PRUNUS VIRGINIANA L

A BIOCHEMICAL STUDY

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A Thesis Submitted for the Degree of

BACHELOR OF SCIENCE

COURSE IN PHARMACY

University of Wisconsin

1915

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PREFACE

Of the five subfamilies of the rose family, representing a total of about 2000 species, viz.,the

(1) Spiraeoideae
(2) Pomoideae
(3) Rosoideae
(4) Prunoideae and
(5) Chrysobalanoideae

all but the last yield economically more or less important products.[1] Thus the first yields soap bark; the second the quince, apple and pear; the third the strawberry, raspberry, black berry, kusso, the roses and their valuable oils, the fourth the almond, peach, apricot, prune, plum, and the cherries. It is but natural that these products which have added to man's comfort for a thousand years and more, should have been made the subject of chemical research. But this investigation has not been restricted altogether to those plants yielding the more important economic products. Thus Ulmaria has made important contributions to plant chemistry and the mountain ash berry has been the subject of numerous investigations. Even many of those genera and species which have not been otherwise examined have been tested for cyanogenetic glucosides, i. E. for their capacity to yield hydrogen cyanide.

Inasmuch as the bark of the wild cherry is used for medicinal purposes on the American continent and has been official in eight editions of the U. S. Pharmacopoeia, the bark of Prunus virginiana L. (P. serotina Ehrh.) has been examined repeatedly.

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1. Wehmer, Die Pflanzenstoffe, pp. 273 to 355.

The leaves have been tested for the hydrogen cyanide which they yield upon distillation, and the seeds have been extracted for fatty oil. It seemed desirable, therefore, to learn something more about the chemistry of this species, more particularly of the fruit which grows so abundantly in different sections of this country. With this object in view the following examination was undertaken.

Bibliography.

Bastin, E. S. 1895

Some further observations on cherry barks.

Am. Journal Pharmacy, 67, p. 595. (Proc. A. Ph. A., 44, p. 623)

The author submits microscopic drawings of the bark of Prunus demissa Walters and agrees with Sargent that this western tree is but a "form", not even a distinct "variety" of the choke cherry, Prunus Virginiana. He also comments on the bitter taste, starch, and crystales of calcium oxalate, but does not otherwise refer to chemical constituents.

Cooley, G. E. 1897.

An investigation of the official Prunus Virginiana, to distinguish it from barks collected at other seasons.

Am. Journal Ph., 69, p. 414. (Proc. A. Ph. A., 46, p. 845)

The U. S. P. calls for the bark collected in fall. Inasmuch as the bark collected at other seasons cannot be distinguished histologically, chemical methods of distinction were tried. The starch content was found to be at its maximum in fall, the tannin content (color test) in spring. Tests for hydrocyanic acid and emulsin gave negative results.

Dohme, A. R. L., and Engelhardt, H. 1896.

Further experiments with wild cherry bark.

Pharm. Review, 14, P. 13. (Jahresb. d. Pharm., 31, p. 193.

The authors correct an error occuring in a previous publication on the hydrocyanic acid content and describe their laboratory method for assaying the bark.

Dohme, A. R. L., and Engelhardt, H. 1895.

Untersuchungen der Cortox Pruni Virginianae.

Pharm. Rundsch., 13, p. 260 (Jahresb. d. Pharm., 30, p. 154.

The authors found that, in conformity with the common assumption, and contrary to the statement of A. B. Stevens, the young green bark contained considerably more hydrocyanic acid than the thick brown bark.

Hawkins, L. W. 1889

Wild Cherry Bark and its Preparations.

Pharm. Journal, 49, p. 355. (Jahresb. d. Pharmacie, 24, p. 10.

The author made an investigation of the hydrocyanic acid content of commercial wild cherry bark and found that its content from various sources varied considerably.

Lemberger, J. L. 1871.

On Wild Cherry Bark.

Proc. A. Ph. A., 19, p. 503. (Year-Book Ph., 9, p.46.

The author found that the variation of the color of infusion of wild cherry bark is due to the existence of tannin in greater or less quantity.

Pawer, R. and Weimar, H. 1887.

On the Constituents of Wild Cherry Bark.

Pharm. Rundsch., 5, p. 203. (Jahresb. d. Pharm., 22, p. 24.

The authors report on the following substances:-

Amygdalin Bitter principle

Emulsin Fluorescent principle

Rother, R. 1887.

The Fluorescent Principle of Wild Cherry Bark.

Am. Journ. Pharm., 59, p. 286. (Pharm. Ztg., p. 484.

The author reports on the results of his investigations into the nature of the fluorescent principle.

Schimmel ' Co. 1890.

Oel der Rinde von Prunus Virginiana.

Bericht von Schimmel & Co., April, p. 48. (Jahresb. d. Pharm., 25, p. 35.)

An oil, which very much resembles the oil of bitter almonds, was obtained from the bark.

Stevens, A. W. 1900.

Wild Cherry Bark and its Preparations.

Am. Journ. Pharm., 72, p. 300. (Jahresb. d. Pharm., 35, p. 36;

The author finds that the inner layer of the bark contains practically all of the glucoside.

Stevens, A. B. 1899.

Wild Cherry Bark.

Pharm. Review, 17, p. 445. (Jahresb. d. Pharm., 35, p. 36.

As a result of numerous assays the author finds that wild cherry bark deteriorates with age and arrives at the following conclusions:

1.) The bark is best preserved in air tight "retainers".

2.) That only the fresh bark should be used in the manufacture of galenical preparations.

Stevens, A. B. and Judy, J. N. 1895.

The relative hydrocyanic acid content of the thick and thin bark of Pruna Virginiana.

Proc. A. Ph. A., 43, p. 226. (Pharm. Rundsh., 13, p. 204; Jahresb. d. Pharm., 30. p. 154;

The authors conclude that the thick bark has the higher hydrocyanic acid content.

Williams, J. L. 1875.

Bitter principle of wild cherry bark.

Am. Journ. Pharm., 47, p. 53. (Proc. A. Ph. A., 23, p. 209;

The author reports an attempt to isolate the bitter principle.

Review of the Chemistry of Prunus Virginiana.

The earliest chemical statement on record appears to be that made by Lemberger in 1871 who points out that the variation in the color of the infusion of wild cherry bark is due to the presence of tannin in greater or lesser extent.

In 1875 Williams attempted to isolate the bitter principle from the bark, but did not succeed.

In 1887 Power and Weimar report on the following constituents: amygdalin, emulsin, bitter principle, fluorescent principle, but none of these were isolated in pure or crystalline form.

No more successful were Rother's investigation into the nature of the fluorescent principle reported on the same year.

In 1889, Hawkins examined into the hydrogen cyanide content of commercial wild cherry bark and found that it varied considerably.

In 1890 Schimmel & Co., distilled from the bark an oil which very much resembled that of bitter almonds.

As the result of a microsocopic examination of the bark, Bastin in 1895 reported on the observation of starch and of crystals of calcium oxalate.

In 1895 and 1896, the hydrogen cyanide content of the bark was further investigated by stevens and Judy on the one hand and by Dohme and Engelhardt on the other. Stevens concludes that the thicker bark has the higher hydrogen cyanide content, whereas Dohme arrives at the conclusion that the

young, green bark, contains more than the thick brown bark. The latter describe (1896) in detail the assay process employed by them.

In 1897 Grace E. Cooley looked into the demand made by the U. S. Pharmacopoeia, that wild cherry bark should be collected in fall. She found the starch content to be at its maximum in fall, the tannin content (color test) in spring. Tests for the more important hydrogen cyanide and emulsin gave negative results.

A. B. Stevens, resuming his work in 1899, arrives at the conclusion that as to its hydrogen cyanide or glucoside content wild cherry bark deteriorates with age and that only fresh bark should be used in the manufacture of galenical preparations.

A year later A. W. Stevens reports that the inner layer of the bark contains practically all of the glucoside.

It thus becomes apparent that our knowledge of the chemistry of this species is practically restricted to the dried bark, the drug of the U. S. Pharmacopoeia and of the shops; that even of this no immediate constituents have been isolated in a pure state; and that our principal knowledge is restricted to the volatile oil, products of hydrolysis of the glucoside, which the bark unquestionably contains.

The Root

The only chemical examination thus far of the root is the hydrogen cyanide assay carried out by Dahme and Engelhardt in 1896, who found that a sample of root bark obtained from J. U. Lloyd contained 0.3423 p.c. of HCN. During the months of June, August and September, 1914, root bark was collected near Webster, S. D. The bark was thoroughly air dried. Later, in October, all three samples were ground to a No. 20 powder and the powder kept in glass stoppered bottles until assayed. The assay was carried out according to the method described by Dohme and Engelhardt in 1896,[1] five grams being used for each assay. The amounts of hydrogen cyanide computed on the percentage basis are herewith recorded:

	HCN Content	Computed as amygdalin
Bark collected in June.....	0.268 p.c.	4.533 p.c.
" " " August.....	0.333 "	5.632 "
" " " September..	0.375 "	6.356 "

It thus becomes apparent that the amygdalin content of the root bark increases with the advance of the season. It might be rash, however, to base a general conclusion on this single observation.

The above results were supplemented by the assays of root material, both wood and bark, collected near Madison, Wisconsin, in December, 1914, and April, 1915. The results

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1 Pharmacy Review, 14, p. 13.

obtained are herewith tabulated for comparison.

		HCN Content of Drug	HCN Content of dry material	Computed as amygdalin
Wood of root collected in December	(a)	0.064 P.C.	0.066 p.c.	1.128 p.c.
	(b)	0.069 "	0.073 "	1.235 "
Bark of root collected in December	(a)	0.375 "	0.391 "	6.617 "
	(b)	0.386 "	0.403 "	6.818 "
Wood of root collected in April	(a)	0.075 "	0.078 "	1.32 "
	(b)	0.075 "	0.078 "	1.32 "
Bark of root collected in April	(a)	0.375 "	0.391 "	6.617 "
	(b)	0.375 "	0.391 "	6.617 "

From these results the tentative conclusion may be drawn that the bark of the root contains much more glucoside than the wood of the root, and that the amygdalin content of the root bark increases with the advance of the season.

The Stem and Branches

Inasmuch as it is the bark that is used for medicinal purposes, it is this part that has received the greatest attention on the part of chemists.

That a cyanogenetic glucoside is present in the bark must have been recognized at an early date, for its use in the preparation of a syrup was undoubtedly based upon the formation of hydrogen cyanide in the preparation of this galenical. Nevertheless, the isolation of such a glucoside in crystalline form[2] was not accomplished by Power and Weimar in

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2 Comp. old editions of U. S. P. and U. S. Dispensatory.

1887, neither has it been accomplished since then.[3] Our belief in its presence, however, is well substantiated by the isolation of hydrocyanic acid by a number of investigations, and by the isolation of benzaldehyde by Schimmel & Co., in 1890.

Inasmuch as the hydrogen cyanide content of the bark (naturally in glucosidal combination) is regarded as an indication of the medicinal value of the bark, this aspect of the subject has possibly received most attention. It is of importance not only for the assay of commercial bark, but should likewise throw light on the best time of collection of the bark to be used as drug. Moreover, it may serve as a convenient means of ascertaining both qualitatively and quantitatively the amount of cyanogenetic glucoside in the different parts of the tree, and in the same part at different seasons of the year. Inasmuch as the conclusions drawn from the work have been more or less contradictory, it seemed best to tabulate all of the experimental data available.

Experimentor	HCN content	Remarks
Dohme & Engelhardt 1895	a)0.21 p.c. b)0.216 "	"Thin green bark."
	a)0.165 " b)0.167 "	"Thick brown bark."
	a)0.162 " b)0.100 " c)0.183 "	"Thin green bark."
	a)0.140 " b)0.156 " c)0.158 "	"Thick brown bark."

3 Comp. Hist. of publications of Wellcome Research Laboratory.

Experimentor	HCN content		Remarks
Stevens & Judy 1895	a)0.35 p.c.	Method I	
	b)0.345 "		
	c)0.34 "		
	d)0.323 "		"Thick brown bark"
	e)0.348 "		
	a)0.319 "	Method II	
	b)0.323 "		
	a)0.24 "	Method I	
	b)0.264 "		
	c)0.27 "		"Thin bark"
	d)0.268 "		
	a)0.229 "	Method II	
	b)0.238 "		
Dohme & Engelhardt 1896	a)0.1760 "	Lloyd,"Bark of Tree"	
	b)0.1736 "		
	a)0.1150 "	Lloyd,"Bark of twigs"	
	b)0.1170 "		"Thick and brown"
	a)0.0782 "	Squibb,"Common bark"	
	b)0.0831 "		
	a)0.0636 "	Muth,"Brown bark"	
	b)0.0738 "		
	a)0.1760 "	Lloyd,"Bark of tree"	
	b)0.1736 "		
	a)0.1565 "		
	b)0.1565 "	Higgins & Waters, "Rossed bark"	
	a)0.22 "	Squibb,"Young bark"	
	a)0.1418 "	Muth,"Virgin bark"	
	a)0.115 "	Lloyd,"Bark of twigs"	
	b)0.117 "		"Thin and green"
	a)0.116 "	Higgins & Waters "Green"	
	b)0.167 "		
	a)0.1565 "	Gilpin,Langdon & Co. "Green"	

Experimentor	HCN content	Remarks
Hawkins 1899	1)0.079 p.c. 2)0.082 " 3)0.137 " 4)0.107 " 5)0.160 " 6)0.133 "	Samples from different drug houses.

For the hydrogen cyanide assays recorded below, the material was collected partly near Webster, S. D. during the months of June, August and September, partly near Madison, Wisconsin, in December of 1914, and in April, 1915. The results are again tabulated.

	HCN Content	Computed as amygdalin.
Stem bark collected in June	0.150 p.c.	2.538 p.c.
Stem bark collected in August	0.137 "	2.311 "
Stem bark collected in Sept.	0.129 "	2.174 "
Outer bark of stem collected in December	a)0.078 " b)0.078 "	1.519 "
Inner bark of stem collected in December	a)0.335 " b)0.335 "	5.669 "
Wood of stem collected in December	a)0.022 " b)0.020 "	0.372 "
Bark of stem collected in April	a)0.186 "	3.147 "
	b)0.192 "	3.248 "
Wood of stem collected in April	a)0.039 "	0.659 "
	b)0.039 "	0.659 "
Bark of twigs collected in December	a)0.268 "	4.536 "
	b)0.279 "	4.622 "

	HCN content	Computed as amygdalin
Wood of twigs collected in December	a)0.034 p.c.) b)0.034 ")	0.575 p.c.
Bark of twigs collected in April	a)0.185 " b)0.186 "	3.130 " 3.147 "
Wood of twigs collected in April	a)0.039 " b)0.039 "	0.659 " 0.659 "

Not all of the above results are absolutely comparable without slight correction for material collected in June, August and September. Whereas the percentages computed for the materials are based on the ordinary drugs, all of the percentages computed for the December and April materials are based on absolutely dry material. For this purpose the moisture content was determined by the xylene method.

A comparison of the results for both ordinary drug and absolutely dry material is made possible by the following tabulation of data of the December material.

(See following page)

Part of tree used	Wt. of drug	Wt. of Moisture	Wt. of dry material	No. of cc of N/10 $AgNO_3$ req.	Pc. of HCN computed for Dry material	Drug
Outer bark	5.0 g	0.2 g	0.48 g	1.4 c.c.	0.075 p.c.	0.078 p.c.
Of stem	5.0 g	0.2 g	0.48 g	1.4 "	0.075 "	0.078 "
Inner bark	5.0 g	0.2 g	0.48 g	6.0 "	0.322 "	0.335 "
Of stem	5.0 g	0.2 g	0.48 g	6.0 "	0.322 "	0.335 "
Wood of	5.0 g	0.2 g	0.48 g	0.4 "	0.021 "	0.022 "
stem	5.0 g	0.2 g	0.48 g	0.36 "	0.019 "	0.020 "
Bark of	5.0 g	0.2 g	0.48 g	4.8 "	0.257 "	0.268 "
twigs	5.0 g	0.2 g	0.48 g	5.0 "	0.268 "	0.279 "
Wood of	5.0 g	0.2 g	0.48 g	0.6 "	0.032 "	0.034 "
twigs	5.0 g	0.2 g	0.48 g	0.6 "	0.032 "	0.034 "

A comparison of the South Dakota material would seem to indicate that the amygdalin content of the stem bark diminishes slightly with the advance of the season, whereas that of the root bark seemed to advance with the season. The record of earlier investigations do not reveal that the seasonal factor has been taken into consideration by them.

The wood, whether of the stem or of the twigs, evidently contains much less amygdalin than does the bark. This agrees with the observation made in connection with the wood and bark of the roots.

The inner bark of the stem contained much more amygdalin than the outer bark or cortical layer. This observation agrees with that previously made by Stevens.

Inasmuch as most of the earlier records are based on different varieties of commercial drug material, rather than with

reference to stem, branches, etc., any and all generalizations drawn must be taken with considerable allowance. The stem from which the Madison material was obtained was 31 mm. in diameter and revealed 13 annual rings.

Fruits.

So far as phytochemical literature throws any light on the subject, no chemical investigation of the fruit or any part thereof has been made. The fruit, however, is used for culinary purposes, more particularly for the preparation of home-made wine. The material for the investigation of the fruit was collected near Webster, S. D. The material for the preliminary investigation was collected in August, 1912. Larger amounts were obtained in 1914. The latter lot of material included pits that had been collected as a waste product in wine making. For the purpose of this investigation the pits (see plus hard endocarp) were separated from the pulp (mesocarp) and skins (epicarp). In one instance the seeds were removed from the cracked pits, i. e. the seeds were separated from the endocarp of the fruit. In one instance also the separation of the fruit into its parts was effected quantitatively.

Preparation of the Material.

The first lot of choke cherries used for the experimental part of this thesis was collected in August, 1912, near Webster, S. D. Being collected by hand, only the ripe or nearly ripe

fruits were taken. These were placed in small bags and allowed to stand for a day to insure thorough ripening. The berries were then separated from the leaves and twigs and thoroughly washed, dried and weighed. Fifteen pounds of the well ripened cherries were thus obtained.

Small portions of the cherries were thrown into hot water, which process softened them thoroughly, without, however, rupturing the skins. They were then thoroughly macerated by squeezing with the fingers, after which they were extracted for one half hour with boiling water.

The aqueous extract was separated from the pits and skins by straining and expressing through muslin bags, after which it was evaporated to a syrupy extract and this weighed.

The pits and skins were allowed to dry by exposure to sunlight, after which they were separated by hand and each weighed.

The results thus obtained from the 15 lbs. of cherries (6804 grams) are herewith tabulated.

Evaporated extract.....	1048 grams	(15.3 p.c.)
Pits.....	1531 "	(22.5 ")
Skins.....	369 "	(5.4 ")
Loss due to evaporation of water....	3857 "	(56.8 ")

The material collected in 1914, as already indicated, was of two kinds. The first kind consisted of about 50 lbs. of fresh fruits which were treated in like manner as those collected in 1912, but the quantities of the parts were not ascertained.

The second kind consisted of pits which had been separated from dregs (pits, skin and insoluble pulp) of choke cherry wine.

Ratio of Seeds to Pits.

In order to ascertain the ratio of seeds to pits, ten grams of the pits were carefully cracked and the seeds removed and weighed. The seeds constituted 27.6 p.c. of the pits.

Determination of the Hydrocyanic Acid Content of the Pits.

Fifteen grams of the pits were ground to a No. 40 powder in a drug mill. In order to insure the complete removal of the particles of seeds adhering to the sides of the mill, a small amount of ground marc of the pits (from which the cyanogenetic glucoside had been removed) was run through. The resulting powder represented exactly fifteen grams of the pits. This powder was carefully weighed and divided into three equal portions, each representing five grams of the pits. Each of these portions was then placed in an Erlenmeyer flask of 250 cc. capacity and macerated with 100 cc. of distilled water for forty-eight hours. Each sample was subjected to steam distillation in a special form of apparatus suggested by Dohme & Engelhardt (Pharmaceutical Review 14, p. 13). The distillate was collected in a solution of potassium hydroxide and titrated with $\frac{N}{10}$ $AgNO_3$. The number of cc. of $AgNO_3$ used, multiplied by two equal the actual amount of silver nitrate solution necessary, as one half of the silver nitrate solution was used in dissolving

the double salt which was formed. Sodium chloride was used as indicated.

The following tabulation shows the results obtained:

	I	II	III
Weight of pits taken	5 gms.	5 gms.	5 gms.
Amount of N/10 A_gNO_3 required for titration.....	1.3 cc.	1.3 cc.	1.3 cc.
Percentage of HCN in seeds.....	0.1395 p.c.	0.1395 p.c.	0.1395 pc.
Percent. of glucoside in seeds..	2.36 "	2.36 "	2.36 "

Extraction of seeds with ether.

A small portion of the pits were carefully cracked and exactly ten grams of seeds were separated therefrom. The seeds were ground finely in a mortar, transferred to a Soxhlet extraction apparatus and percolated with ether until exhausted. Upon evaporation of the ethereal extract 4.45 grams of oil were obtained, corresponding to 44.5 p. c. of the seeds, or 11.28 p.c. computed with reference to the pits, and 2.7 p.c. when computed with reference to the entire fresh fruit.

Examination of Seed and Endocarp (Pits).

The separation of pits into seed and endocarp being so unsatisfactory a task that demanded so much time without seeming to afford any particular advantage, practically all of the work here recorded was done on the pits.

Extraction of pits with ether: One hundred (100) grams of the pits were ground to a No. 70 powder in a drug mill. Ninety grams of this powder placed in a Soxhlet extraction apparatus and percolated with ether until exhausted of their oil

content. The ethereal extract was then set aside in a tared open dish and allowed to evaporate spontaneously until the weight was constant. The weight of oil thus obtained was 13.5 grams corresponding to 151 p.c. of the pits, or 54.34 p.c. when computed with reference to the seeds. This yield is much more in harmony with the yield of oil of other rosaceous seeds than the yield of 5 p.c. obtained by Betz according to the reports of Czapek.[1] The oil thus obtained was of a viscid consistency with a slightly benzyaldehyde odor and has a specific gravity of 0.916 at 20 degrees.

The iodine value was determined in accordance with the directions of the U. S. Pharmacopoeia.

Amount of oil used.....	0.3098 gms.
Amount of Iodine T.S. used.....	12.5 cc.
Amount of Mercuric Chloride TS. used...	12.5 cc.
Amount of Sodium Thiosulphate (Factor 1.15) used.....	17.6.cc.
Amount of Sodium Thiosulphate (factor 1.15) used in blank.....	40.7 cc.
Difference (= equivalent of amt. of iodine absorbed).....	23.1 cc.
Iodine number.....	107.9

Extraction of pits with heptane.

Seven hundred and fifty grams of the dry pits were ground to a No. 40 powder, the powder packed in a continuous perculator and extracted with heptane.[2] The extraction was

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1 Biochemie, Vol. 1, p. 119.

2 The heptane used for this experiment had been obtained from Jeffrey pine (fraction 16 of the investigation by Harmon) and had been purified in the following manner: 950 cc. of the fraction mentioned (sp. gr. 0.7004 at 20 degrees) were shaken repeatedly with 75 cc. of sulphuric acid until the acid no longer became colored. To bring this about the hydrocarbon fraction had to be shaken

continued for 18 hours. At the end of this time, the fatty oil content was found to be exhausted. The heptane solution of the oil was then subjected to a careful distillation for the recovery of a part of the heptane. The remainder was allowed to evaporate spontaneously, leaving the pure fatty oil. Seventy-six grams of oil were thus obtained corresponding to 10.01 per cent of the pits, or 36.7 p.c. when computed with reference to the seeds.

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eight times with the amount of acid mentioned. Thus purified, the hydrocarbon had a specific gravity of 0.6844 at 20 degrees. Twenty grams of sodium hydroxide were then added, the contents of the flask shaken occasionally during several hours and then set aside for forty-eight hours. Rectified by steam distillation, the hydrocarbon was found to have a specific gravity of 0.6842 at 20 degrees. When shaken with 50 cc. of a one per cent solution of patassium permanganate, the color of the latter disappeared. The process was repeated until the hydrocarbon showed no further action on the permanganate solution. The specific gravity of the heptane thus purified was found to be 0.6813 at 20 degrees, whereas according to Morley and Muir (Dictionary of Chemistry, Vol. II, p. 675) that of pure heptane is 0.6814 at 25 degrees.

The chemical constants of the oil were then determined: i.e. saponification number, iodine number and oxygen absorption value. The saponification number and iodine number were determined according to the method given in the United States Pharmacopoeia, p. 535 (eighth revision). The oxygen absorption value was determined by the Lewkowitsch method which consists in spreading thin films of oil on tared glass plates and exposing to air for a given length of time (in this case 16 days) or until the gain in weight remains constant.

The specific gravity of the oil determined by means of a pycnometer at 20 degrees C was found to be 0.9182. The refractive index (Abbe white light refractometer) at 23 degrees C was found to be 1.480. The oil did not congeal at a temperature of -15 degrees C.

The following tabulations show the results obtained:-

Saponification Number (Koettstorfer)	I	II
Amt. of oil used	2.1134 gms	1.5656
Amt. of N/2 alc. KOH for saponificat...	25 cc.	25 cc.
Amt. of HCl, factor 1.56 for back titration	10.6 "	11.8 "
Amt. of N/2 KOH consumed in saponificat.	9.4 "	7.0 "
Saponification value	193	193

Iodine Absorption Number	I	II
Amt. of oil used	0.3038 gms	0.3618 gms
Amt. of iodine T.S. used	12.5 cc.	12.5 cc.
Amt. of $HgCl_2$ T.S.	12.5 cc.	12.5 cc.
Amt. of sodium thiosulphate, factor 1.12 used	18.9 cc.	15 cc.
Amt. of sodium thiosulphate used in blank test	39.8 cc.	39.8 cc.
Difference (= equivalent of the amt. of iodine absorbed)	20.9 cc.	24.8 cc.
Iodine number	96.9	96.5

Oxygen Absorption Value	I	II
Amt. of oil used	0.1324 gms.	0.1164 gms.
amt. after exposure	0.1340 "	0.1179 "
Gain in weight	0.0016 "	0.0015 "
Oxygen absorption value	0.11+ p.c.	0.12+ p.c.

Isolation of Oleic Acid.

The oleic acid was isolated from the oil in the following manner: 56 grams of the oil were mixed with one half their weight of litharge and twice their volume of water added. The mixture was digested on a water bath until oily globules

disappeared. The mixture of lead salts of the fatty acids was dried and the dry mixture extracted with hot and cold ether to dissolve the lead oleate. The ethereal solution was decanted and shaken with dilute hydrochloric acid until the aqueous layer remained distinctly acid. Lead chloride was thus precipitated, leaving free oleic acid in the ethereal solution. The ethereal solution was separated, washed with water, and evaporated at a low temperature. The weight of oleic acid thus obtained was 28 grams corresponding to 50 p.c. of the oil. The specific gravity of the oleic acid was found to be 0.9008 at 20 degrees C.

The acid was subjected to low temperature and no separation occurred until -2 degrees C., when a small amount of a whitish solid separated therefrom. At -5 degrees C., it was completely solidified to a yellowish white mass.

The pits collected in 1914 were likewise extracted with heptane. Twenty-four hundred and ninety grams of ground pits (No. 40 powder) yielded upon extraction in a cylindrical percolator and subsequent recovery by distillation of the solvent, 270 grams of a fatty oil, i.e. 10.8 p.c. with reference to the pits. The viscid oil has a golden yellow color, an almond-like taste, and a decidedly fatty odor (?). Its specific gravity determined at 18 degrees was 0.9190 and its index of refraction, determined at 15 degrees with an Abbe refractometer, 1.4771.

Free acids.

The amount of free acids in the 1914 oil was ascertained by a method suggested by Lewkowitsch[1], which consists in titrating a mixture of the oil and alcohol with N/10 potassium hydroxide. Five grams of the oil required 1.5 milligrams of potassium hydroxide for neutralization, corresponding to 0.30% of free fatty acids, computed as oleic acid. In another determination five grams of the oil required 1.78 milligrams of N/10 potassium hydroxide corresponding to 0.36% of free fatty acids.

Saponification Value (Koettstorfer number)

This is the number of milligrams of potassium hydroxide required for saponifying one gram of the fat and is a measure of the glycerides of fatty acids which the oil contains. The saponification was conducted in the usual manner by heating a weighed quantity of the fixed oil with a definite volume of standard alcoholic potassium hydroxide and titrating back the excess of alkali with N/2 hydrochloric acid solution. Four determinations of the saponification value of the oil were made for comparative results. The usual time allotted for saponification values is thirty minutes, but for the sake of comparison, one of the saponifications was allowed fifteen minutes while another was allowed forty-five minutes. The saponification value of the latter compared favorably with those that were conducted for the usual length of time, while the value of the former

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1 Technology of oils, fats and waxes, Vol. I, p. 345.

was slightly lower showing that at least thirty minutes must be allowed for a complete saponification.

Saponification Value.

15 m.	185.8
30 m.	191.5
30 m.	190.4
45 m.	189.9

Iodine Absorption Value

The iodine value is a measure of the unsaturated fatty acids or their glycerol esters contained in fatty oil. It is the property of all unsaturated fatty acids to take up iodine by direct addition the amount absorbed depending upon the number of double bonds which they contain.

The iodine value of the cherry pit oil was determined by the method suggested by Hubl, which is recognized as official by the U. S. Pharmacopoeia.[1] Two determinations were made, the time limit in each case being four hours. In one case the iodine value (Hubl) number was found to be 109.3 and in the other 109.5.

The Volatile Acids.

The volatile acid number represents the number of cubic centimeters of N/10 caustic potash required for neutralizing the volatile acids liberated from five grams of a sample of fat or oil under special conditions.

The determination was conducted by the Leffman-Beam method

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1 8th revision, p. 527.

suggested by the Association of Official Agricultural Chemists.[1] This method consists in saponifying a weighed portion of the oil with glycerol soda solution, then adding sulphuric acid solution to liberate the free fatty acids and finally distilling and titrating 100 cc. of the distillate with N/10 potassium hydroxide. The number of cubic centimeters of N/10 potassium hydroxide required for neutralizing the volatile acids from 5 grams of the fatty oil was 1.42 cc. in one case and 1.54 cc. in the other. The results indicate that the oil contains but a small percentage of the lower volatile acids.

Acetyl Value.

This is a measure of the amount of hydroxyl groups which a fat contains; its value depends upon the fact that compounds containing alcoholic hydroxyl groups react with acetic anhydride to replace the H of the hydroxyl by the acetyl group (CH_3CO). Upon saponifying this ester with caustic potash, potassium acetate is formed and by determining the number of milligrams of potassium hydroxide thus combined, the acetyl value is obtained. The method employed is that recommended by the Association of Official Agricultural Chemists[1] which consists in heating the oil with an equal volume of acetic acid anhydride until acetylization has taken place and washing the acetylized product until free from acetic acid. A weighed amount of the acetylized product is then saponified with a definite volume of standard alcoholic potash and the fatty

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1 Bureau of Chemistry, United States Department of Agriculture, Bulletin 107, 1910, p. 141.

acids liberated by the addition of a known amount of sulphuric acid. After filtering off the fatty acids the filtrate is treated with N/10 potassium hydroxide.

The results of the determination are herewith recorded.

Exper.	Amt. of oil used	cc. of KOH consumed.	Acetyl value
I	2.1442 gm.	34.2 cc.	81.6
II	2.1432 "	35.3 "	84.2

Unsaponifiable Residue.

In addition to the triatomic alcohol, glycerol, all fats contain small quantities of the monatomic alcohols cholesterol or phytosterol, the former being found in animal fats and the latter in fatty oils obtained from plants. To determine the amount of phytosterol, the method suggested by Haas & Hill[1] was employed. This consists in saponifying 5 grams of the oil with alcoholic potassium hydroxide, and after evaporating the alcohol the residual soap is dissolved in hot water and shaken out with ether. The ethereal solution is then separated and transferred to a weighed flask and the ether evaporated. The gain in weight of the flask was 0.125 grams; corresponding to 2.36 p.c. of unsaponifiable residue calculated as phytosterol.

Soluble Acids.

The percentage of soluble acids was also determined by the method suggested by the Association of Official Agricultural

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1 An introduction to the Chemistry of Plant Products, 1912, p.15.

Chemists.[1] This consists in liberating the fatty acids from the saponified oil by the addition of a definite volume of N/2 Hcl. After washing the fatty acids several times with hot water, they are separated and the aqueous filtrate titrated with N/10 potassium hydroxide. The oil contained no soluble acids.

Insoluble Acids.

The insoluble acids (Hehner Value) was determined by the method suggested by the Association of Official Agricultural Chemists.[2] It consists in separating and drying of the insoluble acids which separated on the addition of the N/2 acid and in the determination of the soluble acids. To insure a complete removal of the fatty acid adhering to the sides of the flask, in which the soluble acid determination is conducted, it should be rinsed with absolute alcohol and the washings added to the weighed crystallizing dish containing the insoluble acids. After the evaporation of the alcohol, it was found that the crystallizing dish gained 0.708 grams in weight corresponding to 94.87 p.c. of insoluble acids.

The pits obtained from the production of wine were not extracted for lack of time.

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1 Ibidem, p. 138.
2 Ibidem, p. 139.

Extraction of the Pits with Alcohol.

After the fatty oil had been extracted from the pits with heptane, the marc was exhausted with alcohol by repeated extraction. The 1912 material thus extracted yielded 13 grams of solid extract corresponding to 1.867 p. c. of the pits or 6.28 p. c. of the seeds. Two saponification experiments revealed saponification values of 229 and 230 respectively.

The preliminary saponification test having revealed the presence of considerable ester or free acid and the entire alcoholic extract obtained was saponified with alcoholic potassium hydroxide. The alcohol was evaporated, leaving a non-alcoholic saponified aqueous alkaline solution which was shaken with ether until the non-acid constituents were completely removed. The ethereal solution was separated from the aqueous solution and allowed to evaporate spontaneously.

The aqueous alkaline solution was acidified with hydrochloric acid and again shaken out with ether to remove the liberated acids. This ethereal solution was also allowed to evaporate spontaneously.

The first ethereal solution, upon evaporation yielded a small amount of minute prismatic crystals, yellow in color and having an agreeable odor. A melting point was attempted but both purity and quantity of material were insufficient for a successful determination.

The second ethereal solution upon evaporation resulted in some small brown crystals, cubical in shape, having an acid

reaction. As in the first case, the quantity of material was insufficient for its successful characterization.

The 1914 material, after the fatty oil had been removed with heptane, was likewise extracted with alcohol 2.77 p. c. of solid extract, with reference to the pits being obtained. The saponification value of the solid extract was found to be 156.7, hence considerably less than that of the previous experiment. The entire amount of extract was then saponified and treated as before. The residue supposed to contain the alcoholic constituents of the resin was a thick "oily" mass with a sweet but penetrating odor. The residue supposed to contain the resin acids was thick, brown, and semicrystalline.

Both the higher percentage of the alchholic extract and the lower saponification value (compare saponification value of resin of first experiment with that of fatty oil extracted by heptane) seem to indicate that the marc subjected to alcoholic extraction had not been previously exhausted completely with ether. The sticky nature of the ethereal resudues seem to substantiate this interpretation. Unfortunately, the impure condition of the ethereal residue prevented the further study of crystalline substances that promised to be of no little interest.

SUMMARY.

These results seem to indicate that the root bark contains much more amygdalin than the wood of the root. This is in harmony with the amygdalin content of bark and wood respectively of the stem. The results of the assays of the June, August and September material collected in South Dakota seemed to admit of the conclusion that the amygdalin content of the bark increases with the advance of the season. The low results obtained for the December Wisconsin material, however, seems to throw a doubt upon this conclusion, though this difference may be due to other reasons. Noteworthy in this connection is the similarity in the glucosidal content of the December and April Wisconsin material. Hence further studies will be necessary to ascertain differences brought about by season and consequent change in the biochemical processes of the plant, also by geographic differences, and even individual differences for the same geographic and even local conditions.

Cyanogenetic Glucoside.

Several hypotheses have been offered by plant physiologists concerning the biochemical role played by glucosides in plants. These need not be reviewed here. Inasmuch, however, as the cyamogenetic glucosides offer ready means of ascertaining their distribution in the several organs of the plant at different seasons, a series of quantitative data seemed very desirable.

These might not only prove of value in the study of wild cherry as a drug plant, but might possibly prove of interest in throwing general light on the physiological role of glucosides in general. Hence, in this preliminary study was not directed toward the isolation of the glucoside as such, but the effort was made to learn as much as possible in the limited time, about the glucosidal content of the several parts of the plants, and, wherever possible, of the same part in different seasons. The results of the numerous assays made with this object in view are herewith tabulated.

		HCN Content of Drug	HCN Content of dry Material	Computed as amygdalin
ROOT				
a. Bark				
1.) June 1914.....		0.268 p.c.	--------	4.533 p.c.
2.) August 1914.....		0.332 "	--------	5.632 "
3.) Sept. 1914.....		0.375 "	--------	6.356 "
4.) December.....	a)	0.375 "	0.391 p.c.	6.617 "
	b)	0.386 "	0.403 "	6.818 "
5.) April.....	a)	0.375 "	0.391 "	6.617 "
	b)	0.375 "	0.391 "	6.617 "
b. Wood				
1.) December 1914....	a)	0.064 "	0.066 "	1.128 "
	b)	0.069 "	0.073 "	1.235 "
2.) April 1915... ...	a)	0.075 "	0.078 "	1.319 "
	b)	0.075 "	0.078 "	1.319 "
STEM				
a. Bark				
1.) June 1914.....		0.150 "		2.538 "
2.) August, 1914.....		0.137 "		2.311 "
3.) Sept. 1914.....		0.129 "		2.174 "
4.) December 1914....	a)	0.078 "	0.081 ")	
(outer bark)	b)	0.078 "	0.081 ")	1.519 "

		HCN Content of Drug	HCN Content of dry Material	Computed as amygdalin
(Stem - Continued).				
5.) December 1914....	a)	0.335 p.c.	0.348 p.c.)	
(inner bark)	b)	0.335 "	0.348 ")	5.669 p.c.
6.) April, 1915.....	a)	0.177 "	0.186 "	3.147 "
	b)	0.181 "	0.192 "	3.248 "
b. Wood				
1.) December, 1914...	a)	0.022 "	0.0228 ")	
	b)	0.020 "	0.0228 ")	0.372 "
2.) April, 1915.....	a)	0.037 "	0.039 ")	
	b)	0.037 "	0.039 ")	0.659 "
TWIGS				
a. Bark				
1.) December 1914....	a)	0.268 "	0.2787 "	4.536 "
	b)	0.279 "	0.290 "	4.629 "
2.) April 1915.....	a)	0.176 "	0.185 "	3.130 "
	b)	0.181 "	0.190 "	3.214 "
b. Wood				
1.) December 1914....	a)	0.034 "	0.0353 ")	0.575 "
	b)	0.034 "	0.0353 ")	
2.) April.1915.....	a)	0.037 "	0.039 ")	
	b)	0.037 "	0.039 ")	0.659 "
LEAVES				
1.) Sept. 1914.....	a)	0.107 "		1.810 "
	b)	0.107 "		
2.) April 1915.....	a)	0.214 "	0.223 ")	
	b)	0.214 "	0.223 ")	3.773 "
PITS				
1.) August 1914.....	a)	0.0965 "		1.626 "
	b)	0.0965 "		
2.) August 1914.....	a)	0.115 "		1.945 "
(res.of wine)	b)	0.115 "		
FLOWERS (fresh)				
1.) April 1915.....	a)	0.0456 "		0.7716 "
	b)	0.0469 "		0.7935 "

From the above data the following conclusions may be drawn at least temporarily. It should go without saying that numerous

other observations will be necessary, to be made with different materials and in a series of years, before a final conclusion along any of the following lines can be indulged in.

(1) In the fruit the cyanogenetic glucoside may be regarded as being restricted to the seed when it is present in relatively appreciable amount (about 1.8 p.c.). As a reserve material, however, the corresponding amount of carbohydrate (about 1.4 p.c.) i.e. of sugar made available upon hydrolysis of the glucoside, is very small indeed as compared with the amount of fatty oil (about 45 p.c.). It will be of interest to ascertain the role played by the glucoside as well as the fatty oil in germinating seed.

(2) The wood and bark of the root, stem and branches are in a measure comparable, yet afford striking differences that are noteworthy. In all these instances the glucosidal content of the wood is much lower than that of the bark.

This might be assumed to indicate that the object of the cyanogenetic glucoside is to protect the tree against animals to whom hydrogen cyanide is a poison. If this interpretation be indulged in, one would expect the bark of the twigs, presumably the more choice morsel because of the leaves, to contain more glucoside than the stem bark. This is true, but the apparent advantage to this hypothesis is offset by the highest glucosidal content in the root bark when presumably no such protection is needed. Moreover, the glucosidal content of the root bark appears to increase with the advance of the

season, which seems to indicate that the theory of glucosides as reserve material may after all play a role though but a relatively unimportant one.

Noteworthy in this connection is also the striking difference in the glucosidal content of the inner and outer bark of the stem; the cortical layer containing much less than the woody portion of the bark.

The few assays made of the leaves seem to indicate that early in the season they contain more cyanogenetic glucosides than those later in the season. This might be assumed to agree in favor of the protective feature since the early leaves are more important as laboratory units for the plant than the later ones. It might also be used as argument in favor of the reserve material hypothesis, for as the glucosidal content of the leaves diminishes that of the root bark increases. This apparently satisfactory generalization, however, is not as satisfactory when one observes a still higher glucosidal content, for the April root bark than for the December root bark. The observation, however, is of sufficient interest to invite further investigation, for as previously stated, the data collected is still insufficient for more than speculative generalization.

(4) The flowers contain but little cyanogenetic glucoside. This statement, however, is based upon a single experiment. It may well be that the glucosidal content may change rapidly from the expanding buds to the full blown blossoms. Hence this statement must at present be taken cautiously.

While all of the above temporary deductions must be taken

with a considerable grain of allowance they appear of sufficient interest to warrant a further investigation of this subject. It would be of interest indeed to start with the seed and trace the rise and fall of the cyanogenetic glucoside not only through the several organs of the plant but in the same organ for a series of years.

Approved Edward Kremers

Prof. of Pharm. Chemistry

Date June 10, 1915.