition of concentrated sulphuric acid drop by drop to the solution, a pink color appeared which upon the addition of more of the acid changed to blue and finally green.

These color tests indicate the presence of sterols.

Ether Extract.

The material previously extracted with petroleum ether was completely extracted with diethyl ether. The ether solution

was evaporated spontaneously in a tared beaker. The extract was a greenish-brown, thick liquid with an agreeable odor.

Weight of the beaker and extract	26.6400 Gm.
Weight of the beaker	26.0760 Gm.
Weight of the ether extract	0.5640 Gm.

Saponification value of the ether extract:

An accurately weighed sample of the extract was saponified in the same manner as the petroleum ether extract had been. The results of this determination were:

Weight of sample used	0.5640 Gm.
cc. N/2 alcoholic KOH added	36.54
cc. N/2 HCl added	34.29
cc. N/2 alcoholic KOH actually used	2.25
Saponification value	111.94

Alcoholic extract:

The material previously extracted with petroleum ether and diethyl ether was then extracted with alcohol. It was found experimentation and an examination of the results of previous work that best results could be obtained by extracting with approximately 75% alcohol. The resulting extract was a chocolate brown solid in which were embedded colorless rosette crystals. These crystals had a sweet taste and without recrystallization melted at 181° C..

Extraction of a second sample:

Extraction with petroleum ether:

875 grams of the powderedroot wood were packed in a percolator arranged for continuous percolation and extracted with Skelly Sol B as was the first sample. The extract was again a brown syrupy liquid with an agreeable odor. An accurately weighed sample was saponified and the saponification value was determined. The results were:

Weight of sample of extract	2.0700 Gm.
cc. N/2 alcoholic KOH added	24.84
cc. N/2 HCl used	17.10
cc. N/2 alcoholic KOH necessary to saponify the sample	7.74
Saponification value	104.92

The remainder of the extract was saponified and the non saponifiable portion was separated as previously.

Weight of material saponified	7.62 Gm.
Weight of beaker and nonsaponifiable matter	94.0920 Gm.
Weight of the beaker	89.5750 Gm.
Weight of non saponifiable matter	4.5170 Gm.
Percentage of nonsaponifiable matter	59.28%

Extraction with di-isopropyl ether:

The material previously extracted with Skelly SolB was extracted with di- isopropyl ether. The solution of the extract contained minute amounts of white fluffy.material. When the solution was evaporated to dryness, the extract resulting was a gray-green, fatty solid. Too small a quantity of the material was obtained to allow further investigation.

Extraction with Alcohol.

The sample was then extracted with 95% alcohol. When most of the extraction was completed, distilled water wass added in sufficient quantity to make the menstruum approximately 50% alcohol by volume because it had been noted that the crystals obtained from the previous extraction were more readily soluble in water than in alcohol.The solution was then placed into a distilling flask, and the alcohol was distilled from the solution by heating on a water bath. When most of the alcohol had been distilled off, the solution began to froth and was covered with a foam resembling soal bubbles. This action may have been due to the presence of saponins.

The solution thus condensed was placed into an evaporating dish and evaporated to dryness on a water bath. The dried extract was chocolate brown in color and was studded with small colorless, needle-shaped crystals. These crystals possessed a sweet taste.

Several crystals of the material were dissolved in water. The resulting solution did not reduce Fehling's solution indicating that the crystalline matter was not a reducing sugar.

About 0.5 Gm. of the crystalline matter was dissolved in a quantity of distilled water sufficient to make 100 cc. of the solution. The solution was hydrolized with a few drops of concentrated hydrochloric acid. The hydrochloric acid was neutralized with sodium carbonate. The solution was added to hot Fehling's solution, but again the Fehling's solution was not reduced.

Molisch's test was carried out upon a solution of the crystalline material. A few drops of 15% alcoholic solution of alpha naphthol were added to 5 cc. of the solution in a test tube. Concentrated sulphuric acid was carefully added to the mixture. No green or purple color appeared at the juncture of the liquids, indicating the absence on a carbohydrate in the crystalline material.

0.2 Gm. of the crystalline material, accurately weighed was dissolved in sufficient distilled water to make 20 cc. of solution. The optical activity of the solution was taken and the material was found to be optically inactive.

The remainder of the crystalline material had much of the brown colored material adhering to it and could not be obtained inpure form by recrystallizing from 95% alcohol or absolute

alcohol. It was found by experimentation that when the material waswwashed with methyl alcohol most of the colored material was dissolved and the crystalline material was not noticeably affected. The methyl alcohol solution was allowed to evaporate spontaneously and the residue obtained was reserved for further investigation. The crystalline material thus purified was dried and the melting point was determined. It melted at 188° C..

Thus far the material appears to be dulcitol. Several attempts were made to make the dibenzal derivative.[17] 2 grams of the crystalline material were mixed with 7 grams of benzaldehyde. Hydrochloric acid gas, previously dried by passing through sulphuric acid, was passed through the mixture, A reaction appeared to take place, the material went into solution in the benzaldehyde, and the reaction mixture became warm. The mixture was allowed to stand in the cold but no crystalline materiam separated.

Another method for forming the derivative was attempted. 1 gram of the material was mixed with 3.5 grams of benzaldehyde. 2cc. of concentrated hydrochloric acid were added to the mixture. The resulting mixture was well shaken and allowed to stand at subfreezing temperatures for several weeks, but no crystalline material separated.

1 gram of the crystalline material and 10 cc. of acetic anhydride were placed into an acetylization flask and carefully heated for a period of about an hour at which time all of the

17) Rosenthaler, L., "Der Nachweis organischer Verbindungen," p.109;

solid material had gone into solution. The hot solution was placed in a crystallizing dish but no acetyl derivative crystallized from the solution. The solution was evaporated to dryness on a water bath. The semi solid material was placed in a small amount of methyl alcohol and thoroughly mixed with it by the use of a spatula. A white crystalline material separated. This precipitate was filtered from the solution and the material washed with methyl alcohol. The white precipitate was dried and its melting point was found to be 163° C.. This melting point so closely approached galactose, identified by Kimbel[18] in the plant, that a small amount of the crystalline material was placed into some Fehling's solution and the mixture was heated. The Fehling's solution was not reduced. This indicated that the material was not a reducing sugar, so could not be galactose.

An accurately weighed sample of the dried material obtained above was refluxed with standard potassium hydroxide solution for ½ hour and the residual alkali was titrated with standard sulphuric acid. A blank titration was run, and the results showed that the material was saponified, indicating that some acetyl derivative of dulcitol had been obtained.

Weight of weighing bottle and sample	8.9685 Gm.
Weight of weighing bottle	8.8705 Gm.
Weight of sample	0.0980 Gm.
cc. of KOH solution added	24.9
cc. of .41908 N H_2SO_4 used	20.4
Blank Titration:	
cc. of KOH solution added	24.9
cc. of .41908 N H_2SO_4 used	22.7
cc. of KOH .41908 N for above reaction	2.3

18) Jr. A.Ph.A., 23, p. 873;

2.3 cc. KOH .41908N = 0.054083 Gm. of KOH

Thus 0.054 gram of potassium hydroxide were required to hydrolize 0.098 gram of the acetate. By means of a proportion it was computed that to hydrolize an equal weight of triacetyl dulcitol 0.0535 gram would be required.

$$0.098 : 308.22 :: x : 168$$
$$308.22 = 16.49634\ x$$
$$x = 0.05352 \text{ Gm.}$$

Calculating the molecular weight of the ester from the weight of potassium hydroxide required to hydrolize it gave a molecular weight of 305.02. The computed molecular weight of triacetyl dulcitol is 308.22.

From these results it is assumed that the product obtained is triacetyl dulcitol, M.P. 163° C.. No description of this derivative of dulcitol could be found in the literature.

The material obtained from the spontaneous evaporation of the methyl alcohol solution from the purification of the crystalline material was a reddish brown, thick syrupy mass. It possessed a sweet taste and was readily soluble in water. A small amount of the material was dissolved in distilled water and was added to a few cubic centimeters of hot Fehling's solution. The Fehling's solution was readily reduced indicating the presence of a reducing sugar.

Molisch's test was tried upon the material. A few drops of a 15% alcoholic solution of alpha naphthol were added to a solution of the material. A few cubic centimeters of concentrated sulphuric acid were added to the mixture and at the juncture of the liquids there appeared a green ring which upon gentle agitationturned purple. The test indicated the presence of a carbohydrate.

1.0 gram of the material was dissolved in 10 cubic centimeters of distilled water. To this solution there was added 1 cubic centimeter of freshly distilled phenylhydrazine and 1 cubic centimeter of glacial acetic acid. The mixture was heated on a water bath until a yellow precipitate was formed. This precipitate when examined under a microscope consisted of burr- shaped crystals. These crystals were carefully dried and the melting points of several samples were determined as:

sample 1	197° C.
sample 2	193°-194° C.

The crystalline form and the melting point of the osazone are similar to those of galactosazone and indicate the presence of galactose. The melting point of galactosazone is given as 193°-197° C..

APPROVED BY Nellie Lakeman

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