70. Tokunosuke Kanzawa: Application of the Countercurrent Distribution Method. I. Purity of Diacetone Sorbose and β-Methylnaphthalene.

(*Research Laboratory, Takeda Pharmaceutical Industries, Ltd.**)

The countercurrent distribution method devised by Craig[1] has been applied to many problems including purity test and isolation of substances. The author also used this method for the separation of penicillin[2] and of vitamin D[3] and obtained some results. In the present paper the isolation of the nonelectrolytes such as diacetone sorbose and β-methylnaphthalene is reported. In the isolation of a substance by means of countercurrent distribution method it is desired that the partition ratio of the substances to be separated is made larger by selecting a suitable combination of the solvents. The selection of the solvent is fairly difficult in the case of nonelectrolytes where the kinds of the solvent are limited, while the electrolytes such as organic acids or bases are treated conveniently by changing the value of the partition coefficient, which is dependent on the nature of organic solvents and pH of the aqueous phase.

Experimental

Materials—Diacetone sorbose prepared in this factory was recrystallized from benzene several times. m.p. 78°. Purity, 99.5%. This is used in the determination of partition coefficient. The sample used in the distribution experiment colored slightly brown, which melted at 73°. Purity, 95%. $[\alpha]_D$: −6.6°. The values reported by Reichstein, *et al.*[4] were as follows: m.p. 77~78°. $[\alpha]_D$: −4.9° (H_2O, c=5.0). Monoacetone sorbose was prepared in the same manner and melted at 92°.

Methylnaphthalene was fractionally distilled from commercial products and thought as a mixture of α- and β-methylnaphthalene, and its composition could not be determined.

The organic solvents such as benzene, methanol, and petroleum benzine (b.p. 40~80°) were purified in the usual manner.

Determination of the Partition Coefficients—About 400 mg. of diacetone sorbose was placed in a separatory funnel of 50-cc. capacity, containing 5 cc. each of light and heavy solvents and shaken for 2 minutes at room temperature. After complete separation into two phases each layer was poured into flasks of 50-cc. capacity and concentrated on a water bath under a reduced pressure. The residue in the flasks was washed into glass-stoppered weighing tubes with acetone, which was evaporated in the dessicator under a reduced pressure. The residues were weighed after drying over P_2O_5. The sum of the contents in both layers did not exactly agree with the original weight of samples distributed, but its deviation was within 5%.

Apparatus—A distributing machine was made of stainless steel in the same type as Craig's first model. The instrument was provided with 20 tubes, each of which contains 7 cc. each of both heavy and light solvents and a spare room of 4.5 cc. To mix the two solvent layers the instrument was laid down in a box mounted on a shaking machine and shaken laterally with reciprocating frequency of 100 times per minute.

Procedure—The organic solvent (benzene or petroleum benzine) and distilled water were shaken and mutually saturated at a room temperature before the experiment. The aqueous layer was introduced into the lower drum of the instrument to fill each tube to its brim. Then 7 cc. of the organic solvent was introduced into each tube of the upper drum. The solution in organic solvent of the material to be distributed was poured into tube 0. Stepwise countercurrent distribution was conducted as described by Craig.[1] Time of shaking and standing were 1 and 4 minutes, respectively. Upon completion of a run the content in each tube of the instrument was pipetted out into flasks of 50-cc. capacity. Removal of the solvent and weighing of the content in each tube were carried out in the same way as described above. The quantity of the sample distributed was 1.2 to 1.4 g. and their recoveries after the run were 92 to 94%.

* Juso-nishino-cho, Higashiyodogawa-ku, Osaka (神沢得之助).

1) L. C. Craig: J. Biol. Chem., **155**, 519(1944).
2) T. Kanzawa, H. Kawai, T. Ito: Ann. Repts. Takeda Research Lab., **9**, 57(1950).
3) T. Kanzawa, H. Mima: *Ibid.*, **12**, 95(1953).
4) T. Reichstein, A. Gruessner: Helv. Chim. Acta, **17**, 314(1934).

β-Methylnaphthalene was weighed in the same way, but gave no constant recovery. To the content in some tubes was added 5% solution of picric acid in methanol, and the precipitates were filtered, washed with minimum quantity of distilled water, dried, and subjected to melting point measurement.

Results and Discussion

Diacetone Sorbose Diacetone sorbose is generally prepared by the condensation of sorbose with acetone. Such a product might be contaminated with sorbose, monoacetone sorbose, and condensation products of acetone. The determination of the partition coefficient will give a useful information about isolation of diacetone sorbose from the reaction mixture and the homogeneity test of the purified materials. The solubilities of mono- and diacetone sorbose were described by Reichstein, *et al.* as follows. The former is soluble in water, ethanol, and acetone, and slightly in ether and benzene, and the latter soluble in organic solvents such as benzene and fairly soluble in water.

The partition coefficients were determined in the systems shown in Table I. Since the system including butanol makes the separation of the two layers difficult, and for

Table I. Partition Coefficients of Mono- and Diacetone Sorbose at 27°

System		K (org./aq.)	Concentrations in light liquid layer (mg./cc.)
M. A. S.	Benzene–water	0.088	18.1*
D. A. S.	Benzene–water	1.51	50.8
	Benzene (45)–water (20)–methanol (50)	0.52	24.3
	Butanol–water	7.06	66.1
	Butanol (62)–water (70)–methanol (15)	1.97	24.1

M. A. S. : Monoacetone sorbose
D. A. S. : Diacetone sorbose
* Concentration in aqueous layer

the purpose of testing homogeneity of a substance it is favorable to use a system giving a partition coefficient having a value close to unity, the system composed of benzene and water was chosen in the distribution studies.

The distribution curve of slightly impure sample is shown by the solid line in Fig. 1.

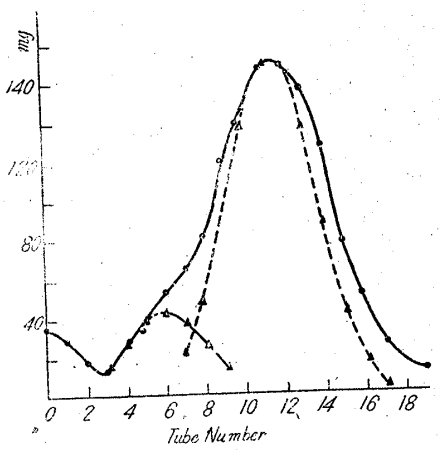

Fig. 1.
19-Plate Distribution of Diacetone Sorbose
System : benzene and water
——— Experimental curve
– – – – Theoretical curve
—•—•— Subtracted curve

The position of the maximum of the experimental curve is between tubes 11 and 12 as expected from the measured partition coefficient. The partition coefficient calculated by the method of Williamson and Craig[5] is 1.526 and shows good agreement with the measured one. However, the theoretical curve obtained from this value is not exactly consistent with the experimental one. The disagreement between the two curves may be attributed to the incomplete separation of the two layers, the error of weighing resulting from inadequate drying of the contents in each tube, or some impurities. Colored

5) B. Williamson, L. C. Craig : J. Biol. Chem., **168**, 687 (1947).

substances were concentrated at the two extreme sides of the curve (i.e. in tubes 0 and 1, and tubes 18 and 19), and none was observed in the middle part of the curve. Accordingly the colored substances in the original sample are of two kinds, of which the one is soluble in water and the other in benzene. The former may be a compound converted from sorbose and the latter condensation product of acetone, such as mesitylene or phorone.

When monoacetone sorbose is contained in the original sample it is expected from the calculation using the partition coefficient of 0.088 that 98% of it is distributed in tubes 0～4. A small rise in the curve from tubes 3 to 0 may be partly due to monoacetone sorbose. From these results it can be said that the separation of diacetone sorbose from monoacetone sorbose is almost complete. The residues in the tubes near the maximum of the curve (tubes 12 to 14), after evaporation of the solvents, are white cyrstalline substances and seem to be pure diacetone sorbose. However, the shape of the left side slope of the curve (tubes 4 to 8) suggests the presence of a substance other than diacetone sorbose, and subtraction of the theoretical curve (broken line) from the experimental one leaves the curve (chained line) having a small maximum at tube 6. The theoretical curve for this small maximum was calculated with the value of 0.482 as a partition coefficient and showed fairly good agreement with the subtracted one.

If this is an impurity, it must be a substance having a similar molecular structure to that of diacetone sorbose and different from 2,3-monoacetone sorbose. Although Ohle[6] reported the formation of another kind of monoacetone sorbose, the presence of such a compound appears to be improbable considering the purity of the original sample.

Anothar explanation may be given by the presence of a decomposition product of diacetone sorbose or the incomplete equilibration. Diacetone sorbose is decomposed into acetone and monoacetone sorbose in aqueous and acetone solution by the catalytic action of acid as Asahi[7] described. In the present experiment the decomposition may occur by the presence of minute amount of acids or salts remaining in the original sample.

A further experiment was conducted in order to confirm the above result. The benzene solution of the original sample was extracted three times with water, and after evaporation of the benzene, the residue (m.p. 78.5°, $[\alpha]_D$: −6.0) was subjected to a 19-plate distribution. Time of shaking and standing was two and eight minutes, respectively. The result is shown in Fig. 2.

Nothing was recovered from tubes 0 to 2 owing to preliminary washing with water. From the contents in tubes 12 to 16 crystalline substances were obtained and the content in tube 19 increased in amount. The right side of the maximum in the distribu-

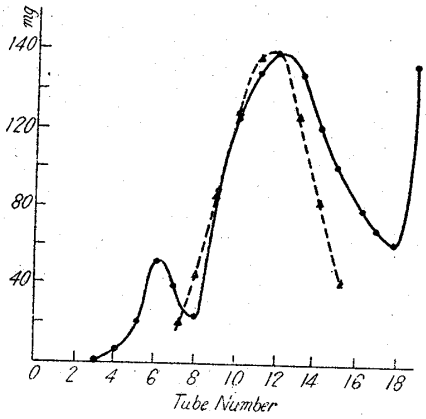

Fig. 2.
19-Plate Distribution of Diacetone Sorbose after Removal of Hydrophilic Impurities
——— Experimental curve
– – – – Theoretical curve

6) Ohle : Ber., **71**, 562 (1938).
7) Y. Asahi : Ann. Repts. Takeda Research Lab., **11**, 16 (1951).

tion curve shows distinct deviation from the theoretical one and suggests the presence of impurities. This is confirmed by the fact that $[\alpha]_D$ of the contents in tubes 11, 15, and 19 are −4.8, −8.4, and −11.3, respectively. In this experiment there appears a small maximum at tube 6, but its shape seems to be not in good agreement with the theoretical one.

It is concluded from the above results that diacetone sorbose is readily separated from monoacetone sorbose and condensation products of acetone, but shows a distribution curve which suggests the presence of some impurities. These impurities may be formed by the decomposition of diacetone sorbose, and may not be apparent ones due to incomplete equilibration.

β-Methylnaphthalene β-Methylnaphthalene is a main starting material in the synthesis of vitamin K_3 and difficult to isolate from α-methylnaphthalene by means of fractional distillation because of the only slight difference in boiling points. The isolation of such a substance by solvent extraction is one of the difficult problems because the selection of the combination of the solvent is limited and the partition ratio is small owing to the similarity in the molecular structure.

As pure β-methylnaphthalene was not in hand, a mixture of α- and β-methylnaphthalene was subjected to a 19-plate distribution in a system composed of petroleum benzine and 97% aqueous methanol. The brown colored substances in the sample remained in tubes 0 to 7 after the run. The amount of the residue in each tube indicates the presence of two maxima of the distribution curve at tubes 3 or 4 and tubes 13 or 14, respectively. The residues in tubes 14 to 16 solidified in the ice box at 0°, but the ones in the other tubes remained oily. The literature value of m.p. of α- and β-methylnaphthalene is −22° and 37°.

Melting points of the picrate of the contents in some of the tubes are shown in Table II. The picrate obtained from tubes 14 to 16 showed melting points close to that of the picrate of β-methylnaphthalene.

Although it may be said that the mixtures are separated into two parts, no definite conclusion on the purity of β-compound in tubes of larger tube numbers could be obtained, because the melting point of the mixture of picrates of α- and β-compound has not been determined and accordingly the values shown in Table II may not be that of pure β-compound, but that of the mixtures. The literature values of picrates of α- and β-compound are m.p. 116~117° and 141°, respectively. However, the fact that the contents in tubes 13 to 15 readily solidify and their picrates show a high melting points may suggest the existence of fairly pure β-methylnaphthalene. It is also probable that separation of α-methylnaphthalene from β-compound is not complete, because if all α-compound is contained in tube 3 or 4, the partition ratio of α- and β-compound becomes about 10, which is too large a value as that between isomers.

TABLE II.

Tube No.	Picrate, m.p., °C	Tube No.	Picrate, m.p., °C
9	102~107	14	113~115
10	112~113	15	114~115
11	112~114	16	114~115
12	113~114	17	111~112
13	113~115		

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Summary

Diacetone sorbose was separated from monoacetone sorbose and colored substances

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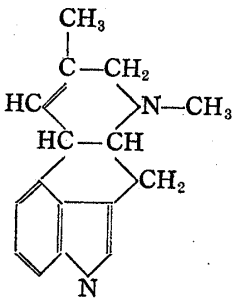
71. **Tokunosuke Kanzawa**: Application of the Countercurrent Distribution Method. II[1]. The Colorimetric Determination and Partition Coefficient of Agroclavine.

(*Research Laboratory, Takeda Pharmaceutical Industries, Ltd.**)

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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* Juso-nishino-cho, Higashiyodogawa-ku, Osaka (神沢得之助).

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).