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Remarks on the Synthesis of Pure Mesomorphic Compounds

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Abstract—The effect of impurities in sterol derivatives upon the mesomorphic behavior cannot be estimated or extrapolated from measured data. Therefore, it is imperative to use pure materials for meaningful measurements. All possible sources for impurities in mesomorphic sterol derivatives are assessed, and methods feasible for the detection of contaminents at levels of 1% or less are described.

Thin layer chromatography (TLC) is the common method for purity determination of mesomorphic sterol derivatives. Most impurities such as unreacted starting materials and side products can be detected by this method. This technique also allows comparison of identical materials which were synthesized by independent and different reaction paths, and is demonstrated for the homologous series of 5α-cholestanyl *n*-alkyl carbonates. However, TLC is not sensitive enough to detect small amounts of impurities, which are very similar in structure and behavior to that of the mesomorphic compound. Therefore, a more sensitive analytical method for the detection of impurities in sterol derivatives is needed.

Gas chromatography is an analytical approach which offers a big increase in sensitivity over TLC. Unfortunately, mesomorphic sterol derivatives are not suited for direct gas chromatographic analysis. Nevertheless, this method is essential for the quality control of starting materials. Furthermore, the detection of homologues in mesomorphic compounds is possible by suitable modification of gas chromatographic procedures. For example, a transesterification was adapted for esters of sterols and is demonstrated in detail for cholesteryl hexadecanoate. Other methods for the analysis of carbonates and thiocarbonates of sterols are proposed.

Background

One of the major problems for the study of mesomorphic compounds is the preparation of pure materials.[1] Purification of organic compounds usually is handled in a routine manner with

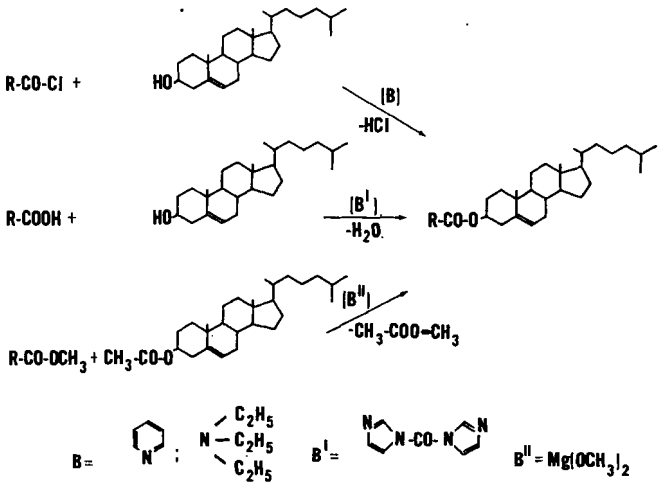
the main effort concentrated on a high yield. An organic compound is considered pure, when it has a sharp melting point which cannot be increased by repeated recrystallizations. However, this criterion is not sufficient for a mesomorphic compound because its melting point depends on the thermal history of the sample,[2] and is less characteristic of the purity. The clearing (mesomorphic → isotropic) and transition (mesomorphic^{I} → mesomorphic^{II}) points are a better indication of purity for a mesomorphic material because they are more closely related to the chemical constitution of the compound,[3] and therefore more susceptible to impurities. Discrepancies in transition temperatures[4,5] reported for the same compound might be explained by different impurities.

Being primarily interested in cholesteric compounds we have concentrated on the esters, the carbonates, and thiocarbonates of 3β-sterols. For these derivatives we differentiate between dissimilar impurities formed by side reactions, and impurities similar in structure which were formed in an analogous fashion to the main products.

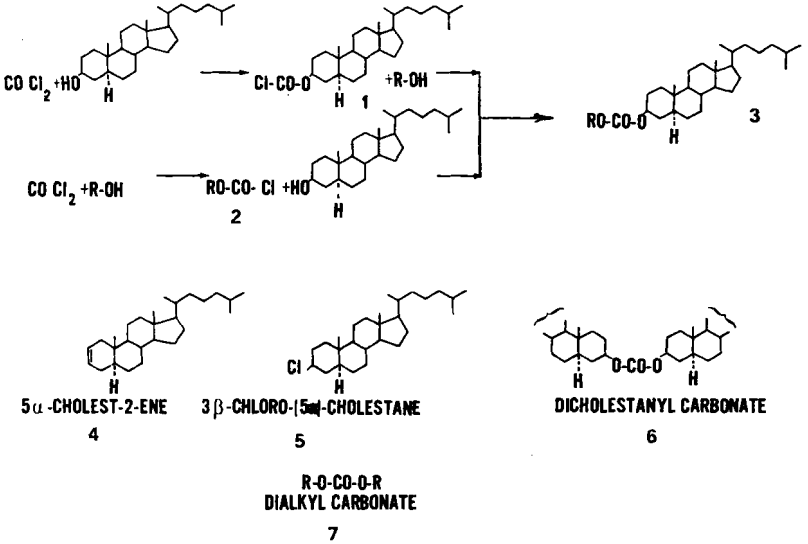
The former impurities can be removed by ordinary means and thus require little discussion. But complete analysis of the problems associated with similar impurities must be considered since they are both difficult to detect and to remove.

Impurities Obtained by Side Reactions

The dissimilar impurities in mesomorphic compounds include unreacted starting materials, side products of the reaction, and all decomposition products. These may stem from any deviation of the reaction or from mistreatment of sensitive compounds in the course of preparation. The amount of side products is reduced by using high-purity starting materials, and by selecting a mild and efficient reaction method for the synthesis. If a mesomorphic material is prepared by different and independent reaction schemes, the chance of overlooking an impurity is further reduced.



SCHEME I



SCHEME II

For the preparation of cholesteryl esters we prefer the second reaction path of scheme I carried out in an adapted version of Staab's esterification.[6,7] This reaction allows the direct utilization of acids and produces a high yield with very little side products accompanying the esters. It can also be used for the preparation of thioesters.

The preparation of carbonates or thiocarbonates is demonstrated for the case of 5α-cholestanyl *n*-alkyl carbonates. According to the reaction scheme two intermediates are possible. Depending which of the chloroformates is prepared in the first step, the amount and type of side products can be influenced. In the given example, the preparation of 5α-cholestanyl chloroformate (1) in the first step increases the formation of 5α-cholest-2-ene (4), 3β-chloro-5α-cholestane (5), and dicholestanyl carbonate (6). These side products are of less importance if the alkyl chloroformate (2) is selected as intermediate. In this case one has to anticipate primarily dialkyl carbonate (7). Both methods give comparable yields of pure carbonate (3). For the corresponding homologous series of 5α-cholestanyl S-alkyl thiocarbonates only the first reaction path is practical. Due to their unpleasant properties, the mercaptans always should be introduced into the reaction at the latest point.

The impurities thus far mentioned can be removed by ordinary purification methods combined with chromatographic procedures. A careful preparation can yield mesomorphic compounds with a purity near 99%. Since these methods are standard procedures, they will not be discussed in greater detail.

Impurities Introduced by Starting Materials

The impurities remaining after ordinary purification are the derivatives of homologues and isomers, which are present in the starting material. Since all homologues and some isomers undergo the identical reaction, the resulting impurities are carried together with the main product through all steps of purification. They are almost identical in structure and properties to the

attempted mesomorphic compound and cannot be separated by ordinary means or more often not by any means. This is demonstrated for the homologous series of 5α-cholestanyl *n*-alkyl carbonates in Fig. 1. While the dissimilar impurities are well

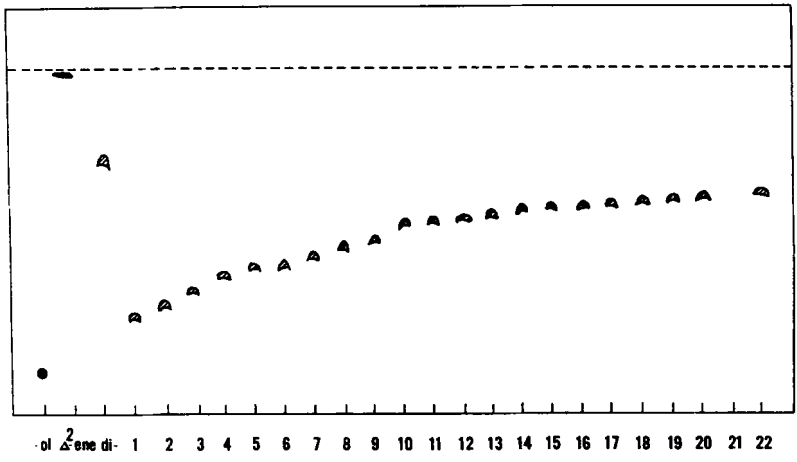


Figure 1. Cholestanyl *n*-alkyl carbonates

separated, the R_F-values for the homologues are very close. Therefore, it is rather hopeless to detect by TLC homologous impurities of 1% or less accompanying the mesomorphic compound.

Since the present state-of-the-art does not allow a direct detection of similar impurities in mesomorphic compounds of the sterol type, the only way to assure pure compounds for meaningful measurements is a careful control of the impurities which accompany the starting material. In this connection, it is important to know the source of a chemical, because this will determine the purification method and the likely remaining contaminants. For these impurities the most sensitive detection methods have to be applied.

Sterols are obtained from biological materials and are isolated in purities rarely exceeding 95%. For cholesterol Fieser[8] worked

out an effective purification method. This preparative procedure yields a material which is uniform as judged by TLC and which does not reveal a single impurity in carefully developed partition chromatograms. For the other sterols analytical and preparative methods are needed. The best analytical method available for sterols is gas chromatography which offers a sensitivity exceeding that of TLC. Sterols can be analyzed in the form of their trimethyl silanyl ethers[9,10] or even in free form when phases of the OV-series are used.[11]

Alcohols come either from synthesis or originate from biological materials. The lower members in general are of very high purity, with the exception of *n*-hexanol. An isomer of *n*-hexanol could only be separated by distillation over a spinning band column at a reflux ratio of 100 : 1. The higher alcohols of even-numbered chain length are isolated from biological materials and are contaminated with homologues. Unfortunately, they also represent the most convenient starting material for the synthesis of odd-numbered alcohols. The most sensitive analytical method for impurity detection in alcohols is gas chromatography. Even tridecanol and higher alcohols can be analyzed on phases of the OV-series, which are stable to 300°.

Mercaptans have the same origin as the corresponding alcohols, thus the above purification and detection methods apply with very little modification.

Acids and acid chlorides originate from the same sources as alcohols. The lower members are obtained by synthesis, the higher even-numbered are isolated from biological materials, and the higher odd-numbered are prepared synthetically from the even ones. The direct analysis by gas chromatography is generally not possible. However, since it is no problem to prepare the methyl esters,[12] reliable results can be obtained indirectly. We used columns with the phase EGSS-X operated isothermally or with temperature program.

Figure 2 illustrates the trace of methyl ester standards (A), the trace obtained from a practical grade (B), and from 99.5% pure hexadecanoic acid (D). By increasing the sensitivity of the

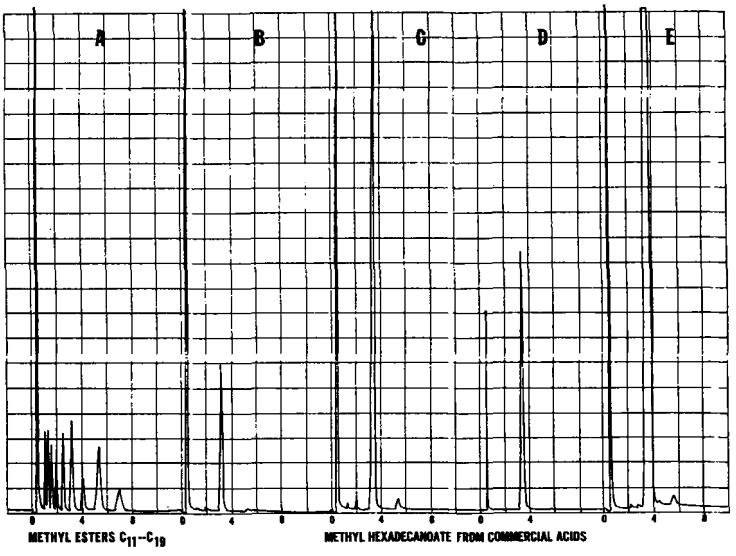


Figure 2

instrument the impurities can be identified. The practical grade hexadecanoic acid (C) contains dodecanoic, tetradecanoic, and octadecanoic acid; the 99.5% pure acid (E) contains octadecanoic acid and traces of three other contaminants still unidentified.

Impurity Detection in Mesomorphic Sterol Derivatives

The analytical methods discussed for the starting materials greatly increase the chance for the preparation of pure mesomorphic sterol derivatives. But they also point out the need for a sensitive method by which the final product can be analyzed in regard to homologues and other hidden impurities. TLC lacks the sensitivity, and gas chromatography cannot be used in a direct manner. Thus, the best promise lies with indirect gas chromatographic procedures.

To compare the purity of commercially obtained cholesteryl

esters with those prepared in our laboratory we adapted Metcalfe's transesterification[13] and analyzed the obtained methyl esters by gas chromatography. This method does not introduce new side products and leads to reproducible results. Next to the methyl ester standards, Fig. 3 depicts traces obtained from

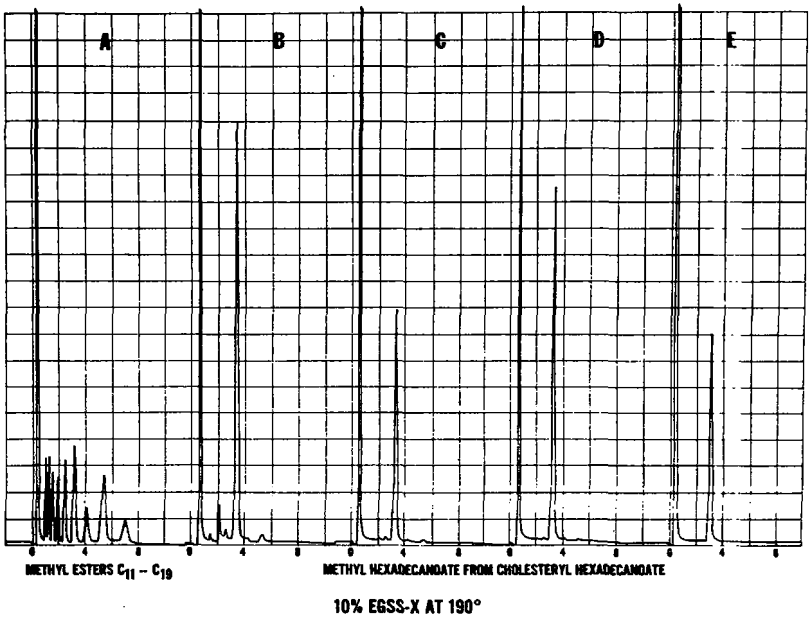


Figure 3

commercial cholesteryl hexadecanoate. Trace B is from purchased product, trace C from the same product after three recrystallizations, and trace D after column chromatographic purification. In comparison, trace E was obtained from cholesteryl hexadecanoate, which had been prepared from purified cholesterol and a 99.5% pure hexadecanoic acid by Staab's method,[3] and followed by column chromatography. These traces indicate that recrystallization removes the more distant homologues to an acceptable degree. However, for close homologues also column chromatography is not a perfect method of separation. Thus, it

is again exhibited that the best assurance for pure mesomorphic derivative is the use of pure starting materials.

The sensitivity of the employed analytical method can be readily appreciated from Fig. 4, which shows the traces of methyl

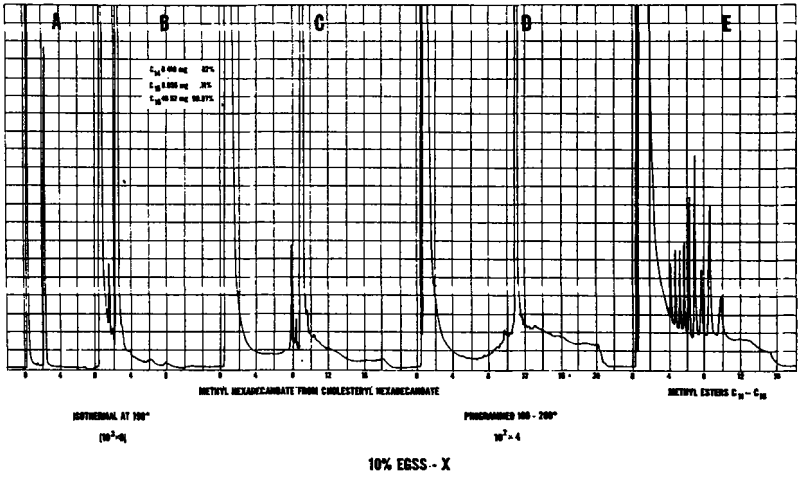


Figure 4

esters obtained from mixtures of cholesteryl esters. A matrix of cholesteryl hexadecanoate contained about 1% of cholesteryl tetradecanoate and 0.1% of cholesteryl pentadecanoate. Comparison of traces C and D show that even the 0.1% impurity can be detected.

Although all starting materials for carbonates and thiocarbonates of sterols can be well analyzed, we still need a reliable method to detect impurities in the final mesomorphic compound. Similar to the transesterification method, an acetylation procedure might be adopted by which the acetates of alcohols and mercaptans can be obtained and gas chromatographically analyzed. Another possible method is a modified pyrolysis as it is used for the gas chromatographic analysis of polymers. This latter method we propose would be a " controlled fragmentation ". In analogy to the Tschugaeff reaction[14] the carbonates should thermally

break down into carbon dioxide, aliphatic, and sterol component. The last two most likely will be in the form of olefines and unsaturated steranes. Using a general purpose column with a phase stable to at least 300 °C, the fragments should all be analyzable with a single run.

Summary and Conclusion

The experimental evidence points out that reasonably pure mesomorphic sterol derivatives can be obtained by employing the purest starting materials, very mild reaction conditions, independent synthesis via different reaction schemes (if necessary and possible), and subsequent chromatographic purification. This preventative care is reflected in the regular dependence of transition temperatures for homologous series. However, the data for a few compounds do not fit the smooth curves, and thereby give a strong indication that undetected impurities might still be present in the mesomorphic material. This clearly suggests that the purity of the starting materials, especially that of the sterols, has to be improved. Furthermore, a sensitive analytical method has to be devised for the final testing of mesomorphic compounds.

TLC, which is successfully used for the detection of contaminants with different structure than the mesomorphic compound, is not sensitive enough for homologous impurities of less than 1%. Gas chromatographic procedures offer a big increase in sensitivity over TLC but are not suitable for the direct analysis of mesomorphic sterol derivatives. Indirect analytical methods were sought which would bypass its shortcomings without losing resolution. A transesterification adapted for the cholesteryl esters was so successful that similar indirect methods for carbonates and thiocarbonates of sterols are presently under development.

Acknowledgments

The authors are indebted to Drs. R. D. Ennulat and L. M. Cameron for many stimulating discussions, and to Messrs. A. J.

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Experimental Part

All preparations were carried out in standard laboratory glassware. The reactions were run in an atmosphere of purified nitrogen which was free of oxygen. One example for each reaction type is given in detail. All temperatures listed are in centigrades.

5α-Cholestanyl *n*-alkyl Carbonates

Route A: 5α-Cholestanyl *n*-hexadecyl carbonate. To a solution of 1.95g (8 mmoles) *n*-hexadecanol and 3.61g (8 mmoles) 5α-cholestanyl chloroformate in about 80 ml dry benzene was added 1 ml (12.5 mmoles) pyridine. After four hours heating under reflux, the precipitated pyridinium chloride was filtered off and the benzene was removed. Purification of the crude carbonate was achieved by column chromatography, using silica gel as adsorbent and a 15/85 mixture of benzene and *n*-hexane as eluent. 5α-cholestanyl *n*-hexadecyl carbonate obtained from the main fractions was twice recrystallized from butanone/acetone; yield 4.15g (79%), mp. 83–84.5°.

Route B: 5α-Cholestanyl *n*-hexadecyl carbonate. In 80 ml of dry benzene was dissolved 2.45g (8 mmoles) *n*-hexadecyl chloroformate and 3.11g (8 mmoles) cholestanol. While stirring, 1 ml (12.5 mmoles) pyridine was added at room temperature causing a white precipitation. After 4 hours of reflux, the precipitated pyridinium chloride was filtered off and the solvent was removed. 5α-Cholestanyl *n*-hexadecyl carbonate was purified as above; yield 3.85g (73%), mp. 83–85°.

Analytical data for 5α-cholestanyl *n*-hexadecyl carbonate are given in Table 1 together with data for all homologous carbonates of this series. Data of calorific measurements have been reported in another connection by R. D. Ennulat of our Laboratory. In order to give a complete accounting of the properties of 5α-cholestanyl *n*-alkyl carbonates their transition temperatures are given again.

TABLE 1 5α-Cholestanyl *n*-Alkyl Carbonates

R (alkyl)	Rt.	yield %	mp., °C	formula	mol. wt.	Analytical Values % calculated C	calculated H	calculated O	found C	found H	found O
methyl	A	91.5	97–100	$C_{29}H_{50}O_3$	446.7	77.97	11.28	10.74	78.05	11.11	11.05
ethyl	B	85.5	104–106.5	$C_{30}H_{52}O_3$	460.7	78.21	11.37	10.42	78.11	11.37	10.53
propyl	A	86	107–109	$C_{31}H_{54}O_3$	474.8	78.43	11.46	10.11	78.38	11.25	9.94
butyl	B	85	85–87	$C_{32}H_{56}O_3$	488.8	78.63	11.55	9.82	78.69	11.38	9.96
amyl	A	89.5	71–73	$C_{33}H_{58}O_3$	502.8	78.83	11.63	9.55	79.00	11.60	9.44
hexyl	B	81	69–71	$C_{34}H_{60}O_3$	516.9	79.01	11.70	9.29	79.20	11.74	9.48
heptyl	A	88.5	52 (cp 65)	$C_{35}H_{62}O_3$	530.9	79.19	11.77	9.04	79.36	11.54	8.70
octyl	B	86.5	62.5–64	$C_{36}H_{64}O_3$	544.9	79.35	11.84	8.81	79.16	11.79	8.93
nonyl	A	85.5	65–67	$C_{37}H_{66}O_3$	558.9	79.51	11.90	8.59	79.71	11.66	8.69
decyl	B	85.5	69.5–71	$C_{38}H_{68}O_3$	573.0	79.66	11.96	8.38	79.57	11.80	8.72
undecyl	A	86	66–68	$C_{39}H_{70}O_3$	587.0	79.80	12.02	8.18	79.74	11.96	8.27
dodecyl	B	86.5	76–77.5	$C_{40}H_{72}O_3$	601.3	79.95	12.07	7.98	80.09	11.84	8.08
tridecyl	A	90	71–74	$C_{41}H_{74}O_3$	615.0	80.07	12.13	7.80	79.99	12.15	7.55
tetradecyl	B	76.5	80.5–82.5	$C_{42}H_{76}O_3$	629.1	80.19	12.18	7.63	80.33	12.05	7.65
pentadecyl	A	87	78–80	$C_{43}H_{78}O_3$	643.1	80.31	12.23	7.46	80.04	12.03	7.38
hexadecyl	A, B	79	83–84	$C_{44}H_{80}O_3$	657.1	80.42	12.27	7.30	80.46	12.32	7.42
heptadecyl	A	83.5	79–82	$C_{45}H_{82}O_3$	671.1	80.53	12.32	7.15	80.45	12.43	7.09
octadecyl	B	73	88.5–90.5	$C_{46}H_{84}O_3$	685.2	80.64	12.36	7.00	80.90	12.39	7.06
nonadecyl	A	75	75–78	$C_{47}H_{86}O_3$	699.2	80.74	12.40	6.86	80.54	12.30	6.85
eicosyl	A, B	80.5	85–87	$C_{48}H_{88}O_3$	713.2	80.83	12.44	6.73	80.95	12.43	6.68
docosyl	A, B	82.5	84–86	$C_{50}H_{92}O_3$	741.3	81.02	12.51	6.48	80.96	12.33	6.45

Note: Microanalyses were carried out by Schwarzkopf Microanalytical Laboratory, Woodside, New York 11377.

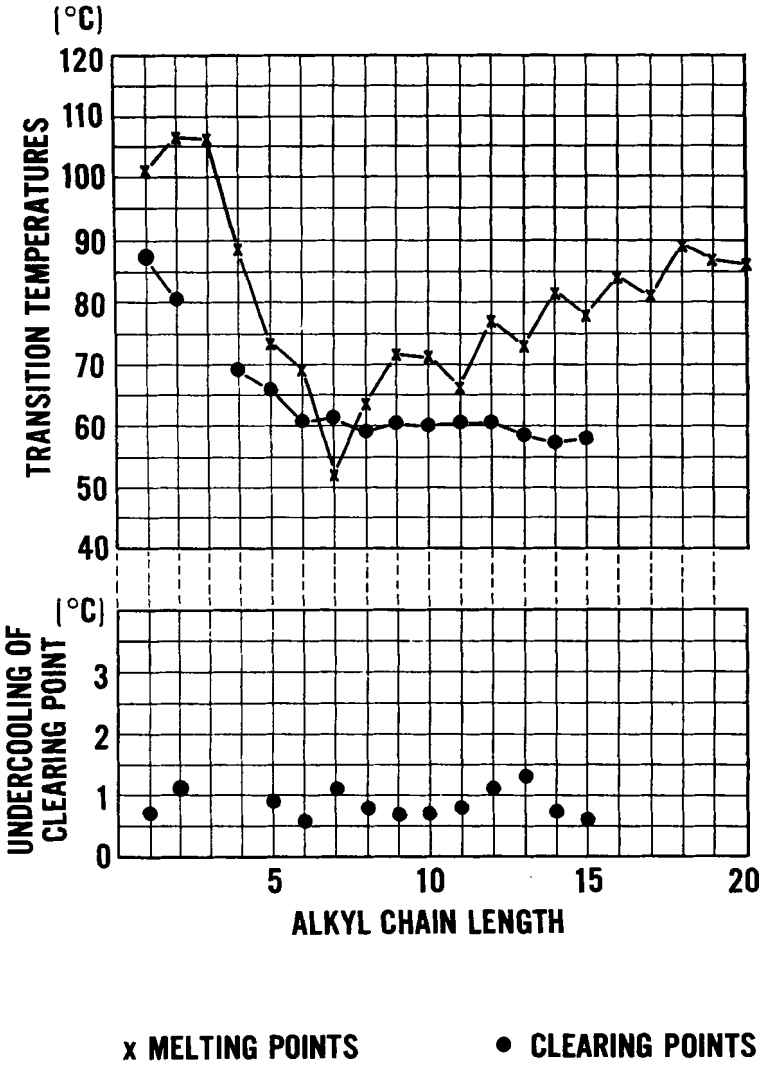

Figure 5. Transition temperatures of cholestanyl *n*-alkyl carbonates

Di-5α-cholestanyl Carbonate

To a solution of 3.89 g (10 mmoles) 5α-cholestanol and 4.51 g (10 mmoles) 5α-cholestanyl chloroformate in 100 ml dry benzene was added a solution of 1 ml pyridine (12.5 mmoles) in 10 ml benzene. A white precipitation formed at room temperature. After 4 hours heating under reflux pyridinium chloride was filtered off and benzene was distilled off. The crude dicarbonate was purified by column chromatography using silica gel as adsorbent and a 3/7 mixture of benzene/ligroine for elution. The purified di-5α-cholestanyl carbonate was twice recrystallized from butanone, yield 7.3 g (91%), mp. 173–175°, cp. 250–253°.

$C_{55}H_{94}O_3$	(803.4)	calcd.	C 82.23	H 11.79	O 5.98
		found	C 82.15	H 11.69	O 6.05

Transesterification

In 5 ml methanol containing 2 mmoles sodium hydroxide was heated under reflux 50 mg (80 micro moles) cholesteryl hexadecanoate. After 30 minutes the ester had completely dissolved. To the cold solution was added 5 ml methanol containing (14%) 6 mmoles boron trichloride. After 5 minutes at room temperature the sample was again heated under reflux for 15 minutes.

Saturated sodium chloride solution and 2 ml *n*-hexane were added to the flask. The methyl ester mixture was extracted with more *n*-hexane. A white solid separated from the neutralized and washed organic phase. TLC proved this compound to be cholesterol. The filtered organic phase was concentrated. 1 μl of the remaining solution was injected into an analytical gas chromatograph.

Gas Chromatographic Analysis of Esters

Gas chromatographic analyses were carried out with a Hewlett-Packard Model F & M 5756–B. The presented traces were obtained with the following settings of the instrument:

Methyl Hexadecanoate (Figs. 2 and 3)

Column: 6 ft, 4 mm I.D.; 10% EGSS–X on Gas-Chrom P,

100/120 mesh; carrier gas He, 50 ml/min; on-column injection; injector 230°, oven 190°, TC-detector 270°; attenuation: 4 or 2.

METHYL HEXADECANOATE (Fig. 4)

Column: 6 ft, 4 mm I.D.; 10% EGSS–X on Gas-Chrom P, 100/120 mesh; carrier gas He, 50 ml/min; on-column injection; injector 230°; temperature program 100° (1 min), heating 20°/min to 200° (4 to 8 min). Dual flame detector 320°, H_2 30 ml/min, air 450 ml/min; attenuation: 8×10^3 or 2×10^2

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