THE OPTICAL CRYSTALLOGRAPHIC IDENTIFICATION OF STRYCHNINE

By

J. E. Sellers, B. S., M. S.
University of Colorado, 1921 ; 1928.

A Thesis Submitted to the Faculty of the Graduate School of the University of Colorado in Partial Fulfillment of the Requirements for the Degree Ph.D.

Department of Chemistry.

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This Thesis for the Ph.D. degree

By

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for the

Department of Chemistry

By

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INTRODUCTION

This investigation was undertaken with the idea of preparing a number of the salts of strychnine, determining their optical properties, and then, if possible, using the optical properties of the most suitable salts as a basis for the identification of strychnine. It was felt that some definite and measurable physical property might be more satisfactory as a basis for the test than color reactions or the general appearance of microscopic crystals produced by various reagents. Since strychnine forms a number of well crystallized salts, it lends itself well to a test involving the optical properties of crystals. For this investigation only the well crystallized, water-soluble salts of inorganic and simple aliphatic organic acids were included.

There are at present a rather large number of tests for strychnine. These may be divided into three groups, as follows: physiological, color reactions, and microchemical or microscopic tests. There is only one test in the physiological group and this consists of injecting the strychnine into a frog and obtaining a characteristic reaction. The test is fairly satisfactory. There are many color reactions for strychnine. Mandelin's test, Otto's test, and Malaquin's test are the most important. Color reactions are open to the objection that other substances may either give colors which are the same or similar to those produced by Strychnine, or impurities may be present which will prevent the formation of the characteristic color when strychnine is present. In the case of Mandelin's test, O'Day (1) reports eleven substances which give erroneous positive results and a large number of substances which, when present, will interfere with the production of the characteristic color of strychnine with Mandelin's reagent. Bailey (2) found similar interferences with Otto's test for strychnine.

The microchemical-microscopic tests consist of adding some reagent to a solution of strychnine or its salts on a microscope slide and thus producing characteristic crystals or groups of crystals. These tests are open to the objection that they are based only on general appearance of the crystals, and it is well known that small amounts of substances in solutions may affect the development of crystal faces and hence change markedly the appearance of the crystals.

HISTORICAL

The use of optical properties for the identification of alkaloids has been suggested by Wright (3), by Wherry (4), and by Wherry and Yanovsky (5). Keenan (6) suggests the use of refractive index in two directions for the identification of the following alkaloids or alkaloid salts: Brucine sulphate, morphine sulphate, atropine, heroin, codeine sulphate, morphine, quinine sulphate, and cocain hydrochloride.

Only a very meager amount of data on the optical properties of strychnine salts could be found in the literature. Wright (3) gives the optical constants of strychnine alkaloid along with those of eleven other alkaloids. Bolland (7) gives the following data on three strychnine salts:

Strychnine iodide	1.66	1.69	-03
" nitrate	1.62	1.67	-05
" salicylate	1.72	1.69	-03

Groth (8) gives the crystallographic data and only a few of the optical constants of the following strychnine salts studied in this thesis: strychnine hydrochloride, sulphate pentahydrate, sulphate hexahydrate and selenate. For the sulphate pentahydrate he gives $\gamma = 1.594$ red. No other refractive indices are given.

The literature contains records of the preparation and composition of a rather large number of salts of strychnine, but as it was desired to limit this study to the water soluble, well crystallized salts of inorganic or simple non-aromatic organic acids only a small number of these salts are included. Of the eighteen salts studied the composition of the following were found to check with that published in the literature: $StHClO_4H_2O$, $StHNO_3$, $StHBrH_2O$, $StHIH_2O$, $StTr3H_2O$, $St_2H_2C_2O_44\frac{1}{2}H_2O$, $St_2H_2SO_46H_2O$, $St_2H_2SO_45H_2O$, $StHCl\ 1\frac{3}{4}H_2O$, $StH_3PO_42H_2O$ ($St = C_{21}H_{22}O_2N_2$).

The literature reports $StH_2C_2O_4$. The salt studied has the composition $StH_2C_2O_41\frac{1}{2}H_2O$. The literature reports $St_2H_2SeO_46H_2O$ and $St_2H_2SeO_45\ H_2O$. The salt studied here seemed to have only $4\frac{1}{2}$ H_2O. The literature reports the normal malonate with $3\frac{1}{2}$ H_2O. The salt studied here had 6 H_2O. The literature reports strychnine thiocyanate, but the analysis does not check that of the salt studied here. No report could be found in the literature for acid succinate, glutarate, malëate, or chlorate.

MATERIALS

The strychnine used was Mallinckrodt's Strychnine

Alkaloid N.F. Powdered and N.F. crystals. The acids were chemically pure and with the exception of a few were either Baker's Analyzed or Eastman's chemicals.

EXPERIMENTAL

With the exception of the chlorate and thiocyanate, the salts were all prepared in the similar fashion. In the case of the salts of mono-basis acids and the acid salt of di-or tri-basic acids, the acid and strychnine alkaloid, in the proportions of one gram molecule of acid to one gram molecule of alkaloid, were weighed out and mixed together in water. The mixture was then heated to hasten the reaction. Sufficient water was added to dissolve the salt to form a nearly saturated solution when hot. The solution was then filtered and the salt allowed to crystallize out. It was then filtered and purified by recrystallization twice, or in some cases three times, from water. The salts were then dried in air except in cases where the salt lost water of crystallization in air.

The chlorate and thiocyanate were prepared by dissolving strychnine alkaloid in dilute acetic acid and then adding potassium chlorate and thiocyanate, respectively. The solution was then heated and the concentration adjusted so as to make a nearly saturated solution when hot. The solution was filtered hot and allowed to cool and crystallize. The salt which separated out was then purified by recrystallization.

The composition of the salts was found by determining nitrogen, strychnine, and water of crystallization.

Nitrogen was determined by Kjeldahl - Gunning - Arnold method.

Strychnine was determined by dissolving an accurately weighed amount of the salt in water, adding a few drops of dilute acetic acid when necessary to prevent hydrolysis, and adding an excess of saturated picric acid solution (9). The yellow precipitate of strychnine picrate was stirred several times while hot, allowed to stand for three hours or more, filtered through a Gooch crucible, dried at 110° C., and weighed. The calculated factor is

$$\frac{\text{Mol.wt. of strychnine}}{\text{Mol.wt. of strychnine picrate}} \approx \frac{334.3}{563.5} \approx 0.5933$$

but since the strychnine picrate is not completely insoluble, an empirical factor was determined by carrying out the analysis with pure strychnine alkaloid. The empirical factor which was used for all calculations was 0.5955 and was calculated from the following data:

Wt. of Strychnine	Wt. of Strychnine Picrate
0.1500	0.2519
0.1500	0.2519

Wt. of Strychnine	Wt. of Strychnine Picrate
0.1494	0.2512
0.1494	0.2514
0.1512	0.2542
0.1508	0.2528
0.9008	1.5126

$$\frac{0.9008}{1.5126} = 0.5955$$

Water of hydration was determined by the method of Wilson (10) modified by Germann and Muench (11) with some further modifications. The method is based on the principle that each hydrate has a definite aqueous tension and will therefore lose water, and hence weight, if exposed to an atmosphere which has an aqueous tension less than the aqueous tension of the hydrate, thus forming either the next lower hydrate of the anhydrous salt. If exposed to an atmosphere having an aqueous tension equal to or higher than the next higher hydrate, if one exists, it will gain water and hence weight forming the next higher hydrate, or will go into solution. If exposed to an atmosphere whose aqueous tension is less than the next higher hydrate and higher than, or equal to, the aqueous tension of the hydrate, the given hydrate is stable and will neither lose or gain weight, except that small amounts of moisture may be absorbed on the surface.

If a sample of a salt hydrate is exposed to atmospheres of decreasing aqueous tension (the intervals being small), and allowed to come to equilibrium in eabh case, the weight of the sample should remain constant until exposed to the first atmosphere which has a lower aqueous tension than the salt hydrate. Then the weight should decrease to that of the next lower hydrate, or the anhydrous salt. Practically, this is affected by absorption of small amounts of moisture on the surface of the salt and also by the extreme slowness of the loss of water when the aqueous tension of the atmosphere is only slightly below that of the salt hydrate.

The weights were taken on an assay balance which was sensitive to about 0.01 mg. and the aqueous tension of the atmosphere was regulated by means of sulphuric acid solutions of various concentrations.

The first set of determinations made was carried out as follows: a sample of the moist salt hydrate was placed on a tared microscope cover glass, the whole placed in a desiccator with a sulphuric acid solution of low concentration (15% to 20% of acid being about the lowest concentration it was practical to use although lower concentrations and even water were tried) and allowed to come to equilibrium. The sample was transferred rapidly to the balance and weighed.

The exposure and weighing were repeated until the weight seemed constant, when it was assumed that equilibrium had been established. The acid concentration was then increased (usually by 5%) and the procedure repeated. The transfer of the sample through the atmosphere of the room in some cases introduced very large errors. Data obtained by this method will be designated by the figure I in the following tables.

The later determinations were carried out by placing the sulphuric acid solution inside the balance case as Germann and Muench recommend. It was found, however, that the balance case could not be made tight enough to get either an atmosphere of moderately low or high aqueous tension. In order to improve this condition, a square bottle of suitable size was placed on its side in the balance case with the large mouth pointing outward and covered with a glass plate ground to fit and sealed with vaseline. A hole was drilled in the bottle through which the pan support of the balance passed so that the left pan of the balance hung freely inside the bottle. The sample was placed on a tared microscope cover glass and placed on this pan of the balance and did not require further manipulation except when it was removed for heating to drive off the last traces of moisture.

The sulphuric acid solutions contained in small dishes were put in and out through the mouth of the bottle. The same concentration of acid was placed both inside the bottle and in the balance case. With the glass plate closing the mouth of the bottle the only opening in the bottle was the small hole (about 1/4 inch) through which the pan support passed. With the balance case closed, the bottle was surrounded by an atmosphere which more or less approximated that in the bottle, and hence there was much less likelihood of a loss or gain of moisture by the system inclosed in the bottle than in the case where the whole system was only inclosed in the rather open balance case. Data obtained by this method will be designated by the figure II in the following tables.

The optical data was obtained using a Petrographic microscope and the usual methods of Optical Mineralogy. Refractive index was determined by the immersion method in all cases.

In those cases where the sign ± is found following the axial angle, the angle was estimated from the curvative of the isogyre in a centered optic axis figure. In the other cases the axial angle was determined by measuring the distance between the optic axes in a centered acute bisectrix figure by means of a calibrated scale and calculating the axial angle from this data.

Since system and optical orientation are difficult to determine with certainty on microscopic crystals, the data recorded is what seemed most reasonable after careful observation of extinction, dispersion, appearance under the microscope, etc.

In connection with the determination of optical properties, use was made of the following texts and publications: Winchell (12), Johannsen (13), Chamot and Mason (14), Larsen (15), Groth-Jackson (16), Wright (17), and others.

In the following pages each salt is taken up separately, and the analytical and optical data given.

STRYCHNINE DIOXALATE

The salt was prepared by mixing oxalic acid and strychnine alkaloid in the proportions of one molecule of acid to one of strychnine and purifying the salt thus formed by recrystallization. The purified salt consisted of white needles of moderate size and fairly soluble in water.

Determinations of nitrogen and of strychnine on the hydrous and anhydrous salts gave the following results:

	Calculated for $StH_2C_2O_4\ 1\frac{1}{2}H_2O$ Percent	Found Percent
N	6.20	6.22;6.18

	Calculated for $StH_2C_2O_4\ 1\frac{1}{2}H_2O$ Percent	Found Percent
St(Anhyd)	78.79	78.61;78.71
St(Hyd)	74.07	74.11;74.03

Determinations of water of hydration gave the following results:

I

Acid Concentration Percent	Mols. of Water
0	1.45
10	1.55
20	1.55

II

Acid Concentration Percent	Mols. of Water 1	Mols. of Water 2
15	1.63	
25	1.57	
50	1.52	1.51
55		1.51

This data indicates that the salt has the formula $St\ H_2C_2O_4\ 1\frac{1}{2}H_2O$. The only acid salt of oxalic acid found in the literature was $StH_2C_2O_4$.

The optical data found was as follows: crystals biaxial, sign +, elongation ± usually −, $\alpha = 1.592-$, $\beta = 1.603$, $\gamma = 1.669+$, $2V = 75^\circ \pm$, $\rho > v$, system monoclinic, extinction angle $9\frac{1}{2}^\circ$.

STRYCHNINE DISUCCINATE

The salt was prepared from equimolecular quantities of succinic acid and strychnine alkaloid, and was purified by recrystallization. Fine, rather fluffy needles were obtained, which were fairly soluble in hot water.

ANALYSIS

	Calculated for $StC_4H_6O_4H_2O$ Percent	Found Percent
N	5.95	5.96; 5.98
St (Anhyd)	73.90	73.94; 73.49
St (Hyd)	71.07	71.09; 71.21

Determination of water of hydration gave on the sample dried in air 1.00 H_2O.

I

Acid Concentration Percent	Mols. of Water
10	1.05

II

Acid Concentration Per cent	Mols. of Water	
	1	2
15	1.14	
35		1.15
45		1.15
50	.92	1.15
55	.78	1.15
60		1.01
65		.98
75		.96
95	.76	
P_2O_5		.96

The above data indicates that the formula is $StC_4H_6O_4H_2O$. No record of an acid succinate could be found in the literature.

Optical data was as follows: crystals biaxial, sign -, elongation -, $\alpha = 1.592$, $\gamma = 1.670$, $\beta = 1.646 \pm$, system orthorhombic?.

STRYCHNINE DITARTRATE

The salt was prepared from equimolecular quantities of strychnine alkaloid and tartaric acid, and was purified by recrystallization. The salt consisted of small white needles, which are much more soluble in hot water than in cold.

ANALYSIS

	Calculated for $St(CHOH)_2(COOH)_2 3H_2O$ Percent	Found Percent
N	5.20	5.16; 5.18
St (Anhyd)	69.02	69.46;69.47
St (Hyd)	62.09	62.11;62.41

Determination of water of hydration gave on the original sample dried in air 2.95 H_2O.

I

Acid Concentration Percent	Mols. of Water
10	3.05
30	3.05

II

Acid Concentration Percent	Mols. of Water	
	1	2
15	3.22	
40		
56	3.11) 2.95)	2.98

II (Cont.)

Acid Concentration Percent	Mols. of Water 1	2
55		2.98
60		2.98
75	2.78	

The formula from the above data must be $StC_4H_6O_6 3H_2O$.

The optical data was as follows: crystals biaxial, sign +, elongation indeterminate, $\alpha = 1.592$, $\beta = 1.603$, $\gamma = 1.625$, 2 V large 75° ± , dispersion marked $\rho < V$, system monoclinic.

STRYCHNINE CHLORATE

The salt was prepared by dissolving strychnine in a slight excess of acetic acid and adding potassium chlorate in the proportion of one mol of strychnine to one of potassium chlorate. On the first crystallization the solution was cooled rapidly, and small needles were obtained. On the final crystallization the solution was cooled slowly, and fairly large clear crystals were obtained. These crystals explode when heated to temperatures above 180°C. on a melting point plate.

ANALYSIS

	Calculated for $StHClO_3H_2O$ Percent	Found Percent
St (Anhyd)	79.83	79.97;80.11
St (Hyd)	76.54	76.52;76.64

Determination of water of hydration gave:

II

Acid Concentration Percent	Mols of Water
40	.99
50	.96
55	.91
60	.77
65	< .17
P_2O_5	0

This data indicates that the formula is $StHClO_3H_2O$. No record of this salt was found in the literature.

The optical properties were: crystals biaxial, sign +, elongation -, $\alpha = 1.605$, $\gamma = 1.669$, $\beta = 1.611$, $2V = 18^\circ$, dispersion horizontal, system monoclinic, $X = b$.

STRYCHNINE GLUTARATE

The salt was prepared from equimolecular quantities of the acid and strychnine alkaloid and purified by recrystallization.

The crystals before filtering the mother liquor were small flakes with a beautiful pearly lustre. When filtered out and dried, they formed a white powder.

Analysis gave:

	Calculated for $STC_5H_8O_4$ Per cent	Found Per cent
N	6.00	5.95; 5.89
St (Anhyd)	71.68	71.91;71.79

The analysis indicates that the salt has no water of crystallization. No record of this salt could be found in the literature. The salt decomposes when heated.

The optical data was as follows: crystals biaxial, sign -, elongation -, $\alpha = 1.575$, $\gamma = 1.655$.

STRYCHNINE HYDROBROMIDE

The salt was prepared from equimolecular quantities of strychnine alkaloid and hydrobromic acid and was purified by recrystallization. The needle-shaped crystals were of moderate size and fairly soluble in water.

ANALYSIS

	Calculated for $StHBrH_2O$ Percent	Found Percent
N	6.46	6.44; 6.52
St (Anhyd)	80.51	80.08;79.89
St (Hyd)	77.17	77.39;77.45

Determination of water of hydration gave:

I

Acid Concentration Percent	Mols. of Water
20	.89

Material from the sample bottle without exposure over acids gave .89 H_2O.

II

Acid Concentration Percent	Mols. of Water 1	2
25	1.27	
50	1.11	.99
55		.99
60		.99

This data indicates that the formula is $StHBrH_2O$.

The optical data was as follows: crystals biaxial, sign ±, elongation -, $\alpha = 1.646$, $\beta = 1.650$,

$\gamma = 1.73 \pm$ 2V = 10°, dispersion weak, system monoclinic.

STRYCHNINE HYDROCHLORIDE

The salt was prepared from equimolecular quantities of hydrochloric acid and strychnine, and was purified by recrystallization. The crystals obtained were light fluffy needles with a silky sheen.

ANALYSIS

	Calculated for StHCl $1\frac{3}{4}$ H_2O Percent	Found Percent
N	6.96	7.02; 6.97
St (Anhyd)	90.17	90.31;90.17
St (Hyd)	83.09	83.27;83.21
Cl	8.83	8.82; 8.86

Determinations of water of hydration gave on a sample dried in air 1.55 H_2O.

II

Acid Concentration Percent	Mols. of Water
10	2.03
20	1.97
35	1.80
50	1.75
60	1.64
75	1.50

The results of the water of hydration data do not agree with the result of analysis. This salt, however, is difficult to dehydrate completely, and since all calculations are based on the weight of anhydrous salt, this may account for the low valve for the mols of water of hydration.

The literature reports StHCl $1\frac{1}{2}H_2O$ and StHCl $1\frac{3}{4}H_2O$. The latter is the more recent and is in accord with the data of the strychnine and nitrogen determinations.

The optical data was as follows: crystals biaxial, sign +, elongation -, α=1.609, γ = 1.667, β = 1.627, 2V = 26°, dispersion weak $\rho > v$, system orthrhombic. Groth (8) gives the system as rhombic.

STRYCHNINE IODIDE

The salt was prepared from equimolecular quantities of strychnine alkaloid and hydriodic acid and was purified by recrystallization. Large needles (3-5 mn long) having a very slight amber color were obtained. They are a little less soluble than most of the other salts in water.

ANALYSIS

ANALYSIS

	Calculated for $StHI\ H_2O$ Percent	Found Percent
N	5.83	5.78; 5.86
St (Anhyd)	72.32	71.96;72.01
St (Hyd)	69.61	69.58;69.69

Water of hydration determinations gave:

I .89 H_2O when water was used instead of a solution of sulphuric acid in the desiccator.

II

Acid Concentration Percent	Mols. of Water 1	Mols. of Water 2
15	1.09	
25	1.04	
50	(1.04) (1.01)	.99
55		.99
60		.99

The parentheses indicate different determinations on the same sample over the same concentration of acid. That is, the sample was dehydrated, then hydrated, and in some cases dehydrated again.

From the above data the formula must be $StHIH_2O$.

The following optical data was obtained: crystals biaxial, sign +, elongation -, $\alpha = 1.661$, $\gamma = 1.73 \pm$, $\beta = 1.665$, $2V = 13^\circ$, $\rho < v$, system monoclinic.

The literature gives: $\alpha = 1.66$, $\gamma = 1.69$.

STRYCHNINE MALËATE

The salt was prepared from equimolecular quantity of malëic acid and strychnine alkaloid and was purified by recrystallization. The crystals were small needles with an amber tinge.

ANALYSIS

	Calculated for $StC_4H_4O_4H_2O$ Percent	Found Percent
St (Anhyd)	74.23	74.18;74.30
St (Hyd)	71.38	71.07;71.31

Determination of water of hydration gave:

II

Acid Concentration Percent	Mols. of Water 1	2
25	1.55	
30	1.44	1.46
35	1.28	1.23
40		1.28
45		1.21
50	(1.04) (1.05)	1.07

The analysis indicates that the formula should be $StC_4H_4O_4H_2O$. No record of this salt could be found in the literature.

Optical data was as follows: Crystals biaxial, sign - ?, elongation -, $\alpha = 1.544$, $\gamma = 1.667$, $\beta = 1.598$, $2V = 90° \pm$, system orthorhombic.

STRYCHNINE MALONATE

The salt was prepared from malonic acid and strychnine in the proportion of one molecule of acid to two of strychnine and was purified by recrystallization. The crystals thus prepared were white rectangular plates.

ANALYSIS

	Calculated for $St_2C_3H_4O_4 3\frac{1}{2}H_2O$ Percent	Calculated for $St_2C_3H_4O_4 6H_2O$ Percent	Found Percent
N		6.36	6.28; 6.27
St (Anhyd)	86.53	86.53	85.87;86.28
St (Hyd)	80.01	75.91	75.41;75.57

Determination of water of hydration gave:

On a sample dried in air 5.90 H_2O.

I

Acid Concentration Percent	Mols of Water
20	6.14

I (Cont.)

Acid Concentration Percent	Mols. of Water
30	6.12
40	(6.07) (5.56)

II

Acid Concentration Percent	Mols. of Water 1	Mols. of Water 2
30	6.37	6.48
35	6.28	
40	6.27	
45	6.23	
50	6.12	
55	5.97	6.08
60	3.18	2.87
65		Anhyd.

This data as well as the analysis indicates 6 H_2O. The literature records only $St_2H_4C_6O_5 3\frac{1}{2}H_2O$.

The optical data was as follows: crystals uniaxial, sign -, $\epsilon = 1.608$, $\omega = 1.610$, system tetragonal.

STRYCHNINE NITRATE

The salt was prepared by mixing equimolecular quantities of strychnine alkaloid and nitric acid, and was purified by recrystallization. White, moderately soluble, needles were obtained.

Analysis gives the following data:

	Calculated for $StHNO_3$ Percent	Found Percent
N	10.57	10.42;10.45
St(Anhyd)	84.14	84.24;84.54

The salt contains no water of hydration. The analysis indicates that the formula must be $StHNO_3$.

The optical data was as follows: crystals biaxial, sign +, elongation −, $\alpha = 1.610$, $\gamma = 1.675$, $\beta = 1.624$, $2V = 22^\circ$, $\rho > v$, system monoclinic.

The literature gives $\alpha = 1.62$, $\gamma = 1.67$ and system monoclinic.

STRYCHNINE OXALATE (Normal salt)

The salt was prepared by mixing oxalic acid and strychnine alkaloid in the proportions of one gram mol. of the acid to two of strychnine. The salt was purified by recrystallization. It consisted of fine white needles or coarse needles, depending on the rate at which the final crystallization took place.

ANALYSIS

Calculated for $St_2H_2C_2O_4\,4\frac{1}{2}H\ O$ Percent	Found Percent

N	6.67	
St (Anhyd)	88.14	88.20;88.14
St (Hyd)	79.62	79.74;79.65

Determination of water of hydration gave the following data:

II

Acid Concentration Percent	Mols. of Water 1	2
15	(4.84) (4.83)	
25	4.72	
35	(4.55) (4.50)	
40	4.50	
45	4.38	
50	(4.11) (4.11) (4.17)	
55	4.08	
60	(4.00) (3.61) (3.83)	
65	1.89	

Acid Concentration Percent	Mols. of Water 1	2
75	(1.78) (1.66)	
95	1.44	
P_2O_5	1.34	

This data indicates hydrates with 4½, 4 and 2 molecules of water. Analysis for strychnine however, shows that the salt has 4½ H_2O. Recrystallization of the salt near the boiling point produced somewhat broader needles, but the refractive index in the lengthwise direction was the same as for the analyzed sample, so it was assumed that the crystals were the same.

The literature reports 4½ H_2O. The optical data was as follows: crystals biaxial, sign +, elongation ±, $\alpha = 1.592\pm$, $\gamma = 1.666$, $\beta = 1.598$, $2V = 14^\circ$, $\rho > v$, system monoclinic.

STRYCHNINE PERCHLORATE

The salt was prepared from equimolecular quantities of strychnine alkaloid and perchloric acid after which it was purified by recrystallization. The salt thus prepared consisted of rather fine white needles, which are not very soluble in cold water and only a little more so in hot water; being much less soluble in hot water than other strychnine salts.

	Calculated for $StHClO_4H_2O$ Percent	Found Percent
N	6.18	6.08; 6.13

St (Anhyd)	76.89	76.38;76.38
St (Hyd)	73.83	73.94;74.11

Determinations of water of hydration gave the following results: The sample of salt from the sample bottle without exposure over acids gave .95 H_2O and the same sample dried in the air of the room until constant gave 1.00 H_2O.

II

Acid Concentration Percent	Mols. of Water 1	2
15	1.24	
25	1.14	
50	1.10	1.01
55		1.01
60		1.01

This indicates that the salt has the formula $StHClO_4H_2O$. The literature reports $StHClO_4H_2O$.

The following optical data was obtained: crystal biaxial, sign+, elongation±, $\alpha = 1.589$, $\gamma = 1.654$, $\beta = 1.598$, $2V = 45^\circ$, dispersion inclined, system monoclinic, extinction angle 22°.

STRYCHNINE PHOSPHATE

The salt was prepared from phosphoric acid and strychnine alkaloid by using slightly more phosphoric acid than was required by the ratio of one mol of acid to one of base. Small white needles were obtained.

ANALYSIS

	Calculated for $StH_3PO_4 2 H_2O$ Percent	Found Percent
N	5.98	6.11; 6.17
St (Anhyd)	77.32	77.63;77.40
St (Hyd)	71.36	71.42;71.39

Determination of water of hydration gave:

II

Acid Concentration Percent	Mols. of Water
40	
45	1.98
50	1.98
55	1.98
60	1.98

The data indicates that the formula is $StH_3PO_4 2H_2O$ which is in agreement with that published in the literature.

The optical data was as follows: crystals biaxial, sign +, elongation ±, $\alpha = 1.589$, $\gamma = 1.660$, $\beta = 1.597$, $2_V = 21°$, dispersion inclined?, system monoclinic, Y = b.

STRYCHNINE SELENATE

Strychnine alkaloid (12.5 gr.) and crystaline selenic acid $H_2SeO_4H_2O$(6.1 gr.) were mixed together and the resulting salt purified by recrystallization in the first attempt to prepare a selenate. Since these materials were in the proportion of one molecule of acid to one of alkaloid, it was expected that an acid salt would result. Analysis indicated, however, that the product was the normal and not the acid salt. It was also noted that while the purified crystals were stable over a period of about a year, the mother liquor on standing for some time evaporated to dryness and left some crystals and a quantity of red powder, which was probably selenium.

Apparently the alkaloid and the acid must react to form the normal salt leaving the excess selenic acid in the mother liquor. The selenic acid subsequently decomposes, depositing selenium. No attempt was made to repeat the preparation of the normal salt from amounts of acid and base which should produce the acid salt.

Later the normal salt was prepared from selenic acid and strychnine in the proportions of one molecule of acid to two of base.

ANALYSIS

	Calculated for $St_2H_2SeO_4 5 H_2O$ Percent	Calculated for $St_2H_2SeO_4 4\frac{1}{2}H_2O$ Percent	Found Percent
N		6.26	6.22; 6.18
St (Anhyd)	82.16	82.16	81.97;82.18
St(Hyd)	73.97	74.17	74.64;74.58

A weighed sample of the air-dried crystals, dehydrated in a vacuum over P_2O_5, gave data which calculated to 4.45 molecules of water.

Water of hydration determined by the second method was as follows:

II

Acid Concentration Percent	Mols. of Water 1	2
20		5.13
25		5.12
30		5.10
35	5.00	4.95
40	5.00	4.95
45	4.92	4.87

(Cont.)

Acid Concentration Percent	Mols. of Water 1	2
50	4.78	4.71
55	4.58	4.54
60	4.35	4.36
65	1.89	1.82
75	1.89	1.79
P_2O_5	1.25	1.53

The weight of the anhydrous salt was determined by analysis for strychnine because the salt could not be dehydrated without decomposition.

The literature reports $St_2H_2SeO_46H_2O$ and $St_2H_2SeO_45H_2O$ which are analogous to the strychnine sulphates. Determinations of nitrogen and strychnine indicate that the salt is $St_2H_2SeO_44\frac{1}{2}H_2O$. The water of hydration indicates a salt having 4½ molecules of water as shown by the sharp break between 60% and 65% acid. There is also indication of a salt with something between 1 and 2 H_2O. Recrystallization of the salt from nearly boiling solution, from solution at room temperature, and from solution in the ice box at about 5° C. produced crystals which gave the same refractive index as that determined on the analyzed sample, so it was assumed that the composition was the same.

One would expect to obtain $St_2H_2SeO_46H_2O$ by crystallization at lower temperatures, and if a salt with 1 to 2 H_2O exists, it should crystallize out at the higher temperatures. Although the hydrate over 20% acid contains more than 5 H_2O and indicates that such a hydrate is possible, there is no definite break in the dehydration to show that the hydrate exists.

The above data would seem to indicate that the formula was $St_2H_2SeO_4 4\frac{1}{2}H_2O$ rather than $St_2H_2SeO_4 5H_2O$ reported in the literature.

The optical data is as follows: crystals biaxial, sign $+$, elongation $-$, $\alpha = 1.598$, $\gamma = 1.661$, $\beta = 1.600$, $2V = 7^\circ$, $2E = 10\frac{1}{4}^\circ$, $\rho < \nu$, system monoclinic.

The literature gives for $St_2H_2SeO_4 5H_2O$ sign very strong, axial plane perpendicular to b(010), $2E = 14^\circ$, dispersion strong $\rho < \nu$, system monoclinic.

STRYCHNINE SULFATE (Normal salt)

The salt was prepared from sulphuric acid and strychnine alkaloid in the proportions of one mol of acid to two of strychnine and was purified by recrystallization.

For the preparation of the hexahydrate the crystals were allowed to form by evaporation at room temperature. The crystals were large square plates with an amber color. The same hydrate was obtained by allowing the crystals which separate from hot solution to stand in contact with the mother liquor for a day or more. The crystals thus formed were smaller and thicker but otherwise the same.

For the preparation of the pentahydrate, the crystallization was carried out in hot solution, above 60°. The crystals were broad needles or flakes with an amber tinge, although the crystals were not very large.

Analysis of the hexahydrate gave:

	Calculated for $St_2H_2SO_46H_2O$ Percent	Found Percent
N	6.40	6.46; 6.43
St (Anhyd)	87.20	86.83;86.80
St (Hyd)	76.42	76.04;75.96

Analysis for the Pentahydrate gave:

	Calculated for $St_2H_2SO_45H_2O$ Percent	Found Percent
St (Hyd)	78.03	78,02;78.04

Determination of the water of hydration gave:

I

Acid Concentration Percent	Mols. of Water
20	5.95
Dried in air	5.02

II

Acid Concentration Percent	Mols. of Water 1	2
10		(6.00) (5.95) (5.89)
15	5.72	5.89
25	5.67	5.68
35	5.55	
50	5.17	(5.08) (5.12) (5.11)
60	5.00	(4.75) (4.95) (4.88)
75	nearly anhydrous	

The literature reports $St_2H_2SO_4 6H_2O$, $St_2H_2SO_4 5H_2O$ and $St_2H_2SO_4 5\frac{1}{2}H_2O$. The latter, however, has been shown to be a mixture of the other two.

The optical data for the hexhydrate: crystals biaxial, sign -, $\alpha = 1.595$, $\gamma = 1.614$, $\beta = 1.613$, $2V = 3^{\circ}$, system orthorhombic or pseudo tetragonal.

Groth's Chemische Krystallographie gives system

Tetragonal Trapezohedral, sign -, 2E up to 30°.

The optical data for the pentahydrate: crystals biaxial, sign+, elongation -, $\alpha = 1.592$, $\gamma = 1.661$, $\beta = 1.597$, $2V = 10°$, $2E = 16°$, dispersion weak, system monoclinic.

Groth (8) gives: system monoclinic, sign+, $2E = 16° 30'$, dispersion distinct $\rho < v$, $\gamma = 1.594$ red.

STRYCHNINE THIOCYANATE

The salt was prepared by dissolving strychnine in acetic acid and adding potassium thiocyanate in the proportion of one mol of strychnine to one of potassium thiocyanate. The salt was purified by recrystallization and looked very much like the chlorate. It was much less soluble in hot water than the other salts except the perchlorate and normal oxalate.

ANALYSIS

	Calculated for $StHCNSH_2O$ Percent	Found Percent
St (Anhyd)	84.98	85.35;85.30
St (Hyd)	81.26	81.29;81.19

Determination of water of hydration gave:

II

Acid Concentration Percent	Mols. of Water
45	1.01

This data indicates the formula $StHCNSH_2O$.

Strychnine thiocyanate has been reported in the literature but the analysis does not check with the above.

The optical properties were: crystals biaxial, sign+, elongation±, $\alpha = 1.651$, $\gamma = 1.695$, $\beta = 1.654$, $2V = 15^\circ$, dispersion inclinde, $Y = b$.

TEST FOR STRYCHNINE

Strychnine is extracted with petroleum ether from alkaline solution in the usual way and the petroleum ether evaporated. The residue is then dissolved in a small amount of dilute acetic acid, the volume of solution depending on the amount of strychnine present. The solution is then divided into four parts, each of which should contain at least 1/4 cc. of solution and 1/2 milligram of strychnine. In case the original amount of strychnine is less than 2 milligrams, the number of subsequent tests may be reduced accordingly. The four solutions are placed in 50 cc beakers tilted so that the solution will collect at the side ~~so as~~ in order not to lose too much of the solution by spreading over the surface of the beaker. The solutions are then heated nearly to boiling, and the appropriate reagent added to each while hot.

To the first $HClO_4$ is added. The solution is allowed to cool and crystallize. The liquid is removed, and the crystals washed with 1 drop of water. The liquid may be soaked up in blotting paper, or filter paper, or may be filtered off by means of a micro-filter made by sticking a small piece of cotton or filter paper in the tip of an ordinary medicine dropper, sucking the liquid into the dropper through the filtering medium by means of the rubber bulb. The crystals are allowed to dry in air and are mounted in oil of refractive index 1.598. These will show the same index of refraction as the oil (will disappear) when the long direction of the crystal is parallel to the direction of vibration of the lower Nicol prism. (The 6 o'clock 12 o'clock direction in the Leitz Petrographic and in the B & L microscopes)

To the second solution add enough KCl to make the solution fairly concentrated, but not enough to separate out when cold. Allow the strychnine hydrochloride to crystallize. Filter and wash as before and mount in oil of refractive index 1.609. The crystals will show the same refractive index lengthwise as the oil.

To the third solution add KBr and proceed as with KCl, mounting in oil of refractive index 1.646. The crystals will show this index of refraction for light vibrating lengthwise.

To the fourth solution add KI and proceed as with KCl, mounting in oil of refractive index 1.661. The crystals will show this index of refraction when light is vibrating parallel to their long direction.

If desired, the test may be extended to include other salts, such as nitrate and thiocyanate. The four salts chosen seemed to be the most satisfactory because their solubility was not so great as most of the other salts, and the refractive index in the lengthwise direction is easy to determine.

The concentration of strychnine in the solution may require adjusting either by concentration or dilution, for, if the quantity is too small, no strychnine salt will separate out and if too concentrated, the salt will separate out in very small crystals which are difficult to work with. In case the strychnine salt separates out immediately on adding the reagent to the nearly boiling solution of strychnine in acetic acid, it is best to dilute the solution slightly and heat to get the salt all in solution when hot, because otherwise the crystals are apt to be quite small. The solutions should be allowed to cool slowly.

An excess of potassium halide is added because it decreases the solubility of the strychnine halide, but it is necessary to avoid crystallizing out the potassium halide from the solution. If too much KBr is added, KBr or possibly a double salt crystallizes from this solution in large needle shaped crystals which, however, are isotropic and can be easily distinguished from the strychnine halide.

In the standard procedure for the separation of alkaloids by extraction with various solvents, there are a rather large number of alkaloids which are extracted by petroleum-ether from alkaline solution along with the strychnine. Of this number only the more common ones were available.

Tests were carried out with each of these alkaloids with the perchloric acid reagent in order to see if they gave results the same or similar to those given by strychnine. In a second set of experiments the test was carried out on a mixture of strychnine with each of the above alkaloids to determine whether their presence would interfere with, or prevent, the formation and observation of the characteristic crystals of strychnine perchlorate.

In the first set of experiments where the test was run on each alkaloid separately, the quantity of material used was in all cases sufficiently large so that even quite soluble substances would crystallize out when the solutions were concentrated by evaporation. The results were as follows:

Aconite: On evaporation gave extremely small, stubby rods. Mounted in oil 1.598 do not even faintly resemble strychnine perchlorate.

Atropine: No crystals at all. Viscous liquid when evaporated almost to dryness.

Berberine (Dilute), Gives a yellow insoluble precipitate containing some small rods with parallel extinction. These have an index lower than 1.598.

Brucine: Gives a precipitate of good crystals from only moderately concentrated solution. The index of refraction for these crystals lengthwise is less than 1.598 and crosswise is greater than 1.598.

Codeine: Only an oil separates on concentration almost to dryness.

Emetin: From moderately concentrated solutions gives oily drops.

Homoatropin: Gives an oil only when concentrated nearly to dryness.

Hydrastin: Gives an oil in moderately concentrated solutions.

Hyoscyamine: Gives an oil when quite concentrated.

Papaverine: Gives an oil when moderately concentrated.

Pilocarpine: Gives no precipitate and no oil when concentrated.

Quinine: Yields a very little oil when very concentrated.

Scopolamin: Gives the same results as quinine.

Sanguinarin: Yields a copious red precipitate which contains some very tiny rods whose index of refraction is distinctly different from 1.598.

Physostigmin: Gives no precipitate or oil on concentration.

In the second set of experiments the test was run on mixtures of each alkaloid with strychnine to find out whether there was any interference. The alkaloids used were the same as in the preceeding set.

Except in the following three cases there was no interference:

Brucine gives a precipitate which interferes if the quantity of Brucine is large in proportion to the strychnine.

With berberine the precipitate which forms can be filtered from the hot solution. The strychnine perchlorate than crystallizes from the filtrate without interference.

Sanguinarin acts like berberine and can be removed in the same way.

Of the substances tried brucine, in large quantities, is the only one which really interferes with the test for strychnine.

CONCLUSION

1. Eighteen salts of strychnine have been prepared and their composition proved.
2. Four of these salts, the disuccinate, glutarate, maleäte, and chlorate, have not previously been reported in the literature.
3. The following salts were found to have a different composition than that reported: dioxalate, selenate, malonate and thiocyanate.
4. The optical properties of the eighteen salts are reported.
5. A proposed test for the identification of strychnine, based on refractive index, is given.
6. Tests on substances which might interfere with the test for strychnine are given.

REFERENCES

1. O'Day, David W. 1928. "The Study of Organic Substances which Interfere with Mandelin's Test for Strychnine." Thesis University of Colorado

2. Bailey, John F. 1931. "A Study of Organic Substances which Interfere with Otto's Test for Strychnine." Thesis University of Colorado.

3. Wright, F. E. 1912. "The Petrographic Miscroscope in Analysis." Journal American Chemical Society. 38, 1647.

4. Wherry, E. T. 1918. "The Application of Optical Methods of Identification to Alkaloids and Other Organic Compounds." U. S. Dept. of Agriculture Bulletin 679.

5. Wherry, E. T. and Yanovsky, E., 1918. "The Identification of the Cinchona Alkaloids by Optical-Crystallographical Measurements." Journal American Chemical Society 40, 1063.

6. Keenan, J. L. 1927. "Optical Identification of Alkaloids." Journal Amer. Phar. Assoc. 16, 837.

7. Bolland, A. 1910 "Mikrochemische Studien IV" Monatsch. f. Chem. 31, 387.

8. Groth, P. 1906-1919 Chemische Krystallographie.

REFERENCES (Cont.)

9. Elmore, J. W. 1926. "Determination of Strychnine in Poisoned Grain." Journal Assoc. Official Agric. Chem. 9, 224.

10. Wilson, R. E. 1921. "Some New Methods for the Determination of Vapor Pressure of Salt Hydrates." Journal American Chemical Society. 43, 704.

11. Germann, F. E. E. and Muench, O. B. 1928. " A Microchemical Method for the Determination of Vapor Pressure and Water of Hydration of Solid Compounds." Journal Phy. Chem. 32, 1380.

12. Winchell, N. H. and A. N. 1922. "Elements of Optical Mineralogy. Part I. Principles and Methods." John Wiley & Sons, New York.

13. Johannsen 1914. "Manual of Petrographic Methods."

14. Chamot, E. M. and Mason, C. W. 1930. "Handbook of Chemical Microscopy." John Wiley and Sons, New York

15. Larsen, Esper S., 1921. "The Microscopic Determination of the Nonopaque Minerals." U. S. Geological Survey, Bull. 679.

16. Groth-Jackson 1910. "Optical Properties of Crystals."

17. Wright, F. E. 1911. "The Methods of Petrographic Microscopic Research." Carnegie Inst., Washington Pub. 158.