On the Use of Monolayer Phase Diagrams for Determining the Composition of Mixtures of Homologous Long Chain Compounds of High Molecular Weight

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The determination of the composition of mixtures of homologous long chain compounds, e. g. of the type often found in natural waxes, is usually rather difficult, especially when only small amounts of material are available. As a rule, molecular distillation gives only a partial separation of the components, while crystallization from solvents is quite an ineffective method of separation when dealing with a mixture of closely related homologues of high molecular weight. Chromatographic methods [Cf. 1, 2] may ultimately prove capable of analyzing such mixtures, but the technique is not yet sufficiently developed. Chibnall, Piper and their collaborators, in the course of extensive work on the composition of natural waxes [Cf. e. g. 3, 4], determined the composition of binary and ternary mixtures of long chain compounds by thermal and X-ray methods. The behaviour of a large number of artificial mixtures of known composition was investigated, and the composition of naturally occurring mixtures determined by comparing their behaviour with that of mixtures of known composition.

The object of the present paper is to show that monolayer phase diagrams may be advantageously used for the quantitative analysis of mixtures of homologous long chain compounds of high molecular weight.

In a previous communication [5] we have described the monolayer phase diagram for n-docosanoic (behenic) acid. The phase diagram was constructed from force-area curves obtained with an automatically recording Wilhelmy-Dervichian type surface balance with symmetrical compression of the monolayers [6, 5]. The results obtained with this balance have since been checked by means of a recording horizontal balance, employing the 'mikrokator' prin-
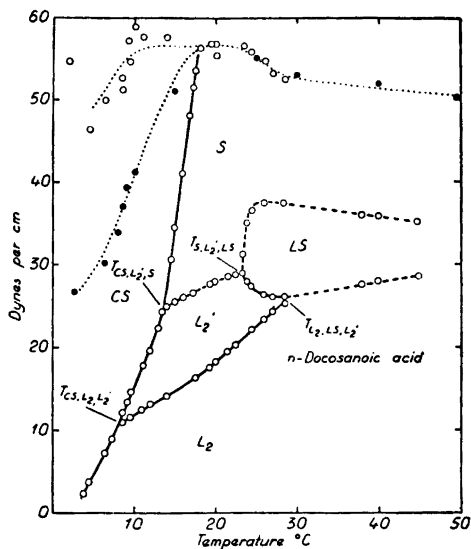
Fig. 1. Phase diagram for n-docosanoic (behenic) acid on 0.01 N hydrochloric acid substrate. Unbroken lines represent first order transitions, and broken lines second order. The stability limit is given as a dotted line. Filled circles represent collapse pressures obtained with a Wilhelmy-Dervichian type balance with symmetrical compression.

ciple [7]. The phase diagram for n-docosanoic acid obtained by the use of the latter instrument is reproduced in Fig. 1. Differences between the results given by the vertical and the horizontal balances, outside of experimental errors, are found only for the collapse pressures at low temperatures (CS phase). The lower stability of the monolayers, when the vertical balance is used, is probably due to mechanical breaking of the solid monolayer during the upward movement of the glass slide. The monolayer is not subjected to any strain of this type when the mikrokator balance is used.

The monolayer phase diagram for a long chain acid such as n-docosanoic acid shows five different phases and four triple points (a detailed description of the phase diagrams, and a discussion of the symmetry relations between the different phases will be given in another communication) [8]. Of primary importance in the present connection are the changes in the monolayer phase diagrams brought about by an increase in the length of the hydrocarbon chain of the carboxylic acid, and the effects of admixture of homologues. The phase diagrams for monolayers of the C_{20}, C_{22}, and C_{24} normal chain carboxylic acids are shown superimposed on each other in Fig. 2. An increase in chain length brings about a shift of the phase diagrams towards higher temperatures and slightly higher pressures. One CH_2-group causes a shift of about 5°, but the distance between the diagrams decreases with increasing chain-length. The odd-numbered acids (C_{21} and C_{23}) fall in between the evennumbered homologues, no odd-even alternation being observed.
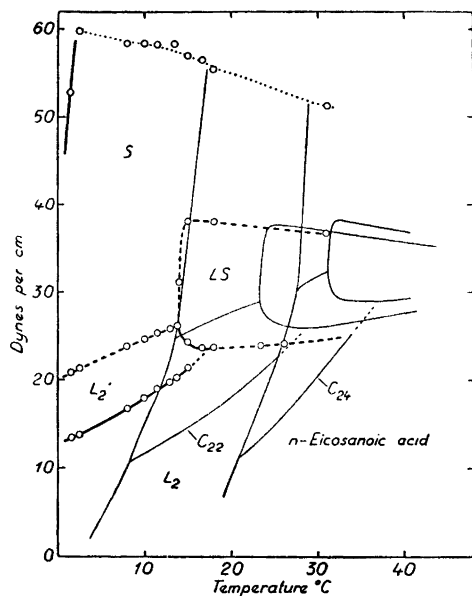
Fig. 2. Monolayer phase diagrams for n-eicosanoic acid, n-docosanoic acid and n-tetracosanoic acid respectively spread on 0.01 N hydrochloric acid substrate.
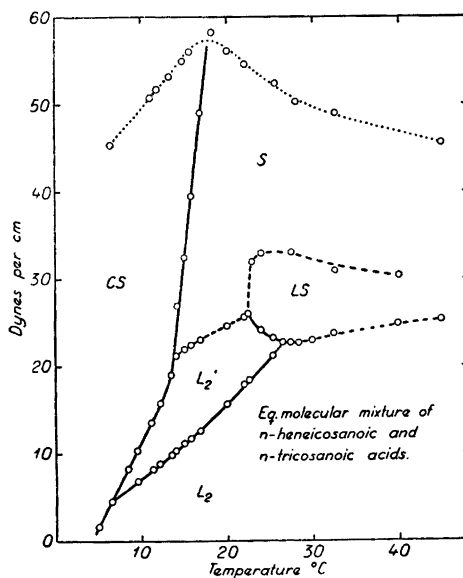
Fig. 3. Phase diagram for the equimolecular mixture of n-heneicosanoic acid and n-tricosanoic acid.

The shifts of the different triple points are not equal, and this changes the form of the diagrams slightly when the chain-lengths are altered. The nature of the phases themselves, however, and the type of transition between them remain unaltered.

Phase diagrams for the monolayers of two artificial binary mixtures, each having a mean molecular weight equal to the molecular weight of the C_{22} acid, are shown in Figs. 3 and 4. When the chain-lengths of the components differ by two CH_2-groups (Fig. 3), the phase diagram is very similar to that of the pure C_{22} acid, but the triple points are shifted towards lower pressures. This shift is most pronounced for the triple point T_{CS, L_2, L'_2}, which lies about 7 dynes per cm lower for the mixture. The shift of this triple point is still more pronounced for the mixture whose phase diagram is shown in Fig. 4. In this case the chain-lengths of the components differ by 4 CH_2-groups, and the triple point in question is now displaced below zero pressure. The three other triple points are also displaced towards lower pressures compared with those of Fig. 3. The phase diagram in Fig. 4 is still of the type found for pure acids, but this is no longer the case when the components of the binary
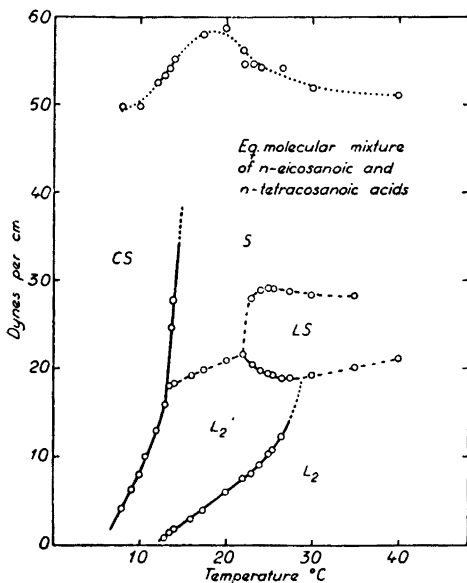
Fig. 4. Phase diagram for the equimole-
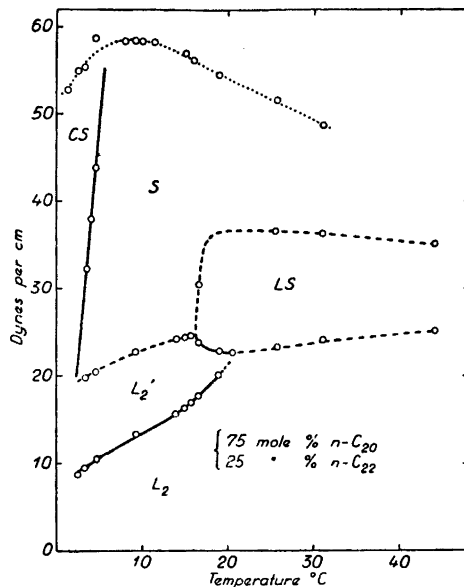cular mixture of n-eicosanoic acid and
n-tetracosanoic acid.

Fig. 5. Phase diagram for mixture con-
taining n-eicosanoic acid and n-docosanoic
acid in the molecular proportions 3 : 1.

mixture differ in chain-length by six carbon atoms or more, as in the equi-
molecular mixtures of n-nonadecanoic and n-pentacosanoic acids, or n-octa-
decanoic (stearic) and n-hexacosanoic acids. The phase transitions now be-
come blurred, and the phase diagrams can no longer be plotted with any
degree of accuracy.

Figs. 5—7 show the monolayer phase diagrams for binary mixtures con-
taining n-eicosanoic and n-docosanoic acids in varying proportions. It appears
that there is a gradual shift of the diagram from that of the pure n—C_{20} acid
to that of the pure n—C_{22} acid (cf. Fig. 2). The relation between the tem-
perature at which a certain phase transition occurs, and the composition of the
binary mixture appears to be approximately linear, while the corresponding
pressures are slightly lower than those obtained by linear interpolation.

It is evident, from the above results, that the monolayer phase diagram
will, in general, be a characteristic property of a given mixture. If a mixture
of unknown composition gives a monolayer phase diagram agreeing in detail
with that of a mixture of known composition, it may be assumed, with some
confidence, that the two mixtures are identical. The chance that two different
mixtures give the same result is probably less for the rather complicated
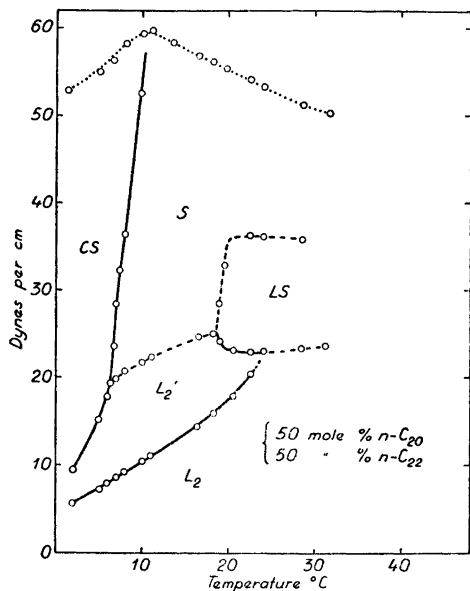monolayer phase diagrams than for thermal and X-ray data.

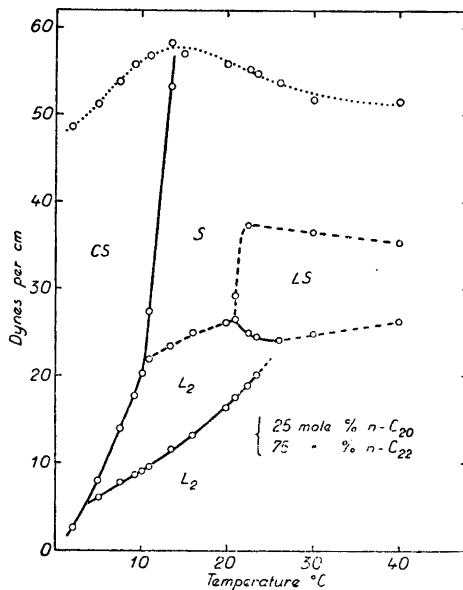Fig. 6. Phase diagram for the equimolecular mixture of n-eicosanoic acid and n-docosanoic acid.

Fig. 7. Phase diagram for mixture containing n-eicosanoic acid and n-docosanoic acid in the molecular proportions 1 : 3.

The new method of analysis has been applied to the quantitative analysis of a mixture of acids obtained by Dr. J. Gripenberg from the heart wood of *Thuja plicata* D. Don. A series of force-area curves for a specimen of this mixture, kindly put at our disposal by Professor H. Erdtman, are reproduced in Fig. 8 a. The curves are of the usual type given by normal chain carboxylic acids of high molecular weight. The various phase transitions occur at higher temperatures, and the transitions $L_2 \rightleftharpoons CS$, $L_2 \rightleftharpoons L_2'$, and $L_2' \rightleftharpoons CS$ are not as distinct as in the case of n-docosanoic acid [5]. This indicates that the mean molecular weight of the mixture is higher than the molecular weight of the C_{22} acid. As area values are not needed for the construction of the phase diagrams, a previous knowledge of the mean molecular weight of the mixture is not necessary for the analysis.

The phase diagram for the mixture indicates that the difference in chain lengths of the components cannot be large, as the diagram is very similar in form to that of the n-C_{24} acid (Fig. 9). The fact that the diagram for the *Thuja plicata* mixture is shifted towards higher temperatures shows that the mean molecular weight of this mixture is higher than that of the C_{24} acid. This, finding — combined with the fact that there can be no large difference in the
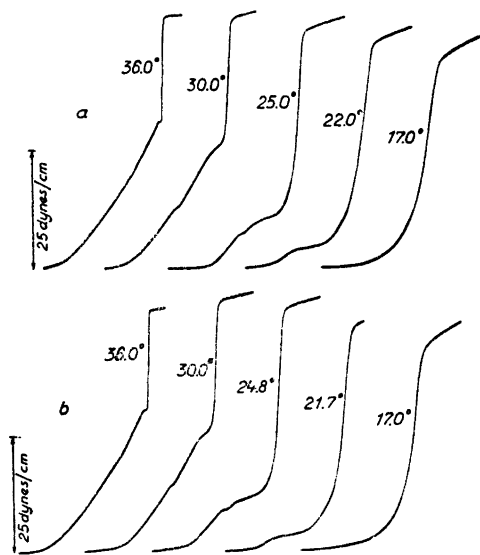
Fig. 8. Force-area curves on 0.01 N hydro-
chloric acid substrate. a) mixture of acids
derived from heart wood of T h u j a p l i-
c a t a. b) artificial mixture containing
n-tetracosanoic acid and n-hexacosanoic
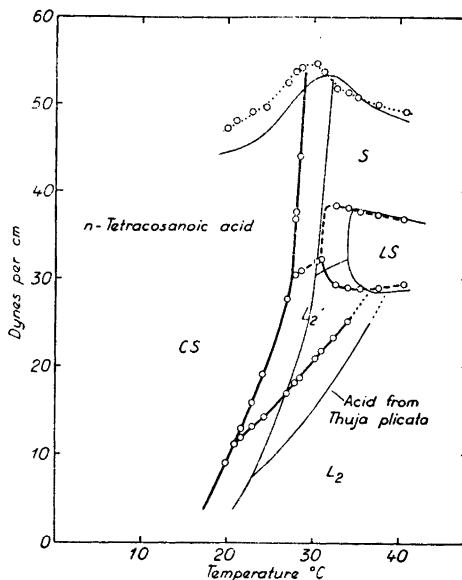acid in the molecular proportions 7 : 3.

Fig. 9. Comparison between the phase dia-
gram for the mixture of acids derived from
T h u j a p l i c a t a and that given by
n-tetracosanoic acid.

chain lengths of the components and the rule that naturally occurring mixtures
consist of even-numbered homologues — suggested a mixture of n-tetra-
cosanoic acid and n-hexacosanoic acid. The phase diagrams of several mixtures
of these two acids were therefore determined for comparison. Fig. 10 shows
that the diagram for a mixture containing equimolecular proportions lies to
the right of that of the natural product, indicating that the mean molecular
weight of the latter is lower than that of this mixture. This diagram, together
with that of Fig. 11, shows that the mixture from Thuja plicata must contain
between 50 and 23 % of the higher (C_{26}) acid, and finally Fig. 12 shows that
a mixture containing 30 mol-% of the C_{26} and 70 mol-% of the C_{24} acid gives
a monolayer phase diagram coinciding with that of the natural product.
Fig. 8 b shows a series of force-area curves for the synthetic mixture, which
should be compared with those in Fig. 8 a. A very small amount of a third
component cannot, of course, be excluded; but appreciable amounts (of the
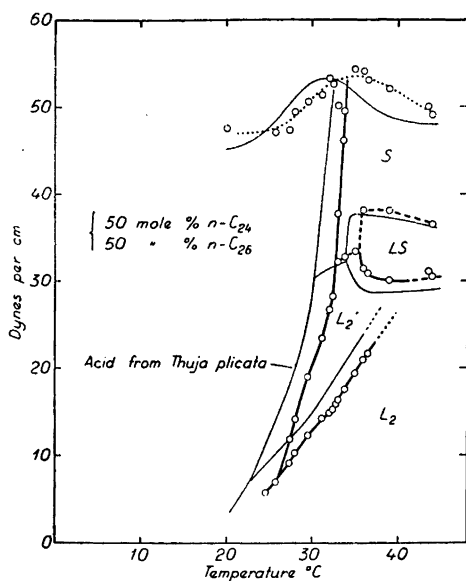order of 3 to 5 %) should have caused a shift of the triple point $T_{CS, \ L_2, \ L'_2}$

Fig. 10. Comparison between the phase diagram for the mixture of acids derived from *Thuja plicata* and that of an artificial mixture containing equimolecular proportions of n-tetracosanoic acid and n-hexacosanoic acid.
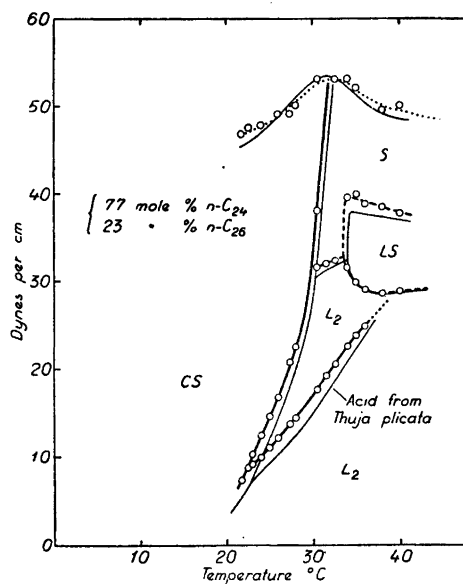
Fig. 11. Phase diagram for mixture of acids derived from *Thuja plicata* compared with that of an artificial mixture containing n-tetracosanoic acid and n-hexacosanoic acid in the molecular proportions 77 : 23.

towards lower pressures. The effect is clearly shown in the phase diagram for a mixture of C_{24}, C_{26} and C_{28} acids reproduced in Fig. 13.

Thermal and X-ray data * confirm the results of the above analysis. The m. p. of the mixture from *Thuja plicata* was found to be 78.2—78.4°. The melt showed a very faint brownish tinge. A synthetic mixture of the $n\text{-}C_{24}$ and $n\text{-}C_{26}$ acids containing 70 mol % of the former melted at 78.4—78.7°, and the mixed m. p. with the mixture from *Thuja plicata* was 78.3—78.7°. The melting point curve for the binary system n-tetracosanoic acid — n-hexacosanoic acid given by Piper, Chibnall and Williams [4] shows that the mixture containing 70 mol % of the lower acid corresponds to the eutectic mixture. This explains the sharp m. p. given by the *Thuja plicata* mixture, as well as the fact that its properties remained unchanged in spite of several recrystallizations.

The long X-ray spacing given by the natural specimen (as received, or after recrystallization from acetone) was 60.1 Å, calculated from a diffraction

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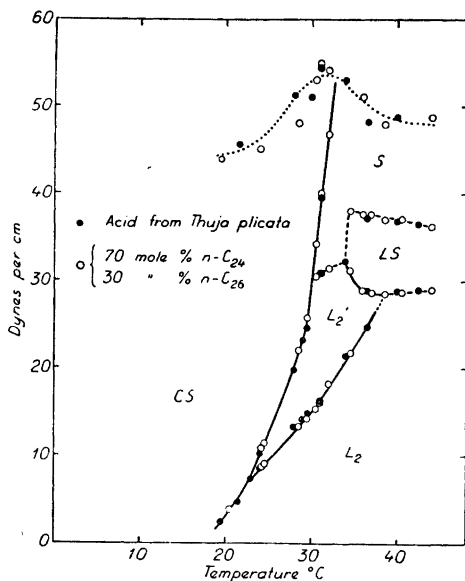* For the technique used cf. e. g. ref. 10.

Fig. 12. Comparison between the phase diagram for the mixture of acids derived from *T h u j a   p l i c a t a* and that of an artificial mixture containing n-tetracosanoic acid and n-hexacosanoic acid in the molecular proportions 7 : 3.
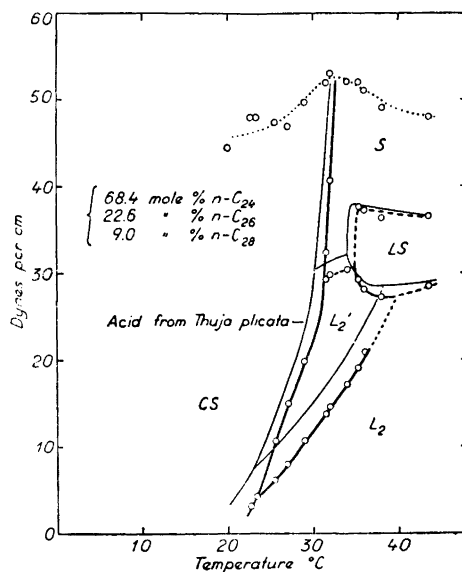
Fig. 13. Comparison between the phase diagram for the mixture of acids derived from *T h u j a   p l i c a t a* and that of an artificial mixture containing n-tetracosanoic acid, n-hexacosanoic acid and n-octacosanoic acid in the molecular proportions 68.4 : 22.6 : 9.

pattern of the type expected for a mixture. The value recorded corresponds to a B spacing [Cf. 4]. A multilayer of the barium salt gave a long spacing of 67.5 Å, calculated from a fairly good photograph. Multilayers of the barium salt of n-tetracosanoic acid gave a spacing of 65.3 Å [8], and the value calculated for the barium salt of the n-C_{26} acid is 70.3—70.4 Å. The X-ray data just mentioned supports the results derived from monolayer and thermal data.

Finally, the mean molecular weight of the mixture from *Thuja plicata* was determined by titration. However, owing to the solubility difficulties and the small amount of material available, this determination could not be performed with great accuracy. The value found was 381 ± 5. The value calculated for the eutectic mixture (70 % of the lower acid) is 377.0.

The method of analysis described can be regarded as a micro-method, as the amount of material needed for the analysis is less than 10 mg. For comparison, pure specimens of the appropriate homologues are, of course, necessary.

EXPERIMENTAL

The monolayer phase diagrams in this paper have all been plotted from force-area curves on 0.01 N hydrochloric acid substrate obtained with the recording mikrokator balance [7]. Spreading was effected from a solution in benzene-chloroform (9 : 1 by volume) [9].

Materials used. The specimen of the mixture from *Thuja plicata* had been obtained * by alkaline hydrolysis of the neutral fraction of the acetone extract of the heart wood. The alcoholic fission product consisted mainly of a mixture of sterols ('phytosterol'). The free 'acid' obtained by the hydrolysis had been recrystallized 10 times from ethanol, without appreciable change in melting point. The specimen received had a white, micro-crystalline appearance.

For comparison, original samples of the normal chain fatty acids described by Francis and Piper [11] were available.

SUMMARY

It has been shown that monolayer phase diagrams may be used for the quantitative analysis of the composition of mixtures of homologous long-chain compounds of the type often derived from natural sources. The new method of analysis is applied to a mixture of acids from the heart wood of *Thuja plicata*. The analysis requires less than 10 mg of sample.

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* We are indebted to Professor Erdtman for this information.