[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

The Veratrine Alkaloids. XVIII. Surface Film Studies

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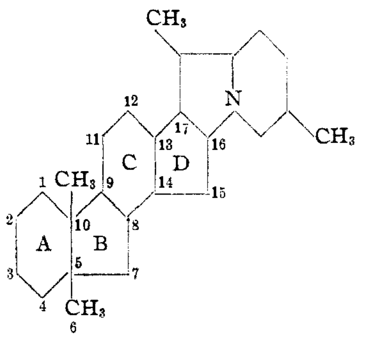
More than thirty degradation products have been isolated thus far from the veratrine alkaloids but these alone have not made it possible to write a full structural formula with certainty. Nevertheless, a tentative ring structure, shown in Formula I,[1] has been proposed as a working hypothesis based partly on a suspected relationship to the sterols. Recently[2] a relationship between the veratrine group and the solanine alkaloids has been demonstrated and since the latter are considered to be sterol derivatives[3] Formula I now appears to be more certain although the possibility still persists that ring B may be six-membered.



Formula I

Although such a formula as I explains more or less satisfactorily all the degradation products thus far encountered, several features were not directly indicated by experimental data or by direct analogy but were assumed on the basis of biogenetic possibilities and the limitations of other possible arrangements. Thus one of the assumptions made was the position of attachment of the condensed nitrogen heterocyclic ring system to the hydrogenated cyclopentenofluorene ring system. Although analogy to the sterols appeared to indicate position 17 as one of the two positions, since the nitrogen heterocyclic part must be presumed to be molded from the sterol side-chain, direct evidence for the other position was lacking. However, position 16 appears most likely, for in certain sapogenins[4] the oxygen of an oxide ring is attached there. Such an arrangement necessarily produces a formula of maximum length and minimal cross-sectional area for the six condensed rings known to be present in cevine.[5]

With the sterols the study of surface films has proved unusually helpful in deciding how the various rings were attached and supported the view that the four rings have a linear arrangement.[6] The present problem is of similar nature and it therefore appeared desirable to carry out a parallel study in the hope that supporting evidence either for or against the arrangement shown in Formula I would be forthcoming.

Although the eight hydroxyl groups present in cevine apparently make this base entirely too soluble for such a study, another veratrine alkaloid, rubijervine, contains only two hydroxyl groups and has been found to have suitable properties when spread on alkaline solution. Diacetylrubijervine, isorubijervine and solanidine were found to have similar properties. This paper accordingly reports the results of the study with these substances and the conclusions drawn therefrom in regard to the molecular dimensions of the alkaloids.

Experimental

Apparatus and Procedure.—Preliminary experiments showed that while the bases had a tendency to spread on pure water they appeared to pass into solution rather easily. These substances have a basic nitrogen and the solution tendency could either be due to inherent solubility in water or to the formation of a carbonate from the carbon dioxide of the air. In any case, subsequent experiments showed that the presence of alkali in the solution permitted a maximum and reproducible area to be obtained. Buffer solutions of glycine–sodium hydroxide, *p*H 12.9 (0.36% sodium hydroxide and 0.075% glycine) or solutions of 0.2 *N* sodium hydroxide were satisfactory. Benzene solutions of the bases of concentrations varying from 0.25 mg./cc. to 0.085 mg./cc. were prepared from freshly distilled benzene previously tested for possible spreading impurities. The required amounts were added from a pipet with a fine capillary tip. A fresh buffer solution was used for every film.

The apparatus for measurement consisted of a paraffined trough 50 × 14 cm. made of plexiglass[7] and chrome plated barriers moving perpendicular to the length of the

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(1) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **141**, 253 (1941).

(2) L. C. Craig and W. A. Jacobs, *Science*, **97**, 122 (1943).

(3) A. Soltys and K. Wallenfels, *Ber.*, **69**, 811 (1936).

(4) C. W. Shoppee, *Ann. Rev. Biochem.*, **11**, 103 (1942).

(5) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **139**, 293 (1941).

(6) O. Rosenheim and H. King, *Ann. Rev. Biochem.*, **3**, 87 (1934).

(7) A. Rothen, B. F. Chow, R. O. Greep and H. B. van Dyke, *Cold Spring Harbor Symposia on Quantitative Biology*, **9**, 272 (1941).

trough, for compression. Changes in pressure were measured by the Wilhelmy balance method, as described by Harkins and Anderson.[8] This method has proved very satisfactory, its only disadvantage being that the glass plate is sometimes likely to become contaminated with some impurity and thus render the contact angle of liquid and glass greater than zero. It has been our experience, however, that if buffer solutions are used instead of pure water the contact angle remains zero for long intervals of time and this holds true over the whole *p*H range. The sensitivity of the apparatus was of the order of ±0.1 dyne (0.1 dyne corresponding to 1 mm. of the scale). Numerous determinations were made of the pressure–area relationships both at room temperature (25°) and in a cold room (3°).

For the optical thickness measurements the technique of Blodgett and Langmuir[9] was employed. Preliminary experiments showed that the forces of adhesion between the barium stearate–stearic acid layer and the alkaloid were not strong enough to prevent the slippage of the film back into the trough. However, if the chrome-plated slides covered with stearic acid layers were first conditioned in 0.001 molar thorium nitrate[10] perfect transfers could be obtained (except in the case of isorubijervine) with only one layer being deposited. Films withdrawn in this way from the buffered solutions were wet and could not be directly dried since an amount of dry buffer salt would interfere and washing, unfortunately, removed the film. The following procedure with the use of ammoniacal solution instead of the buffer gave satisfactory results. Four plates were used for each experiment. These were covered with the optical gage of the stearic acid layers and conditioned for one minute in 0.001 molar thorium nitrate. After washing and before drying they were dipped into the well of the trough filled with the buffer, no film being on the surface. The film was then spread and compressed to at least 6 dynes before withdrawing the slides. The wet slides were dried and the optical thickness was measured.

For the calculation of the thickness the value 1.495 was assumed for the refractive index of the film. The thickness was derived from the formula[11]

$$t = 24.3\left[N\frac{\cos r}{\cos r'} - N'\right]$$

where t is the thickness in Å., N the number of stearic acid layers which give a minimum intensity in the reflected light for the angle of refraction r before the deposition of the film. N' is the corresponding number of stearic acid layers after the film has been deposited and r' is the corresponding angle of refraction. N was generally equal to N' in the present study because of the small thickness increment.

It was found preferable not to dry the plates after conditioning. Such conditioning gave an increased thickness to the films due to absorption of the thorium salt (and possibly other impurities), and was measured by submitting to the same treatment control plates which were removed from the tray before spreading the film of the alkaloid. These controls exhibited an increase in thickness equivalent to 7 Å. on the basis of an index of refraction of 1.495. Seven Å. were accordingly subtracted from all thickness increments obtained in measuring the thickness of the alkaloid films.

Transfer ratios were made on a brass cylinder in similar fashion to that used by Langmuir.[12] The cylinder was of 2-cm. diameter and terminated at one end by a cone 1.4 cm. high and was entirely platinized. The area covered on such a plummet was in all experiments 25.3 sq. cm. The plummet was subjected to the same treatment used for the slides in the above thickness measurements and then dipped into the tray. A somewhat smaller tray 50 × 9 cm. made of paraffined enameled brass was employed for these measurements. The film of the alkaloid was then spread and compressed to a given pressure which was maintained constant while the film was deposited by withdrawing the plummet slowly from the trough. The difference in the covered area before and after transfer was then recorded.

Two small corrections were applied to this measurement. One was to correct the change of the zero of the balance due to the higher level of the solution when the plummet was dipped into the trough and the other was to take into account the drop of pressure with time resulting from the collapsing of the rubijervine films under high pressure.

Discussion of Results and Conclusions.—Representative determinations of the many experiments made are given graphically in Figs. 1–4. The curves showing the pressure–area relationship have been plotted in the conventional way, pressure in dynes against area in Å.^{2} per molecule. The limiting area (area extrapolated to zero pressure) has a physical significance only if all the material is deposited on the surface and none goes into solution. Such a condition has apparently been realized in our experiments since the results obtained at different temperatures and with different buffers were all the same.

Curve 1 of Fig. 1 represents the compressibility of rubijervine at *p*H 12.9 and at 25°. The film proved to be incompressible and quite stable up to pressures of 18 dynes. At higher pressures it collapses and pressures greater than 26 dynes cannot be measured. In ammoniacal solution the film starts collapsing at somewhat lower pressures.

It readily can be seen from the curve that the film is of the incompressible or so-called solid type to which belong so many members of the sterol family. The limiting area is approximately 46 to 48 Å.^{2} and is definitely larger than the limiting area given for the sterols which form the solid type of film (38 to 42 Å.^{2}) and more like

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[8] W. D. Harkins and T. F. Anderson, THIS JOURNAL, **59**, 2189 (1937).

[9] K. B. Blodgett and I. Langmuir, *Phys. Rev.*, **51**, 964 (1937).

[10] I. Langmuir and V. J. Schaefer, THIS JOURNAL, **59**, 1762 (1937).

[11] A. Rothen and K. Landsteiner, *J. Exptl. Med.*, **76**, 437 (1942).

[12] I. Langmuir, V. J. Schaefer and H. Sobotka, THIS JOURNAL, **59**, 1751 (1937).

the solid films of the triterpenes, such as lupeol[13] (48 to 52 Å.^{2}).

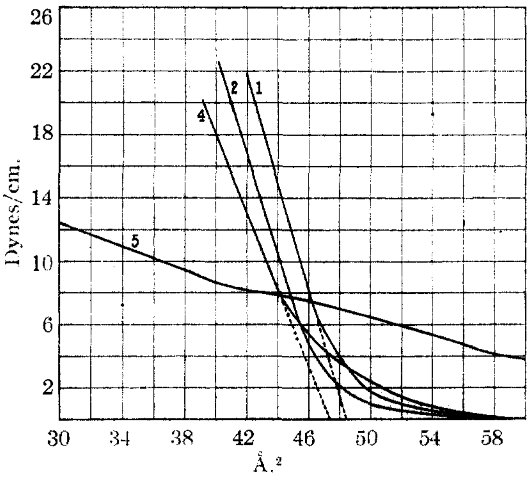

Fig. 1.

The compressibility of the film did not prove reversible. For example, curve 2 of Fig. 1 was obtained with the same film used for curve 1, after releasing the pressure to zero by moving back the barrier. Recompression then gave curve 2. Curve 4 (Fig. 1) was obtained for the fourth cycle about ten minutes after the initial spreading. The film was then left standing at zero pressure for one hour after which curve 5 was obtained. It is evident from the curve that the film has been so much altered as to change its nature entirely and has now become highly compressible.

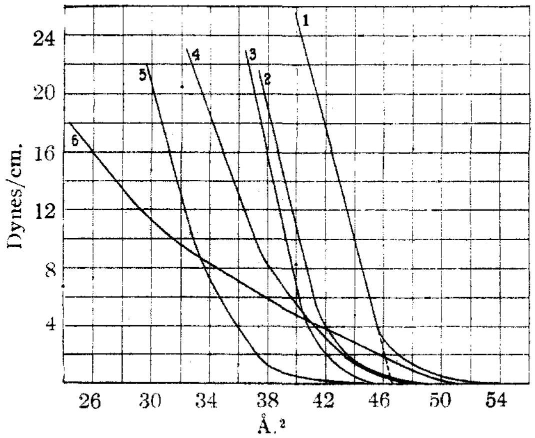

Fig. 2.

Further study of the conditions influencing this transformation was made as shown in Fig. 2. Curve 1 is the same as in Fig. 1 and was obtained immediately after spreading. Curves 2 and 4 were obtained fifteen and ninety minutes, respectively, after spreading. Curves 3 and 5 were obtained fifteen minutes after spreading during which time the film was held under 9.5 and 20 dynes pressure, respectively. Curve 6 was obtained by holding the film under 20 dynes pressure, then decompressing for twenty minutes and making two compression cycles, in all a time of approximately sixty minutes.

It appears from the relatively smaller limiting area that at room temperature approximately 10% of the material has disappeared from the film after fifteen minutes standing at zero pressure. Since the compressibility curve is of the same general type as curve 1 but only displaced the film is probably of unchanged material. After longer standing, however, it seems that the transformation products which are present in larger amount diffuse back into the film and make it no longer homogeneous but of a much more compressible type as seen in curve 5, Fig. 1, or curve 6, Fig. 2. Pressure apparently accelerates the transformation, as shown in curves 3 and 5 of Fig. 2.

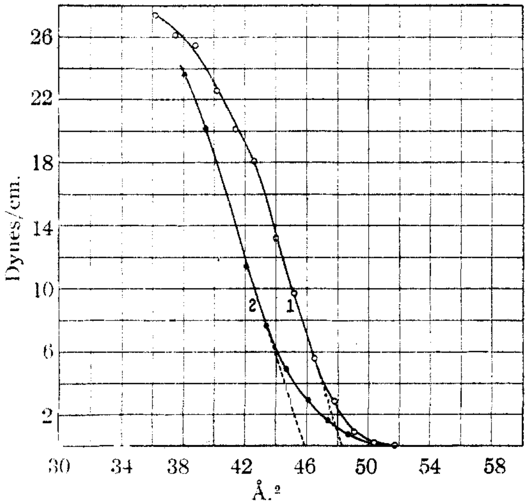

Fig. 3.

Temperature also influences the rate of transformation. Films spread at 3° were found to be much more stable and would withstand pressures of 20 dynes without rapid collapse. Curve 1, Fig. 3, was run on rubijervine at 3° immediately after spreading while curve 2 was obtained after standing at zero pressure for fifteen minutes. There appears to be no doubt but that rubijervine when spread as a film undergoes some kind of chemical change the nature of which cannot be predicted from these data. It seems most

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(13) F. A. Askew, *J. Chem. Soc.*, 1585 (1936).

likely that this change could be either a hydrolytic transformation or oxidation of some sort.

Figure 4, curve 1 represents the results obtained with diacetylrubijervine. The film proved to be of the compressible type, appeared very stable and showed a limiting area of about 160 Å.^{2} With this derivative the same type of curve was obtained at either 6 or 25° and the transformation noted above with the unacetylated alkaloid was not observed. Curve 3 is the unacetylated alkaloid given here for reference.

Curve 4, Fig. 4, is the type of film shown by isorubijervine. It is a much more compressible film than rubijervine and has a considerably larger limiting area. No tendency for alteration was noted with this isomer.

Curve 2, Fig. 4, is the type of film shown by solanidine. It proved to be a very stable film entirely similar in type to that of rubijervine except there was no tendency to undergo transformation under the conditions of the experiments. The limiting area was very close to that of rubijervine.

From the above data it can be said definitely that the molecules of rubijervine and of solanidine are oriented in the film with the long axis perpendicular to the water. In order to permit this, in the case of rubijervine at least one of the two hydroxyls could be situated on an end ring in position 3 since in solanidine, a sterol derivative, the single oxygen is considered to be in position 3 of the nucleus.[12] The second hydroxyl in all probability is not far removed from the first in order to explain the incompressible nature of the film although it has been remarked by Askew[13] that large molecules with more than one hydrophilic group "may so orient themselves on the water surface that they are attached by only one of the groups and behave as if the others were not present." It seems entirely possible that the strongly alkaline nature of the medium largely represses the hydrophilic property of the heterocyclic nitrogen or that its relative affinity for the water surface is much less than that of a hydroxyl group or the two hydroxyl groups together so that these bases behave as if the N had little attraction for the water. Otherwise it would seem likely that the diacetyl derivative would also give a solid type film instead of the compressible type found.

In the case of the diacetyl derivative the molecules undoubtedly lie with the long axis parallel with the surface since the limiting area is greater than twice that of rubijervine or solanidine and is approximately 150–160 Å.^{2}. Acetylated hydroxyls are known to be much less hydrophilic than free hydroxyls and it is possible that their hydrophilic properties are now more of the same degree as that of the nitrogen so that the molecule lies extended at zero pressure. Compression apparently tilts the molecules which, when the pressure reaches 20 dynes, have been again reoriented with the long axis perpendicular to the surface as in the case of the unacetylated alkaloid.

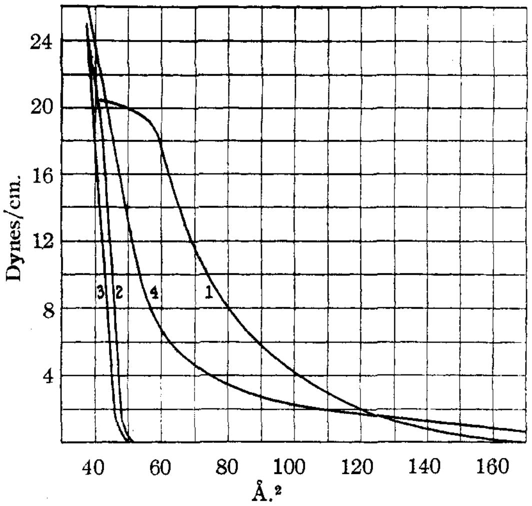

Fig. 4.

Optical Measurements.—Representative results of the thickness measurement for the rubijervine and diacetylrubijervine film from optical measurements are given in Table I. The accuracy in the determination of the thickness of the film (±2 Å.) is not so great as that obtained by Langmuir, Schaefer and Sobotka[12] in their measurements of sterol films since only one layer of the alkaloid could be deposited on the plate.

TABLE I

	Rubijervine			Diacetylrubijervine				
Pressure, dynes/cm.	8	10	20	6	8	12	18	20
Thickness, Å.	16	19	21	7	9	13	18	18

From the thickness measurement of the film it can therefore be deduced that the molecule of rubijervine has a length of approximately 19 Å. The thickness of the film of the diacetyl derivative, however, varies greatly with the pressure but approaches that of the unacetylated alkaloid when under high pressure. This is confirmatory evidence of reorientation during compression. Under low pressures somewhat under half this

TABLE II

TRANSFER RATIOS OF FILMS OF RUBIJERVINE AND ITS DIACETYL DERIVATIVE

	Rubijervine				Diacetylrubijervine					
Pressure, dynes/cm.	6	8	10	15	6	8	9	10	11	15
Area taken, sq. cm.	25.2	25.6	24.9	25.6	19.0	20.7	22.5	23.5	24.3	25.6
Area covered, sq. cm.	25.3	25.3	25.3	25.3	25.3	25.3	25.3	25.3	25.3	25.3
Ratio	0.99_5	1.01	0.98_5	1.01	0.75	0.82	0.89	0.93	0.96	1.01

value was obtained which might be expected if the molecules lie extended on the surface rather than perpendicular.

These results are significant only if the film area taken from the trough is equal to the area covered on the plate and expansion or contraction of the film during the transfer is excluded. That such is the case is shown by the data in Table II. They indicate that the transfer ratio is 1 within experimental error for rubijervine. In the case of diacetylrubijervine it seems that the molecules which are slightly tilted under a pressure of 6 dynes/cm. on the water surface reorient themselves with the long axis parallel to the surface of the slide on which they are transferred.

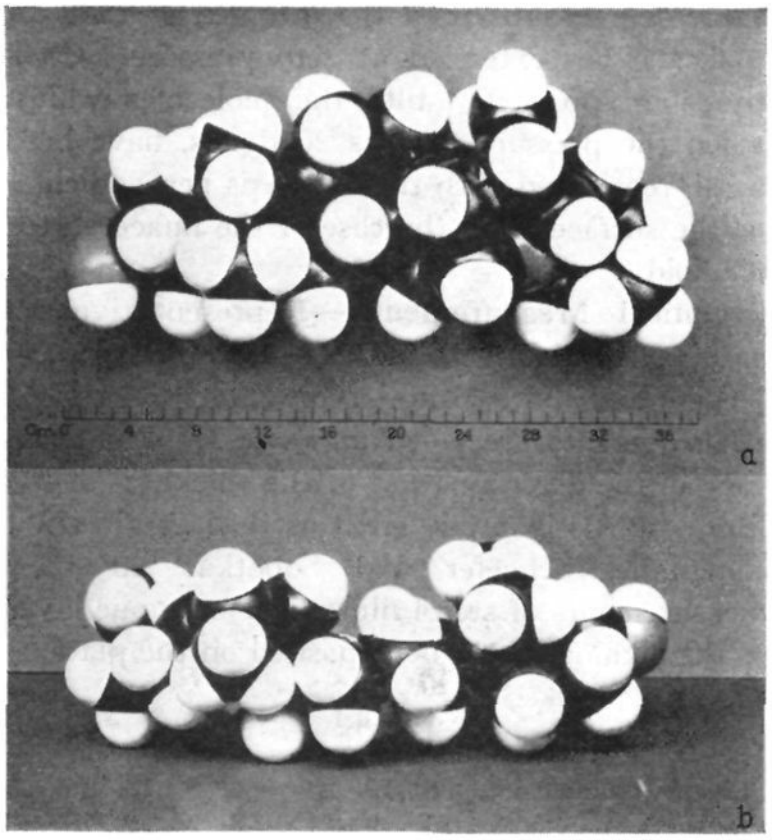

Fig. 5.

However, when under high compression the molecules are oriented perpendicular to the water surface, and lateral cohesion apparently prevents reorientation after transfer.

When the structure given in Formula 1 was constructed from Stuart models[14] no difficulty was encountered as far as steric hindrance was concerned although no attempt was made to construct all the theoretically possible optical isomers. Photographs of one of the possible isomers which appeared to be that of the most extended form are shown in Fig. 5, a and b. Fig. 5a shows the broader side and corresponds to a theoretical rectangle approximately 16 × 38 cm. in size, which represents the length and breadth of the molecule (1 Å. = 2 cm.). Fig. 5b represents the length and thickness and corresponds to a theoretical rectangle 12 × 38 cm. in size. The models, therefore, indicate a somewhat irregular volume approximately 38 cm. in length, 16 cm. in width, and 12 cm. in depth. From these dimensions the molecule would be 19 × 8 × 6 Å. which is in excellent agreement with the measurements obtained from the film study. The latter indicate, as we have seen, the molecules of rubijervine to have a length of approximately 19 Å. and a cross-sectional area of 46–48 Å.^{2}. Should the molecule lie extended on the surface, with the largest possible area as in diacetylrubijervine (found 150 Å.^{2}, as seen above), an area of $8 \times 19 \simeq 150$ Å.^{2} would be expected.

The measurements recorded in this paper therefore appear to exclude any other arrangement than the linear arrangement in which the maximum length is obtained for the six condensed rings present in rubijervine.

Summary

A study of the surface films of certain veratrine alkaloids has been made. Molecular dimensions calculated from the film measurements indicate the structure to be such that the six hydroaromatic rings indicated to be present in the alkaloids are so arranged that a maximum length (18 Å.) and minimal cross-sectional area (47 Å.^{2}) are obtained. The dimensions found are in good agreement with the theoretical values for the proposed linear structural formula.

Solanidine, the potato alkaloid, which is now known to be closely related to the veratrine group was included in the study. Its molecular dimensions proved to be approximately the same.

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[14] H. A. Stuart, *Z. physik. Chem.*, **B27**, 350 (1934).