SEPARATION OF SABADILLA ALKALOIDS BY COUNTER-CURRENT EXTRACTION

by

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CHAPTER I

Introduction

Interest has been revived recently in the sabadilla alkaloids because of their use in insecticides and because of advancements in the chemistry and pharmacology of related alkaloids. Little is known about the individual alkaloids which make up the alkaloidal mixture obtained from sabadilla and commonly known as "veratrine"(1).

The dried ripe seeds of Schoenocaulon officinale A. Gray (Asagroea officinalis Lindley) were recognized by the British Pharmacopoeia of 1885. In the earlier literature sabadilla is referred to as Veratrum Sabadilla. There are at least twenty species of this plant, three of which occur in the United States. Nineteen species have been found in Mexico and one species is indigenous to Costa Rica, El Salvador, Guatemala, Honduras, Peru and Venezuela. In general all species of Schoenocaulon are referred to as sabadilla. The name sabadilla is derived for the Spanish cebadilja, which refers to barley, probably due to the similarity between this plant and barley, particularly in the formation of the seed which resembles this grain (3).

Sabadilla belongs to the Liliaceae family. Many

common names have been used in the designation of this plant in various countries. It has been referred to in English as sabadilla, sebadilla, cevadilla and Indian caustic barley; in French as Cebadille, cevadille, sebadilla, petite orge and sevadille; in German as Cebrauchliche sabadilla, g. sabadille, and lausekraut; in Dutch as Cebadilla and severzaadkruid; and as Cebadilla, cebadilla Mexicana, barley of Mexico, ceballeja, coballeta, cintul, cebadilla de Tierra Caliente, quimiodipatli, Itzcuimpatli, Cunicho and etzemo by the Spanish and Mexicans (3).

The fruit is a capsule having three locules in each of which there are from three to four seeds. The seeds are elongated, pointed at each end, flat on one side and convex on the other, and somewhat curved. They are in general five to eight mm. long, wrinkled, slightly winged and appear black or dark brown on the outside and whitish within (3).

Sabadilla seed usually yields from three to six percent total alkaloids and from fifteen to twenty percent fixed oil. The total alkaloids are made up of a mixture of alkaloids which has been reported to consist of cevadine (crystallized veratrine), veratridine (amorphous veratrine), cevadilline (sabadilline), sabadine and cevine (sabadinine). Undoubtedly other unidentified alkaloids occur in this mixture (3).

Of the five components reported to make up the sabadilla alkaloidal mixture only cevadine, veratridine and cevine have been studied to any degree, and details on these three compounds are meager. Some doubt exists as to the purity of veratridine and the actual existence of cevadilline and sabadine has not been confirmed (3).

In the present work, the material used was the extract IV-D of Chester E. Poetsch (3). Water solutions of various pH's were made, in which some of the extract IV-D was dissolved, and shaken out with chloroform to determine the amount of extraction by the chloroform at various pH's. This was repeated using counter-current extraction methods. Finally, a large scale counter-current extraction was performed where the product obtained was submitted to a chromatographic absorption column.

CHAPTER II

Theory of Counter-Current Extraction

This method of separation is based upon an observation made by Berthelot in 1872. If a third substance is added to a system consisting of two immiscible or slightly miscible components and this third substance is soluble in both layers, it will distribute or divide itself between the two layers in a definite manner. Suppose C_1 and C_2 are the concentrations in the layers 1 and 2 respectively, then at a constant temperature;

$$\frac{C_1}{C_2} = \text{constant (K)},$$

independent of the total amount of dissolved substance present; that is, the third substance distributes itself between the two layers in a constant ratio. This constitutes the distribution law or partition law, and the constant K is referred to as the distribution, or, partition coefficient. In the strict sense this is only an approximation and to be exact the equation should read;

$$\frac{a_1}{a_2} = \text{constant (K)},$$

where a_1 and a_2 are the activities of substance three in layers 1 and 2 respectively (2).

In counter-current extraction the substance three

is subjected to repeated systems of the immiscible or slightly miscible layers 1 and 2. To illustrate this procedure, the method used with separatory funnels will be described.

In this method, five separatory funnels are arranged in a row, and numbered consecutively from 1 to 5. The layers are chloroform and a buffered water solution, each mutually saturated with each other. 75 cc. of the buffered water solution is placed in each of the five separatory funnels; with the substance three (extract IV-D) dissolved in the buffered water solution in separatory funnel #1. 75 cc. of the chloroform is then added to funnel #1, and the funnel is shakened for two minutes. After the layers are allowed to settle, the chloroform layer (bottom layer) is withdrawn and transferred to funnel #2, and 75 cc. of fresh chloroform is added to funnel #1. Both funnels are shakened for two minutes and the layers again allowed to settle. The chloroform layer in funnel #2 is then withdrawn and placed in funnel #3, while the chloroform layer in funnel #1 is withdrawn and placed in funnel #2, and 75 cc. of fresh chloroform is added to funnel #1. This process is repeated, collecting the chloroform layers removed from funnel #5 until the desired number of chloroform layers are removed. These layers removed from funnel #5 are then evaporated to determine the amount of extract present

in them.

In this procedure, if a single component is present, the first layer removed from funnel #5 will contain little of the compound. As the successive layers are removed, the layers will contain more and more of the compound, rising to a maximum, and then finally dropping off again to a minimum. In a two component mixture, if the distribution constants of the two components are widely different, quanitative separation can be achieved. However, when working with an unknown mixture, like the extract IV-D, where the number of compounds and their respective partition coefficients are unknown, the separation becomes more difficult. By varying the pH of the buffered water solution in the method just described, the amount of removal by the chloroform layer can be regulated, and considerable separation for the resolution of the extract's constituents can be achieved.

CHAPTER III

Experimental Results

Before the counter-current extraction method was employed, it was necessary to determine the correct pH of a buffered water solution, in which some of the extract is dissolved, that chloroform would remove the right amount of constituents of the extract to obtain a good separation of the extract's components. This was accomplished by using two kinds of buffer solutions; Phosphate buffer (pH 5.0-8.0), and McIlvaine's buffer (pH 4.0-5.0). The solutions were made and adjusted to correct pH by means of a Beckman pH Meter and appropriate solutions. After the various pH solutions were made, a known amount of extract was dissolved in 25 cc. of each of the buffered solutions and each of the solutions placed respectively in a 100 cc. separatory funnel. 25 cc. of chloroform was then added to each funnel, the funnels shakened for two minutes and allowed to settle. After the layers have separated, the chloroform layer of each funnel was withdrawn and evaporated in a Al-dish to determine the amount of extract in each chloroform layer. Table I gives the results of this procedure.

TABLE I

Effect of pH on Distribution Law

Buffer Used	pH	% Removed by Chloroform
McIlvaine's	4.0	7.44
"	4.5	12.25
"	5.0	16.70
Phosphate	5.0	15.25
"	6.0	26.62
"	7.0	43.96
"	8.0	71.69

From the results of Table I, the counter-current extraction procedure was started as described on page 5. The first buffered solution used was at pH 6.0 (26.62% removal by chloroform), with 2.0234 Gms. extract used. The weight of extract removed from each successive layer of chloroform from funnel #5 is given in Table II.

TABLE II

Counter-Current Extraction at pH 6.0

No. of Layers Removed from Funnel #5	Gms. Removed in 75 cc. Chloroform Layer	% Removed
1	0.3883	19.2
2	0.0900	4.44
3	0.0629	3.11

No. of Layers Removed from Funnel #5	Gms. Removed in 75 cc. Chloroform Layer	% Removed
4	0.0390	1.93
5	0.0262	1.30
6	0.0218	1.08
7	0.0188	0.927
8	0.0165	0.815
9	0.0172	0.853
10	0.0232	1.15

From the results in Table II, it was noted that as each layer was removed from funnel #5, the weight of extract removed steadly decreased to layer #8, then started to increase with 26.75% of the extract removed in the first 8 layers.

On the basis of the foregoing, the procedure was repeated using a buffer solution at pH 7.0, and using 2.0457 Gms. of extract. The weight of extract removed from each successive layer of chloroform from funnel #5 is given in Table III.

TABLE III

Counter-Current Extraction at pH 7.0

No. of Layers Removed from Funnel #5	Gms. Removed in 75 cc. Chloroform Layer	% Removed
1	0.6750	33.0

No. of Layers Removed from Funnel #5	Gms. Removed in 75 cc. Chloroform Layer	% Removed
2	0.1691	8.26
3	0.1063	5.21
4	0.0870	4.25
5	0.0750	3.66
6	0.0675	3.29
7	0.0682	3.34
8	0.0562	2.75
9	0.0750	3.66
10	0.0600	2.93
11	0.0367	1.80
12	0.0360	1.76
13	0.0322	1.58
14	0.0696	3.40
15	0.0232	1.14
16	0.0165	0.805
17	0.0240	1.17
18	0.0112	0.550
19	0.0247	1.21

From the results in Table III, it was noted that 33.0% of the extract added was removed in the first layer, with 54.38% of the extract added removed in the first five layers.

With the information obtained from Table II and III above, a large scale counter-current extraction was performed using 25.0110 Gms. of extract and the buffer solution at a pH 6.0. The first layer removed from funnel #5 contained 4.4620 Gms. (17.81%) of the extract added, the second layer removed from funnel #5 contained 1.0340 Gms. (4.13%) of the extract added. These two layers were combined, making a total of 5.4960 Gms. (21.94%) of the extract added.

To make sure that only alkaloids were present, the combined layers were placed in a liter separatory funnel and 0.05N sulfuric acid added to shake out the alkaloids. After removal of the chloroform layer, fresh chloroform was added and the acid solution made alkaline with ammonium hydroxide. Thus the chloroform layer after shaking should contain only alkaloids. This chloroform layer was removed and evaporated down.

This dried, extracted alkaloids were then tested as to their solubility in various solvents. The dried alkaloids were found to be soluble in: Chloroform, Ethyl acetate, N-butyl alcohol (hence the lower mol. wt. alcohols also), Carbon tetrachloride, Benzene, Acetone, and Trichloroethylene. The dried alkaloids were insoluble in: Ether, Water, and Skelly C.

At this point, a chromatographic column of aluminum oxide was used to try to separate the components of the alkaloidal mixture a little further. The solvent used was a alcohol (absolute)-chloroform mixture, using 5% alcohol for the first 13 layers and 15% alcohol for the last 2 layers. The weight of the dried alkaloids used was 0.1582 Gms. The results are indicated in Table IV.

TABLE IV

First Chromatographic Column

No. of Layers Removed	Wt. (mgms.) Removed in 10 cc. Layers	% Removed
1	1.3	0.82
2	54.2	34.3
3	33.5	21.2
4	11.2	7.06
5	4.3	2.72
6	2.5	1.58
7	1.8	1.14
8	1.3	0.82
9	0.7	0.44
10	0.6	0.38
11	0.7	0.44
12	0.6	0.38
13	0.3	0.19

No. of Layers Removed	Wt. (mgms.) Removed in 10 cc. Layers	% Removed
14	0.3	0.19
15	0.8	0.51

Because 98.9 mgm. or 62.4% of dried alkaloids added were removed in layers 2, 3, and 4, the procedure was repeated using reagent chloroform as the solvent for the first 26 layers, then using 10% alcohol-chloroform mixture as the solvent for the next 10 layers. The wt. of dried alkaloids used was 0.1410 Gms. The results are indicated in Table V.

Table V

Second Chromatographic Column

No. of Layers Removed	Wt. (mgms.) Removed in 10 cc. Layers	% Removed
1	0.4	0.284
2	0.1	0.0710
3	0.2	0.142
4	0.0	0.000
5	0.2	0.142
6	0.1	0.071
7	1.3	0.923
8	1.5	1.06
9	1.0	0.710

No. of Layers Removed	Wt. (mgms.) Removed in 10 cc. Layers	% Removed
10	0.5	0.355
11	0.6	0.426
12	0.6	0.426
13	0.4	0.284
14	0.5	0.355
15	0.6	0.426
16	0.1	0.0710
17	0.4	0.284
18	0.3	0.213
19	0.3	0.213
20	0.4	0.284
21	0.3	0.213
22	0.3	0.213
23	0.2	0.142
24	1.4	0.992
25	0.4	0.284
26	0.3	0.213
27	0.3	0.213
28	0.3	0.213
29	50.3	35.6
30	10.9	7.72
31	4.3	3.05
32	2.9	2.06
33	2.0	1.42

No. of Layers Removed	Wt. (mgms.) Removed in 10 cc. Layers	% Removed
34	1.4	0.992
35	1.4	0.992
36	1.1	0.780

CURVE I

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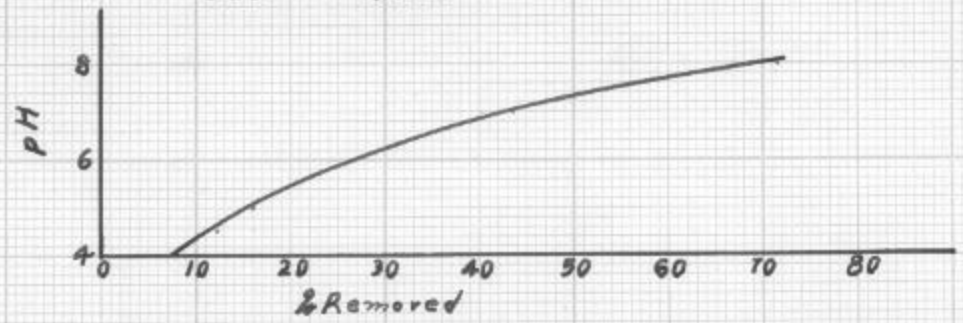
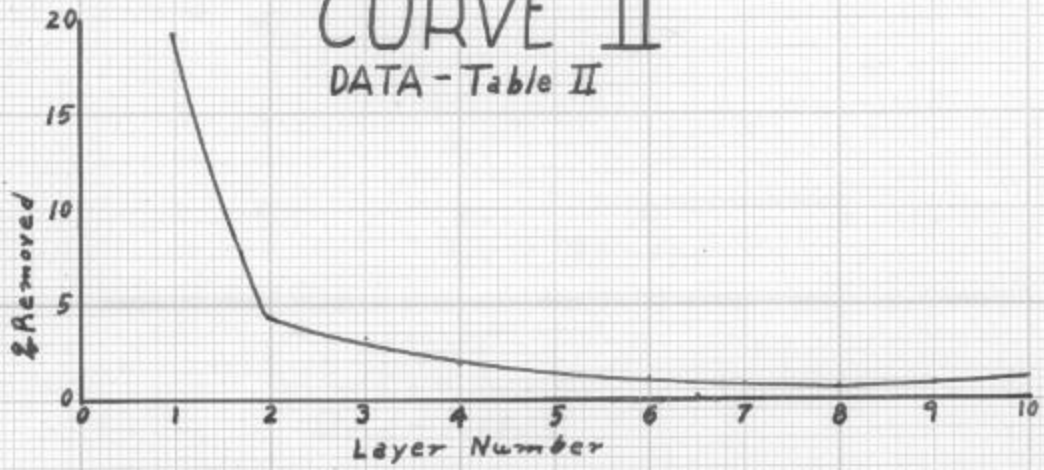


CURVE II

DATA - Table II



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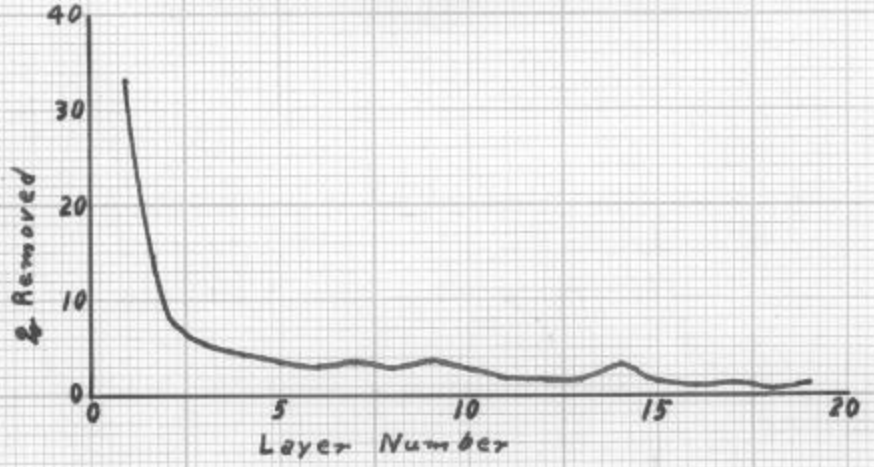

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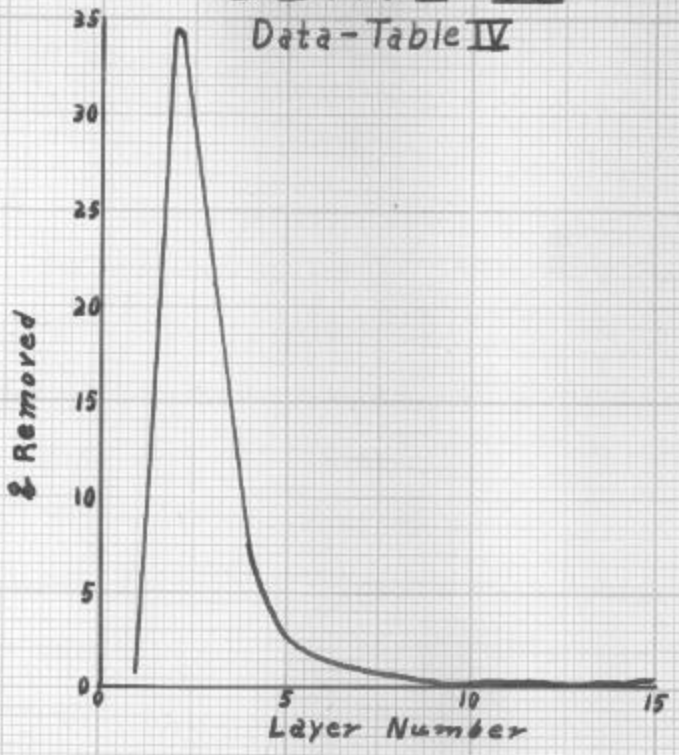
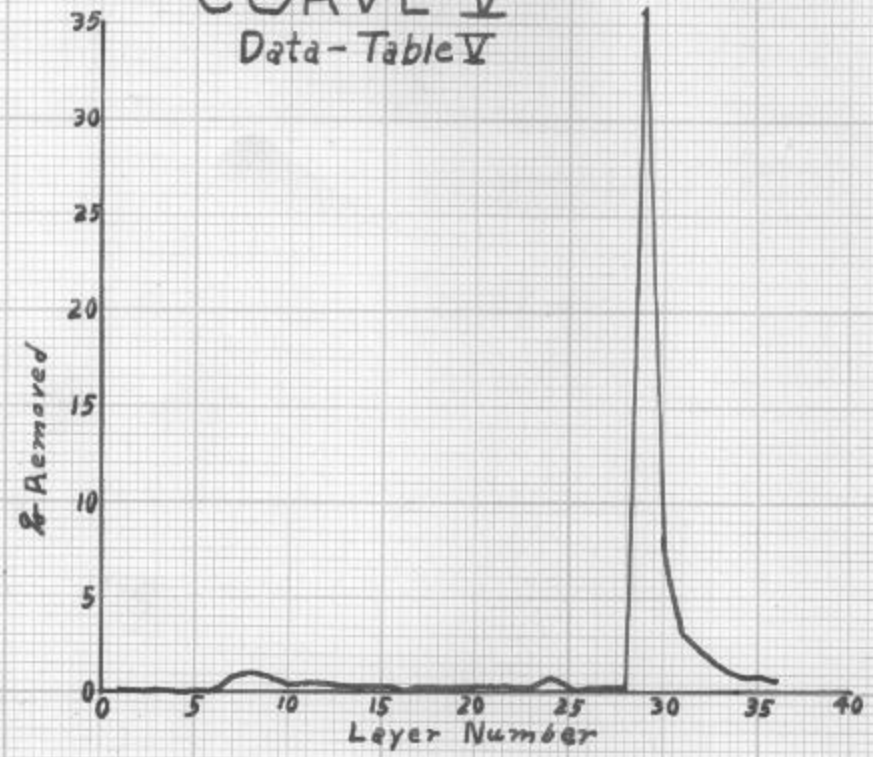


CURVE V

Data - Table V



CHAPTER IV

Conclusions

Counter-current extraction methods, as the one described on page 5, offers a practical method of resolution of complicated mixtures. It is of value especially when large quanities of material have to be broken down into simpler mixtures.

The extract IV-D, as shown in the different tables, can be broken down into simpler mixtures by this method. These mixtures can then be readily used for other methods of isolation, such as the chromatographic column. It is suggested that future work in this method be applied by using a number of different pairs of solvents in the layers, and analyzing the resulting components more thoroughly with the chromatograph column.

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Approved Lloyd M. Parks

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