

**The constituents of the rhizome of *Iris versicolor* / by Frederick B. Power and Arthur H. Salway.**

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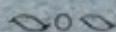
THE CONSTITUENTS  
OF THE RHIZOME OF  
IRIS VERSICOLOR

BY

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## THE CONSTITUENTS OF THE RHIZOME OF IRIS VERSICOLOR.

BY FREDERICK B. POWER and ARTHUR H. SALWAY.

A Contribution from the Wellcome Chemical Research Laboratories, London.

The rhizome and roots of *Iris versicolor*, Linné (Nat. ord. *Iridaceæ*), commonly known as the "Larger Blue Flag," were recognized for several decades by the Pharmacopœia of the United States (Revisions of 1870, 1880 and 1890) under the official title of *Iris*, together with liquid and solid extracts prepared therefrom. In the eighth revision (1900), however, of the above-mentioned work the drug was deleted, probably on account of its limited use or the variability and uncertainty of its action. Under the names of "Irisin" or "Iridin" preparations have been introduced, and are still somewhat employed medicinally, which consist either of the resin or of an ethereal or alcoholic extract of the drug.

Although in the literature<sup>1</sup> it has been recorded that the rhizome of *Iris versicolor* contains starch, gum, tannin, sugar, an acid resin, and fatty oil, together with some volatile matter and possibly an alkaloid, its constituents have never been thoroughly investigated. As the rhizome, at least in the fresh state, evidently possesses considerable potency, acting as a cathartic and emetic, although its medicinal activity is stated to become impaired by age, it was deemed desirable to subject it to a complete chemical examination.

### EXPERIMENTAL.

The material employed for the present investigation consisted of commercial "Blue Flag Root," the identity of which was established, and it appeared to be of good quality.

As a preliminary experiment, a small portion of the ground material was tested for the presence of an alkaloid, but with a negative result. A further quantity (25 grammes) of the ground material was successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:

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<sup>1</sup> This *Journal*, 1876, 48, p. 406; 1881, 53, p. 601; 1884, 56, p. 616.

Petroleum (b. p. 35-50°)	extracted	0.30 Gm.	=	1.2 per cent.
Ether	"	0.80 "	=	3.2 " "
Chloroform	"	0.22 "	=	0.9 " "
Ethyl acetate	"	0.42 "	=	1.7 " "
Alcohol	"	4.13 "	=	16.5 " "

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Total 5.87 Gm. = 23.5 per cent.

For the purpose of a complete examination 62.82 kilogrammes of the ground material were extracted by continuous percolation with hot alcohol. After removing the greater portion of the alcohol, there remained a viscid, dark-colored extract, amounting to 15.72 kilogrammes.

*Distillation of the Extract with Steam. Separation of an Essential Oil.*

Two kilogrammes of the above-mentioned alcoholic extract were mixed with a little water, and subjected to distillation in a current of steam for several hours. The distillate contained a small quantity of oil floating on the surface, which was extracted with ether. The ethereal liquid was dried, and the solvent removed, when there remained 2 grammes of an *essential oil*, this amount corresponding to 0.025 per cent. of the weight of the drug. The essential oil possessed a yellow color, a strong, somewhat unpleasant odor, and contained traces of furfuraldehyde, as indicated by the aniline color test. Its density was 0.9410 at 20°/20°; when examined polarimetrically, no appreciable optical rotation could be observed.

*Non-volatile Constituents of the Extract.*

After removal of the essential oil, as above described, there remained in the distillation flask a deep brown, aqueous liquid (A) and a brown resin (B). These were separated by filtration, and the resin well washed with water, the washings being added to the main portion of the aqueous liquid.

*Examination of the Aqueous Liquid (A).*

The aqueous liquid was concentrated to a convenient volume under diminished pressure, and then repeatedly extracted with ether, the ethereal extracts being united, washed with a little water, and dried with anhydrous sodium sulphate. On removing the solvent,

15 grammes of an oily residue were obtained, which did not solidify on keeping. For the further examination of this product it was redissolved in ether, and the ethereal liquid shaken successively with solutions of ammonium carbonate (a), sodium carbonate (b), and sodium hydroxide (c).

*Isolation of isoPhthalic Acid, C<sub>6</sub>H<sub>4</sub>(CO<sub>2</sub>H)<sub>2</sub>.*

The ammonium carbonate extract (a) of the above-mentioned, ethereal liquid was acidified with dilute sulphuric acid, when an oil was precipitated which was taken up by ether. This ethereal solution was then washed, dried, and the solvent removed, when 10.5 grammes of a brown, viscid oil remained. The latter was next heated with methyl alcohol in a current of dry hydrochloric acid gas, and thus converted into its methyl ester, which, by treatment with dilute, aqueous sodium hydroxide could be separated into phenolic and non-phenolic portions. The portion insoluble in alkali, comprising the non-phenolic esters, amounted to 7 grammes. It distilled at 140–260°/20 mm., the greater portion, however, passing over at 150–180°/20 mm. This ester was then converted into the corresponding acid by heating for a short time with alcoholic potassium hydroxide. After hydrolysis was complete, the alcohol was removed, and the alkaline liquid acidified with dilute sulphuric acid, when a semi-solid, oily product was precipitated. The latter was removed by filtration, dried on a porous tile, and purified by crystallization from dilute alcohol. It was thus obtained in colorless, glistening leaflets, which did not melt below 300°.

0.0919 required for neutralization 10.85 c.c.  $\frac{N}{10}$  Ba(OH)<sub>2</sub>.

Neutralization value = 66.2

C<sub>6</sub>H<sub>4</sub>(CO<sub>2</sub>H)<sub>2</sub> requires Neutralization value = 66.8

The crystalline acid, above described, was only sparingly soluble in hot water, but readily soluble in alcohol. When heated strongly in an ignition tube, it sublimed, forming colorless prisms on the cooler portions of the tube. Its barium salt was readily soluble in water. These properties, together with the result of its titration, as given above, indicated it to be *isophthalic acid*. In order to confirm its identity, a portion of the substance was heated with phosphorus pentachloride in the presence of benzene, and the product vigorously agitated with concentrated ammonia. In this manner an acid amide was formed, which was collected and well washed with

water. The latter compound sintered at  $260^{\circ}$  and melted completely at  $275^{\circ}$ . A known specimen of *isophthalic acid*, when similarly treated, also yielded an acid amide sintering at  $260^{\circ}$  and melting at  $275^{\circ}$ , and when the two specimens of amide were mixed the melting point remained unaltered.

The above-described substance was thus identified as *isophthalic acid*, the occurrence of which in nature does not appear to have been hitherto observed. The amount of this acid obtained from 2 kilogrammes of the alcoholic extract of "Blue Flag Root" was about 0.15 gramme, thus corresponding to 0.0019 per cent. of the weight of the drug.

The phenolic methyl esters, which had been separated from the non-phenolic portion by means of sodium hydroxide, as above described, were hydrolyzed by heating for a short time with an aqueous solution of this alkali. The liquid was then acidified, when a resinous precipitate was deposited, which was extracted with ether. The ethereal liquid was washed and dried, and, on removing the solvent, yielded about 1 gramme of a brown oil. Since the latter did not show any indication of crystallizing, it was agitated with light petroleum, the petroleum extract decanted from insoluble oil, and the solvent allowed to evaporate. A trace of a crystalline solid was thus obtained, which gave a violet coloration with ferric chloride as well as the characteristic odor of oil of wintergreen when heated with methyl alcohol and a few drops of concentrated sulphuric acid. The presence of salicylic acid was thus indicated, but the amount was too small to admit of complete identification. That portion of the oil which was insoluble in light petroleum was again converted into its methyl ester, and the latter benzoylated by the Schotten-Baumann method. The product was oily, but gradually deposited a few colorless crystals. These were separated from adhering oil by spreading them on a porous tile, and then recrystallized from alcohol, when they separated in stellar aggregates of fine needles, melting at  $110^{\circ}$ . The amount of this substance was too small for further examination.

The sodium carbonate (*b*) and the sodium hydroxide (*c*) extracts of the original ethereal liquid, when acidified, yielded only small quantities of gummy substances from which no definite compound could be isolated. On finally evaporating the ethereal liquids,

which had thus been completely extracted with alkalies, 1.5 grammes of a yellow, amorphous mass remained.

The original aqueous liquid (A), which had been extracted with ether as above described, was next repeatedly shaken with amyl alcohol. The united amyl alcoholic extracts were washed with water, and then extracted successively with aqueous solutions of ammonium carbonate, sodium carbonate, and sodium hydroxide. The ammonium carbonate extract, when acidified with dilute sulphuric acid, yielded a resinous precipitate, which was taken up by amyl alcohol, the solution being washed, dried, and the solvent removed. The brown syrup thus obtained, which showed no tendency to crystallize, gave an intense black coloration with ferric chloride, and evidently contained tannin. For the further examination of this syrup it was esterified, but no definite substance could be isolated by this treatment.

The sodium carbonate and sodium hydroxide extracts of the amyl alcoholic liquid yielded only small quantities of brown, amorphous resins, which were specially examined for glucosides, but no positive evidence of the presence of such compounds was afforded. The amyl alcoholic liquid, which had been extracted with the various alkalies, was finally washed, and the solvent removed under diminished pressure. A thick, gummy mass, amounting to about 2 grammes, was thus obtained, but no crystalline compound could be isolated from it.

The aqueous liquid which had been extracted with ether and with amyl alcohol, as above described, was dark brown in color, and gave a copious brown precipitate on the addition of a slight excess of basic lead acetate. The lead precipitate was collected, thoroughly washed, then suspended in water, and decomposed by hydrogen sulphide. After removing the lead sulphide by filtration, the filtrate was concentrated under diminished pressure to a small volume. The concentrated liquid was light yellow, gave a faint brown coloration with ferric chloride, and, after standing for some time, deposited a brown, amorphous solid, but nothing crystalline was obtained from it.

The filtrate from the basic lead acetate precipitate, after removal of the excess of lead, gave a slight precipitate with potassium mercuric iodide, and when heated with potassium hydroxide a strong ammoniacal odor was developed, thus indicating the probable pres-



ence of soluble protein products. It readily reduced Fehling's solution, and contained a large amount of sugar, which yielded *d*-phenylglucosazone, melting and decomposing at 212°. It also contained some potassium salts, since the addition of an aqueous solution of picric acid to a portion of the liquid caused a gradual separation of crystals of potassium picrate. The main portion of the aqueous liquid was concentrated to the consistency of a syrup and kept for a considerable time, but nothing crystalline was deposited.

#### *The Resins (B).*

The resinous material which had been separated from the original alcoholic extract, as previously described, was a dark-colored, soft solid, and amounted to 694 grammes, thus representing about 8.7 per cent. of the weight of the drug. It was digested with hot alcohol, the liquid brought on to purified sawdust, and the thoroughly dried mixture then extracted successively in a large Soxhlet apparatus with various solvents. The amounts of the respective extracts, dried at 100°, were as follows:

Petroleum (b. p. 35-50°)	extracted	296.0 Gms.	=	42.6	per cent.
Ether	"	226.0 "	=	32.6	" "
Chloroform	"	38.2 "	=	5.5	" "
Ethyl acetate	"	16.4 "	=	2.4	" "
Alcohol	"	30.2 "	=	4.4	" "

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Total 606.8 Gms. = 87.5 per cent.

From these results it would appear that a portion of the resin had been rendered insoluble during the process of extraction.

#### *Petroleum Extract of the Resin.*

The petroleum extract of the resin consisted of a dark-colored, oily product, and amounted to 296 grammes. It was dissolved in a large volume of ether, and the ethereal liquid shaken with aqueous ammonium carbonate, which, however, removed only traces of gummy matter. An attempt subsequently to extract the ethereal liquid with aqueous sodium hydroxide resulted in the formation of an emulsion, which did not separate after several days. The ether was therefore completely removed from the mixture, and the residue hydrolyzed by heating for an hour with an excess of alcoholic potassium hydroxide. After removing the greater portion of the alcohol,

water was added, and the alkaline mixture shaken repeatedly with ether. These ethereal extracts were united, washed, dried, and the solvent removed, when a yellowish-brown, semi-solid mass was obtained. This amounted to 35 grammes, and represented the un-saponifiable portion of the petroleum extract of the resin.

*Isolation of a Phytosterol, C<sub>27</sub>H<sub>46</sub>O, Myricyl Alcohol, C<sub>31</sub>H<sub>64</sub>O, and Heptacosane, C<sub>27</sub>H<sub>56</sub>.*

The above-described, brown, semi-solid mass was dissolved in a mixture of alcohol and ethyl acetate, and subjected to a process of fractional crystallization. The more soluble fraction ultimately yielded a crystalline substance, which separated from alcohol in colorless needles. This substance, when air-dried, melted at 133°, but, when dried at 100°, it lost water of crystallization, and then melted at 148°.

0.1714, when heated at 110°, lost 0.0076 H<sub>2</sub>O. H<sub>2</sub>O = 4.4

0.1052, dried at 110°, gave 0.3236 CO<sub>2</sub> and 0.1144 H<sub>2</sub>O.

C = 83.9; H = 12.1

C<sub>27</sub>H<sub>46</sub>O, H<sub>2</sub>O requires H<sub>2</sub>O = 4.5 per cent.

C<sub>27</sub>H<sub>46</sub>O requires C = 83.9; H = 11.9 per cent.

The above-described substance has thus been shown to agree in composition with a phytosterol of the formula C<sub>27</sub>H<sub>46</sub>O, and it yielded the color reactions of that class of compounds. Its optical rotatory power was determined with the following result:

0.1638 of anhydrous substance, made up to 25 c.c. with chloroform, gave  $a_D - 0^\circ 28'$  in a 2 dcm. tube, whence  $[a]_D - 35.6^\circ$ .

The more sparingly soluble crystalline deposits obtained by the above-mentioned fractionation melted indefinitely over a considerable range of temperature, namely from 63 to 77°, and apparently consisted of a mixture of a hydrocarbon and a fatty alcohol. They were therefore heated for an hour at 130° with an equal weight of phthalic anhydride in the presence of xylene. The product of the reaction was then dissolved in a mixture of ether and chloroform, and the solution shaken with aqueous sodium carbonate, when the sodium salt of an acid phthalic ester was immediately deposited. The latter was collected, and washed with a mixture of ether and chloroform, and then hydrolyzed by heating with alcoholic sodium hydroxide. After removing the alcohol from the reaction product, water was added, and the fatty alcohol thus precipitated was collected and crystallized from ethyl acetate, when minute, colorless

needles, melting at  $84^{\circ}$ , were obtained. This substance was analyzed with the following result:

0.0913 gave 0.2742  $\text{CO}_2$  and 0.1154  $\text{H}_2\text{O}$ .  $\text{C} = 81.9$ ;  $\text{H} = 14.1$   
 $\text{C}_{31}\text{H}_{64}\text{O}$  requires  $\text{C} = 82.3$ ;  $\text{H} = 14.2$  per cent.

The compound was thus identified as myricyl alcohol.

The ethyl acetate mother-liquors, remaining after the separation of the myricyl alcohol, yielded a small quantity of a crystalline substance melting at  $77-78^{\circ}$ . The melting point of this compound, together with the method of its isolation, would indicate that it consisted of ceryl alcohol, but the amount of substance was too small for an analysis.

The ether-chloroform solution which had been shaken with aqueous sodium carbonate to remove the myricyl acid phthalate, as above described, was evaporated, and the residue heated with alcoholic sodium hydroxide to remove the excess of phthalic anhydride. After evaporating the alcohol, and adding water to the residue, a small quantity of a solid was obtained, which was collected and recrystallized from ethyl acetate. It separated from this solvent in small, colorless leaflets, melting at  $64-66^{\circ}$ .

0.0931 gave 0.2903  $\text{CO}_2$  and 0.1230  $\text{H}_2\text{O}$ .  $\text{C} = 85.0$ ;  $\text{H} = 14.7$   
 $\text{C}_{27}\text{H}_{56}$  requires  $\text{C} = 85.3$ ;  $\text{H} = 14.7$  per cent.

It is evident from the analysis and the melting point determination of this substance that it was heptacosane.

#### *Isolation of Ipuranol, $\text{C}_{23}\text{H}_{38}\text{O}_2(\text{OH})_2$ .*

The alkaline liquid obtained by the hydrolysis of the petroleum extract of the resin, having been extracted with ether to remove unsaponifiable material, as above described, was next acidified with dilute sulphuric acid, when the fatty acids were precipitated as a dark-colored oil. The mixture was then shaken with ether, which dissolved the fatty acids, leaving, however, a small quantity (0.7 gramme) of a green solid undissolved. The latter was collected, washed with hot alcohol, and recrystallized from pyridine containing a little alcohol. It separated from this solvent in stellar aggregates of small, colorless needles, which decomposed at  $285-295^{\circ}$ .

0.0648 gave 0.1718  $\text{CO}_2$  and 0.0600  $\text{H}_2\text{O}$ .  $\text{C} = 72.3$ ;  $\text{H} = 10.3$   
 $\text{C}_{23}\text{H}_{40}\text{O}_4$  requires  $\text{C} = 72.6$ ;  $\text{H} = 10.5$  per cent.

This compound, when dissolved in chloroform with a little acetic anhydride, and a drop of concentrated sulphuric acid subsequently added, gave a transient pink coloration, rapidly changing to blue and green. It yielded an acetyl derivative, which crystallized in

colorless leaflets melting at 162–163°, and when this was mixed with a known specimen of diacetylipuranol the melting point remained unaltered.

The above-described compound was thus definitely identified as ipuranol (compare this *Journal*, 1908, 80, pp. 264, 576; *Journ. Chem. Soc.*, 1908, 93, p. 907; 1909, 95, p. 249; 1910, 97, pp. 7, 1102; *Pharm. Journ.*, 1910, 84, p. 327).

#### *Examination of the Fatty Acids.*

The ethereal solution of fatty acids, from which the ipuranol had been separated, was washed with water, dried with anhydrous sodium sulphate, and the solvent removed. The residue, amounting to about 210 grammes, was dissolved in absolute ethyl alcohol, and esterified by passing a current of dry hydrochloric acid gas into the boiling solution. The excess of alcohol was then evaporated, the residual oil taken up by ether, and the ethereal solution washed, first with aqueous sodium hydroxide, which extracted a quantity of resinous substance, and then with water until free from alkali. After drying the solution, and removing the ether, the residual ethyl ester was fractionally distilled under diminished pressure, when 5 fractions were ultimately obtained, possessing the following constants:

Fraction	B. p.	Saponification Value	Iodine Value
1.	140–155°/20mm.	249	2.5
2.	155–175°/20mm.	242	4.7
3.	175–195°/20mm.	224	15.3
4.	195–225°/20mm.	189	51.7
5.	above 225°/20mm.	...	...

*Fraction 1.*—This fraction, amounting to 12 grammes, distilled under atmospheric pressure at 260–270°. It possessed a fruity odor, and, from a consideration of its boiling point and saponification value, appeared to consist chiefly of ethyl laurate (b. p. 269°; saponification value 246). In order to confirm the presence of lauric acid, the entire fraction was hydrolyzed with alcoholic potassium hydroxide, and the mixture then acidified and distilled in a current of steam. A quantity of volatile fatty acid was thus obtained, which melted at 40°, and was further identified as lauric acid by means of its neutralization value.

0.3150 required for neutralization 15.7 c.c.  $\frac{N}{10}$  Ba(OH)<sub>2</sub>.

Neutralization value = 280.

C<sub>12</sub>H<sub>24</sub>O<sub>2</sub> requires Neutralization value = 280.

*Fractions 2 and 3.*—These fractions, which were small in amount, were united and hydrolyzed with alcoholic potassium hydroxide, in order to obtain the free fatty acids. The latter were then dissolved in hot alcohol, and separated into four different fractions by the successive addition of small quantities of concentrated aqueous barium acetate. These fractions of barium salt yielded acids which melted at  $33-35^{\circ}$ ,  $33-35^{\circ}$ ,  $35-36^{\circ}$ , and  $39^{\circ}$ , and whose neutralization values were 260, 261, 268, and 275 respectively. It was evident from these results that fractions 2 and 3 consisted of a mixture of lauric acid (neutralization value = 280) and the acids of higher carbon content contained in the succeeding fraction.

*Fraction 4.*—This fraction contained the greater portion of the total fatty acids, and amounted to 120 grammes. Its iodine value (51.7) indicated the presence of a considerable quantity of unsaturated acid. For its further examination the acids were regenerated by hydrolysis, and then fractionally precipitated in hot alcoholic solution with small portions of concentrated aqueous barium acetate. In this manner the saturated acids were precipitated in the first 4 fractions, while the final fraction, which was oily, contained the unsaturated acids, and was therefore put aside for subsequent examination, as described below. The barium salts of the saturated acids thus obtained were treated separately with dilute hydrochloric acid, and the liberated fatty acids recrystallized from alcohol. The several fractions were then found to melt at  $54-56^{\circ}$ ,  $54-56^{\circ}$ ,  $54-55^{\circ}$ , and  $53-55^{\circ}$  respectively, while the corresponding neutralization values were 209, 212.5, 216, and 217. It was evident from these results that the saturated acids contained in fraction 4 consisted of a mixture of stearic and palmitic acids, whose neutralization values are 198 and 219 respectively.

The unsaturated acids, which were regenerated from the above-mentioned oily barium salt, still contained small quantities of saturated acids. The mixture was therefore converted into its lead salt, and the latter treated with cold ether. The portion insoluble in ether was found upon examination to consist of the lead salts of lauric and palmitic acids. The portion soluble in ether, when treated with dilute hydrochloric acid, yielded the unsaturated acids as a pale yellow oil, which possessed the following constants: neutralization value = 208; iodine value = 111.

In order to ascertain the constituents of the unsaturated acids, 12 grammes of the mixture were oxidized with dilute alkaline per-

manganate, as described by Lewkowitsch (*Chemical Technology and Analysis of Oils, Fats, and Waxes*, 1904, vol. i, p. 360), when the chief oxidation product was dihydroxystearic acid, melting at 128°.

0.3412 required for neutralization 10.95 c.c.  $\frac{N}{10}$  KOH.

Neutralization value = 180.

$C_{18}H_{36}O_4$  requires Neutralization value = 178.

A small quantity of tetrahydroxystearic acid (sativic acid), melting at 156°, was also isolated.

0.3164 required for neutralization 9.1 c.c.  $\frac{N}{10}$  KOH.

Neutralization value = 164.

$C_{18}H_{36}O_6$  requires Neutralization value = 164.

No linusic or isolinusic acid was present in the product of oxidation, and it may therefore be concluded that the unsaturated acids consisted of a mixture of oleic and linolic acids, the former predominating.

#### *Isolation of Cerotic Acid, $C_{26}H_{52}O_2$ .*

*Fraction 5.*—The final fraction obtained by the distillation of the esters of the total fatty acids solidified in the receiver as a colorless solid. It was recrystallized several times from alcohol, when it separated in glistening leaflets, melting at 55–56°. This ester was then hydrolyzed, and the liberated fatty acid recrystallized from ethyl acetate. It was deposited from this solvent in colorless leaflets, melting at 78°, and was identified as cerotic acid.

0.1110 gave 0.3185  $CO_2$  and 0.1295  $H_2O$ . C = 78.3; H = 13.0

0.1822 required for neutralization 4.75 c.c.  $\frac{N}{10}$  KOH.

Neutralization value = 146.

$C_{26}H_{52}O_2$  requires C = 78.8; H = 13.1 per cent. Neutralization value = 142.

#### *Ethereal Extract of the Resin.*

This was a hard, black, brittle mass, and amounted to 226 grammes. It was digested with a large volume of ether, when a small portion of the resin (2 grammes) was found to be sparingly soluble in that solvent. The ethereal liquid was therefore filtered, and the undissolved portion separately examined. It was found to consist of ipuranol,  $C_{23}H_{40}O_4$ , which was identified by means of its diacetyl derivative, melting at 161–162°. This substance had already been isolated from the petroleum extract of the resin.

The ethereal liquid, from which the ipuranol had been removed by filtration, was then successively extracted with aqueous solutions

of ammonium carbonate, sodium carbonate, and sodium hydroxide respectively. The ammonium carbonate and sodium carbonate solutions extracted only small quantities of amorphous, brown resins, from which no definite compound was isolated. The aqueous sodium hydroxide, on the other hand, extracted practically the whole of the resin as a thick, black liquid, a small quantity of the alkali being sufficient to extract a comparatively large amount of the resin, thus indicating that the latter possesses a very high molecular weight. On acidifying the alkaline extract, a brown, amorphous solid was precipitated, which was readily soluble in ether and alcohol. All attempts to isolate a crystalline compound from it were, however, unsuccessful. In order to ascertain whether the resin was glucosidic in character, a portion of it was heated for some time in alcoholic solution with dilute aqueous sulphuric acid. On removing the alcohol in a current of steam a quantity of resinous matter separated, which was collected and examined, but it yielded nothing crystalline. The aqueous liquid, after being freed from sulphuric acid by means of baryta was concentrated and tested for the presence of sugar, but with a negative result. The portion of resin extracted by ether is therefore non-glucosidic.

The ethereal liquid which had been shaken with alkalies, as above described, was washed, dried, and the solvent removed, when a small quantity of a thick oil was obtained. This was found to be similar in character to the petroleum extract of the resin, which had already been exhaustively examined.

*Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.*

These were all black, brittle resins, and were small in amount. They were each carefully examined, but nothing of a crystalline character could be isolated from them. Both the ethyl acetate and alcohol extracts of the resin were specially tested for the presence of a glucoside, but with a negative result.

SUMMARY AND PHYSIOLOGICAL TESTS.

The results of the present investigation of "Blue Flag Root" (the rhizome and roots of *Iris versicolor*, Linné), together with some physiological tests and the deductions therefrom, may be summarized as follows:

The material employed was the commercial drug, the genuineness of which was established, and it appeared to be of good quality. A preliminary test with a small portion of the material showed the

absence of an alkaloid, and in the course of the investigation no evidence was obtained of the presence of a glucoside.

An alcoholic extract of the ground material, when distilled with steam, yielded a small amount of an essential oil, which possessed a yellow color, a strong, rather unpleasant odor, and had a density of 0.9410 at 20°/20°.

The portion of the extract which was soluble in water contained a little *isophthalic* acid,  $C_6H_4(CO_2H)_2$ , which has not previously been observed to occur in nature, and apparently a trace of *salicylic* acid, together with tannin, and a sugar which yielded *d*-phenylglucosazone (m. p. 212°).

The portion of the extract which was insoluble in water consisted chiefly of a dark colored, soft resin, amounting to about 8.7 per cent. of the weight of the drug. From this resin the following definite products were isolated: A phytosterol,  $C_{27}H_{46}O, H_2O$  (m. p. when anhydrous, 148°;  $[a]_D - 35.6^\circ$ ); myricyl alcohol,  $C_{31}H_{64}O$ , heptacosane,  $C_{27}H_{56}$ ; ipuranol,  $C_{23}H_{38}O_2(OH)_2$ ; and a mixture of fatty acids, consisting of lauric, palmitic, stearic, cerotic, oleic, and linolic acids.

As no definite substance had been isolated in the course of this investigation to which the reputed properties of "Blue Flag Root" could be attributed, the following crude products therefrom were kindly tested for us by Dr. H. H. Dale, Director of the Wellcome Physiological Research Laboratories, to whom we desire here to express our thanks:

I. The total alcoholic extract.

II. The total resinous material.

III. The portion of the alcoholic extract which was soluble in water.

IV. An aqueous extract of the drug, prepared without heat.

Each of these preparations was given to a dog by the mouth, Nos. I and II in amounts of 1 gramme, and III and IV in considerable quantity. No vomiting or modification of the fæces was produced, nor could any other sign of activity be detected.

Although the potency of "Blue Flag Root," in the fresh state, is evidently well established, it has also been recorded that its medicinal activity is impaired by age. From the results of the above experiments, it would appear that it is possible for the drug completely to lose its physiological activity.





