

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY,¹ ALBANY, CALIFORNIA]**Method of Isolation of Usnic Acid from *Ramalina reticulata***

By J. B. STARK, E. D. WALTER AND H. S. OWENS

On the basis of results of antibiotic studies by Marshak^{2,3} and other investigators^{4,5,6} the U. S. Public Health Service requested this laboratory to isolate several kilograms of usnic acid to be used in further studies.

This paper describes preliminary experiments and the method developed for this work.

Experimental

Personal communication from Marshak disclosed that iron caused destruction of usnic acid and that storage of wet lichen markedly decreased its usnic acid content. Samples of moss stored for forty days at normal relative humidity and extracted according to the method of Marshak² showed no loss of usnic acid. Extraction of the moss in stainless steel equipment yielded the same percentage of usnic acid (0.08%) as in glass.

To simplify the extraction and purification process a study was made of the solubility of usnic acid in various solvents. These results are given in Table I.

TABLE I

SOLUBILITY OF USNIC ACID IN VARIOUS SOLVENTS AT 25, 40 AND 60°

Solvent	Solubility in g./100 ml.		
	25°	40°	60°
Acetone	0.77	1.19	..
Ethyl acetate	0.88	1.29	2.41
Ethanol	0.02	0.06	0.22
Methyl cellosolve	0.22	0.30	0.76
Ethyl cellosolve	0.32	0.42	0.77
Furfural	7.32
Furfuryl alcohol	1.21
Water	<0.01

A series of qualitative experiments gave sufficient information to settle upon the following method of isolation. A unit of 100 kg. of moss is used for descriptive purposes.

One hundred kg. of moss ground to pass a half-inch screen was placed in each of two stainless steel tanks. The moss in the first container was covered with acetone, which was allowed to remain one-half hour. Approximately the first 10 gallons was drained and evaporated to recover product; the remainder was drained and poured on the moss in the second container. The drained moss was washed with sufficient acetone to make the total volume of extractant on the second batch equal to the initial volume used. The moss in the second tank was treated in the same manner with the washings used on a fresh batch of moss in the first tank. The solution containing usnic acid was evaporated at 35° and 260 mm. until precipitation began in the concentrate, which was then allowed to stand overnight at room temperature. The total weight of precipitate amounted to about 1% of the weight of the moss.

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946. Article not copyrighted.

(2) A. Marshak, *Public Health Reports*, **62**, 3 (1947).

(3) A. Marshak, G. T. Barry and L. C. Craig, *Science*, **106**, 394 (1947).

(4) A. Stoll, A. Brack and J. Renz, *Experientia*, **3**, 115 (1947).

(5) V. C. Barry, L. O'Rourke and D. Twomey, *Nature*, **160**, 800 (1947).

(6) F. Bustinza and A. C. Lopez, *Anales Jardín Bot. Madrid*, **7**, 1 (1948).

The precipitate from 100 kg. of moss was filtered and suspended in about 6 liters of boiling methanol. The dispersion was cooled to 45° and filtered. This step removed about 12% of the solids. The residue was triturated with three separate 2-liter portions of petroleum ether (b. p. 30–60°).

The residue was dissolved in a minimum volume of boiling acetone, and ethanol was added in the ratio of 1 part to 10 parts of acetone. The mixture was allowed to cool in order to crystallize the usnic acid. The precipitate was treated with small quantities of methanol and petroleum ether as before. Final purification was accomplished by recrystallization from acetone. The yield of usnic acid was 0.08% or about 8% of the total acetone soluble solids.

The crystals from this treatment are yellow and have a specific rotation, measured in a saccharimeter with a sodium lamp, of 491–495° (2% in CHCl₃ at 25°). After re-solution in acetone and treatment with charcoal, the specific rotation is increased to 503°. This value compares with 495° and 508° given in the literature.^{7,8} The analysis showed C, 62.9; H, 4.7, calculated for C₁₈H₁₆O₇; C, 62.8; H, 4.71.⁷ Optical and X-ray measurements are to be reported separately by F. T. Jones and K. J. Palmer of this Laboratory.⁹

In the course of this work some of the impurities were isolated, which might be of interest to future workers in this field. An acetone extract of moss, concentrated until freed of usnic acid and a white material, yielded white crystals which were identified as *p*-arabitol.

A white material, which showed slight birefringence between crossed Nicols, interfered with the purification of usnic acid. When hydrolyzed with alkali and acidified, two acids could be isolated. One was identified as α -crotonic acid from its anilide and its *p*-bromophenacyl ester. The Rast molecular weight of the original material was 886, indicating that it represented a polymer of α -crotonic acid or a precursor, such as β -hydroxybutyric acid. No further work is planned in this field.

The biological properties of usnic acid are under investigation by A. Marshak and his co-workers of the U. S. Public Health Service. Some preliminary tests made by D. M. Reynolds, formerly of this Laboratory, in conjunction with other antibiotics show that usnic acid is effective in dilutions between 1 part in 100,000 and 1 part in 1,000,000 against such Gram-positive organisms as *Sarcina lutea*, *Bacillus mycoides*, *B. subtilis*, *Streptococcus faecalis*, *Micrococcus lysodeikticus* and *Staphylococcus aureus* and the acid-fast organism *Mycobacterium tuberculosis*, strain 607.

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(7) E. Abderhalden, *Biochemisches Handlexikon*, Vol. VII, Julius Springer, Berlin, 1912, p. 115.

(8) I. M. Heilbron and H. M. Bunbury (editors), "Dictionary of Organic Compounds," Vol. III, Oxford University Press, New York N. Y., 1938, p. 905.

(9) F. T. Jones and K. J. Palmer, *THIS JOURNAL*, in press.

Summary

A method satisfactory for production of kilogram lots of usnic acid has been described. The solubility of usnic acid in several solvents at 25,

40 and 60° has been determined. D-Arabitol has been identified in the acetone extract of *Ramalina reticulata*.

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[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY²]

Optical, Crystallographic and X-Ray Diffraction Data for Usnic Acid¹

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Crystals of usnic acid ($C_{18}H_{16}O_7$) isolated from the lichen *Ramalina reticulata* by the method of Stark, Walter and Owens³ have been examined by the methods of optical crystallography and X-ray diffraction and the results obtained are reported in this paper.

Optical and Crystallographic Properties.—The product isolated at this Laboratory by Stark, Walter and Owens³ consisted of a yellow felted mass of long, thin, ribbon-like crystals out of an acetone solution. This material was recrystallized by cooling a warm saturated solution in chloroform. The resulting crystals were rectangular tablets having beveled ends and sides

(Fig. 1). The refractive indices were determined by immersion methods as described in Chamot and Mason.⁴ The crystals dissolved slowly in some of the immersion liquids used, but a good value could be obtained by examining the sample immediately after adding the immersion liquid or by saturating the immersion liquid with the usnic acid and redetermining the index of the resulting solution. The indices of the liquids used were checked on a refractometer. Ordinary views of the crystals show a centered obtuse bisectrix interference figure. The alpha and beta refractive indices can be obtained from this view, alpha being lengthwise. The end and edge views were obtained from crystals mounted on a stage goniometer. The orientations were determined by means of interference figures. Figure 2 shows the three principal views and optic orientation. The angles shown were measured by means of the rotating stage. Sodium light was used for all refractive index determinations.

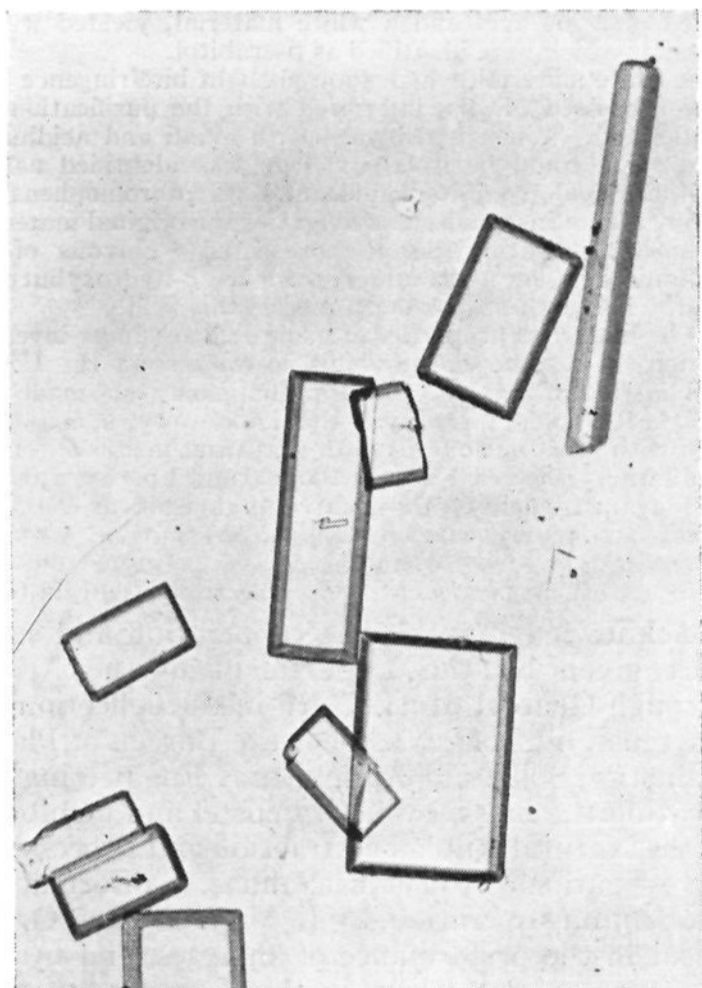


Fig. 1.—Usnic acid from chloroform 100 \times .

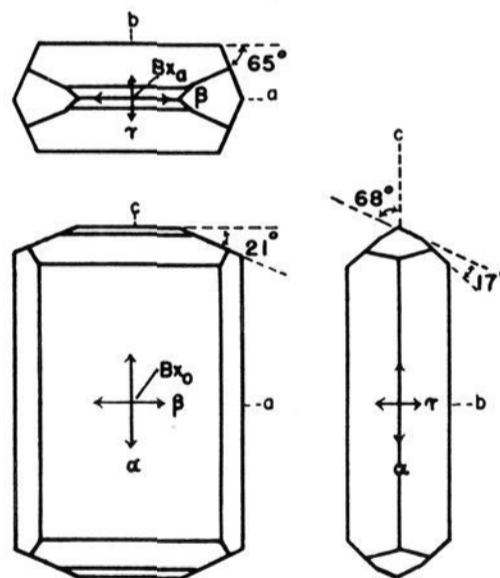


Fig. 2.—Optic orientation of usnic acid.

The dispersion of the optic axes was determined on a crystal mounted in a liquid which matched the beta refractive index for sodium light. The optic axial angles were measured on the goniometer scale by turning the crystal from one melatope to the other and taking the angular difference between the two settings. The wave

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Report of a study made under the Research and Marketing Act of 1946.

(3) J. B. Stark, E. D. Walter and H. S. Owens, *THIS JOURNAL*, **72**, 1819 (1950).

(4) E. M. Chamot and C. W. Mason, "Handbook of Chemical Microscopy," Vol. I, John Wiley and Sons, New York, N. Y., 1938, pp. 358-375.