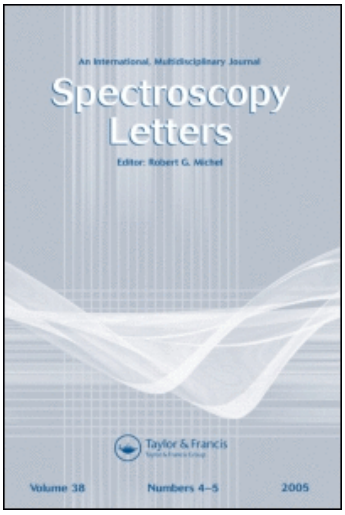

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

^{1}H NMR Spectral Simplification with Achiral and Chiral Lanthanide Shift Reagents. 2-Methyl-3-(2'-Hydroxymethylphenyl)-4(3*H*)-Quinazolinone, A Major Metabolite of Methaqualone

Andrea Poropatich[a]; Robert Rothchild[a]
[a] Department of Science Toxicology Research and Training, The City University of New York John Jay College of Criminal Justice, New York, NY

To cite this Article Poropatich, Andrea and Rothchild, Robert(1990) ''H NMR Spectral Simplification with Achiral and Chiral Lanthanide Shift Reagents. 2-Methyl-3-(2'-Hydroxymethylphenyl)-4(3*H*)-Quinazolinone, A Major Metabolite of Methaqualone', Spectroscopy Letters, 23: 1, 29 — 43
To link to this Article: DOI: 10.1080/00387019008054034
URL: http://dx.doi.org/10.1080/00387019008054034

SPECTROSCOPY LETTERS, 23(1), 29-43 (1990)

^{1}H NMR SPECTRAL SIMPLIFICATION WITH ACHIRAL AND CHIRAL LANTHANIDE SHIFT REAGENTS. 2-METHYL-3-(2'-HYDROXYMETHYLPHENYL)-4(3H)-QUINAZOLINONE, A MAJOR METABOLITE OF METHAQUALONE.

Key Words: Europium, Optical Purity, Enantiomer, $Eu(FOD)_3$, $Eu(HFC)_3$, Biaryl Hindered Rotation, Analysis

Andrea Poropatich and Robert Rothchild*

The City University of New York
John Jay College of Criminal Justice
Department of Science
Toxicology Research and Training Center
445 West 59th Street
New York, NY 10019-1199

ABSTRACT

The 60 MHz ^{1}H NMR spectra of 2-methyl-3-(2'-hydroxymethylphenyl)-4(3H)-quinazolinone, 1, a major metabolite of methaqualone, have been studied at 28° in

*To whom correspondence should be sent

$CDCl_3$ solution with the achiral reagent tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), 2, and the chiral reagent tris[3-(heptafluoropropylhydroxy-methylene)-d-camphorato]europium(III), 3. Lanthanide induced shifts, $\Delta\delta$, are consistent with major LSR binding at the hydroxyl, and the diastereotopic hydrogens of the CH_2 are clearly anisochronous. Substantial $\Delta\delta$ values and spectral simplification are achieved with 2 or 3. Significant enantiomeric shift differences, $\Delta\Delta\delta$, are observed with 3 that should provide direct optical purity determinations of 1. Results are discussed in terms of LSR binding sites and hindered rotations about selected bonds in 1.

INTRODUCTION

The drug methaqualone has been a sedative/hypnotic and a strictly controlled substance of abuse of considerable importance; over two hundred articles on the chromatographic, spectroscopic and analytical aspects of the compound have been cited in Chemical Abstracts from 1972 to the first half of 1988. Recently, extensive NMR studies of methaqualone employing 1-D and 2-D ^1H and ^{13}C techniques as well as lanthanide shift reagents (LSR) have been completed (1) that encouraged us to undertake further investigations

of related compounds. These present studies involve 2-methyl-3-(2'-hydroxymethylphenyl)-4(3H)-quinazolinone, 1, which has been found to be a major metabolite of methaqualone (2-14). The stereochemistry of the parent compound, methaqualone, has lately been of particular interest. Because of an energy barrier to rotation about the single bond joining the two aromatic moieties, this drug can exist as a pair of stable enantiomers that are chromatographically resolvable (15-19) and which exhibit unequal pharmacological activity (17). Drug stereochemistry can have important implications on pharmacological activity, toxicity and legal classification and has been of ongoing interest in our laboratories. The conversion of methaqualone to 1 was expected to raise the barrier to the biaryl rotation because of the effective increase in group size on going from methyl to hydroxymethyl. We therefore anticipated that the enantiomers of 1 should also be stable at ambient temperatures. In considering studies with achiral and chiral LSR, we were particularly interested in being able to compare lanthanide binding sites in methaqualone and in 1, because of the presence of an added potential binding site in 1, i.e., the hydroxyl group. In addition to examining lanthanide induced shifts, $\Delta\delta$, we considered

the use of a chiral LSR to obtain possible enantiomeric shift differences, $\Delta\Delta\delta$, that might permit direct optical purity determinations of 1. Enantioselectivity in the metabolism of methaqualone might thereby be explored if a method were available for measuring enantiomeric excess of 1. We employed the achiral reagent tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III), 2, known as $Eu(FOD)_3$, and the chiral reagent tris[3-heptafluoropropylhydroxymethylene)-d-camphorato]europium(III), 3, known as $Eu(HFC)_3$ or $Eu(HFBC)_3$. Techniques and principles for the use of achiral and chiral LSR have been described (20-26).

EXPERIMENTAL

Racemic 1 free base was obtained from the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD) through the Research Triangle Institute (Research Triangle Park, NC). The sample, batch no. 2563-1022-58, was used as supplied without further purification. Chloroform-d, (99.8 atom % D), obtained from Aldrich Chemical Corp., Milwaukee WI 53201 or from Norell, Inc., Landisville NJ 08326, was dried and stored over 3A Molecular sieves. Shift reagents were obtained from Aldrich and were stored in

a desiccator over P_2O_5. Materials were used as received except as noted.

For runs with shift reagents, an accurately weighed portion of drug was added to $CDCl_3$ [containing about 0.5% tetramethylsilane (TMS) as internal standard] in an NMR sample tube and dissolved by shaking; increments of solid shift reagent were added directly to the sample, dissolved by shaking, and the spectra immediately obtained. Drug concentrations were typically from 0.25-0.56 molal. The presence of particulates in some solutions made filtration necessary; some precipitate was observed during LSR additions. Because of some losses on filtration, actual drug concentrations and LSR:substrate molar ratios must be considered approximate. Nominal values are presented in the text and Figures. Filtering through $CDCl_3$-rinsed cotton (Pasteur filter pipet) resulted in significantly sharper signals.

RESULTS AND DISCUSSION

The 60 MHz ^1H spectrum of 1 in $CDCl_3$ at 28° showed signals as follows (δ, ppm): 8.20 (approx. d, J=8 Hz, 1H, H_5); ca. 7-7.9 (complex mult., 7H, other aromatic H); 4.38 (s, 2H, $H_{a,b}$); 3.7 (br s, 1H, OH); 2.15 (s, 3H, CH_3). The doublet at lowest field is assignable to H_5 because of carbonyl anisotropy. The assignment is

consistent with our studies of methaqualone (1). All of the seven remaining aryl hydrogens, $H_{6,7,8,3',4',5',6'}$, appear as part of a complex envelope; only H_5 is distinct from the aryl signals. The two diastereotopic hydrogens of the methylene group, $H_{a,b}$ of CH_2OH, are coincidentally isochronous at 60 MHz, appearing as a slightly broadened singlet at 4.38 ppm. The broad singlet of the exchanging OH appeared near 3.7 ppm, somewhat concentration dependent. The CH_3 appeared as a sharp singlet at 2.15 ppm. Increments of 2 were added resulting in considerable spectral simplification as a result of the lanthanide induced shifts, $\Delta\delta$(LIS). The LIS is defined as the chemical shift of a nucleus with LSR present minus the chemical shift of that nucleus for the unshifted substrate. Results with 2 added to 1 are summarized in Fig. 1. The nonequivalence of the diastereotopic H_a and H_b is clearly observed with their appearance as an AB quartet with a characteristic geminal coupling constant of approx. 13 Hz. This splitting allows assignment of the $H_{a,b}$ signals, distinct from other doublets (at higher 2:1 molar ratios) which exhibit the smaller vicinal coupling of adjacent hydrogens on the aryl rings. While the distinction of the expected approximate doublets of $H_{5,3',6'}$ is tentative since their ^3J couplings should be similar within experimental error, the

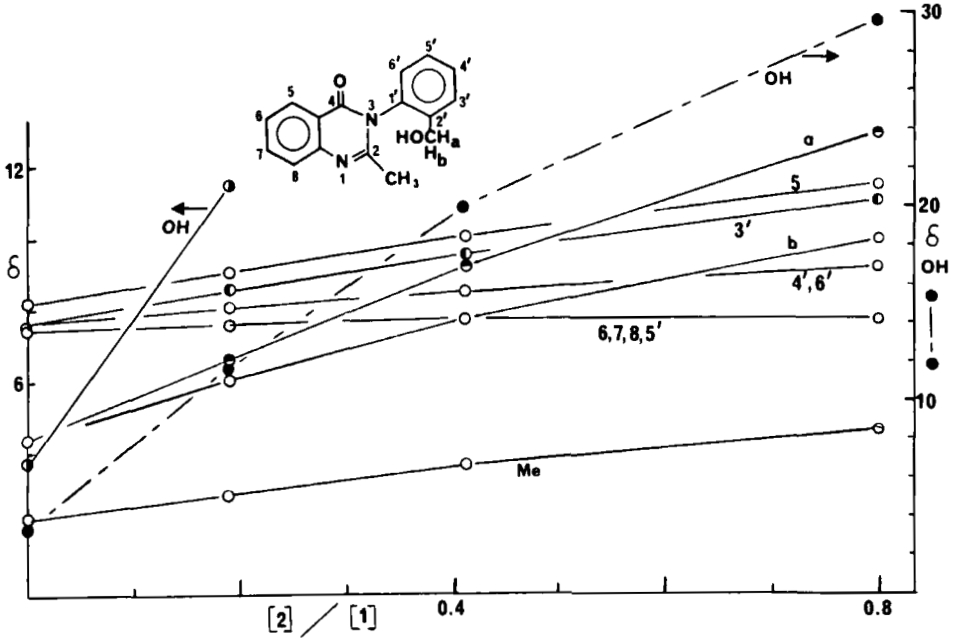

Fig. 1. Variation of chemical shift, δ (in ppm), with molar ratio of 2:1. Note: Where plotted points designate two or more nuclei, the indicated chemical shift generally corresponds to the approximate center of a complex multiplet. See text.

indicated assignments appear relatively consistent with the linear slopes of the corresponding lines in Fig. 1. Most striking is the dramatic difference compared to results with methaqualone (1) in which predominant LSR binding occurred at the carbonyl. While some LSR complexation at the carbonyl of 1 or at N-1 cannot be ruled out, the major binding site for 1 appears to be at the hydroxyl. The relatively unhindered primary OH group would probably be preferred to the alternative

sites both on steric and electronic grounds (20,22,25,26). The strongest evidence is seen in the relative slope magnitudes of Fig. 1, OH >> H_a > H_b > H_5 ≈ $H_{3'}$ > CH_3 > $H_{4'}$≈$H_{6'}$, with the remaining aryl protons, $H_{6,7,8,5'}$, showing the smallest magnitudes. The metabolic oxidation of methaqualone by which the 2'-methylphenyl is converted to the 2'-hydroxymethylphenyl of 1 thus results in a compound with a significantly altered LSR binding site.

Because of the existence of methaqualone as a pair of resolvable enantiomers of differing pharmacological activity, and the efficacy of chiral LSR to permit direct optical purity determinations, we extended our studies of 1 using the chiral reagent, 3. These results are summarized in Figs. 2 and 3. Induced shift magnitudes are generally similar to results with 2, with some differences. For example, at higher molar ratios of 3:1, the $H_{3'}$ resonance appears to move downfield past that of H_5. The spectra at these higher levels of 3 are more complex than with added 2 because of enantiomeric shift differences, $\Delta\Delta\delta$, seen for several nuclei when the chiral 3 is used. The enantiomeric shift difference is the magnitude of the difference in chemical shifts for corresponding nuclei of a pair of enantiomers in the presence of a chiral LSR. Substantial $\Delta\Delta\delta$ values are clearly observed for

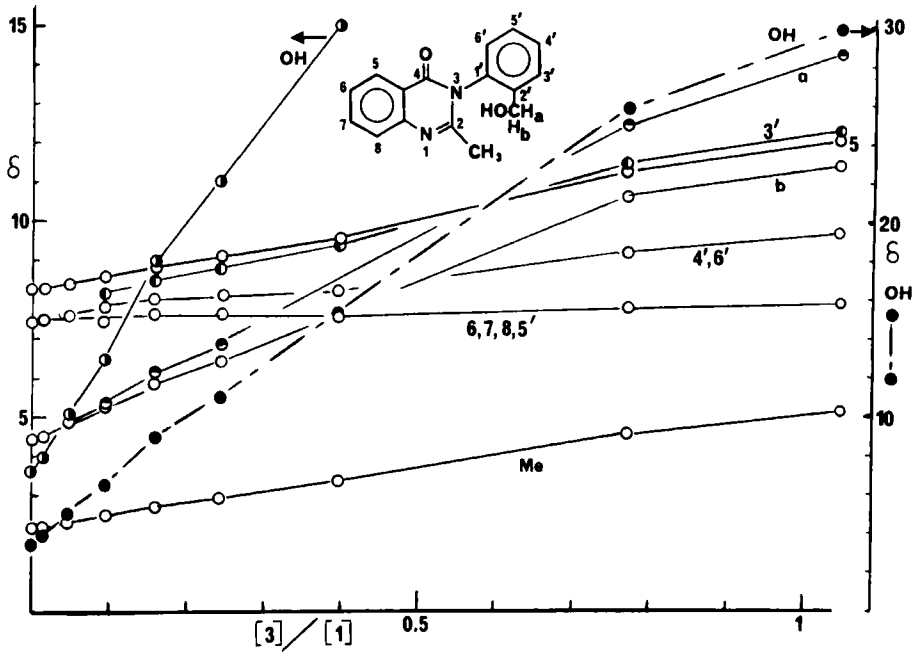

Fig. 2. Variation of chemical shift, δ (in ppm), with molar ratio of 3:1. Note: Average values are plotted where antipodal differences occur. See Note for Fig. 1.

the OH, CH_3 and H_a nuclei, which would be close to the LSR bound to the hydroxyl oxygen and also close to the region of molecular chirality, i.e., near the N_3-$C_{1'}$ bond. Observation of $\Delta\Delta\delta$ confirms that rotation about this hindered bond of 1 must be slow on the NMR time scale (at 60 MHz and 28°). Location of a nucleus close to a chiral center and to bound LSR has commonly been found to favor $\Delta\Delta\delta$. It is of interest that at lower 3:1 molar ratios, the upfield half of the

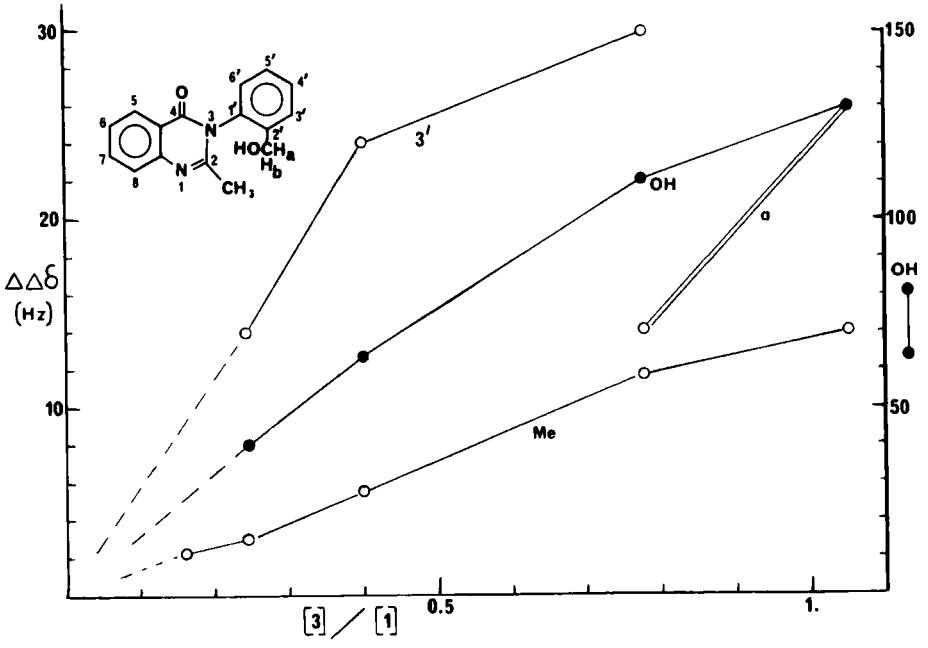

Fig. 3. Variation of enantiomeric shift difference, $\Delta\Delta\delta$ (in Hz), with molar ratio of 3:1. Values shown for some nuclei (e.g., $H_{3'}$) are considered tentative and are not shown for other nuclei (e.g., H_b) because of uncertainties due to overlaps with interfering peaks. See text.

approximate AB quartet of the diastereotopic methylene protons appears substantially broadened (with resultant lower peak heights) relative to the downfield half. The downfield and upfield signals have been assigned to H_a and H_b, respectively. Presumably H_b is broadened by some $\Delta\Delta\delta$, more than H_a. At higher 3:1 molar ratios, the H_b signals are overlapped with peaks assigned to H_5

and H_3, and the $\Delta\Delta\delta$ values cannot rigorously be assigned. Tentative $\Delta\Delta\delta$ values for H_3, are shown in Fig. 3. Assuming that the plots of $\Delta\Delta\delta$ for H_a and H_b increase monotonically with added 3 , then H_b should actually display larger $\Delta\Delta\delta$ magnitudes than H_a.

The observation of very large $\Delta\Delta\delta$ for an exchangeable hydrogen, OH, is quite striking. Despite some line broadening, potential analytical utility for direct optical purity determinations should be possible. For example, with a 3:1 ratio of 0.401 and $\Delta\Delta\delta$ of 63 Hz, the valley height above the baseline was only 16% of the average height of the peaks for each enantiomer's OH signal. The H_a signal is also potentially useful. However, the methyl signal appears to be most suitable for determining enantiomeric excess, particularly because of its favorable signal-to-noise ratio. We observed valley heights of 13 and 17% of the average peak heights for the methyl signals at 3:1 ratios of 0.774 and 1.05, respectively. Detection of as little as 5-7% of a minor enantiomer should be feasible. The absence of interfering signals would make this a non-critical technique. The use of this chiral LSR method appears to be the first report of a potential technique for direct optical purity determinations of 1.

CONCLUSIONS

We have reported the use of the achiral LSR, $Eu(FOD)_3$, 2, and the chiral LSR, $Eu(HFC)_3$, 3, for ^1H NMR spectral simplification of racemic 1. The potential utility of 3 for direct optical purity determinations of samples of 1 has been shown. Predominant binding of the europium, for either 2 or 3, appears to occur at the hydroxyl; relative magnitudes of $\Delta\delta$ and $\Delta\Delta\delta$ are consistent with this as the favored binding site. The availability of a direct method for measuring enantiomeric excess of 1 should allow studies of enantioselectivity in the metabolism, distribution and excretion of methaqualone with respect to a major metabolite, 1.

ACKNOWLEDGMENTS

Samples of 1 were kindly provided by the Research Technology Branch of the National Institute on Drug Abuse (Rockville MD) through the Research Triangle Institute (Research Triangle Park, NC). These studies were supported, in part, by the National Science Foundation Instrumentation and Laboratory Improvement Program grant no. USE-8851684, U.S. Department of Education Minority Science Improvement Program grant

no. G008641165, Hewlett-Packard Co. grant no. 0017-80769, Hoffmann-La Roche Inc. and the Sandoz Research Institute (to R.R.)

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Date Received: 08/15/89
Date Accepted: 09/14/89