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Contributors

Jowett, H. A. D.

Potter, C. E.

Wellcome Chemical Research Laboratories.

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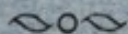
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OF
SALICIN AND SALINIGRIN
IN
DIFFERENT WILLOW AND POPLAR BARKS

BY
H. A. D. JOWETT, D.Sc.,
AND
C. E. POTTER, B.Sc.



THE WELLCOME CHEMICAL RESEARCH LABORATORIES,
FREDERICK B. POWER, PH.D., *Director*,
6, King Street, Snow Hill,
LONDON, E.C.



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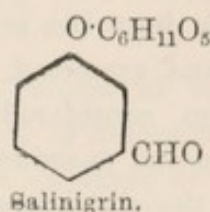
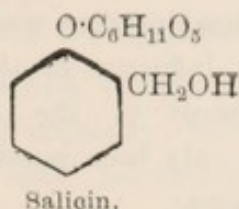
VARIATIONS IN THE OCCURRENCE
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In a paper communicated to the Chemical Society two years ago (*Journ. Chem. Soc.*, 1900, **77**, 707) it was shown by one of us that the glucoside obtained from an unknown species of *Salix* was not salicin, but a new substance, which was named salinigrin. It differed from salicin by yielding on hydrolysis, besides glucose, meta-hydroxy-benzaldehyde, instead of ortho-

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hydroxy-benzyl alcohol. The chemical relationship existing between the two glucosides is shown by their constitutional formulæ:—



The name salinigrin was chosen because the bark from which it was obtained had been supplied as "black willow," though its identity with *Salix nigra* was doubtful. In order to determine, if possible, the exact botanical source of salinigrin, a considerable number of different species of *Salix* and *Populus*—both European and American—were collected and examined. At the same time the opportunity was taken to determine which species contained salicin or populin. For the very considerable care and trouble taken in the collection of this large number of authentic specimens of *Salix* and *Populus* we wish to express our hearty thanks to the following:—Mr. E. M. Holmes, F.L.S., the Directors of the Botanic Gardens of Cambridge, Kew, and Edinburgh, and the Director of the Missouri Botanical Garden, St. Louis, Mo., U.S.A. For a full account of the work done by former chemists on salicin, and a complete list of references, we would refer those interested to 'Die Glykoside,' by van Rijn, pp. 143 *et seq.* From this it appears that salicin has been found in thirteen species of *Salix* and four species of *Populus*, whilst it was absent in eight species of *Salix* and seven of *Populus* examined. In these experiments, however, it would appear that only one factor was considered, viz., that of different species. There are at least three other factors which may influence the presence of salicin in the tree, viz., locality, time of collection, and sex of the tree. In the present investigation the species selected for examination were the chief European and American ones, hybrids, except in one or two cases, being neglected, and they were collected from different localities. In the course of the investigation results were obtained which rendered it desirable

that the two latter factors above-mentioned should be taken into consideration. Accordingly, two species were collected at different seasons of the year, and, in the case of one species, the bark from the male and female trees was separately collected and examined. As the influence of these factors had not been considered when most of the specimens were collected and examined, it has only been possible to determine this in the above-mentioned cases.

METHOD OF EXAMINATION OF THE BARK.

In order to ascertain whether the bark contained salicin or a similar glucoside, it was examined according to the following method. A convenient quantity of the bark, not exceeding 1000 Gm., was extracted by boiling with water for from three to four hours. The infusion was strained, the marc pressed, and the whole liquid then evaporated to a low bulk, generally about one litre. To the concentrated extract a quantity of lead acetate, equal in weight to 10 per cent. of the bark taken, was added, the mixture boiled for a few minutes, and set aside. After standing for half-an-hour it was strained through calico, and the precipitate well washed with water. The filtrate was then saturated with hydrogen sulphide to remove excess of lead acetate, the lead sulphide filtered off, and well washed with hot water. The filtrate and washings were next evaporated to a low bulk under diminished pressure, and the concentrated liquid then set aside to crystallise. In those cases where an appreciable amount of salicin was present, it crystallised out very readily, and was then drained on the filter pump, washed with a very little cold water, dried on a porous tile, and weighed. In some cases the product obtained was too impure to be considered satisfactory; it was, therefore, recrystallised from the least possible quantity of hot alcohol, the purified product weighed, and the mean of the two weights taken as the amount of salicin contained in the bark examined. When no crystals separated even after inoculation with a crystal of salicin, the syrupy extract was taken up with dry sawdust (which had previously

been extracted with alcohol), dried in the water oven, and extracted with 90 per cent. alcohol in a Soxhlet apparatus. The excess of alcohol was removed by distillation, and the residue set aside to crystallise.

As this method would not detect traces of salicin, the residue was, if necessary, further treated to determine whether any evidence could be obtained of the existence of salicin by indirect means. This was accomplished by first hydrolysing the residue with acid and ascertaining whether any glucose was formed, and then by oxidising the hydrolysed residue with chromic acid, and testing for salicylic acid. The following method was employed:—The alcoholic extract was diluted with hot water, filtered, and the filtrate diluted to 250 C.c. with water. The amount of glucose in this solution was then determined volumetrically by Fehling's solution in the usual manner. The remainder of the solution was hydrolysed by boiling with dilute sulphuric acid in a reflux apparatus for one to one and a half hours, and, after cooling, the amount of glucose again determined in the solution. In this way it was possible to ascertain whether hydrolysis had taken place. The remainder of the liquid, not used for the sugar determination, was boiled with an oxidising mixture of sulphuric acid and potassium dichromate for three hours, cooled, filtered, and then extracted twice with ether. The ethereal extract was washed with water, dried, and the ether removed by distillation. The residue was then taken up with a little water and tested for salicylic acid with ferric chloride, the violet coloration being produced if a mere trace of the acid was present.

The accuracy of this method was proved by applying it to some of the mother liquid from which salicin had crystallised, and also to a very dilute solution of pure salicin; in both cases well marked reactions were obtained.

In a few cases, on long standing, crystals were obtained which, however, proved to be inorganic.

The results of this examination are given in the following tables:—

EUROPEAN WILLOW

Species Examined.	Source and Date of Collection
<i>Salix alba</i> , Linn.	Kew, March, 1901
<i>S. alba</i> , var. <i>vitellina</i> , Linn.	Sevenoaks, May, 1901
Ditto	Sevenoaks, August, 1901
<i>S. babylonica</i> , Linn.	Kew, March, 1901
<i>S. fragilis</i> , Linn.	Cambridge, July, 1901
<i>S. hippophaifolia</i> , Thuill.	Kew, March, 1901
<i>S. nigricans</i> , Sm.	Kew, March, 1901
<i>S. pentandra</i> , Linn.	Edinburgh, May, 1901
<i>S. phylicifolia</i> , Linn.	Edinburgh, August, 1901
<i>S. purpurea</i> , Linn.	Cambridge, July, 1901
<i>S. rubra</i> , Huds.	Kew, March, 1901
<i>S. Russeliana</i> , Sm.	Cambridge, August, 1901
<i>S.</i> [species?] (narrow-leaved)	Sevenoaks, August, 1901
<i>S. triandra</i> , Linn.	Cambridge, August, 1901
<i>S. viminalis</i> , Linn.	Cambridge, August, 1901
<i>S. viridis</i> , Fries.	Kew, March, 1901
<i>Populus alba</i> , Linn.	Cambridge, July, 1901
<i>P. canescens</i> , Sm.	Kew, March, 1901
<i>P. nigra</i> , Linn.	Kew, March, 1901
<i>P. nigra</i> , var. <i>pyramidalis</i> , Spach.	Cambridge, July, 1901

AMERICAN WILLOW

Species Examined.	Source and Date of Collection
<i>Salix cordata</i> , Muhl.	Ithaca, N.Y., Spring, 1901
<i>S. discolor</i> , Muhl.	Ithaca, N.Y., Spring, 1901
<i>S. longifolia</i> , Muhl.	St. Louis, Mo., March, 1901
<i>S. Missouriensis</i> , Bebb.	Courtney, Mo., March, 1901
<i>S. nigra</i> , Marsh.	Courtney, Mo., March, 1901
<i>S. nigra</i> , Marsh.	Washington, D.C., May, 1902
<i>S. Wardi</i> , Bebb.	Allenton, St. Louis Co., Mo., A.
<i>Populus angustifolia</i> , James	Unknown
<i>P. balsamifera</i> , Linn.	Ithaca, N.Y., Spring, 1901
<i>P. Fremonti</i> , S. Wats.	San Bernardino Co., S. Calif.
<i>P. grandidentata</i> , Michx.	Ithaca, N.Y.
<i>P. monilifera</i> , Aiton. (Cottonwood) ..	Jefferson Barracks, Mo., Ma.
<i>P. tremuloides</i> , Michx.	Unknown

POPLARS.

Used.	Salicin.	Reaction for—		Remarks.
		Glucoside.	Salicylic Acid.	
00	nil	trace	trace	salicin present.
00	nil	nil	nil	—
00	nil	nil	nil	—
00	nil	trace	nil	—
00	nil	nil	trace	—
00	nil	trace	trace	salicin present.
00	nil	nil	trace	—
00	nil	nil	trace	—
00	nil	well marked	well marked	salicin present.
00	nil	trace	trace	Ditto.
00	0.4 per cent.	0.4 per cent.	—	Ditto.
00	nil	nil	nil	—
00	nil	nil	trace	—
00	nil	nil	trace	—
00	nil	nil	trace	—
00	nil	nil	trace	—
00	nil	nil	nil	—
00	nil	trace	nil	—
00	nil	nil	nil	—
00	nil	trace	trace	{ salicin or populin present.

POPLARS.

Used.	Salicin or Salinigrin.	Reaction for —		Remarks.
		Glucoside.	Salicylic Acid.	
1	nil	nil	nil	—
20	0.2 per cent.	0.2 per cent.	salinigrin	salinigrin present.
1	nil	nil	trace	—
1	nil	nil	nil	—
1	nil	trace	nil	—
1	nil	—	—	—
1	nil	trace	nil	—
1	nil	nil	nil	—
1	nil	nil	nil	—
1	nil	trace	trace	{ salicin or populin present.
1	nil	nil	nil	—
1	nil	nil	nil	—
1	nil	nil	nil	—

In those cases in which salicin and salinigrin were isolated their identity was established by the determination of the following constants:—

Salicin, melted at 200° corr., gave a red colour with sulphuric acid, and in aqueous solution $[\alpha]_D = -64.4^{\circ}$. Beilstein gives melting point, 201° and $[\alpha]_D = -65^{\circ}$.

Salinigrin, melted at 193° corr., gave no colour with sulphuric acid, in aqueous solution $[\alpha]_D = -85^{\circ}$, and gave crystalline metahydroxybenzaldehyde on hydrolysis. Previously found for salinigrin (*J.C.S., loc. cit.*), melting point 195° corr., and $[\alpha]_D = -87.3^{\circ}$.

For the purpose of comparison it seems desirable to give here a list of the species of *Salix* and *Populus* which have been stated by previous investigators to contain salicin and those in which salicin has not been detected. (cf. 'Die Glykoside,' *loc. cit.*)

Salicin has been found in *Salix helix*, **S. purpurea*, **S. alba*, *S. Lambertina*, *S. incana*, *S. amygdalina*, *S. fissa*, *S. hastata*, *S. præcox*, **S. pentandra*, *S. polyandra*, **S. fragilis*, **S. Russeliana*, *Populus tremula*, **P. alba*, *P. græca*, and **P. balsamifera*.

The following species have been examined and did not yield salicin:—**S. vitellina*, *S. caprea*, **S. viminalis*, *S. daphnoides*, **S. babylonica*, *S. bicolor*, **S. triandra*, *S. argentea*, **P. nigra*, **P. monilifera*, *P. fastigiata*, *P. balsamea*, *P. virginica*, *P. angulosa*, and *P. grandiculata*.

It will thus be seen that salinigrin has been found in only one species—viz., *S. discolor*, and that it is not present in the two samples of *S. nigra* examined. Furthermore, out of thirty-three specimens examined, there were only eight which gave positive evidence of the existence of salicin or an analogous glucoside, and only in two instances were the glucosides present in sufficient quantity to be separated and identified.

As the results obtained were not in harmony with those of previous observers, it was thought that perhaps this might be

* These species were examined in the present investigation.

due to a variation in the amount of salicin in bark collected at different seasons of the year. Accordingly, two species were collected at different seasons of the year and examined.

Species.	Locality.	Time of Collection.				
		July, 1901.	Oct. 1901.	Jan., 1902.	April, 1902.	July, 1902.
<i>S. purpurea</i>	Cambridge....	traces	2·2	2·2	3·	0·2 p.c.
<i>S. rubra</i> *	Kew	0·4	3·7	—	—	— p.c.

These results prove conclusively that the amount of salicin contained in willow bark varies according to the time of its collection, hence an accurate comparison with previous determinations cannot be made, as this factor does not appear to have been previously taken into account. The determinations in the case of *S. purpurea* are, however, strictly comparable, as the bark examined in July and October, 1901, and January and April, 1902, was from the same tree (female), and that in July, 1902, from another tree (also female) of the same species from the same locality.

When the material for examination was received in April, 1902, it was noticed that it was delivered in two bundles, marked male and female respectively. The bark from the female tree was used for the first experiment, and a yield of 3·5 per cent. of salicin obtained. For the purpose of repetition the bark from the male tree was examined, when, to our surprise, only 1·2 per cent. of salicin was obtained.

It was therefore arranged that the bark collected in July, 1902, should be obtained from both the male and female trees. Inquiry also showed that the bark examined previously had been obtained from the same female tree.

* Unfortunately the tree at Kew was destroyed in the autumn of 1901, so that the series of experiments with this bark could not be completed.

The results of the examination of the bark from the male and female trees may thus be tabulated:—

Season of Collection.	July, 1901.	Oct. 1901.	Jan. 1902.	April, 1902	July, 1902.
Male Tree	—	—	—	1·2*	3·9* p.c.
Female Tree.....	traces	2·2†	2·2†	3·5†	0·2‡ „

These results are remarkable and entirely unexpected; we have, therefore, carefully checked and repeated the experiments, with confirmatory results. The most remarkable fact to which we wish to draw attention is that in April the bark from the female tree contained about three times as much salicin as that from the male, whereas three months later the conditions were reversed, and the bark from the male tree contained 3·9 per cent. salicin, whilst that from the female tree contained only 0·2 per cent. The results obtained with the bark of the female tree collected in July of 1901 and of 1902 are concordant, as only small amounts of salicin were obtained from two different female trees of the same species and collected on the above dates in the same locality.

It is certain, therefore, that not only does the season of collection affect the amount of salicin contained in the bark, but that the bark from the male and female trees contains different amounts of salicin.

Whilst these experiments have shown that the presence of salicin in willow bark depends not only on the species examined but on the sex of the tree from which the bark is obtained and the time of the year at which it is collected, it is possible that there are other conditions not yet determined which also influence the amount of glucoside present. The cause of this variation opens up a very interesting field of inquiry, which belongs, however, more to the domain of the botanist than that of the chemist. An inquiry into the influence of these various factors on the amount of salicin contained in the bark would probably

*Same tree (male). †Same tree (female). ‡A different tree, but same species.

throw considerable light on the function of the glucoside in the metabolism of the plant. Whilst recognising that further experiments on this point are desirable, we desire to make the following suggestion—that the salicin acts as a reserve food material, being stored away in the winter for use in the coming spring. The increase in the amount of salicin present in both *S. purpurea* and *S. rubra* in the autumn is thus explained. In the spring and early summer the reserve food material is drawn upon to a different extent by the male and female trees, owing to their special functions. It is possible that the glucoside is first hydrolysed by the ferment which seems to accompany every glucoside, and that it is the glucose thus formed which is used by the plant. The glucoside thus would perform a somewhat similar function to starch.

SUMMARY.

The results of this investigation may thus be briefly summarised:—

(1) That of the thirty-three samples of willow and poplar examined, salinigrin was only found in one, *Salix discolor*, Muhl, which may therefore be considered to be the source of salinigrin.

(2) That the amount of salicin contained in the bark of a willow or poplar depends not only on the species, but on the season of the year at which it is collected, the sex of the tree, and possibly other factors.

The investigation has shown, therefore, that for practical purposes chemical assay alone can decide whether a willow bark does or does not contain salicin.

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