METHYL SALICYLATE.

Accurately weigh about 0.500 Gm., transfer it to a 500-cc. Erlenmeyer flask and add 25 cc. of alcohol and 25 cc. of about 10 per cent sodium hydroxide solution. Boil until the alcohol is expelled, cool and transfer to a separator and proceed as with sodium salicylate.

One commercial sample of methyl salicylate gave 98.99, 98.88, 98.91 and 98.69, average 98.87 per cent methyl salicylate.

PHENYL SALICYLATE.

An assay of this substance based upon its saponification with alcoholic potash is complicated by the fact that the phenol formed in the saponification is more difficult to remove than the methyl alcohol formed in the saponification of methyl salicylate. By applying the principle used by Puckner and Clark[1] in the estimation of phenol in pharmaceutical mixtures it seemed quite possible to accomplish this. After saponification of the salol, carbon dioxide is passed through the alkaline mixture until it no longer reddens phenolphthalein. This mixture is then extracted with chloroform to remove phenol, then made acid with hydrochloric acid and the liberated salicylic acid extracted with chloroform. Several trials gave results varying from 99 to 100.7 per cent on a commercial sample of salol. Because of a lack of time the technic of the method could not be developed to a point giving better results. Further work will be done on it.

Since the melting point is a good indication of the purity of salol a method of assay may not be required in the U. S. P. but any method is valuable which might be of service in estimating salol in pharmaceutical products such as tablets, pills, etc., and this method is being studied with a view to its possible use in this connection.

Acknowledgment is made to Prof. C. C. Glover for advice and encouragement during the progress of the work.

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THE STABILITY OF ATROPINE AND HYOSCYAMINE DURING PROCESS OF ANALYSIS.*

BY S. PALKIN AND H. R. WATKINS.

The problem of accurate quantitative determination of hyoscyamine and atropine has in spite of apparent simplicity remained a perplexing matter. No small part of the voluminous literature (1) on the solanaceous alkaloids is devoted to their extraction and determination, as well as to the study of the stability of the various members. Nevertheless, the reader is left with a vague impression as to the degree of their stability or instability. The exact conditions, for purposes of analysis, under which these alkaloids can be depended upon to remain stable throughout the process of evaluation are yet to be established.

* Contribution from Drug Control Laboratory, Bureau of Chemistry, U. S. Department of Agriculture.

[1] PROCEEDINGS OF THE A. PH. A., Vol. 56, p. 824 (1908).

Hyoscyamine (2) is readily converted into its racemic modification, atropine, and thence may be hydrolyzed to tropeine and tropic acid.

$$\underset{\text{Atropine}}{C_{17}H_{23}O_3N} + H_2O \qquad C_8H_{15}ON + \underset{\text{Tropeine + Tropic Acid}}{C_9H_{10}O_3}$$

When atropine is thus hydrolyzed it may be considered from an analytical standpoint as undergoing self neutralization, so that a given quantity of atropine base when completely converted to equivalent tropeine and tropic acid would yield a neutral solution.

The racemization and hydrolysis of hyoscyamine have been studied by a number of investigators. Will and Bredig (3) studied the effect of various alkaline agents on hyoscyamine by way of the progressive loss of optical rotating power. Hyoscyamine is optically active, while both atropine and tropeine are inactive. The conditions under which loss of optical activity is at a minimum are particularly significant. According to these authors hyoscyamine in alcohol water solution containing ammonia loses only about 5% (162.31′ to 153.61′) in rotation in a period of $5^1/_2$ hours. As this slight loss in optical activity represents the total change, it is improbable that any change beyond slight racemization took place and that the splitting action into tropeine and tropic acid under these conditions is negligible.

Will and Bredig made their examinations in a 2:1 alcohol water solution, but did not study the effect of water alone on hyoscyamine.

Gadamer (4) made a very exhaustive study of the behavior of hyoscyamine and atropine under varying conditions tracing both racemization and hydrolysis simultaneously by rotation measurements and titration. He shows conclusively that water has a marked hydrolytic action on hyoscyamine and atropine.

Dott (5), in working with atropine and total belladonna alkaloids in water solutions, concludes that sodium bicarbonate, sodium carbonate and ammonia all effect hydrolysis.

In the investigation here reported it has been shown experimentally that the tolerance of these alkaloids to water, ammonia and heat is not wholly in accord with views generally held,[1] and definite limits of tolerances have been ascertained which make possible accurate and dependable determination of atropine and hyoscyamine.

PHYSIOLOGICAL METHOD OF EVALUATION.

In addition to the chemical method for estimating these alkaloids, quantitative physiological determinations were made in a number of instances on the basis of their mydriatic power. The method was developed by Dr. J. C. Munch (6), of the Pharmacological Laboratory of the Bureau of Chemistry, who has determined the "threshold" effect of the various mydriatic alkaloids[2] as observed on cat's eyes. Tropeine is reported to have no mydriatic power. For "threshold" values (6) he found 0.05 cc. (1 drop) of a solution containing the following quantities of alkaloid per liter of solution: Hyoscine, 0.4 mg. per liter; hyoscyamine, 4 mg. per liter; atropine, 12 mg. per liter.

[1] U. S. Pharmacopœia, p. 453, 4th paragraph.

[2] The same alkaloids were used in mydriatic standards as in chemical study.

AUTOMATIC EXTRACTOR.

A continuous automatic extraction method frequently referred to in this paper is described in a previous publication (7). It involves the continuous passage for about two hours of hot solvent (chloroform or benzene as the case may be) through the aqueous liquid containing the alkaloid. Subsequent operations involving removal of solvent and titration of alkaloidal residues are the same as in hand separatory funnel methods or as indicated in the experiments recorded in the tables where this procedure is designated "A. E."

EXPERIMENTAL.

STABILITY OF ATROPINE IN AQUEOUS, AMMONIACAL AND PURE ALCOHOL.

The experiments reported in Table I were made to determine the influence on ultimate results by varying the conditions in what is usually the final step in alkaloidal assay including the titration of the alkaloid. In 8 experiments 83.5 mg. pure atropine was dissolved in absolute alcohol; water or ammonia was then added as indicated and the whole evaporated to dryness. The alkaloid

TABLE I.—STABILITY OF ATROPINE.

In Aqueous, Ammoniacal and Pure Alcoholic Solution.

Expt. No.	Atrop. used. Mg.	Abs. alcohol used. Cc.	Water used. Cc.	NH_4OH used. Cc.	Treatment prior to titration.	Alkaloid found by titration. Mg.	%
1	83.5	40	...	.....	Evap. to dryness on steam-bath	83.72	100.26
2	83.5	40	...	1 cc. N.NH_3	Evap. to dryness on steam-bath	83.72	100.26
3	83.5	39	...	1 cc. N.NH_3	Evap. to dryness on steam-bath	82.94	99.33*
4	83.5	39	...	1 cc. 5N.NH_3	Evap. to dryness on steam-bath	82.66	99.0
5	83.5	35	...	5 cc. N.NH_3	Evap. to dryness on steam-bath	83.52	100 0
6	83.5	35	...	5 cc. 5N.NH_3	Evap. to dryness on steam-bath	84.07	100.68*
7	83.5	20	20	.....	Evap. to dryness on steam-bath	68.45	81.97
8	83.5	20	20	1 cc. N.NH_3	Evap. to dryness on steam-bath	62.95	75.39
9	83.5	27	13.5	.....	Stood at room temperature 24 hrs.	83.78	100.34
10	83.5	27	13.5	.....	Stood at room temperature 24 hrs.	83.78	100.34
11	83.5	27	13.5	.....	Stood at room temperature 24 hrs.	83.5	100.00
12	83.5	27	13.5	.....	Heated on steam-bath 1 hr.	75.1	89.94
13	83.5	27	13.5	.....	Heated on steam-bath 1 hr.	82.33	98.61
14	83.5	27	13.5	.....	Heated on steam-bath 1 hr.	78.57	94.10

* Tested on cat's eyes, mydriatic power found to be that of atropine.

residue was taken up in a measured volume of standard 0.02 *N* sulphuric acid and the excess acid titrated back in the usual way.

Complete recovery to within the errors of titration (1%) was shown in all cases (even on heating) where the water content was not greater than 12%. In the cold a much higher percentage of water is tolerated. The presence of ammonia up to 5% in 5 of the 6 experiments, provided the alcoholic strength was high, did not effect the least hydrolysis.

STABILITY OF ATROPINE SULPHATE IN WATER AND ACID.

The stability of atropine sulphate in acid solutions is incidentally demonstrated by the results shown in Table I. Solutions of atropine sulphate were boiled one hour without affecting the titration value (Table II, Expt. 22) (8). Further experiments in which atropine sulfate was heated in varying concentration of acid show the stability of this salt under those conditions, both at elevated temperature and over a long period of time at room temperature (Table II).

TABLE II.—STABILITY OF ATROPINE SULPHATE IN AQUEOUS AND DILUTE ACID SOLUTIONS.

Expt. No.	Atropine sulfate taken. Mg.	H_2O. Cc.	Total vol. 30 cc. Stand. H_2SO_4 added. *N*/50. Cc.	*N*/10. Cc.	Treatment.	Stand. alkali required *N*/50. Cc.	Atropine hydrolyzed.
15	100	20	...	...	Heated 1 hr. on steam-bath.	1 drop	None
16	...	17.5	2.5	...	Heated 1 hr. on steam-bath	2.5	None
17	...	15	5.0	...	Heated 1 hr. on steam-bath.	5.05	None
18	...	10	10.0	...	Heated 1 hr. on steam-bath.	9.9	None
19	...	10	...	10.0*	Heated 1 hr. on steam-bath	50.5	None
20	...	...	...	20.0	Heated 1 hr. on steam-bath	101.0	None
21	100	40	0.4	...	Room temperature 1 hr.	0.4	None
22	...	40	0.4	...	Boiled 1 hr.	0.4	None

* 10.0 cc. *N*/10 H_2SO_4 = 50.5 cc. "*N*/50" NaOH.

HYDROLYTIC ACTION OF WATER ON THE FREE BASE.

The splitting effect of water earlier pointed out by Gadamer (9) is evident in Experiments 7 and 8 (Table I) and more strikingly in the experiments reported in Table III.

The hydrolytic action of water on atropine often manifests itself when least expected. For example, on evaporating to dryness a chloroform solution of atropine as obtained by extracting the atropine from an aqueous medium, some moisture remains after the chloroform has been evaporated, this is sufficient to damage the atropine residue when an attempt is made to drive it off in order to obtain the alkaloidal residue dry and ammonia free. This moisture is partly brought over mechanically by the shaking process during extraction, unless filtered, and occasionally results from condensation of atmospheric moisture, owing to cooling while evaporating the solvent. The error can become even more serious

when the operator, fearing the deleterious effect of heat, evaporates the solvent at low temperature, thus exposing the atropine to water action over a longer period. This error is here shown in simple form by experiments in which the solvent containing weighed quantities of atropine was evaporated in the presence of water or ammonia water and also where moisture was excluded (Table V).

In view of the protecting influence of alcohol against hydrolysis of atropine (as already evidenced in Table I) experiments were made in which alcohol was added to chloroform extract before evaporating to dryness (Experiments 34 and 35, Table III). The addition of 10 cc. of absolute alcohol to 50 cc. of chloroform, even where 1 cc. of ammonia water was present in the mixture, permitted the evaporation of the last remnant of water without hydrolyzing the alkaloid.

TABLE III.—HYDROLYTIC ACTION OF WATER ON ATROPINE.

Expt. No.	Atropine taken. Mg.	Solvent. 40 cc.	Treatment.	Atropine found by titration. Mg.	%.
23	83.5	Water*	Stood 24 hrs.	37.83	45.31
24	83.5	Water*	Heated 1 hr.	22.57	27.03
25	83.5	Water*	Heated 1 hr.	21.99	26.35
26	83.5	Water*	Heated 1 hr.	22.15	26.53
27	83.5	Water*	Heated 1 hr.	20.88	24.93
28	83.5	Chlorof.	1 cc. H_2O added and evap. to dryness.	80.31	96.80
29	83.5	Chlorof.	1 cc. H_2O added and evap. to dryness.	81.47	97.60
30	83.5	Chlorof.	1 cc. H_2O added and evap. to dryness.	80.89	96.88
31	83.5	Chlorof.	1 cc. 5 *N* NH_4OH added and evap. to dryness.	81.47	97.60
32	83.5	Chlorof.	1 cc. 5 *N* NH_4OH added and evap. to dryness.	82.33	98.61
33	83.5	Chlorof.	1 cc. 5 *N* NH_4OH added and evap. to dryness.	81.47	97.60
34	83.5	50 cc. $CHCl_3$	10 cc. abs. alc. added and evap. to dryness.	83.32	99.78
35	83.5	50 cc. $CHCl_3$	10 cc. abs. alcohol 1 cc. 5 *N* NH_4OH added and evap. to dryness.	83.14	99.57
36	83.5	50 cc. $CHCl_3$	$CHCl_3$ shaken with 2 cc. 5 *N* NH_4OH before dissolving atropine and evap. to dryness.	79.73	95.5

* Alkaloid was brought in water solution by first taking up in 1 cc. alcohol and then diluting with the requisite quantity of water.

INFLUENCE OF AMMONIA ON CHLOROFORMIC EXTRACT.

The practice of evaporating a chloroformic solution of alkaloid (obtained by extraction) completely to dryness is based on the assumption that there is danger of retention of ammonia unless the solution is brought completely to dryness. That this is a fallacy and that this extreme precaution is unnecessary are brought out in the following way:

Chloroform solutions and benzene solutions containing ammonia were evaporated from a 50-cc. volume to a 5- or 10-cc. volume. A measured volume of standard acid was then added and the rest of the solvent evaporated. On ti-

tration in the usual way the acid required not less than the equivalent alkali, thus showing complete absence of ammonia at the low volume point. The chloroformic and benzene solutions were obtained as in the usual way for alkaloid extraction by shaking the solvent with aqueous ammonia, both by hand and by prolonged automatic continuous hot extraction.

Experiments where known quantities of atropine were added before evaporating the chloroform show conclusively that this procedure is safe and accurate. In

TABLE IV.—ELIMINATION OF NH_3 IN CHLOROFORM OR BENZENE EXTRACT.

Expt. No.	Atropine taken. Mg.	Solvent.	5 *N* NH_4OH used. Cc.	Method or treatment.	*N*/50 acid consumption by solvent.* Cc.	Atropine found. Mg.	Atropine found. %.
37	None	Chlorof.	2	Aut. extract 2 hrs.	None	....	.....
38	None	Chlorof.	2	Solvent evap. to low	None	....	.....
39	None	Benzene	2	vol. but not to dry-	None	....	.....
40	None	Benzene	2	ness before titration.	None	....	.....
41	83.5	Chlorof.	2	Solvent shaken with	14.31	83.26	99.71
42	83.5	Chlorof.	2	NH_4OH alkd. added	14.32	83.32	99.79
43	83.5	Chlorof.	2	and evap. to low	14.32	83.32	99.79
44	83.5			volume, etc., but	14.32	83.32	99.79
45	83.5			not to dryness be-	14.37	83.61	100.13
46	83.5			fore titration.	14.32	83.32	99.79
47	83.5				14.32	83.32	99.79

* After total solvent was evaporated to 10 cc. volume.

TABLE V.—HYDROLYSIS OF ATROPINE.

(Error due to evaporation of solvent completely to dryness.)

Expt. No.	Atropine taken. Mg.	Method of extraction.	Solvent used.	Treatment of extract.	Atropine found. Mg.	Atropine found. %.
48	83.5	A. E. 2 hrs.	Benzene	Evap. to low volume not to dryness.	83.57	100.08
49	83.5	A. E. 2 hrs.	Benzene	Evap. to low volume not to dryness.	83.29	99.75
50	83.5	A. E. 2 hrs.	Chlorof.	Evap. to low volume not to dryness.	83.29	99.75
51	83.5	A. E. 2 hrs.	Chlorof.	Evap. to low volume not to dryness.	82.71	99.06
52	83.5	A. E. 2 hrs.	Chlorof.	Evap. to dryness before titration.	80.92	97.14
53	83.5	A. E. 2 hrs.	Chlorof.	Evap. to dryness before titration.	78.37	94.08
54	83.5	A. E. 2 hrs.	Chlorof.	Evap. to dryness before titration.	80.28	96.38
55	83.5	A. E. 2 hrs.	Chlorof.	Evap. to dryness before titration.	79.01	94.85

the experiments reported in Table VIII, known weights of atropine were added to chloroformic ammonia solutions and evaporation was carried to 10 cc. before adding acid, etc. (Table IV).

The precaution of evaporating the chloroformic alkaloidal solution to the point of low volume (5 or 10 cc.) and not to dryness has been employed in all subsequent experimental studies of variants where true alkaloidal values were sought. In some instances extractions were made by the hand separatory funnel method, but in most cases they were made by the continuous automatic procedure

described previously (10). The latter experiments have particular significance, inasmuch as the conditions to which the alkaloids are subject are more severe than when the hand separatory funnel method is used. The constancy of results obtained by this extraction procedure is even more indicative of the remarkable stability of atropine and hyoscyamine when the fundamental precautions here deduced were observed.

In the experiments reported in Table V weighed portions (83.5 milligrams) of atropine, after being dissolved in excess acid and then titrating back with alkali, were made alkaline with ammonia and extracted by continuous automatic extraction. The chloroform or benzene solutions were evaporated to a low volume (but not to dryness), and the standard acid was added before evaporating the rest of the solvent. Complete recovery is evident (Experiments 48, 49, 50, 51).

TABLE VI.—INFLUENCE OF AMMONIA ON HYDROLYSIS OF ATROPINE.*

Expt. No.	Ammonia added.	Atropine found by A. E. 2 hours. Mg.	%.
56	None	77.30	92.63
57	None	78.46	93.96†
58	1 cc. *N*/10	76.43	91.53
59	1 cc. *N*/10	77.30	92.57
60	1 cc. *N*	79.04	94.66
61	1 cc. *N*	79.90	95.70
62	2 cc. *N*	81.64	97.77
63	2 cc. 5 *N*	80.77	96.73
64	5 cc. 5 *N*	82.45	98.74
65	5 cc. 5 *N*	79.62	95.35†
67	5 cc. 5 *N*	83.78	100.32
69	5 cc. 5 *N*	84.07	100.69
66	None	81.76	97.92‡
68	None	81.18	97.22

* 83.5 mg. atropine alkaloid taken up in 1 cc. alcohol to facilitate solution, diluted with water or water and ammonia to 40 cc. and solution heated 1 hr. on steam-bath.

† Tested on cat's eyes. Mydriatic effect found to be only about one-fourth that of atropine.

‡ Period of extraction 1 hr. longer.

TABLE VII.—EXTRACTION OF TROPEINE-ATROPINE MIXTURES.

	Titratable base found.								
	By direct titration after hydrolysis.		(*a*) By extraction A. E. 2 hrs.		(*b*) Reextraction by hand of titrated solution from (*a*).		(*c*) By extracting residual aqueous layer from (*b*).		
Expt. No.	Mg.	%.	Mg.	%.	Mg.	%.	Mg.	%.	*b* + *c*. %.
70 (24)	22.57	27.03	82.62	98.95	53.81	64.45	24.19	28.97	93.42
71 (25)	21.99	26.35	82.62	98.95	57.57	68.95	21.00	25.25	94.2

Similar experiments in which this precaution was not observed and the chloroformic extract merely evaporated to dryness show very clearly the loss entailed (Experiments 52, 53, 54, 55).

CHARACTER OF HYDROLYTIC ERROR.

From a purely "titration value" standpoint the hydrolytic action at any point prior to the extraction and ultimate determination would be of little analytical consequence, as the resultant equivalent tropeine base formed should give a value equivalent to the original atropine unless some other factors entered, such as

actual destruction of the alkaloid or incomplete extraction of tropeine if the solubility of the latter is markedly less than that of atropine. In Table VI are shown duplicate sets of experiments in which hydrolytic action was effected by heating equal quantities of atropine, using water alone and water containing varying quantities of ammonia. The resulting hydrolyzed products were extracted by continuous automatic method, and the extracted alkaloid determined as described. As seen from Table VI, the loss in titratable bases is appreciable. That a very considerable hydrolysis took place is evidenced by experiments which show that only a little over one-fourth of the extracted base was atropine. In view of the substantial agreement of Experiments 57 and 65 in mydriatic strength, it is of

TABLE VIII.—BEHAVIOR OF HYOSCYAMINE UNDER VARYING CONDITIONS.

Expt. No.	Hyoscyam. taken. Mg.	Treatment.	NH_4OH used.	"Hyoscyamine" found. By direct titration. Mg.	%	By extraction A. E. 2 hrs. Mg.	%
72	83.3	Dissolved in 1 cc. alcohol, *added* 39 cc. water, heated one hour.	.....	20.88	25.01	....	.....
73	83.3	Dissolved in $N/50$ acid excess, steam-bath 1 hr.	.....	84.15	100.78	....	.....
74	15.88	Extracted A. E. 2 hrs. using varying quant. of NH_3 for making alkaline.	3 cc. $N/50$	....	.....	15.8	99.50†
75	15.88		3 cc. $N/50$	....	.....	15.52	97.73
76	15.88		1 cc. 5 N	....	.....	15.8	99.50
77	15.88		1 cc. 5 N	....	.....	15.52	97.73
78	15.88	All precaution observed as described for atropine.	2 cc. 5 N	....	.....	15.8	99.50
79	15.88		2 cc. 5 N	....	.....	.....	.....
80	15.88		3 cc. 5 N	....	.....	15.8	99.50
81	15.88		3 cc. 5 N	....	.....	15.8	99.50
82	15.88		5 cc. 5 N	....	.....	15.09	101.33
83	15.88		5 cc. 5 N	....	.....	15.96	100.68†
84	10		2 cc. 5 N	....	.....	10.12	101.2 *

* 160 mg. "commercially pure" hyoscyamine, titrated 158.8 mg., was diluted to 200 and 20 cc. aliquots (15.88 mg. each), used in Experiments 74–83 inclusive.

† Tested on cat's eyes and found to be full mydriatic strength of hyoscyamine.

further interest to note that ammonia plays a very small part in the hydrolysis (Table VI).

It would also appear that the presence of ammonia favors more rapid and complete extraction (compare Experiments 56 and 57 with 64 and 65). Similar experiments (67 and 69) indicate even more clearly that practically all the error involved is due to hydrolytic action and that with sufficiently exhaustive extraction full equivalent base can be recovered.

The difficulty of extracting tropeine is well illustrated in Experiments 70 and 71 (Table VII). Where the hydrolyzed solutions were first extracted by the continuous automatic extraction they showed about 99% titratable bases. These titrated solutions on liberation of the alkaloid, when again extracted by way of

the hand separatory funnel, showed only about 65% recovery. The rest of the tropeine base, however, was again nearly completely recovered when continuous automatic extraction was used on the residual aqueous liquid.

HYOSCYAMINE.

In the light of what has been experimentally deduced for atropine, the series of experiments given in Table VIII show the hydrolytic action of water and the conditions favoring stability of this alkaloid. The mydriatic test shows that when proper precautions are observed hyoscyamine remains unchanged throughout the analytical process.

From a purely analytical standpoint, racemization of the hyoscyamine to atropine would not alter the alkaloidal assay, as the resulting atropine value would still be a true index of the original hyoscyamine content.

PRECAUTIONS FOR METHODS OF DETERMINATION.

Whenever occasion demands that either atropine or hyoscyamine be brought in aqueous solution it should be borne in mind that at least equivalent acid or preferably excess (about 2 cc. $N/10$ per 30 cc. solution) should be present. It is then safe to heat in order to effect complete solution. For the liberation of the alkaloid preparatory to extraction the aqueous solution should be cooled and *immediately shaken* with a solvent to bring the bulk of alkaloid into the organic solvent.

The extraction process may then be continued in the usual way by the hand funnel or the automatic continuous extraction separator. Subsequent elevation of temperatures (as is the case in the automatic extractor) has no noticeable effect on the final results. The chloroform or benzene solution containing the alkaloid may then be evaporated on the steam-bath (using air blast) to a low volume, 5 to 10 cc. *but not to dryness*, after which standard 0.02 N sulphuric acid in slight excess is added before proceeding to evaporate the remainder of the solvent. When cool, back titration with 0.02 N alkali (methyl red indicator) may then be carried in the usual way. Evaporation to dryness may be accomplished with safety by adding to the chloroform solution of alkaloid at least one-fifth the volume of absolute alcohol (neutral), the whole evaporated to dryness, and the alkaloidal residue taken up in standard acid and titrated as usual. In no case should the free alkaloid be allowed to stand in solution of, or in contact with, water for any length of time. It is unsafe to allow the evaporation of the chloroform or benzene solution of the alkaloid to proceed to dryness without the precaution of alcohol addition. In no case should the free alkaloid be taken up in water solution without acid for what may be termed "direct titration" nor, if taken up in a little alcohol, should dilution with water be made before titration with acid.

CONCLUSIONS.

It has been experimentally shown that hyoscyamine and atropine in the form of salts of a strong mineral acid, particularly sulfate, are stable in water solution and also in the presence of a reasonable concentration of acid. Such solutions may even be boiled without danger of hydrolysis of the alkaloid.

In the form of free base, hyoscyamine and atropine are stable in organic

solvent, such as chloroform, benzene, even at their boiling points, and in alcohol, provided the alcohol is reasonably free from water. In the case of the former solvents it is essential that no water will condense in the vessel during evaporation. Such solutions may be safely evaporated to dryness and the dry alkaloid subjected to low heat with impunity.

Hyoscyamine and atropine are unstable in water alone or in aqueous alkaline solution even in the cold, and heat materially hastens the process of hydrolysis. Ammonia appears to exert much less activity as a hydrolytic agent than has been supposed.

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ONE REASON WHY MANY ALKALOIDAL ASSAYS COME LOW.

NOTES ON THE ASSAY OF HYOSCYAMUS.

BY ALBIN STIKAROFSKY.

I read with much delight W. F. Reindollar's article on "A Note on the Assay of Tincture of Hyoscyamus" in the September[1] number of the JOURNAL, because of my own interesting experience.

A sample of Hyoscyamus, assayed by the writer according to the U. S. P. method, was found to contain 0.078 per cent of alkaloids. Check analyses were run in three other disinterested laboratories.

Laboratory No. 1 (U. D. Co.), 0.0782; 0.0758; 0.0790
Laboratory No. 2 (Consulting chemist), 0.048; 0.050
Laboratory No. 3 (Consulting chemist), 0.078
Laboratory No. 4 (Manufacturing chemist), 0.053.

Surely, a director of a laboratory must be (?) pleasantly impressed when the submitted figures differ so widely.

Of course, it was now up to the writer to determine, for his own satisfaction, who was right in this "Tower of Babel." As I suspected overheating to be responsible for these losses, a fourth portion of the drug was assayed, allowing the dry alkaloidal residue to remain five minutes longer on the steam-bath. This time the estimated alkaloidal per cent was 0.0478. Thus checking reports of Laboratories No. 2 and No. 4.

[1] JOUR. A. PH. A., September 1925, p. 789.