Journal of Thermal Analysis, Vol. 33 (1988) 1033–1039

CHARACTERIZATION OF CHIRAL BUILDING BLOCKS FOR VITAMIN D METABOLITES BY DSC

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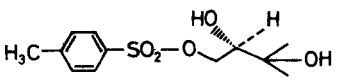
The enantiometric purity of a vitamin D_3 metabolite was determined more exactly by means of DSC than by ^{1}H-NMR. The melting curve was analysed by the partial area method based on the Schröder-van Laar equation. In order to find a suitable method for separation (R)-2,3-dihydroxy-3-methylbutyl *p*-toluenesulfonate from the racemate, the phase diagram of the enantiomers was evaluated from DSC results. The occurrence of a racemic compound was confirmed by an X-ray diffraction investigation of the racemate and the enantiomers. The conclusions are discussed in comparison with the results of previous investigations.

Both the drug activities and the undesired side-effects of enantiomers can differ extremely. The enantiomeric purity of bioactive components is therefore highly important, as was demonstrated by the Contergan disaster, for example.

The enantiomeric purity of (R)-2,3-dihydroxy-3-methylbutyl *p*-toluenesulfonate



for the synthesis of (24R)-24, 25-dihydroxy-vitamin D_3, and also that of the (S) enantiomer, were determined by ^{1}H-NMR spectroscopy [1]. An enantiomeric purity of 94% or higher was determined. This result was not satisfactory. Therefore, the problem arose of finding a method of determining the purity more exactly. DSC seemed to be an interesting method.

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Determination of purity

For a more precise determination of the enantiomeric purity, we tested the applicability of DSC. The DSC method offers two variants:

1. A direct method is applicable to samples with enantiomeric purities that are neither too large nor too small, for which the eutectic melting point is not too close to that of the eutectic temperature or to the termination of fusion. This analysis is valid only if the peaks (eutectic and enantiomer) are well separated and if the peak area is measured precisely.
2. An indirect method, based upon the analysis of a melting scan by the general procedure applicable to nearly pure crystalline substances; this can furnish enantiomeric purity information of fair precision [2–5].

Our problem was to determine the enantiomeric purities of the two basic products, (R)-sulfonate and (S)-sulfonate. These are nearly pure products. The conditions for the direct method are not fulfilled. Therefore, only the indirect method is applicable.

Application of the indirect method

It is very important to produce good melting curves in order to permit precise measurements of the total area Q and the partial areas q. Moreover, the temperatures T_{1-3} on the fusion scans must be located exactly (Fig. 1).

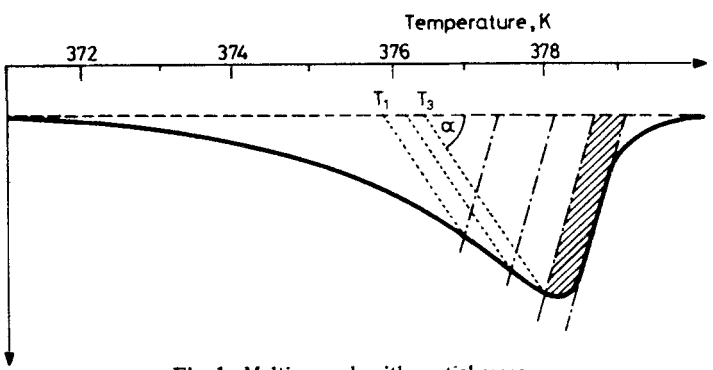

Fig. 1 Melting peak with partial areas

Therefore, a sample of an unknown mixture is melted under conditions such that the fusion peak is defined exactly. The area Q of the peak gives the enthalpy of fusion of the mixture.

Heat conduction effects necessitate two corrections:

a) T correction: determination of the slope of the leading edge of the melting peak of a very pure substance.

b) Area correction: from the slope of the back edge of the actual melting peak, we define the partial areas; through application of this procedure to the fusion curves, we determine purities.

The measurements of q towards the end of the melting process are given by the differences from planimetric area determinations corresponding to the nonmelted substance. One obtains Q-q and, since Q is known, the value of q follows.

An experimental problem is the determination of the temperature T corresponding to the arbitrary points 1-3 of the fusion curve. The method used is to transfer the angle α made by the slope of the peak obtained in a DSC melting scan of a very pure organic standard (naphthalene), measured under identical conditions, to the trace of the unknown sample. This angle incorporates the final time necessary for melting.

In fact, the temperature of the pure sample in the process of melting remains constant and equal to T_S along the leading edge of the peak. Though the use of the same angle for different samples may appear unjustified, the method nevertheless works.

From the foregoing, it follows that the fraction of the substance melting at temperature T is given by $F = q/Q$. The plot of T *vs.* Q/q furnishes a straight line, the slope of which yields the value of T.

Application of the Schröder-van Laar equation [2] requires two additional data, which are likely to be unknown: the melting point T_R and the enthalpy of fusion H of the pure substance. The melting point of the pure substance may be taken as $T_R = T' + \Delta T$, where T' is the temperature of the termination of fusion, which corresponds to the top of the melting curve. The mole fraction X_M of the preponderant enantiomer in the unknown sample is calculated as follows:

$$\ln X_M = \frac{H}{R}\left(\frac{1}{T'+\Delta T} - \frac{1}{T}\right)$$

The measurements were made with a Perkin-Elmer DSC-1B instrument at a heating rate of 4 deg min^{-1}. The results of the determination of purity were:

(R)-sulfonate: $x = 99.38\% \pm 0.43\%$

(S)-sulfonate: $x = 99.58\% \pm 0.25\%$

Determination of the melting diagram

In a general way, phase diagrams describe the behaviour of systems that may consist of one or more components distributed in one or more phases as a function of variables such as temperature, pressure and composition. Consequently, important information is obtained by evaluation of the phase diagram. The evaluation of preliminary studies suggested the simple behavior of our substances. (R,S)-2,3-Dihydroxy-3-methylbutyl *p*-toluenesulfonate should exist in the form of a conglomerate separable by preference crystallization because:

– The melting points of the (R) and (S) enantiomers (106°) are essentially different from that of the (R, S) compound.

– The solubility of the (R, S) material is twice that of the (R) and (S) enantiomers.

– By the Kofler contact method, a simple eutectic was observed in the contact area of melting of the (R) and (S) enantiomers.

– Visual observation of the process of melting of the mixture of the (R) and (S) enantiomers indicates a phase diagram corresponding to a type of conglomerate.

By mixing the (R) and (S) sulfonates in a definite way, we produced samples for determination of the melting processes, in order to obtain a phase diagram of this system.

DSC measurements are not easy, because of the tendency of the material to supercool. Therefore, only the first heating curves were evaluated. The fusion peaks of the mixtures are very different (Fig. 2), depending on their composition.

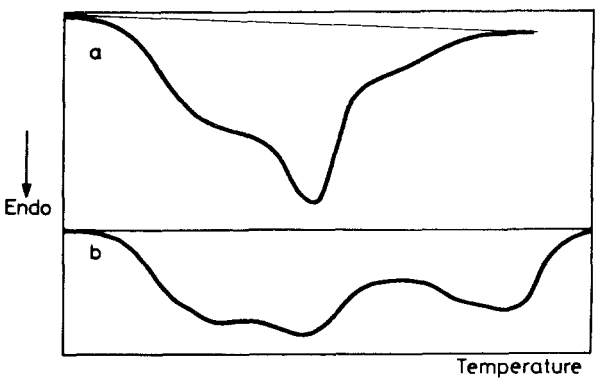

Fig. 2 Melting peaks of mixtures, a) 70% (S)-enantiomer, b) 80% (S)-enantiomer

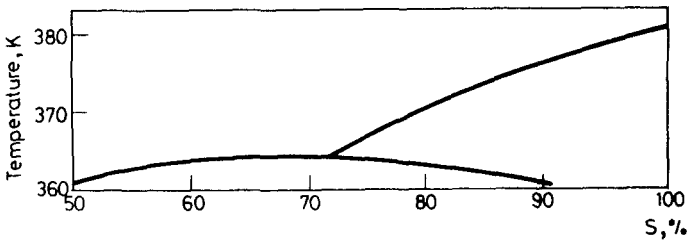

Fig. 3 Phase diagram of the system (R)-and (S)-sulfonate

The melting curves show at least two overlapping peaks for the compositions $70\% \leqslant S \leqslant 90\%$. The existence of three overlapping peaks for these compositions is very probable. The different shapes point to different melting processes of the components of the mixtures. The existence of a compound is possible. To achieve a more effective evaluation, melting points resulting from the maximum positions of the peaks are transferred to a phase diagram (Fig. 3). The phase diagram of the two enantiomers is symmetric. Therefore, the diagram determined by means of DSC is definite for an (S) enantiomer proportion of 50–100%.

It is seen from this diagram that at above 65% (S) enantiomer the melting peak changes to a double peak resulting from the mentioned melting process. Above 85% there is again only a single peak.

The conclusions from this phase diagram are that the components in the (R,S)-sulfonate do not crystallize separately; the (R,S)-sulfonate is a racemic compound and not a mixture. To confirm this result, we made X-ray diffraction measurements on the (R,S)-sulfonate, on its components, the (R) and (S)-sulfonate, and additionally on a mechanical mixture of the two components.

These measurements were made with an X-ray diffractometer and CuK_α radiation. Figure 4 presents some diffractograms. The diffractograms of the (R) and (S)-sulfonates exhibit no differences relative to the intensity and to the angles of all reflexes, as does the mechanical mixture, because the two components differ only in their steric structures.

However there are differences in the diffractogram of the (R,S)-sulfonate (Fig. 4 c) compared with those of the two components (Fig. 4 a,b). This result confirms the conclusion from the DSC examination that in the (R, S)-sulfonate the components do not crystallize separately. This also means that in this case the (R,S)-sulfonate is a compound. The results impressively demonstrate that DSC and X-ray diffraction measurements complement each other.

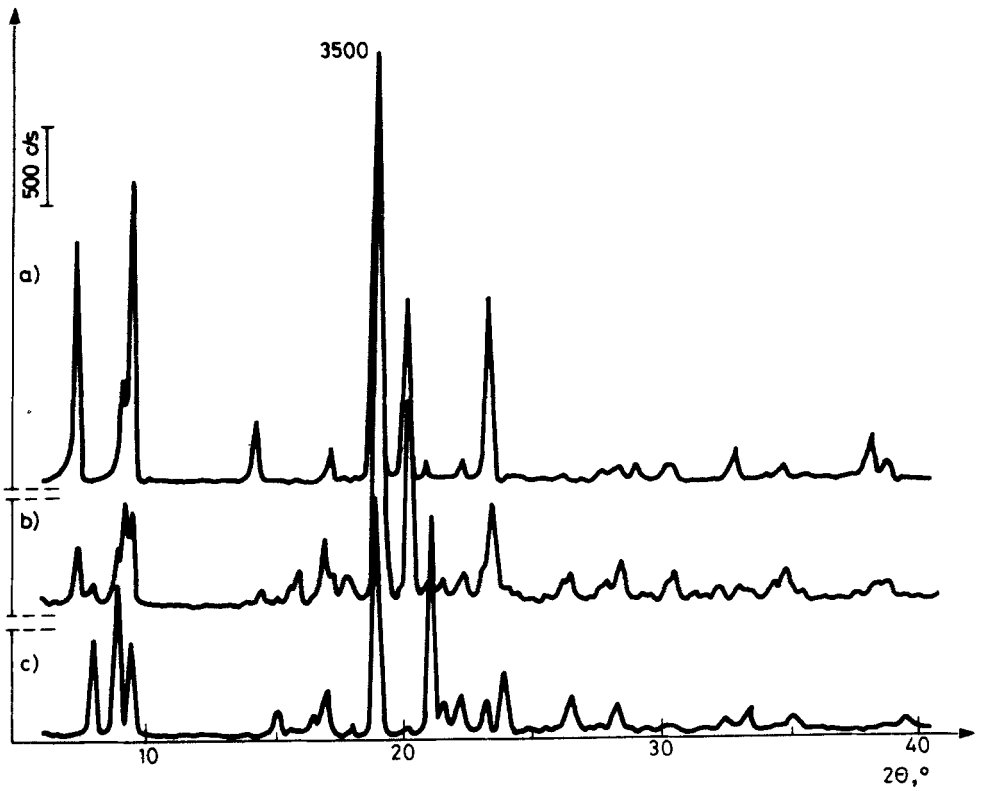

Fig. 4 Diffractograms of the sulfonates a) (R)-sulfonate, b) mechan. mixture of (R)-and (S)-sulfonate, c) (R,S)-sulfonate)

Conclusions

The results of these examinations demonstrate that the applicability of DSC for the characterization of enantiomers is very important for the development of new bioactive materials. By means of DSC we can gain characteristic data in a very short time.

The results of the measurements may be summarized in two points;

1. The determination of enantiomeric purity by means of DSC is more exact that by means of ^{1}H–NMR spectroscopy.
2. Evaluation of the DSC phase diagram demonstrates that the (R,S)-sulfonate is a racemic compound and not a mixture.

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Zusammenfassung – Die Enantiomeren-Reinheit des Vitamin D3-Metaboliten (R)-2,3-Dihydroxy-3-methylbutyl-*p*-toluensulfonat (R-I) lässt sich durch DSC genauer als mittels ^{1}H-NMR ermitteln. Dazu wird die Schmelzkurve nach der Partialflächenmethode ausgewertet, die auf der Schröder-van Laar'schen Gleichung beruht.

Zum Auffinden einer geeigneten Methode für die Abtrennung des R-Enantiomeren aus dem Racemat wurde das Phasendiagramm der Enantiomeren aus DSC-Messungen konstruiert. Das Vorliegen einer racemischen Verbindung wurde durch Röntgenbeugungsuntersuchungen von Racemat und reinen Enantiomeren bestätigt. Die Ergebnisse werden mit denen früherer Untersuchungen verglichen.

РЕЗЮМЕ — Энантиомерная гистота метаболита витамина D_3 была определена методом ДСК более точно, чем ЯМР спектроскопией. Кривая плавления обрабатывалась методом частичных поверхностей, основанном на уравнении Шрёдера-ВанЛаара. С целью нахождения приемлемого метода выделения (R)-2,3-диокси-3-метилбутил-п-толуолсульфоната из рацемической смеси, была определена фазовая диаграмма энантиомеров на основе ДСК измерений. Наличие рацемического соединения было подтверждено рентгеноструктурным исследованием отдельно рацемической смеси и энантиомеров. Проведено сравнительное обсуждение полученных заключений с результатами предыдущих исследований.