by the countercurrent distribution method. The distribution curve of diacetone sorbose showed the presence of some impurities. The isolation of β-methylnaphthalene from a crude material was attempted, but no satisfactory result was obtained.

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71. **Tokunosuke Kanzawa**: Application of the Countercurrent Distribution Method. II[1]. The Colorimetric Determination and Partition Coefficient of Agroclavine.

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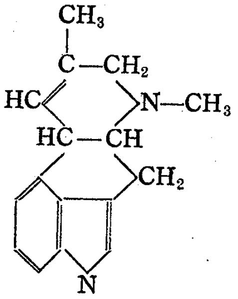
Agroclavine (I), which is a kind of ergot alkaloid, was discovered by Abe[2] in natural ergot and in saprophytic culture. In connection with the vomiting action of agroclavine, it was desirable to test its homogeneity. Organic bases such as (I) give different partition coefficients depending on the kind of combination of organic solvents and pH of aqueous solution. Accordingly such a test seems to be a simple case of the application of the countercurrent distribution method.



(I)

In the distribution studies a suitable analytical method is needed for the interpretation of the results. In this case a colorimetric determination was adopted. The ergot alkaloids such as ergometrine have been colorimetrically determined in the following manner[3]. The bases are dissolved in 1% aqueous solution of tartaric acid and to 1 cc. of this solution is added 2 cc. of *p*-dimethylaminobenzaldehyde reagent. After five minutes the deep indigo blue color developed is measured by the Lovibond tintometer. The author modified this method to be carried out with the Pulfrich photometer and determined the optimum conditions for the analytical procedures. Then the partition coefficients in systems composed of organic solvents and phosphate buffer and stability of agroclavine in acid solution were determined colorimetrically.

Method of Determination. One cc. of an acid solution containing less than 60 γ/cc. of agroclavine is taken into a glass-stoppered tube of 20-cc. capacity and 2 cc. of *p*-dimethylaminobenzaldehyde reagent is added. The mixture is heated at 60° for 30 minutes in a water bath, cooled, and diluted with 5 cc. of 10% sulfuric acid. Within 10 minutes after the dilution the indigo blue color is measured with the Pulfrich photometer using filter No. 5 in a cell 0.5 cm. thick. From values thus obtained the quantity of agroclavine is determined by a calibration curve or a conversion equation.

Partition Coefficient The partition coefficient of organic electrolytes such as agroclavine is a so-called "apparent" one and generally is not due to the partition of monomer because of the presence of ionization and association phenomena. The partition coefficients of agroclavine in several systems of organic solvents and 0.1 *M* phosphate buffer were determined as shown in Fig. 1.

The systems including chloroform gave such a large value at pH 6.6 as to show the transference of almost entire amount of agroclavine to the chloroform layer. This may be related to the fact that the solubility of agroclavine in chloroform is large compared

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[1] Part I: This Bulletin, **2**, 308(1954).

[2] M. Abe: J. Agr. Chem. Soc. Japan, **22**, 61, 85(1948); Ann. Repts. Takeda Research Lab., **10**, 145, 162(1951).

[3] N. L. Allport: "Colorimetric Analysis", 303(1947).

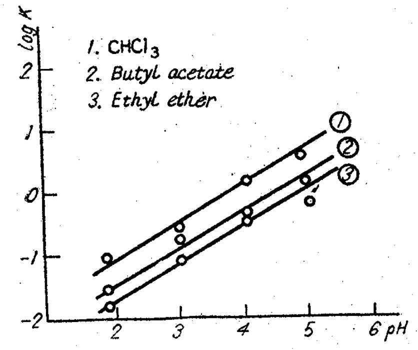

Fig. 1.
Partition Coefficients of Agroclavine

to that in other organic solvents.

In Fig. 1 a linear relationship between $\log k$ and pH is shown, especially in a region of lower pH values. Golumbic, *et al.*[4] derived the following relation

$$\log k' = \mathrm{pH} + \log k - \mathrm{pK},$$

where k' and k are partition coefficients of ionized and nonionized base, respectively. In the derivation of this relation it is postulated that pH $\leqslant$ pK and no association occurs. Since pK of agroclavine is 5.2[5], it may be said that the above relation holds in the region of pH 2~3 and therefore no association occurs. However, at pH 5 the values of $\log k$ deviates somewhat from linearity.

Though Craig[6] discussed the effect of concentration of the buffer upon partition coefficient, no marked change of the partition coefficient of agroclavine was observed in 0.5, 0.25, and 0.125 *M* phosphate buffers.

Stability of Agroclavine in Aqueous Solution Since it has not been determined whether agroclavine is stable or not in aqueous solution, and it takes several hours to determine the content in each tube after completion of a countercurrent distribution run, the rate of decomposition of agroclavine in aqueous solution was measured. The results are shown in Table V. In a solution of higher acid concentration agroclavine already decomposes immediately after preparation of the solution. In tartaric acid and 0.1*N* phosphoric acid solution, however, the decomposition is negligible within five hours. After one day, the concentration decreases to 5~10% of the initial one.

Experimental

Colorimetric Determination

Materials—Agroclavine. White crystalline substance prepared in the Takeda factory was recrystallized twice from AcOEt, m.p. 206~208°(decomp.). *Anal.* Calcd. for $C_{16}H_{18}N_2$: C, 80.67;H, 7.56. Found: C, 80.86; H, 7.61.

Reagent for Color Reaction—To H_2SO_4 (65 w/v%) containing 0.005 w/v% of $FeCl_3$, 0.125 w/v% of *p*-dimethylaminobenzaldehyde was added. H_2SO_4 (analytical grade) was diluted with distilled watar to a suitable concentration. $FeCl_3$ and *p*-dimethylaminobenzaldehyde were of reagent grade.

Absorption Maximum of the Color—The color of agroclavine produced by the addition of *p*-dimethylaminobenzaldehyde reagent was indigo blue and had maximum absorption in the range of No. 5 (S 57) and No. 6 (S 61) filter of Pulfrich Photometer. Hence, No. 5 filter was used in the following experiments.

Effect of Dilution—The blue color was so intense that the colored solution had to be diluted. Since the color reaction is a sort of condensation reaction, dilution by distilled water might have some effect upon the colored products. Hence, the dilution by addition of 5 cc. of distilled water and some mineral acids to the reaction mixture, after heating at 55° for 1 hour, was tested. The results are shown in Table I. The table shows that 10% H_2SO_4 is suitable as a diluent. This choice was supported in another experiment by the result that the colored solution in various concentrations

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4) C. Golumbic, M. Orchin, S. Weller : J. Am. Chem. Soc., **71**, 2624 (1949).
5) Y. Asahi : Unpublished data.
6) C. Craig, C. Golumbic, H. Mighton, F. Titus : J. Biol. Chem., **161**, 321 (1945).

Table I. Effect of Dilution

Diluent	Optical density	Diluent	Optical density
distd. water	0.531	$1\,N$ H_3PO_4	0.507
$1\,N$ HCl	0.529	10% H_2SO_4	0.543

in a range from 100 γ to 1 mg. per cc. of agroclavine showed a straight optical density–concentration line when diluted with 10% H_2SO_4, but a bending point in a line with H_2O.

Effect of Heating—The intensity of the color was markedly increased by heating the reaction mixture and gave higher values of optical density. The results are shown in Fig. 2. The upper curve was obtained by heating at 50° for 30 mins. and the lower one, without heating.

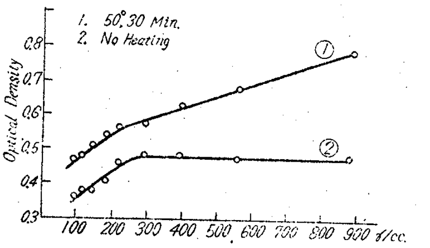

Fig. 2.
Effect of Heating on Color Intensity

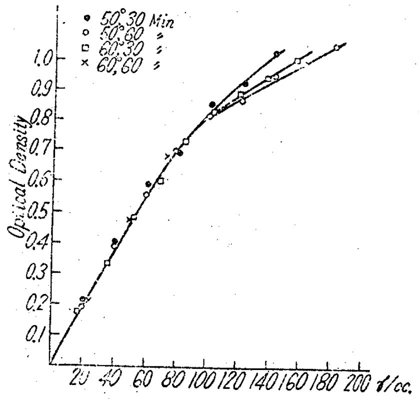

Fig. 3.
Effect of Different Heating Conditions

To determine the optimum heating condition the reaction mixtures were heated at 50° and 60° for 30 and 60 mins. The optical density–concentration curve became a straight line to a concentration of about 60 γ/cc., and over 100 γ/cc. its slope became less accute and different depending on heating condition. The results are shown in Fig. 3. From these results the condition of heating at 60° for 30 mins. was adopted.

Calibration Curve—The optical density of the color produced by agroclavine at various concentrations below 60 γ/cc. was measured repeatedly according to the method described above. From these values a regression equation was obtained as follows:

$$y = 0.00464x + 0.012,$$

where y is the optical density and x is the concentration in γ/cc. A ratio of standard error of estimate[7] to a mean of y, corresponding to coefficient of variation, was 4.1%.

Stability of the Color—The change of optical density after dilution was measured at different intervals to observe the stability of the color. The results are shown in Table II. The data show that the color is stable within 30 mins. and therefore should be measured within that time.

Table II. Change of the Color Intensity

Time(min.)	Optical density	Time(min.)	Optical density
5	0.29	30	0.29
10	0.29	45	0.30
20	0.29	60	0.31

The Presence of Impurities—The aqueous solution of agroclavine turned brown by partial decomposition during standing. In order to test the effect of such decomposition products, a fresh solution (I) of agroclavine was determined before and after the addition of a solution containing partially decomposed agroclavine (II). The experiments were conducted at two different concentrations of (II), 116 and 50 γ/cc. If there is no disturbance by the decomposition product, the amount in the mixture (III) of equal volumes of (I) and (II) must be equal to one-half the sum of the values of (I) and (II), that is, 85 and 52 γ/cc. Since (III) gave the values of 86 and 58 γ/cc., as shown in Table III, it may be said that the disturbance is negligibly small.

Table III.

Solution	Concn.	(γ/cc.)	Solution	Concn.	(γ/cc.)	Solution	Concn.	(γ/cc.)
I	54	54	II	116	50	III(I+II)	86	58

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7) Snedecor: "Statistical Methods," 4th Ed., 117(1950).

Determination of Partition Coefficient

Solvent and Buffer—Ether, CHCl$_3$, and AcOBu were purified in the usual manner for spectrographic purpose. Buffers were 0.1 M phoshate buffer (KH$_2$PO$_4$+Na$_2$HPO$_4$). In the lower value of pH, a solution composed of 0.1 M H$_3$PO$_4$ and 0.1 M KH$_2$PO$_4$ was used, pH of which may change in the presence of a large amount of bases because of its poorer buffer action. pH of buffers was measured with the Beckmann Model G pH-meter.

Equilibration—As a preliminary experiment disturbance of color reaction by organic solvents was examined. Four cc. each of distilled water saturated with ether, CHCl$_3$, and AcOBu, respectively, was taken into a test tube, into which was added 1 cc. of 1% tartaric acid solution of agroclavine. One cc. of each solution gave exactly the same value as the one diluted with distilled water. Hence, there was no disturbance.

The weighed samples were dissolved in the buffers at respective pH to make solutions of a concentration of about 400 γ/cc. To 5 cc. of each buffer solution, equal volume of the organic solvent was added in separatory funnel of 50-cc. capacity, which was shaken at a room temperature. After complete separation into two phases, the aqueous layer was poured into a glass-stoppered test tube. Diluting the aqueous layer with the same buffer, the amount of agroclavine in 1 cc. of the solution was colorimetrically determined. The difference of the initial and the final concentration of agroclavine in aqueous layers is the amount transfered into the organic layers. The partition coefficients were obtained by dividing the concentration in the organic layers by that in the aqueous layers. The results are showh in Table IV.

TABLE IV. Partition Coefficient of Agroclavine

pH	Ether	CHCl$_3$	AcOBu
1.9	0.0156	0.0937	0.0264
3.05	0.0754	0.264	0.178
4.05	0.353	1.422	0.435
5.0	0.493	3.22	1.10

Stability of Aqueous Solution—Agroclavine was dissolved in 1% tartaric acid solution, 2, 1, 0.5, and 0.1 N phosphoric acid solution, respectively, to make a concentration of 50 γ/cc., and 1 cc. of each solution was colored at several time intervals. The results are shown in Table V.

TABLE V. Stability of Agroclavine (γ/cc.)

Time (hr.)	1% Tartaric acid	Phosphoric acid			
		2 N	1 N	0.5 N	0.1 N
0	50	40.5	44.5	44.5	48.5
1	〃	〃	〃	42.5	〃
2	〃	〃	〃	〃	〃
3	〃	〃	42.5	〃	〃
5	〃	〃	〃	〃	〃
24	46.5	38.5	40.5	〃	46.5

Summary

The method of the colorimetric determination of agroclavine was modified to be carried out with the Pulfrich photometer. The partition coefficients of agroclavine in some systems and its stability in aqueous solution were determined.

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