Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXIV, No. 2

NUCLEAR OVERHAUSER EFFECTS IN TRITIUM NMR

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SUMMARY

The accuracy of the quantification of the tritium distribution in labelled compounds may be reduced by differential nuclear Overhauser effects, especially for compounds in which the different tritiated positions differ in the number of protons surrounding them.

Keywords: steroids, tritiation, quantification of distribution of label

INTRODUCTION

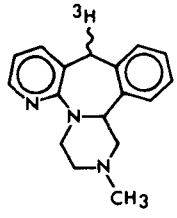
^{3}H NMR spectroscopy is a fast and non-destructive method of localizing tritium in a labelled molecule[1]. The integral of NMR signals is in general proportional to the number of contributing nuclei, so ^{3}H NMR is also suited for the quantification of the tritium distribution. ^{3}H NMR spectra are generally recorded using ^{1}H broad-band decoupling which simplifies the ^{3}H signals (by removing ^{3}H-^{1}H couplings) and enhances the intensity of the ^{3}H signals; the enhancement, the so-called nuclear Overhauser effect (NOE), has a theoretical maximum of 47%[2].

Since the ^{3}H signals from different tritiation situations (different site and/or number of ^{3}H-atoms) will in principle undergo different NOE's, the normal, continuous, ^{1}H decoupling will introduce errors in the quantification of the ^{3}H-distribution.
If the NOE's are unknown, which is generally the case, these errors can only be avoided by suppressing the NOE[3] by the so-called inverse-gated ^{1}H decoupling experiment. In this experiment[4] additional delays without decoupling are inserted between the signal acquisitions. However, these

0362-4803/87/020219-07$05.00

Received July 10, 1986

inverse-gated decoupling experiments are very time-consuming and it has been argued[5] that since the NOE-differences are generally small, many quantitative ^3H distribution measurements are not essentially improved this way. In order to quantify such errors we determined the (differential) NOE's for a number of model compounds.



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H-Thr-Ser-Glu-Lys-Ser-Gln-Thr-
Pro-Leu-Val-Thr-Leu-OH

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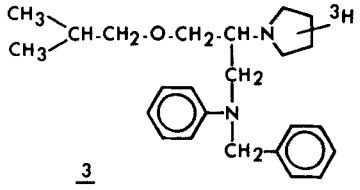

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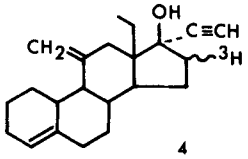

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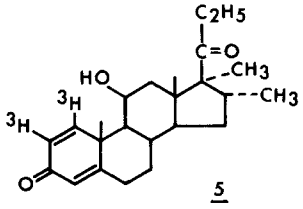

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Figure 1

EXPERIMENTAL

^{1}H-NMR spectra were recorded with a Bruker AM360 NMR spectrometer operating at 384,138 MHz always in the presence of 5 mg unlabelled material as carrier. Chemical shifts are referred to "ghost-TMS" the value of which was obtained by multiplying the ^{1}H frequency of TMS by 1,06663975. Generally the following spectrum parameters were applied: sweep width 4000 Hz, time domain 16K, size 32K, ^{1}H broad band decoupling (1,2 W), acquisition time 2 s and in case of NOE suppression a delay of 3 s.

F.D.-mass spectra were measured by Dr. W. Lehmann, University Hospital Eppendorf, Hamburg, Federal Republic of Germany.

[10-^{3}H]-Org 3770 (mepirzepine, proposed usan) (1) was prepared by exchange of 6-azamianserin with ^3H_2O catalyzed by $NaOCH_3$.

[lysine-^{3}H]-des-enkephalin-γ-endorphin (2) was prepared as described elsewhere[7].

[pyrrolidine-^{3}H]-bepridil (3) was synthesized as described elsewhere[9].

[16-^{3}H]-desogestrel (4) was prepared as described elsewhere[6].

[1,2-^{3}H]-rimexolone (5) was prepared by reduction of rimexolone with $^3H_2$ and reintroduction of $\Delta^{1,2}$ with thallium acetate.

All labelled compounds were purified by HPLC and had radiochemical purities of ≥98% as checked by TLC and HPLC.

RESULTS

The compounds for which we measured the NOE's are given in Figure 1. For [10-^{3}H]-Org 3770 (1) with a specific activity of about 1 Ci·mmol^{-1} two signals are obtained at 3,38 ppm (^{3}H(eq)) and 4,50 ppm (^{3}H(ax)). The

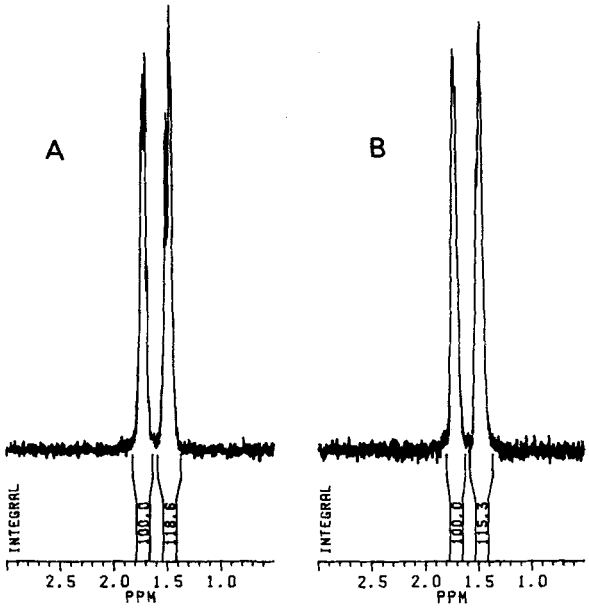

Figure 2: ^{1}H-decoupled ^{3}H NMR spectra of [lysine-^{3}H]-des-enkephalin-γ-endorphin (2); solvent ^2H_2O

A: NOE B: NOE-suppressed

10eq/10ax ratio without ^{1}H decoupling is 6,87 and this changes to 6,74 in the case of ^{1}H-coupled spectrum illustrating that differential NOE's are not important for this compound. We also measured compounds of higher spec. activity such as ^{3}H-des-enkephalin-γ-endorphin (2) tritiated as the β and γ-positions of the lysine residue (specific activity 43 $Ci \cdot mmol^{-1}$; mixture of mono-, di- and tritritiated material[8]) and [pyrrolidine-^{3}H]-bepridil (3), a mixture of mono-/ditritiated material[9]. For ^{3}H-des-enkephalin-γ-endorphin the spectra are shown in Figure 2. The signals at 1,40 and 1,65 ppm confirm the tritiation at the Cβ and Cγ of the lysine residue; the complex pattern is due to the presence of different mono-, di- and tritritiated materials.

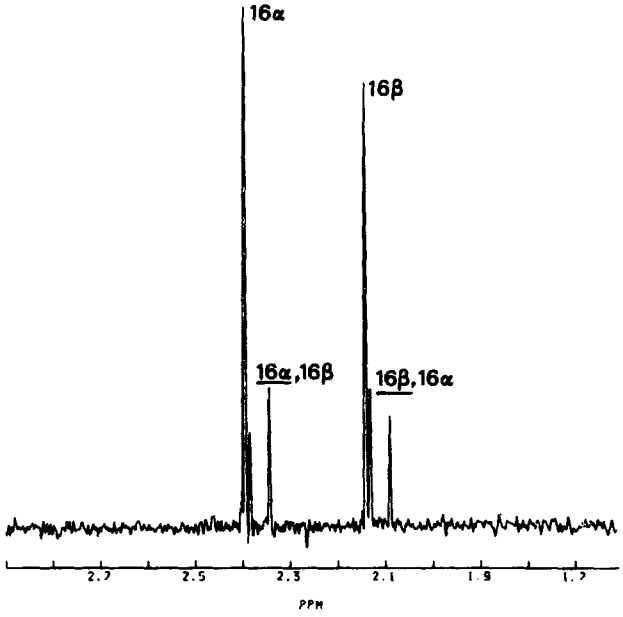

Figure 3: ^{1}H-decoupled ^{3}H-NMR spectrum in $C^{2}HCl_3$ of [16-^{3}H]-desogestrel (4)

Under NOE-conditions a ratio for $^{3}H(\beta)/^{3}H(\gamma)$ of 1,19 is obtained while under NOE suppressed conditions this changes to 1,15, again an illustration for the absence of significant differential NOE's. For bepridil we measured the ratio [mono-3-^{3}H]-/[cis-3,4-$^{3}H_2$]-material both under NOE and NOE suppressed conditions and again no significant differences were observed (the ratio of 1,19 changes to 1,21).

On the other hand, substantial differential NOE's have been measured for [16-^{3}H]-desogestrel (4) and [1,2-^{3}H]-rimexolone (5). The ^{1}H decoupled ^{3}H spectrum of [16-^{3}H]-desogestrel (4) is shown in Figure 3. The singlets at 2,14 and 2,39 ppm point to [16β-^{3}H]- and [16α-^{3}H]-desogestrel respectively

while the two doublets at 2,11 and 2,36 ppm are due to [16-$^3H_2$]-desogestrel. The measured absolute NOE's (with standard deviations) are: [16α-^{3}H]: 36% (3,6%), [16β-^{3}H]: 38% (2,3%) and [16α-$^3H_2$]: 14% (5,8%). The ratio monotritiated/ditritiated material as measured from the NOE-suppressed ^{3}H spectrum is 3,95 which is in good agreement with the ratio 3,92 obtained by F.D.-mass spectrometry. On the other hand, the "normal" ^{3}H spectrum gives a value of 4,76 which is about 20% too high.

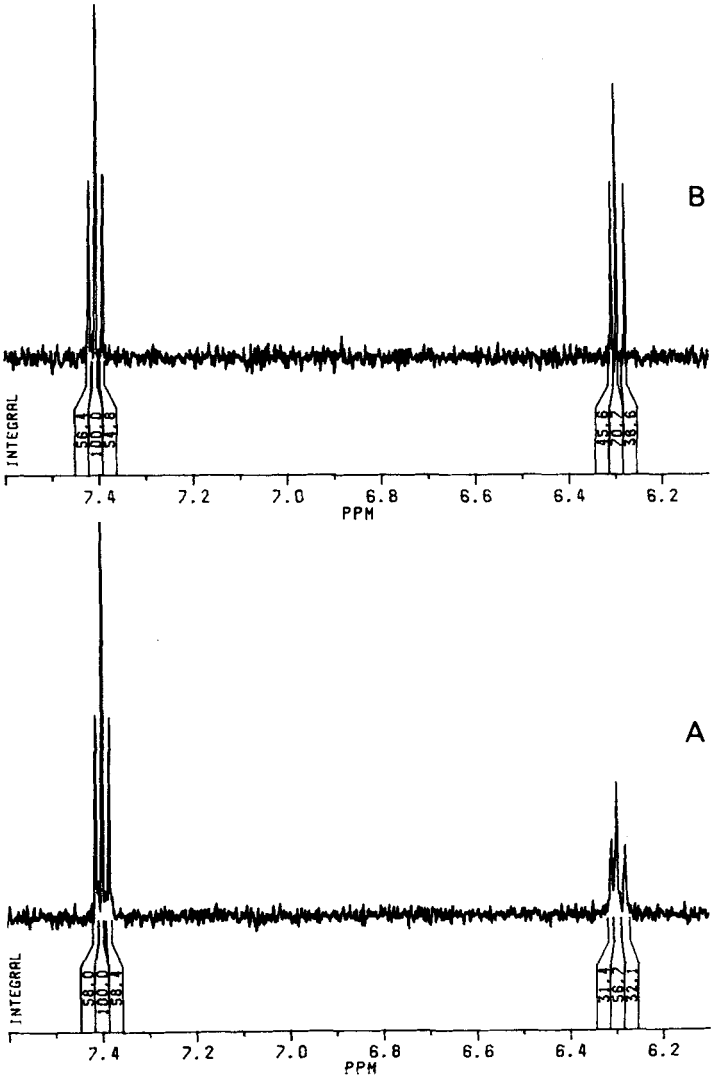

Figure 4: ^{3}H NMR spectra (^{1}H-decoupled) in $C^2HCl_3/C^2H_3O^2H$ of [1,2-^{3}H]-rimexolone (5)

A: with NOE B: NOE-suppressed

The ^{3}H NMR spectrum of [1,2-^{3}H]-rimexolone (5) is shown in Figure 4. The singlets at 7,43 and 6,31 ppm are due to molecules monotritiated at C(1) and C(2), respectively while the doublets at these positions represent the 1,2-ditritiated material. In the "NOE" spectrum the ratio $^{3}H(1)/^{3}H(2)$ is 1,87 while in the NOE-suppressed spectrum a ratio of 1,56 is observed. Apparently the tritium signals at C(1) are favoured by the NOE from the protons at C(19). This is further illustrated by the inequality of the doublets in the NOE spectrum; of course they should show the same intensity. Even in the NOE-suppressed spectrum the intensity of the doublet at 6,3 ppm is about 20% too low, indicating the presence of some residual NOE-effects. The ratio of monotritiated/ ditritiated material calculated from the NOE-suppressed spectrum is 1,82 which is in reasonable agreement with the value of 2,02 obtained by F.D. mass spectrometry.
It can be concluded that the accuracy of quantitative measurements by ^{3}H NMR of distribution of label and labelled species (such as for the determination of specific activies[6,10]) can be influenced by NOE. Particularly for the tritium atoms without nearby protons the spectra should be measured under NOE-suppressed conditions. Naturally, the advantage of more accurate measurements should be balanced against the longer recording times needed.

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