

Sabadilla Alkaloids IX

Isolation of Unreported Minor Constituents and Their Relationship to the Known Sabadilla Alkaloids

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

Additional minor alkaloidal constituents of sabadilla have been isolated from veratrine. Four of these were obtained as symmetrical countercurrent distribution peaks. A fifth, sabadene, was obtained in crystalline form. Sabadene possessed no hypotensive properties. The investigation also notes the existence of extremely minor quantities of unresolved constituents. A review of the relationship of all reported sabadilla alkaloids is presented.

AS INDICATED in previous reports (1, 2), additional unreported alkaloidal constituents of sabadilla have been isolated by countercurrent distribution of commercial veratrine. One of these, for which the name "sabadene" is proposed, was obtained in crystalline form. Sabadene was shown by infrared analysis to contain an isolated carbon-carbon double bond structure, previously unknown for any sabadilla alkaloid. In addition, four other amorphous alkaloidal materials were obtained as symmetrical distribution peaks and two additional distribution peaks were obviously of a heterogeneous nature.

Fortunately, the isolation procedure resulted in the isolation of previously known sabadilla constituents for which pH assignments have been made at which these constituents demonstrate a partition coefficient of unity in aqueous buffers *versus* chloroform. The constants represented by partition coefficients equal to one have been called alkaloidal spectra (A.S.) values (3). These values allowed the accurate characterization of the new components with respect to their relative A.S. values and gave partition coefficient data for each unknown.

None of the new alkaloidal constituents described herein exhibited a characteristic ultraviolet spectrum. However, vanilloyl veracevine which had been reported previously (4) did exhibit its characteristic ultraviolet spectrum.

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† Present address: Freeman Chemical Corp., Port Washington, Wis.

‡ Present address: Barnes-Hind Laboratories, Inc., Sunnyvale, Calif.

§ Present address: College of Pharmacy, Ohio State University, Columbus.

The description of the original isolation of vanilloyl veracevine did not cite its partition characteristics relative to the total sabadilla constituents as is done in the present work.

EXPERIMENTAL

Apparatus and Reagents.—These were the same as described previously (2). The isolation techniques and distribution determinations have been similarly described (2).

Constituents of Fraction I.—The distribution

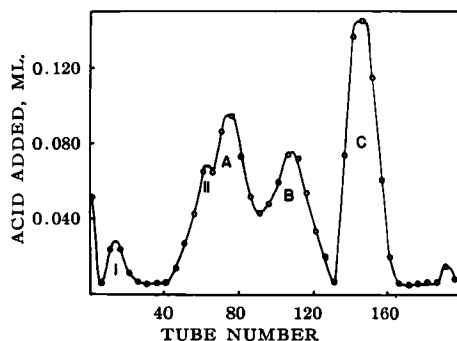


Fig. 1.—Countercurrent distribution of a 1.0-Gm. sample of Fraction I at pH 6.35 for 433 transfers *vs.* chloroform.

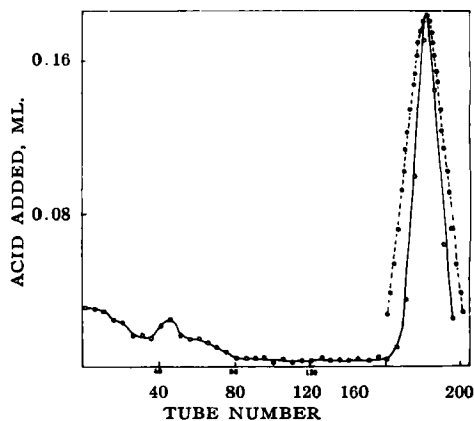


Fig. 2.—Countercurrent distribution of Peak I, Fraction I at pH 5.13 for 427 transfers *vs.* chloroform. Key: O—experimental, ●—theoretical.

conditions which resulted in the best resolution of the constituents of consecutive 1.0-Gm. samples of Fraction I were a pH of 6.35 and about 400 transfers *versus* chloroform. Figure 1 shows the results obtained, all of the alkaloidal constituents being present in the first 200 tubes. The alkaloidal material which constituted each of the individual peaks was collected and treated as follows.

Peak I, Fraction I, was collected and distributed at pH 5.13 for 427 transfers *versus* chloroform, all of the alkaloidal constituents being present in the first 200 tubes (Fig. 2). The calculated A.S. value of the single symmetrical peak was 5.00. Peak I, Fraction I, exhibited the following melting characteristics: melted at 123–131° and remelted at 153–158°.

The distribution of Peak II, Fraction I, at pH 5.47 for 403 transfers *versus* chloroform (Fig. 3) showed that the original peak was actually composed of at least three materials as well as a residual quantity of Peak I, Fraction I. In this distribution, all of the alkaloidal material was present in tubes 120 to 280. The identity of residual Peak I, Fraction I, as an unknown was confirmed by the calculated A.S. value (5.25) of Peak I (Fig. 3) compared to the value obtained for Peak I, Fraction I (5.20), of Fig. 2.

Peak A, Fraction I, was distributed at pH 5.88 for 464 transfers *versus* chloroform (Fig. 4). Peak A had a calculated A.S. value of 5.89 compared to 5.88 for cevacine and exhibited an infrared spectrum similar to that of authentic cevacine.

The treatment of Peak B, Fraction I, from which sabadilline I was isolated has been described previously (2).

The fractions which constituted Peak C (Fig. 1) were combined and yielded 159 mg. of crystalline

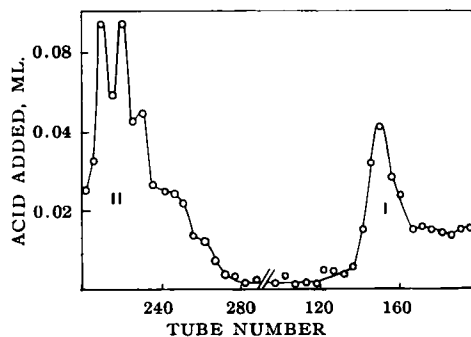


Fig. 3.—Countercurrent distribution of Peak II, Fraction I at pH 5.47 for 403 transfers *vs.* chloroform.

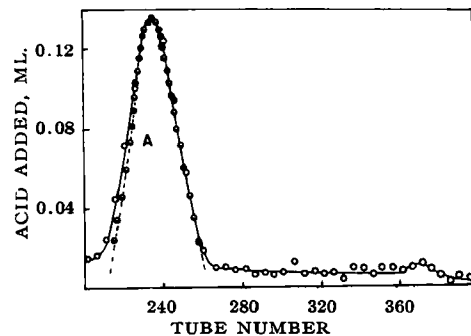


Fig. 4.—Countercurrent distribution of Peak A, Fraction I at pH 5.88 for 464 transfers *vs.* chloroform. Key: ○—experimental, ●—theoretical.

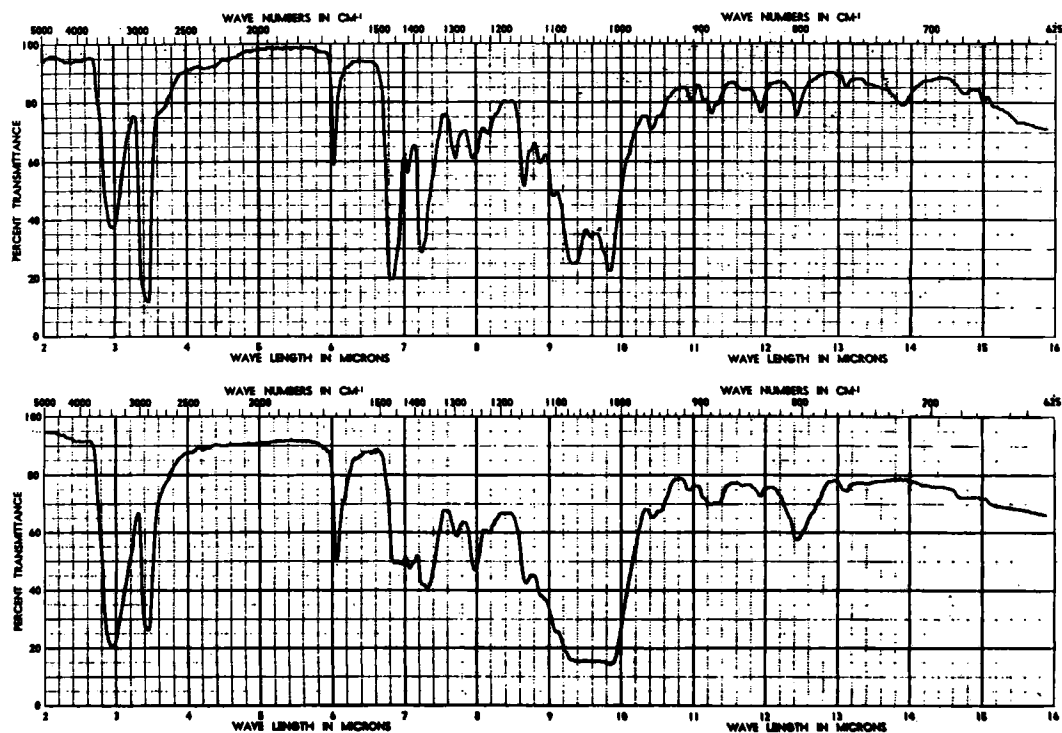


Fig. 5.—(a) Infrared spectrum of sabadene in Nujol. (b) Infrared spectrum of sabadene in a potassium bromide pellet.

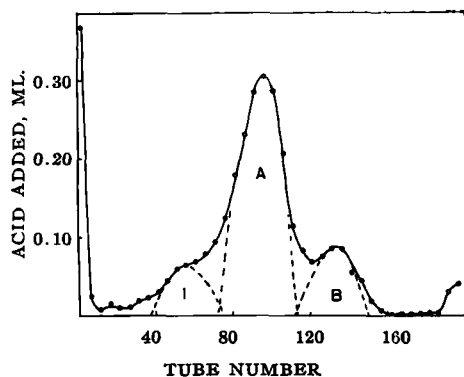


Fig. 6.—Countercurrent distribution of a 1-Gm. sample of Fraction II at pH 6.70 for 297 transfers *vs.* chloroform.

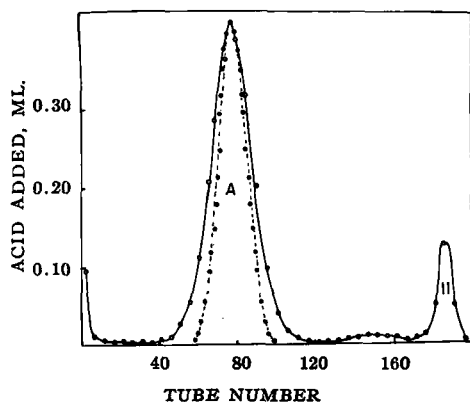


Fig. 7.—Countercurrent distribution of Peak A, Fraction II at pH 6.70 for 204 transfers *vs.* chloroform. Key: ○—experimental, ●—theoretical.

sabadene from acetone-water. Crystalline sabadene melted from 218 to 219.5° after drying at 0.01 mm. for 24 hours. The silky mass of white needles lost their shiny appearance upon drying.

Elemental analyses best agreed with the formula $C_{22}H_{41-46}O_7N$. Insolubility of the material in chloroform required that the infrared spectrum be determined in Nujol (Fig. 5a) or as a potassium bromide pellet (Fig. 5b). Sabadene has a calculated A.S. value of 6.22.

Constituents of Fraction II.—Distribution of Fraction II (1-Gm. samples) at pH 6.70 for 200–300 transfers *versus* chloroform resulted in the resolution of three peaks (Fig. 6). Peak I, Fraction II, had a calculated A.S. value of 6.16 which suggested that it consisted of sabadene (6.22). Peak B, Fraction II, had a calculated A.S. value of 6.76 which suggested its identity as sabadilline II (6.72). Peak A, Fraction II (calculated A.S. value = 6.50), appeared to be another new alkaloidal constituent. The tube contents which constituted Peak A, Fraction II, were collected and distributed at a pH of 6.70 for 204 transfers *versus* chloroform (Fig. 7). Peak A, Fraction II, had a calculated A.S. value of 6.42 and Peak II, 7.64. The infrared spectrum of Peak II was identical to that of authentic veracevine although the A.S. values did not agree (7.64 *versus* 8.15). However, Peak II could only have resulted from the distribution conditions. This suggests

that the alkaline of Peak A, Fraction II, is a veracevine type. The melting characteristics of amorphous Peak A, Fraction II, were as follows: swelled from 128–134°, melted 152–157°, remelted 152–157°, and decomposed slowly from 230–290°.

Constituents of Fraction III.—The distributions of Fraction III (1.0-Gm. samples) at pH 6.65 *versus* chloroform resulted in the isolation of sabadilline II from Peak A, Fraction III, and what appeared to be its alkaline isomerization product (Peak B, Fraction III) which has been discussed previously (2). In addition, three very minor distribution peaks were observed. Peak I, Fraction III (A.S. value 6.52), was probably identical to Peak A, Fraction II (A.S. value 6.50). Peak II, Fraction III (A.S. value 7.23), was probably identical to sabatine (A.S. value 7.33). Peak III, Fraction III (A.S. value 7.56), was probably identical to Peak A, Fraction V (A.S. value 7.51).

Constituents of Fraction IV.—The material which comprised Fraction IV had the same calculated A.S. value (7.33) as did sabatine. Crystalline sabatine

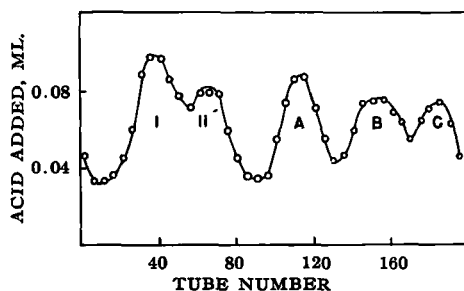


Fig. 8.—Countercurrent distribution of a 500-mg. sample of Fraction V at pH 7.80 for 210 transfers *vs.* chloroform.

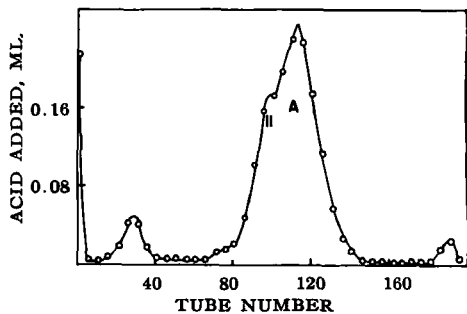


Fig. 9.—Countercurrent distribution of Peak A, Fraction V at pH 7.50 for 215 transfers *vs.* chloroform.

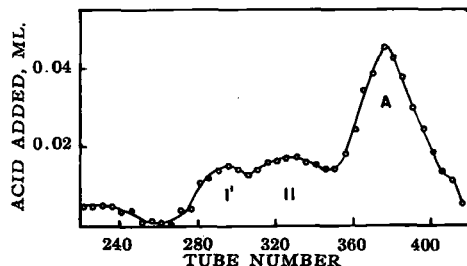


Fig. 10.—Countercurrent distribution of Peak A and Peak II, Fraction V at pH 7.50 for 697 transfers *vs.* chloroform.

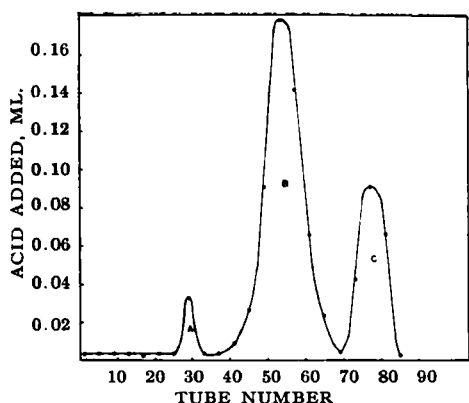


Fig. 11.—Countercurrent distribution of Fraction VI at pH 8.00 for 90 transfers *vs.* chloroform.

was isolated from this peak material. In addition, Fraction IV exhibited infrared absorption characteristics similar to sabatine.

Constituents of Fraction V.—The conditions which resulted in the best resolution of 0.5–1.0-Gm. samples of Fraction V were a pH of 7.80 and about 210 transfers *versus* chloroform. A typical distribution pattern obtained is shown in Fig. 8. Peak I, Fraction V, had an average calculated A.S. value of 6.77 which corresponded to the A.S. value of sabadilline II (6.72). Peak II, Fraction V, had an average A.S. value of 7.52. Peak A, Fraction V, appeared to be composed of at least two constituents in one distribution (not shown), but was generally obtained as a single symmetrical peak. The material which comprised Peak A and Peak II was collected and distributed at pH 7.50 for 215 transfers *versus* chloroform. As can be noted from Fig. 9, a poorly resolved peak of A.S. value 7.43 was obtained. This was believed to be similar to Peak II, Fraction V. The material which constituted Peak A was again collected and distributed at pH 7.50 for 697 transfers *versus* chloroform (Fig. 10). All of the alkaloidal material was contained in tubes 220 to 400. Again, material other than Peak A was observed. These minor peaks, I and II, were not further investigated. Amorphous Peak A (A.S. value 7.51) was collected and the following melting characteristics were obtained: swelled 105–115°, melted 140–150°, reswelled and became opaque from 222–229°, decomposed slowly above 310.

As noted previously (2), Peak B, Fraction V, was found to consist of sabadilline III.

Peak C, Fraction V, had an average calculated A.S. value of 8.12. This corresponded quite closely to the A.S. value of veracevine (8.15). All attempts to crystallize this material were unsuccessful. The infrared spectrum of Peak C was identical to that of authentic veracevine.

Constituents of Fraction VI.—A single distribution of Fraction VI (100-mg. sample) was made at pH 8.00 for 90 transfers *versus* chloroform. Three peaks were obtained (Fig. 11). Peak A had a calculated A.S. value of 7.68, but the small amount of material obtained precluded further study. Peak B had a calculated A.S. value of 8.15 and was undoubtedly veracevine (A.S. value 8.15). Peak C had a calculated A.S. value of 8.79, which is inter-

TABLE I.—TOTAL KNOWN AND UNKNOWN ALKALOIDAL COMPOSITION OF SABADILLA

Compound	Source (1, 2)	A.S. Value
Veratridine	P _{6,7,8}	3.65
Cevadine	P _{6,7,8}	4.27
Vanilloyl veracevine	P _{3,4,5}	4.87
	P _{3,4,5}	
Unknown	P _{6,7,8}	5.00
Unknown (mixture of three constituents)	Peak I, Fraction I	5.00
Cevacine	Peak II, Fraction I	5.47
Sabadilline I	Peak A, Fraction I	5.88
Sabadene	Peak B, Fraction I	6.05
Unknown (single constituent)	Peak C, Fraction I	6.22
Sabadilline II	Peak A, Fraction II	6.42
Alkaline isomerization product of Sabadilline II	Peak A, Fraction III	6.72
Sabatine	Peak B, Fraction III	6.98
Unknown	Fraction IV	7.33
Unknown	Peak I, Fraction V	7.43
Unknown	Peak II, Fraction V	7.52
Unknown	Peak A, Fraction V	7.51
Sabadilline III	Peak B, Fraction V	7.83
Veracevine	Peak C, Fraction V	8.15
	Peak B, Fraction VI	
Cevagenine	8.6
Unknown	Peak C, Fraction VI	8.79
Sabine	8.9

mediate between the values for cevagenine (8.6) and sabine (8.9). Peak C was not further investigated.

Pharmacological Evaluation of Sabadene.—In the dog under phenobarbital anesthesia doses up to 1 mg./Kg., intravenously, this had no significant effect on blood pressure, respiration, gut motility, or EKG.

SUMMARY AND CONCLUSIONS

In addition to the previously known sabadilla alkaloids, the three sabadillines and the aqueous isomerization product of sabadilline II, the six fractions of the hydrophilic sabadilla alkaloids have been shown to contain additional minor alkaloids. The total known and unknown alkaloidal composition of sabadilla is presented in Table I in increasing order of A.S. value.

It is possible that Peak C, Fraction VI, may consist of several materials which include cevagenine and sabine, although these materials were not directly noted in this study.

A procedure such as cited in our several papers on the countercurrent separation of the sabadilla alkaloids is to be recommended in the analysis of complex mixture of natural products which possess slightly different partition characteristics. Isolation of products in crystalline form or known chemical identity is not a prerequisite to identification of the constituents.

REFERENCES

- (1) Svoboda, G. R., *Anal. Chem.*, **34**, 1559(1962).
- (2) Svoboda, G. R., Mitchner, H., and Parks, L. M., *THIS JOURNAL*, **52**, 772(1963).
- (3) Mitchner, H., Ph.D. Thesis, University of Wisconsin, Madison, 1956.
- (4) Stuart, D. M., and Parks, L. M., *THIS JOURNAL*, **45**, 252(1956).