Solvent Effects on the Nuclear Magnetic Resonance Spectra of Methyl Hydroxystearates[1,2]

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Abstract

High resolution NMR spectra of all the isomeric methyl hydroxystearates have been measured in carbon tetrachloride, pyridine, and quinoline. As a result of association with the solvent, spectra in pyridine, and particularly in quinoline, are considerably different from those in carbon tetrachloride. The two pairs of methylene groups on either side of the CHOH grouping are deshielded by the associated solvent molecule giving a new signal at 1.58 ppm in pyridine, and at 1.75 ppm in quinoline. Deshielding of the terminal CH_3 occurs in isomers with hydroxyl near the methyl end, and of the methylene alpha to the ester group in isomers with hydroxyl near the ester end of the chain. When the spectra of isomers substituted near the center of the chain are observed in quinoline, the major methylene signal is split into two peaks due to differential shielding by the solvent. The differences between the spectra of each isomer measured in quinoline are sufficiently large for use as a method of identification.

Introduction

THE USE OF PHYSICAL methods to identify and separate the isomeric methyl hydroxystearates was discussed previously (1). Nuclear magnetic resonance (NMR) is another valuable technique giving information about fatty acids which would be difficult to obtain otherwise. NMR spectra of a number of fatty acids were examined by Hopkins and Bernstein (2, 3). Applications of NMR to specific fatty acid problems such as estimation of oleic, linoleic, linolenic, and other unsaturated acids have been made by Johnson and Shoolery (4), Glass and Dutton (5), and Hashimoto et al. (6). The method has been reviewed by Harlan (7) and by Hopkins (8) who extended his earlier work by the inclusion of spectra of isomeric acids. Most of the work has been on nonoxygenated fatty acids, but Hopkins (3,8) examined 12-hydroxystearic acid and assigned the NMR signals to the different protons.

In the majority of applications of NMR to organic chemistry for determination of compound structure as distinct from theoretical studies with simple compounds, spectra have been measured in aliphatic solvents such as carbon tetrachloride and deuterochloroform, which do not interact appreciably with the solute. However, recently studies have been made in the steroid and terpene fields using aromatic solvents (9–11). The present work shows that more information can be obtained by using solvents which form complexes with the solute. Also, by studying the spectra of all 17 isomeric hydroxystearates, long range interactions of functional groups with each other and with methylene groups of the fatty acid chain have been investigated.

Materials and Methods

Hydroxy esters were prepared as previously described (1) except for methyl 13-hydroxystearate which was derived from methyl 13-oxostearate obtained by the 12-carbon chain extension method of Hünig and Buysch (12); methyl 13-hydroxydocosanoate was prepared in an analogous manner. 7-Hydroxyoctadecane was synthesized by anodic coupling of 12-hydroxystearic acid with acetic acid. Spectra were recorded at 32C using a Varian HA-100 spectrometer and 5% solutions of the esters. Analytical grade solvents were used; pyridine and quinoline were further dried by distillation from barium oxide but equally satisfactory spectra are obtained without this treatment. Chemical shifts are in parts per million on the *delta* scale where $\delta = 0.00$ for tetramethylsilane (added as internal standard) with positive values to low field. The carbon tetrachloride solutions were shaken with D_2O to confirm the position of the hydroxylic proton.

Results and Discussion

Spectra of the 17 isomeric methyl hydroxystearates were recorded in carbon tetrachloride, pyridine, and quinoline. The spectra of methyl 12-hydroxystearate in these solvents are shown in Fig. 1 and the chemical shifts of the principal signals are listed in Table 1. Those obtained in carbon tetrachloride will be discussed first, since the signals observed in this solvent have been assigned previously (2, 3). The principal differences are between isomers in which the hydroxyl group (OH) is near one end of the chain. Thus for 2-hydroxystearate (2-OH), and for the 3- and 4-OH isomers, the proximity of OH and ester groups results in a downfield displacement of the signal for CH of the CHOH group (carbinolic CH) and of the OMe signal.

In 3-OH, the 2 protons of the αCH_2 group (unless otherwise stated αCH_2 refers to methylene α to the ester group) are magnetically nonequivalent, since they are adjacent to an asymmetric center (13). They form the AB portion of an ABX system and should give rise to two quartets (14), which in this case are overlapping. The difference between the chemical shifts of these geminal protons is small compared to their coupling constant (J) of 15 cps (which, as is shown later, can be measured from the lines of the spectrum in quinoline), so that the outer pairs of the quartets are very weak and are barely observable in carbon tetrachloride. The signal thus appears to consist of only three very sharp peaks, the one at lowest field being approximately twice as intense as the other two. For convenience, only the positions of the three strong lines are recorded in Table 1, since the exact chemical shift of each proton cannot be determined without accurate intensity measurements of the lines (14). The α-CH_2 signal in 4-OH is 0.1 downfield from that of 5-OH, and for each isomer from 5- to 10-OH this signal occurs at slightly higher field.

When the OH is near the terminal CH_3 group, changes are observed in the signal of this group. In

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16-OH, the CH_3 signal is a deeply split triplet with an apparent J of 7 cps, whereas in the other isomers the apparent J is 5 cps. This is similar to the appearance of CH_3 in linolenate in which it is affected by the proximity of the 15,16-double bond (5). In 17-OH, the CH_3 signal appears as a doublet, since it is coupled to only one proton, and is also at lower field. In 18-OH, the terminal CH_3 signal is absent, and the carbinolic CH of the other spectra is replaced by a well-defined triplet, for two protons, at slightly lower field.

The other interesting features of the spectra in carbon tetrachloride are the shapes of the signals of the methylene chain CH_2 for the 6- to 10-OH isomers which are shown in Fig. 2. Starting with 6-OH a low shoulder is seen at 1.35 on the low field side of the major peak and, along the series, this appears at higher field and higher up on the side of the large signal. Thus, in 7-OH it is at 1.33, at 1.30 in 8-OH, and 1.28 in 9-OH, the shoulders in these last two isomers being quite sharp and distinct. However, 10-OH, and later isomers, have just single sharp peaks. The OH proton signal also appears in Fig. 2 as a small separate peak, except in 9-OH in which it causes an inflexion on the high field side of the CH_2 signal. The shoulder on the major peaks may be due to the CH_2 adjacent to, and on the carboxyl side of, the CHOH; its environment is probably modified by the ester group since the alcohol 9-hydroxyheptadecane shows only a single large methylene signal without splitting. The influence of the ester group should be smaller when it is further away, accounting for the appearance of the shoulder at higher field.

Since the spectra in carbon tetrachloride were only of limited value for structure determination, a number of other solvents were examined. Benzene, which has been useful in the steroid field (11), was tried first and the chemical shifts for the 12-OH isomer were CH_3, 0.91; CH_2, 1.28; αCH_2, 2.10; CHOH, 3.40; and OCH_3, 3.35. In agreement with other work (11) the signals of groupings in the vicinity of ester carbonyl are displaced to higher field due to complex formation with the solvent so that these groups are shielded by the aromatic ring (16,11). However, benzene did not distinguish between isomers any better than carbon tetrachloride.

The solvents pyridine and quinoline caused much greater changes in the spectra, as shown in Fig. 1B and C. The most striking change is the appearance of a large broad signal in the spectra of isomers 5- to 16-OH, at 1.58 in pyridine and at 1.75 in quinoline, which corresponds to about 10 protons. The low signal at about 1.55 in the spectrum in carbon tetrachloride (Fig. 1A) is due to the CH_2 β to the ester group (17), and since the spectra of methyl stearate in pyridine and quinoline show that this signal is not displaced in these solvents it accounts for 2 of the 10 protons. Since each hydroxyester molecule is probably associated with a solvent molecule through an O-H . . . . N hydrogen bond, the CH_2 groups adjacent to the CHOH group would be in a region in which the applied field has been augmented by the ring current effect of the pyridine or quinoline molecule, and so would resonate at lower field (16). The 8-proton signal would then be that of the 2 pairs of CH_2 groups on either side of the CHOH; that is, the

TABLE I

Chemical Shifts (ppm) of Principal Signals of NMR Spectra of Methyl Hydroxystearates in Carbon Tetrachloride (C), Pyridine (P), and Quinoline (Q)

Hydroxyl position		Terminal methyl	CH_2 of carbon chain	CH_2 α and β to CHOH	CH_2 α to ester group	CH of CHOH	Methoxyl
	C	0.86	1.23	....	....	3.93	3.67
2	P	0.85	1.26	1.60, 1.88	....	4.45	3.63
	Q	0.85	1.18	1.80, 2.10		4.75	3.72
	C	0.86	1.24	....	2.24, 2.28, 2.34(i)[a]	3.80	3.60
3	P	0.85	1.26	1.56	2.58(i), 2.64, 2.66[a]	4.30	3.55
	Q	0.85	1.18	1.72	2.75, 2.80(i), 2.88[a]	4.58	3.61
	C	0.86	1.24	1.55–1.75	2.33	3.48	3.57
4	P	0.84	1.25	1.55; 1.79–2.03	2.65	3.76	3.54
	Q	0.85	1.17	1.72; 2.18	2.88, 2.92	4.04	3.62
	C	0.85	1.23	1.64	2.22	3.40	3.54
5	P	0.87	1.26	1.60, 1.98	2.42	3.77	3.57
	Q	0.87	1.17	1.74, 2.16	2.50	3.98	3.60
	C	0.86	1.24	....	2.20	3.42	3.54
6	P	0.85	1.24	1.60	2.32	3.75	3.56
	Q	0.87	1.18 (Sh 1.28)	1.75	2.37	3.95	3.58
	C	0.85	1.24 (Sh 1.33)	....	2.19	3.40	3.53
7	P	0.85	1.24	1.58	2.28	3.75	3.56
	Q	0.86	1.15 (Sh 1.32)	1.72	2.29	3.96	3.59
	C	0.85	1.24 (Sh 1.30)	....	2.18	3.40	3.53
8	P	0.85	1.23	1.58	2.27	3.76	3.57
	Q	0.86	1.15, 1.30	1.74	2.26	4.00	3.61
	C	0.85	1.24 (Sh 1.28)	....	2.18	3.40	3.53
9	P	0.85	1.26	1.58	2.27	3.78	3.58
	Q	0.85	1.16 (Sh 1.21)	1.74	2.25	4.04	3.62
	C	0.85	1.28	....	2.16	3.38	3.52
10	P	0.84	1.24	1.58	2.28	3.78	3.58
	Q	0.85	1.16	1.77	2.25	4.04	3.63
	C	0.86	1.26	....	2.17	3.39	3.52
11	P	0.84	1.23	1.58	2.28	3.78	3.57
	Q	0.84	1.11, 1.17	1.77	2.26	4.05	3.62
	C	0.86	1.27	....	2.18	3.40	3.53
12	P	0.84	1.22 (Sh 1.26)	1.58	2.28	3.76	3.57
	Q	0.85	1.11, 1.27	1.77	2.27	4.03	3.64
	C	0.87	1.25	....	2.17	3.40	3.53
13	P	0.83	1.21 (Sh 1.27)	1.58	2.27	3.76	3.56
	Q	0.88	1.10, 1.30	1.77	2.28	4.04	3.64
	C	0.87	1.24 (Sh 1.30)	....	2.17	3.40	3.52
14	P	0.89	1.24 (Sh 1.28)	1.58	2.29	3.76	3.58
	Q	0.95	1.11 (Sh 1.33)	1.76	2.28	4.01	3.64
	C	0.88	1.24 (Sh 1.30)	....	2.17	3.42	3.52
15	P	0.95	1.24	1.60	2.31	3.78	3.59
	Q	1.04	1.12 (Sh 1.30)	1.72	2.27	3.99	3.63
	C	0.88	1.24	....	2.17	3.34	3.53
16	P	1.08	1.25	1.60	2.30	3.70	3.58
	Q	1.26	1.12	1.72	2.27	3.90	3.63
	C	1.07	1.23	....	2.17	3.58	3.53
17	P	1.32	1.26	1.58	2.30	3.94	3.53
	Q	1.53	1.13 (Sh 1.18)	1.60	2.27	4.18	3.63
	C	.....	1.23	....	2.17	3.46	3.52
18	P	.....	1.25	1.60	2.27	3.77	3.55
	Q	.....	1.20 (Sh 1.16)	1.60, 1.86	2.28	4.12	3.63

[a] Only the positions of the three strong lines are recorded; the most intense are indicated by (i).

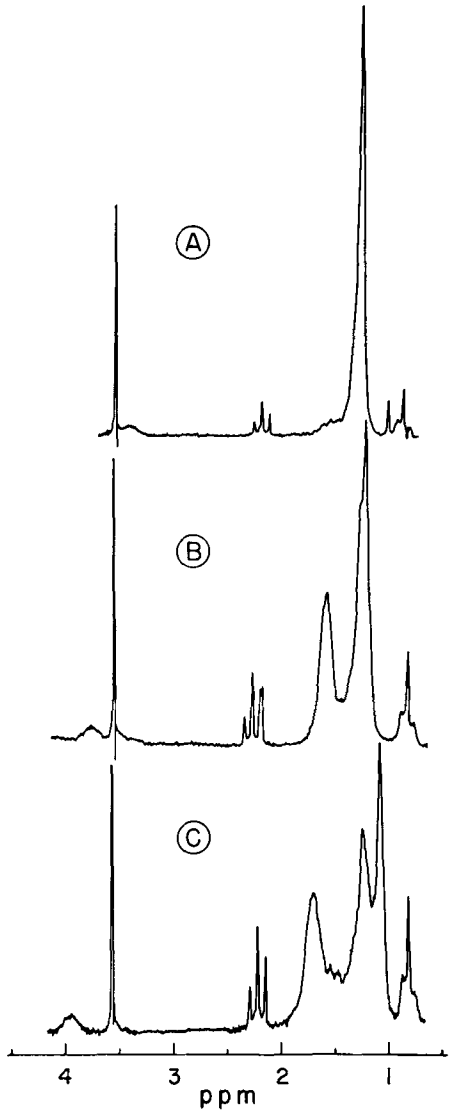

FIG. 1. NMR spectrum of methyl 12-hydroxystearate. A) in carbon tetrachloride. B) in pyridine. C) in quinoline. [The extra signal, in B), superimposed on the high field portion of the αCH_2 triplet is due to an impurity in the pyridine].

protons on carbons 10, 11, 13, and 14 in the case of 12-hydroxystearate. The carbinolic CH signal is also at lower field by 0.4 in pyridine and 0.65 in quinoline.

The absence of OH proton signals in the zero to 6 ppm region of the spectra in these solvents is further evidence that association between solute and solvent has occurred, since complex formation by OH groups, results in displacement of the OH signal to low field (14). The large signal at 1.58 (Fig. 1B) is not observed in the spectrum of 12-acetoxystearate in pyridine, also suggesting that it is the result of association involving the OH group. In isomers in which, due to proximity of OH to the ends of the chain, there are fewer CH_2 groups adjacent to CHOH, this signal is correspondingly smaller. Downfield displacements of the CH_3 signal of acetoxime were also observed by Hatton and Richards (18) when γ picoline and quinoline were used as solvent, and here, too, quinoline had the greater effect.

Besides the appearance of the 8 proton signal in the spectra in pyridine and quinoline, the chemical shifts and shapes of most of the other signals were modified. However, only the changes in spectra recorded in quinoline will be discussed, since those found using pyridine were in the same direction but smaller.

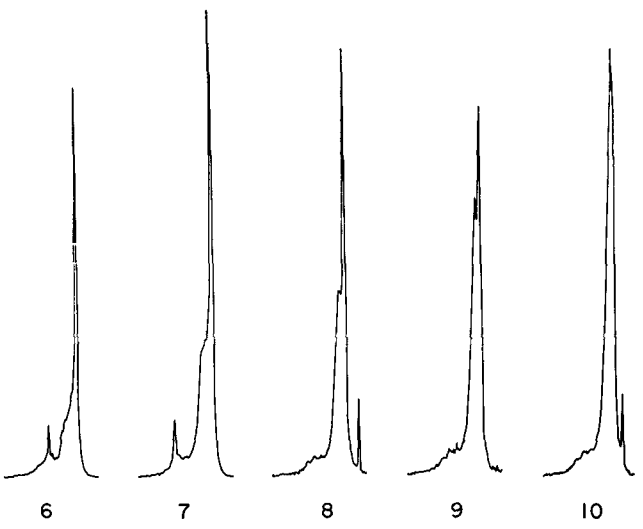

FIG. 2. 1–2 ppm region of NMR spectra of methyl 6-, 7-, 8-, 9-, and 10-hydroxystearates in carbon tetrachloride.

2-OH

The signal for CH_2 α and β to CHOH consists of two broad 2-proton peaks at 1.80 and 2.10. The lower field peak is presumably that of the protons at carbon 3 since these are β to the ester group as well as α to the CHOH.

3-OH

Seven signals are observed for the α CH_2 group, the outer pairs are stronger than in the other solvents (due to a greater difference in the chemical shifts of the two protons) and two of the intense lines are overlapping. The geminal coupling constant (15 cps) was obtained by measuring the spacing of the lines (14). The signal at 1.72 is due to only 4 protons.

4-OH

The 2 protons of carbon 3 which, as in 2-OH, are α to CHOH and β to the ester group give rise to an apparent quartet at 2.18. A broad signal of the 4 protons on carbons 5 and 6 is found at 1.72. The αCH_2 group gives rise to two triplets, the centers of which are 4 cps apart; presumably the 2 protons are magnetically nonequivalent due to differential shielding by the quinoline rings. Bowman et al. (19) have shown that protons separated from an asymmetric center by several carbon atoms can still display magnetic nonequivalence, and that the degree of nonequivalence is augmented by attachment of aromatic groups to the asymmetric center. The assignment of these groups of signals was confirmed by spin-decoupling. In pyridine, the protons on carbon 3 give a broad multiplet and the splitting of the αCH_2 triplets is smaller.

5-OH

A broad multiplet centered at 2.16 is probably due to the protons of carbon 3 which are β to both ester and CHOH groups, and the signal at 1.74 for CH_2 α and β to CHOH is consequently due to only 6 protons. The αCH_2 gives a very distorted triplet (the low field portion is much smaller than usual) at 2.50; again probably caused by unequal shielding.

6-OH

The methylenes α and β to CHOH together with CH_2 β to the ester group give a 10 proton signal.

The αCH_2 signal is again distorted from the normal triplet with apparent J = 6 cps instead of J = 7 cps as in most of the isomers, and is 0.1 downfield from the αCH_2 of 7-OH and later isomers.

7- to 13-OH

The spectra of these isomers differ mainly in the shape of the signal in the 1–1.5 region, due to the CH_2 groups of the chain, as illustrated in Fig. 3; (the 1.5–2 regions contain the signals of CH_2 β to ester and α and β to CHOH). In 7- to 10-OH the shape of the major CH_2 peak is similar to that observed in carbon tetrachloride, with a shoulder on the low field side, which disappears at 10-OH; but for 7- and 8-OH, the splitting is more marked here than in the other solvent. The CH_2 peak of 9-OH is fairly similar to that of 10-OH but is shorter and broader (relative to the signal at 1.75) and has a definite inflexion on the low field side. The CH_2 peak of 11-, 12-, and 13-OH (unlike the behavior in CCl_4) is split into two signals, the larger at 1.10 and the smaller at 1.20–1.30. In 14- and 15-OH, very little splitting occurs, and the main CH_2 peak is consequently narrower and higher.

The varied signal shapes in Fig. 3 indicate that each isomer is oriented in the solute-solvent complex in a specific way, with the CH_2 groups shielded to different extents by the associated quinoline molecule. The ester group may in some way stabilize a particular configuration, since the CH_2 signal of the spectrum (in quinoline) of 7-hydroxyoctadecane, which is the alcohol analog of 12-hydroxystearate, is not split in the 1–1.5 region but only has a shoulder at 1.23. The portions of the chain further from the CHOH group can presumably bend round so that some of the CH_2 groups enter the area of diminished field above or below the quinoline rings (16,20). The number of shielded CH_2 groups, shown by the relative size of the highest field part of the signal, appears to depend on the separation of the CHOH and CO_2CH_3 groups. Splitting in the 1–1.5 region was not observed in the spectra in pyridine solution (Fig. 1B), perhaps because the molecule is smaller or because it does not contain a benzene ring. Hatton and Richards (18) also found that one of the CH_3 signals of mesityl oxide was displaced to higher field and the other to lower field in quinoline solution, but in γ picoline the changes were much smaller.

14-OH

The terminal CH_3 signal appears at 0.95, 0.1 downfield from that of the previous isomers, the lowfield portion of the triplet is seen in spectrum 14, Fig. 3, just to the right of the CH_2 signal. The CH_3 group, separated from the CHOH group by only 3 CH_2 groups, must be close enough to the associated quinoline molecule for its magnetic environment to be modified.

15-OH

The CH_3 signal is displaced to 1.04 and the center portion of the triplet can be seen in spectrum 15, Fig. 3, as a sharp peak on the side of the large CH_2 signal.

16-OH

The CH_3 signal is further displaced to 1.26 and the shape is modified as in CCl_4.

17-OH

The CH_3 doublet is at 1.53 and the carbinolic CH displays similar relative changes of chemical shift

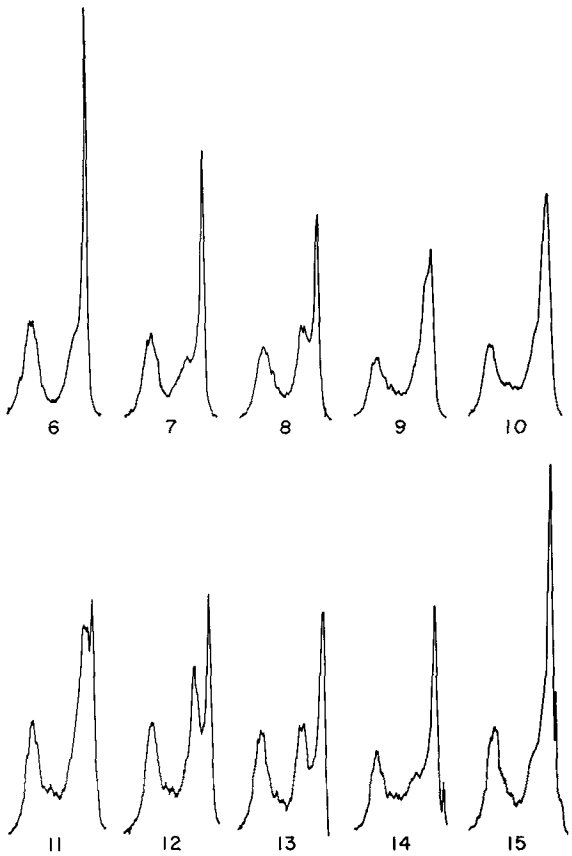

FIG. 3. 1–2 ppm region of NMR spectra of 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, and 15-hydroxystearates in quinoline.

to those observed in CCl_4. The peak at 1.60–1.70 now corresponds to only about 6 protons.

18-OH

The signal due to the protons on carbon 17 is probably at 1.86 and that of CH_2 β to CHOH and to CO_2CH_3 together at 1.60 (both broad multiplets). Since the quinoline molecule is associated with the end of the chain it may affect CH_2 α and CH_2 β to the CH_2OH group to different extents. The spectrum of 1-octadecanol shows similar signals.

Thus by using quinoline and (to a lesser extent) pyridine as solvents, more structural information can be obtained from the NMR spectra. In effect quinoline extends the range of modification of the environment of neighboring groups produced by an OH substituent. Changes in chemical shifts of neighboring groups can only be detected, in carbon tetrachloride, when the groups are separated from the CHOH groups by 1 or 2 CH_2 groups, in pyridine when the separation is 2 CH_2 groups, but in quinoline when the separation is 3 CH_2 groups. In addition quinoline results in extensive spreading and splitting of the major CH_2 signal.

Nitrobenzene and chlorobenzene were also tried as solvents, using 9-hydroxyheptadecane as solute, but did not have as much effect on the spectra as did pyridine and quinoline. An 8 proton signal was observed at 1.43 in nitrobenzene, but in chlorobenzene there was only a low shoulder at 1.35.

Application to Identification of Isomers

Isomers 2- to 4-OH and 16- to 18-OH can be easily distinguished in any of the solvents examined. Isomers 5- and 6-OH can be recognized from the chemical shifts and shapes of the αCH_2 signals in quinoline. Isomers 7- to 13-OH can be distinguished by the

shapes of the signals of the CH_2 chain measured in quinoline (Fig. 3); 9- and 10-OH are the most similar but there seems to be just enough difference for positive identification. Isomers 14- and 15-OH are identified by the chemical shift of the terminal CH_3 group in quinoline. Mixtures of isomers can be usefully examined only when the characteristic signals are partly or completely separated; but when the spectra are quite similar as in the case of 9- and 10-OH no information can be obtained about the composition of the mixture.

The spectra of 6- and 7-OH myristates and 8-OH palmitate (15) also resemble those of the C_{18} homologs; in general it is likely that similar differences would be found between the spectra of other shorter chain hydroxy esters. The spectra of longer chain hydroxy esters should be similar to those discussed here when the OH group is within 5 carbon atoms of either end of the chain. However, when the OH is near the center, smaller differences in the methylene signal might be expected, since there would be more CH_2 groups and the signals might not split in a characteristic manner. In agreement with this expectation the CH_2 portion of the spectrum of methyl 13-hydroxydocosanoate in quinoline is a large broad peak without the splitting found in the same region of the spectrum of 13-hydroxystearate.

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