Prediction of the Absolute Configuration of Chiral Drugs by NMR Spectroscopy

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Abstract. An NMR-based method was developed for the prediction of the absolute configuration of chiral carboxylic acids exemplified by over-the-counter drug naproxen. In this method, a pair of diastereomers were generated by purification and chiral derivatization of naproxen, whose absolute configuration was predicted by the difference in chemical shifts due to the magnetic shielding effects of phenyl functional group. The paper further illustrated the role of the related methods of Mosher ester analysis in the determination of absolute stereochemistry of carboxylic acids in addition to the derivatization and analysis of chiral secondary alcohols and amines.

Keywords: Chirality; NMR spectroscopy; enantiomer; absolute configuration; naproxen.

1. Introduction

Chirality was introduced by Lord Kevin in the year 1904 [1], referring to the characteristic of a molecule to form non-superimposable mirror images. Such molecules differ in their ability to rotate plane-polarized light. A chiral molecule and its mirror image are called enantiomers, named after the Greek word enantios [1].

The importance of chirality has long been recognized, for the significant differences in toxicity [2] and other biological aspects exhibited by enantiomeric forms of a drug [3]. The best known among all chiral biomacromolecules is levorotatory amino acids [4,5]. Pharmacologists are placing an increasing emphasis on the isolation and identification of enantiomerically pure drugs prior to the conduction of pharmaceutical experiments since even though some are pharmacologically active, others can perform extraordinary toxicity and side effects [4,6]. Examples include (R)-thalidomide, notoriously renowned for its teratogenic effect, whereas its (S)-isomer has desired antinausea efficacy [5]. Currently, the assignment of the absolute configuration of compounds could be achieved by the following methods: (i) correlating compounds with known conformations using interconversion followed by a comparison of optical rotations [7], (ii) X-ray crystallographic-based methods [8], and (iii) NMR spectroscopy-based methods [9]. Although X-ray crystallographic methods are the most commonly applicated, the requirement of crystal formation comes as a setback [7]. In such circumstances, NMR spectroscopy could be applied which originates from Mosher ester analysis, first reported by Dale and Mosher [9,10], where a chiral derivatizing agent is used to transform the chiral secondary alcohols into species that can be differentiated by spectroscopy (i.e., diastereomers and conformers).

Naproxen 1 [11], (2S)-2-(6-methoxynaphthalen-2-yl) propanoic acid, sold under the brand name Aleve, is a non-steroidal anti-inflammatory drug (NSAID) [12] used for the treatment of painful conditions including postoperative pain, often administered as sodium salt to improve solubility in vivo [13] with a carboxyl substituent on the stereocenter where chiral derivatization via amide formation is suitable for a method applied similar to Mosher ester analysis (Scheme 1) [11].

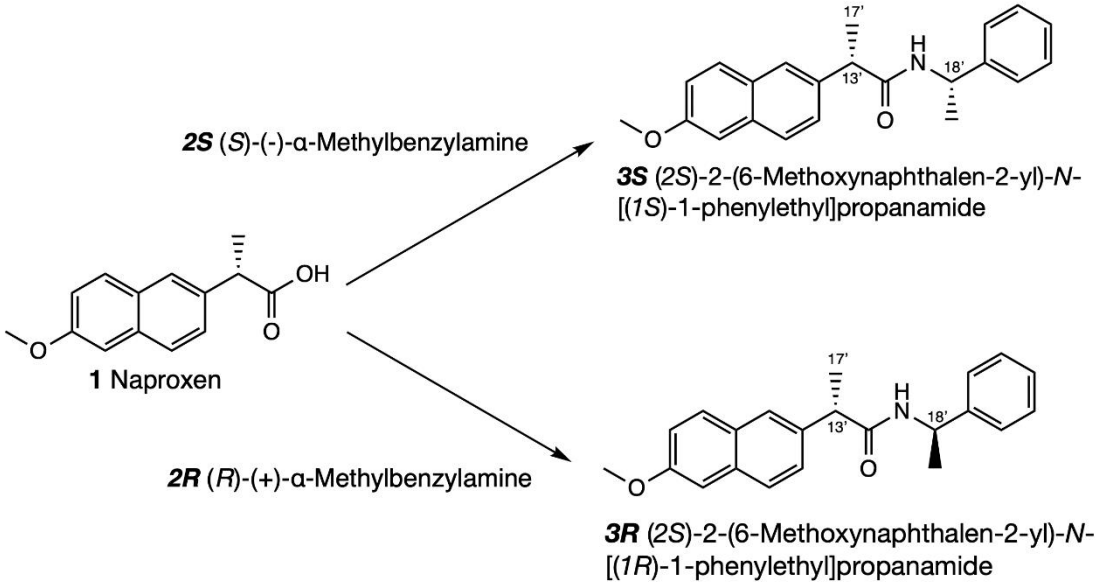

Fig. 1 Synthesis of diastereomer 3R and 3S from naproxen 1

2. Research status

The concept of Mosher ester analysis was first established by James A. Dale and Harry S. Mosher in the year 1972 [10]. They explained the use of NMR non-equivalence in the configuration of diastereomer and the chiral alcohols and amines, and α-methoxy-α-trifluoro-methylphenylacetic acid (MPTA) was mainly used as an example. A pair of diastereomeric (*R*,*R*)-methyl-tert-butylcarbinyl MTPA ester 5R and (*R*,*S*)-methyl-tert-butylcarbinyl MTPA ester 5S were generated by reaction of a chiral secondary alcohol. They then assigned the ^{1}H NMR signals and under configurational correlation model for isomers 5R and 5S (Figure 2), they had discovered differences in chemical shifts of specific protons ($\Delta\delta^{RS} = \delta^R - \delta^S$) of two substituents in the secondary alcohol moiety were different. By assigning the $\Delta\delta^{RS} < 0$ substituent to the left of the Mosher ester plane [10] and $\Delta\delta^{RS} > 0$ substituent to the right of it they were able to confirm the configuration of the chiral secondary alcohol.

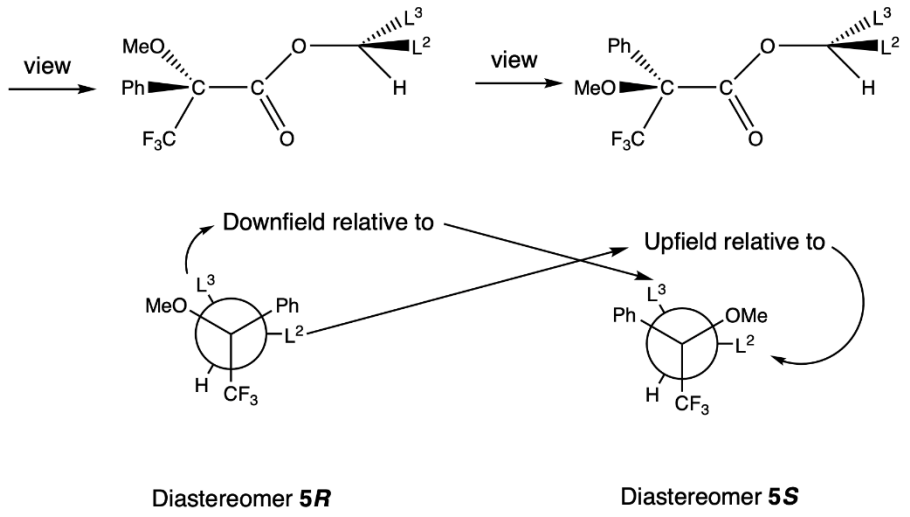

Fig. 2 Configurational correlation model for (R)-MPTA derivative 5A (5R) and (S)-MPTA derivative 5B (5S), taken from the original report [10]

Further research [14,15] conducted found that the antimagnetic shielding effect occurs for distant protons in the non-β-position of the opposing secondary alcohol substituent of the phenyl group as well as for further away protons on the same side as the β-H in MTPA esters, though the strength of the effect varies.

The phenyl group has a magnetic shielding effect that reaches further-away protons as well as the chiral secondary alcohol substituent's -H location. When the positive and negative values of each proton on the chiral secondary alcohol substituent in (R)-MTPA ester and (S)-MTPA ester are calculated, it is discovered that they are nicely aligned on both sides of the Mosher pattern diagram (Figure 2). It can be observed that the anti-magnetic shielding effect causes the H-NMR signals of protons on same side of phenyl group to be stronger in the (R)-MTPA esters than in the (R)-MTPA esters. Since the group with the negative $\Delta\delta^{RS}$ on the chiral secondary alcohol group is on the left side of the MTPA plane and the group with the positive $\Delta\delta^{RS}$ on the chiral secondary alcohol group is on the right side of the MTPA plane, it is possible to determine the absolute configuration of the chiral secondary alcohol using the Mosher pattern. In comparison to the traditional Mosher approach, which simply considers the sign of β-H to determine the absolute configuration of the chiral carbon, the results obtained by the modified ^{1}H-NMR Mosher method are more trustworthy.

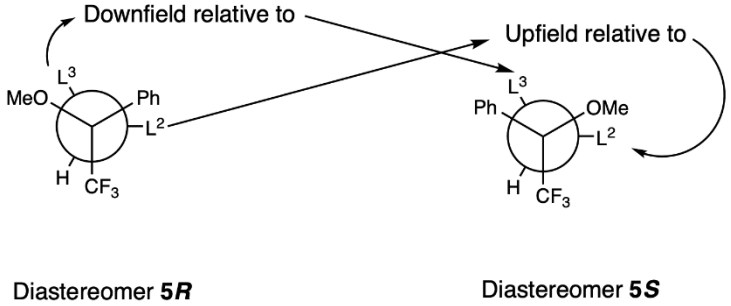

Fig. 3 Configurational correlation model for (R)-MPTA derivative 5A (5R) and (S)-MPTA derivative 5B (5S), taken from the original report [10]

For more complicated chemicals, it can be challenging to precisely assign signals to protons when using low-field NMR. Because of the ^{19}F-NMR signals' clarity and simplicity, Professor Mosher created the ^{19}F-NMR Mosher's approach [16]. According to the conformational pattern of the MTPA esters (Figure 2), the anisotropic deshielding action of the carbonyl group on ^{19}F in the higher MTPA esters is the primary cause of the variation in the ^{19}F-NMR shift signals. The anisotropic deshielding action of the carbonyl group on ^{19}F in the top MTPA ester is primarily to blame for the discrepancy in the NMR signal. Now assume that group volume of R^1 is greater than R^2. If group R^1 is larger than group R^2, the trifluoromethyl group in the (R)-MTPA ester should be closer to the carbonyl group in a planar position as a result of which ^{19}F is more paramagnetically shielded by the carbonyl group and its ^{19}F-NMR signal should be at a lower field; conversely, in the (S)-MTPA ester, the trifluoromethyl group and the carbonyl group should be further apart. ^{19}F-NMR signal rests in a higher field.

3. Experimental Section

3.1. Materials

OTC naproxen 1 from local pharmaceutical shop, (*S*)-(-)-*α*-methylbenzylamine 2S, (*R*)-(+)-*α*-methylbenzylamine 2R, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide 4, chloroform, pentane, hexane and ethyl acetate bought from Rhawn were used without further purification unless otherwise stated.

3.2. Purification of OTC naproxen 1 [17]

One whole bottle (10.00 g, 100 tablets) of commercially available naproxen 1 was crushed using mortar and pestle and transferred to a 100 mL beaker. 20 mL of 1:1 ethyl acetate and pentane were added to the powder. Filtration followed by column chromatography (1:1=Pentane:EtOAc) and solvent removal in vacuo afforded the desired naproxen 1 as a white solid (142.3mg).

^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.72 – 7.66 (m, 3H), 7.41 (dd, J = 8.4, 1.9 Hz, 1H), 7.15 – 7.09 (m, 2H), 3.91 (s, 3H), 3.88 (q, J = 7.2 Hz, 1H), 1.59 (d, J = 7.1 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ: 179.7, 157.7, 134.8, 133.8, 129.2, 128.8, 127.2, 126.1, 126.1, 119.0, 105.5, 55.3, 45.1, 18.1.

3.3. Preparation of diastereomer (2S)-2-(6-methoxynaphthalen-2-yl)-N-[(1R)-1-phenylethyl] propanamide 3R [18] and (2S)-2-(6-methoxynaphthalen-2-yl)-N-[(1S)-1-phenylethyl] propanamide 3S [19]

To a stirred solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide 4 (1mmol, 191.7mg dissolved in 3mL chloroform) and liquid (*S*)-(-)-*α*-methylbenzylamine 2S (1mmol, 121.2mg) at room temperature, purified naproxen 1(1mmol, 230.3mg dissolved in 5mL chloroform) was added dropwise. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel (1:1=Hexane:EtOAc). After completion, reaction mixture was quenched by hexane. Washing with 0.1 M aq. ammonia, 2.0 M HCl and H_2O were followed by solvent removal in vacuo, affording (2*S*)-2-(6-methoxynaphthalen-2-yl)-*N*-[(1*S*)-1-phenylethyl] propanamide 3S (175.7 mg, 76.3%) as a faint yellow solid.

3S: ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.93 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.83 (dd, J = 4.1, 1.5 Hz, 2H), 7.55 (dd, J = 8.4, 1.8 Hz, 1H), 7.46 – 7.33 (m, 5H), 7.31 – 7.28 (m, 2H), 6.01 (d, J = 7.9 Hz, 1H), 5.31 (p, J = 7.1 Hz, 1H), 4.14 (s, 3H), 3.95 (q, J = 7.2 Hz, 1H), 1.80 (d, J = 7.1 Hz, 3H), 1.60 (d, J = 6.9 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 173.6, 157.7, 157.3, 143.2, 136.4, 135.0, 133.7, 133.5, 129.2, 129.2, 129.0, 129.0, 128.6, 128.5, 127.5, 127.1, 126.9, 126.8, 126.2, 126.1, 125.8, 125.8, 125.7, 121.7, 119.1, 118.7, 105.7, 105.6, 55.3, 55.3, 48.8, 47.0, 21.9, 19.0, 18.5.

Following the above procedure, (2*S*)-2-(6-methoxynaphthalen-2-yl)-*N*-[(1*R*)-1-phenylethyl] propanamide 3R (123.3 mg, 64.3%) was prepared using (*R*)-(+)-*α*-methylbenzylamine 2R as a faint yellow solid.

3R: ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.94 (dd, J = 16.2, 8.7 Hz, 2H), 7.92 – 7.88 (m, 1H), 7.85 (s, 1H), 7.61 (dd, J = 8.4, 1.9 Hz, 1H), 7.57 – 7.49 (m, 3H), 7.48 – 7.42 (m, 3H), 7.41 – 7.34 (m, 2H), 6.04 (d, J = 8.0 Hz, 1H), 5.32 (s, 1H), 4.16 (s, 3H), 3.92 (q, J = 7.2 Hz, 1H), 1.81 (d, J = 7.2 Hz, 3H), 1.56 (d, J = 6.9 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ: 173.7, 157.7, 157.3, 143.1, 137.8, 136.4, 135.0, 133.7, 133.4, 129.2, 129.0, 129.0, 128.6, 128.6, 127.6, 127.3, 127.1, 126.9, 126.8, 126.2, 126.1, 126.0, 125.8, 125.7, 121.6, 119.2, 118.7, 105.7, 105.6, 55.3, 55.3, 51.2, 48.9, 47.2, 47.0, 21.6, 19.0, 18.6.

4. Results and Discussion

Starting with naproxen 1, a pair of diastereomers 3R and 3S with unidentical NMR spectra are produced by chiral derivatization, which can be differentiated by differences in chemical shifts of protons in different chemical environments. This difference is originated from the shielding effect of the phenyl groups' conjugated π-electron system [20], as applicated initially in Mosher ester analysis. Protons on the same side of the phenyl group are further shielded and the chemical shift moves upfield compared to protons on the opposite side of the phenyl group.

In this method, the absolute configuration of the chiral center in the naproxen 1 molecule is assumed to be unknown. R^1 and R^2 were used to denote methyl groups and protons on chiral carbon atoms whereas R was used to represent the aromatic substituent. A model of naproxen 1 was built concerning the chiral center (Figure 4).

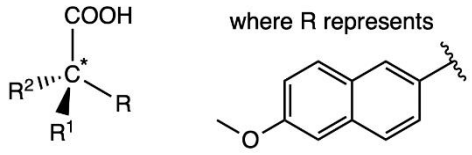

Fig. 4 Naproxen 1

The absolute configuration of naproxen 1 was confirmed by derivatization with a pair of enantiomerically pure chiral derivatization agents (*R*)-(+)-*α*-methylbenzylamine 2R and (S)-(-)-*α*-methylbenzylamine 2S to obtain two amide derivatives (2*S*)-2-(6-methoxynaphthalen-2-yl)-*N*-[(1*R*)-1-phenylethyl] propanamide 3R and (2*S*)-2-(6-methoxynaphthalen-2-yl)-*N*-[(1*S*)-1-phenylethyl] propanamide 3S with stereo-dominant conformations as shown in Figure 5. Comparing the chemical shifts between these two isomers gives information on the chemical environment difference between these diastereomers. The amides adopt the usual s-trans arrangement about its N-CO bond with the aromatic R substituent of the original naproxen moiety and the C(20') methyl substituent in the diastereomer moiety being syn-coplanar with the amide group, known as the Mosher amide plane.10

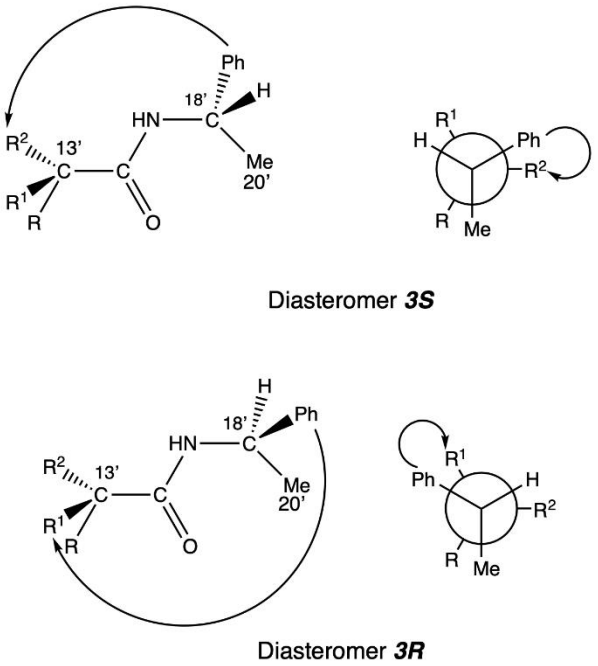

Fig. 5 Stereo-dominant conformations for the analysis of diastereomeric 3R and 3S

Figure 5 also illustrates configurational correlation models represented by Newman projections. In 3R derivative, the substituent R^1 is located on the same side of the Mosher amide plane as the phenyl group and is therefore relatively shielded, whereas in the 3S isomer, R^1 is located on the opposite side of the Mosher amide plane to the phenyl group and is therefore less shielded compared to the 3R diastereomer. Thus, the R^1 protons are further downfield in the 3R diastereomer compared to 3S derivative and conversely the R^2 proton is further upfield. Therefore, the ^{1}H NMR chemical shifts were obtained, and the chemical shift between the C(17') protons in both the 3R and 3S isomers were compared. The difference in chemical shift, denoted as $\Delta\delta^{RS}$=0.01ppm, is positive (Figure 6). The signals of C(13')H of the derivatives were compared in a completely analogous fashion and the difference was $\Delta\delta^{RS}$=-0.04ppm, which is negative (Figure 7). R^1 group is hence deduced to be the C(13')H and the R^2 group is C(17') methyl. Assignment of known substituents to naproxen 1 confirms it to be S configuration.

Fig. 6 Comparison of chemical shifts of C(17')H ^{1}H NMR spectra of compounds 3R and 3S

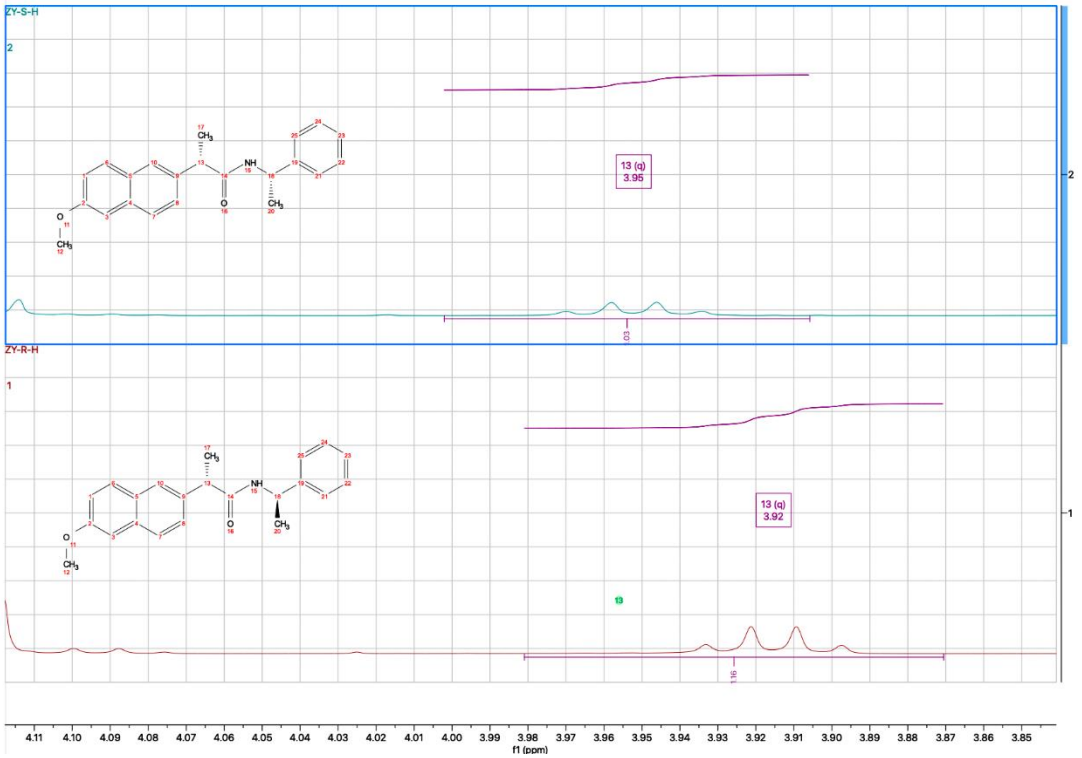

Fig. 7 Comparison of chemical shifts of C(13')H ^{1}H NMR spectra of 3R and 3S

5. Conclusion

Enantiomerically pure OTC drug naproxen 1 was derivatized by (*S*)-(-)-*α*-methylbenzylamine 2S and (*R*)-(+)-*α*-methylbenzylamine 2R to produce amides (2*S*)-2-(6-methoxynaphthalen-2-yl)-*N*-[(1*R*)-1-phenylethyl] propamide 3R and (2*S*)-2-(6-methoxynaphthalen-2-yl)-*N*-[(1*S*)-1-phenylethyl] propamide 3S. Performance in ^{1}H NMR spectroscopy showed difference in $\Delta\delta^{RS}$ in C(13')H and

C(17')H, confirming that naproxen is *S* configurated, hence providing an experimental and analytical approach that further extends the application of NMR as a non-chiral spectrum in stereochemistry.

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