TRANSACTIONS OF THE WAGNER FREE INSTITUTE OF SCIENCE OF PHILADELPHIA

VOLUME XI

APRIL, 1927

THE BIOCHEMISTRY OF THE AMERICAN PITCHER PLANTS

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WAGNER FREE INSTITUTE OF SCIENCE OF PHILADELPHIA

MONTGOMERY AVE. AND SEVENTEENTH STREET
PHILADELPHIA

BIOCHEMICAL STUDIES OF THE NORTH AMERICAN SARRACENIACEAE

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INTRODUCTION

THE observations and experiments forming the basis of the following papers on the North American *Sarraceniaceæ* had their beginning in 1892 when *Sarracenia flava, S. rubra,* and *S. purpurea* came under observation in Richmond County, North Carolina. In succeeding years all the species have been under frequent observation in their native habitats, and have been made the subject of several papers on insect-plant relations by one of us (Jones).

Field and laboratory experiments on the biochemistry of the *Sarraceniaceæ* were commenced in 1917, and have been continued until the present time. These researches form part of a general study of insectivorous plants, work on *Nepenthes* having been begun by Hepburn and St. John in 1914. *Dionaea* has been made the subject of a recent paper.

Observations and field experiments have been made or material for laboratory examination collected chiefly near the following localities for each species:

Darlingtonia californica.—Keddie, Plumas Co., and Mt. Eddy (the type locality), Siskiyou Co., Cal.

Sarracenia minor.—Summerville, Berkeley Co., S. C.; Jacksonville, Duval Co., Fla.

Sarracenia Sledgei.—Mobile, Theodore, and Bayou La Batre, Mobile Co., Ala.; Biloxi, and Wiggins, Harrison Co., Miss.

Sarracenia flava.—Southern Pines, Moore Co., Hamlet, Richmond Co., and Wilmington, New Hanover Co., N. C.; Summerville, Berkeley Co., S. C.; De Funiak Springs, Walton Co., Fla.; Bay Minette, Baldwin Co., Ala.

Sarracenia Drummondii.—De Funiak Springs, and Freeport, Walton Co., Fla.; Bayou La Batre, and Theodore, Mobile Co., and Bay Minette, Baldwin Co., Ala.

Sarracenia rubra.—Southern Pines, Moore Co., and Hamlet, Richmond Co., N. C.; De Funiak Springs, Walton Co., Fla.

Sarracenia purpurea.—Tolland, Tolland Co., Conn.; Whitings, and Toms River, Ocean Co., N. J.; Pocono Pines, Monroe Co., Pa.; and many other localities from Maine to Mississippi.

Sarracenia psittacina.—De Funiak Springs, Walton Co., Florida; Bayou La Batre, Mobile Co., and Bay Minette, Baldwin Co., Ala.; and Ocean Springs, Jackson Co., Biloxi, and Wiggins, Harrison Co., Miss.

Samples of pitcher liquor and aqueous solutions of the nectar, which were collected in the field for laboratory examination at Philadelphia, were preserved by addition of 0.2 percent of trikresol. The collection of material for bacteriological study and for chemical examination of the plant tissues is described in detail in the papers on these respective subjects.

In this series of papers the authors have followed Macfarlane[95] in the nomenclature of the *Sarraceniaceæ*. They are indebted to Dr. Macfarlane for many helpful suggestions in the course of these researches.

The authors record their indebtedness to the Franklin Institute of the State of Pennsylvania for permission to use portions of a paper on absorption of nutrients and allied phenomena in the pitchers of the *Sarraceniaceæ*, originally published in the *Journal of the Franklin Institute*, February, 1920. The Institute has also permitted the reprinting of several illustrations from its *Journal*.

The American Museum of Natural History has granted permission to reprint three illustrations from its journal, *Natural History*.

A CHEMICAL STUDY OF THE NECTAR OF THE SARRACENIACEÆ

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Either 25 or 50 pitchers of a given species were collected in the field while secreting nectar. They were cut from the plant near the base of the pitcher, and their tops were washed in succession with the same portion of distilled water (approximately 75 cc.). In this way an aqueous solution of the nectar was obtained free from pitcher liquor, prey, and plasma of the plant cells. The solution was filtered through filter paper to remove any insoluble particles, chiefly dust. The solution was immediately tested, by means of Benedict qualitative alkaline copper solution, for the presence of reducing sugar, and was then preserved by addition of sufficient trikresol to render the concentration of that bactericide 0.2 percent. Further tests were made in the laboratory at Philadelphia.

Separate series of tests were made on each of the following species: *Darlingtonia californica*, *Sarracenia minor*, *S. Sledgei*, *S. flava*, *S. Drummondii*, and *S. purpurea*.

The aqueous solution of the nectar of each of these species gave the following reactions:

(1) It reduced Benedict qualitative alkaline copper solution, showing the presence of a reducing sugar.

(2) It yielded the characteristic crystals of phenylglucosazone in the osazone test. This test was performed in two ways. The aqueous solution of the nectar was digested at the temperature of boiling water with phenylhydrazine hydrochloride and sodium acetate, then permitted to stand at the temperature of the room. Or the aqueous solution of the nectar was heated for one minute over a low flame after the addition of free phenylhydrazine and acetic acid; the solution was rendered almost, but not quite, neutral by addition of an approximately 14 percent aqueous solution of sodium hydroxide, was again heated for 1 minute, then permitted to stand at the temperature of the room. In either case crystals of an osazone were formed, and were identified as those of phenylglucosazone by microscopic examination.

(3) It gave a distinct crimson color in the Seliwanoff test. In this test, a portion of the aqueous solution of the nectar was mixed with its own volume of Seliwanoff reagent and then heated at the temperature of boiling water for exactly 20 minutes. The Seliwanoff reagent was an aqueous solution containing 0.05 percent resorcinol and approximately 12 percent hydrochloric acid. The development of the crimson color under the conditions of the test was evidence of the presence of a ketose sugar.

These reactions indicated that the nectar contained either fructose (levulose) or invert sugar.

The aqueous solutions of the nectar of *Darlingtonia californica* and *Sarracenia flava* were sufficiently concentrated to permit quantitative examination, both chemically and optically. In the *chemical* examination, the solution was titrated against Benedict quantitative alkaline copper solution to determine its reducing sugar content which was then calculated as glucose. This Benedict solution was standardized by titration with a freshly prepared solution of glucose of the highest purity. For the *optical* examination, the solution of the nectar was first clarified by addition of solid lead subacetate (Horne), thorough mixing, and filtration through a dry filter paper; this treatment removed all optically active compounds other than sugars. The filtrate was treated with solid neutral potassium oxalate to remove the excess of lead; and the resulting solution was filtered through a dry filter paper. This filtrate was used for the optical examination in a Peters saccharimeter.

The solution of the nectar of *Darlingtonia californica* was found, by the Benedict method, to contain 0.22 percent of reducing sugar, calculated as glucose. Examined in a standard tube, 200 mm. in length, it gave a reading of $-0.9°$ in the saccharimeter.

According to Browne,[69] the relative copper reducing values of the sugars in the volumetric method are:—Glucose 1.000, fructose 0.924, invert sugar 0.962. This author gives the following normal weights of sugars for saccharimeters with a Ventzke scale:—Fructose 18.592 grams, invert sugar 86.450 grams.

From the relative copper reducing values $\frac{\text{Glucose}}{\text{Frutcose}} = 0.924$ and $\frac{\text{Glucose}}{\text{Invert Sugar}} = 0.962$, it follows that the same copper reducing value is possessed by solutions containing 0.22 percent glucose, 0.24 percent fructose, and 0.23 percent invert sugar respectively. Therefore, the aqueous solution of the nectar contained either 0.24 percent fructose or 0.23 percent invert sugar.

From the normal weights, it follows that a solution producing a rotation of −0.9 saccharimeter degree may contain 0.009 times 18.592 or 0.17 gram of fructose in 100 cc., *i. e.*, 0.17 percent fructose. Or it may contain 0.009 times 86.450 or 0.78 gram of invert sugar in 100 cc., *i. e.*, 0.78 percent invert sugar.

Since the values for fructose, obtained by copper reduction (0.24 percent) and by the optical method (0.17 percent), show a far closer agreement with each other than do those for invert sugar (0.23 percent by copper reduction and 0.78 percent by the optical method), it follows that the reducing sugar present in the nectar of *Darlingtonia californica* is fructose (*d*−fructose, levulose) and not invert sugar.

The solution of the nectar of *Sarracenia flava* was found, by the Benedict method, to contain 0.17 percent of reducing sugar, calculated as glucose. Examined in a tube 400 mm. in length, it gave a reading of −1.4° in the saccharimeter; this corresponded to a reading of −0.7° for a standard tube 200 mm. in length.

From the relative copper reducing values, it follows that the same reducing value is possessed by solutions containing 0.17 percent glucose, 0.18 percent fructose, and 0.18 percent invert sugar, respectively.

From the normal weights, it follows that a solution producing a rotation of −0.7 saccharimeter degree may contain 0.13 percent fructose or 0.61 percent invert sugar.

Since the values for fructose, obtained by copper reduction (0.18 percent) and by the optical method (0.13 percent), show a far closer agreement with each other than do those for invert sugar (0.18 percent by copper reduction and 0.61 percent by the optical method), it may be concluded that in *Sarracenia flava* also, the reducing sugar present in the nectar is fructose and not invert sugar.

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