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THE INFLUENCE OF STRUCTURE ON THE ORIENTATION OF THE VERATRUM ALKALOIDS AT THE AIR/WATER INTERFACE

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SUMMARY

The veratrum alkaloids—veracevine, veratridine, cevadine, and veratramine—have similar chemical structures yet are quite distinct in their pharmacological actions. Veracevine is inactive, veratridine and cevadine are "labilizers" or "unstabilizers", and veratramine is an antagonist for cevadine and veratridine. The properties of these compounds at the air/water interface also reveal marked differences. Surface tension measurements in water show surface activity decreases in the order veratramine $>$ veratridine $>$ cevadine $\gg$ veracevine. A Langmuir-type film balance was used to measure their F-A curves. Films of the alkaloids were unstable because of their slight solubility in the substrate, and a technique was devised which permits reproducible F-A measurements. Despite these precautions, veracevine did not form any films. The F-A curves for veratridine and cevadine show (a) an inflection which corresponds to their areas when oriented horizontally at the a/w interface, and (b) an area in which the molecules are tight-packed corresponding to molecular areas for the vertical orientation of the molecules. Veratramine shows no inflection in the F-A curve, but the tight-packed film occurs at an area in which this molecule is oriented almost horizontal to the a/w interface. Interpretation of these results is in terms of the distribution of hydrophobic and hydrophilic groups on the molecules; their implication for pharmacological action is discussed.

INTRODUCTION

Molecular orientation at the air/water interface consists of the adsorption in the surface of one end or side of a molecule in preference to the other. For polar-nonpolar compounds, *e.g.*, organic paraffin derivatives containing polar groups composed of N, S, O or unsaturated carbon-carbon bonds, the polar groups will be positively adsorbed in the surface, while the nonpolar paraffin region is raised into the energetically more favorable vapor phase above the surface. The adsorption of the polar region of the molecule depends largely on the group's ability to form H-bonds with water; if the formation of these bonds is prevented or decreased, the molecular orientation may be altered[1].

Prediction of molecular orientation at the air/water interface is relatively simple for compounds like the fatty acids and alcohols, where the distribution of polar and nonpolar moieties is distinct[2]. For the structurally more complicated compounds which do not have clearly defined polar and non-polar regions, but are nevertheless surface active, the prediction of their orientation at the air/water interface is necessarily more difficult. This is generally true for the pharmacologically important compounds like the plant alkaloids where subtle changes in molecular structure have profound effects on the pharmacological activity[3], as well as their orientation at the air/water interface.

In this study, a representative group of compounds from the veratrum alkaloids[4] were examined with regard to the effect molecular structure has on the orientation these compounds assume at the air/water interface; the particular compounds were chosen because the broad variety of pharmacological activity which they exhibit[5] would permit us to examine whether a relationship exists between their pharmacological action and molecular orientation at the air/water interface.

EXPERIMENTAL

Surface tension

The drop-weight method[2] was used to measure surface tensions, and the empirical corrections of HARKINS AND BROWN[6] were applied. A stainless steel rod was cut in a lathe to 5 cm in length and 0.5 cm in diameter; a 1 mm diameter hole was bored coaxially through it. One end surface of the tube was ground plane in the lathe so that under 10 × magnification no imperfections were observed in either the rim or the surface. This tube was connected by a short piece of heavy walled rubber tubing to a syringe driven by a micrometer screw, and the entire apparatus was mounted vertically. A tared 2 ml pycnometer with a neck 0.9 cm inside diameter was used to collect the drops. Approx. 90 % of each drop was rapidly preformed, then allowed to reach full size under the force of gravity. Each drop was collected and weighed; a total of ten drops was used to obtain the average surface tension, the deviation from the mean always being less than 0.5 %. All measurements were made at 25° ± 0.5°. The value measured for water by this method was 71.95 dynes/cm compared to the reported value of 71.97 dynes/cm (see ref. 7).

Surface pressures

A Langmuir-type film balance (the Cenco hydrophil balance) was used to measure the surface pressures of the monolayer[8]. The tray was heavily coated with paraffin and the float assembly modified by using polyethylene end-loops (0.002 inch thick) and an aluminum float coated with a thin layer of teflon. Aluminum bars coated with paraffin were used as barriers.

Since the alkaloids studied do not form stable films on water even at pH's greater than 7, the following technique was devised, which permitted reproducible F-A (surface pressure–area) measurements. At each area a new monolayer was spread on water at pH 12.2 and the surface pressure exerted by the film 30 sec after deposition recorded. This time was sufficient for evaporation of the spreading solvent (benzene), but was too short for much film solution to occur. The 1- and 2-min values were also recorded at each area, and from these values the relative change of surface pressure with time was found to be independent of the initial pressure. Extrapolating these values to zero time raises by about 20 % the values of F_S (the surface pressure after 30 sec) reported here. It will be seen shortly that the significant data are the areas at which transitions in the F_S-A curves occur; these will not be significantly altered by the relatively constant and small decline in F in 30 sec in the neighborhood of the transition points. After F_S had been recorded the surface was swept clean by the barriers, and a new monolayer at a different area was prepared and measured. After a completed run the substrate was removed and the run repeated. Since contamination of the surface by the Gibbs' layer formed by alkaloid molecules dissolved in the substrate is a real problem, an adequate check on the cleanliness of the surface was to measure the surface pressure between the spreading of each new film. If a surface pressure greater than 0.1 dyne/cm was developed when the barriers decreased the length of the surface from 50 cm to 5 cm, the substrate was replaced. With these precautions, the results obtained were reproducible to within 0.1 dyne/cm. This technique, utilized exclusively in the present study, will be referred to as "static".

Veratridine, cevadine and veracevine were obtained from Professor KRAYER of the Department of Pharmacology, Harvard Medical School; the veratramine sample from Dr. O. WINTERSTEINER of the Squibb Institute for Medical Research. All the alkaloids were purified, white preparations and were employed without further purification. The drugs dissolved readily in benzene, except veracevine which was dissolved in ethanol.

Benzene (Mallinkrodt, thiophene free) was prepared free of surface active contaminants by filtering through a column of silica gel and florisil.

All aqueous solutions were prepared from water obtained from a quartz still. pH 12.2 water was prepared by adding the appropriate amount of NaOH, pH 7.2 water by adding an all sodium Sørensen phosphate buffer (1 mM).

Molecular areas

These were estimated by first constructing, with Catalin models of the atoms (scaled 1 cm = 1 Å), three dimensional models of the compounds, and then tracing the plane projection of the model in its various favored orientations onto paper. The total area of this plane projection was obtained with a planimeter, and represented the approximate surface area occupied by the drug in a particular orientation.

RESULTS

Structure

The structures of veratridine, cevadine[9], veracevine[10] and veratramine[11] are shown in Fig. 1. They are bases with pK values* of about 9–10 (see ref. 12). The relation between the first three compounds may be further visualized by the following esterifications:

veracevine + veratric acid = veratridine
veracevine + tiglic acid = cevadine

Surface tension

The surface activities of these compounds are shown in Fig. 2, where the surface

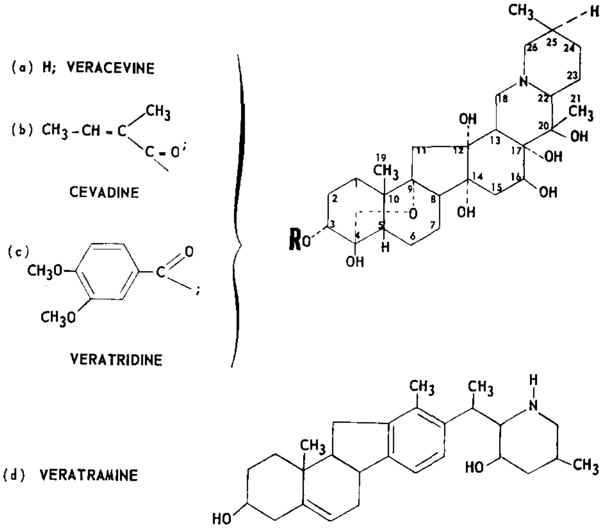

Fig. 1. Structural relationships among the veratrum alkaloids. If R is replaced by (a), (b), or (c) one obtains veracevine, cevadine, or veratridine, respectively.

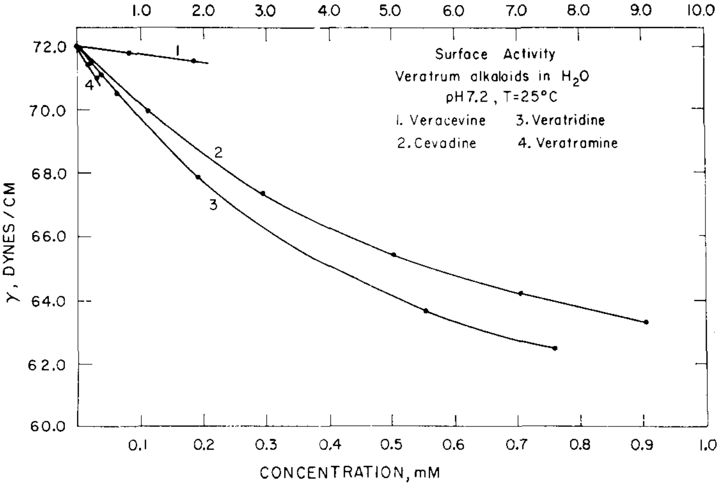

Fig. 2. The effect of lowering the surface tension, γ, of water by the veratrum alkaloids. Curve 1, concentration scale on top; curves 2, 3, and 4, concentrations listed on bottom.

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[*] The pK value for veratridine is presented here and since veracevine and cevadine also have the identical tertiary nitrogen groups we may assume their pK values are similar to veratridine. The pK value for veratramine is estimated from the value for piperidine.

tensions of solutions of the alkaloids in water at pH 7.2 are compared at various concentrations of the alkaloids. While veracevine is not very surface active, esterification of the -OH group in position 3, with either veratric acid or tiglic acid yields the highly surface active veratridine and cevadine, respectively. Veratramine is the most surface active of this group, but its limited solubility prevented measurement of surface tensions beyond the indicated concentrations. If we assume that the initial slopes of these curves are linear, then $-\Delta\gamma/\Delta C$ will be a measure of their surface activities; these values are listed in Table I, and indicate the following order for surface activity: veratramine $>$ veratridine $>$ cevadine $\gg$ veracevine.

TABLE I

PHYSICAL CHEMICAL PROPERTIES OF THE VERATRUM ALKALOIDS

Compound	$-\frac{(\Delta\gamma)^*}{(\Delta C)}$	*Areas estimated from molecular models*		*Observed areas at:*		*Probable orientations at the A/W interface H = horizontal; V = vertical*
		Horizontal	*Vertical*	*"Shoulder"*	*Film "collapse"*	
Veracevine	$0.0194 \cdot 10^4$	85 Å^2	45 Å^2	—	—	No stable film
Cevadine	$1.82 \cdot 10^4$	100 Å^2	45 Å^2	85 Å^2	42 Å^2	H, V
Veratridine	$2.12 \cdot 10^4$	130 Å^2	45 Å^2	110 Å^2	42 Å^2	H, V
Veratramine	$2.74 \cdot 10^4$	76 Å^2	35 Å^2	—	60 Å^2	H

* This value represents the mean of the slope of the first two points of each curve.

F-A curves

Previous attempts to measure *F-A* (surface pressure-molecular area) curves for these veratrum alkaloids were unsuccessful[13]. Much of the difficulty arises from the solubility of these compounds in water which, although slight, leads to film instability[14]. This is true even at elevated pH's which presumably reduce the solubility of these salts by conversion to the free base. Some of the difficulties are obviated with the static method (see EXPERIMENTAL), for *F-A* curves are obtained whose reproducibility under these circumstances compares favorably with standard techniques[3]. The results obtained by the static method are shown in Fig. 3, where F_S, the surface pressure 30 sec after spreading, is plotted against molecular areas, A, in Å^2 for alkaloid films spread from benzene on water at pH 12.2. Even though the values of surface pressure so obtained are not equilibrium values, they are still useful for relative comparison because the rate of fall of surface pressure is very nearly the same for veratridine, cevadine and veratramine.

Veracevine forms no films that were measurable by these techniques. This is in keeping with the low surface activity of this compound in water.

The curve for veratramine (Fig. 3) shows a gradual increase in surface pressure to an area of 60 Å^2; beyond this point F_S, remains constant at about 6 dynes/cm. The latter effect is analogous to film collapse for a stable film. We can better understand this phenomenon by considering the actual processes involved. Each point in the isotherm is obtained by limiting the area for spreading of a constant number of film molecules; when the water surface is completely covered, excess film molecules must be either forced above the adsorbed monolayer, or squeezed into the aqueous sub-phase. In either case no further increase in surface pressure would be expected. This area, 60 Å^2, represents the molecular area of veratramine in a state of close-packing in an orientation which is favorable for adsorption at the air/water interface.

The observed value is close to the molecular area of 76 $Å^2$ for veratramine in a horizontal orientation estimated from molecular models (see Table I). The -OH group in the 3-position and the piperidine nucleus are strong hydrophilic centers, each capable of forming H-bonds with water. Since these groups are at either end of the major axis of veratramine, their strong adsorption in water would necessitate a horizontal molecular orientation at the air/water interface.

The curves for cevadine and veratridine at the larger areas parallel the curve for veratramine in Fig. 3. But at about 6 dynes/cm where F_S remains constant for veratramine, "shoulders" appear in the cevadine and veratradine curves at 85 $Å^2$, and 110 $Å^2$, respectively; beyond the "shoulders" the curves rise to a limited area of 42 $Å^2$ and values of 9 and 10.5 dynes/cm, respectively, where F_S then remains constant.

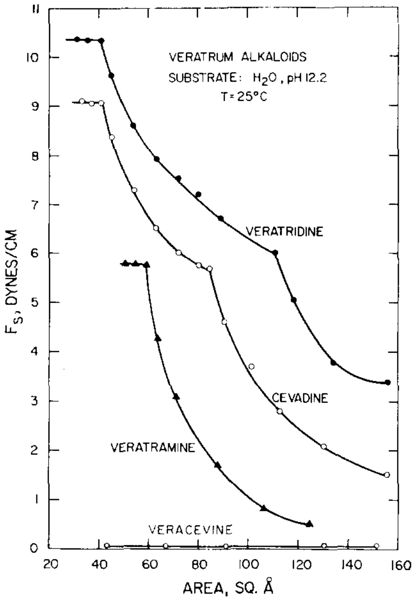

Fig. 3. F_S–A (static **surface** pressure-area) curves of the veratrum alkaloids on pH 12.2 water. For explanation of F_S see text.

From Table I, we see that the areas at which the "shoulders" occur approximate the areas of cevadine and veratridine estimated from models horizontally oriented in the interface, *i.e.*, 100 $Å^2$ and 130 $Å^2$ respectively; and the areas at which F_S remains constant (42 $Å^2$) are in good agreement with the estimated value of 45 $Å^2$ for the vertical orientation of these compounds with the ring N embedded in the aqueous sub-phase.

These results are consistent with a reorganization of the film molecules from a horizontal to a vertical orientation. At areas greater than the "shoulder" the molecules are horizontally oriented, much like in the veratramine film. But unlike veratramine at areas smaller than the "shoulder" a moiety of the veratridine and cevadine molecules can be raised into the vapor phase to permit an accommodation of more film molecules per unit surface area; as the areas become smaller increased numbers become vertically oriented. The vertical orientation also enhances the extent of Van der Waal's interactions between the film molecules, and this is reflected in the rise in F_S. When the vertically oriented molecules are close-packed, the area observed will be for that molecular cross-section which presents the most hydrophilic surface to the interface. That both molecules give the same cross-sectional area for their vertical orientation indicates the ring N as the most hydrophilic group in the molecule, since this group is the only one which is common to both and has the proper dimensions.

DISCUSSION

An important question arises regarding the role of –OH groups in the orientation of the molecules. Thus, if the –OH group in the 3 position in veratramine is so hydrophilic that it prevents the vertical orientation of this molecule, ought the several –OH groups distributed on veratridine and cevadine also prevent their transition from horizontal to vertical orientation? Since the –OH groups depend largely on their ability to form H-bonds with water for their hydrophilic properties, any decrease in this capacity would decrease the interaction of the molecule with water. This may arise from either a competitive intramolecular H-bonding* or by the steric blocking of the approach of water by a bulky group adjacent to the hydrophilic center. The –OH groups in positions 12, 14, 16, 17 and 20 are situated very favorably for intramolecular H-bonding and the veratric or tiglic ester group on position 3 may very well either shield or form H-bonds with the –OH group on position 4. The ester linkage itself and the lactone bridge between position 4 and 9 are much less hydrophilic than –OH[15]. The net effect is to decrease the hydrophilic properties of these groups to the extent that when the films become close-packed, additional molecules may be accommodated in the film only by a reorganization of the film where the less hydrophilic portions of the molecules may be raised into the vapor phase by pivoting around the N atom. When all the molecules are close-packed in the vertical orientation no further reorganization in the film is possible and excess molecules are forced into the sub-phase or on the surface of the film. The pressure remains constant in this case.

While many attempts have been made to relate the surface activity of a compound with its pharmacological action[16–19], they have for the most part dealt with the distribution of the chemical agent between the "biophase" and the bathing solutions. What has been largely overlooked is the possibility that molecular orientation of the chemical agent at the cell/plasma interface may also contribute to its ultimate action as a drug.

The results of the present experiments indicate that the veratrum alkaloids, though they are structurally similar, exhibit contrasting behavior at the air/water interface. Veracevine is only slightly surface active in water; veratridine, cevadine, and veratramine are very surface active, with the first two capable of orienting

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* A striking example of this effect is found by comparing the solubilities of the *o*- and *m*-hydroxybenzoic acids in water, the former being 0.18 and the latter 0.92 g/100 ml at 20°. (Handbook of Chemistry and Physics, 38th Edition, Chemical Rubber Publishing Co.) Intramolecular H-bonding between the –COOH and –OH groups reduces the solubility of the *ortho* compound while intermolecular H-bonds between –OH and –COOH and water enhances the solubility of the *meta compound*.

horizontally as well as vertically, while veratramine shows only a horizontal orientation at the air/water interface.

Pharmacologically, we find they also exhibit diverse behavior[5]. Veracevine is inactive*, veratridine and cevadine are "labilizers" or "unstabilizers"[20], and veratramine is an antagonist of veratridine and cevadine[21]. Little is known of the actual mechanism for action of these drugs, but their seemingly parallel physical and pharmacological properties suggest that the orientation of these compounds at the cell surface may at least be partially responsible for their pharmacological activity.

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* The low surface activity and pharmacological inactivity (see ref. 5) of veracevine would indicate for the veratrum alkaloids at least, that surface or interfacial activity is necessary to concentrate the drug at or near its site of action.