TRITIATION OF MIANSERIN

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Summary

Labelling of mianserin in the N-methyl group or the piperazine ring results in changes in the physical properties of the molecule. Using deuterium it is shown that mianserin can be labelled at metabolically stable positions without introducing isotope effects by exchange under alkaline conditions (at C_{10}) or by reductive dechlorination (at C_{12} or C_{13}). [13-^{3}H]-mianserin with a specific activity of 16 Ci.mmol^{-1} was synthesized by reductive dehalogenation of 13-chloromianserin using $^{3}H_{2}$. The position of the label was confirmed by ^{3}H-NMR spectroscopy.

Keywords: mianserin, tritiation, deuteration, ^{3}H-NMR

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INTRODUCTION

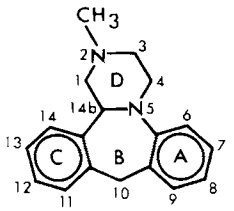
Mianserin (1,2,3,4,14b-hexahydro-2-methyldibenzo(c,f)-pyrazino(1,2-a-)-azepine, Figure 1, 1) is a tetracyclic compound intended for the treatment of depressive illness and depression associated with anxiety. Clinical experience with depressed patients has established that it is more potent than tricyclic antidepressants with fewer side-effects[1].

[13-^{3}H]-mianserin was synthesized for metabolism studies[2]. Mianserin tritiated in the methyl group[3] or at C_8[4] has recently become commercially available and this prompted us to summarize in this paper our results and problems with the synthesis with tritiated mianserin.

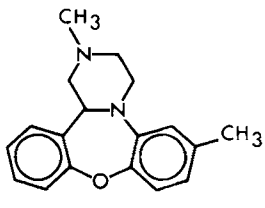
RESULTS

Position of the label

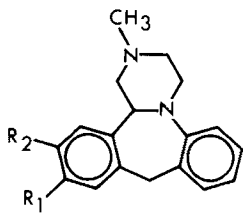
As was shown in experiments with deuterium, mianserin can be labelled at almost every position of the molecule[5]. In the structurally related compound Org GC 94 (Figure 1, 2) deuteration at C_3 and C_4 in the piperazine ring resulted in an unusual isotopic fractionation of labelled and unlabelled compounds during HPLC[6]. For mianserin deuterated at positions 1, 3 or 4 (by reduction of the corresponding keto-mianserin with $LiAl^2H_4$) or deuteration in the N-methyl group (by reaction of desmethylmianserin with C^2H_3I) similar isotope effects were observed during HPLC (Table I). Isotopic fractionation is also reported by Filer et al.[3] for mianserin deuterated or tritiated in the N-methyl group.



1: mianserin



2: Org GC 94 (free base)



3: R_1 = Cl, R_2 = H (12-chloromianserin)

4: R_1 = H, R_2 = Cl (13-chloromianserin)

Figure 1

Because of the observed isotope effects, together with the fact that metabolism studies[2] had revealed that degradation of the piperazine moiety occurred during the biotransformation of mianserin, we limited our studies to labelling in the A-, B- and C-ring. As can be seen in Table I no isotope effects, at least in this chromatographic system, could be detected for mianserin deuterated at these positions[7].

Table I: HPLC selectivity coefficients (α) of deuterated mianserin towards unlabelled mianserin.

Column: LiChrosorb Si 60-5; length 2 x 25 cm, I.D. 4,6 mm; eluent: n-hexane/propanol-2 (9:1, v/v) containing 0,1% aq. NH_4OH (24%); flow: 2 ml.min^{-1}; UV detection: 280 nm.

compound	α	compound	α
[1,1-$^{2}H_2$]-mianserin	1,08	[6,8-$^{2}H_2$]-mianserin	$\leq$ 1,02
[N-C$^{2}H_3$]-mianserin	1,12	[10,10-$^{2}H_2$]-mianserin	$\leq$ 1,02
[3,3-$^{2}H_2$]-mianserin	1,10	[12-^{2}H]-mianserin	$\leq$ 1,02
[4,4-$^{2}H_2$]-mianserin	1,03	[13-^{2}H]-mianserin	$\leq$ 1,02
[3,3,4,4-$^{2}H_4$]-mianserin	1,12	[14b-^{2}H]-mianserin	$\leq$ 1,02
[8-^{2}H]-mianserin	$\leq$1,02		

$\alpha = t_{N_i}/t_{N_o}$ with t_{N_i} being the net retention time for the deuterated compound and t_{N_o} the net retention time for the unlabelled compound. In all experiments anthracene was used as the unretained compound. At an α-value of 1,02 or lower no distinct peak maxima will be detected.

Exchange under acidic conditions

Since the A-ring of mianserin is activated towards electrophilic substitution it should be possible to induce isotopic exchange by protonation/deprotonation. Indeed, using $CF_3COO^{2}H$ at 55°C deuteration of mianserin was observed (Table II).

Table II: Deuteration of mianserin in CF_3COO^2H at 55°C.

Reaction time (h)	Isotopic compound (%)			
	$^2H_0$	$^2H_1$	$^2H_2$	$^2H_3$
16	45,3	43,7	10,8	0,1
22	37,5	45,9	16,6	not determined
42	16,0	47,9	34,6	1,5

However, since labelling occurred not only at position C_6 but to a large extent at the metabolically unstable C_8-position[2] (about 35% according to ^1H-NMR) no tritiation studies were performed with this method.

Exchange under alkaline conditions

Since mianserin contains three benzylic positions exchange (through benzylic ionization) using concentrated NaO^2H was studied. Hexamethylphosphoric triamide (HMPT) was chosen as solvent since the hydrogens of this solvent are almost unreactive to base[8]. The results are given in Figure 2.

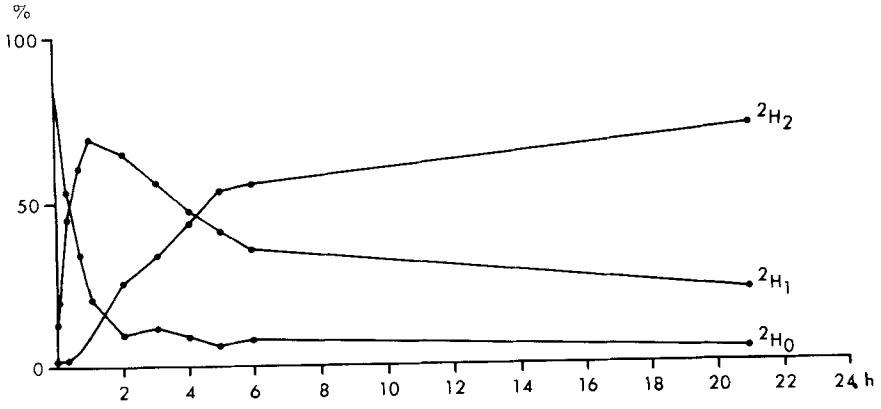


Figure 2

Deuteration of mianserin in HMPT/NaO^2H/^2H_2O at 80°C.

NMR analysis of deuterated mianserin showed that the exchange occurred exclusively at C_{10} (after 24 hours a total incorporation of 1,7 atoms ^{2}H was obtained). Since the axial H_{10} and equatorial H_{10} have distinct chemical shifts (in $C^{2}HCl_3$ 4,82 ppm and 3,30 ppm, respectively) it was observed that the exchange at the equatorial position was favoured (by a factor of about 5) over exchange at the axial position.
The space-filling model of mianserin based on X-ray analysis[12] revealed the axial C_{10} hydrogen oriented to the N_5 lone pair and thus more shielded. Obviously, the equatorial hydrogen would be more prone to deuterium exchange.

In another approach deuteration of mianserin was achieved through quenching of the lithium salt of mianserin (prepared by reaction with butyllithium in diethyl ether) with $^{2}H_2O$. These results are given in Table III.

Table III: Deuteration of mianserin in the system n-BuLi-$^{2}H_2O$ at ambient temperature.

Reaction time (h)	Isotopic compound (%)			
	$^{2}H_0$	$^{2}H_1$	$^{2}H_2$	$^{2}H_3$
3	30,0	48,2	21,6	0,2
70	33,8	42,8	23,4	<0,1

The position of the deuterium introduced under these conditions was not determined but it is expected that, similar to the exchange catalyzed by $NaO^{2}H$, deuteration occurs at C_{10}.
Using $NaO^{3}H/^{3}H_2O$ and HMPT the base-catalyzed exchange was carried out with mianserin at Amersham International plc , U.K. The tritiated mianserin had a specific activity of 13 $mCi.mmol^{-1}$. This incorporation was significantly less than observed for the deuteration experiments, which may be partly explained by the lower ^{3}H content of the [^{3}H]-water. Since [^{3}H]-mianserin with a higher specific activity was obtained by reductive dehalogenation

of 13-chloromianserin (see below) no further tritiation experiments were performed using this method.

Reductive dehalogenation

The labelling of mianserin by reductive dehalogenation was investigated using 12-chloro- and 13-chloromianserin (Figure 1, 3 and 4) as substrates. The reactions were carried out with Pd/C as catalyst in dioxan while triethylamine was added to neutralize the ^{2}HCl formed. In agreement with the results of Cerny and Hanus[10] an exchange of ^{1}H for ^{2}H was observed in the deuteration experiments when unpurified triethylamine was used; this exchange could be suppressed by distillation of the triethylamine over sodium.

With 12-chloro- and 13-chloromianserin, [^{2}H]-mianserin with a deuterium content of 75-80% was obtained. For 13-chloromianserin the reaction was completed in 4 hours, while the reductive dechlorination of 12-chloro-mianserin took 20 hours; thus 13-chloromianserin was selected for the tritiation reaction.

With this substrate two batches of [^{3}H]-mianserin were prepared by reaction with $^{3}H_{2}$ gas in dioxan/triethylamine using Pd/C as catalyst. The reaction was slow and even after 24 hours there was incomplete dehalogenation. Besides [^{3}H]-mianserin considerable amounts of by-products (in about the same amounts as the labelled mianserin) were formed. After purification by preparative TLC [^{3}H]-mianserin was isolated in radiochemical yields of 5-10% (50-190 mCi) with a radiochemical purity of $\geq$98% and a specific activity of 15-16 $Ci.mmol^{-1}$.

The ^{3}H-NMR spectrum shown in Figure 3 is in agreement with tritiation exclusively at C_{13}.

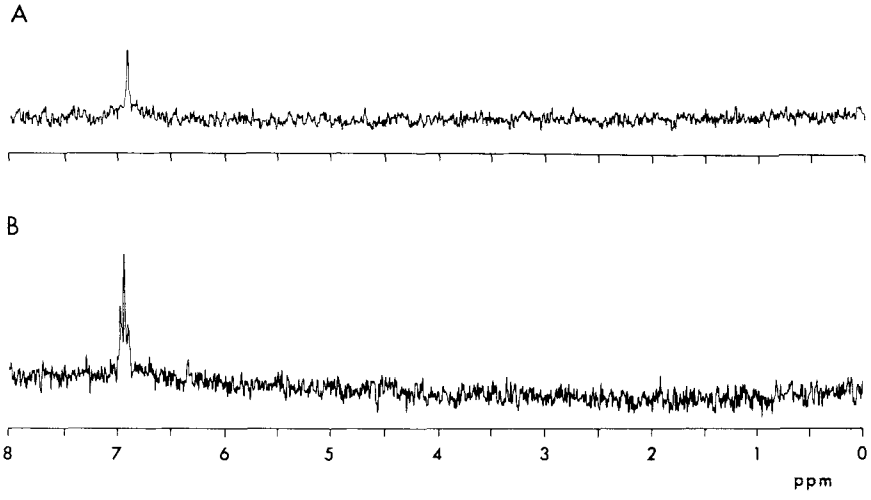


Figure 2

^{3}H-NMR spectra of [^{3}H]-mianserin (in $C_6{}^2H_6$) prepared by dechlorination of 13-chloromianserin. A) proton decoupled, B) proton coupled.

DISCUSSION

Tritiation of mianserin in the N-methyl group, the piperazine moiety or at C_8 leads to compounds with the label at metabolically unstable positions, while labelling of the N-methyl group and at positions 1, 3 or 4 results in drastic changes in the physical properties of the molecule. These disadvantages are found with commercially available [^{3}H]-mianserin.

It has been shown in this paper, using deuterium, that exchange under alkaline conditions or reductive dechlorination of mianserin substituted with chlorine in the C-ring lead to mianserin labelled in metabolically stable positions, while unwanted isotope effects are absent. Reaction of mianserin with ^3H_2O in the presence of NaO^3H resulted in tritiated mianserin of low specific activity, but reductive dechlorination with $^3H_2$ of 13-chloromianserin resulted in [13-^{3}H]-mianserin of high specific activity.

EXPERIMENTAL

Melting points were determined in a Büchi apparatus and were not corrected. Radioactivity was determined in Packard Liquid Scintillation counters (models 211 and 3375) using Insta Gel (Packard) as the counting medium. The radiochemical purity was determined by fluorography (on X-Omat RP-film, Kodak) and radiochromatogram scanning (Berthold, model LB 2721) of the plates (silica gel 60 F_{254}, Merck).

Mass spectra were determined using a Varian MAT 311-A spectrometer and a Varian CH-7 instrument both in the E.I. mode.

The carbon-13, proton, deuterium and tritrium resonance spectra were obtained in the Fourier transform mode on a Bruker WP-200 instrument equipped with an Aspect 2000 computer with 32K memory; the spectra were obtained at an observing frequency of 50,28 MHz for ^{13}C, 30,31 MHz for ^{2}H and 213,46 MHz for ^{3}H. The ^{3}H-NMR sample was transferred by syringe into a 3 mm diameter combination tube (Wilmad, SK 1374) which was then sealed and mounted in Teflon spacers inside standard 5 mm NMR tubes[11]. Tetramethylsilane was used as an internal reference; chemical shifts are given in parts per million (s = singlet, d= doublet, m= multiplet).

Deuteration and tritiation studies

The deuterium contents were determined by mass spectrometry while the position of deuterium was established by NMR (^{1}H, ^{2}H and ^{13}C). The tritiation reactions were carried out at Amersham International plc, U.K.

The specific activity of [13-^{3}H]-mianserin was determined by gc-ms analysis on a Varian CH-7 instrument (using selected ion-monitoring). Gas-liquid chromatography was on 1% OV-1 at 230°C.

Mianserin was analyzed by TLC with ethyl acetate/methanol (8:2, v/v), toluene/pyridine (8:2, v/v) or n-butanol/acetic acid/water (8:2:1, v/v organic phase) as eluent.

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Exchange under acidic conditions

Mianserin (50 mg) was dissolved in 1 ml of CF_3COO^2H (prepared from ^2H_2O and trifluoroacetic acid anhydride). The solutions were heated in sealed tubes at 55°C. After the reaction the mixtures were poured into 10% aq. $NaHCO_3$ and extracted with CH_2Cl_2. Deuterated mianserin was isolated by chromatography over SiO_2 with ethyl acetate/methanol (8:2,v/v).

Exchange under alkaline conditions

a) Mianserin (50 mg) was dissolved in HMPT (4,5 ml) and 40% solution of NaO^2H in ^2H_2O (0,1 ml) and ^2H_2O (0,4 ml) were added. The mixture was stirred at 80°C under nitrogen. Deuterated mianserin was isolated by extraction of the reaction mixture with diethyl ether.

b) Mianserin (5 mg) was dissolved in diethyl ether (2 ml) containing 2 M n-butyllithium. The mixture was stirred at room temperature and subsequently quenched with ^2H_2O (50 µl).

Reductive dehalogenation

13-Chloromianserin (50 mg) (Figure 1, 4) dissolved in 2 ml of a mixture of dioxan/triethylamine (9:1, v/v; dioxan was purified by distillation over sodium wire) was stirred with $^2H_2$ gas under atmospheric pressure in the presence of 100 mg Pd/C (10%). After removal of the catalyst and the solvent deuterated mianserin was isolated by chromatography over SiO_2 (ethyl acetate/methanol (8:2, v/v)).

13-Chloromianserin (Figure 1, 4) was prepared starting from 2-(4-chlorobenzyl)-aniline similar to the synthesis described for mianserin[12].
Colourless crystals, m.p. 125°-127°C.
^{1}H-NMR (C^2HCl_3): 2,34 (s, N-CH_3); 2,10-2,55 (m, H_{1ax}, H_{3ax}); 2,70-3,50 (m, H_{1eq}, H_{3eq}, H_{4eq}, H_{4ax}); 3,28 and 4,78 (two d, H_{10}); 4,05 (double d, H_{14b}); 6,70-7,45 (m, aromatic H's)
Mass spectrum: 298/300 (93 and 31% M^+); 227/229 (78 and 25%, $(M-C_4H_{10}N)^+$) 72 (100%, $H_5C_2-N^+-(CH_3)=CH_2$).
Chlorine content: 11,40% (calculated 11,90%).