TRITIUM NMR SPECTROSCOPY OF STEROIDS

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SUMMARY

The ^{3}H NMR spectra of a series of 7 steroids tritiated at C(1), C(2), C(6), C(7), C(9) and C(16) yield quantitative information on the ^{3}H distribution in these compounds. Deuterated chloroform is not a good solvent to examine the regio- and stereospecificity of the labelling process for acid labile positions such as C(2) in a 3-oxosteroid and C(6) in a 3-oxo-Δ^{4}-steroid.

Keywords : stereochemistry, detritiation.

INTRODUCTION

Precise knowledge of the distribution of the label in labelled compounds, is of importance for the correct interpretation of metabolic and pharmacokinetic studies.

Organon has been involved in the synthesis and analysis of tritiated compounds for more than 15 years[1,2]. The distribution of the label in these products was determined by degradation or indirectly on the basis of labelling experiments with deuterium.

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Recent publications[3,4] on ^{3}H NMR spectroscopy, pointed to the superiority of this method, since both the position and extent of ^{3}H labelling can be measured quickly and non-destructively.

Therefore we included ^{3}H NMR in our standard analyses[5] (HPLC, TLC, mass spectrometry). In the present paper we report our ^{3}H NMR results for a number of steroids (Figure 1).

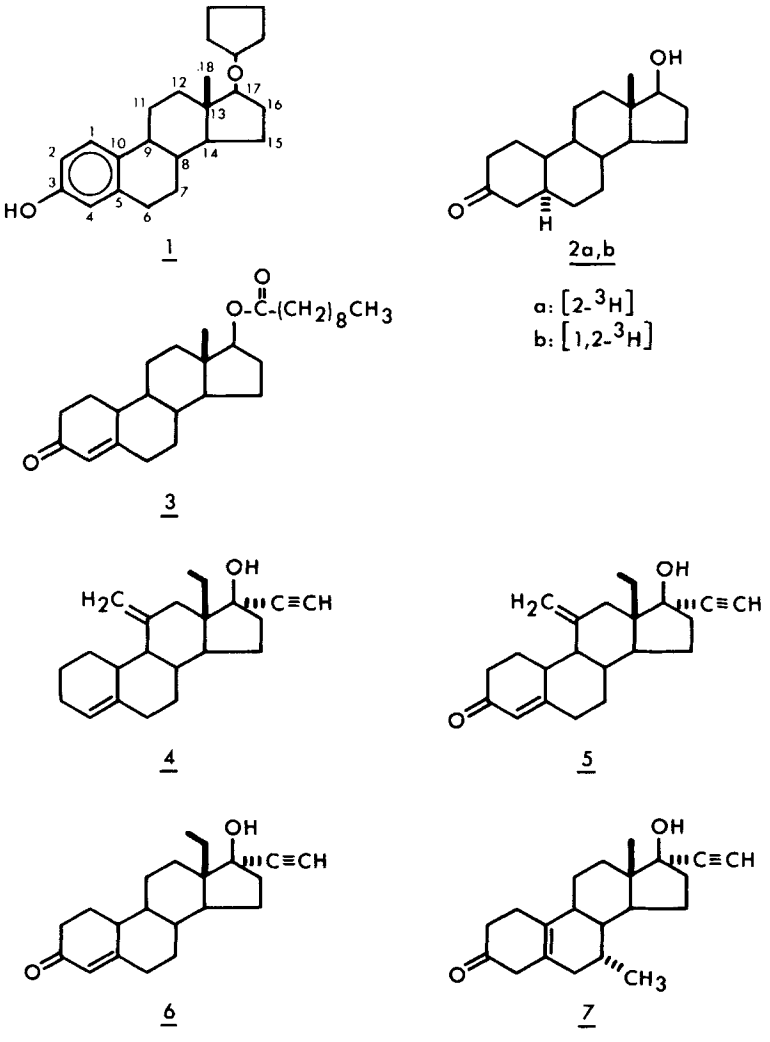


Fig. 1

RESULTS AND DISCUSSION

Synthesis

The tritiated steroids in Figure 1, were synthesized via three routes :

1) exchange with $^3H_2$ gas at benzylic positions catalyzed by Pd/C for the 17β-cyclopentylether of oestradiol (1) according to the method of Evans et al.[6].
2) reduction of a double bond with $^3H_2$, in case of dihydronortestosterone (2b) the 1,2-double bond and for nandrolone decanoate (3) the 6,7-double bond in their corresponding precursors.
   In one experiment the labile C(2) tritons of 2b were removed by reaction with dilute alkaline resulting in 2a.
3) exchange with excess ^3H_2O of the 17-oxo precursors of compounds 4-7 followed by ethynylation analogous to the method described for lynestrenol[1,2]. The 17-oxosteroids- in case of 5-7 as 3-(thio)acetals - were heated in DMF at 80°C with ^3H_2O for 48 hours. By adding a catalytic amount of sodium methoxide, the reaction time could be reduced to 2 hours at 80°C. Initially ethynylation was carried out with ethynylmagnesium bromide in THF[2], but application of the commercially available ethynyllithium ethylenediamine complex resulted in more simple and mild conditions. Hydrolysis of the (thio)acetal functions resulted in the tritiated compounds 5-7.

^3H NMR

The ^3H NMR spectra are summarized in Table 1; typical spectra are given in Figures 2-4.

Recent one-and two-dimensional ^1H NMR studies on steroids[7-9], allowed complete assignments of both chemical shifts and coupling constants. These ^1H data agree with the ^3H data given in Table 1, considering that $J(^3H,^1H) = 1.07\ J(^1H,^1H)$, $J(^3H,^3H) = 1.14\ J(^1H,^1H)$ and $\delta(^3H) \simeq \delta(^1H)$[10]. Table 1 confirms further, that ^3H isotope effects are c. 0.01 ppm for vicinal gauche ^3H's and c. 0.03 ppm for geminal ^3H's, both to higher field[10].

Table 1. ^{3}H NMR spectra of compounds 1 - 7.

Compound	Solvent	Chemical shift (δ)[a]	Assignment[b]	Relative intensity[c]		$J(^{3}H, ^{3}H)$ [d]	$J(^{3}H, ^{1}H)$ [e]
1	$C^{2}HCl_{3}$	1.25	7α	1		--	f
		2.13	9α	66		--	11, 11, 4
		2.80	6β	33		--	18, 12, 6
2a[g]	$C^{2}HCl_{3}$	1.21	1α	27		--	14, 14, 12, 5
		2.25	1β	73		--	14, 6, 6, 3
2b	$C^{2}HCl_{3}$	1.18	1α(2α)	6		4.4	f
		1.21	1α	54		--	f
		1.57	$^{3}H^{1}HO$	17		--	--
		2.24	1β	20		--	f
		2.35	2α(1α)	6		4.4	f
2b	$C_{6}^{2}H_{6}$	0.80	1α(2α)	46		4.9	f
		0.81	1α	26		--	f
		1.76	1β(2β)	14		7.1	f
		1.77	1β	10		--	f
		1.85	2β(1β)	14		7.1	14, 14
		1.87	2β	2		--	f
		2.28	2α(1α)	46		5.0	14, 4, 2
		2.29	2α	2		--	f
3	$C^{2}HCl_{3}$	1.02	7α(6α)	14	10^{h}	4.6	f
		1.03	7α	14	14^{h}	--	14, 14, 4
		1.64	$^{3}H^{1}HO$		10^{h}	--	--
		1.78	7β(6β)	31	18^{h}	4.9	f
		1.80	7β	26	36^{h}	--	14, 3, 3, 3
		2.21	6β(7β)	31	18^{h}	5.6	14, 14, 2
		2.23	6β	4	6^{h}	--	f
		2.43	6α(7α)	14	10^{h}	4.0	14, 4
		2.44	6α	2	4^{h}	--	f
3	$^{2}H_{6}$-DMSO	0.94	7α(6α)	18		4.8	f
		0.95	7α	9		--	f
		1.72	7β(6β)	45		5.0	14, 3, 3
		1.73	7β	16		--	f
		2.22	6β(7β)	45		5.6	14, 14, 2
		2.23	6β	10		--	f
		2.39	6α(7α)	18		4.3	14, 4
		2.40	6α	2		--	f
4	$C^{2}HCl_{3}$	2.04	16β(16α)	21		15.6	12, 4
		2.07	16β	34		--	15, 12, 4
		2.29	16α(16β)	21		15.1	9, 6
		2.33	16α	43		--	15, 10, 6
4[i]	$^{2}H_{6}$-DMSO	1.90	16β(16α)	22		15.3	f
		1.94	16β	38		--	f
		2.09	16α(16β)	22		15.3	f
		2.12	16α	40		--	f

5	C^2HCl_3	2.08	16β(16α)	21	15.7	f
		2.11	16β	32	--	f
		2.32	16α(16β)	21	15	f
		2.35	16α	47	--	f
6	C^2HCl_3	2.08	16β	47	--	15, 12, 4
		2.30	16α	53	--	15, 9, 6
7	C^2HCl_3	2.00	16β	50	--	f
		2.30	16α	50	--	f

a In ppm from the ghost reference, determined from the ^1H decoupled spectra.

b Based on chemical shifts and on ^3H-^3H splitting patterns.

c Calculated from (continuously) ^1H decoupled spectra.

d In Hz, determined from ^1H decoupled spectra.

e In Hz, estimated from - often degraded - ^3H- ^1H splitting patterns.

f Not determined.

g C(2) tritons removed by dilute alkaline.

h 24 hours later.

i Spectrum recorded by Dr. J.A. Elvidge, University of Surrey.

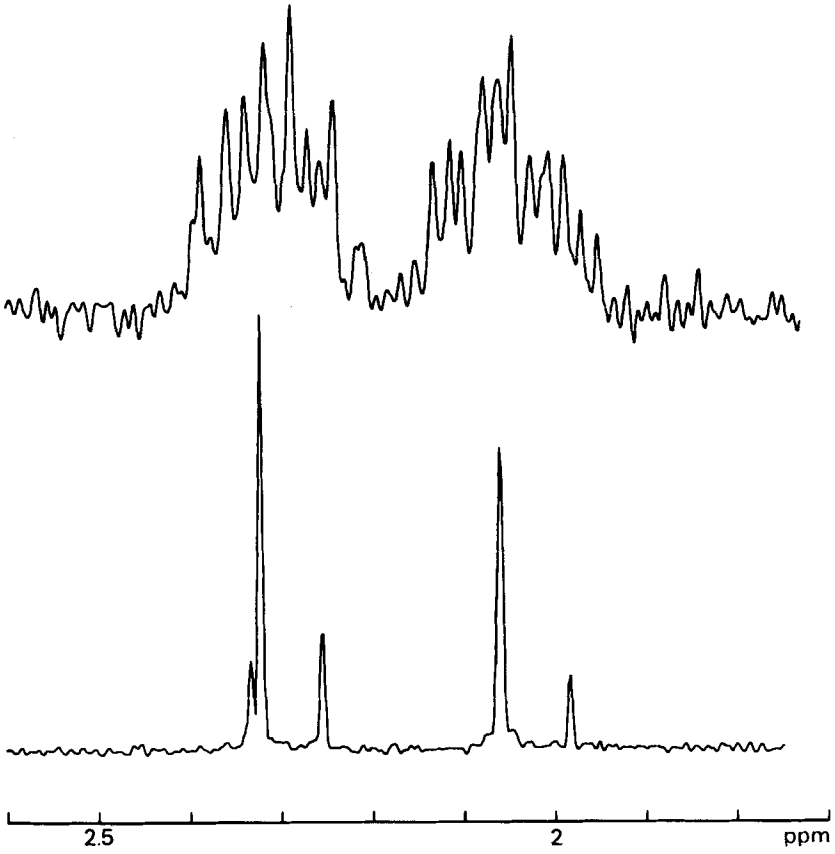


Fig. 2 ^3H NMR spectra of 4 in C^2HCl_3
Lower trace: ^1H decoupled; upper trace: ^1H coupled

Fig. 3 ^{3}H NMR spectrum of 1,
A: ^{1}H decoupled
B: ^{1}H coupled
C: expansion of B

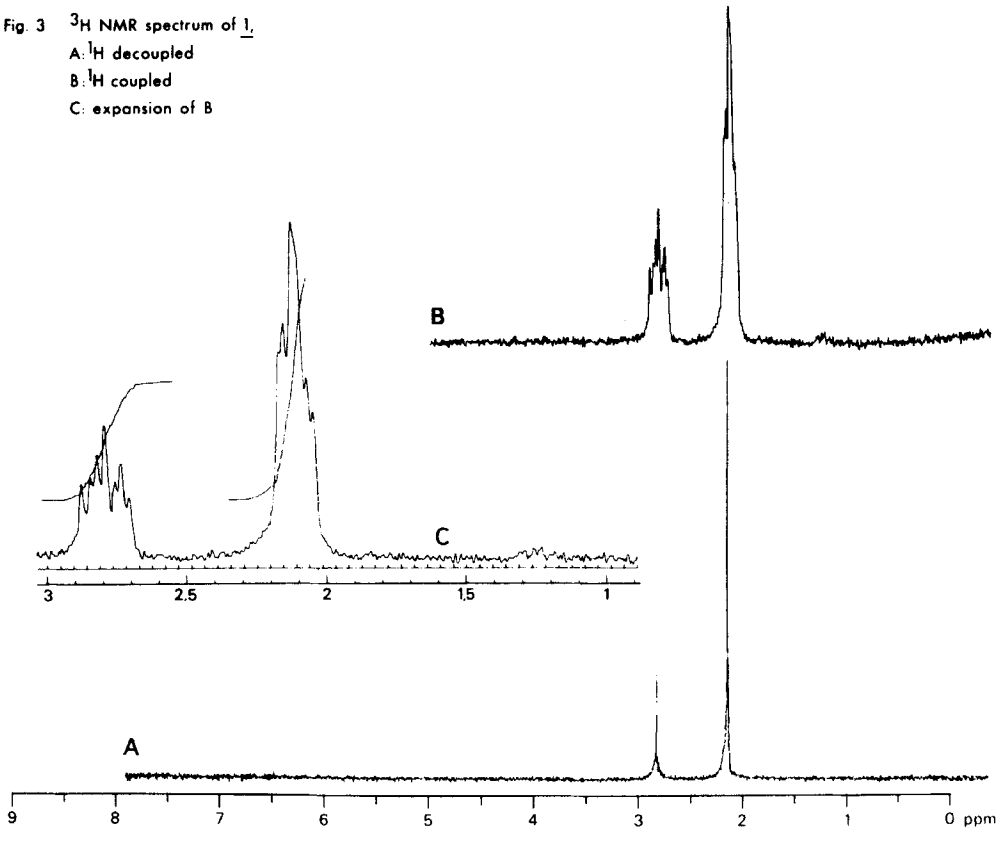
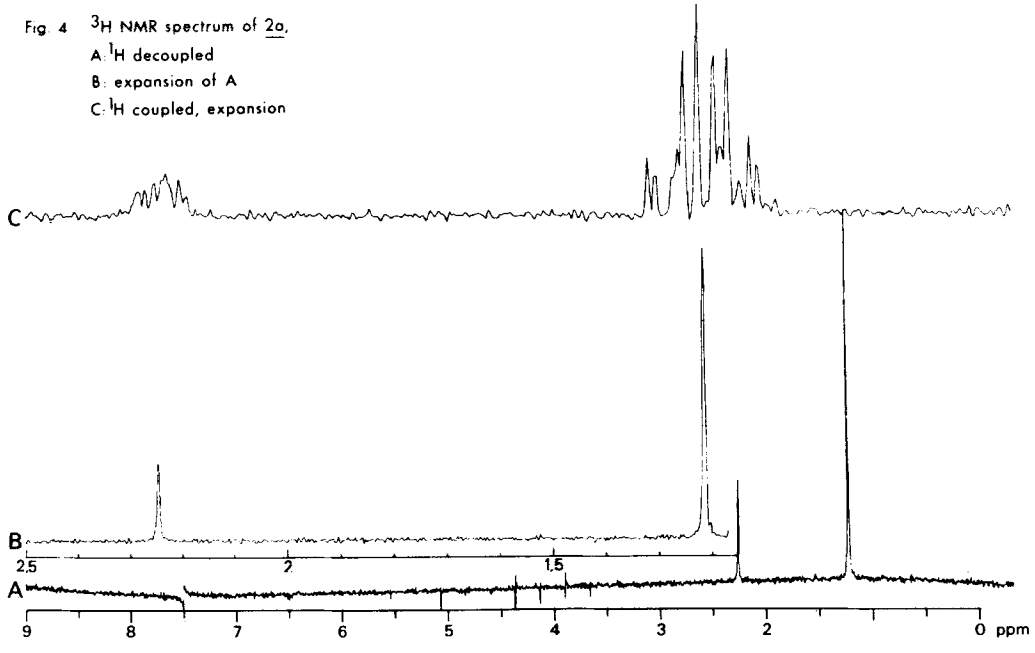


Fig. 4 ^{3}H NMR spectrum of 2a,
A: ^{1}H decoupled
B: expansion of A
C: ^{1}H coupled, expansion



The $^{3}H(6\beta)$ assignment for 1 is in conflict with another study[11], but the approximately 12 Hz vicinal coupling leaves no doubt that the ^{3}H at C(6) is in the axial β-position.

The dependence of the ^{3}H distribution on the solvent for 2b and 3, and on time for 3, points to detritiation processes in $CDCl_3$. The $^{3}H^{1}HO$, also found in these $CDCl_3$ solutions, suggests that the C(2) tritons of 2b and the C(6) tritons of 3 exchanged with protons from ubiquitous water. These processes are probably induced by acidic impurities in $CDCl_3$. Hence, for compounds tritiated at enolic or allylic positions, other solvents such as deuterated benzene or ditto DMSO should be preferred.

The ^{3}H-distribution over the steroids is as expected for the reaction conditions, viz.: exchange of the benzylic protons in 1[3] and exchange exclusively of the C(16) protons in 4-7. On reduction of the double bond in 2b and 3 no allylic exchange was observed, although asymmetrical reduction[12] is obviously also operative with these steroids.
In the [16-^{3}H]-steroids 4-6 a slight preference of the label for the α-position is observed. This could be attributed to steric hindrance by the 13-ethyl group.

In principle it should be possible to determine the specific activities of the tritiated steroids from the decrease of the signals in the ^{1}H NMR spectra. However, these spectra are often obscured by the impurities present, such as plasticizers, paraffinic materials and water. Moreover, for compounds 1-7 the signals of interest overlap with other ^{1}H signals, which prevents the determination that way.
With the steroids 4-7, which are tritiated at C(16), the ^{3}H signals of both mono-and ditritiated molecules can be observed (Table 1). If we ignore isotope effects in the enolisation reaction (which is not correct[13]) it is possible to calculate the specific activity from the equation $S.A. = 58.3\ [1-(2R+1)^{-1}]$ in which S.A. is the specific activity and R is the ratio between di- and monotritiated material. Comparison of the specific activities and R-values obtained directly from mass spectra of both tritiated and deuterated steroids supports the validity of this equation.

The specific activities calculated from the R-values determined by ^{3}H NMR are compiled in Table 2, together with the measurement of the specific activities by mass spectrometry. The specific activities calculated from NMR seem to be slightly too low, i.e. the ratio ditritiated/monotritiated should be too low. This may be due to a differential nuclear Overhauser effect[15] (NOE) produced by ^{1}H decoupling, favouring the ^{3}H NMR-intensities of the monotritiated steroids over the ditritiated ones.

Table 2. Specific activities (S.A.) for the tritiated steroids 4 - 7.

steroid	$[^{3}H_2]/[^{3}H_1]$ from ^{3}H NMR	S.A.[a] from ^{3}H NMR	S.A.[a] from mass spectrometry
desogestrel 4	0.27	20.7	23.0
3-oxodesogestrel 5	0.26	20.1	25.0
norgestrel 6	0.09	9.0	8.5
7α-methyl-norethinodrel 7	0.08	8.1	8.0

[a] in Ci mmol^{-1}

EXPERIMENTAL

All tritiation reactions were carried out at Amersham International plc, U.K. ^{1}H (200 MHz) and ^{3}H (213 MHz) NMR spectra were obtained with a Bruker WP 200 Fourier transform spectrometer. The ^{1}H spectra were referred to internal TMS; multiplication of this ^{1}H frequency by 1.06663975 (the Larmor ratio for $^{3}H/^{1}H$), yielded a ghost reference for the ^{3}H spectra[3]. Most ^{3}H spectra have been taken with and without broad-band ^{1}H decoupling. Specific activities were determined by E.I.-mass spectrometry on a CH7 spectrometer using selected ion-monitoring, or by F.D.-mass spectrometry[14] by Dr. Lehmann, University Hospital Hamburg, Federal Republic of Germany.

[6,9-^{3}H]Oestradiol-17-cyclopentylether (1) was prepared by exchange with $^{3}H_2$ in ethyl acetate with Pd/C as catalyst. It was purified by preparative

HPLC on LiChrosorb 10 RP 18 with methanol/water (9:1, v/v) as eluent. Radiochemical purity > 98%. Spec. act. 9.9 Ci/mmol.

5α-Dihydro-19-nor[1,2-^{3}H]testosterone (2) was prepared by reduction of the Δ 1,2-analogue with $^3H_2$ on Pd/C (10%) in ethyl acetate. The label at C(2) was exchanged in 0,1 N NaOH in methanol/water at 50°C. The final product was purified by preparative HPLC on LiChrosorb Si-60-5 with n-hexane/propanol-2 (95:5, v/v) as eluent. Radiochemical purity >99%. Spec. act. 25.5 Ci/mmol.

19-Nor[6,7-^{3}H]testosterone decanoate (3) was prepared by reduction of the Δ 6,7-analogue with $^3H_2$ in pyridine catalyzed by $Pd/CaCO_3$. It was purified (at Amersham International plc) by preparative TLC. Radiochemical purity > 98%. Spec. act. 38 Ci/mmol.

[16-^{3}H]Desogestrel (4)

13-Ethyl-11-methylene-18,19-dinorandrost-4-ene-17-one was tritiated with ^3H_2O in DMF at 140°C for 48 hours. The resulting crude product was ethynylated with ethynylmagnesium bromide in THF and the reaction product was purified on TLC (silica gel with toluene/ethyl acetate (95:5, v/v)). Radiochemical purity > 98%.

3-Oxo[16-^{3}H]desogestrel (5)

13-Ethyl-11-methylene-18,19-dinorandrost-4-ene-3,17-dione-3-cyclic-1,2-ethanediyl-acetal was tritiated by reaction with ^3H_2O in DMF at 80°C catalyzed by sodium methoxide. The crude product was ethynylated in dioxan at room temperature with LiC≡CH-Ethylenediamine comolex and the acetal group was removed by reaction with HCl in acetone. The product was purified by HPLC on Cp^{tm}—Spher C_{18} LiChrosorb in methanol/water (6:4, v/v). Chemical purity > 98%.

[16-^{3}H]D-Norgestrel (6)

13-Ethyl-18,19-dinorandrost-4-ene 3,17-dione-3-cyclic-1,2-ethanediyl--thioacetal was tritiated by reaction with ^3H_2O in DMF at 145°C for 45 hours.

The resulting product was purified by TLC (silicagel toluene ethyl/acetate 8:2, v/v) and was ethynylated with ethynylmagnesium bromide in THF. The thioacetal group was removed by reaction with methyl iodide in ethanol. The crude steroid was purified by TLC on silica gel; toluene/ethyl acetate (8:2, v/v). Chemical purity > 99%.

7α-Methyl[16-^{3}H]norethinodrel (7)

(7α)-7-Methylestr-5(10)-ene-3,17-dione-3,3-dimethylacetal was tritiated with $^{3}H_2O$ catalyzed by sodium methoxide by reaction in DMF at 80°C for 2 hours. The resulting product was ethynylated in dioxan with LiC≡CH--ethylene diamine.comolex and the acetal function was hydrolyzed by reaction with oxalic acid in ethanol. The final product was purified by preparative HPLC on μ Bondapak C_{18} with methanol/water (6:4, v/v) as eluent. Radiochemical purity >95%.

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