Pharmaceutical Research, Vol. 6, No. 8, 1989

Report

A Nuclear Magnetic Resonance (NMR) Method for the Determination of the *cis/trans* Isomeric Content of Chlorprothixene

Karl-Heinz A. Rosler,[1] Jeremy Wright,[1] Kristine M. Fox,[1] Rolland M. Waters,[2] and Patrick S. Callery[1,3]

Received November 18, 1988; accepted March 6, 1989

Proton NMR spectroscopy was applied to the assignment of the isomeric identity of commercially available chlorprothixene. Nuclear Overhauser effect studies confirmed that the clinically useful isomer is the *cis* (Z) configuration. An NMR method for determining the isomeric content of chlorprothixene was developed based on integration of the ratio of areas of signal strength of the *cis-N-methyl* in comparison to the *trans-N-methyl* resonances.

KEY WORDS: chlorprothixene; geometric isomers (*cis* and *trans* isomers); nuclear magnetic resonance (NMR); nuclear Overhauser effect (NOE).

INTRODUCTION

The *cis* isomer (**1a**) (Fig. 1) of chlorprothixene [*Z*-2-chloro-9-(3-dimethylaminopropylidene)thioxanthene] has been clinically useful for many years as an antipsychotic drug. The *trans* isomer (**1b**) possesses less pharmacological activity than the *cis* isomer and is considered a contaminant in chlorprothixene dosage formulations (1). Although a number of authors have referred to chlorprothixene in the literature as the *trans* isomer (2–8), X-ray crystallographic studies indicate that the assignment of the *cis* isomer as the therapeutically active isomer is correct (9).

Li Wan Po and Irwin (10) showed that exposure of chlorprothixene to uv light rapidly induces *cis–trans* isomerization. Measurement of the isomeric composition of chlorprothixene was accomplished by a high-performance liquid chromatographic (HPLC) method (11). The isomeric content of chlorprothixene batches has also been monitored by an infrared spectroscopic method (12) and a high-performance thin-layer chromatographic (HPTLC) procedure (13).

In this communication, a high-field proton NMR spectroscopic method is described for the estimation of the *cis/trans* isomeric content of chlorprothixene.

MATERIALS AND METHODS

^{1}H-NMR spectra were recorded in $CDCl_3$ with tetramethylsilane (TMS) as internal reference on a General Electric QE300 FT spectrometer operating at a resolution of 0.5–1.0 Hz. Chemical shifts are reported as parts per million relative to the TMS standard.

Chlorprothixene isomers were kindly provided by Hoffmann LaRoche Co., Nutley, N.J. Taractan tablets were supplied by the University of Maryland Robert L. Swain Model Pharmacy.

RESULTS

Confirmation of Isomer Identity

It was possible to distinguish between the *cis* and the *trans* isomers of chlorprothixene by measuring the interaction between the 2′-methylene and the *peri* protons at C-1 or C-8, respectively. In the case of the *cis* isomer (**1a**), a nuclear Overhauser effect (NOE) between the H-1 and the 2′-methylene protons is possible, whereas in the *trans* isomer (**1b**) the NOE should be apparent only between the 2′-methylene protons and H-8.

The NMR spectrum of the *cis* isomer of chlorprothixene showed a singlet at 2.24 [$N(CH_3)_2$], an apparent triplet at 2.4 (3′-CH_2), and apparent quartet at 2.6 (2′-CH_2), a triplet at 5.94 ppm (methine proton), and complex aromatic signals between 7.1 and 7.6 ppm consistent with a literature spectrum (14). Although several of the aromatic signals overlapped, the proton of interest, H-1, was readily characterized. The H-1 proton, *meta* coupled to H-3 (J = 2.2), appeared at 7.41 ppm. Irradiation of the 7.46 signal (H-3, the *o*- and *m*-coupled doublet of doublets; J = 7.2 and 1.6, respectively) resulted in collapse of the H-1 proton *meta* coupling, confirming the assignment of H-1.

An NOE experiment was then performed by first irra-

[1] Department of Medicinal Chemistry/Pharmacognosy, School of Pharmacy, University of Maryland, 20 North Pine Street, Baltimore, Maryland 21201.

[2] United States Department of Agriculture, Agricultural Research Service, Insect Chemical Ecology Laboratory, Beltsville, Maryland 20705.

[3] To whom correspondence should be addressed.

0724-8741/89/0800-0706$06.00/0

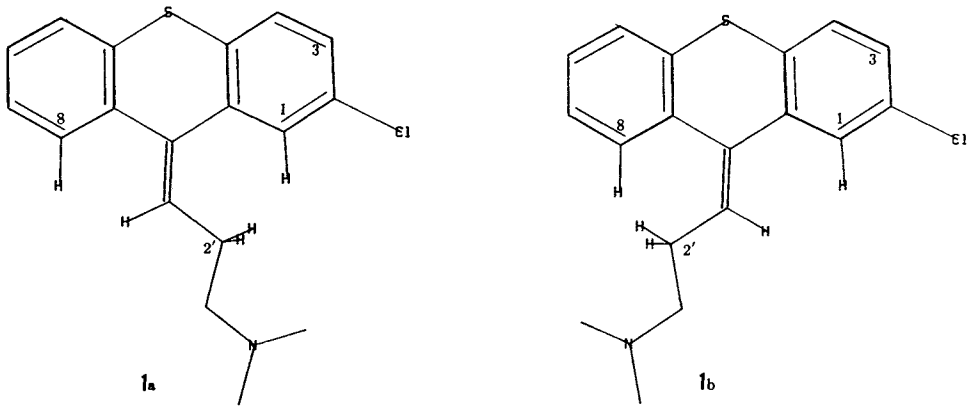

Fig. 1. *cis*-Chlorprothixene (**1a**) and *trans*-chlorprothixene (**1b**).

diating at 2.61 (2'-methylene protons) and then by irradiating off resonance at 12.0 ppm. An apparent increase in the size of the signal at 7.41 was observed. Subtraction of the control spectrum that had been obtained by irradiation at 12.0 ppm from the spectrum obtained by irradiation at 2.61 ppm resulted in reduction of the aromatic signals. The only signal not reduced was that of the H-1 proton at 7.41 appearing as a doublet (J = 2.2). A dipolar interaction between the 2'-methylene protons and the H-1 ring proton was thus indicated for the *cis* isomer (Fig. 2).

The spectrum of the *trans* isomer of chlorprothixene (**1b**) was similar to that of **1a**, except for the aromatic region. In this case, the H-1 proton appeared as a *meta*-coupled doublet at 7.47 ppm. The H-8 proton, which would be expected to demonstrate an NOE, appeared as a doublet of doublets (*o* and *m* coupled; J = 5.4 and 1.2, respectively) at 7.41 ppm. Irradiation of the signal at 2.61, (2'-methylene protons), then irradiation at 12.0, followed by subtraction of the latter spectrum from the former resulted in the retention of the signal at 7.41 ppm. All other aromatic signals were eliminated. Therefore, for **1b**, a dipolar interaction was demonstrated between the side-chain methylene and the H-8 *peri* proton consistent with the *trans* assignment.

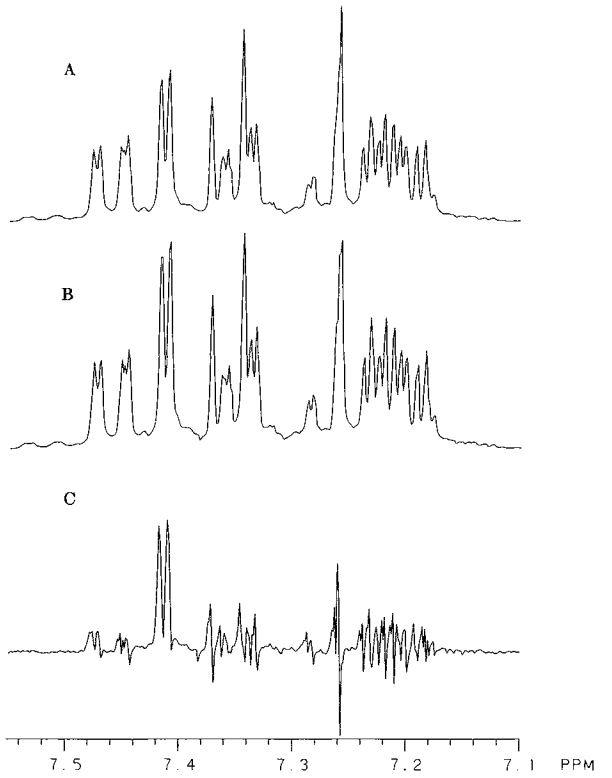

Fig. 2. (A) NMR spectrum of the aromatic region of *cis*-chlorprothixene irradiated off-resonance at 12.0 ppm. (B) After irradiation at the resonance of the 2'-CH_2 (2.61 ppm), the signal at 7.41 (H-1) was enhanced. (C) Difference spectrum obtained by subtraction of Spectrum A from Spectrum B.

Calculation of *cis/trans* Ratios

For quantification, the singlets for the *N*-methyl protons at 2.24 ppm were selected for *cis*-chlorprothixene and at 2.23 ppm for *trans*-chlorprothixene. Spectra were plotted from 2.26 to 2.20 ppm. Two methods were employed to integrate the region. First, the integrals of the areas +7 Hz upfield and −7 Hz downfield from the exact center between the two singlets were calculated using the peak area quantification

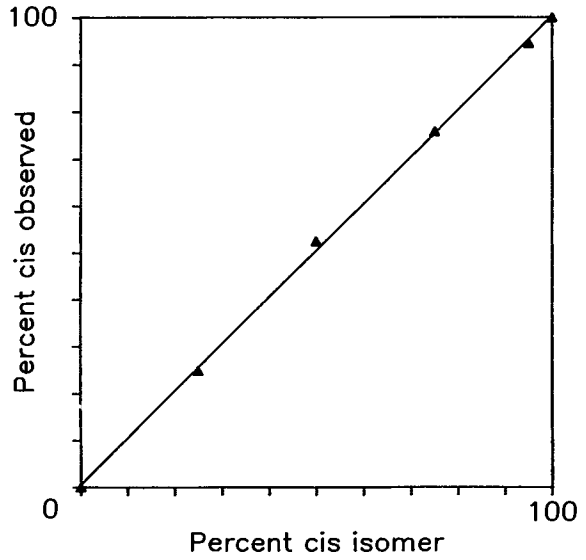

Fig. 3. Calculated isomeric content of chlorprothixene from NMR signal integration data.

software provided by the QE300 data system. Second, a method based on the QE300 deconvolution routine was used. Two simulated lines were superimposed on the two lines of the recorded spectrum until a best fit was achieved. The results obtained from the standard machine integration were the same values as those generated by the more time-consuming deconvolution procedure. Sampling of isomer concentration ratios indicated a linear relationship of observed and calculated isomer percentages of *cis*- and *trans*-chlorprothixene at ratios of 0:100, 25:75, 50:50, 75:25, 95:5, and 100:0 (Fig. 3). The limit of detection is easily below 5% of the *trans* isomer as shown in the spectrum of the 95:5 *cis:trans* mixture (Fig. 4).

Analyses of three lots of Taractan (chlorprothixene) tablets (25 mg, Lot No. 0510-1; 50 mg, Lot No. 0413; and 100 mg, Lot No. 0814-1) for the presence of *trans*-chlorprothixene were negative. In the higher strengths, only one-half or one-quarter of the tablets were utilized. The sample was prepared by crushing the tablet in a mortar and macerating the powder with 1.5 ml of chloroform for 5 min. The resulting slurry was then filtered through a cotton plug inside a Pasteur pipet and washed with chloroform (1 ml), and the filtrate collected in a V-shaped microvial. The solvent was removed in a heated stream of nitrogen (15). The oily residue, which solidified on standing, was dissolved in deuterated chloroform. NMR analysis of samples was not delayed after breaking the tablets, since photochemically induced isomerization of chlorprothixene was observed after exposing solutions of chlorprothixene in NMR tubes to daylight for 8 hr. Integration of the spectra indicated that the amount of *trans*-chlorprothixene in the tablets was below the level of detection of the method.

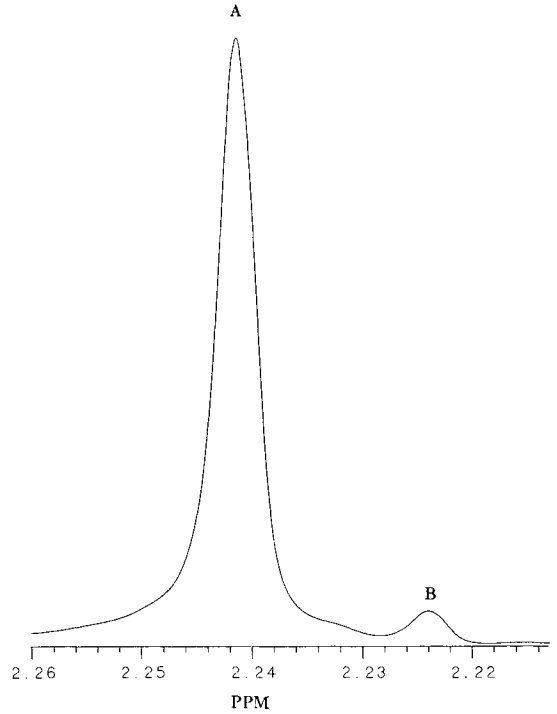

Fig. 4. NMR spectrum of the *N*-methyl region generated with a 95:5 mixture of *cis*- and *trans*-chlorprothixene. Peak A, *cis*-isomer. Peak B, *trans*-isomer.

DISCUSSION

The isomer assignment based on the NOE results confirms that the therapeutically active isomer of chlorprothixene is *cis*, in contrast to several references to a *trans* assignment that have appeared in the literature (2–8).

Analysis of the NMR spectrum of a mixture of chlorprothixene isomers obtained at 300 MHz revealed the presence of cleanly separated singlets assigned to the *N*-methyl signals arising from each isomer. Rough calculations indicate that at least a 100-MHz instrument would be required to resolve these singlets adequately. Lower-field strength instruments would yield less well-resolved singlets, and the difficulties encountered in integration of overlapping peaks would complicate isomer quantification.

As a quantitative method for the determination of the isomeric content of chlorprothixene samples, NMR analysis provides a method with a short setup time without the need to establish proper chromatography conditions with appropriate internal standards as required for the existing HPLC methods. An NMR method would be especially advantageous compared to the development and validation of HPLC methods for occasional determinations.

In summary, integration of high-field NMR signals can be considered as a convenient method for the measurement of the *cis* and *trans* isomeric composition of chlorprothixene.

ACKNOWLEDGMENT

Support was provided by Hoffmann-La Roche Inc., Department of Drug Metabolism.

REFERENCES

1. S. J. Enna, J. P. Bennett, D. R. Burt, I. Creese, D. U'Prichard, D. A. Greenberg, and S. H. Snyder. *Nature* 267:184 (1977).
2. L.-G. Allgen, B. Jonsson, B. Nauckhoff, M.-L. Andersen, I. Huus, and I. Moller Nielsen. *Experientia* 16:325 (1960).
3. I. Huus and A. Rauf Khan. *Acta Pharmacol. Toxicol.* 25:397–404 (1967).
4. J. E. Wallace. *J. Pharm. Sci.* 56:1437–1441 (1967).
5. S. P. Agarwal and M. I. Blake. *J. Pharm. Pharmacol.* 21:556 (1969).
6. H. Christensen. *Acta Pharmacol. Toxicol.* 34:16–26 (1974).
7. H. Oelschlager and R. Spohn. *Arch. Pharm.* 314:355–359 (1981).
8. J. Masse and A. Chauvet. *Ann. Pharm. Franc.* 41:579–589 (1983).
9. A. S. Horn, M. L. Post, and O. Kennard. *J. Pharm. Pharmacol.* 27:553–563 (1975).
10. A. Li Wan Po and W. J. Irwin. *J. Pharm. Pharmacol.* 32:25–29 (1980).
11. A. Li Wan Po and W. J. Irwin. *J. Pharm. Pharmacol.* 31:512–516 (1979).
12. E. Svatek. *Ceskoslov. Farm.* 14:332–334 (1965).
13. S. Tammilehto, M. Sysmalainen, and P. Makinen. *J. Chromatogr.* 285:235–241 (1984).
14. B. C. Rudy and B. Z. Senkowski. In K. Florey (ed.), *Analytical Profiles of Drug Substances, Vol. 2,* Academic Press, New York, 1973, pp. 63–84.
15. K.-H. A. Rosler. *J. Chem. Ed.* 63:1006 (1986).