Determination of the enantiomeric purity of mevalonolactone via NMR using a chiral lanthanide shift reagent

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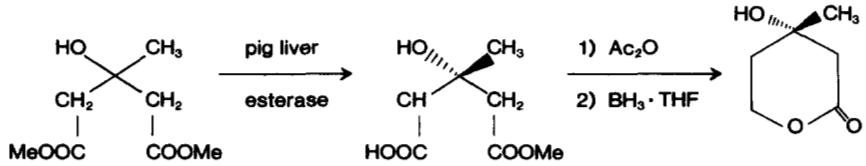
Abstract Simple methods for determining the enantiomeric purity of mevalonolactone and linalool by NMR using the chiral shift reagent $Eu(hfc)_3$ are reported. These methods are more reliable than polarimetry and require only a few milligrams of sample to detect as little as 2% of the minor enantiomer. The accuracy of these methods is limited primarily by spectral resolution for samples of high enantiomeric excess and by errors inherent in measuring peak intensities for samples of low enantiomeric excess. The Cornforth synthesis (Cornforth, R. M., J. W. Cornforth, and G. Popják. 1962. *Tetrahedron*. **18:** 1351–1354) of (S)-mevalonolactone from (R)-linalool has been improved and shown to proceed with negligible racemization.—**Wilson, W. K., T. J. Scallen, and C. J. Morrow.** Determination of the enantiomeric purity of mevalonolactone via NMR using a chiral lanthanide shift reagent. *J. Lipid Res.* 1982. **23:** 645–652.

Supplementary key words synthesis of (S)-mevalonolactone • $Eu(hfc)_3$ • $Eu(fod)_3$ • enantiomeric purity of (R)-linalool • CrO_3/pyridine/CH_2Cl_2

The central importance of (R)-mevalonolactone as an intermediate in the biosynthesis of cholesterol is well established (1, 2). More recent investigations have been devoted to examining the effect of mevalonate on the regulation of cholesterol biosynthesis (3–7). In this regard, one of us has recently reported (6) that intragastric administration of (R,S)-mevalonolactone leads to significant inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the key regulatory enzyme in the biosynthesis of cholesterol. In extending this work, it would be desirable to establish the contribution, if any, of the unnatural (S)-component in the racemic mevalonolactone to the regulatory process. For such a study, a sample of several grams of (S)-mevalonolactone of high and precisely known enantiomeric purity is required.

Of several preparations (8–14) and potential preparations (15, 16) reported for (S)-mevalonolactone, **1**, two chemical syntheses (10, 11) appeared suitable for our large sample requirements. Huang et al. (11) have described the preparation of (S)-mevalonolactone in three steps from achiral starting materials (equation *1*).



1 Eq. *1*)

However, despite our experience in reducing monoesters to lactones with boranes (17), we have been unable to duplicate the final reduction step. Thus, we turned to a longer synthetic route (equation *2*), described by Cornforth, Cornforth, and Popják (10), to obtain a sample of (S)-mevalonolactone, which we proceeded to analyze for enantiomeric purity.

Analysis of mevalonolactone for enantiomeric purity has been reported by polarimetry, a technique which we (see below) and others (12, 18, 19) have found unreliable,

Abbreviations: ee, enantiomeric excess; $[\alpha]_{max}$, specific rotation of chemically and enantiomerically pure material; $Eu(hfc)_3$, tris[3-heptafluoropropylhydroxymethylene)-d-camphorato],europium(III) derivative; $Eu(fod)_3$, tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III); NMR, nuclear magnetic resonance; FT-NMR, Fourier transform nuclear magnetic resonance; GLC, gas–liquid chromatography; s, singlet in description of NMR spectrum; d, doublet; t, triplet; m, multiplet; ppm, parts per million; TMS, tetramethylsilane; HPLC, high performance liquid chromatography.

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and by an enzymatic method (14, 20).[4,5] Optical rotations are difficult to reproduce because of their sensitivity to solvent, concentration, temperature, and trace impurities of high specific rotation. Moreover, polarimetry cannot reliably and accurately establish the enantiomeric purity of a sample unless the rotation values are calibrated by an independent method (19). Optical purity has been presumed for mevalonolactone samples when: *1*) no higher rotation could be obtained, *2*) the same specific rotation of opposite sign was reported for the other enantiomer, or *3*) a chemical or biological synthesis was assumed to occur with complete stereospecificity. Not surprisingly, a large discrepancy in the rotations reported for the benzhydrylamide derivative of mevalonolactone has recently been pointed out (12), and the optical purity of (S)-mevalonolactone prepared by the synthesis of Huang et al. (11) has been called into question.

The chiral shift reagent NMR technique (22, 23) is one of the simplest and most accessible of the several non-optical methods available for assay of enantiomeric purity. In the presence of a chiral lanthanide shift reagent such as $Eu(hfc)_3$, the NMR spectra of each of a pair of enantiomers may differ slightly. If the corresponding peaks of each enantiomer can be resolved, the ratio of enantiomers can be measured as relative peak heights or areas. This method is applied here to determine the enantiomeric purity of a sample of (S)-mevalonolactone.

EXPERIMENTAL

Materials

(R)-Linalool, **2**, (90% chemically pure) was obtained from ICN Pharmaceuticals and was further purified by spinning band distillation. The distilled material was found to be >99% chemically pure by GLC and ^{13}C NMR, and 94–96% enantiomerically pure by ^{1}H NMR in the presence of a chiral shift reagent (vide infra). The specific rotation was $[\alpha]_D^{23} - 17.04°$ (neat). (RS)-Mevalonolactone was obtained from ICN Pharmaceuticals and found to be >99% pure by ^{13}C NMR and GLC. $Eu(fod)_3$ and $Eu(hfc)_3$ were purchased from Norell Chemical Co. and Aldrich Chemical Co. and stored in a desiccator over P_2O_5.

Methods

^{1}H and ^{13}C NMR spectra were obtained as $CDCl_3$ solutions on a Varian FT-80A spectrometer (80 MHz for ^{1}H, 20 MHz for ^{13}C). ^{1}H NMR spectra in the presence of shift reagents were obtained using an 80 MHz receiver coil with an acquisition time of 4 sec or 8 sec (16000 data points), a 45° tip angle, and a pulse repetition time of 10 sec. Other ^{1}H NMR spectra were recorded with an acquisition time of 8 sec and a pulse delay of 20 sec unless otherwise stated. Peaks are reported as ppm (δ) downfield from TMS, which was used as an internal standard. ^{13}C NMR chemical shifts are reported with respect to TMS, as determined from $CDCl_3$, assuming a chemical shift of 76.9 ppm for deuterochloroform. Carbon multiplicities were determined from single frequency off resonance decoupling experiments.

Optical rotations were measured on a Jasco DIP-181 polarimeter.

GLC. Gas–liquid chromatography analyses were carried out on a Varian 3700 gas chromatograph equipped with a 6 ft × ⅛″ stainless steel column packed with 5% SE-30 on Chromosorb-W-HP (80/100 mesh). Unless otherwise stated, the column temperature was 130°C, and the injector temperature was 200°C. Helium was used as the carrier gas at a flow rate of 30 cm^3/min.

Moisture-sensitive reactions were carried out in oven-dried glassware cooled in a stream of nitrogen. Solvents were dried according to standard procedures.

Chemical syntheses

3,7-Dimethyloctane-1,3,6-triol, **3**, (diastereomeric mixture) was prepared according to Wolinsky and Bedoukian (24). Distillation fractions were analyzed by GLC at 160°C for relative amounts of diol impurities (retention time 0.8 min) and **3** (retention time 3.0 min). ^{13}C NMR δ 76.5 (d, C-6), 72.3 (s, C-3), 58.7 and 57.2 (t, C-1), 42.1 (t), 41.3, 38.4, 33.2, 27.7, 26.0 (q, 3-CH_3), 18.4, 17.7, 17.2: most peaks representing diastereomers are not well resolved at 20 MHz.

2,4-Dimethyl-4- (3-hydroxy-4-methylpentyl) -1,3-dioxane, **4**, (diastereomeric mixture) was prepared according to Cornforth et al. (10) and analyzed by GLC; ^{1}H NMR δ 4.9 (m, 1H, $CH_3C\underline{H}O_2$), 3.95 and 3.75 (finely split peaks, 2H, CH_2O), 3.3 (m, 2H, C$\underline{H}$O$\underline{H}$), 2.5–1.3 (m, 19H which includes 1.25 (d, J = 6Hz, 3H, $C\underline{H}_3CHO_2$), 1.2 (s, 3H, $C\underline{H}_3C(OH)(CH_2)_2$ and 0.9 (d, J = 7Hz, 6H, $(C\underline{H}_3)_2CH$); and ^{13}C NMR δ 91.7 and 91.5 (C-2), 76.0 and 75.9 (CH-OH), 72.5 and 72.3

[4] Diastereomers prepared by derivatizing mevalonolactone with an optically active amine have been resolved by HPLC and by NMR (21). However, the authors of the report did not explore the potential of that method as a procedure for determining the enantiomeric purity of mevalonolactone.

[5] After we submitted this paper, a report appeared describing an assay of the benzhydrylamide derivative of (R)-mevalonolactone for enantiomeric purity using the chiral shift reagent $Eu(hfc)_3$, but in that work the amount of the minor isomer appeared to be only roughly approximated (36).

(C-4), 62.1 and 16.9 (C-6), 40.4, 40.1, 34.8, 34.6, 33.5, 33.0, 29.2, 27.8, 27.0, 26.7, 20.9, 19.2, 18.3, 16.8.

2,4-Dimethyl-4-(3-oxo-4-methylpentyl)-1,3-dioxane, **5**, (diastereomeric mixture). Following a procedure by Ratcliffe and Rodehorst (25), a flask containing 2850 ml of dichloromethane and 163 g of dry pyridine was cooled to 0°C and 103 g of chromium trioxide was added in 25-g portions. The resulting reddish-brown mixture was stirred 15 min and 37.0 g of acetal **4** was added. A black precipitate formed immediately. The reaction mixture was stirred for 1 hr at 25°C and then was washed several times with 200 ml of 5% NaOH and once with 200 ml of saturated brine. The organic layer was dried ($MgSO_4$) and concentrated by rotary evaporation to an oil which, upon distillation, gave a forerun, two fractions totaling 7.5 g and containing 3% to 6% starting alcohol, and a main fraction of 17.2 g containing less than 2% of **4** by GLC. Ketone **5** was characterized by GLC, ^1H NMR δ 4.9 and 4.8 (quartets, J = 5Hz, 1H, $CH_3C\underline{H}O_2$), 4.0 and 3.85 (finely split peaks, 2H, CH_2O), 2.85 to 2.35 (m, 3H, $C\underline{H}C(O)C\underline{H}_2$), 2.2 to 0.9 (m including strong sharp peaks at 1.26, 1.20, 1.17, 1.15, 1.14, 1.07, and 1.06, 16H) and ^{13}C NMR δ 212.6 and 212.4 (C=O), 91.5 and 91.4 (C-2), 71.5 (C-4), 61.7 (C-6), 40.1, 39.9, 37.0, 34.7, 33.0, 27.6, 26.1, 20.7, 17.5.

2,4-Dimethyl-4-(2-hydroxymethylene-3-oxo-4-methylpentyl)-1,3-dioxane, **6**, (diastereomeric mixture) was prepared according to Cornforth et al. (10) and analyzed as a mixture of dicarbonyl and enol species by GLC and ^{13}C NMR δ 198.5, 183.5, 179.6, 112.4, 99.2, 91.8, 89.1, 88.7, 62.0, 57.2, 42.5, 39.7, 39.1, 38.0, 37.7, 32.4, 32.2, 25.8, 20.5, 19.2, 18.8.

(S)-mevalonolactone, **1**, was prepared from **6** using Cornforth's procedure (10). During the acetal hydrolysis, temperature and time of distillation were carefully controlled in order to avoid dehydration or racemization. The course of this hydrolysis was followed by periodically analyzing aliquots of the reaction mixture by ^1H NMR using the selective gated saturation technique (irradiation of the water peak by the homodecoupler only during the pulse delay) to suppress the strong H_2O peak. After 1 hr at 50°C on a rotary evaporator, the reaction mixture gave a ^1H NMR spectrum showing only traces of methanol and having the characteristic resonances for mevalonolactone.

The product (S)-mevalonolactone was analyzed by GLC and ^{13}C NMR δ 170.6 (s, C-1), 66.7 (s, C-3), 65.3 (t, C-5), 43.5 (t, C-2), 34.6 (t, C-4), 28.2 (q, 3-CH_3). The assignments of the methylene carbons were confirmed by a series of single frequency off resonance decoupling experiments (26), which showed that carbon atoms with chemical shifts 65.3, 43.5, and 34.6 are bonded to protons with chemical shifts δ 4.4, 2.6, and 2.0, respectively. The product was analyzed for enantiomeric purity by polarimetry ($[\alpha]_D^{23}$ + 24.0° (c = 0.83, 95% EtOH) and chiral shift reagent techniques (see below).

Shift reagent experiments

General. All samples were filtered through a tight plug of glass wool in a Pasteur pipet using oven-dried glassware. Series of spectra with varying amounts of Eu(hfc)$_3$ or Eu(fod)$_3$ were obtained by successive additions of (RS)-mevalonolactone or (RS)-linalool to a solution of shift reagent in $CDCl_3$. Observed induced shifts were approximately halved when 0.5 mg of H_2O was added to a solution of 5 mg of (RS)-mevalonolactone and 5 mg of Eu(hfc)$_3$ in $CDCl_3$. Proton relaxation times were measured by the inversion-recovery technique for a non-degassed solution of (RS)-mevalonolactone and Eu(hfc)$_3$ in $CDCl_3$. In this sample, all T_1 were less than 0.9 sec, and T_1 for H_b was about 0.7 sec.

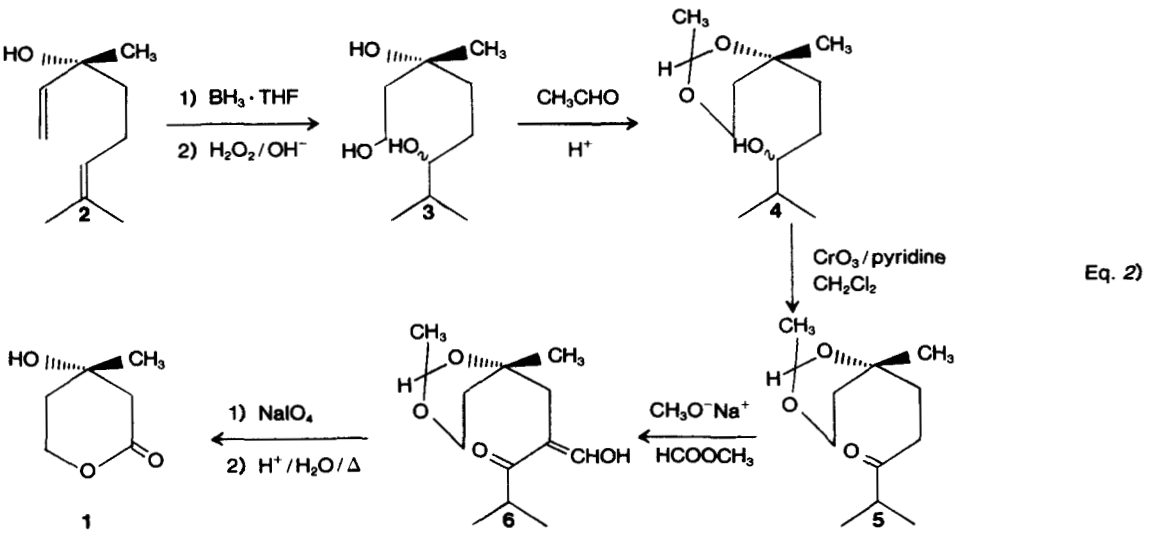
Measurement of enantiomeric purity. A solution of 17.4 mg of (S)-mevalonolactone, prepared by the Cornforth synthesis as described above, and 46.5 mg of Eu(hfc)$_3$ in 390 mg of $CDCl_3$ was prepared, and the ^1H NMR spectrum was measured. The vertical scale was increased 20-fold and the relative heights of the downfield pair of H_b peaks (Fig. 3a) were found to be 38:1 (95% ee). To this solution was added 1.7 mg of (RS)-mevalonolactone, and relative peak heights were found to be 21:1 (91% ee). Further addition of 4.4 mg of (RS)-mevalonolactone changed the peak height ratio to 5.6:1 (70% ee) (Fig. 3b).

Similarly, the ^1H NMR spectrum of a solution of 6.2 mg of distilled (R)-linalool and 39.5 mg Eu(hfc)$_3$ in 450 mg $CDCl_3$ was obtained, and the relative peak heights were measured (Fig. 4a). Peak height measurements were repeated on spectra obtained after adding small portions of (RS)-linalool to estimate the smallest amount of (R)-linalool detectable in the sample.

RESULTS

Preparation of (S)-mevalonolactone

(S)-Mevalonolactone, **1**, was prepared from commercially available (R)-linalool, **2**, by a modified version of the Cornforth synthesis (10) as outlined in equation *2*. The modifications, which are described in the experimental section, include an analysis of triol **3** for troublesome diol impurities, a much-improved procedure for oxidizing **4** to ketone **5**, and a technique for monitoring the formation of mevalonolactone in the last step so as to minimize dehydration and racemization.



Eq. 2)

Determination of the enantiomeric purity of (S)-mevalonolactone and (R)-linalool

The proton NMR spectrum of racemic mevalonolactone was determined in the presence of varying amounts of the chiral shift reagent Eu(hfc)$_3$ and the expected downfield shifts were observed. The AB quartet of the diastereotopic hydrogens labeled H_a and H_b in **Fig. 1** exhibits a large induced shift. The downfield half (labeled H_a) appears as a broad doublet. The upfield half (labeled H_b) is a sharp doublet, each peak of which is further split into a doublet.[6] We attribute this further splitting to the differential induced shifts of the two enantiomers of mevalonolactone on the following grounds. *1)* With the achiral shift reagent Eu(fod)$_3$, a series of spectra that were very similar to those in Fig. 1 was obtained except that the H_b doublet was not further split. (Compare **Fig. 2** and Fig. 1e). *2)* In the spectrum of the (S)-enantiomer of mevalonolactone, the H_b doublet is also not further split (**Fig. 3a**). *3)* In spectra of mixtures containing a known ratio of (RS)- and (S)-mevalonolactone, each peak of the H_b doublet is further split into two doublets in which the relative heights reflect the ratio of (R)- and (S)-mevalonolactone in the samples (Fig. 3b). In each experiment, the upfield peak was taller and, therefore, corresponds to the (S)-enantiomer.

We found that a molar ratio (Eu(hfc)$_3$/mevalonolactone) of 0.3 gave the optimal separation of the H_b doublets into the two peaks corresponding to the two enantiomers while positioning the H_b absorption in the range δ 5.3 to δ 4.3, a spectral region that was free of other absorptions. Lower molar ratios gave inadequate enantiomeric shift differences while higher ratios either caused the H_b peaks to shift under another absorption or become significantly broadened. Other absorptions in the spectrum did not show usable enantiomeric shift differences even when solvents other than $CDCl_3$ were tried. In $CDCl_3$, the downfield doublet due to H_a showed broadening even when peaks elsewhere in the spectrum were sharp, and the enantiomeric H_a peaks were not resolved. The absorptions due to the methyl hydrogens in each enantiomer were, at best, only partially resolved at 80 MHz. The remaining two pairs of diastereotopic methylene hydrogens comprise an ABXY spin system and, in all of the spectra, were seen as complex multiplets in which enantiomeric shifts were not fully resolved. Solvents other than $CDCl_3$ were unsatisfactory due to the insolubility of mevalonolactone (CCl_4, $CFCl_3$) or inadequate enantiomeric shift differences (CD_3CN). Mevalonolactone is slightly soluble in C_6D_6, but the enantiomeric shift differences were no larger than in $CDCl_3$.

Using the chiral shift reagent Eu(hfc)$_3$ in $CDCl_3$, the synthetic (S)-mevalonolactone was found to be 95% enantiomerically pure. In view of the apparent partial racemization of the synthetic (S)-mevalonolactone, it became desirable to determine the enantiomeric purity of the (R)-linalool used in the synthesis. Through a series of experiments analogous to those described above, it was found that, at an optimal linalool to Eu(hfc)$_3$ molar ratio of 0.6, the absorption appearing at about 10 ppm is a doublet for (R)-linalool but is split into a pair of doublets for (RS)-linalool (**Fig. 4**). Other enantiomeric pairs are partially resolved in the spectrum of racemic linalool, but these proved less useful for determining the enantiomeric

[6] While the question as to which of the diastereotopic hydrogens gives rise to which doublet has no bearing on use of the absorptions in establishing the enantiomeric purity of the mevalonolactone, it should be noted that the greater line-broadening and larger induced shifts for the downfield doublet are consistent (27) with the previous assignment (28) of this absorption to the proton *syn* to the hydroxyl group.

purity of linalool under the conditions examined. A series of experiments in which the (R)-linalool was doped with known amounts of (R,S)-linalool showed that, due to better resolution of the peaks in this case, detection of <1% of the minor enantiomer is possible. The starting linalool was found to contain 94–96% enantiomeric excess of the (R)-isomer. This result suggests that virtually no racemization occurs during the Cornforth synthesis (10) of (S)-mevalonolactone.

DISCUSSION

The enantiomeric purity of small, possibly impure samples of mevalonolactone and linalool can be quickly and reliably determined by NMR in the presence of $Eu(hfc)_3$. Using the 10 to 15-mg samples of mevalonolactone or linalool, we could detect as little as 2% of the minor isomer after about 15 min of NMR data acquisition. Acquisition of more transients, optimization of pulse width and pulse repetition time, or use of high field NMR could permit the experiment to be performed on much smaller samples. Polarimetry, by contrast, requires 800 mg of (S)-mevalonolactone to duplicate the conditions reported by Cornforth using a standard 10 mm ID by 100 mm cell. Moreover, using polarimetry we could not confidently determine the enantiomeric purity of the starting linalool because of discrepancies in reported $[\alpha]_{max}$ values and our difficulty in obtaining reproducible rotations.

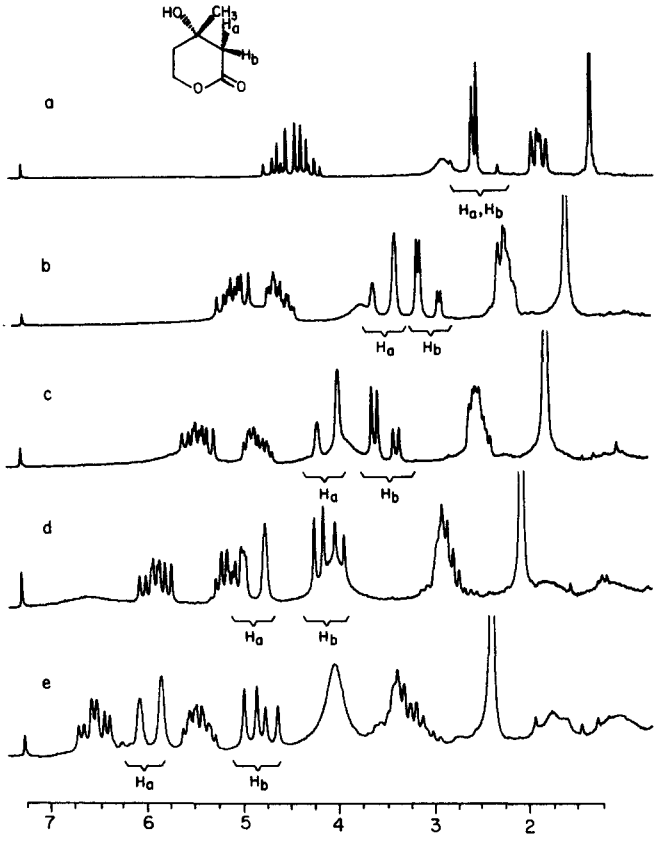


Fig. 1. 80 MHz proton NMR spectra of (RS)-mevalonolactone in the presence of $Eu(hfc)_3$ at various molar ratios: (a) 0.00, (b) 0.14, (c) 0.19, (d) 0.26, (e) 0.32. Spectrum (e) is similar to Fig. 2 except that the doublet at δ 4.7 in Fig. 2 is split into a pair of doublets in (e) due to the presence of the chiral shift reagent.

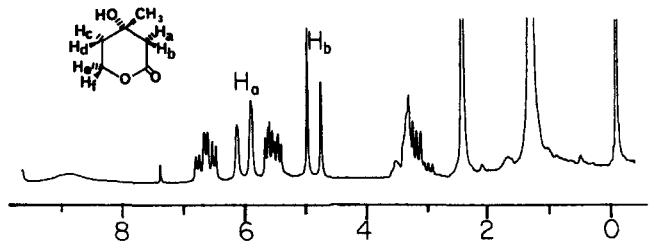


Fig. 2. 80 MHz NMR spectrum of (RS)-mevalonolactone in the presence of $Eu(fod)_3$ at a molar ratio of 0.46 in $CDCl_3$. Assignments are as follows: δ 7.3 (s, $CHCl_3$), 6.6 (t of d, H_c), 6.0 (d, H_a), 5.5 (m, H_f), 4.8 (d, H_b), 3.3 (m, H_c, H_d), 2.5 (s, CH_3). (Broad absorption at 8.8 and 1.4 are due to $Eu(fod)_3$.) In the presence of a higer proportion of the shift reagent, the multiplet at δ 3.3 is resolved into a broadened d for H_c and a crude t of d for H_d. (Previous workers (28) have assigned the upfield portion of the resonance at δ 1.8 in mevalonolactone to H_c and the downfield portion of that resonance to H_d. In analyzing the spectra recorded in the presence of $Eu(fod)_3$, we have assigned to H_c a resonance downfield of the resonance assigned to H_d. These assignments are not necessarily inconsistent with those made previously since the resonance for H_c may have shifted to a greater extent when the $Eu(fod)_3$ was added or the conformation of mevalonolactone may be different in the presence of $Eu(fod)_3$.)

We have found NMR spectral resolution of mevalonolactone in the presence of $Eu(hfc)_3$ to be rather capricious. Allowing a solution to stand for an hour occasionally makes the spectral resolution far better than that shown in Figs. 1–3, whereas carelessness in sample preparation may result in very poor resolution. Poor resolution may also result from a gradual decline in lanthanide-induced shifts during lengthy NMR data acquisition. We have not encountered resolution problems of such severity in any other work with shift reagents or with mevalonolactone/$Eu(fod)_3$ solutions. However, by rigorous adherence to the usual precautions (23, 29) of excluding water (see experimental) and mevalonic acid, filtering the shift reagent solutions, and carefully adjusting magnetic homogeneity, the spectral resolution displayed in Figs. 1–3 can be routinely obtained.

Although peak areas are the true measure of the relative amounts of enantiomers, measurement of peak heights is simpler and often more accurate when peaks overlap or differ greatly in area (30, 31). In the present work, we have chosen to estimate the relative amounts of the two enantiomers using peak heights. The validity of this method rests on our demonstration that peak

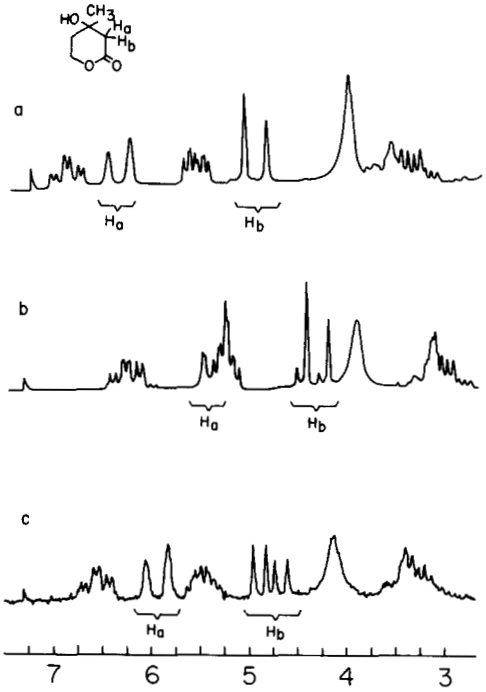


Fig. 3. 80 MHz proton NMR spectra of mixtures of (R)- and (S)-mevalonolactone in the presence of approximately 0.3 equivalents of $Eu(hfc)_3$. The molar percentages of (R)-mevalonolactone in the samples are: (a) 3%, (b) 15%, (c) 50%. Spectrum (b) shows how use of slightly less than 0.3 equivalents of $Eu(hfc)_3$ causes the H_a absorption to move upfield under the multiplet at δ 5.4 and the H_b absorption to move upfield towards the broad singlet and suffer a decrease in enantiomeric shift differences.

heights provide acceptable estimates of enantiomeric purity for mixtures containing known ratios of (R)- and (S)-mevalonolactone or (R)- and (S)-linalool, but it also has a sound theoretical basis.

The assumption that the ratio of peak areas measures the ratio of the enantiomers requires that several conditions be met (30–33) including that the nuclei be allowed to relax between pulses, and that the peaks of interest be accurately phased and digitized. Careful phasing, attention to possible digitizing errors, and use of a pulse repetition time (10 sec) and tip angle (45°) adequate for the T_1 relaxation times (0.7 sec for H_b) insured that these conditions were met. The approximation of relative peak areas by peak heights requires that all peaks of interest have the same line width and be defined by sufficiently many data points (30, 32). Adequate density of data points was assured by zero-filling to 16k data points, and a peak simulation program was used to subtract the small peak height enhancements resulting from peak overlap and baseline slope. In contrast to the differential broadening of the H_a and H_b peaks, in the presence of $Eu(hfc)_3$, the line widths of the two enantiomeric H_b peaks differed by less than 5%. Since, for a fixed peak area, the peak height is inversely proportional to the line width, this corresponds to an error of 0.5% (absolute) when less than 10% of the minor isomer is present.

For samples of high enantiomeric excess, the accuracy of the method described in this report is limited primarily by the resolution of the spectral lines, which in turn is determined by how the sample is prepared and by the field strength and magnetic homogeneity of the NMR spectrometer used. The resolution available in the experiments reported here allowed us to estimate that the sample of (S)-mevalonolactone contained 3% ± 1% of the (R)-enantiomer. However, for samples of low enantiomeric excess, the accuracy of the method is limited primarily by errors inherent in the measurement of peak heights or areas. Since the integration of peaks in NMR is generally considered to be accurate to at best 1–2% (31, 33), and it is our observation that peak heights of corresponding protons of enantiomers are reproducible with comparable accuracy, caution must be exercised in applying this method to the detection of an enantiomeric excess of less than 5%.

In summary, we have described in this report some significant improvements in what we feel is the best available enantioselective synthesis of (S)-mevalonolactone. More importantly, however, we have described a procedure that allows an accurate estimate of the enantiomeric purity of a relatively small quantity of mevalonolactone to be made. Because of the central importance of (R)-mevalonolactone in the biosynthetic pathway lead-

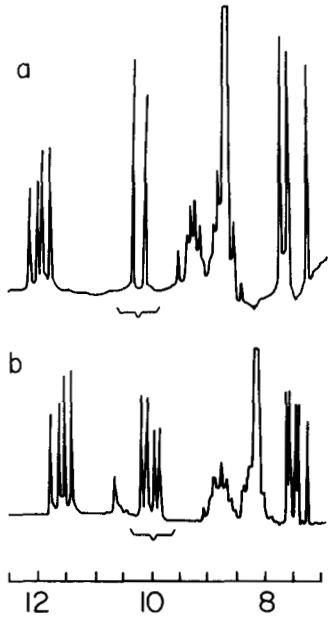


Fig. 4. 80 MHz proton NMR spectra of (a), (R)-linalool, and (b), (RS)-linalool in the presence of approximately 0.6 equivalents $Eu(hfc)_3$.

ing to sterols, terpenes, and other natural products, this procedure should find wide application in assessing the outcome of enantioselective synthetic routes to mevalonolactone as well as for evaluating the enantiomeric purity of mevalonolactone from other sources such as the products of enzymatic reactions (14, 20, 34, 35) and natural product degradations (13).

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REFERENCES

1. Tavormina, P. A., M. H. Gibbs, and J. W. Huff. 1956. The utilization of β-hydroxy-β-methyl-δ-valerolactone in cholesterol biosynthesis. *J. Am. Chem. Soc.* **78:** 4498–4499.
2. Clayton, R. B. 1965. Biosynthesis of sterols, steroids, and terpenoids. Part I. Biogenesis of cholesterol and the fundamental steps in terpenoid biosynthesis. *Q. Rev.* **19:** 168–200.
3. Edwards, P. A., G. Popják, A. M. Fogelman, and J. Edmond. 1977. Control of 3-hydroxy-3-methylglutaryl coenzyme A reductase by endogeneously synthesized sterols in vitro and in vivo. *J. Biol. Chem.* **252:** 1057–1063.
4. Mitropoulos, K. A., S. Balasubramaniam, S. Venkatesan, and B. E. A. Reeves. 1978. On the mechanism for the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase, of cholesterol 7α-hydroxylase and of acyl-coenzyme A: cholesterol acyltransferase by free cholesterol. *Biochim. Biophys. Acta.* **530:** 99–111.
5. Erickson, S., A. Shrewsbury, R. Gould, and A. Cooper. 1979. Rapid modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (R) in the intact liver. Evidence for dual mechanisms. *Circulation.* **60-II:** 110.
6. Arebalo, R. E., J. E. Hardgrave, B. J. Noland, and T. J. Scallen. 1980. In vivo regulation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase: enzyme phosphorylation as an early regulatory response after intragastric administration of mevalonolactone. *Proc. Natl. Acad. Sci. USA.* **77:** 6429–6433.
7. Havel, C., E. Hansbury, T. J. Scallen, and J. A. Watson. 1979. Regulation of cholesterol synthesis in primary rat hepatocyte culture cells. *J. Biol. Chem.* **254:** 9573–9582.
8. Shunk, C. H., B. O. Linn, J. W. Huff, J. L. Gilfillan, H. R. Skeggs, and K. Folkers. 1957. Resolution of DL-mevalonic acid and the synthesis and biological activities of DL-3-hydroxy-3-methylglutaraldehydic acid. *J. Am. Chem. Soc.* **79:** 3294–3295.
9. Eberle, M., and D. Arigoni. 1960. Absolute Konfiguration des Mevalolactons. *Helv. Chim. Acta.* **43:** 1508–1513.
10. Cornforth, R. H., J. W. Cornforth, and G. Popják. 1962. Preparation of R- and S-mevalonolactones. *Tetrahedron.* **18:** 1351–1354.
11. Huang, F-C., L. F. H. Lee, R. S. D. Mittal, P. R. Ravikumar, J. A. Chan, C. J. Sih, E. Caspi, and C. R. Eck. 1975. Preparation of (R)- and (S)-mevalonic acids. *J. Am. Chem. Soc.* **97:** 4144–4145.
12. Irwin, A. J., and J. B. Jones. 1977. Asymmetric synthesis via enantiotopically selective horse liver alcohol dehydrogenase catalyzed oxidations of diols containing a prochiral center. *J. Am. Chem. Soc.* **99:** 556–561.
13. Ohloff, G., W. Giersch, K. H. Schulte-Elte, P. Enggist, and E. Demole. 1980. Synthesis of (R)- and (S)-4-methyl-6-2′-methylprop-1-enyl-5,6-dihydro-2H-pyran (nerol oxide) and natural occurrence of its racemate. *Helv. Chim. Acta.* **63:** 1582–1588.
14. Ngan, H-L. and G. Popják. 1975. Stereochemistry of the reaction catalyzed by mevaldate reductase. *Bioorg. Chem.* **4:** 166–180.
15. Cornforth, R. H., K. Fletcher, H. Hellig, and G. Popják. 1960. Stereospecificity of enzymic reactions involving mevalonic acid. *Nature.* **185:** 923–924.
16. Abushanab, E., D. Reed, F. Suzuki, and C. J. Sih. 1978. Stereospecific microbial oxidation of thioethers to sulfoxides. Application to the synthesis of R-mevalonolactone. *Tetrahedron Lett.*: 3415–3418.
17. Wilson, W. K., C. J. Morrow, and T. J. Scallen. 1978. An improved method for the conversion of cyclic anhydrides to lactones. *In* Abstracts of Papers, 3rd Rocky Mountain Regional Meeting of the ACS. Abstract 199.
18. O'Donnell, G. W., and M. D. Sutherland. 1966. Terpenoid chemistry. XIII. Derivatives of optically pure (+)-citronellal. *Aust. J. Chem.* **19:** 525–528.
19. Jacques, J., A. Collet, and S. H. Wilen. 1981. Enantiomers, Racemates, and Resolutions. John Wiley & Sons, New York. 405–406.
20. Levy, H. R., and G. Popják. 1960. Studies on the biosynthesis of cholesterol. *Biochem. J.* **75:** 417–428.
21. Bergot, B. J., F. C. Baker, E. Lee, and D. A. Schooley. 1979. Absolute configuration of homomevalonate and 3-hydroxy-3-methylglutaryl coenzyme A, produced by cell-free extracts of insect corpora allata. A cautionary note on prediction of absolute stereochemistry based on lipid chromatographic elution order of diastereomeric derivatives. *J. Am. Chem. Soc.* **101:** 7432–7434.
22. Whitesides, G. M., and D. W. Lewis. 1970. Tris[3-(*tert*-butylhydroxymethylene)-d-camphorato]europium(III). A reagent for determining enantiomeric purity. *J. Am. Chem. Soc.* **92:** 6979–6980.
23. Sullivan, G. R. 1978. Chiral lanthanide shift reagents. *Top. Stereochem.* **10:** 287–329.
24. Wolinsky, J., and R. H. Bedoukian. 1976. Hydroboration of monoterpene alcohols. *J. Org. Chem.* **41:** 278–281.
25. Ratcliffe, R., and R. Rodehorst. 1970. Improved procedure for oxidation with the chromium trioxide-pyridine complex. *J. Org. Chem.* **35:** 4000–4002.
26. Johnson, L. F. 1979. Spin-decoupling methods in ^{13}C NMR studies. *In* Topics in Carbon-13 NMR Spectroscopy. Volume 3. G. C. Levy, editor. John Wiley & Sons, New York. 2–16.
27. Willcott, M. R., R. E. Davis, and R. W. Holder. 1975. Interpretation of the pseudocontact model for nuclear magnetic shift reagents. VI. Determination of the stereoisomeric

relationships of four structurally isomeric methyl bicyclooctenols. *J. Org. Chem.* **40:** 1952–1957.

28. Johnson, R. N., and N. V. Riggs. 1971. Proton magnetic resonance spectra and conformation of mevalonolactone and 3,3-dimethylvalerolactone. *Aust. J. Chem.* **24:** 1659–1666.
29. McCreary, M. D., D. W. Lewis, D. L. Wernick, and G. M. Whitesides. 1974. The determination of enantiomeric purity using chiral lanthanide shift reagents. *J. Am. Chem. Soc.* **96:** 1038–1054.
30. Kasler, F. 1973. Quantitative Analysis by NMR Spectroscopy. Academic Press, New York. 78–88.
31. Becker, E. D. 1980. High Resolution NMR. Second edition. Academic Press, New York. 41–42, 260–265.
32. Lindon, J. C., and A. G. Ferrige. 1980. Digitisation and data processing in Fourier transform NMR. *Prog. Nucl. Magn. Reson. Spectrosc.* **14:** 27–66.
33. Leyden, D. E., and R. H. Cox. 1977. Analytical Applications of NMR. John Wiley & Sons, New York. 17–19, 258–271.
34. Schlesinger, M. J., and M. J. Coon. 1961. Reduction of mevaldic acid to mevalonic acid by a partially purified enzyme from liver. *J. Biol. Chem.* **236:** 2421–2424.
35. Donninger, C., and G. Popják. 1966. The stereochemistry of mevaldate reductase and the biosynthesis of asymmetrically labeled farnesyl pyrophosphate. *Proc. R. Soc. London Ser. B.* **163:** 465–491.
36. Eliel, E. L., K. Soai, and W. R. Kenan. 1981. Highly stereoselective asymmetric synthesis of (R)-(−)-mevalonolactone. *Tetrahedron Lett.* **22:** 2859–2862.