Bisbenzyltetrahydro*iso*quinoline Alkaloids

I. A General Method for Determining Partition Coefficients and Testing Homogeneity

ESKIL HULTIN*

Department of Pharmacology, University of California Medical Center, Los Angeles 24, California, U.S.A.

Partition coefficients can be computed from solute concentrations measured in a series of extracts obtained in such a way that one portion of a phase of two immiscible solvents is extracted with several portions of the other phase. The logarithms of the concentrations are plotted *versus* the numbers of the corresponding extractions. If the solute is homogeneous, and if the partition coefficient is independent of the concentration, the points lie about a straight line, the inclination of which equals the logarithm of the fraction q of the solute remaining in the extracted phase after an extraction. If the partition coefficient is defined as the ratio of the concentration in the less polar phase to that in the more polar phase, and if the volumes of the phases are V_l and V_m, respectively, then the partition coefficient equals (V_m/V_l) $(1-q)/q$ when the more polar phase is extracted with the less polar phase, and $(V_m/V_l)q/(1-q)$ when the less polar phase is extracted with the more polar phase. If the solute is a mixture of two components, the partition coefficient of one component can be computed by using the asymptote of the points from one experimental series in which the less polar phase is extracted, and the partition coefficient of the other can be similarly obtained from another experimental series in which the more polar phase is extracted. Both partition coefficients can also be computed from one series by using the differences between the concentrations observed and those computed for one component from the asymptote, for making another plotting, valid for the other component.

Although tertiary bisbenzyltetrahydro*iso*quinoline alkaloids have been investigated extensively, efficacious methods still have not been adopted for the separation of many naturally occurring mixtures of these alkaloids nor for the separation of such mixtures of their isomers as are obtained by partial methylation or demethylation. A study of these alkaloids was taken

---

* Present address: Institutionen för organisk kemi, Tekniska högskolan, Stockholm 70, Sweden.

up in this laboratory, and the importance of working out counter current separation methods for them soon became obvious. It may be pointed out here that the separation problem arising at the beginning of this investigation and dealt with in this paper only concerns separation of substances whose partition coefficients differ by a factor of about 3 or more. It was highly desirable that the separation procedure be suitable for amounts varying at least between 0.1 and 100 g, and that the separation could be done by hand with separatory funnels.

For planning a counter current separation of a mixture of two substances it is of some importance to know the partition coefficients for each of the substances, both for choosing the best volume ratio and for choosing the appropriate number of transfers.

A general equation for the relation between optimum volume ratio and partition coefficients was derived by Baker [1]. He found that a symmetrical counter current separation of two substances is most efficacious, if the ratio of the volume of the less polar phase of two immiscible solvents to the volume of the more polar phase equals the inverse value of the square root of the product of the partition coefficients of the two substances.

The number of transfers necessary to give a certain degree of separation depends [2,3] on the ratio of the two distribution coefficients.

When the partition ocefficients of the two components in a mixture are not known and the components are not available separately as pure substances, it is usually recommended [4-6] that the value obtained from a simple determination of the distribution of the mixture between the two solvent phases be used for the counter current distribution separation. This may work fairly well if the amounts of the two substances are about equal, but if one of the substances is in considerable excess, the value found for the partition coefficient of the mixture will be near the value valid for the substance in excess and differ considerably from the geometric mean of the values for the two substances. Hence, the separation will not be as efficient as would otherwise be possible. If an apparatus permitting a large number of transfers is available this may not be a serious problem in most cases. However, if the separation is carried out by hand with a few separatory funnels and with the convenient method [7] for finally obtaining two fractions called "diamond separation" and "completion of squares", a large amount of the substance in excess in the original mixture will still be found in the fraction containing most of the substance present only in small amounts, and hence a second determination of the partition coefficient of this mixture may give a value still too near that of the substance originally in excess.

Another fairly common difficulty in determining distribution coefficients in a conventional way is that the two solvent phases may contain other substances, *e.g.*, buffer salts, which may complicate the analysis of the two phases.

In the case of bisbenzyltetrahydro*iso*quinoline alkaloids the concentration can easily be determined spectrophotometrically. However, these alkaloids decompose readily, partly to substances with strong absorption. Most of these substances have partition coefficients which are considerably lower than those of the pure alkaloids. Hence, partition coefficients computed from a simple determination of the light absorption in both phases may be highly erroneous.

These difficulties can, however, in many cases be overcome by a simple procedure which will be described here, and by which it is possible to determine the partition coefficients of each of two substances in a mixture. This procedure is also useful in the determination of the distribution coefficient of a pure substance and of a substance with some impurities. It is also useful for checking the purity of a preparation and for determining whether or not a partition coefficient is independent of the concentration of the substance.

Stevenson [8] independently found the same general method carrying out another investigation in this laboratory.

THEORETICAL

In this procedure for determining partition coefficients appropriate volumes of the two phases are used for dissolving the preparation. These volumes do not need to be equal. One of these phases is extracted successively with portions of the other phase. The volumes of each portion must be the same. Either the phase which is extracted or the extracts are analyzed for their content of the preparation, and the values obtained are used for computing the partition coefficients, checking the purity, *etc.*

In this paper the two phases of the solvent system are referred to as the more polar phase and the less polar phase instead of the upper and the lower phase. This is advantageous for comparing partition coefficients, *e.g.*, between ether and buffer and between chloroform and buffer. Partition coefficients are consequently calculated as concentration in the less polar phase divided by concentration in the more polar phase.

The following symbols are used.

K partition coefficient (distribution constant)

V_l volume of the less polar phase

V_m volume of the more polar phase

c_{l1} concentration of preparation in the less polar phase after the first equilibration

c_{m1} concentration of preparation in the more polar phase after the first equilibration

c_{l2} concentration of preparation in the less polar phase after the second equilibration

c_{m2} concentration of preparation in the more polar phase after the second equilibration

q fraction of the solute remaining in the extracted phase after equilibration

With these symbols the distribution law [9,10] can be written

$$K = c_{l1}/c_{m1} = c_{l2}/c_{m2} \qquad (1)$$

Depending on the value of the partition coefficient (if considerably larger or smaller than 1) it may be advantageous either to extract one portion of the more polar phase with several portions of the less polar phase or *vice versa*. In many cases it is not too inconvenient to run both extraction series. Methods for calculation of distribution coefficients of pure substances and of a two-component mixture will be presented.

The more polar phase is extracted with several portions of the less polar phase

It is assumed in the following method that the concentration of the substance is measured in the more polar phase. However, there will be no difference in the final result if the substance is measured in the other phase because the concentration in one phase is proportional to the concentration in the other.

After the first equilibration the amount of solute in the more polar phase is $V_m c_{m1}$. If this phase is extracted with a second portion of the less polar phase, the amounts of solute present in the two phases are $V_m c_{m2}$ and $V_l c_{l2}$, respectively. Thus,

$$V_l c_{l2} + V_m c_{m2} = V_m c_{m1} \tag{2}$$

Because the concentration is not measured in the less polar phase, c_{l2} must be eliminated, which is done as follows. According to eqn (1), $c_{l2} = K c_{m2}$, and eqn (2) can thus be written

$$V_l K c_{m2} + V_m c_{m2} = V_m c_{m1} \tag{3}$$

Hence,

$$c_{m2} = \frac{V_m}{V_l K + V_m} c_{m1} \tag{4}$$

and, generally,

$$c_{mn} = \left(\frac{V_m}{V_l K + V_m}\right)^n \cdot c_{m1} \tag{5}$$

This equation is equivalent to Craig and Craig's [11] eqn (8), but a corresponding equation was first published by Herz [12].

By writing this equation in logarithmic form

$$\log c_{mn} = n \cdot \log \frac{V_m}{V_l K + V_m} + \log c_{m1} \tag{6}$$

we arrive at the main improvement suggested in this paper for the calculation of partition coefficients *.

This equation implies that if the logarithms of the values for the solute concentration in successive extracts are plotted in a graph *versus* the numbers of the extractions, the points are expected to lie about a straight line, the inclination of which is $\log V_m/(V_l K + V_m)$, provided the substance is homogeneous and provided no reaction occurs which is not accounted for in the method of calculation (association or dissociation).

The partition coefficient is computed from the value of the inclination of the line which is measured. The fraction of substance remaining after equilibration is c_{m2}/c_{m1}, if we consider the amounts present in the more polar phase before and after the second extraction, and this quotient is in our denotation called q. Eqn (4) can then be written

---

* After this manuscript was prepared, a similar equation has been published by Rudstam [13].

$$\frac{V_m}{V_l K + V_m} = q \tag{7}$$

In the procedure of computing the value of the partition coefficient, q is the antilogarithm of the inclination of the line fitted as just described. By solving eqn (7) we get

$$K = \frac{V_m}{V_l} \frac{1 - q}{q} \tag{8}$$

It would, of course, be possible to compute K from two extractions, as eqn (4) can be written

$$K = \frac{V_m}{V_l} \frac{c_{m1} - c_{m2}}{c_{m2}} \tag{9}$$

but the use of more than two extractions makes it possible to check the solute for homogeneity. If the solute is homogeneous, and if there is no association or dissociation, the points fit a straight line; then several extractions make the assay of the partition coefficient more accurate.

If the points do not fit a straight line, and if this is caused by the presence in the substance of some impurity or by the presence in the substance of two components with different partition coefficients, one may fit an asymptote, from which it is possible to compute the partition coefficient of the component which is less readily extracted from the more polar phase.

The less polar phase is extracted with several portions of the more polar phase

It is assumed also in the following method that the concentration of the solute is measured in the more polar phase, but the result of the computation of the partition coefficient will be the same if the concentration is measured in the less polar phase, as the concentration in one phase is proportional to the concentration in the other.

After the first equilibration the amount of substance present in the less polar phase is $V_l c_{l1}$. The more polar phase is removed, and the less polar phase is extracted with a fresh portion of the more polar phase. After equilibration the amount of solute present in the less polar phase is $V_l c_{l2}$ and in the more polar phase $V_m c_{m2}$. Thus,

$$V_l c_{l2} + V_m c_{m2} = V_l c_{l1} \tag{10}$$

Because the concentration is measured in the more polar phase, c_{l1} and c_{l2} must be eliminated. Considering eqn (1), we can rewrite eqn (10) in the following form

$$V_l K c_{m2} + V_m c_{m2} = V_l K c_{m1} \tag{11}$$

Hence,

$$c_{m2} = \frac{V_l K}{V_l K + V_m} c_{m1} \tag{12}$$

and, generally,

$$c_{mn} = \left(\frac{V_l K}{V_l K + V_m} \right)^n c_{m1} \tag{13}$$

This equation is rewritten in logarithmic form

$$\log c_{mn} = n \cdot \log \frac{V_l K}{V_l K + V_m} + \log c_{m1} \tag{14}$$

This equation implies that if the logarithms of the concentrations of a homogeneous solute in a series of extracts are plotted *versus* the numbers of the extractions, the points lie about a straight line, the inclination of which is $\log V_l K/(V_l K + V_m)$. Here $V_l K/(V_l K + V_m)$ can be identified as the fraction q of a substance remaining after an extraction in the phase which is extracted. Thus, q is the antilogarithm of the inclination of the line fitted as described, and we get

$$K = \frac{V_m}{V_l} \frac{q}{1 - q} \tag{15}$$

If the solute is impure or is a mixture of two components, the partition coefficients of which differ noticeably, an asymptote may be fitted to the points. The partition coefficient of the component which is less readily extracted from the less polar solvent phase can be computed from the inclination of this line.

Computation of two partition coefficients for a mixture of two components

There are obviously two ways for determining the two partition coefficients for a mixture consisting of two components. One way, already intimated, is to run two distribution series, one series in which the more polar phase is extracted with the less polar, and another series, in which the less polar phase is extracted with the more polar, and then to compute the partition coefficients from the two asymptotes plotted as described in the previous sections. Another way to determine the two partition coefficients is to compute them from one series of extractions in the following way.

The logarithms of the concentrations of the solute in the extracts are plotted *versus* the numbers of the corresponding extracts, and an asymptote is fitted as described. The logarithms of the concentrations of the component, for which the asymptote is valid, are calculated for the various extracts. The corresponding antilogarithms give the concentrations of this compound. The differences between the concentrations actually measured and the concentrations thus computed are the concentrations of the other substance in the series of extracts. The logarithms of these values are plotted *versus* the numbers of the extracts, and a straight line is fitted. The inclination of this line is read, and its antilogarithm q is used for computing the partition coefficient, using eqn (8) or (15), depending on whether the more polar or less polar solvent phase was extracted.
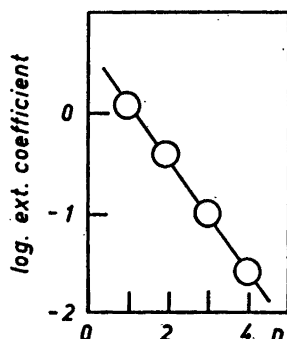
Fig. 1. A preparation of pure d-chondro-curine gives points, all of which lie closely about a straight line even over a wide concentration range. The logarithms of the extinction coefficients are plotted *versus* the numbers of the corresponding extracts.
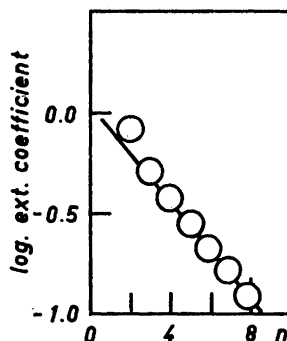
Fig. 2. A preparation of monodemethylated l-curine (not crystallized); the diethyl ether phase was extracted with buffer. The points indicate the presence of an impurity with considerably lower partition coefficient but do not indicate any heterogeneity of the monodemethylated l-curine.

EXPERIMENTAL

A few examples will be given to illustrate the suggested method. The examples have been chosen from various determinations of partition coefficients for some bisbenzyl-tetrahydroisoquinoline alkaloids: d-chondrocurine, and monomethyl and monodemethylated derivatives of l-curine. The results from more determinations will be reported in forthcoming publications [14],[15].

Determination of the partition coefficient for d-*chondrocurine between diethyl ether and water at pH 11.6.* The water phase consisted of a buffer solution containing 2 g of potassium metabisulfite $K_2S_2O_5$ and 0.5 g of K_3PO_4 and somewhat more than 1 g of KOH in 100 ml of water. The amount of KOH was chosen so that the pH value of the buffer was 11.6. A high concentration of sodium ions was avoided because a glass electrode was used for measuring the pH values. The solution was equilibrated with diethyl ether. 25 ml of the ether phase and 10 ml of the water phase were added to a separatory funnel, and a few drops of a solution of d-chondrocurine in a mixture of equal parts of chloroform and methanol were added. The ether phase was successively extracted with 10 ml portions of the water phase, and the extinction coefficient at 289 mμ was read for each extract. The following values were obtained: 1.20, 0.365, 0.098, and 0.027. The measurements thus were extended over a wide range of concentrations. The logarithms of the values were plotted (Fig. 1) *versus* the numbers of the extractions, and a straight line was fitted to the points. The inclination of the line, log q, was read as −0.56; hence $q = 0.275$. The values of the volumes and of q were substituted in eqn (15).

$$K = \frac{10}{25} \cdot \frac{0.275}{0.725} = 0.15$$

Determination of the partition coefficient of one of the isomers of monodemethylated l-*curine for diethyl ether and water at pH 10.3.* The water phase consisted of 1 g of $K_2S_2O_5$, 3 g of $Na_2CO_3 \cdot H_2O$ and 1.5 g of K_3PO_4 in 100 ml of water, with pH adjusted to 10.3, and equilibriated with diethyl ether. The experiment was done both by extracting 25 ml of the ether phase with 10 ml portions of the water phase and by extracting 15 ml of the water phase with 25 ml portions of the ether phase. The following extinction values were obtained when the ether phase was extracted: 0.835, 0.496, 0.363, 0.273, 0.209, 0.161, and 0.120. A straight line was fitted, the inclination of which was −0.12 (Fig. 2). The corresponding value of the partition coefficient was 1.24, computed according to
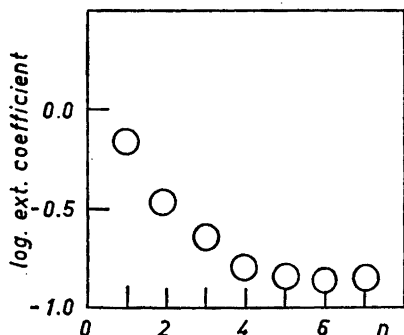
Fig. 3. A preparation of monodemethy-
lated l-curine as in Fig. 2; the water phase
was extracted with diethyl ether. The
points indicate the presence in the water
phase of an impurity with considerably
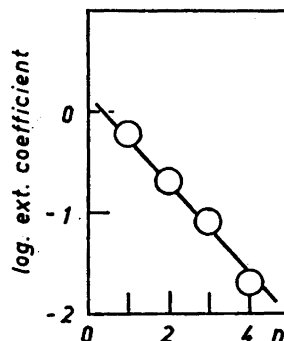lower partition coefficient.

Fig. 4. Monodemethylated l-curine. The
same experiment as in Fig. 3 but with
the extinction coefficient of the impurity
subtracted from the measured values.

eqn (15). When the water phase was extracted, the values obtained were the following:
0.690, 0.333, 0.218, 0.156, 0.145, 0.138, and 0.140. The logarithms of these values are
plotted in Fig. 3. It is obvious that one light absorbing impurity with very low partition
coefficient is present in the water phase. Assuming that the extinction caused by this
impurity is 0.140, and subtracting this value from the measured values, we get an esti-
mate of the extinction caused by the monodemethylated l-curine. The logarithms of
these values were plotted *versus* the numbers of the corresponding extractions (Fig. 4),
and a straight line was fitted. Its inclination, -0.45, corresponded to the value 1.09 of
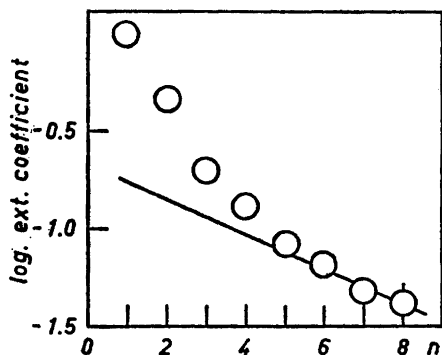the partition coefficient.

Fig. 5. A mixture of the d-chondrocurine
monomethyl ethers. The distribution coeffi-
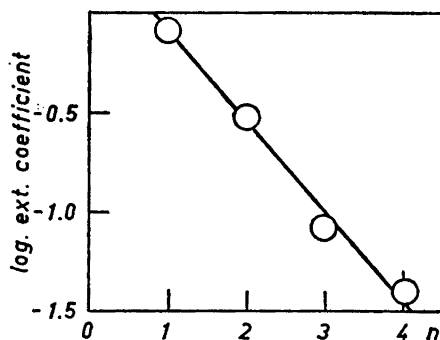cient for one of them is computed from
the asymptote.

Fig. 6. The same experiment as in Fig. 5.
The partition coefficient of the other d-chon-
drocurine monomethyl ether is computed
from the difference between the observed
values for the extinction coefficient and the
values computed from the asymptote The
logarithms of the differences are plotted
versus the numbers of the corresponding
extracts.

Preliminary determination of the partition coefficients of the two d-*chondrocurine monomethyl ethers in a solvent system containing benzene, heptane, methanol, and water (2, 4, 3, and 3 volumes, respectively).* The water phase contained 0.5 % $K_2S_2O_8$ and 0.5 % Na_2CO_3, and the pH-value of this solution was adjusted to 8.0. A preparation of partly methylated d-chondrocurine had been separated [14] into three fractions, one of which consisted of the monomethyl ethers of d-chondrocurine. A few milligrams were dissolved in a few drops of a mixture of chloroform and methanol (1:1) and added to a separatory funnel containing 10 ml of the lower phase and 15 ml of the upper phase of the solvent system. The lower phase was changed. The extinction readings at 280 mμ for the extracts were 0.995, 0.440, 0.198, 0.131, 0.085, 0.065, 0.047, and 0.041. The logarithms of the values are plotted in Fig. 5, and an asymptote fitted to the tail values. The partition coefficient value corresponding to this line is 2.7. The antilogarithms of the ordinata values of this line at abscissa values corresponding to the numbers of the successive extractions give the calculated concentrations of the substance with the partition coefficient 2.7 in the various extracts. These values were subtracted from the original readings, and the logarithms of the remainders for the first four extracts were plotted *versus* the number of the corresponding extractions (Fig. 6). A straight line was fitted to the points, and its inclination read. The corresponding partition coefficient was 0.35. This value is somewhat too low; the value 0.5 was later obtained from a crystallized preparation of this substance. The values found are, however, sufficiently accurate to enable a rational planning of a counter current separation. It can be concluded from these figures, that one should use about equal volumes of the two solvent phases for a counter current separation, and that the ratio of the partition coefficients is so large that a fair separation of the two d-chondrocurine monomethyl ethers can be carried out, using a conveniently small number of separatory funnels. Such a separation was also carried out, using five funnels. The separation can be considerably improved by repeating the separation with each fraction.

REFERENCES

1. Bush, M. T., Goth, A. and Dickison, H. L. *J. Pharmacol. Exp. Therap.* **84** (1945) 262, footnote 6.
2. Hecker, E. *Verteilungsverfahren im Laboratorium*, Verlag Chemie, Weinheim/Bergstr. 1955, p. 78.
3. Rauen, H. M. and Stamm, W. *Gegenstromverteilung*, Springer-Verlag, Berlin-Göttingen-Heidelberg 1953, p. 4.
4. Hecker, E. Ref.[2], p. 140.
5. Rauen, H. M. and Stamm, W. Ref.[3], p. 22.
6. Jübermann, O. in Müller, E. *Methoden der organischen Chemie* I/1, Georg Thieme Verlag, Stuttgart 1958, p. 303.
7. Bush, M. T. and Densen, P. M. *Anal. Chem.* **20** (1948) 121.
8. Stevenson, G. *To be published.*
9. Riecke, E. *Z. physik. Chem.* **7** (1891) 97.
10. Nernst, W. *Z. physik. Chem.* **8** (1891) 110.
11. Craig, L. C. and Craig, D. in Weissberger, A. *Technique of organic Chemistry* III:I, Interscience Publishers, Inc., New York 1956, p. 162.
12. Herz, W. *Sammlung chemischer und chemisch-technischer Vorträge* **15** (1910) 1.
13. Rudstam, G. *Anal. Chem.* **32** (1960) 1664.
14. Hultin, E. *Acta Chem. Scand.* **15** (1961). *In press.*
15. Hultin, E. *Acta Chem. Scand. In press.*