

ALKALOIDS FROM *SANGUINARIA CANADENSIS* AND THEIR
INFLUENCE ON GROWTH OF *PHYMATOTRICHUM*
OMNIVORUM

G L E N N A . G R E A T H O U S E

(WITH ONE FIGURE)

Introduction

Sanguinaria canadensis L. is reported by TAUBENHAUS and EZEKIEL (6) to be highly resistant to the *Phymatotrichum* root rot. It has long been of interest on account of its alkaloidal content. DANA (1) isolated the alkaloid, sanguinarine, from this species in 1827. Since that time chelerythrine, protopine, and β - and γ -homochelidonine have been isolated from the rhizome and roots (5). For these reasons this plant seems an exceptionally good object for investigation in connection with the theory of alkaloids as a possible factor in the resistance of some plants to *Phymatotrichum omnivorum* (Shear) Duggar (3, 4). In the work reported here certain alkaloids were isolated from crude extracts of roots and rhizomes of *S. canadensis* and incorporated in various concentrations into the substrates ordinarily used for the pure culture of *P. omnivorum*. The effect of these substances on the growth and development of the fungus are discussed.

Materials and methods

Powdered rhizome and root tissue of *Sanguinaria canadensis* was purchased from Eimer and Amend Co. Two kilograms of this ground tissue was thoroughly extracted with acidified (acetic acid) alcohol, following the method of FISCHER (2) for the separation of the alkaloids. The procedure of extraction, separation, etc., is shown diagrammatically in figure 1. The suggestions of the original paper were followed in separating protopine and chelerythrine from sanguinarine.

Results

Three alkaloids were separated from fractions BC and BS (figure 1). FISCHER found β - and γ -homochelidonine in fraction BSC, but in this study nothing of a crystalline form was secured; however, this fraction did yield a small amount of syrup of alkaloidal nature.

Alkaloids with the following physical properties and chemical composition were separated and studied for their effects on the growth of *P. omnivorum*. Alkaloid no. 1 (fraction BC and BS) crystallized from alcohol in colorless groups of needles, melting point 212° C.¹; dissolved in alcohol, ether, chloro-

¹ All melting points are corrected.

form, forming solutions that were fluorescent. The hydrochloride was deep red in color. Chemical composition: Found,² C, 68.48 per cent.; H, 4.30 per cent.; N, 3.94 per cent. Calculated for sanguinarine ($C_{20}H_{15}O_4N \cdot H_2O$), C, 68.34 per cent.; H, 4.31 per cent.; N, 3.99 per cent. The concentration found for this alkaloid was approximately 0.2 per cent.

Alkaloid no. 2 (fraction BS) crystallized from alcohol in colorless prismatic leaflets, melting point $206^\circ C$.; readily soluble in chloroform, sparingly soluble in alcohol and ether. The hydrochloride formed by the addition of

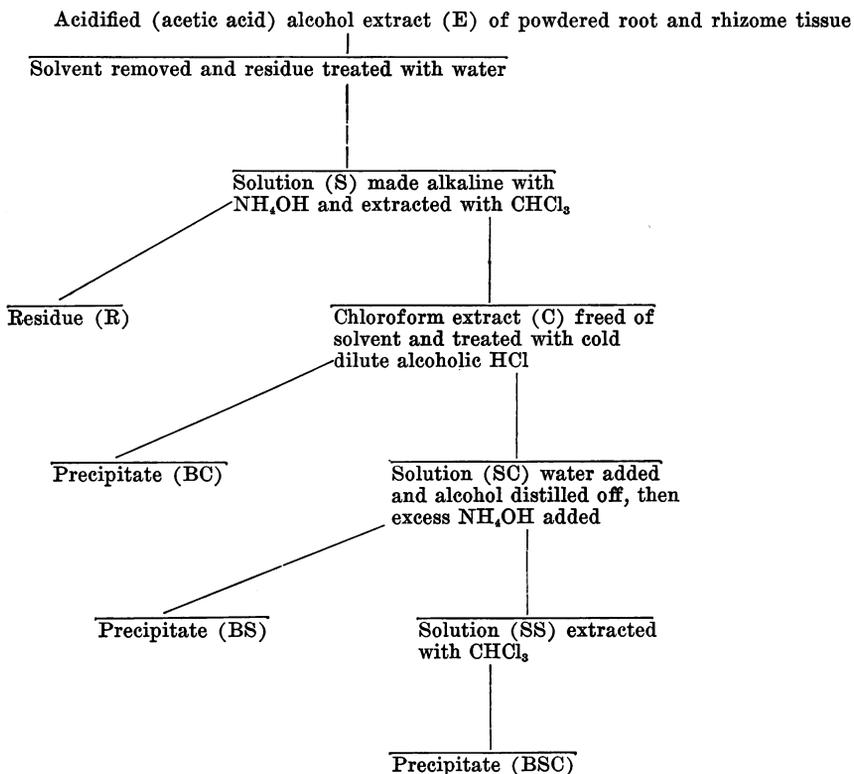


FIG. 1. Method of FISCHER for the separation of the alkaloids of *Sanguinaria canadensis*.

hydrochloric acid was citron-yellow in color, with needle-shaped crystals. Chemical composition: Found, C, 63.22 per cent.; H, 5.05 per cent.; N, 3.63 per cent. Calculated for chelerythrine hydrochloride ($C_{21}H_{17}O_4N \cdot HCl \cdot H_2O$), C, 62.73 per cent.; H, 5.02 per cent.; N, 3.49 per cent. The concentration of this alkaloid was 0.1 to 0.2 per cent.

² Average of duplicate determinations made by Arlington Laboratories, Arlington, Virginia.

Alkaloid no. 3 (fraction BC) crystallized from chloroform by addition of a small amount of alcohol, formed monoclinic crystals, melting point 208° C.; very slightly soluble in alcohol, ether, benzene, and ammonium hydroxide; and soluble in acetone. Chemical composition: Found, C, 67.85 per cent.; H, 5.45 per cent.; N, 4.09 per cent. Calculated for protopine (C₂₀H₁₉O₅N), C, 67.96 per cent.; H, 5.42 per cent.; N, 3.97 per cent. The concentration of this alkaloid was 0.01 to 0.05 per cent.

The effect of these alkaloids on the growth of *P. omnivorum* was tested in the manner reported by GREATHOUSE and WATKINS (4). The results are presented in table I.

Discussion

Alkaloids having the physical properties and chemical composition of sanguinarine, chelerythrine, and protopine were isolated from *Sanguinaria canadensis*. All of these alkaloids, with the exception of protopine, were found to be present in the rhizome and root tissues in far greater concentration than that which prevents growth of *P. omnivorum*. The concentration of protopine found in the tissue was great enough to decrease fungous growth from 369 mg. to 81 mg. Sanguinarine completely prevented growth of the organism at a concentration of 2.5 p.p.m. Chelerythrine was not so toxic, permitting a growth of the fungus in three weeks of 3.4 mg. at a concentration of 10 p.p.m. Protopine under similar conditions yielded 81 mg. of

TABLE I
INFLUENCE OF ALKALOIDS ISOLATED FROM *Sanguinaria canadensis* ON
GROWTH OF *Phymatotrichum omnivorum*

COMPOUND	DRY WEIGHT (MG.) OF FUNGUS IN NUTRIENT SOLUTION + FOLLOWING CONCENTRATIONS OF ALKALOID			
	100 P.P.M.	50 P.P.M.	10 P.P.M.	2.5 P.P.M.
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Sanguinarine	0	0	0	0
Chelerythrine	0	0	3.4	73
Protopine	81	137	308	356
Control = 369 mg.				

fungous growth at 100 p.p.m. This study clearly shows that alkaloids, even from the same plant, are not equally inhibitive to the growth of *P. omnivorum*.

Experimental evidence suggests strongly that the alkaloids in roots and rhizomes of *Sanguinaria canadensis* constitute an important factor in the resistance of this plant to *Phymatotrichum* root rot.

Summary

Sanguinarine, chelerythrine, and protopine have been isolated from roots and rhizomes of *Sanguinaria canadensis* by the method of FISCHER. Sangui-

narine has been shown experimentally to prevent the growth of *Phymatotrichum omnivorum* at a concentration as low as 2.5 p.p.m. Chelerythrine and protopine were less toxic than sanguinarine. There is considerable indication from this work that alkaloids play an important rôle in the resistance of *Sanguinaria canadensis* to Phymatotrichum root rot.

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