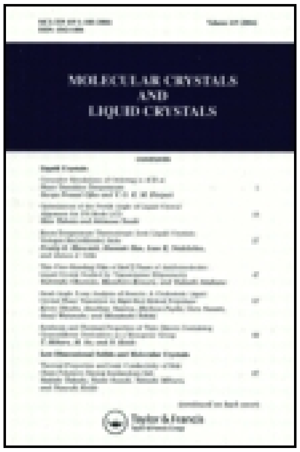

Molecular Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl15

Some Solubnility Characteristics of Cholesteryl Esters

G. Jerry Davis [a] & Roger S. Porter [a]
[a] Polymer Science and Engineering, University of Masssachusetts, Amherst, Massachusetts, 01002

Version of record first published: 21 Mar 2007.

To cite this article: G. Jerry Davis & Roger S. Porter (1969): Some Solubnility Characteristics of Cholesteryl Esters, Molecular Crystals, 6:3-4, 377-391

To link to this article: http://dx.doi.org/10.1080/15421407008083473

Molecular Crystals and Liquid Crystals. 1970. Vol. 6, pp. 377–391

Printed in Great Britain

Some Solubility Characteristics of Cholesteryl Esters

G. JERRY DAVIS and ROGER S. PORTER

Polymer Science and Engineering,
University of Masssachusetts,
Amherst, Massachusetts 01002

Received July 16 1969; *in revised form September* 23, 1969

Abstract—Heretofore, ethanol has been the most widely used solvent for the recrystallization of cholesteryl esters. In this study of some solubility characteristics of cholesteryl esters, *n*-pentanol is shown to be a more effective solvent than ethanol in which to remove impurities and/or crystalline structural defects from cholesteryl esters that can cause additional transitions on initial heating. *n*-Pentanol is shown to be a regular solvent for cholesteryl esters, whereas ethanol is not. This could be a major reason why *n*-pentanol is better able to discriminate between compound and impurity than ethanol. Contrary to previous reports, after crystallization from *n*-pentanol, in contrast to ethanol, cholesteryl acetate and cholesteryl laurate have only one transition on first heating. A detailed study of cholesteryl benzoate recrystallized from *n*-pentanol has confirmed that there are two transitions, one at 146.0 °C, the second at 179.6 °C. The heats of transitions are found to be 14.7 cal./gm. and 0.26 cal./gm. respectively.

Introduction

The importance of obtaining high purity esters of cholesterol for the study of their inherent properties, such as heats and temperatures of transition, cannot be overemphasized. Experience has demonstrated that impurities can create transitions, as for instance the appearance of a mesophase where none truly exists in a purer sample, or can inhibit the formation of mesophases which would be present in a purer sample. An example of transitions created by impurity is presented in Fig. 1. Published Perkin-Elmer Differential Scanning Calorimetry data on cholesteryl oleate show five transitions on heating from − 3 to + 57 °C (270 to 330 °K)[1],

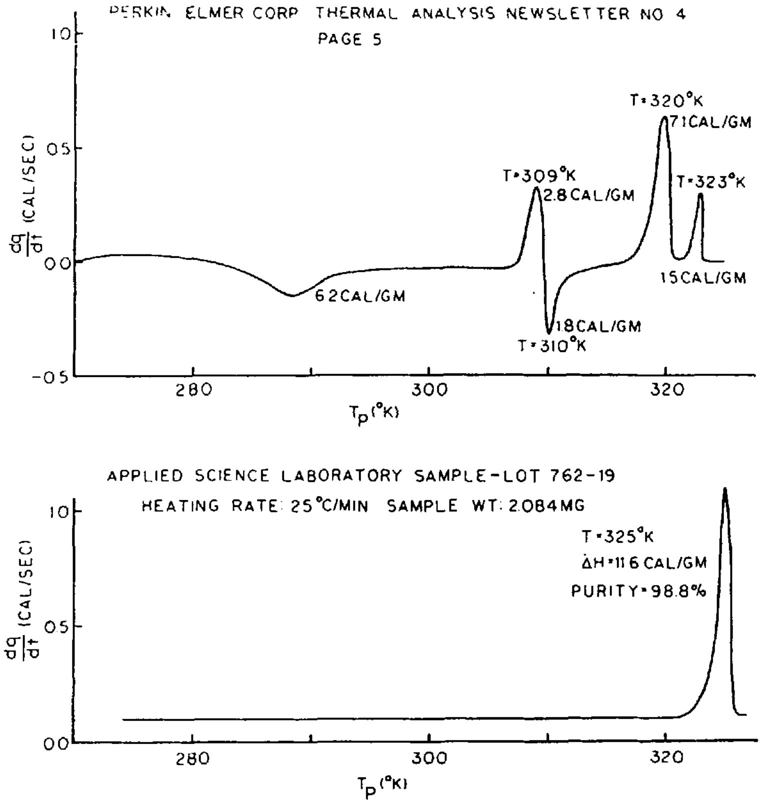

Figure 1. Cholesteryl oleate DSC traces.

whereas a 98.8%‡ pure sample from Applied Science Laboratories tested in this study on a Perkin-Elmer Differential Scanning Calorimeter (DSC) gives only a single transition on heating over the same temperature interval. An Imperial Chemical Industry, Ltd. study of cholesteryl oleate by DSC indicated three endothermic transitions on heating.[2] On cooling from the isotropic melt, the Applied Science cholesteryl oleate showed mesophases transitions at 46.4 °C and 41.8 °C of 0.18 cal/gm and 0.37 cal/gm respectively. Perkin-Elmer reported these mesophase transitions

---

‡ All purities quoted in this paper were estimated from the shape of the DSC traces. This method is extremely sensitive to impurities.

on cooling at 37 °C and 32.5 °C of 0.7 cal/gm and 1.2 cal/gm respectively.[1] The transitions of the Applied Science sample displayed on heating from the crystalline solid and cooling from the isotropic melt indicates that cholesteryl oleate is a montropic liquid crystal.

An example of mesophase removal by impurity can be illustrated with data on cholesteryl myristate. A 93% pure sample, obtained from Eastman Chemicals, gives only a suggestion of the two well-established smectic-cholesteric and cholesteric-isotropic liquid mesophase transitions for purer materials.

Cholesteryl benzoate is another important case of an ester whose reported transitions have been affected by impurities. The earliest investigators claimed two transitions (meaning one mesophase)[3]; a recent work has indicated four.[4] This solubility study will help explain why experimenters have expressed varied views on supposedly pure compounds.

The purity problem is understandable when one considers that cholesterol is a natural animal product which has to be separated from many similar steroids. Esterfied cholesterol samples supplied by a variety of manufacturers vary widely in purity, from 92.0 to 99.5%. The virtually exclusive purification practice involves solvent recrystallization. The solvent most widely used is ethanol. This purification procedure was followed at first, but only small improvements in cholesteryl ester purity were achieved, as measured by DSC trace shape. For more definitive studies, a more effective recrystallization process became necessary. Thus, a new purification solvent had to be chosen and a better procedure developed.

Development of Recrystallization Procedure

A. THEORY

The choice of an effective solvent for recrystallization is often stated as being a matter of trial and error. However, certain solvent characteristics, such as a high temperature coefficient of solubility for the substance to be purified, an absence of solvation, and an affinity for impurities are generally indicated as being desirable.[5] A high temperature coefficient of solubility permits either a good yield or a selective recovery of higher purity crystals, without excessive temperature change. The above characteristics would be fulfilled by a solvent which forms a regular solution with the esters of cholesterol, yet has a polarity different enough so that impurities of opposite polarity remain in solution.

Regular solutions are defined as those that possess ideal entropy of mixing, just as for ideal solutions, but have a positive enthalpy of mixing. In other words, a regular solution is one in which there is sufficient thermal energy to overcome the tendency of solute and solvent to segregate due to different molecular fields.[5] From this concept, the following relationship is set forth by Hildebrand and Scott for regular solutions, assuming negligible heat capacity effects:[5]

$$\mathrm{Log}\,(1/x_2) = \frac{\Delta H^f_m(T_m - T)}{4.575T_mT} + \frac{V_2}{4.575T}(\delta_1 - \delta_2)^2\phi_1^2 \qquad (1)$$

where:

x_2 = mole fraction of solute dissolved in solvent
ΔH^f_m = molar heat of fusion of solute in cal/mole
T_m = melt temperature of solute, °K
T = temperature of solution, °K
V_2 = molar volume of solute, c.c.
δ_1 = solubility parameter of solvent, $(\mathrm{cal/c.c.})^{1/2}$
δ_2 = solubility parameter of solute, $(\mathrm{cal/c.c.})^{1/2}$
ϕ_1 = volume fraction of solvent.

The first term in the equation represents the change in entropy when a solute and solvent are mixed together for an ideal solution in which the heat of mixing is zero and the molar volumes of the solute and solvent are the same. However, it is quite common for volume differences to be at least as large as a factor of three and ideality is still followed, e.g. Iodine-$\mathrm{SnI_4}$.[5] The second term represents the internal energy change from the variation in solvent-

solvent, solvent-solute, and solute-solute potential energy interaction. These effects are derived from London forces but exclude such interactions as hydrogen bonding.

B. EXPERIMENT

The concept of regular solution behavior was applied to the solubility of cholesteryl myristate in four common solvents—ethanol, toluene, acetone, and *n*-pentanol. Of particular interest was whether any of these four solvents functioned as a regular solvent, as solved by Eq. (1), in dissolving cholesteryl myristate.

The procedure applied to determine the mole fraction of solute x_2 dissolved in the solvent, was as follows:

1. Heat solvent to boiling in presence of an excess of ester.
2. Cool solution and the excess of solute to 28 °C.
3. Filter off the excess of insoluble solute.
4. Determine weight of filtrate.
5. Evaporate to dryness under vacuum.
6. Weigh solute.

The equilibrium solubilities for each solvent at 28 °C were as follows:

1. Toluene: $x_2 = 0.138$
2. Acetone: $x_2 = 0.00076$
3. *n*-Pentanol: $x_2 = 0.0026$
4. Ethanol: $x_2 = 0.00008$.

Note that precision and accuracy are considerably reduced at the lower concentrations. The solubility of cholesteryl myristate in ethanol should be considered to be equal to or less than the 0.00008 figure stated.

The solubility of cholesteryl myristate in toluene was so high that calculations by Eq. (1), using only the first term on the right hand side, as would be done for an ideal solution, predicted the solubility within 1.8%. This result indicated that the solubility parameter of cholesteryl myristate had to be within ± 0.35 of the 8.9 value for toluene. With this information, the second term of Eq. (1) could now be utilized to determine whether one of the other three solvents produced a regular solution. However, Eq. (1) requires the molar volume of the cholesteryl ester, in this case the myristate ester. This was estimated as follows:

1. The molar volume of cholesterol was calculated from its molecular weight of 386.66 and density of 1.067[6] to be 362 c.c.
2. The molar volume of myristic acid was calculated from its molecular weight of 228.36 and density of 0.8439[6] to be 270 c.c.
3. As an estimation of the molar volume of cholesteryl myristate, cholesterol was assumed to combine with the myristate acid by losing one molar volume of water of 18 c.c. Then the molar volume of cholesteryl myristate would be $362 + 270 - 18 = 614$ c.c.

This estimation is considered accurate only to ± 10%. Such an error in V_2, however, would lead to only a ± 3% error in δ_2.

n-Pentanol has a solubility parameter of 10.9.[5] Substitution of this value and the measured solubility of the myristate ester in *n*-pentanol into Eq. (1) gives a calculated solubility parameter of 8.9 for cholesteryl myristate. This value is so close, within experimental error, to the 8.9 ± 0.35 suggested by toluene that *n*-pentanol can be considered to function as a regular solvent. A similar substitution into Eq. (1) for acetone predicts a solubility parameter for cholesteryl myristate which is much too low, and for ethanol, a solubility parameter which is much too high. Acetone and ethanol appear to be highly non-regular solvents.

With a value of 8.9 taken as the solubility parameter for cholesteryl myristate, acetone should dissolve 0.04 mole percent and ethanol only 10^{-9} mole percent as regular solvents. The fact that ethanol appears to have about a 10^5 higher solubility capability than Eq. (1) would predict either that ethanol will be relatively insensitive as a purification solvent or worse, that ethanol is a poorer solvent for certain impurities than for the ester itself.

The solubility parameters for cholesteryl nonanoate and cholesteryl propionate, as determined from their solubility in *n*-pentanol, are 8.85 and 8.75 respectively. The similar solubility parameter values for these three esters of widely differing acid chain lengths indicates that the whole series of esters of aliphatic acids have close to the same solubility parameter.

Purification Procedure

With the identification of *n*-pentanol as a regular solvent for cholesteryl esters, the following recrystallization procedure was applied to obtain purified esters of cholesterol:

1. For a known volume of *n*-pentanol, add cholesteryl ester in the amount of 140 to 150% of saturation by weight based on calculations from Eq. (1).
2. Heat to dissolve; this temperature will be well below the *n*-pentanol boiling point of 138 °C.
3. Filter the hot solution through a standard No. 1 filter paper to remove undissolved particles.
4. Reheat to approximately 80 °C to insure solution.
5. Permit recrystallization to occur during cooling to room temperature. (For samples with low transition temperatures, recrystallization may be carried out at lower temperatures. 5 °C has been used.)
6. Filter off mother liquor.
7. Wash crystals with a solution of ethanol and water in a ratio of 3 : 1 to 9 : 1 by volume, depending on ester acid chain length; the longer the chain length, the less water is used. The wash solution is important for the removal of residual mother liquor containing impurity. These wash solutions are chosen to be relatively poor solvents for the crystals but not so incompatable that the crystals congeal and reduce wash efficiency. The water is necessary to avoid dissolving a substantial portion of the crystals to be washed. Note that as the size of the acid chain length increases, the solubility of the cholesteryl ester in ethanol decreased, and less water is needed.

8. Slurry crystals in the above wash solution. Crystals and water must be mingled to insure quantitative removal of impurities.
9. Refilter through standard No. 1 filter paper.
10. Place recrystallized sample in clean container and dry at 50 °C and 28 inch Hg vacuum (a lower temperature with a higher vacuum would have been more desirable if proper equipment were available).

The above procedure emphasizes the recovery of high purity esters at the expense of yield, since the sample size required for the DSC analysis of transition heats and temperatures is only 3 to 4 mg per run. All samples were tested on a Perkin-Elmer, Norwalk, Connecticut, Differential Scanning Calorimeter, Model DSC-1B.

Purification Results

A. Cholesteryl Laurate

In Fig. 2, DSC traces of three cholesteryl laurate samples, areas normalized to 2.000 mg, are shown as they occurred upon heating from 67 to 107 °C (340 to 380 °K) at a rate of 2.5 °C per minute. Note that the sample as received and the ethanol recrystallized compound both display an endotherm followed by an exotherm prior to the major transition. Another investigator[7] has reported a similar result for samples recrystallized from boiling ethanol. With the *n*-pentanol recrystallized sample, only the major transition occurred, indicating the ability of the *n*-pentanol recrystallization procedure to eliminate extraneous transition.

On cooling from the isotropic melt, cholesteryl laurate displays an isotropic liquid—cholesteric transition at 87.2 °C of 0.31 cal/gm and a cholesteric-smectic transition at 80.2 °C of 0.40 cal/gm. Gray also reports first one transition at 93 °C on heating cholesteryl laurate and the above two mesophase transitions at 90 °C and 83.5 °C on cooling.[8] These data indicate that cholesteryl laurate is a monotropic liquid crystal.

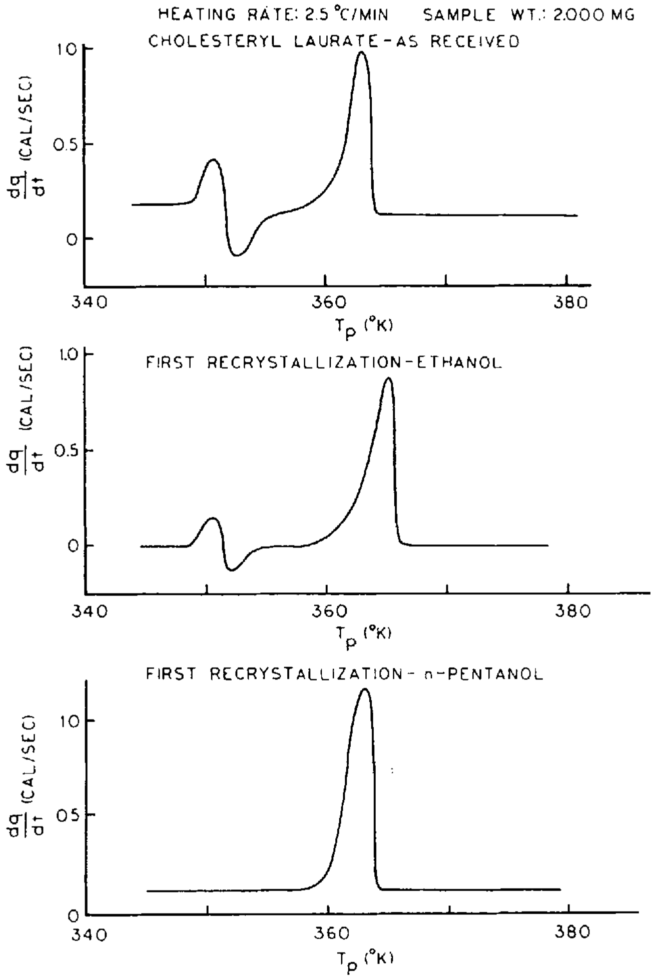

Figure 2. Cholesteryl laureate DSC traces.

B. Cholesteryl Benzoate

The effect of twice recrystallizing cholesteryl benzoate from *n*-pentanol is presented in Fig. 3. These are the normalized traces of an " as received," a first, and a second recrystallized sample, as they were heated from 127 to 187 °C (400 to 460 °K) at 5 °C per

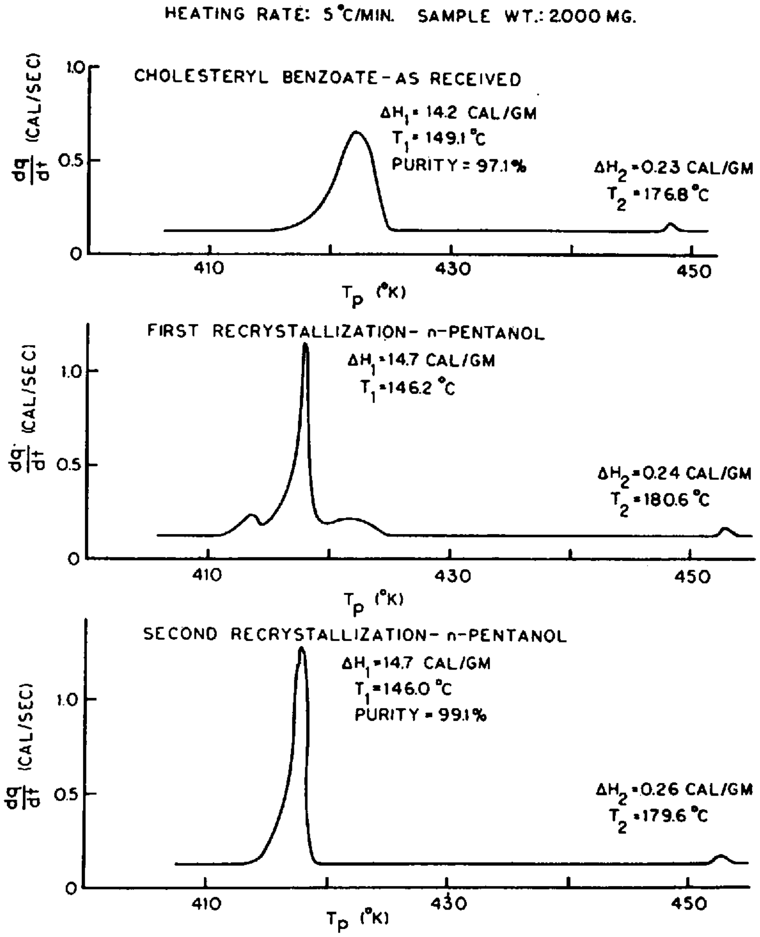

Figure 3. Cholesteryl benzoate DSC.

minute. After the first recrystallization, the major transition gave three peaks, approximately equal in area to the one previous peak. This same effect was found with an ethanol recrystallized benzoate ester. This behavior also has been previously obtained on samples recrystallized three times from boiling ethanol with the spurious inference that there were four transitions on heating cholesteryl benzoate.[4] However, the trace of the second *n*-

pentanol recrystallized sample shows a return to just two transitions. Each of the three samples presented in Fig. 3 gave only two transitions on cooling and reheating, as did the ethanol recrystallized samples of the present investigators and in an earlier study.[4] In the experience of this investigator, a cholesteryl ester transition is not meaningful unless the transitions that appear when a crystalline sample is heated also occur on heating that sample from the recrystallized melt. The second transition is quite small, about 0.26 cal/gm, and is spread out over 2.5 °C at a heating rate of 5 °C per minute, a wider temperature span than the typical cholesteryl ester of an aliphatic acid. This second transition appears to be extremely sensitive to heating rate. At a DSC heating rate of 2.5 °C per minute, the second transition could not be detected unless the size of the sample under test was increased from 1.6 to 2.6 mg.

Cholesteryl benzoate transition heats and temperatures for this investigation are presented in Fig. 3. The two transition temperatures, after the second recrystallization, of 146.0 and 179.6 °C agree reasonably well with those listed in the International Critical Tables[9] of 146.0 ± 1.0 °C and 178.5 ± 0.3 °C. The first heat of transition ever reported for the change of cholesteric mesophase to isotropic liquid was a value of 0.32 cal/gm (not a calorimetric measurement) by Schenck on cholesteryl benzoate in 1905[10] and is relatively close to the 0.26 cal/gm value of this study. Cholesteryl benzoate was the first liquid crystal ever confirmed[3].

C. OTHER CHOLESTERYL ESTERS OF ALIPHATIC ACIDS

The presence of impurities in cholesteryl esters can either create transitions or decrease heats and temperatures of transition. In Table 1, presenting a comparison of cholesteryl ester transition values, cholesteryl acetate is an example of the first type caused by impurities. The samples as received had two substantial transitions separated in temperature by 30–35 °C on first heating. After the first recrystallization in *n*-pentanol, only a single transition appeared at 114.6 °C whose transition heat was approxi-

TABLE 1. A Comparison of Cholesteryl Ester Transition Values, All Measured Recrystallizations from *n*-Pentanol

Sample(1)	Measured ΔH		% Purity	Reference ΔH(2)		% Diff.	Ref.
	Temp. °C	Cal./gm.	Est.(4)	Temp. °C	Cal./gm.	ΔH	Temp.(3) °C
Acetate							
Lower Trans.							
As Received	79.5	3.5					
1st Recry.	None	None	99.4	81–87	4.89		None
Higher Trans.							
As Received	111.2	8.1					
1st Recry.	114.6	11.4		118.2	11.2	1.6	116.5(5)
n-Propionate							
Cry.-Chol. Trans.							
As Received	89.9	7.1	93.2				
2nd Recry.	97.2	13.3	99.6	99.	12.5(6)	2.9	102.
Chol.-Iso. Trans.							
As Received	None	None					
2nd Recry.	113.0	0.17		115.3	0.23	−26.0	116.
n-Nonanoate(7)							
Cry.-Chol. Trans.							
As Received	77.0	9.6	96.6				
1st Recry.	77.8	11.4	98.5	80.8	10.2	12.	80.5
Chol.-Iso. Trans.							
As Received	90.5	0.24					
1st Recry.	91.7	0.25		93.0	0.22	−14.	92.
n-Heptadecanoate							
Cry.-Chol. Trans.							
As Received	75.4	18.6	93.8				
1st Recry.	76.3	22.8	98.4	(8)	(8)		
Chol.-Iso. Trans.							
~~As Received~~	78.3	0.43					
1st Recry.	79.7	0.52					

(1) Cholesteryl Acetate supplied by Coleman, Matheson, and Bell, Cholesteryl Propionate and Cholesteryl Nonanoate by Eastman Chemicals, and Cholesteryl Heptadecanoate by Applied Science Laboratories.

(2) Barrall *et al.*[11] obtained these temperatures by DTA and heats by DSC. The measured transition temperatures are somewhat lower than these values. This result is not attributed to purity but to differences in instrumentation and calibration methods.

(3) Gray[8] recorded these temperatures for purified synthetic samples of the esters (rather than commercial esters) after measurement by optical microscopy in conjunction with a microscope heating stage.

(4) Purity estimate refers to measured samples.

(5) Gray reports a cholesteric transition at ca. 90 °C on cooling.[8] The 99.4% pure cholesteryl acetate of the present investigators had already recrystallized at 102.5 °C on cooling.

(6) Barrall *et al.*[11] detected another transition at 110 °C of 0.43 cal/gm on first heating. The % difference in ΔH is based on the sum of 12.5 cal/gm and this value of 0.43 cal/gm.

(7) Barrall *et al.*[11], Leclerq *et al.*[12], and the present investigators found an additional transition on cooling of 0.11 cal/gm at 66 °C, 0.16 cal/gm at 76.5 °C or 0.14 cal/gm at 74.8 °C respectively. Gray noted this monotropic smectic transition at 77.5 °C.[8]

(8) No previous transition values have been reported for n-heptadecanoate.

mately the sum of the two original transition heats. A sample as received was recrystallized from ethanol. A DSC trace of this recrystallized sample had the same two transitions as the original uncrystallized compound. Previously published DSC data on ethanol recrystallized cholesteryl acetate also indicated two transitions.[11] The single transition value represents an improvement over the two transition values.

The other three esters in Table 1, *n*-propionate, *n*-nonanoate, and *n*-heptadecanoate exhibited increased transition heats and temperatures at higher purities after recrystallization from *n*-pentanol. At 93.2% purity, and lower the reported cholesteric-isotropic liquid transition for the *n*-propionate ester was apparently eliminated. The major heats of transition of these esters (the crystal-mesophase transitions), as recorded by the DSC, were increased anywhere from 20 to 80 percent.

Conclusions

1. Heretofore, ethanol has been the most widely used solvent for the recrystallization of cholesteryl esters. *n*-Pentanol has been shown here to be a more effective solvent than ethanol in which to remove impurities and/or crystalline structural defects from cholesteryl esters that can cause additional transitions on initial heating. In addition, higher purity cholesteryl esters have been observed to exhibit significantly increased heats and temperatures of transition.

2. *n*-Pentanol has been shown here to be a regular solvent for cholesteryl esters, whereas ethanol is not. This could be a major reason why *n*-pentanol is better able to discriminate between compound and impurity than ethanol.

3. After recrystallization from *n*-pentanol, in contrast to ethanol, cholesteryl acetate and cholesteryl laurate have only one transition on first heating. This is contrary to previous reports.[7,11]

4. A detailed study of cholesteryl benzoate has confirmed that there are two transitions, one at 146 0 °C, the second at 179 6 °C. The heats of transition are found to be 14.7 cal/gm and 0.26 cal/gm respectively.

Acknowledgement

The authors express sincere appreciation to the National Institutes of Health, 5-ROI-HE11342, for support of this work.

REFERENCES

1. Perkin-Elmer Corporation, *Thermal Analysis Newsletter* **4**, Norwalk, Connecticut, 5.
2. Personal Communication.
3. Reinitzer, F., *Wiener Monatsschr. Chem.* **9**, 421 (1888).
4. Unpublished data by Barrall, E. M. II, Johnson, J. F. and Porter, R. S.
5. Hildebrand, J. H. and Scott, R. L., *The Solubility of Nonelectrolytes*, Dover Publications, 1964.
6. *Handbook of Chemistry and Physics*, 49th Edition, The Chemical Rubber Company, 1968–69.
7. Barrall, E. M. II, Johnson, J., F. and Porter, R., S., " The Homologous Series of Aliphatic Esters of Cholesterol Thermodynamic Properties," *Thermal Analysis* 1, Schwenker, Jr., R. F. and Gain, P., D., Academic Press, 555 (1969).
8. Gray, G., W., *J. Chem. Soc.*, 3733 (1956).
9. *International Critical Tables*, Volume 1, 319 (1926).
10. Schenck, R., *Crystalline Liquids and Liquid Crystals*, W. Engelmann Publisher, Leipzig, 1905.
11. Barrall, E. M. II, Porter, R., S. and Johnson, J., F., *J. Phys. Chem.* **71**, 1224 (1967).
12. Leclerq, M., Billard, J. and Jacques, J., *Compt. Rend.* **264**, 1789 (1967).