than with sulfathiazole; however, it presented the same trend at higher lubricant concentration that had been found with the sulfonamide.

The reason for the observed behavior may be found in the compressional characteristics of magnesium stearate. Figure 14 shows the curve obtained for the compression of pure magnesium stearate which is qualitatively similar to the curve shown for stearic acid in Fig. 11. The poor transmission behavior under lower pressure might give rise to the effect seen above, but this is not clearly established.

CONCLUSION

Although these studies have been largely preliminary, the results suggest that measurements of lateral pressure developed during formation of pharmaceutical tablets may provide a useful indication of compressional characteristics of various materials. As a broad and possibly too sweeping conclusion, it appears that materials which permit rather good conversion of normal pressure to lateral pressure tend to form good tablets. Substances expected to exhibit poor flow properties under pressure, such as those composed of thin, flat, leaf-like crystals, appear to be shown by this technique to behave in this manner.

The general method of study seems to be adaptable to pilot plant and commercial tablet machines with appropriate modifications. It is relatively simple and rapid. The rubber plug technique seems to provide a ready and quick means of calibration.

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Sabadilla Alkaloids VIII

Isolation of Sabadillines I, II, and III

By GLENN R. SVOBODA†, HYMAN MITCHNER‡, and LLOYD M. PARKS§

The techniques of partition and adsorption chromatography as well as countercurrent distribution have been applied to alkaloidal concentrates from both sabadilla and veratrine. Whereas sabadilline previously was considered to consist of a single alkaloidal constituent, the present work has resulted in the isolation of three different materials which possess the characteristic ultraviolet absorption maximum at 238 mμ. These materials have been named sabadilline I, II, and III. Only sabadilline II was obtained in crystalline form. Attempts to establish a relationship between the three compounds were unsuccessful. Only sabadilline II yielded an alkaline isomerization product and this was not similar to either sabadillines I or III.

THE PRESENCE of an alkaloidal constituent of *Schoenocaulen officinale* (sabadilla) which exhibited an ultraviolet maximum at 238 mμ was first noted by Poetsch (1). This material was obtained from a commercial concentrate sold under the name "sabadilline." This was the name applied to the crystalline material, isolated by Hennig (2), which exhibited a similar ultraviolet spectrum. The work of Stuart (3) and Mitchner (4) confirmed the presence of a sabadilline-like material in both sabadilla and the commercial concentrate, veratrine, an alkaloidal extract of sabadilla.

A material which appeared to be similar to sabadilline was isolated by Auterhoff (5) and partially characterized by Vejdelek, Macek, and Kakac (6). To this material, which was named veragenine, was attributed an $\alpha\beta$-unsaturated ketone structure, unknown for any isolated sabadilla constituent other than sabadilline, for which an $\alpha\beta$-unsaturated ketone structure previously had been postulated by Stuart (3). The possibility of a similarity between the two compounds indicated the necessity of further investigation of the substance which exhibited

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the ultraviolet maximum at 238 mμ first noticed by Poetsch (1). Before this could be done it was necessary to devise a more convenient separation procedure than the chromatographic separation devised by Hennig (2).

EXPERIMENTAL

Materials.—All chemicals except technical chloroform were of reagent quality. Ultraviolet spectra were obtained with a Cary model 11S automatic recording spectrophotometer. Infrared spectra were obtained with a Baird double-beam instrument. Buffers were standardized with a Beckman model H-2 pH meter. The Craig countercurrent distribution apparatus used was a 200 tube, robot driven instrument (manufactured by H. O. Post, Maspeth, N. Y.). Each tube was of 20-ml. capacity, adjusted for 10 ml. upper and lower phase. A 2-ml. microburet was used for the titrations. Samples were titrated in chloroform *versus* perchloric acid in glacial acetic acid using quinaldine red as the indicator. The veratrine used in this investigation was kindly supplied by S. B. Penick and Co.

Purification of Sabadilline Concentrates.—Available from the isolation of sabatine from Poetsch's Fraction D (1) by partition chromatography of successive samples on silicic acid at pH 8.00, were the peak materials eluted immediately prior to the sabatine peak. These corresponded to the material

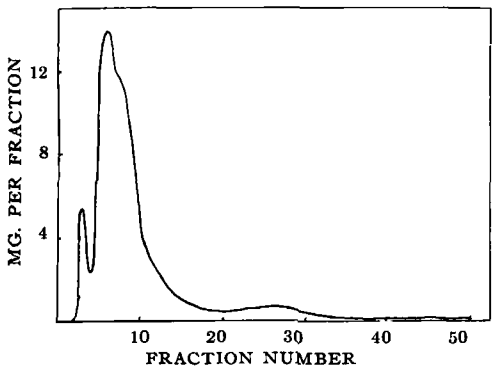

Fig. 1.—Partition chromatogram of a sabadilline concentrate on silicic acid with chloroform *vs.* pH 8.00 phosphate buffer.

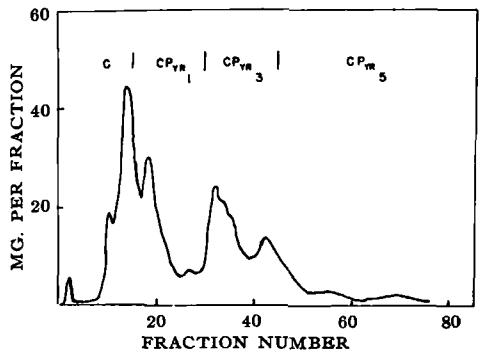

Fig. 2.—Partition chromatogram of a sabadilline concentrate on purified cellulose with chloroform and chloroform containing the designated increments (%) of pyridine *vs.* pH 6.50 phosphate buffer.

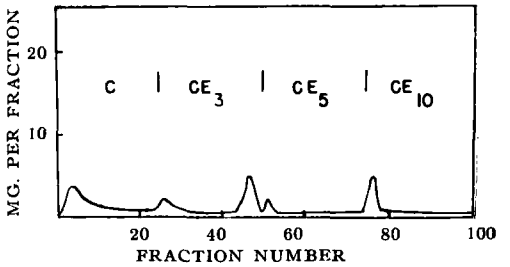

Fig. 3.—Adsorption chromatogram of a sabadilline concentrate on activated (basic) alumina with chloroform and chloroform containing the designated increments (%) of ethanol.

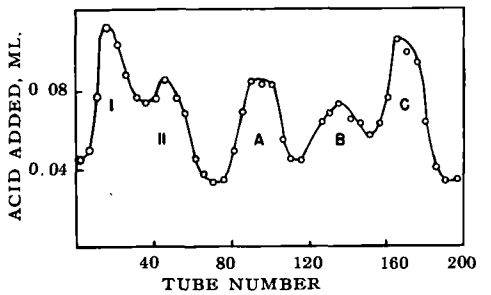

Fig. 4.—Countercurrent distribution of 1 Gm. of Fraction I at pH 6.35 for 406 transfers *vs.* chloroform.

from which Hennig (2) was able to isolate a few milligrams of sabadilline. Ultraviolet absorption analyses of these peak materials confirmed the presence of a maximum at 238 mμ. These sabadilline concentrates were purified by a modified countercurrent distribution using a chloroform–phosphate buffer (pH 7.25) system.

The sabadilline concentrates were dissolved in 50 ml. of chloroform and shaken with an equal volume of pH 7.25 phosphate buffer. The chloroform solution was transferred to the next separator containing fresh buffer at pH 7.25. The process was continued through six transfers. The buffer solutions were combined, made alkaline with ammonium hydroxide, and extracted with 10-ml. portions of chloroform until a negative Mayer's test was obtained. The chloroform extracts were filtered through anhydrous sodium sulfate and evaporated to dryness under reduced pressure. Successive peak materials were treated in a similar manner. Those materials which exhibited an ultraviolet maximum at 238 mμ were combined and used in chromatographic separations.

Partition Chromatography with Sabadilline Concentrates.—Silicic acid columns (30 Gm. silicic acid:30 ml. buffer) were prepared as described by Poetsch (1). A 15-to-1 ratio of length-to-width was maintained for all columns. Samples (about 500 mg.) were dissolved in a minimal volume of chloroform and placed on the column. Ultraviolet analyses of 10-ml. fractions showed that the sabadilline-like material was eluted from the negative slope of the main elution peak. Alkaloidal fractions which exhibited the ultraviolet absorption maximum at 238 mμ from several such columns were combined (125.6 mg.) and placed on a column which consisted of 6 Gm. of silicic acid and 6 ml. of pH 8.00 phosphate buffer. Ultraviolet analysis of the chloroform elu-

tion pattern obtained (Fig. 1) indicated the possibility of two independent materials which exhibited a sabadilline-like absorption spectrum based upon the extinction coefficients obtained.

Another supporting phase used for the partition chromatographic separation of sabadilline concentrates was Solka-Floc BW-200, a purified cellulose (obtained from the Brown Co., Boston, Mass.). A 1.0-Gm. sample of sabadilline concentrate was placed on a column which consisted of 40 Gm. of cellulose and 30 ml. of pH 6.50 phosphate buffer. Figure 2 shows the elution pattern of 10-ml. fractions obtained using chloroform and chloroform-containing increments of pyridine.

Ultraviolet analyses of the eluted fractions indicated the existence of three independent materials which exhibited the sabadilline maximum at 238 mμ. Fractions 13 to 20 were combined to give about 300 mg. of material which had an extinction coefficient ($k \times 100$ at 238 mμ) in excess of 1000. All attempts to crystallize this material were unsuccessful.

Adsorption Chromatography with Sabadilline Concentrates.—The adsorbents investigated in this study included silicic acid, acid-washed alumina, activated alumina, light and heavy magnesium oxides, Florisil, and Celite. Of these, only activated alumina gave results worth further consideration. Ultraviolet absorption analyses of the 10-ml. fractions obtained by the chromatographic separation of a 100-mg. sample of a sabadilline concentrate on 5.0 Gm. of activated alumina using chloroform and chloroform-containing increments of ethanol (Fig. 3) indicated the existence of three sabadilline materials.

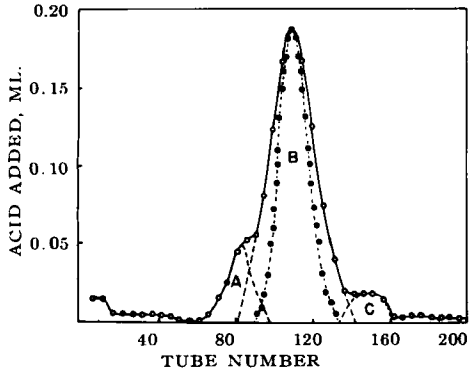

Fig. 5.—Countercurrent distribution of Peak B, Fraction I at pH 6.35 for 400 transfers *vs.* chloroform. Key: O, experimental; ⊖, theoretical.

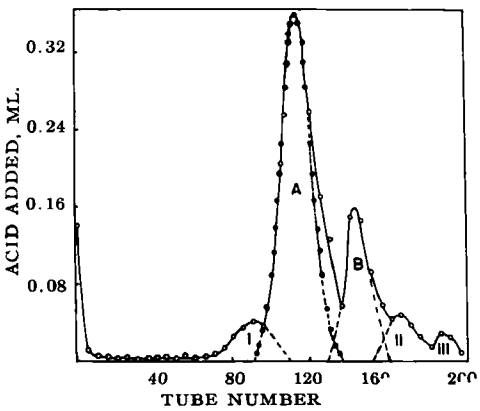

Fig. 6.—Countercurrent distribution of 1.0 Gm. Fraction III at pH 6.65 for 210 transfers *vs.* chloroform. Key: O, experimental; ⊖, theoretical.

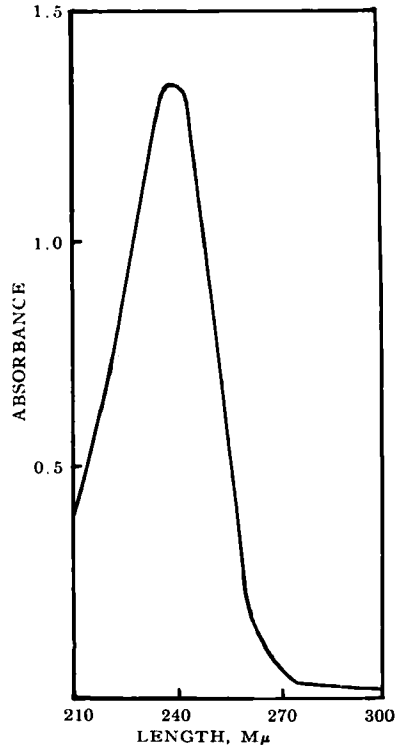

Fig. 7.—Ultraviolet spectrum of sabadilline II in absolute ethanol ($k \times 100 = 3004$).

The other adsorbents cited gave either too large a sample holdup or a lack of constituent resolution.

At this point it was deemed advisable to investigate other means of separation.

Countercurrent Distribution of Sabadillines Obtained from Veratrine.—Previous work (7) describing the quantitative determination of the known alkaloidal constituents of commercial veratrine described the isolation of the hydrophilic alkaloids in $P_{0.1.2}$. Successive 5-Gm. samples of $P_{0.1.2}$ were distributed for about 200 transfers at pH 7.00 *versus* chloroform. Five distribution peaks were obtained. Ultraviolet absorption analyses of the negative slopes of (*a*) Peak A, (*b*) Peak C, and (*c*) the positive slope of Peak E, indicated the presence of a maximum at 238 mμ. Alkaloidal material which was interposed between (*a*), (*b*), and (*c*) did not exhibit ultraviolet maxima at 238 mμ. Infrared absorption analyses confirmed the presence of an unsaturated band for (*a*), (*b*), and (*c*) and the absence of this band for the interposed material. The tubes which corresponded to these areas of the distributions were combined to give Fractions I to VI.

Successive portions of Fractions I, III, and V were subjected to countercurrent distribution until nearly identical theoretical and experimental curves were obtained or until the sample size prevented further purification; then attempts were made to crystallize the material. Distribution patterns were obtained as described previously (7).

Six portions of Fraction I (100 to 1100 mg.) were

distributed at pH values from 5.50 to 6.35 for 100 to 400 transfers. The best resolution of material was obtained with a 1.0-Gm. sample at pH 6.35 *versus* chloroform for 400 transfers. All alkaloidal material was found as shown in the first 200 tubes of Fig. 4. Peak B was the only material which exhibited an ultraviolet maximum at 238 mμ. All of the material of this peak was again distributed at pH 6.35 *versus* chloroform for 400 transfers. All of the alkaloidal material was found as shown in the first 200 tubes of Fig. 5. Although the distribution was symmetrical, there was no agreement between theoretical and experimental points. All attempts to crystallize the material obtained from Peak B, Fraction I were unsuccessful.

Amorphous sabadilline I (Peak B, Fraction I) softened from 126–133°, melted at 133–137°, and remelted from 133–140°. The material decomposed above 250°. Elemental analyses were inconclusive. The infrared spectrum of sabadilline I is shown in Fig. 8a. The alkaloidal spectrum (A.S.) value of sabadilline I was 6.05. The designation of alkaloidal constituents by A.S. value is helpful for unknown constituents. These constants are represented by that pH at which the partition coefficient of the constituent in chloroform *versus* aqueous buffer is unity (4). The A.S. values allow the accurate characterization of new components with respect to their A.S. values and give partition coefficient data for each unknown.

Sabadilline II was obtained from Fraction III in the following manner. Two samples of Fraction III (1.0 and 0.8 Gm.) were individually distributed for 210 transfers at pH 6.65 *versus* chloroform. The distribution pattern of the 1.0-Gm. sample is shown in Fig. 6. The distribution pattern was similar for the 0.8-Gm. sample. The materials found in Peaks A and B both exhibited an ultraviolet maximum at 238 mμ. The material in Peak A had an A.S. value of 6.72. However, the A.S. value of the material in Peak B (6.98) corresponded to the A.S. value of the material obtained by the aqueous alkaline treatment of Fraction III (*vide infra*). Therefore, Peak A was

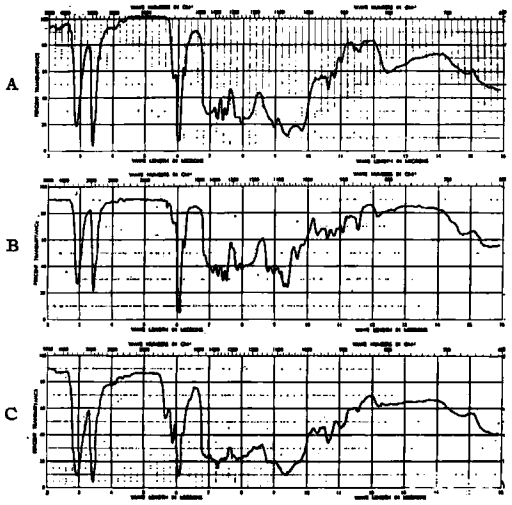

Fig. 8.—(A) Infrared spectrum of amorphous sabadilline I. (B) Infrared spectrum of crystalline sabadilline II. (C) Infrared spectrum of amorphous sabadilline III.

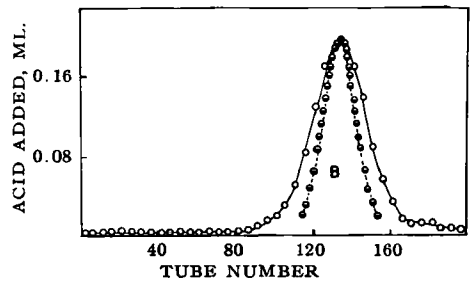

Fig. 9.—Countercurrent distribution of Peak B, Fraction V at pH 7.80 for 214 transfers *vs.* chloroform. Key: ○, experimental; ◒, theoretical.

considered to consist of naturally occurring sabadilline II. Crystalline sabadilline II was obtained from the combined Peak A materials of the two distributions. A total of 100 mg. of water-white, needle-shaped prisms was obtained by crystallization in ethanol-water. The crystals were dried at 100° (0.05 mm.) and exhibited the following melting characteristics: swelled and became opaque from 141–146°, decomposed slightly from 210–225° and melted from 235–252°. The ultraviolet spectrum of sabadilline II is shown in Fig. 7 and its infrared spectrum in Fig. 8b. Elemental analyses were inconclusive.

Sabadilline III was obtained from Fraction V. Five portions of Fraction V of from 100-1000 mg. were distributed at pH values of 7.90 to 8.00 *versus* chloroform for 200 transfers. Only Peak B of Fraction V exhibited an ultraviolet maximum at 238 mμ. The material which comprised Peak B from all of the distributions was combined and distributed for 214 transfers at pH 7.81 *versus* chloroform (Fig. 9). Sabadilline III had a calculated A.S. value of 7.83. There was no agreement between the theoretical and experimental distribution values. Attempts to crystallize sabadilline III were unsuccessful. Amorphous sabadilline III exhibited the following melting characteristics: swelled 126–136°, no true melt to 170°, reswelled from 123–140°, melted from 140 180°, and decomposed slowly above 210°. The infrared spectrum of sabadilline III is shown in Fig. 8c.

Attempts to Determine a Relationship Between Sabadillines I, II, and III.—Countercurrent distribution of the products of methanolysis (8) of Fraction III showed that the reaction conditions were too mild since only the original material appeared in the distribution pattern of the treated material. A similar analysis of the products of ethanolic potassium hydroxide treatment (8) of Fraction III indicated by their number that these reaction conditions were too severe. The conditions finally selected for the methanolic alkaline treatment of Peak B, Fraction III were as follows: A 59-mg. sample of Peak B, Fraction III was dissolved in a solution of 4 ml. of methanol and 1 ml. of 1 *N* potassium hydroxide. The solution was refluxed for 15 minutes, cooled, and made just acid with 1:1 hydrochloric acid. The methanol was removed under reduced pressure and the residue was extracted with four 25-ml. portions of chloroform after adding sufficient ammonium hydroxide to give a pH of 9.00. The combined chloroform extracts were dried by filtration through anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The prod-

uct obtained was distributed for 106 transfers at a pH of 7.00 *versus* chloroform. The single distribution peak obtained was collected and distributed again at pH 7.50 *versus* chloroform. The single peak obtained from the latter distribution had a calculated A.S. value of 6.98 compared to 6.72 for sabadilline II. The material was considered to be an alkaline isomerization product of sabadilline II and was similar to the material obtained from Peak B, Fraction III which had a calculated A.S. value of 6.97.

Peak B, Fraction I and Peak B, Fraction III (sabadilline I and III) were treated in a similar manner. Infrared analysis of the materials obtained in the respective distribution peaks of the reaction products showed that they consisted of impure starting materials. Therefore, the results of the aqueous alkaline treatment of sabadillines I and III were inconclusive, in that they were not changed under the reaction conditions to which they were subjected. The small amount of material available precluded further investigation.

Pharmacological Evaluation of Sabadilline II.— In the dog under phenobarbital anesthesia, 1.0 mg./Kg. intravenously depressed respiration for approximately 10 minutes, depressed gut motility, and produced a slight transitory rise in blood pressure. The EKG record showed depression of the S-T segment with an increase in T-wave amplitude similar to that observed with increased blood potassium level. At 2.19 mg./Kg. intravenously it produced a fall in blood pressure for approximately 5 minutes and a temporary inhibition of respiration and gut motility. After 20 minutes, the heart rate slowed and the dog died. EKG changes were the same as with the 1-mg./Kg. dose.

Because of the amorphous nature of sabadilline I and III, their physiological activity as potential hypotensives was not checked.

DISCUSSION AND CONCLUSIONS

A prerequisite to the successful conclusion of this investigation was to devise separation and purification techniques for sabadilline concentrates. Early chromatographic studies were laborious and confusing, indicating the presence of several materials which possessed the sabadilline ultraviolet maximum at 238 mμ. Additionally, the elution of the possible single compound was discontinuous and the apparent presence of separate compounds of this nature was because of a change in elution solvent composition.

Countercurrent distribution analyses of commercial veratrine had shown that between 1.8 and 4.8% of sabadilline material (as unknown X_1) was present in the total alkaloids (7) This work involved the separation of veratrine into hydrophobic, hydrophilic, and intermediate solubility fractions. Further countercurrent distributions of the hydrophilic alkaloids indicated three of the six fractions contained material which exhibited the sabadilline ultraviolet absorption spectrum. Further separation of the sabadilline-like materials in these fractions utilized additional countercurrent separations as described in this work.

The first of these sabadilline-containing concentrates (Fraction I) was shown to consist of at least five different constituents. The first two peaks obtained (Fig. 4) were not homogeneous. The third peak, Peak A, was known to be cevacine. It was Peak B which yielded sabadilline I. Peak C was shown to be another previously unknown Sabadilla constituent and was obtained in crystalline form. Further description of this material will be the subject of a later publication.

Sabadilline II and a compound believed to be its alkaline isomerization product were isolated by the countercurrent distribution of Fraction III (Fig. 5). Peak A corresponded to sabadilline II; Peak B corresponded to a product obtained by the alkaline treatment of sabadilline II. Sabadilline II was the only sabadilline obtained in crystalline form. The infrared spectrum of sabadilline II suggests a structure which more closely resembles sabine (4) than veracevine (6), thus suggesting that the parent alkamine of sabadilline is structurally most like sabine.

The third of the sabadilline compounds was obtained by countercurrent distribution of Fraction V (Fig. 6). Peak B was shown to consist of amorphous sabadilline III.

There was no question of the independent nature of the three sabadillines. It was then of interest to determine if there was some simple relationship between these alkaloids. The fractions from which the sabadillines were isolated were subjected to aqueous alkaline treatment. Infrared spectra before and after such treatment showed no significant structural changes resulting from the conditions of such treatment. Herein is sufficient evidence that the $\alpha\beta$-unsaturated ketone structure attributed to the sabadillines does not arise from the isolation procedures inasmuch as these procedures are far more mild than the aqueous alkaline treatment.

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