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 LABORATORY[2]]

Optical, Crystallographic and X-Ray Diffraction Data for Usnic Acid[1]

BY FRANCIS T. JONES AND KENNETH J. PALMER

Crystals of usnic acid ($C_{18}H_{16}O_7$) isolated from the lichen *Ramalina reticulata* by the method of Stark, Walter and Owens[3] 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 Owens[3] 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.

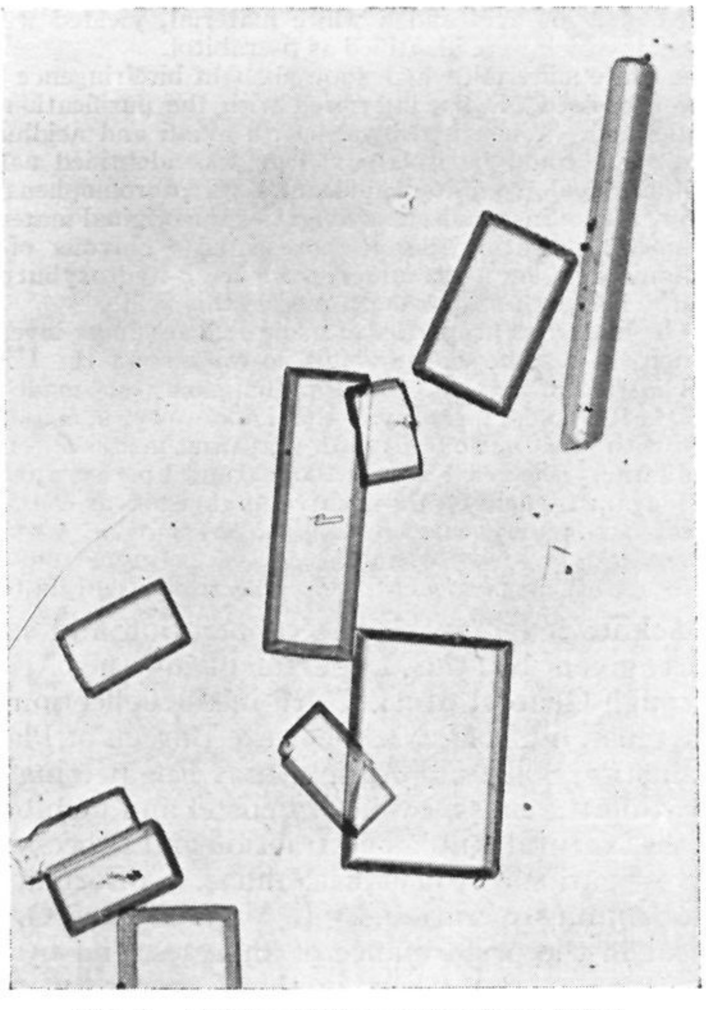

Fig. 1.—Usnic acid from chloroform 100×.

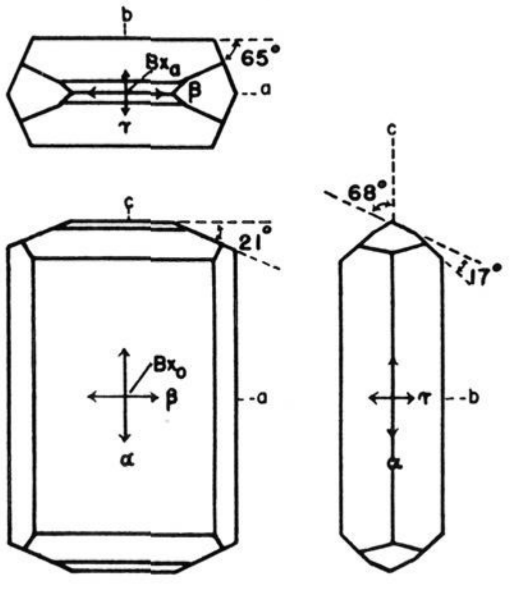

Fig. 2.—Optic orientation of usnic acid.

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

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(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.

lengths used in addition to sodium light (5890 Å.) were those transmitted by the Wratten filters F (approx. 6500 Å.), B (approx. 5500 Å.) and H (approx. 4750 Å.). Table I summarizes the optical data, and compares our results with the values recorded in Winchell.[5] The combination of properties requires that this compound belong to the orthorhombic crystal system. A product isolated from *Parmelia moliuscula* at the Wyoming Agricultural Experiment Station has been compared with our usnic acid and has been found to have identical properties.

TABLE I

OPTICAL PROPERTIES OF USNIC ACID

Author		F. T. Jones	A. N. Winchell[a]
$n^{25}\text{D}$	α	1.611 ± 0.001	1.4627
	β	1.710 ± 0.002	1.6531
	γ	1.772 ± 0.002	1.7798
$2V$	Obs.[b]	72.1	....
	Calcd.	73[c]	60
Dispersion[b]		(v > r) strong	(v > r)
Optical character		(−)	(+)
Pleochroism		None	Green-yellow
Crystal system		Orthorhombic	Orthorhombic

[a] Data of Strüver[9] and Kappen[10]. [b] With Wratten F (red) 71.2°, Wratten B (green) 73.8° Wratten H (blue) 76°. [c] $\text{Cos}^2 V = \gamma^2(\beta^2 - \alpha^2)/\beta^2(\gamma^2 - \alpha^2)$.

X-Ray Diffraction Analysis.—Usnic acid crystals suitable for X-ray diffraction analysis were obtained by recrystallization from chloroform. Weissenberg photographs were obtained of the zero and second layer lines when the long axis of the crystal was parallel to the axis of rotation. These photographs were identical with those obtained by Dr. I. Fankuchen from the usnic acid furnished him by Dr. A. Marshak.[6] Zero and second layer line Weissenberg photographs were also taken of a crystal mounted with the axis of rotation parallel to the beta index direction. The Weissenberg photographs confirm the orthorhombic symmetry of the crystal.

The unit cell dimensions have been determined by measurement of layer line separation and (h00), (0k0) and (00l) reflections on zero layer line Weissenberg photographs. The results obtained are given in Table II. From the unit cell dimensions the axial ratios are calculated to be $a{:}b{:}c$ = 0.937:1:0.397. The axial ratios reported by Strüver[5,9] are $a{:}b{:}c$ = 0.9322:1:0.794. The agreement is very good if Strüver's value for the c axis is halved.

TABLE II

X-RAY DIFFRACTION RESULTS FOR USNIC ACID

$a \parallel \beta$	$b \parallel \gamma$	$c \parallel \alpha$
19.10 Å.	20.39 Å.	8.09 Å.

Space group D_2^4 − $P2_12_12_1$ (may be D^3 − $P2_12_12$)
Density 1.46 g./cc.
Eight molecules ($C_{18}H_{16}O_7$) per unit cell
Axial ratio $a{:}b{:}c$ = 0.937:1:0.397

The only systematic absences are (h00) when $h \neq 2n$, (eighteenth order observed), (0k0) when $k \neq 2n$ (twentieth order observed), and (00l) when $l \neq 2n$ (only out to the sixth order observed). The space group is probably D_2^4 − $P2_12_12_1$ but may be D_2^3 − $P2_12_12$.

The density of an usnic acid crystal was measured by flotation in a mixture of ethylene bromide and toluene and found to be 1.46 g./cc. There are, therefore, eight molecules with the empirical formula $C_{18}H_{16}O_7$ in the unit cell.

Since the space group deduced for usnic acid has only four general positions there must be either two crystallographically different sets of four molecules each with the formula $C_{18}H_{16}O_7$ or one set of four molecules with the formula $2(C_{18}H_{16}O_7)$. To choose between these two alternatives, the molecular weight was determined by the method of Signer as described by Clark[7] using acetone as a solvent. The molecular weight so determined agrees with the formula $C_{18}H_{16}O_7$ and there must be, therefore, two crystallographically different sets of four molecules each in the unit cell of usnic acid. X-Ray powder data on usnic acid have been obtained but have not been included.

Discussion

The X-ray data indicate that our usnic acid is the same as that originally used by Marshak,[6,8] in his experiments on inhibition of *Mycobacterium tuberculosis*, but the optical data do not agree with those recorded in Winchell[5] for the compound originally isolated and characterized under the name usnic acid.

We are forced to conclude either that the original characterization was incorrect in spite of the fact that indices were reported to four places, or that one of the compounds characterized is not usnic acid. There are the further possibilities that we are dealing with polymorphs, or perhaps isomers. No evidence of polymorphism has been observed, and the optical rotation[3] and melting point[3] agree well enough with those reported to make isomerism seem unlikely. The fact that the axial ratios and the habit of the crystals obtained from our usnic acid by recrystallization from the solvent (chloroform) used in the original work[9] is the same as that reported,[5,9] supports the idea that the refractive indices may have been incorrectly determined. The fact that Strüver[9] and Kappen[10] both give the optical character as positive in spite of the fact that the indices reported require that it be negative casts doubt on the accuracy of their work. They report that $\gamma = c$ but we find that $\gamma = b$.

The question can be answered unequivocably

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(5) A. N. Winchell, "Optical Properties of Organic Compounds," University of Wisconsin Press, Madison, Wisconsin, 1943, p. 231.

(6) Private communication.

(7) E. P. Clark, *Ind. Eng. Chem., Anal. Ed.*, **13**, 820 (1941).

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

(9) J. Strüver, *Z. Krist.*, **6**, 538 (1882).

(10) H. Kappen, *ibid.*, **37**, 151 (1903).

only by redetermining the optical properties of crystals obtained from the original source. Strüver[9] obtained his material from the lichens *Zeora sordida* and *Usnea barbata*. Kappen[10] obtained *l*-usnic acid from *Usnea barbata* and the *d*-usnic acid from *Usnea longissima*.

A lichen collected by one of us (F.T.J.) and identified by the University of California Botany Department as *Usnea californica* Herre has yielded crystals of usnic acid[11] having optical and crystallographic properties identical with those of the material obtained from *Ramalina reticulata* reported above. This evidence makes it practically certain that Kappen's values for the refractive indices of usnic acid are in error. His method for determining the indices is not clearly stated but the implication is that it was a goniometric method utilizing the usnic acid crystal as a prism. The sample from *Usnea californica* was too small to test for optical rotation but the material is probably the dextrorotatory isomer. Abderhalden[11] lists only the *d* isomer from any of the *Usneas;* consequently Kappen must have obtained his *l*-usnic acid from a lichen mistakenly identified as *Usnea barbata*.

Acknowledgments.—We wish to express our thanks to Merle Ballantyne for taking the X-ray diffraction photographs, to Oliver Emerson for determining the molecular weight, and to N. Floy Bracelin for help in the preparation of Fig. 2 and for assistance in finding a source of *Usnea californica*. We also thank Phyllis Gardner, herbarium botanist of the University of California, and C. W. Dodge of Washington University and the Missouri Botanical Garden for their assistance in finding and identifying the *Usnea californica* Herre.

Summary

The optical properties of usnic acid isolated from the lichens *Ramalina reticulata*, *Parmelia moliuscula*, and *Usnea californica* Herre have been determined. The crystals obtained from chloroform solution are orthorhombic with indices $\alpha = 1.611$, $\beta = 1.710$, and $\gamma = 1.772$. These values differ markedly from those originally reported for usnic acid. X-Ray diffraction photographs of these crystals give $a = 19.10$ Å. parallel to β, $b = 20.39$ Å. parallel to γ, and $c = 8.09$ Å. parallel to α. The space group is probably $D_2^4 - P2_12_12_1$ but may be $D_2^3 - P2_12_12$, and the density is 1.46 g./cc. There are two crystallographically unrelated sets of four molecules each in the unit cell.

ALBANY, CALIFORNIA RECEIVED SEPTEMBER 3, 1949

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[11] E. Abderhalden, "Biochemisches Handlexikon," Vol. 7, J. Springer, Berlin, 1912, p. 116.

[CONTRIBUTION FROM THE VENABLE CHEMICAL LABORATORY OF THE UNIVERSITY OF NORTH CAROLINA]

2,4,6-Trinitrostyrene[1]

BY RICHARD H. WILEY[2] AND LYELL C. BEHR

Polynitrostyrenes have not been previously reported in the literature. It was felt that it would be of interest to attempt preparation of such compounds, and to observe their polymerizability, in view of recent reports of the successful polymerization of *m*-nitrostyrene[3] and *p*-nitrostyrene,[4] and of the fact that 1,3,5-trinitrobenzene is an efficient inhibitor of vinyl polymerization.[5] In this paper, we wish to report a successful synthesis of 2,4,6-trinitrostyrene and some attempts to polymerize it.

A method which has been successful in the synthesis of many substituted styrenes consists in the decarboxylation of the corresponding cinnamic acid. Attempts to prepare 2,4,6-trinitrocinnamic acid for use in this reaction have been unsuccessful in these laboratories. We have, however, obtained 2,4,6-trinitrostyrene by the series of reactions diagrammed below. Mannich condensations with 2,4,6-trinitrotoluene have been previously carried out using the free amine and formaldehyde solution in the presence of alkali,[6] but our experience was that this technique produced undesirable by-products (probably due to the action of the alkali) which were difficult to remove and which tended to make the product less stable. We therefore carried out the condensation in absolute alcohol, using the amine hydrochloride and paraformaldehyde. The hydrochloride (I) thus obtained was converted, without isolation, into the free base and thence to the methiodide (II). The over-all yields in this reaction varied from 15–40% of the theory. It should be noted that the free amine of the hydrochloride (I) was unstable, particularly in alkaline medium. Use of sodium carbonate, rather than the calcium salt, to free the base from the hydrochloride often resulted in profound decomposition. The methiodide (II) is also somewhat unstable on standing. Conversion to the styrene was carried out in an aqueous methanol solution, using silver oxide. Yields up to 65% of theoretical were obtained. Decom-

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[1] Work done under a contract with the Bureau of Ordnance, Department of the Navy.

[2] Present address: University of Louisville, Louisville, Kentucky.

[3] Wiley and Smith, *J. Polymer Sci.*, **3**, 444 (1948).

[4] Strassburg, Gregg and Walling, THIS JOURNAL, **69**, 2141 (1947).

[5] Frank and Adams, *ibid.*, **68**, 908 (1946).

[6] Bruson and Butler, *ibid.*, **68**, 2348 (1946).