

course the desired information could not be furnished in every instance, either because no method had been edited for our files or because no satisfactory method was known. However, the bibliographic files of the Unit contain several thousands of references to sources of information pertaining to analytical methods for drugs, so that in many cases the inquirer could be referred to the original sources for the information wanted.

"At present there is a large sale for medicines in ampules. There is a great deal of confusion in the manner in which these ampules are labeled. Undoubtedly a great deal of coöperative work between physicians, the industry and the regulatory forces will have to be done before these difficulties can be eliminated. Some time ago the Joint Contact Committees of the two associations considered the advisability of taking up the study of ampules in a collaborative way. The regulatory forces of the Department are also considering this class of products. In anticipation of this expected action I have prepared methods for the assay of six drugs which are sold in ampules. Methods for the assay of others are under investigation. This information is in the files subject to call from anyone interested. The ampules for which tentative methods of assay are available are as follows:

Sodium Iodide	Potassium Iodide
Caffeine and Sodium Benzoate	Iron Cacodylate
Sodium Cacodylate	Quinine Dihydrochloride

"Now, Mr. President, I have too long taxed the patience of this audience. I am indeed very grateful that your members have listened with such forbearance to this outline of what the Drug Research Unit is trying to do to aid the industry. In closing there are three principal thoughts that I would leave with you: First, that the officials of the Food, Drug and Insecticide Administration are very desirous that the work of the Joint Contact Committees shall continue. We believe that the contacts established are of mutual benefit to the industry and to the enforcement officials. I think that I may assure you that the resources of the Drug Control will continue to be at your service for coöperative studies. Second, that the Drug Research Unit is collecting information from a great many sources concerning the analysis of drugs. Such information as we have is yours for the asking. Third, that the Government is spending a great deal of time and energy in an earnest, whole-hearted attempt to ascertain what are the best methods for the analysis of drugs and to devise shorter methods for chemical control. It is trying to aid the industry by supplying these methods. This policy is the outcome of the belief of the Administration officials that the most effective way to insure compliance with the law is by striving by every means within their power to aid the industry in the production of medicines of controlled purity and accuracy.

"I thank you for your attention."

CORNIN: A GLUCOSIDE FROM *CORNUS FLORIDA* L.

BY EMERSON R. MILLER.

The dogwood family, *Cornaceæ*, comprises fifteen genera and about one hundred species which are shrubs and trees growing principally in temperate regions. Two of these genera, *Cornus* and *Nyssa*, are represented in the United

States by ten or twelve species. The best known of these is *Cornus florida* L., commonly called dogwood or flowering dogwood, but known also as American dogwood, Florida dogwood, Virginia dogwood, American boxwood, New England boxwood, false boxwood, white cornel and flowering cornel. Its range is from Maine to Minnesota and southwest to Florida and eastern Texas. It is especially abundant in the Southeastern States. It is the only American species of *Cornus* which attains the size of a tree, sometimes reaching a height of 35 to 40 feet. The flowers are small, greenish yellow in a crowded head which is surrounded by four large conspicuous, petal-like, cream-colored or white bracts.

When in bloom this is one of the most striking and handsome of our native trees and is sometimes cultivated for its remarkable beauty. It is also of considerable interest because of its hard, compact, fine-grained wood which is capable of receiving a high polish.

The bark of the trunk and root was for a long time used as a medicinal agent. As a tonic and antiperiodic it was considered an excellent substitute for Peruvian bark. Some physicians even claimed it was successful when Cinchona failed. "Dr. Gregg states that after employing it for twenty-three years in the treatment of intermittent fevers, he was satisfied that it was not inferior to Peruvian bark."¹

The bark was recognized by the United States Pharmacopœia from 1820 to 1870 inclusive, and the root bark in the revision of 1880. The scarlet fruit has also been used medicinally.

Other species which have been used medicinally are *Cornus Sericea* L., swamp dogwood or red osier which is said to be more astringent and less bitter than *Cornus florida* L.; and *Cornus circinata* L'Hér, the round-leaved dogwood which, according to Gibson, is similar chemically to *Cornus florida* L.

Although *Cornus florida* L. has been the subject of several reports very little is known concerning its chemistry.

According to Walker² the bark contains extractive matter, gum, resin, tannin and gallic acid.

Carpenter³ separated a bitter substance which he considered to have basic properties. To this he gave the name cornin.

Dr. S. Jackson,⁴ from experiment, was satisfied it contained a principle analogous to quinia.

Geiger,⁵ in 1836, separated a bitter compound which he also named cornin. He considered Carpenter's basic substance to be cornin mixed with calcium salts. Geiger had for his experiments only a few drachms of the bark. This he extracted with water by the method of percolation. To the percolate he added freshly prepared lead hydroxide and after thoroughly mixing filtered off the precipitate and the excess of lead hydroxide. The filtrate was concentrated and extracted with alcohol. In order to remove color the extract was digested with animal charcoal and crystallized from alcohol. He obtained it in the form of fine silky needles.

¹ F. P. Porcher, "Resources of Southern Fields and Forests" (1863), 59.

² "Inaug. Diss." (1803).

³ *Magazine für Pharm.*, 15, 146.

⁴ "Porcher," 60.

⁵ *Liebig's Annalen*, 14 (1836), 206.

Due to impurities, Geiger's bitter principle was acid in reaction and as it failed to show any behavior of a base he considered it should be classed with the acids rather than the bases. To determine if it contained nitrogen he heated it with potassium hydroxide, but was in doubt as to the result and considered an elementary analysis necessary to settle the question.

Maisch,¹ in 1861, undertook to separate the bitter principle in a pure state but, apparently, did not succeed, since he did not obtain it in the crystalline form. However, his experiments showed that in aqueous solution the compound is easily decomposed by acids or alkalis.

Frey,² following in part the method of Geiger, separated a bitter principle in white or nearly white silky crystals. His results agreed essentially with those of Geiger.

In 1869, Bowman³ and, in 1882, Kramer⁴ found the bark to contain 3% of tannin. In both cases Müller's aluminated gelatin solution was used.

EXPERIMENTAL.

Extraction and Purification.—In 1902 the writer first prepared pure cornin, essentially by Geiger's method as follows: The dried root bark was coarsely ground and extracted by cold percolation with water. In order to extract the material with a minimum of water the percolate from one portion of bark was allowed to percolate through other portions, which had been previously moistened, until the percolate was of a syrupy consistence and a very deep (almost black) reddish brown color. This percolate was then mixed with an excess of freshly prepared lead hydroxide and the mixture filtered. The filtrate was limpid and almost colorless, never having more than a pale straw-yellow color unless the percolate had been concentrated by heating on the water-bath before the treatment with lead hydroxide.

The decolorized filtrate was then concentrated on a water-bath to a pillular consistence and extracted with boiling acetone under a reflux condenser.⁵ Geiger extracted the aqueous extract with boiling alcohol but acetone works much better and especially so if the extract is of the right consistence.

If the extract is too firm the acetone will not penetrate it any better than alcohol would and if too soft the acetone will be diluted by the water to such an extent that a great deal of impurity will be extracted along with the bitter principle and will interfere with crystallization. Several extractions were made with fresh quantities of acetone. These were united and the greater part of the acetone recovered by distillation on a water-bath. The residue was poured into broad shallow dishes and allowed to stand a short time. The more or less highly colored crystalline mass was then filtered by means of a Buchner and washed with a little acetone.

Appearance, Melting Point, Solubility.—Usually one recrystallization from acetone yields a perfectly white product consisting of very fine soft needles having

¹ *Chem. News*, 4 (1861), 198.

² *Am. J. Pharm.*, 51 (1879), 390.

³ *Ibid.*, 41 (1869), 194.

⁴ *Ibid.*, 54 (1882), 388.

⁵ From 500 Gm. of bark 18 Gm. of nearly colorless cornin were obtained.

an extremely bitter taste. When crystallized from more dilute solutions the crystals are hard and much larger.

After long exposure to dry air these crystals showed no signs of efflorescence and when heated in an oven to 102–103° there was no loss in weight. On ignition it burned without residue.

In appearance it is homogeneous, although the melting point is not sharp. The first material obtained melted at 176–178° with the production of slight color. After standing about a year and a half it was very faintly tinged with yellow. It was then recrystallized, some from alcohol, some from absolute alcohol, some from acetone and some from ethyl acetate. One recrystallization gave a substance free from color. From hot alcohol, hot acetone or hot ethyl acetate it forms rectangular plates. In each case the melting point was 182–183°. Another sample, heated rapidly to about 175° and then very slowly melted rather sharply at 180° C.

The substance is readily soluble in water, sparingly soluble in alcohol and acetone, almost insoluble in ether, chloroform and petroleum ether, very sparingly soluble in hot ethyl acetate and hot benzene, moderately soluble in hot acetone and hot alcohol.

Chemical Reactions.—In aqueous solution it is neutral to litmus. Geiger's product had an acid reaction, due to impurities. The aqueous solution does not form a precipitate either with silver nitrate or lead subacetate. Geiger reported precipitates with both of these reagents. Also, no precipitate was formed with any of the following alkaloidal reagents: Gold chloride, platinum chloride, mercuric chloride, phosphotungstic acid, potassium mercuric iodide, potassium bismuth iodide and molybdic acid reagent. However, with an old sample of cornin the last-named reagent quickly formed a green solution, with phenyl hydrazine hydrochloride, and sodium acetate a lemon-yellow or a deep orange-red, flaky, amorphous substance was formed which has so far not been obtained in crystalline form.

Results obtained with acetic anhydride and benzoyl chloride are, likewise, so far unsatisfactory.

Tested in the usual way with metallic potassium it gave no indication of the presence of nitrogen; heated with soda-lime no ammonia could be detected and when ignited no odor like that of burning nitrogenous matter was noticed.

The presence of a carbohydrate nucleus in the molecule of cornin was shown by its responding to the Molisch reaction and by the fact that with the Pettenkofer reagent it gives a cherry-red color. To a small amount of purified ox-gall concentrated sulphuric acid was added and the temperature kept below 60° C.; to this a small amount of an aqueous solution of cornin was added, after a few minutes the liquid had acquired a cherry-red color which became deeper red on standing.

After heating with acids or alkalies and then adding Fehling's solution and again heating, cuprous oxide is precipitated. At ordinary temperature, it slowly reduces Fehling's solution on standing. An aqueous solution reduces an ammoniacal solution of silver nitrate. It also reduces an alkaline solution of bismuth. To an aqueous solution of cornin an equal volume of a saturated solution of sodium carbonate was added; to this was added some bismuth subnitrate, and the mixture was then boiled. After a few minutes a grayish, finally a black precipitate was formed.

There was no apparent reaction with sodium bisulphite, semicarbazide hydrochloride, hydroxylamine hydrochloride or Schiff's reagent for aldehydes.

Stability.—When pure cornin is heated rapidly it may be melted to a colorless liquid, but when heated slowly more or less decomposition has taken place by the time the temperature of the melting point has been reached. This, no doubt, is the cause of the variation in melting point.

Cornin may be boiled in water a short time without any indication of decomposition, but if allowed to stand some time, in aqueous solution, the liquid becomes more or less brown. The pure, white substance also becomes somewhat yellowish or brownish when exposed to the air for some time.

Optical Activity.—Like most of the naturally occurring glucosides cornin is lævorotatory, but differs from most of them in having a much greater rotatory power. Perhaps only one other known natural glucoside has a higher rotation. *Gentiopicrin*¹ is said to have a specific rotation of -201.2° , and *verbena*² a value of -180.32° .

A solution of *cornin*, prepared by dissolving 3 Gm. in 27 Gm. of water, turned the plane of polarized light 18.64° to the left (temperature 25° C.) in a 100-mm. tube. A Laurent polariscope was used. This gives for $[\alpha]_D^{25^\circ} -180.6^\circ$.

In another determination, using a 10% aqueous solution the rotation in a 220 mm. tube was -41.2° , the average of 16 readings. This gives for $[\alpha]_D^{25^\circ} -181.4^\circ$.

Action of Emulsin.—According to Em. Bourquelot³ all glucosides which are hydrolyzable by emulsin are lævorotatory derivatives of dextro-glucose and by the action of this enzyme have the rotation of their aqueous solutions changed to dextrorotatory. Emulsin is generally considered to be the specific enzyme for β -glucosides although it should be borne in mind that emulsin is possibly a mixture of β -glucosidase (a lactase) and amygdalase.

From the following experiments, then, it would seem that *cornin* is a β -glucoside, having dextro-glucose as its carbohydrate nucleus.

To a 10% aqueous solution of *cornin* having a rotation of -41.2° in a 220-mm. tube there was added 0.75-Gm. emulsin. After shaking and allowing to stand 24 hours the solution was filtered. Since the filtrate was too highly colored to permit the passage of sufficient light the liquid was mixed with an equal volume of distilled water. Even with this dilution a sharp reading could not be made but it could be seen that the rotation in a 100-mm. tube was not more than -2.5° . Undiluted and in a 220-mm. tube the rotation would have been approximately -11° ; 24 hours later the solution was again diluted and examined in a 100-mm. tube and found to have a rotation which was the equivalent of -3.6° in a 220-mm. tube. Hence, the action of 0.75 Gm. of emulsin in 48 hours had reduced the rotation from -41.2° in a 220-mm. tube to -3.6° , showing the formation of a considerable amount of a dextrorotatory substance. Due to the coloration of the liquid it was not possible to determine whether or not the emulsin would carry the hydrolysis far enough to produce a dextrorotatory solution. As will be seen later, however, dextro solutions were obtained when sulphuric, hydrochloric and oxalic acids were used as hydrolytic agents.

Action of Alkali.—One Gm. of cornin and 1.5 Gm. of crystalline $\text{Ba}(\text{OH})_2$ were dissolved in water and the volume made up to 30 cc. In a 100-mm. tube the rotation was -7.2° . After standing three weeks the solution was deep reddish brown. When 5 cc. of this solution was diluted to 50 cc. with water, the rotation in a 100-mm. tube was about -0.45° , equivalent to -4.5° undiluted. Hence hydrolysis had occurred with the formation of a dextrorotatory substance.

¹ "Abderhalden's Biochemisches Handlexikon," 2 (1911), 660.

² Bourquelot, *Arch. Pharm.*, 246 (1908), 276.

³ *J. pharm. chim.*, 14 (1901), 481.

Action of Acids.—1. A solution of cornin in 4.5% sulphuric acid had an initial rotation of -8.6° in a 100-mm. tube. After boiling under a reflux condenser about four hours the rotation was $+1.16^\circ$. After standing several hours the rotation was $+0.65^\circ$.

2. A solution of cornin in 20% sulphuric acid had an initial rotation of -7.16° . Boiling 20 minutes changed the rotation to $+1.0^\circ$.

3. Three Gm. of cornin dissolved in 45 cc. of water, 105 cc. of alcohol and 9 cc. of conc. HCl had an initial rotation of -3.68° in a 100-mm. tube. After heating about 8 hours on a boiling water-bath the rotation was $+0.2^\circ$.

4. One Gm. of cornin dissolved in 30 cc. of a 10% aqueous solution of oxalic acid had an initial rotation of -6.41° . After boiling about nine hours and allowing to stand 3 hours the rotation was $+0.68^\circ$. Boiling an additional 5 hours produced very little change in the rotation.

5. A solution of cornin in 25% acetic acid had an initial rotation of -5.88° in a 100-mm. tube. Boiling for about five hours changed the rotation to -5.43° in a 100-mm. tube.

Elementary Analysis.—I. 0.3824 Gm. of cornin crystallized from absolute alcohol gave on combustion 0.2096 Gm. H_2O and 0.7248 Gm. CO_2 . H = 6.09%; C = 51.69%.

II. 0.2086 Gm. of substance crystallized from acetone gave 0.1212 Gm. H_2O and 0.4027 Gm. CO_2 . H = 6.45%; C = 52.64%.

III. 0.2545 Gm. of substance crystallized from acetone gave 0.1402 Gm. H_2O and 0.4838 Gm. CO_2 . H = 6.12%; C = 51.84%.

IV. 0.2410 Gm. of substance crystallized from alcohol gave 0.1334 Gm. H_2O and 0.4685 Gm. CO_2 . H = 6.15%; C = 53.13%.

V. 0.1688 Gm. of substance crystallized from absolute alcohol gave 0.0904 Gm. H_2O and 0.3238 Gm. CO_2 . H = 5.95%; C = 52.31%. Some water was lost.

VI. 0.2653 Gm. of substance gave 0.1442 Gm. H_2O and 0.5070 Gm. CO_2 . H = 6.04%; C = 52.11%.

VII. 0.2530 Gm. of substance gave 0.1433 Gm. H_2O and 0.4884 Gm. CO_2 . H = 6.29%; C = 52.64%.

VIII. 0.2219 Gm. of substance gave 0.1237 Gm. H_2O and 0.4297 Gm. CO_2 . H = 6.19%; C = 52.81%.

IX. 0.1961 Gm. of substance crystallized from acetone gave 0.1088 Gm. H_2O and 0.3785 Gm. CO_2 . H = 6.16%; C = 52.64%.

X. 0.1871 Gm. of substance crystallized from acetic ether gave 0.1014 Gm. H_2O and 0.3642 Gm. CO_2 . Some H_2O was lost. H = 6.02%; C = 53.08%.

XI. 0.2478 Gm. of substance crystallized from acetic ether gave 0.1295 Gm. H_2O and 0.4790 Gm. CO_2 . H = 5.8%; C = 52.71%.

XII. 0.2038 Gm. of substance crystallized from alcohol gave 0.1317 Gm. H_2O and 0.3880 Gm. CO_2 . H = 7.18%; C = 51.92%.

XIII. 0.2684 Gm. of substance crystallized from alcohol gave 0.1592 Gm. H_2O and 0.5148 Gm. CO_2 . H = 6.59%; C = 52.31%.

XIV. 0.1930 Gm. of substance crystallized from absolute alcohol gave 0.1064 Gm. H_2O and 0.3695 Gm. CO_2 . H = 6.12%; C = 52.21%.

XV. 0.1456 Gm. of substance gave 0.0886 Gm. H_2O and 0.2806 Gm. CO_2 . H = 6.76%; C = 52.55%.

Computed for $C_{17}H_{24}O_{10}$: H = 6.185%; C = 52.57%.

Molecular weight determination. Cryoscopic method. Water was used as solvent.

1. 0.2245 Gm. cornin dissolved in 21.863 Gm. water caused a lowering of the freezing point of 0.054° .

2. 0.3513 Gm. cornin dissolved in 21.863 Gm. water caused a lowering of the freezing point of 0.08° .

Calculations:

$$1. \quad M = \frac{Kg}{G\Delta} = \frac{1880 \times 0.2245}{21.863 \times 0.054} = 357.$$

$$2. \quad M = \frac{Kg}{G\Delta} = \frac{1880 \times 0.3513}{21.863 \times 0.08} = 377.$$

The formula, $C_{17}H_{24}O_{10} = 388$.

Methoxyl Determination.—Quantitative estimation of methoxyl was made by the method of Zeisel,¹ using the modified apparatus recommended by Perkins.²

1. 0.2752 Gm. *cornin* gave 0.1524 Gm. AgI = 7.33% OCH_3 .
2. 0.2429 Gm. *cornin* gave 0.1404 Gm. AgI = 7.63% OCH_3 .
3. 0.2966 Gm. *cornin* gave 0.1708 Gm. AgI = 7.59% OCH_3 .
4. 0.5401 Gm. *cornin* gave 0.3256 Gm. AgI = 7.95% OCH_3 .
5. 0.5751 Gm. *cornin* gave 0.3481 Gm. AgI = 7.98% OCH_3 .

The formula $C_{16}H_{21}O_9(OCH_3)_3$ requires 7.98% $(OCH)_3$.

SUMMARY AND CONCLUSIONS.

From the data so far obtained *cornin* appears to be neither an acid nor a base, but a β -glucoside having dextro-glucose as its carbohydrate nucleus.

Analytical data show that its molecule contains one methoxyl group. There are indications that it either contains a free aldehyde group or is easily hydrolyzed with the formation of an aldehyde.

It is an extremely bitter substance, readily soluble in water, but sparingly soluble or nearly insoluble in all of the ordinary organic solvents.

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ANTIVENIN (NEARCTIC CROTALIDÆ).*

BY JOSIAH C. PEACOCK.

The production recently of serum antidotal to the poisons of North American snakes in quantity that permits its distribution through the drug stores of the United States warrants the heralding of that achievement and of other reference to this antivenin that will serve the pharmacist in interpreting to the physician and public the importance and value of this specific treatment.

Since until a short time ago the average annual supply of such serum was only about fifty packages, which were distributed gratuitously by those studying its effectiveness, the present commercial availability of this remedy as well as its adequacy for protection are matters which deserve wide publicity, especially by way of revising and clarifying many impressions and statements that now pertain only to the past.

As a votary of the welfare of health, the pharmacist can render a distinct service to the community by giving an understanding of these facts to all whose pursuits or pastimes take them into rural surroundings.

A brief account of the development and application of anti-snake-bite serums in general and of this North American one in particular seems opportune; but what the pharmacist will certainly want, in order to be prepared to discuss this new addition to his stock of specific medication is a concise comprehension of this serum in its many practical aspects.

¹ *Monatsh.*, 6 (1885), 989; 7 (1886), 406.

² *J. Chem. Soc.*, 83 (1903), 1367.

* Scientific Section, A. P. H. A., St. Louis meeting, 1927.