

was as little as 25 cc., the killing dose of the digitalis was smaller in the exsanguinated cats than it was in the normal animals. What the explanation of this phenomenon is the writer is not prepared to say. It is certainly not due to any irritation or injury to the vagus and sympathetic nerves in the neck because control experiments were made on animals in which these nerves and the carotid arteries were removed without subsequent bleeding of the animal. The difference in toxicity would seem to indicate that the active principles of digitalis may enter into some loose combination with the proteins of the blood and render them less potent, but this has not been definitely established. The writer has always found that the most reliable figures are obtained when the tests for digitalis are performed on cats weighing not less than 2 Kg. and not more than 3.5 Kg. The difference in the killing doses for exsanguinated and normal cats, respectively, is worthy of notice because the greater our knowledge of the factors responsible for variations in digitalis assay, the more reliable will be the figures obtained by investigators who take these factors into consideration.

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METHODS OF IDENTIFICATION OF THE RHIZOMES OF IRIS
VERSICOLOR L. AND IRIS VIRGINICA L.*

BY GEORGE M. HOCKING.¹

Most of the Blue Flag Root in commerce appears to come from the north- and central-eastern counties of Florida. The whole drug appears on the market in two forms, *viz.*, "with fibre" (*i. e.*, with the roots still attached to the rhizome) and "free of fibre" or "stripped." The species, *Iris versicolor* L., has always been named as the official source of the drug, *Iris versicolor*. Another species, *I. caroliniana* Watson, was recognized for the first time in the N. F. V, presumably on the basis of Farwell's statement (1) that in his twenty-five years' experience with crude drugs, Blue Flag Root had come almost entirely from this species. By the rule of priority, the name *I. virginica* L. takes preference over the name *I. caroliniana* Watson, and this revised nomenclature is used in this paper.

The statement is sometimes made (2), (3), (4) that Blue Flag Root is adulterated. Thus, Rusby (5), (6) says that this is probably the case to a large extent with *Iris versicolor* from the south-eastern states. In 1911, he (7) stated that much of the article appeared to come from *I. missouriensis* Nuttall, which is provided with a larger rhizome and was more readily and cheaply collected. The possibility of adulteration from this source now is remote since no Blue Flag seems to be collected in the areas where this species grows. In the south, collectors of and dealers in Blue Flag accept as genuine only the "red root," *i. e.*, the rhizome reddish when

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broken across as opposed to "White Flag Root" which is whitish in section. Farwell¹ states that in forty years of crude drug handling he had never observed adulteration of *Iris versicolor*.

Small (8) groups *Iris versicolor* and *I. virginica* together with *I. Shrevei* Small² into the subgenus *Versicolores*. This closely related group of species is incorporated by Dykes (9) into the subgenus *Lavigata* of section *Apogon* of the genus, and this indicates a close kinship with the European *I. pseudacorus* L. so long known to materia medica. Members of subgenus *Versicolores* are distributed over eastern and central North America. Contrary to the statement which appears in many floras, e. g., Small (8), *I. versicolor* does not occur throughout all of eastern North America, but is restricted to the area, roughly, north and east of a line between Washington, D. C., and northern Wisconsin (10). *I. Shrevei*, if it is to be regarded as a true species and not identical with *I. virginica*, occurs in the Mississippi Valley region, while in the broad strip of territory between, reaching from the Great Lakes in the north to Florida in the south, occurs *I. virginica*.

METHODS.

Because of the importance of Florida as a source of Blue Flag, methods were developed to distinguish rhizomes of the two most abundant Florida species, viz., *Iris hexagona* Walter and *I. savannarum* Small from the somewhat less common species *I. virginica* and from *I. versicolor*, which latter does not grow naturally in the state. In addition, distinctions between the two official species were developed. According to Hume (11), the seven species of *Iris* native to Florida may be classified into three distinct groups comprising: (a) *I. tripetala* Walter; (b) *I. virginica* L.; (c) *I. savannarum* Small, *I. Kimballia* Small, *I. hexagona* Walt., *I. Albispiritus* Small, *I. rivularis* Small.

I. tripetala was the only type not studied, but this species is not one likely to provide adulteration since its rhizome is said (12) to be small and cordlike.

Fresh rhizomes of *I. hexagona*, *I. savannarum*, *I. versicolor* and *I. virginica* were obtained from plants identified by botanists specializing in the genus. The rhizomes were thoroughly examined macroscopically and microscopically, first fresh, then in the dried state. To facilitate sectioning and study, the dried rhizomes were immersed in 15% chloral solution for a few days. It was found possible to distinguish the rhizomes of official species and also to distinguish *between* them on the basis of three or more of the following points: (1) Dimensions; (2) Color (a) external, (b) internal, (c) extract in chloral solution, (d) with vanillin-hydrochloric acid, (3) Fracture; (4) Comparison of cortical and stelar radii; (5) Count of vascular bundles in cross section; (6) Breadth of vascular bundles; (7) Diameter of intra-endodermal parenchyma cells; (8) Odor.

In determining rhizome diameter, cortical and stelar radii, and vascular bundle numbers, pieces of average to maximum thickness rather than those of smaller size (which may be immature) must be selected, whereas for the remaining data, this precaution is unnecessary.

The eight characteristics enumerated above will now be taken up singly:

¹ Personal communication to author.

² Anderson (personal communication) regards *I. Shrevei* Small as at most no more than a variety of *I. virginica* L.

1. *Dimensions*.—Considerable variation exists in the length of pieces of the rhizome in commercial samples of the drug. Likewise, segmental and internodal dimensions are of little value since they vary greatly within the species and even for the individual plant because of varying environmental conditions.

Diametric values are of considerably more value in identification, although these again are affected by external conditions of growth. Of the group studied, *I. virginica* possesses by far the largest rhizome; only *I. savannarum* can compare with it in this respect, and the rhizomes of the two may be distinguished by other means.

AVERAGE MAXIMUM DIAMETERS OF FRESH* RHIZOME (IN MM.).

	<i>I. versicolor.</i>	<i>I. virginica.</i>	<i>I. hexagona.</i>	<i>I. savannarum.</i>
Vertical	16	24	11	12
Lateral	21	32	13	19

* Immersion of dried rhizomes in 15% chloral solution gives comparable values.

2. *Color*.—(a) A casual inspection of a sample of the drug from *I. virginica* gives the impression that the rhizome is distinctly reddish. A more careful examination, however, will show that *I. virginica* and *I. versicolor* are quite similar externally, but that in the former the rhizome has been sliced longitudinally (probably because of its large size), thereby exposing the bright reddish brown of the interior. The rhizome of *I. hexagona* is externally a rich reddish to dark purplish brown, that of *I. savannarum* similar but distinctly grayer.

(b) The rhizome of *I. versicolor* sectioned is dirty yellow to yellowish white to pale pinkish; that of *I. virginica* may be in places yellowish pink, but characteristically sections are dark red to purplish brown. In the other two species examined, sections are light yellowish, almost creamy white, and this is said to be true of the four remaining species of Florida iris (12).

(c) When macerated for several days in 15% chloral solution, the liquid takes on a dark reddish brown coloration with *I. virginica* but becomes no more than greenish yellow with *I. versicolor*.

(d) As discovered for *I. pseudacorus* by von Lingelsheim (13), the rhizome of *I. virginica* is colored a brilliant red when treated with vanillin and strong hydrochloric acid, while that of *I. versicolor* is stained a pale pink. The rhizome of *I. savannarum* is only slightly stained by the same reagent. This test might perhaps be extended to other non-official *Iris* species: if satisfactory, it would be particularly useful for the examination of the powdered drug.

3. *Fracture*.—With the exception of *I. virginica*, the fresh rhizome of Florida *Iris* species break with a snap leaving a clean fracture (12). The fracture of *I. virginica* and *I. versicolor* rhizomes is tough.

4. *Comparison of Stelar and Cortical Radial Values*.—In the table following, the cortical radius is expressed as a percentage of the total radius: minimum and maximum values were found and recorded.

(CORTICAL RADIUS/TOTAL RADIUS) × 100%.

	<i>I. versicolor.</i>	<i>I. virginica.</i>	<i>I. hexagona.</i>	<i>I. savannarum.</i>
Minimum	16%	21%	30%	29%
Maximum	26%	38%	45%	56%
Average	21%	29½%	37½%	42½%

It will be noted that the maximum value for *I. versicolor* is less than the minimum value for the non-official species, whereas for *I. virginica*, although the variation is greater and the higher values tend to overlap values for non-official species, nevertheless the average value is quite distinctly lower. There is sufficient difference in the values for the official species to be of service in distinguishing between them.

5. *Count of Vascular Bundles in Cross Section*.—The material, softened in 15% chloral solution, was cross-sectioned in thin slices and stained with basic fuchsin. Counting bundles was rendered easier and more accurate by mounting on a slide, cutting into several strips, and using a lens magnifying ten diameters.

AVERAGE TOTAL COUNTS OF VASCULAR BUNDLES.

<i>I. versicolor</i>	263	<i>I. hexagona</i>	181
<i>I. virginica</i>	240	<i>I. savannarum</i>	195

I. versicolor and *I. virginica* again show a relationship in the numbers of their vascular bundles, and have a distinctly higher count than in the other species examined.

6. *Dimensions of Vascular Bundles and Parenchyma Cells.*—The vascular bundles of *I. versicolor* and *I. virginica* are similar, both in size and composition, and are materially different from those of the non-official *Iris* species examined. The bundles in all were concentric, with the xylem forming a more or less complete ring around the phloem. In both official species, the individual elements making up the bundles, particularly the tracheae, are considerably smaller in cross-section and more numerous than those of the other species. Only the smallest diameter of a bundle, here called the breadth, was determined.

AVERAGE VASCULAR BUNDLE BREADTHS (IN MICRONS).

<i>I. versicolor</i>	340	<i>I. hexagona</i>	154
<i>I. virginica</i>	336	<i>I. savannarum</i>	152

This again graphically illustrates the resemblance of the official species, and indicates, by a more than 100% increase in vascular bundle diameter, how markedly they differ from the non-official species studied.

7. *Odor.*—The rhizome of *I. virginica* has a very distinctive and aromatic odor, somewhat resembling that of slippery elm; this distinguishes it from *I. versicolor*, in which the odor is slight and not distinctive.

APPLICATION OF METHODS.

The methods just outlined were utilized in the examination of six commercial samples of the dried Blue Flag Root purchased on the open market and of four specimens of the green plants obtained from field collectors who took care to select specimens of the form identical with that which they collected commercially. Three of the latter were identified from the flower as well as from the rhizome (numbers 7, 8, 10).

No. of Sample.	1.	2.	3.	4.	5.	6.	Fresh.			
Rhizome (mm.)	{ aver. lgth. ... 76 115 max. diam. 26 21 22		76	75	56	
(Cort. rad./tot. rad.) × 100%:							25	27	28	19
Horiz.	32	20	17	30	21	16	21	22	30	34
Vert.	37	36	32	38	34	27	31	27	34	41
Aver.	34	26	23	34	28	20	26	25	32	38
Vasc. Bdle. Count	250	308	221	216	244	296	246	289	365	160
Vasc. Bdle. Brdth. (in μ)	238	330	194	295	285	389	305	227	250	190
Diam. intraendoder. parench. cells (in μ)	105	100	83	90	80	65	85	70	95	80
Color with vanillin-HCl	Deep red	Deep red	Deep red	Deep red	Deep red	Slight red	Deep red	Deep red	Deep red	None

The foregoing data afford proof of the value of the methods used in determining identity in commercial samples of Blue Flag. The prime source of each crude drug sample was traced: it was found that four came from Florida (Nos. 1, 2, 3, 4), and one each from the Carolinas (No. 5) and from the middle west, possibly Indiana (No. 6). No. 6 was identified as from *I. versicolor*; the samples from the south as

from *I. virginica*, except in one instance (No. 3), where the article from a northern dealer was found admixed with some *I. versicolor*.

The green plants were obtained from four areas in northeastern Florida; three (Nos. 7, 8, 9) represented *I. virginica*; the fourth (No. 10) was probably *I. savannarum*. The dealer handling this probably did not supply any large firms.

DIFFERENTIATION OF IRIS VERSICOLOR AND IRIS VIRGINICA.

The monograph on *Iris Versicolor*, N. F. V, has been criticized because in it a single description is employed to describe the drugs from two species. Since they differ so markedly in some ways, it is preferable to describe them separately. The following description of the article as it occurs in commerce is suggested as sufficiently distinguishing:

Unground Iris Versicolor.—Rhizome frequently branched, often provided with remnants of flower stalk; segments markedly constricted and thickly developed at intervals, elliptical in cross section at the enlarged portions, cylindrical at the constrictions. Up to 10 cm. in length and 2 cm. in thickness; with an average length of 5 to 6 cm. and an average diameter of a little over 1 cm.; not ordinarily sliced longitudinally; outer surface grayish brown, obscurely annulated with darker colored markings of leaf bases alternating light and dark, the lower with numerous circular root scars, 1 to 2 mm. across, and sometimes root remnants, particularly at the enlarged portions of the rhizome; coarsely wrinkled longitudinally especially upon the upper surface; fracture short, somewhat spongy, the broken surface with yellowish white to pale pinkish brown central cylinder and pale purplish cortex, the central cylinder exhibiting whitish vascular bundles distributed throughout and surrounded by a distinct endodermis; radius of cortex approximately one-fifth of total radius. Odor slight, somewhat unpleasant; taste acrid and nauseous.

Structure.—A more or less exfoliating epidermal layer of suberized cells with brownish walls; a hypodermis of one to three rows of cells with uniformly thickened strongly lignified walls, some of which contain a reddish brown amorphous substance; a relatively narrow cortex of characteristic structure with ovoid or spherical parenchyma cells, large intercellular spaces, some of the parenchyma containing starch, others a reddish brown amorphous substance; an endodermis of a single layer of cells with walls lignified and thickened on the inner and radial surfaces; a central cylinder made up of numerous nearly spherical parenchyma cells, between which are intercellular spaces not as large as those in the cortex; concentric vascular bundles occasional in the cortex, more numerous in the parenchyma of the central cylinder, mostly grouped toward its periphery; between 250 and 300 vascular bundles in cross section of rhizome, individual breadths averaging 340 microns.

Tests for Identity.—In contact with chloral solution, this is colored no more than a greenish yellow; when the powdered drug is treated with vanillin and strong hydrochloric acid, it is colored a faint pink.

Unground Iris Virginica.—Mostly simple, branching much less common than in *I. versicolor*, up to 20 cm., on an average about 8 cm., in length, and averaging 1 cm. in thickness; segments separated by enlargements not as pronounced as in *I. versicolor*; cut into longitudinal pieces by halving or quartering; many broken pieces; outer surface grayish brown to purplish brown, but sectioned surfaces pinkish brown to dull red to purplish brown, giving entire drug a reddish appearance; more deeply annulated than in *I. versicolor*, the upper surface with prominent markings of leaf bases and often showing their fibrous remains, the lower with numerous root scars; fracture short and brittle, the broken surface light to dark reddish brown to dark purplish brown; outer portion of cortex sometimes yellowish; radius of cortex approximately three-tenths of radius of rhizome. Otherwise similar to *I. versicolor*. Odor pronounced, aromatic, reminiscent of slippery elm; taste acrid, astringent, disagreeable, leaving hot sensation in throat.

Structure.—Similar to that of *I. versicolor*. Vascular bundles of similar size, but somewhat less abundant, with an average count of 240 in cross section.

Tests for Identity.—In contact with chloral solution, this is colored a dark reddish brown; when treated with vanillin and strong hydrochloric acid, the powdered drug is colored a brilliant red.

CONCLUSIONS.

1. In revising them onograph for *Iris Versicolor*, N. F. V, the name *Iris caroliniana* Watson should be replaced by that of *Iris virginica* Linné which is more acceptable by the rule of priority.

2. It is suggested that the section describing *Iris Versicolor*, N. F. V, be revised so as to distinguish between the drug from the species *Iris versicolor* L. and *I. virginica* L.

3. In order to distinguish the official from spurious species of *Iris*, it is necessary to include in the monograph more specific histological data, in particular, vascular bundle counts and dimensions, and stelar and cortical ratios, and in addition, color of the drug and color reaction with vanillin and hydrochloric acid.

4. *Iris Versicolor* from the southeastern United States does not seem to be generally adulterated at the present time.

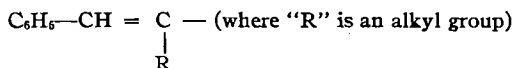
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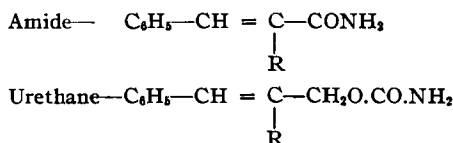
A STUDY OF A NEW SERIES OF URETHANES.*

BY W. A. LOTT AND W. G. CHRISTIANSEN.

As reported in another article, the authors have studied the hypnotic potency of amides and ureides whose acyl residues contain the characteristic grouping:



In order to completely evaluate the grouping in this respect, it was decided to introduce it also into carbinol residues of urethanes; the carboxy group of acids from which the amides (and ureides) are derived being replaced by the carbinol —CH₂OH group. The general formulas for the amides and the corresponding urethanes are as follows:



* Scientific Section, A. P. H. A., Washington meeting, 1934.