

## ON THE ESTIMATION OF ALKALOIDS IN ADMIXTURE WITH VEGETABLE DRUGS.\*

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Watkins and Palkin found<sup>1</sup> that calcium and magnesium-fatty acid soaps when present in alkaloid-bearing materials increase the apparent alkaloidal content when assay is attempted by direct "shake-out" methods, owing to the fact that these soaps are soluble in chloroform and the basic radicle is included with the alkaloid and enters into the titration. They also found that this does not occur with the ammonium, sodium and potassium-soaps of fatty acids. In this laboratory it has often been noted that erroneous results will be obtained in alkaloidal assays of products containing vegetable drugs when an attempt is made to determine the alkaloid by merely dissolving the product in a little water, adding ammonia, extracting the chloroform, evaporating the chloroform to a few cc. in volume, adding a measured excess of standard sulphuric acid, boiling off the chloroform, adding methyl red indicator and titrating back the excess of acid with *N*/50 Sodium Hydroxide.

The following results illustrate the basic effect of ammoniacal, chloroformic extractives of a few different vegetable drugs. Separate portions of powdered rhubarb, gentian, podophyllin, capsicum, cannabis, tannic acid and powdered extract of jalap were shaken occasionally with a mixture of chloroform and ammonia water during 24 hours and the ammoniacal, chloroformic layers then filtered off clearly. Aliquots of these extracts were evaporated to 3 cc., a measured excess of standard sulphuric acid was added, the chloroform was evaporated entirely and the excess of acid titrated back with *N*/50 Sodium Hydroxide, using methyl red indicator. In each case with the exception of the rhubarb and tannic acid, some acid was neutralized and consequently this would have the effect of increasing the apparent proportion of alkaloid if these extractives were in admixture with an alkaloidal residue during titration. The extractives from the rhubarb and tannic acid titrated as though a trifle acid in reaction although the apparent acidity was never equivalent to more than 0.2 cc. *N*/50 Sodium Hydroxide which is within the limits of experimental error, so that it is likely that ammoniacal, chloroformic extractives of rhubarb and tannic acid exert no measurable basic effect. For purposes of comparison with later figures the basic effects yielded by the several extractives are recorded in terms of grams of strychnine alkaloid in the following table.

TABLE I.

Drug.	Blank.	Gentian.	Podophyllum.	Capsicum.	Cannabis.	P. E. Jalap.
Amount of drug		0.66	0.33	See note	0.66	0.33
Basic effect	None	0.00675	0.00735	0.0235	0.0131	0.00388

NOTE: No record was kept of the amount of capsicum used; the ammoniacal, chloroformic extract having a light amber color.

Watkins and Palkin<sup>1</sup> also found that the disturbing influence of these "earthy" soaps could be eliminated by extracting an acidified solution of the impure alkaloids with chloroform to remove the fatty acids and then liberating the alkaloids with

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ammonia, extracting with chloroform, evaporating and titrating as usual. In this laboratory the experience of the above mentioned authors has been essentially duplicated; it is our custom to evaporate the chloroformic solution of crude alkaloids in a flask to about 3 cc.; add 10 cc. of 2% sulphuric acid, shake and transfer the contents of the flask to a separator, draw off the chloroformic layer and wash it with 5 cc. of 2% sulphuric acid, return the acid washings to the main bulk of acidified alkaloidal solution and reject the chloroform, wash the flask in which the evaporation was carried out repeatedly with small portions of 2% sulphuric acid and chloroform as before until all the alkaloid has been transferred to the separator; wash the combined acidified alkaloidal solution repeatedly with small portions of chloroform until all chloroform soluble matter is extracted (usually indicated by absence of color in the chloroformic layer), washing the chloroform extractions in turn with small portions of 2% sulphuric acid and returning the acid extractions to the main bulk of acidified alkaloidal solution while rejecting the chloroformic layer; then liberate the alkaloids with ammonia, extract with chloroform, evaporate the chloroformic extracts in a flask to about 3 cc., add excess of standard sulphuric acid, boil off the chloroform entirely, add methyl-red indicator and titrate back with *N*/50 Sodium Hydroxide. This method, in general, has proven very successful in the assay of such complex combinations as tablets containing  $\frac{1}{120}$  grain of alkaloidal salt each, along with various oily and resinous vegetable drugs, such as gentian, mandrake, capsicum, cactus, cannabis, etc.; although slight modifications are necessary in its application to specific cases. The usefulness of this method in eliminating the disturbing factors introduced by the presence of ammoniacal, chloroformic extractive of the several vegetable drugs is illustrated by the following experiments and results: 10-cc. portions of a standardized solution of strychnine alkaloid in chloroform were added to aliquot portions of ammoniacal, chloroformic extracts of several of the drugs mentioned above, the mixture was then evaporated to about 5 cc. and the alkaloid purified, recovered and titrated according to the general method outlined in the preceding paragraphs, the following results being obtained.

TABLE II.

Drug.	Gentian.	Podophyllum.	Capsicum.	Cannabis.	P. E. Jalap.
Amount of drug	0.66	0.33	See note	0.66	0.33
Strych. alkaloid added	0.0624	0.0624	0.0624	0.0624	0.0624
Strych. alkaloid recovered	0.0634	0.0612	0.0612	0.0622	0.0622

NOTE: No record was kept of the amount of capsicum used, the ammoniacal, chloroformic extract having a light amber color.

The standardized solution of strychnine alkaloid in chloroform was standardized by two methods and the average of several trials by the two methods was taken as standard. One method consisted of evaporating 10 cc. of the solution in a flask to 3 cc. adding excess of standard sulphuric acid, boiling off the chloroform entirely and titrating back with *N*/50 Sodium Hydroxide, using methyl-red indicator. The second method was similar to the first with the exception that 10 cc. of chloroform saturated with ammonia water was added to the 10-cc. aliquot of the standardized strychnine solution before evaporation in order to more closely simulate the method of titrating the alkaloids recovered in the foregoing experiments. Both methods gave almost exactly similar results; repeated trials by the first method ranging

between 0.0620 and 0.0626 Gm. strychnine alkaloid per 10 cc., and by the second method 0.0622 and 0.0626 Gm.; the average of all trials being 0.0624 Gm.

In order to ascertain something of the nature of the basic substance which increased the apparent alkaloidal content in the foregoing experiments, 10-cc. portions of the standardized strychnine alkaloid solution were added to aliquots of ammoniacal, chloroformic extracts of several vegetable drugs of known apparent alkaloidal basicity and the total basicity then determined by the methods used to standardize the strychnine solution. Since the basic substance was thus subjected to the action of the strychnine alkaloid at elevated temperature a total basicity lower than the sum of the basicities of the strychnine alkaloid and apparent alkaloidal basicity of the drugs combined in the terms of strychnine alkaloid was to be expected if the basic substance was the ammonium radicle since strychnine alkaloid readily liberates ammonia from even such "strong" acids as hydrochloric acid under these conditions. The basic substance encountered in these experiments was not ammonia since the total basicity in terms of strychnine alkaloid checked up closely with the calculated total as shown by the following table.

Drug.	TABLE III.				
	Gentian.	Podophyllum.	Capsicum.	Cannabis.	P. E. Jalap.
Amount of drug	0.66	0.33	See note	0.66	0.33
Apparent basicity of drug (in terms of strychnine alkaloid)	0.00675	0.00735	0.0235	0.0131	0.00388
Strychnine alkaloid added	0.0624	0.0624	0.0624	.0624	0.0624
Total basicity expected (in terms of strychnine alkaloid)	0.0692	0.0698	0.0859	0.0755	0.0663
Total basicity found (in terms of strychnine alkaloid)	0.0698	0.0709	0.0844	0.0773	0.0674

NOTE: No record was kept of the amount of capsicum used, the ammoniacal, chloroformic extract possessing a light amber color.

While it seems certain that the ammonium radicle was not the interfering basic substance encountered in these experiments, the exact nature of the basic substance was not determined. It is possible that these interfering substances are "earthy" bases analogous to the calcium and magnesium soaps which were found by Watkins and Palkin<sup>1</sup> to exert a similar influence in alkaloidal assay processes; however, consideration must be given to the fact that most of the drugs in the present study are resinous in nature and may exert an action differing from the materials worked with by Watkins and Palkin.

#### SUMMARY.

While it is known<sup>1</sup> that basic substances other than alkaloids may be included with the alkaloidal residue separated during analysis when "fatty" acids or "soaps" of "earthy" bases are present, unless proper procedure is resorted to to overcome the influence of these interfering substances, in the present study it is shown that the influence of "resinous" and other types of constituents of vegetable drugs also must be guarded against in estimating alkaloids in admixture with these drugs. Means of overcoming the influence of these interfering substances are outlined and it is incidentally pointed out that these methods are applicable to the estimation of small proportions of alkaloids in complex admixture with vegetable drugs.

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