NMR Chemical Shift Reagents in Structural Determination of Lipid Derivatives: III. Methyl Ricinoleate and Methyl 12-Hydroxystearate[1]

JOHN P. WINEBURG and **DANIEL SWERN**,
Fels Research Institute and Department of Chemistry,
Temple University, Philadelphia, Pennsylvania 19122

ABSTRACT

Chemical shift reagents (CSR) can substantially increase the amount of structural information obtainable from NMR studies of saturated and unsaturated lipid derivatives. It is theoretically possible to obtain even more information from CSR studies of unsaturated lipid derivatives by introducing additional CSR-active functional groups into those molecules through derivatization of their double bonds. However additional CSR coordination sites complicate spectral interpretation, because they increase the number of signals that overlap. Therefore two model compounds were investigated to test the feasibility of attempting other CSR analyses of polyfunctional molecules of unknown structure. This paper describes successful CSR studies of methyl ricinoleate and methyl 12-hydroxystearate. A series of complementary interpretive techniques was used to assign proton signals in spectra obtained during incremental $Eu(fod)_3$ addition studies with these compounds. Individual proton signals can be observed and assigned for all the protons in methyl ricinoleate, except those on carbons 5, 6 and 7. Information obtained for methyl 12-hydroxystearate is less specific. Signals are observed for all protons in methyl 12-hydroxystearate, although in some cases several proton signals overlap.

[1]Presented in part at the JOCS-AOCS Joint Meeting, Los Angeles, April 1972.

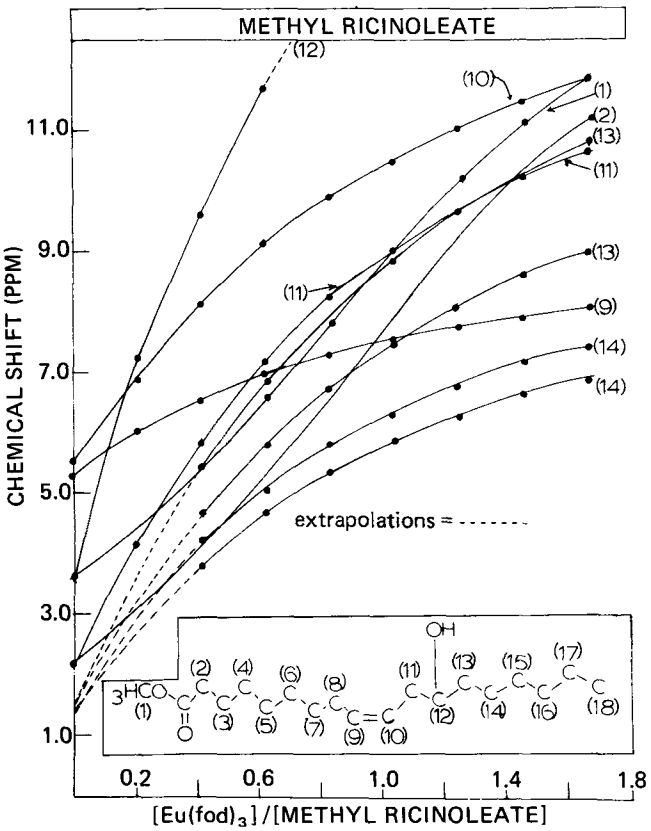


FIG. 1. Proton chemical shift plots for MR I.

INTRODUCTION

Previous reports have demonstrated that NMR chemical shift reagents (CSR) such as *tris*-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato)-europium(III)–$Eu(fod)_3$– can increase considerably the amount of structural information obtainable from NMR studies of lipid derivatives (1-5). CSR complexation can often provide additional spectral data for protons up to eight carbons away from a CSR-active functional group. Sometimes, however, due to overlapping NMR signals, useful information can only be obtained for protons within five carbons of a $Eu(fod)_3$ coordination site (2).

It is theoretically possible to obtain additional CSR information for unsaturated lipid derivatives by introducing additional CSR-active functional groups into those molecules by derivatization of their double bonds. Complete structural elucidation may even be possible if each proton in the molecule is within eight carbons of a CSR coordination site; this condition would be met, for example, if the double bond of methyl oleate (methyl *cis*-$\Delta^{9,10}$-octadecenoate) was derivatized to form methyl 9,10-dihydroxystearate (methyl 9,10-dihydroxyoctadecanoate) or methyl 9,10-epoxystearate (methyl 9,10-epoxyoctadecanoate).

However additional CSR coordination sites complicate spectral interpretation, because they increase the number of proton signals being shifted and the number of signals which overlap. Therefore, before we attempted CSR studies of complicated polyfunctional lipid derivatives of unknown structure isolated from lipid sources, two model compounds were investigated. This paper describes successful CSR studies of methyl ricinoleate (methyl 12-hydroxy-*cis*-$\Delta^{9,10}$-octadecenoate)–MR–, and methyl 12-hydroxystearate (methyl 12-hydroxy-9,10-octadecanoate)–MHS.

Interpretive techniques are described that enable unambiguous proton assignments to be made despite interferences from overlapping signals. These same techniques are applicable to other polyfunctional molecules and have been used in CSR studies of the *erythro*- and *threo*-methyl 9,10-dihydroxystearates and the *cis*- and *trans*-methyl 9,10-epoxystearates. A report of these results is currently being prepared.

EXPERIMENTAL PROCEDURES

The experimental procedures used, have already been described (2). MR (purity >99%) was obtained from the Eastern Regional Laboratory, USDA, Philadelphia, Pa. 19118, and MHS (purity >99%) from Applied Science Laboratories, State College, Pa. 16801.

RESULTS AND DISCUSSION

MR and MHS were selected as model compounds because the carbomethoxy and -OH groups are separated by

11 carbon atoms. This separation of the two CSR-active functional groups (carbon-carbon double bonds are inactive) made it possible for us to use induced shift ratios previously determined for alcohols (2) and esters (2) to facilitate spectral interpretation. In both MR and MHS, shifts of protons on carbons 9 to 18 are induced only by CSR complexation at the -OH group; shifts of protons on the methoxy carbon and on carbons 2 to 4 are induced only by CSR complexation at the carbomethoxy group. This facilitated assignments of overlapping signals, since the only unpredictable induced shift ratios are those observed for protons on carbons 5 to 8. Shifts of these proton signals are induced by interaction with CSR at both coordination sites, since these protons are within eight carbon atoms of both the carbomethoxy and the -OH groups.

The following discussion describes the CSR studies of the title compounds. Our earlier papers (1,2) give a fuller explanation of the complementary interpretive techniques that are discussed below and that were used to assign spectral signals in this study.

Incremental Addition

Only general and limited structural information can be obtained from the 100-MHz NMR spectra of MR and MHS. The 100-MHz NMR spectrum of MR shows five signals: a multiplet for the vinyl protons at δ 5.4; overlapping singlets for the methoxy and -OH protons at δ 3.54; overlapping signals for the allylic protons and protons α to the carbonyl at δ 2.15; a triplet for the terminal methyl protons at δ 0.88; and a singlet for all the other chain methylene protons at δ 1.2. A similar spectrum is observed for MHS; however, since MHS contains neither vinyl nor allylic protons, there is no signal at δ 5.4, and only a triplet is observed at δ 2.15 for protons α to the carbonyl.

The vinyl and allylic protons in the MR spectrum were easy to identify and follow throughout the incremental addition study. They in no way complicated spectral interpretation and actually enabled a more complete structural analysis than was possible for MHS. Many of the protons symmetrically substituted about C-12 in MHS are magnetically equivalent in the presence of Eu(fod)$_3$. The same symmetrical distribution is not possible with MR because of the double bond at C-9. MR therefore contains more magnetically nonequivalent protons than MHS, and this leads to the observation of a greater number of proton signals after Eu(fod)$_3$ addition.

Incremental addition (2) of Eu(fod)$_3$ to the sample facilitates spectral interpretation in CSR studies. Initially assigned signals are easily identified in spectra obtained throughout subsequent Eu(fod)$_3$ additions, since the shifts of proton signals induced by any single 20 mg increment of Eu(fod)$_3$ are small. It is simply a matter of locating similarly shaped signals that have the expected proton integration values. Whenever possible, additional signals that separate following Eu(fod)$_3$ additions are tentatively assigned, based on the considerations discussed below. The chemical shift positions of these tentatively assigned signals are then used to construct proton chemical shift plots.

Proton Chemical Shift Plots—MR

The proton chemical shift plots (2) shown in Figures 1 and 2 summarize data obtained during the incremental addition study of MR. They will be used to illustrate some of the other complementary interpretive techniques, discussed below in connection with the MR CSR study. The figures were constructed by recording the chemical shift positions observed for signals in each of the NMR spectra obtained during the incremental addition study. The identity of these signals was tentatively assigned (see below) and the chemical shifts were plotted vs. the corresponding Eu(fod)$_3$/MR molar ratios. The fact that the proton plots in Figures 1 and 2 are smooth curves is supporting evidence

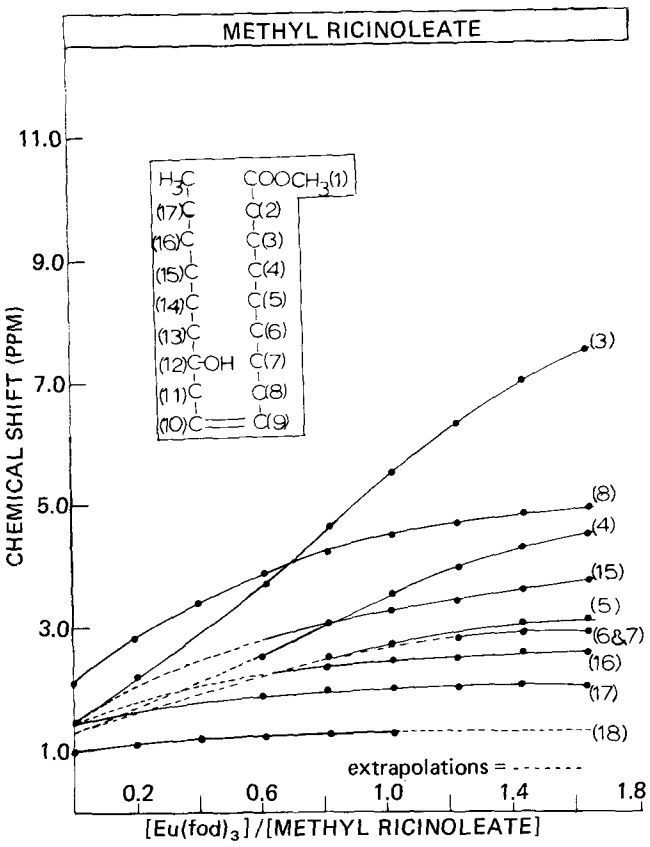


FIG. 2. Proton chemical shift plots for MR II.

that the tentative assignments made on the spectra obtained during the incremental addition study are correct.

Points at which different plots intersect correspond to "signal crossovers" observed in the NMR spectra. For example, signals of protons 1 to 5, which are near the ester function, begin to cross over signals of protons near the -OH group at a Eu(fod)$_3$/MR molar ratio of 0.63. The S-shaped curves observed for protons 1 to 5 substantiate that, at low Eu(fod)$_3$/MR molar ratios, complexation occurs preferentially at the -OH group. This observation has further interpretive implications, discussed below.

Proton chemical shift plots can be used to estimate otherwise unobtainable δ_o values (no CSR present) by extrapolation of the plots to Eu(fod)$_3$/MR molar ratios of 0 (7,8). They can also be used to determine Eu(fod)$_3$/MR ratios, at which proton signals of interest do not overlap. This application is particularly useful when decoupling experiments are to be performed. An absence of signal overlap is essential if double irradiation experiments are to produce optimum results.

CSR Study of MR—Induced Shifts and Induced Shift Ratios

Tentative proton assignments in CSR studies of polyfunctional molecules must be based on the following considerations: relative strengths of complexation, i.e., competition between functional groups for Eu(fod)$_3$; proximity of protons being shifted to a functional group; integration of overlapping proton signals; and calculation of induced shift ratios. This section will explain and illustrate the application of these procedures in the CSR study of MR. Unfortunately these interpretive techniques are complementary and therefore cannot be treated separately. The application of these techniques is not always straightforward. Sometimes apparent deviations from expected behavior are observed. However satisfactory rationalizations of these apparent deviations are possible and are included in the discussion.

The ability of CSR-active functional groups to complex

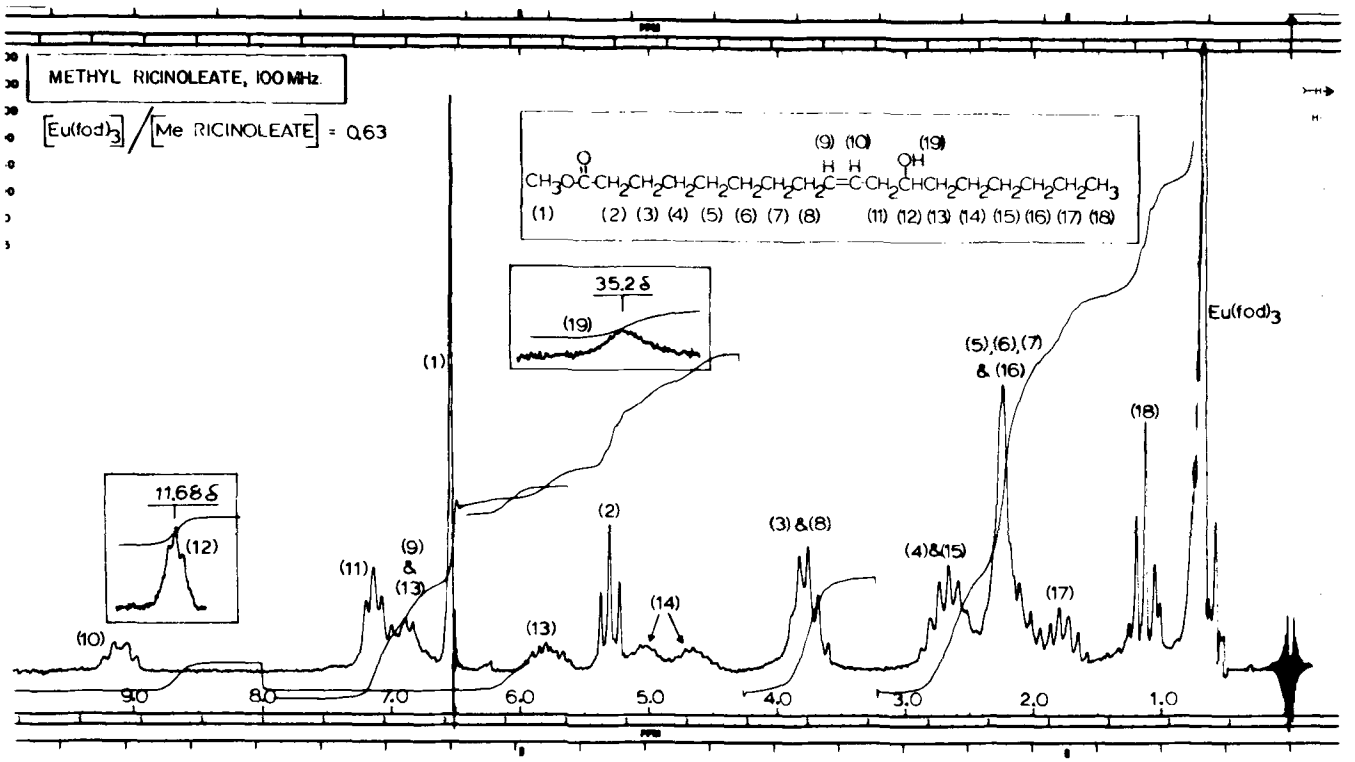


FIG. 3. 100-MHz NMR spectrum of MR. $Eu(fod)_3$/MR molar ratio = 0.63.

$Eu(fod)_3$ is directly related to their Lewis basicity. At low concentrations of $Eu(fod)_3$ the -OH and carbomethoxy groups must compete for the reagent. Since the -OH group is a stronger Lewis base, induced shifts (2) at low $Eu(fod)_3$ concentrations are greater for protons near the -OH group. Figures 1 and 2 demonstrate that, at low $Eu(fod)_3$/MR molar ratios, the induced shifts of protons near the -OH group are more than twice as large as those of protons near the carbomethoxy group. This observation can be used as a basis for tentative proton assignment. Another basis for assignment is that the magnitude of a proton's induced shift diminishes as its distance from the coordination site increases. For example, individual signals are observed for each of the olefinic protons 9 and 10, and their induced shifts are directly related to their proximity to the -OH group.

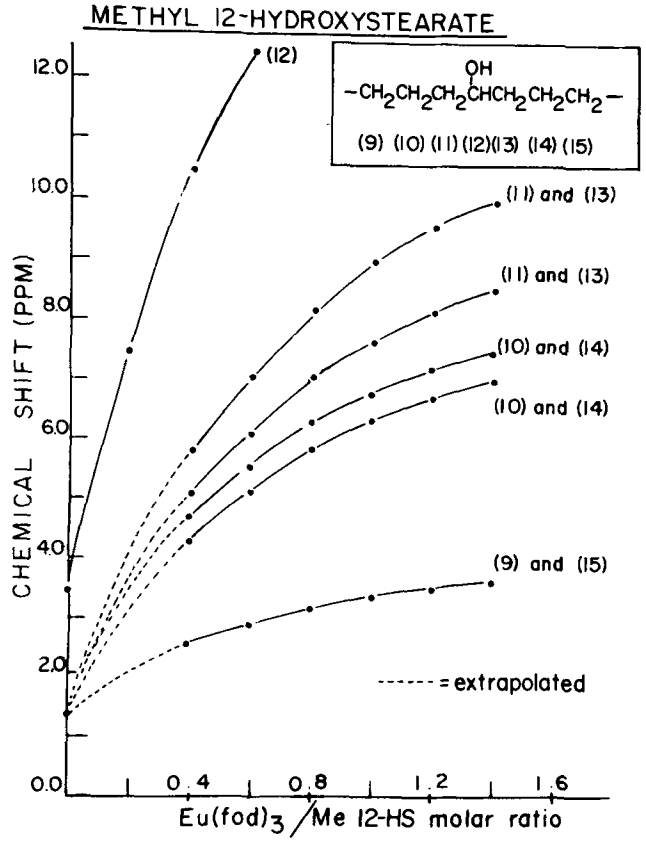


FIG. 4. Proton chemical shift plots for MHS I.

The 100-MHz NMR spectrum of MR following the third 20 mg increment of $Eu(fod)_3$ is shown in Figure 3. The data from this spectrum are plotted in Figures 1 and 2 as points at the $Eu(fod)_3$/MR molar ratio of 0.63. Presentation of this spectrum permits a discussion of the complementary interpretive procedures necessary to make proton assignments in spectra obtained during a CSR incremental addition study. Assignment of peaks 12, 11, 10, 9, 2 and 1 follow from considering relative strengths of complexation and proximity to functional groups. Although the other assignments were more difficult, they were corroborated by proton integration values and calculation of induced shift ratios. Integration shows that the signal at $\delta 3.8$ contains four protons. These are most logically assigned as protons 3 and 8. If an induced shift ratio (2) is calculated on the assumption that signal 3 represents the protons β- to the carbomethoxy group, a value of 0.69 is obtained, which is close to the expected value of 0.63. However, if an induced shift ratio is calculated on the assumption that signal 8 represents the allylic protons closest to the carbomethoxy group, a value of 0.22 is obtained, in contrast to the expected value of 0.09.

The induced shift ratio test fails for protons 8 because of the configuration of the molecule. The *cis* double bond between carbon atoms 9 and 10 forces protons 8 much closer to the -OH group than protons 16, even though both pairs of protons are ϵ- to the -OH group. The abnormal induced shift ratio value obtained for protons 8 is therefore a consequence of the abnormal proximity of these protons to the $Eu(fod)_3$ coordination site. Nevertheless, correct signal identification is possible by following the stepwise shifts induced in protons 8 during the incremental addition study.

Integration shows that the signal at δ2.67 contains four protons. These are most logically assigned as protons 4 and 15. If induced shift ratios are calculated on the assumption that these assignments are correct, the induced shift ratio obtained for protons 4 is 0.43 vs. an expected value of 0.34; the induced shift ratio obtained for protons 15 is the expected value of 0.17. The difference between the actual and expected values for protons 4 may be caused by the configuration of the molecule. The *cis* double bond between carbon atoms 9 and 10 may force protons 4 to be partially influenced by Eu(fod)$_3$ complexation at the -OH group, as well as by complexation at carbomethoxy.

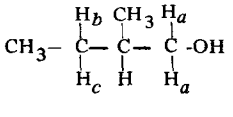
Signals at δ1.80 and δ1.12 are easily assigned to protons 17 and 18, since these protons are farthest from a Eu(fod)$_3$ complexation site. If their induced shift ratios are calculated on the assumption that these assignments are correct, expected values of 0.05 and 0.03, respectively, are obtained.

Individual signals are observed for each of the two pairs of protons labeled 13 and 14 in Figure 3. These assignments can be made on the basis of induced shift ratio values and analogy with other systems. Protons 13 and 14 are α- and β-, respectively, to an asymmetric carbon atom at C-12. Protons α- to an asymmetric carbon atom are magnetically nonequivalent. However magnetic nonequivalence may be so small that separate NMR signals are not observed. Since protons 13 are close to an Eu(fod)$_3$ coordination site, their magnetic nonequivalence is magnified and individual NMR signals can be seen for each C-13 proton (Fig. 1). Induced shift ratios calculated for protons 13 are 0.67 and 0.53, respectively. The average of these values, 0.60, corresponds exactly to the induced shift ratio expected for protons β- to an -OH group.

Eu(fod)$_3$ enhancement of magnetic nonequivalence can evidently extend to protons β- to an asymmetric center as evidenced by the individual signals observed for each of the protons labeled 14 (Fig. 1). Induced shift ratios calculated for signals 14 are 0.45 and 0.41, respectively. The average of these values, 0.43, corresponds exactly to the induced shift ratio expected for protons γ- to an -OH group.

In contrast, individual signals are not observed for protons 11 even though they, like protons 13, are α- to the asymmetric center at C-12. However the same results should not necessarily be expected for protons 11 and 13, since each pair of protons is in a different magnetic environment. C-11 is attached to a vinyl carbon atom, whereas C-13 is attached to a methylene group.

Evaluation of CSR results reported by Rabenstein (6) substantiates that protons α- to an asymmetric carbon do not necessarily absorb at separate chemical shift positions, even in the presence of CSR. Rabenstein reported proton chemical shift positions for 2-methyl-1-butanol in the presence of Eu(thd)$_3$ (*tris*-[2,2,7,7-tetramethyl-3,5-octanedionato] europium [III]).



Although carbons 1 and 3 are both α- to the asymmetric carbon at C-2, NMR signals for protons H_a overlap at δ22.1, whereas individual signals are observed at δ8.01 and δ10.3 for protons H_b and H_c. respectively.

The signal at δ2.24 in Figure 3 is attributed to protons 5, 6, 7 and 16, since these are the only protons not yet assigned.

At low Eu(fod)$_3$ concentrations, protons near the carbomethoxy group do not attain optimum induced shifts because the more basic -OH group is perferentially, although not exclusively, complexed. Carbomethoxy groups

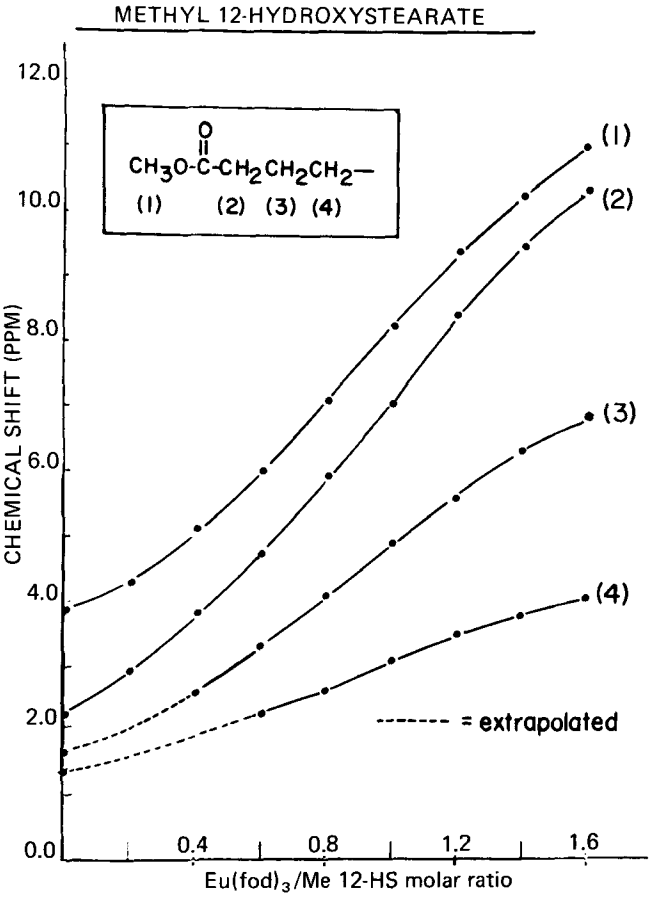


FIG. 5. Proton chemical shift plots for MHS II.

eventually predominate as potential coordination sites during incremental addition studies. The magnitude of the proton shifts induced following the addition of any 20 mg increment of Eu(fod)$_3$ depends, among other things, on the concentration of the functional groups that can be complexed. As the ratio of Eu(fod)$_3$ increases, the concentration of complexable -OH groups decreases faster than the concentration of carbomethoxy groups. Therefore at higher Eu(fod)$_3$ ratios, because of the changing concentrations of the two functional groups, the induced shifts observed for protons near the -OH group gradually decrease and those observed for protons near the carbomethoxy group increase. Compare, for example, the increasing separation of protons 2 and 14 in Figure 1. This behavior facilitates the assignment of several proton signals. The differentiation of protons 3 and 8, protons 4 and 15, and protons 5 (Fig. 2) are possible by analogous reasoning. In each case, at increasing Eu(fod)$_3$/MR molar ratios, the downfield signals are attributable to protons affected by Eu(fod)$_3$ complexation at the carbomethoxy group.

The induced shift ratio calculated for signal 5 at a Eu(fod)$_3$/MR molar ratio of 1.68 (Fig. 2) is 0.19, compared to an expected value of 0.14. The induced shift ratio value obtained may differ from the expected value because of the conformation of the molecule (see the discussion above concerning induced shift ratio value for protons 4 and 8). Further additions of Eu(fod)$_3$ fail to produce any additional signal separation. The only signals in the spectrum not completely separated at some time during the incremental addition study of MR are protons 5, 6 and 7.

Proton Chemical Shift Plots—MHS

The plots shown in Figures 4 and 5 summarize some of the data obtained during the incremental addition study of MHS. Figure 4 shows the plots constructed for some of the protons near the -OH group, while Figure 5 shows the plots

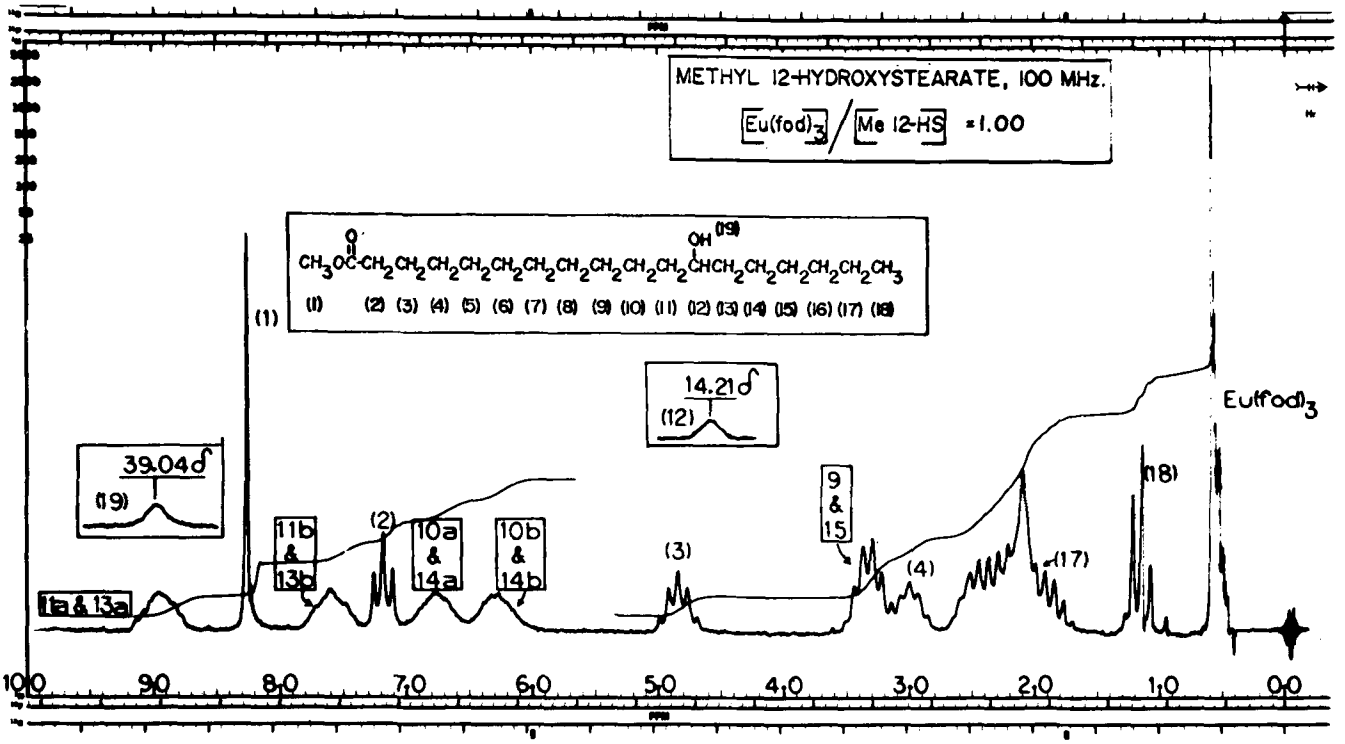
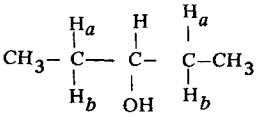


FIG. 6. 100-MHz NMR spectrum of MHS. $Eu(fod)_3$/MHS molar ratio = 1.00.

constructed for some of the protons near the carbomethoxy group. All the curves are smooth and support the tentative proton assignments made during the incremental addition study. The S-shaped curves obtained for protons near the carbomethoxy group substantiate that at low $Eu(fod)_3$ concentrations complexation occurs preferentially at the -OH group. At $Eu(fod)_3$/MHS molar ratios of 0.80 the induced shifts of protons near the carbomethoxy group begin to increase, whereas the induced shifts of protons near the -OH group begin to decrease.

CSR Study of MHS–Induced Shifts and Induced Shift Ratios

NMR spectra obtained during incremental addition studies of MHS are similar to those obtained for MR. However more overlapping proton signals are observed for MHS because of the symmetry about C-12. The assignments for protons symmetrical about C-12 that are shown in Figure 4 are analogous to those reported by Rabenstein (6) for 3-pentanol in the presence of $Eu(thd)_3$.



Carbons 2 and 4 in 3-pentanol are both symmetrically α- to the pseudoasymmetric center at C-3; the induced shift for the H_a protons is δ 11.4, and the induced shift for the H_b protons is δ 13.6. Similarly, signals 11 and 13 in Figure 4 are assigned to protons symmetrically adjacent to the asymmetric carbon at C-12. The assignment of protons 11 and 13 is also supported by their induced shift ratios. The induced shift ratio observed for protons 11a and 13a is 0.64, and that for 11b and 13b is 0.54. The average of these values is 0.59; the expected value for protons β- to an -OH group is 0.58.

Signals 10 and 14 in Figure 4 are assigned by analogy to protons symmetrically γ- to the -OH group. The induced shift ratio observed for signals 10a and 14a is 0.48. The induced shift ratio observed for protons 10b and 14b is 0.42. The average of these values is 0.45; the expected value for protons γ- to an -OH group is 0.43.

Figure 6 shows the NMR spectrum obtained following addition of the fifth 20 mg portion of $Eu(fod)_3$. The following assignments are typical of MHS CSR studies. Signal 2 has crossed over signals 10a and 14a, and 10b and 14b. Peaks 3 and 4 are assigned on the basis of their induced shifts which, since they are shifting away from neighboring signals, indicate that at this $Eu(fod)_3$/MHS molar ratio they are near the carbomethoxy group. In addition, the induced shift ratios for signals 3 and 4 are 0.68 and 0.36, respectively; the expected values for protons β- and γ- to a carbomethoxy group are 0.63 and 0.34. The induced shift ratio of signal 9 and 15 is 0.17, which corresponds exactly to the induced shift ratio expected for protons δ- to an -OH group. Signals 17 and 18 are comparable to those observed for protons 17 and 18 in MR, and they are assigned by analogous reasoning.

It should now be clear that a single spectrum CSR analysis of a polyfunctional molecule is not possible. Unambiguous assignment of overlapping proton signals can be accomplished only through the use of several complementary interpretive techniques including an incremental addition study, the construction of proton plots and the calculation of induced shift ratios.

ACKNOWLEDGMENT

U.S. Public Health Service supported this research in part under Grants CA-07803, 08793 and 07174 of the National Cancer Institute.

REFERENCES

1. Swern, D., and J.P. Wineburg, JAOCS 48:371 (1971).
2. Wineburg, J., and D. Swern, Ibid. 49:267 (1972).
3. Boudreaux, G.J., A.V. Bailey and V.W. Tripp, Ibid. 49:200 (1972).
4. Smentowski, F.J., and R.D. Stipanovic, Ibid. 49:48 (1972).
5. Walters, D.B., and D. Hamm, 163rd National ACS Meeting, Boston, Mass., April 1972, Division of Agricultural and Food Chemistry Paper No. 26.
6. Rabenstein, D.L., Anal. Chem. 43:1599 (1971).
7. Crump, D.R., J.K.M. Sanders and D.H. Williams, Tetrahedron Lett. 1970:4949.
8. Cockerill, A.F., and D.M. Rackham, Ibid. 1970:5153.

[Received August 30, 1972]