

the gases are true gases, and the vapors we are considering are by no means true gases. However, for all vapors which I could follow in tables, the corrections coming from this source were trifling.

I do not mean of course that equation (2) is wrong; I only mean that it does not give results in harmony with our ideas concerning the molecular weights of liquids in concentrated solutions, while equation (1) does give excellent results, and I cannot find sufficient reason why (2) should fail unless we look upon the osmotic laws as ideal laws to be kept out of actual experimental work.

We cannot at present therefore base equation (1) on equation (2), but must seek some other way of justifying it, a conclusion not reached in a previous paper.¹

RUTGERS COLLEGE,
May 6, 1899.

HYDRASTINE HEXAIODIDE, AND THE ASSAY OF HYDRAS- TIS CANADENSIS BY MEANS OF STANDARD IODINE FOR HYDRASTINE AND OF STANDARD POTAS- SIUM IODIDE FOR BERBERINE.

BY H. M. GORDIN AND A. B. PRESCOTT.²

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WHEN a solution of iodine in potassium iodide is added to a solution of a salt of hydrastine a dense precipitate falls out, of a color varying from light brown to a very dark brown. In this order of mixing the alkaloidal solution with the iodine solution, different periodides seem to be formed in mixture, no matter whether the addition of iodine is stopped while the alkaloid is yet in excess or carried till the iodine is in excess. Even in the first case, that is, when the addition of iodine is stopped long before all the alkaloid is precipitated, the body formed only approaches a triiodide in composition, but does not correspond to a triiodide exactly. As will be seen from the accompanying analysis the total iodine of the periodide formed under these circumstances agrees quite well with the theoretical amount required by hydrastine triiodide, but the additive iodine is considerably below the amount required by that body. It

¹ *J. phys. Chem.*, 2, 358 (1898).

² In the work of Research Committee D, Section II., Revision and Publication of the Pharmacopoeia of the United States.

will be noticed that there is a decided difference in this respect between hydrastine on one hand, and strychnine, brucine, and some other alkaloids on the other. The latter always form triiodides when iodine is added to an excess of a solution of their salt.¹

But if the order of mixing be reversed, that is, the weak alkaloidal solution added to a large excess of iodine, a definite and unique periodide, namely, hydrastine hydriodide penta iodide is always formed. This body is prepared according to the general method of making the higher alkaloidal periodides, described by us in the previous paper.² The hexaiodide thus made is a very dark brown powder, very difficultly soluble in ether, benzol, or cold chloroform, more readily in hot chloroform and in alcohol, and very easily in a mixture of alcohol and chloroform, or of alcohol and ether. In hot water it melts to a resinous mass. Attempts to crystallize it have failed, as on evaporation of the solvent it always remains a resinous mass.

Both in the hydrastine hexaiodide and the lower compound, that approaching a triiodide in composition, the so-called additive iodine was estimated by dissolving the substance in a small quantity of a mixture of alcohol and chloroform, and titrating with standard sodium thiosulphate. For the estimation of total iodine in these compounds, the substance was treated with zinc and ammonia, the iodine then liberated by means of a solution of nitrous acid in concentrated sulphuric acid, and taken up with carbon disulphide. The details were carried out exactly as described in our paper on morphine tetraiodide.³

ANALYSIS OF THE HYDRASTINE HEXAIODIDE.

For additive iodine: 0.1371 gram substance was found to contain 0.075833 gram additive iodine, and 0.1623 gram substance, 0.0898344 gram additive iodine.

Calculated for $C_{21}H_{21}NO_6HI_6$.	Found.	
	I.	II.
55.43	55.30	55.34

For total iodine : 0.1491 gram substance contained 0.0996800 gram total iodine, and 0.1429 gram contained 0.0958333 gram total iodine.

¹ This Journal, 20, 708 (1898).

² *Loc. cit.*, 710 (1898).

³ This Journal, 20, 717 (1898).

Calculated for $C_{21}H_{21}NO_6HI.I_2$.	Found.	
	I.	II.
66.52	66.86	66.06

ANALYSIS OF THE LOWER PERIODIDE OF HYDRASTINE,
APPROACHING A TRIIODIDE.

For total iodine: 0.2674 gram contained 0.1337328 gram total iodine, and 0.2396 gram contained 0.1191752 gram total iodine.

Calculated for $C_{21}H_{21}NO_6HI.I_2$.	Found.	
	I.	II.
49.83	50.01	49.74

For additive iodine: 0.09535 gram contained 0.0340975 gram free iodine, 0.1737 gram contained 0.0626681 gram free iodine, 0.1215 gram contained 0.04413953 gram, and 0.2328 gram contained 0.0838545 gram free iodine.

Calculated for $C_{21}H_{21}NO_6HI.I_2$.	Found.			
	I.	II.	III.	IV.
33.22	35.76	36.07	36.33	36.02

In the iodometric estimation of hydrastine the exclusive formation of the hexaiodide is assured by adding a weak solution of the alkaloidal salt to a large excess of iodine. The estimation is carried out in the same way as described by us in a previous paper.¹

The factor for hydrastine is 0.60403.

$$382.14 : 5 \times 126.53 :: 0.60403 : 1$$

In order to test this factor two solutions of hydrastine² in acidulated water were prepared, containing 0.3 per cent. and 0.15 per cent. of the free alkaloid, respectively, and the estimation of the strength of the solutions carried out exactly as described in the above-mentioned paper.

The results were as follows: Twenty cc. of the first (three-tenths per cent.) solution consumed 0.0970001 gram free iodine and twenty-five cc. of the second (0.15 per cent.) solution consumed 0.0611461 gram free iodine.

STRENGTH OF THE SOLUTIONS.

	Actually contained.	Found.
1	0.30	0.29
2	0.15	0.14

¹ This Journal, 20, 722 (1898).

² We are indebted to Prof. John Uri Lloyd, of Cincinnati, for a specially prepared sample of hydrastine. We had also a sample furnished us in a lot of pure alkaloids by Merck & Co. These two samples agreed very well with each other in the quantitative results.

PLAN OF THE ASSAY OF HYDRASTIS CANADENSIS.

The estimation of hydrastine and berberine in this root is based upon the following principles :

1. Hydrastine is quite soluble in absolute ether and forms, as we have just shown, a definite hexaiodide when a weak solution of any of its salts is added to a large excess of iodine dissolved in a potassium iodide solution.

2. Berberine on the contrary is completely insoluble in *absolute* ether.

This can be shown by rubbing up some pure berberine in a mortar with absolute ether, filtering, evaporating the ether and taking up with acidulated water. The liquid thus obtained is perfectly colorless and no trace of turbidity is produced in it, by Mayer's reagent, Wagner's reagent, or picric acid, nor does chlorine water produce the characteristic rose band.

3. Of all the difficultly soluble berberine salts the hydriodide seems to be the least soluble, particularly so in a very large excess of potassium iodide. If to a solution of berberine in water slightly acidulated with acetic or sulphuric acid, a large excess of a potassium iodide solution be added, the precipitation is so complete that the filtrate is almost entirely colorless, and no alkaloid can be detected in it by any of the above-mentioned reagents. Even chlorine water, reacting with a delicacy said to be one in 250,000, does not give any coloration, after removal of potassium iodide from the filtrate by silver nitrate, and of the excess of the latter by hydrochloric acid.

4. If to a very dilute solution of a salt of pure berberine about ten to fifteen times its amount of acetone be added and the solution then made strongly alkaline with solution of sodium hydroxide, berberine-acetone is so fully precipitated, particularly after ten or fifteen minutes' shaking, that the filtrate is almost completely colorless and no traces of alkaloid can be detected in it, after acidulation, by means of the above-mentioned reagents.¹ This berberine-acetone is easily decomposed by boiling with mineral acids, with the liberation of pure berberine.

These statements about berberine are true only when the berberine is perfectly pure, such as can be obtained from berberine-

¹ The compound of berberine with acetone was first made by Gaze: *Arch. d. Pharm.*, (1890), 607.

acetone according to the directions of Gaze¹ and dried in the air without heat. Commercial salts of berberine and particularly commercial berberine itself is generally so impure that some of these reactions will not hold good.²

Basing ourselves upon these facts we have worked out a

¹ *Loc. cit.*

² Beside berberine and hydrastine one other alkaloid has been found in *Hydrastis*, but in proportions quite too small to have any bearing upon the assay percentages, either of berberine or hydrastine. That the assay process here proposed leaves no appreciable quantity of any alkaloid behind we have verified by operating upon a considerable quantity of the drug. After removing the hydrastine with the ether and the berberine with potassium iodide, there was nothing left that gave indications of alkaloidal character. What has been learned of canadine makes it probable that it would adhere to berberine or to hydrastine. In the elaborate research which has established the existence of canadine, Prof. E. Schmidt (*Arch. d. Pharm.*, 232, 136 (1894)) prepared it from a crude hydrastine, and also from a crude canadine hydrochloride made for him by E. Merck, of Darmstadt. In the purification the canadine was precipitated as a salt of nitric acid. The insolubility of its salts with mineral acids would be likely to carry it into the crude salts of berberine, when these are precipitated for the removal of this alkaloid. Prof. J. U. Lloyd ("Drugs and Medicines of North America," Cincinnati, pp. 139-141, 127 (1885)), has shown that strong acidulation with hydrochloric acid is necessary to the full precipitation of the berberine, and that when the filtrate is precipitated by ammonia at the neutral point, any further precipitation caused by an excess of ammonia consists of or contains berberine, as a result of its incomplete removal by acid precipitation. Prof. Lloyd was evidently right in his conclusion that the method of Burt (*Am. J. Pharm.*, 47, 481 (1875), for "a third alkaloid" yields berberine unless this has been more closely removed by the strong acidulation just referred to. But since the research of Schmidt it appears likely that canadine would be precipitated with the berberine by strong acidulation. And the discrepant accounts of the color of the third alkaloid are explained by the discovery that though it is colorless, in purity, it acquires color by exposure to light and air, tetrahydroberberine ($C_{26}H_{21}NO_4$) being oxidized into berberine itself ($C_{26}H_{17}NO_4$) (Schmidt: *Arch. d. Pharm.*, 232, 148 (1894)). However, neither Hale nor Burt worked with enough of the *Hydrastis* to obtain canadine in the purity and amount required for any satisfactory description or conclusion. They did not claim to individualize or name the alkaloid; indeed the note of Hale only raised the question of its existence. Prof. Schmidt, who had, as he said, worked more than ten years with *Hydrastis* alkaloids, found it needful to operate upon fifty kilograms of the drug, and availed himself of a crude product accumulated in a manufactory. This was about five years after Wilhelm had made a report from the laboratory of Prof. Schmidt (*Arch. d. Pharm.*, 226, 350 (1888)), upon a third alkaloid in *Hydrastis*. In this report the finding of this alkaloid was ascribed to Hale (*Am. J. Pharm.*, 45, 247 (1873)), Burt (*loc. cit.*), and Lerchen (*Am. J. Pharm.*, 50, 470 (1878)), and the reader might infer that the name, canadine, had been proposed by Hale and Burt. Consequently in the second edition of "Beilstein" (III, 491) "canadine" is described upon no other authority than that of Hale and of Burt. In Beilstein's third edition, with fuller description, the authority of E. Schmidt, Privatmitth., is prefixed. The alkaloid examined by Wilhelm in 1888, not analyzed because of insufficient quantity, was prepared from ammoniacal solution by extraction with acetic ether. That observed by Hale, by Burt, and by Lerchen was obtained by precipitation with ammonia in some excess. The first elementary analysis was made by L. Deichmann (Inaugural dissertation, Rostock, 1892). In the analysis of Schmidt his figures differ from those of Deichmann, from greater purity of preparation (*Arch. d. Pharm.*, 232, 159 (1894)). Deichmann reported a cryoscopic determination of the molecular weight. Zeisel (Inaugural dissertation, Dorpat, 1892) determined the methoxy groups in canadine.—A. B. PRESCOTT.

method of assaying *Hydrastis Canadensis*, the plan being as follows: The alkaloids of the powdered root are first set free by the action of an ether-ammonia mixture, consisting of stronger ammonia water five cc., alcohol five cc., and ether thirty cc.¹ After drying, the powder is extracted with absolute ether and the ethereal extract, after evaporation of the ether and taking up the residue with acidulated water, is used for the estimation of hydrastine by any suitable method. Through the powdered root left in the extraction apparatus air is passed till it is dry, and then the powder is extracted with alcohol to exhaustion. The alcoholic extract, after dilution with water, evaporation of the alcohol and taking up the residue with diluted acetic acid, is used for the estimation of berberine. The berberine is first precipitated as berberine-acetone, the latter washed, decomposed by the aid of acid, and the purified berberine estimated by standard solutions of potassium iodide, silver nitrate, and ammonium thiocyanate.

DIRECTIONS FOR THE ASSAY.

Ten grams of the finely powdered *Hydrastis* are rubbed up to a paste with a few cc. of the above-mentioned ether-ammonia mixture in an eight ounce, screw-top ointment jar, and a few cc. more of the same mixture are then added so as to have the powder well covered with liquid. The small pestle is then left inside, and the jar well covered is set aside over night. The jar is then opened, put into a good current of air till the odor of ammonia has disappeared, and then in a vacuum over sulphuric acid for about five or six hours. The powder is then put into a filter-paper cell, placed in a Soxhlet extraction apparatus, the jar rinsed out several times with powdered glass or in the absence of this with coarsely powdered barium nitrate, the rinsings added to the Soxhlet, the latter connected with an Erlenmeyer flask containing about forty or fifty cc. absolute ether, and the extraction conducted in the usual way, till a few drops after evaporation of the ether and acidulation give no reaction with Mayer's or Wagner's reagent. The ethereal extract will be found to

¹ This is the same mixture we have used in our general method of extracting alkaloids (*This Journal*, 21, 232 (1899)), with the omission of chloroform. The latter gives with berberine a compound not decomposable by acids (*E. Schmidt. Pharm. Ztg.*, (1887), 542).

have only a very slight yellow color. The Erlenmeyer is then detached from the Soxhlet, the ether poured out into a flat evaporating dish, the Erlenmeyer washed out several times with water containing about two per cent. sulphuric acid, the washings added to the contents of the evaporating dish, and the latter put into a draught at about 30° C. till the ether has disappeared.

The contents of the dish are poured into a 100 cc. flask, the dish washed, the washings added into the flask and the latter filled up to the 100 cc. mark. The solution containing hydrastine sulphate, and of which every ten cc. represent one gram of the root, is used for the estimation of hydrastine.

For the iodometric estimation twenty cc. of the filtered solution (representing two grams of the drug) are run from a burette into a 100 cc. flask containing twenty or thirty cc. of a standardized solution of iodine of any known strength (that in the neighborhood of one per cent. is the best) and the analysis carried out exactly as described in our previous paper.¹ From the amount of iodine consumed the amount of hydrastine is deduced by using the factor of the hydrastine hexaiodide, *i. e.*, 0.60403 gram of hydrastine for one of iodine consumed.

For a gravimetric estimation another portion of twenty cc. of the filtered solution is run into a separator and the hydrastine shaken out with benzene and ammonia. All the coloring-matter remains in the aqueous liquid, and a perfectly colorless solution of hydrastine in benzene is obtained. The benzene solution is then filtered through a small filter into another separator, the first separator and filter washed with benzene and the hydrastine again shaken out with water acidulated with sulphuric acid. At last from the watery solution the hydrastine is shaken out with ether and ammonia, the ether poured into a tared beaker and slowly evaporated in a dark place. After drying in a vacuum over sulphuric acid and paraffin the beaker is weighed. The hydrastine is left in perfectly white crystals, and only a slightly yellowish tint can be seen on the sides of the beaker. This tint is probably due to traces of canadine, which becomes yellow² on exposure to the light. Of course instead of shaking out, the method of perforation may be used if preferred.

¹ This Journal, 20, 722 (1898).

² E. Schmidt: *Arch. d. Pharm.*, 232, 141 (1894).

For the estimation of berberine a current of dry air is passed through the Soxhlet till all the ether is removed, the Soxhlet connected with an Erlenmeyer containing forty or fifty cc. of alcohol, and the extraction continued until the alcohol comes out colorless. The alcoholic extract containing the berberine, and considerable quantities of extractive matter, is poured out into an evaporating dish, the Erlenmeyer washed out with hot water and a little dilute acetic acid, the washings added to the evaporating dish and the latter kept on a water-bath, adding water from time to time till all the alcohol has disappeared. A little more diluted acetic acid is now added, the dish covered and when completely cold its contents are filtered into an Erlenmeyer having the capacity of about three or four hundred cc.¹

Six to eight cc. of acetone are added to the contents of the Erlenmeyer, to which the washings of the dish and the filter have been added, and then a ten per cent. solution of sodium hydroxide is added, drop by drop, till the precipitate first formed ceases to disappear on shaking, and the liquid acquires a strongly alkaline reaction. The Erlenmeyer is then stoppered and shaken in a circular direction for about ten or fifteen minutes, and then set aside in a cool place for two or three hours. The berberine-acetone separates out in crystals, some of which adhere to the sides of the vessel. The supernatant liquid is then poured on a small filter, the precipitate washed once or twice by decantation and then on the filter till the washings are colorless. The filter is then pierced and, by means of the wash-bottle, the precipitate is returned to the same Erlenmeyer in which the precipitation took place. In this way all loss is avoided. To the precipitate about four or five cc. of a five per cent. solution of sulphuric acid is now added and then water enough to make about one or two hundred cc. The Erlenmeyer is now put into hot water when the precipitate will completely dissolve in the course of a few minutes. The solution is now poured out into a long-necked flask, washing the Erlenmeyer several times, the

¹ In the remaining procedure, the simplest way would be to precipitate the berberine with hydrochloric or nitric acid, but in this case a considerable amount of extractive matter contaminates the precipitate and too high a yield would result though the error in this respect might be compensated to some extent by the solubility of the hydrochloride or nitrate in water. But the *best* way is to purify the berberine by converting it into berberine-acetone, regenerate the alkaloid by means of sulphuric acid and then estimate it volumetrically by standard potassium iodide.

flask put on an asbestos plate and kept very gently boiling for about an hour and a half or two hours, adding hot water from time to time if necessary.

The flask is now cooled and its contents poured out into a liter measuring flask¹ into which there has been previously taken from a burette one hundred cc. of twentieth normal potassium iodide. The flask is washed several times, the washings added to the measuring flask and the latter filled up to one liter and set aside over night. Five hundred cc. are now filtered off into another liter flask, fifty cc. of twentieth normal silver nitrate, and nitric acid, added to the flask, which is filled up to one liter, well shaken, filtered, and five hundred cc. of the filtered liquid titrated back with fortieth-normal ammonium thiocyanate, using ferric alum as indicator. Twice the number of cubic centimeters of the thiocyanate solution used is equal to the number of cubic centimeters of the potassium iodide solution consumed by the berberine, representing ten grams of the Hydrastis root. By multiplying the number of cubic centimeters of twentieth normal potassium iodide consumed, by 0.167125, the percentage of anhydrous berberine in the root is obtained, as one cc. of the potassium iodide solution is equal to 0.0167125 gram of berberine.

In our assay of Hydrastis Canadensis three samples of powdered Hydrastis were treated in the way described. The berberine was estimated volumetrically, the hydrastine both iodometrically and gravimetrically.

FOR HYDRASTINE.

	Iodine consumed by two grams of the root.	Iodometric.	Hydrastine. Gravimetric.
1	0.0760015	2.29	2.29
2	0.0772012	2.33	2.30
3	0.0777770	2.35	2.28

FOR BERBERINE.

	Number of cc. of twentieth normal KI consumed by ten grams of the root.	Berberine, anhydrous.
1	15.1	2.52
2	15.3	2.55
3	14.8	2.47

With regard to the precipitation of berberine by potassium

¹ The berberine hydriodide being extremely bulky the error arising from the space occupied by the precipitate is reduced to a minimum by using a large flask.

iodide we wish to draw attention to the fact that even a solution of the free alkaloid without any addition of acid is precipitated by potassium iodide. As there is no acid to combine with the potassium the question is, what becomes of the metal when the iodine of the potassium iodide is taken up by the berberine? That there is no potassium in the precipitated berberine hydriodide was proved by igniting the precipitate with sulphuric acid and ammonium nitrate, when no trace of residue was to be found. All the potassium must then pass into the filtrate and part of it must exist there as a salt of something acting as an acid, as there is no trace of alkalinity in the filtrate. No one of the indicators gives an alkaline reaction. We acidulated the filtrate with sulphuric acid, shook it up with ether, washed the ether till there was no reaction given for iodine by sodium nitrite and starch, nor for sulphates by barium chloride, and evaporated the ether to dryness; a very small amount of a crystalline substance was left, which would seem to indicate the presence of an organic acid, but the quantity was too small for a closer examination. We intend to take a larger quantity of pure berberine into operation and investigate the subject thoroughly in the near future.

UNIVERSITY OF MICHIGAN,
April, 1899.

UPON THE ACTION OF DIAZO COMPOUNDS ON THYMOLPARASULPHO ACID.

[SECOND PAPER.]

BY JAMES H. STEBBINS, JR.

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IN my former paper¹ I described several azo compounds obtained by combining diazobenzene chloride, diazoparatoluene chloride, and diazoxylene chloride with thymolparasulpho acid.

Contrary to precedent, it was observed that the three compounds named, notwithstanding their increasing molecular weight, were all possessed of about the same yellow color, and were all three more or less unstable, being partially decomposed by boiling water.

It therefore seemed of interest to ascertain in what manner the

¹ This Journal, 3, 112.