

2. Certain changes are suggested for improving the Modified Smith-Sorensen method.
3. The U. S. P. and B. P. Amylase assay methods are discussed and compared.
4. Results of lipase content by the B. P. method are given for five samples of Pancreatin U. S. P. X examined.

## LITERATURE CITED.

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- (2) O. Gross, *Arch. expl. Path. Pharmacol.*, 58 (1906), 157, 166.
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- (5) F. E. Willson, *JOUR. A. PH. A.*, 19 (1930), 129-132.

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THE DETERMINATION OF STRYCHNINE AND BRUCINE AS HYDRO-FERROCYANIDES AND THEIR SEPARATION BY MEANS OF FERROCYANIDE.\*

BY I. M. KOLTHOFF AND J. J. LINGANE.

The usual procedure for the separation of strychnine from brucine is based on the fact that brucine is fairly easily destroyed by treatment with nitric acid. In the present work it was found that this method yields approximate results only; either the brucine is not completely destroyed or on more drastic treatment part of the strychnine is decomposed also. Therefore a more exact method would be of value. It is known that ferrocyanide in acid medium gives slightly soluble crystalline hydroferrocyanides with strychnine and brucine; the former crystallizes much faster and is less soluble than the latter. Beckurts and Holtz<sup>1</sup> made use of this difference in behavior and titrated a strongly acid solution of the mixed alkaloids with standard potassium ferrocyanide, using ferric chloride test paper as an outside indicator. The success of the method depends on the slowness with which brucine is precipitated by the slight excess of ferrocyanide after the precipitation of strychnine is complete. The detection of the end-point, however, is not very sharp; after much practice an accuracy of about 5% could be obtained.

Gadreau<sup>2</sup> proposed a much more complicated method. He precipitates the strychnine and part of the brucine by addition of a large excess of ferrocyanide in weakly acid medium. The precipitate is treated with an excess of ammonia and the free alkaloids are extracted with chloroform. After evaporation of the solvent the alkaloids are dissolved in 0.1*N* hydrochloric acid and the precipitation with ferrocyanide repeated as before. The process is repeated three times and the final precipitate of strychnine hydroferrocyanide weighed after drying over sulphuric acid and finally in an oven.

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\* Scientific Section, A. PH. A., Madison meeting, 1933.

<sup>1</sup> Beckurts and Holtz, *Pharm. Zentralhalle*, 28 (1887), 119.

<sup>2</sup> Gadreau, *J. pharm. chim.* [6], 4 (1927), 145.

In this study the properties of strychnine and brucine hydroferrocyanide have been investigated and a simple procedure developed for the determination of strychnine in the presence of brucine.

#### MATERIALS USED.

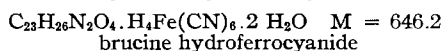
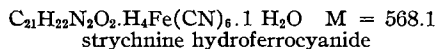
*Brucine Hydrochloride*.—Commercial brucine was dissolved in chloroform and treated with gaseous hydrochloric acid. The hydrochloride was allowed to crystallize and was recrystallized from alcohol containing 20% acetone. The aqueous solution of the salt was standardized by gravimetric chloride determinations. In other experiments a solution of the salt was obtained by dissolving a known amount of brucine, recrystallized from acetone and alcohol, in a slight excess of hydrochloric acid.

*Strychnine Sulphate*.—A Merck & Company product was used without further purification. It appeared to be the normal salt; *i. e.*, containing one molecule of strychnine per one molecule of sulphuric acid. The aqueous solution was standardized by titration with sodium hydroxide, using methyl red as indicator. In addition, the sulphate was determined gravimetrically as barium sulphate; the results obtained in the two methods agreed within 0.2%, and were 0.4% lower than those calculated from the weight of the salt taken, assuming the latter to be pure.

*Potassium Ferrocyanide*.—A C.P. product was twice recrystallized from water and dried over deliquescent sodium bromide. Solutions of this salt in conductivity water were used and stabilized by the addition of 0.2% of sodium carbonate.

#### COMPOSITION OF THE HYDROFERROCYANIDES OF STRYCHNINE AND BRUCINE.

The hydroferrocyanides of both alkaloids were prepared by precipitation from strongly acid medium (*v. i.*) with an excess of potassium ferrocyanide. The precipitates were washed with water and air dried. The water content was determined by heating 0.5- to 0.8-Gm. samples in a vacuum oven at 60° until constant weight was obtained. Upon continued drying at 110° no further loss in weight was found with the strychnine hydroferrocyanide. It is not recommended to heat the air-dry precipitates directly at 110°, since a decomposition, especially of the brucine compound, takes place. The hydroferrocyanide content of the precipitates was determined by titration of the aqueous suspensions with sodium hydroxide, using methyl red as indicator. The results showed that the air-dry hydroferrocyanides have the following composition:



W. M. Cumming<sup>1</sup> reports a similar composition, but with two molecules of water of crystallization for the strychnine salt instead of one. However, four water determinations made by us yielded a water content between 3.1 and 3.5% (theoretical for 1 H<sub>2</sub>O, 3.17%, for 2 H<sub>2</sub>O, 6.15%).

#### SENSITIVITY OF THE PRECIPITATION OF STRYCHNINE AND BRUCINE.

The sensitivity is greatly dependent upon the acid concentration and to a lesser extent on the ferrocyanide content. The original precipitates are white, but the brucine hydroferrocyanide turns green on standing over night in acid medium. In the final procedure 1 cc. 0.5 molar potassium ferrocyanide was added to 10-cc. solution of the alkaloid salt containing the indicated amount of hydro-

<sup>1</sup> W. M. Cumming, *J. Soc. Chem. Ind.*, 44 (1925), 110T.

chloric acid. The results are given in Table I. It is seen that the sensitivity of the precipitation of strychnine in 0.1 to 0.5*N* hydrochloric acid is much larger than that of brucine. At these acidities 0.07-mg. strychnine can be detected in 10 cc. of solution if observed after 30 minutes of standing. The precipitation of this alkaloid, therefore, is quantitative. The sensitivity of the precipitation of brucine is the greatest in 3*N* hydrochloric acid. After 10 minutes of standing 3 mg. of brucine in 10 cc. of solution can be detected. The data in the table also indicate that the precipitation of strychnine is much faster than that of brucine.

TABLE I.—SENSITIVITY OF PRECIPITATION OF STRYCHNINE AND BRUCINE.

Concentration, HCl, <i>N</i> .	Concentration Strychnine, Moles p. l.	Result.	Concentration Brucine, Moles p. l.	Result.
0	0.01	Trace ppt. after 1 hr.	0.02	No ppt. after 2 hours
0.1	0.01	Immediate ppt.	0.02	Ppt. after 25 minutes
0.1	0.004	Ppt. after 10 seconds	0.001	Ppt. after 24 hours
0.1	0.0002	Ppt. after 1–2 minutes		
0.1	0.0001	Ppt. after 3–5 minutes		
0.1	0.00004	Ppt. after 9 minutes		
0.1	0.00002	Slight ppt. after 30 minutes		
0.5	0.0001	Ppt. after 2 minutes	0.003	Ppt. after 10 minutes
0.5	0.00004	Ppt. after 9 minutes		
3			0.0008	Ppt. after 5 minutes
3			0.0006	No ppt. after 24 hours

## SOLUBILITY OF STRYCHNINE AND BRUCINE HYDROFERROCYANIDES.

The solubility was determined by shaking a known weight of the salt for 45 minutes with a known volume of solvent. Longer shaking is not desirable since decomposition may take place. The solid was collected and washed quickly with small portions of alcohol and ether, and weighed after 20 minutes' drying in the air.

TABLE II.—SOLUBILITY OF STRYCHNINE AND BRUCINE HYDROFERROCYANIDE. (TEMPERATURE 25° ± 2°.)

Solvent.	Strychnine Hydroferrocyanide Dissolved, Mg. per 100 Cc.	Brucine Hydroferrocyanide Dissolved, Mg. per 100 Cc.
Water	36	237
0.1 <i>N</i> HCl	3.7	52
1 <i>N</i> HCl	..	50 <sup>a</sup>
3 <i>N</i> HCl	4.2	78

<sup>a</sup> Temperature was 21°.

## GRAVIMETRIC AND VOLUMETRIC DETERMINATION OF STRYCHNINE AS HYDROFERROCYANIDE.

*Procedure.*—To a measured volume of the strychnine solution water and so much hydrochloric acid is added that the concentration is 0.1 to 1*N* in a final volume of 100 cc. Five cc. 0.5 molar potassium ferrocyanide are added slowly from a pipette resulting in the rapid formation of a nicely crystalline precipitate. After 30 minutes of standing and occasional stirring the precipitate is filtered on a sintered glass crucible (1 G.-4), washed 5 to 6 times with approximately 0.1*N* hydrochloric acid, followed by two or three washings with alcohol and two or three washings with ether. Air is aspirated through for a few minutes and the crucible weighed after 20–30 minutes' standing in the air. The strychnine hydroferrocyanide  $C_{21}H_{22}N_2O_2 \cdot H_4Fe(CN)_6 \cdot H_2O$  contains 58.83% strychnine.

TABLE III.—GRAVIMETRIC DETERMINATION OF STRYCHNINE.

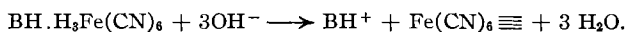
Strychnine Taken, Gm.	HCl, N.	Weight Ppt., Gm.	Strychnine Found, Gm.	Error %
0.3384 <sup>a</sup>	0.1	0.5680	0.3342	-1.3
0.3384 <sup>a</sup>	0.1	0.5673	0.3337	-1.4
0.3384	0.1	0.5764	0.3391	+0.2
0.3384	0.1	0.5756	0.3386	+0.05
0.3384	0.1	0.5760	0.3389	+0.1
0.3384	1	0.5767	0.3393	+0.3
0.1066	1	0.1813	0.1067	+0.1
0.0677	1	0.1150	0.0677	0.0

<sup>a</sup> Washed with water instead of 0.1N hydrochloric acid.

Table III shows that the procedure gives excellent results. The concentration of the strychnine in the last example in the table was only 0.002 molar and still the theoretical yield of precipitate was found.

#### VOLUMETRIC DETERMINATION OF THE STRYCHNINE HYDROFERROCYANIDE.

Since the gravimetric determination of strychnine is so extremely simple, there is not much use for a volumetric determination of the precipitate. If very small quantities of strychnine have to be determined, a volumetric procedure may be of some advantage. The strychnine hydroferrocyanide can be titrated with sodium hydroxide. If methyl red is used as indicator, the end-point is determined by the following reaction:



The color change from red to yellow is not very sharp since strychnine is a relatively weak base and the fourth ionization constant of hydroferrocyanic acid is of the order of  $10^{-4}$ . Still the color change to yellow can be seen with an accuracy of 0.1 cc. 0.05N base.

The same procedure was followed as described for the gravimetric determination except that a Gooch crucible with a paper disc was used to collect the precipitate. After washing with alcohol the precipitate and paper mat were transferred to a flask and treated with 50 cc. of water and the suspension titrated with carbonate free sodium hydroxide, using methyl red as indicator. The precipitate dissolves gradually as the titration proceeds. 1 cc. 0.1N sodium hydroxide is equivalent to 11.14 mg. of strychnine. The data in Table IV show that the results are about 1 to 3% low. In the last example the concentration of the strychnine in the original solution was only 0.0008 molar.

TABLE IV.—VOLUMETRIC DETERMINATION OF THE PRECIPITATE.

Strychnine Taken, Gm.	HCl, N.	0.052N NaOH Used, Cc.	Strychnine Found, Gm.	Error %
0.2714	1	44.91	0.2642	-2.7
0.2714	3	44.70	0.2630	-3.1
0.0678	1	11.38	0.0670	-1.2
0.0678	3	11.30	0.0665	-1.9
0.0271	1	4.38	0.0258	-4.8

#### GRAVIMETRIC DETERMINATION OF BRUCINE.

The same procedure was followed as described for strychnine except that the acidity was 1 to 3N with respect to hydrochloric acid in the final volume, filtration

made after standing for at least one hour, and the precipitate quantitatively transferred to the crucible by means of the first filtrate. If dilute hydrochloric acid is used for this purpose, the results are markedly low on account of solubility loss. After the precipitate is transferred to the crucible, it is washed 2 to 3 times with 2- to 3-cc. portions of 0.1*N* hydrochloric acid and finally with alcohol and ether. The rate of precipitation of brucine is much slower than that of strychnine; in the examples given in the table precipitation began after a few minutes' vigorous stirring. The results in Table V show that the determination of brucine as hydroferrocyanide is not as accurate as that of strychnine.

TABLE V.—GRAVIMETRIC DETERMINATION OF BRUCINE.  
(0.1314 Gm. brucine taken; precipitate contains 61.00% brucine.)

HCl, <i>N</i> .	Time before Filtration, Hours.	Weight Ppt., Gm.	Brucine Found, Gm.	Error %
3 <sup>a</sup>	1/2	0.1994	0.1216	-7.5
3 <sup>a</sup>	1	0.1997	0.1218	-7.3
1	1	0.2103	0.1283	-2.4
3	1	0.2120	0.1293	-1.6
1	20	0.2119	0.1293	-1.6
3	20	0.2108	0.1286	-2.1

<sup>a</sup> Precipitate transferred to crucible with 0.1*N* HCl instead of with filtrate.

It may be mentioned that the results were 2 to 4% high if 25 to 50 cc. of 0.5 molar ferrocyanide was used instead of 5 cc. Coprecipitation of potassium does not account for these high results.

#### DETERMINATION OF STRYCHNINE IN THE PRESENCE OF BRUCINE.

Use has been made of the smaller solubility of strychnine hydroferrocyanide and its greater speed of precipitation as compared with the corresponding brucine compound in the separation of strychnine from brucine.

#### SINGLE PRECIPITATION. APPROXIMATE PROCEDURE.

The mixture of the two alkaloids is diluted with water and hydrochloric acid to a volume of 50 cc. and an acidity of 1 to 2*N*. The solution is titrated with 0.025 molar potassium ferrocyanide until all of the strychnine has been precipitated as indicated by testing with ferric chloride paper. This test is made by immersing a narrow, pointed strip of filter paper into the solution. The end of the paper which has been dipped in the solution is then torn off and a drop of ferric chloride solution placed near the rim of the wet spot. The indicator solution diffuses by capillary action and a bluish zone is formed at the junction in the presence of an excess of ferrocyanide. The end-point is not very sharp, and its detection requires some practice, an excess of 0.5 to 0.6 cc. of the ferrocyanide solution being required to make the color change visible. The mixture is allowed to stand for 10 to 15 minutes. The precipitate is then collected on a sintered glass crucible and further treated as described before in the gravimetric determination of strychnine.

The results in Table VI show that the acidity of the solution may be varied between 1 and 3*N* and that 100 mg. of strychnine can be determined in the presence

of the same amount of brucine with an accuracy of about 1 to 3%. As a rule high results are found owing to precipitation of some brucine. This error increases rapidly with increasing excess of ferrocyanide; for this reason it is safer to apply the double precipitation method as described below.

TABLE VI.—SINGLE PRECIPITATION OF STRYCHNINE IN THE PRESENCE OF BRUCINE.

(0.1066 Gm. strychnine taken in all experiments. Titrated with 0.0238 molar  $K_4Fe(CN)_6$ . Theoretical amount of reagent required for complete precipitation of strychnine is 13.4 cc.)

Brucine Present, Gm.	HCl, N.	$K_4Fe(CN)_6$ Added, Cc.	Time of Standing before Filtration, Minutes.	Weight Precipitate, Gm.	Strychnine Found, Gm.	Error %.
0	1	14.0	5	0.1782	0.1048	-1.7
0	1	14.2	10	0.1782	0.1048	-1.7
0	1	14.2	15	0.1788	0.1052	-1.3
0.066	1	14.0	5	0.1782	0.1048	-1.7
0.066	1	14.2	15	0.1870	0.1100	+3.2
0.066	3	14.2	15	0.1853	0.1090	+2.3
0	3	14.2	15	0.1794	0.1056	-0.9
0.131	3	14.2	15	0.1838	0.1081	+1.4
0.131	3	14.2	15	0.1864	0.1097	+2.9
0.131	3	15.0	15	0.1905	0.1121	+5.2

## DOUBLE PRECIPITATION.

The mixture is brought to a volume of about 50 cc. and a hydrochloric acid concentration of about 3*N*. The solution is then titrated with 0.025 molar potassium ferrocyanide until a distinct excess (about 15 to 20%) is present as indicated by the ferric chloride test. The precipitate is filtered after 15 minutes' standing on a Gooch crucible with paper mat and washed once with a small volume of 0.1*N* hydrochloric acid. It is then transferred back into the original precipitation flask and treated with 40 to 50 cc. water and about 1*N* ammonia until the precipitate is dissolved. A slight excess of ammonia is not harmful but gives rise to the formation of the free alkaloid. Five cc. of concentrated or 10 cc. of 6*N* hydrochloric acid are added. The precipitate formed is allowed to stand for 15 minutes, collected in a sintered glass crucible (1 G.-4) and further treated as described under the gravimetric determination of strychnine.

TABLE VII.—DOUBLE PRECIPITATION OF STRYCHNINE IN THE PRESENCE OF BRUCINE.

(0.1066 Gm. of strychnine and 0.2 Gm. of brucine in all experiments.)

Excess 0.025 Molar $K_4Fe(CN)_6$ in First Ppt., %.	Second Precipitation. Volume.	HCl, N.	Weight Ppt., Gm.	Strychnine Found, Gm.	Error %.
12	50	0.2	0.1711	0.1007	-5.5
12	50	1	0.1780	0.1047	-1.8
20	50	1	0.1832	0.1078	+1.1
20	50	1	0.1785	0.1050	-1.5
20	50	1	0.1806	0.1062	-0.4
20	125	1	0.1823	0.1072	+0.6
20	125	1	0.1806	0.1062	-0.4
20	125	1	0.1767	0.1040	-2.4
20	125	1	0.1764	0.1038	-2.6

The results in Table VII show that 100 mg. of strychnine can be determined in the presence of double the amount of brucine with an accuracy of about 1%.

It is suggested that the methods described may be advantageously applied to the direct determination of strychnine and brucine in strychnos preparations. First the sum of the two alkaloids could be determined by precipitation in acid medium with an excess of potassium ferrocyanide. The weighed precipitate then might be decomposed with ammonia and the strychnine determined as described above.

#### SUMMARY.

1. The sensitivity of the precipitation of strychnine and brucine in hydrochloric acid medium with hydroferrocyanide has been determined.

2. Strychnine can be determined with great accuracy by precipitation as hydroferrocyanide. The precipitate is weighed in the air-dry form. The method yields quantitative results even at great dilutions. The determination of brucine is less accurate owing to the greater solubility of its hydroferrocyanide.

3. A simple method is described for the quantitative determination of strychnine in the presence of brucine. It is based on the fact that the hydroferrocyanide of strychnine is less soluble and is formed more rapidly than that of brucine.

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#### THE ARSENIC CONTENT OF CHONDRUS.\*

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In 1931 and 1932 we conducted an investigation of the sulphur dioxide content of Chondrus, which was reported in the *JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION*, Vol. XXI, No. 11, November 1932, page 1146.

In this paper we showed that sulphur dioxide is not a normal constituent of natural chondrus as had been claimed, and that chondrus of European origin as sold in America was invariably contaminated with sulphur dioxide as a result of the "sulphur bleaching" process to which it is exposed before shipment to America, and that the natural chondrus shows no evidences of sulphur dioxide when subjected to tests for that adulterant by the methods of the A. O. A. C.

In view of the fact that the European samples might possibly have been bleached with arsenic containing sulphur, it seemed advisable to make a further investigation of the samples to ascertain the arsenic content.

As we had sufficient amount of all but one of the samples in question, we undertook the investigation and report the results herewith:

The method followed was that of the A. O. A. C. *Methods of Analysis*, page 306, 3rd Edition.

The preliminary procedure was as follows:

Ten Gm. of chondrus were digested with 25 cc. of sulphuric acid and a total of 30 cc. of nitric acid. After the organic matter had been destroyed and the digestion was complete the remaining liquid was diluted to 100 cc. and 10 cc. of this liquid was used for each determination.

The laboratory numbers, sources of the samples and sulphur dioxide content are repeated from the previous article.

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\* Scientific Section, A. PH. A., Madison meeting, 1933.