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Molecular Dynamics and NMR Analysis of the Configurational ^{13}C Assignment of Epimeric 22,23-Epoxides of Stigmasterol

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The determination of the stereochemistry of brasinosteroid analogs with 22,23-epoxide groups can be easily achieved by means of ^{13}C NMR spectroscopy. Here, we provide a rationalization of the ^{13}C chemical shift pattern found in 22*R*,23*R*- and 22*S*,23*S*-epoxides of stigmasterol, based on the analysis of γ effects. (22*S*,23*S*)- and (22*R*,23*R*)-3β-acetoxystigmast-22,23-epoxy-5,6β-diol were used in the study as model compounds. Our methodology starts with a conformational search by means of molecular dynamics and NMR (NOE contacts) spectroscopy, which is followed by the analysis of the different γ interactions affecting the chemical shift of interest. We demonstrate that the differences between the ^{13}C chemical shift patterns of 22*R*,23*R* and 22*S*,23*S* isomers arise from γ effects as the result of diverging local conformations around the $C_{17}-C_{20}$ and $C_{20}-C_{22}$ bonds.

Introduction

Brassinosteroids (BR) are naturally occurring steroidal phytohormones with a high growth promoting activity.[1−3] In general, the structural requirements postulated for a high BR activity are as follows: 2β,3β-diol, 6-ketone or better 7-oxalactone in B ring, A/B trans fused ring junction, a cis C_{22},C_{23}-diol preferentially with *RR* configurations, and a C_{24} methyl or ethyl substituent. These relationships are more or less stringent depending on the bioassay used to study the biological activity.[4]

The introduction of the cis C_{22},C_{23}-diol moiety in BR analogs has been achieved through oxirane rings as precursors.[5−7] Moreover, some BR analogs with the 22,23-epoxide function displayed significant biological activity in field trials.[8−11] Recently, a procedure to obtain a mixture of 22*R*,23*R*- and 22*S*,23*S*-epoxides from stigmasterol has been reported.[12,13] Some research effort has been devoted to find a simple spectroscopic method for the unequivocal determination of the stereochemistry of these stigmasterol derivatives. A procedure has been developed by Gonzalez Sierra et al., based on the analysis of some representative C_{22},C_{23}-epoxides by ^{13}C NMR spectroscopy.[14] The proposed method is based on the chemical shifts pattern found in 22*R*,23*R*- and 22*S*,23*S*-epoxides (see Figure 1 for nomenclature). They found, in agreement with other reports,[12,13,15] that C_{22} and C_{23} of the *RR*-epoxide display very similar chemical shifts ($\Delta \sim 0.1$ ppm) whereas in the *SS*-epoxide they are clearly distinguishable ($\Delta \sim 4.6$ ppm, C_{23} is more shielded in the *SS* isomer than in *RR*). There is also a shielding effect ($\Delta \sim 2.5$ ppm) for C_{17} in the *RR* isomer, if compared with the *SS* isomer. Gonzalez Sierra et al. attributed these differences in chemical shifts to the prevalence of a gauge conformation for the $H_{20}-C_{20}-C_{22}-H_{22}$ dihedral angle in both, *RR* and *SS* isomers, in view of the striking sensitivity of ^{13}C chemical shifts to steric effects. The proposed gauge conformation, however, disagree with the observed $^{3}J_{H_{20}-H_{22}}$ coupling of 9.6 Hz in (22*S*,23*S*)-3β-acetoxystigmast-22,23-epoxy-5,6β-diol, which clearly indicates the presence of a trans conformation at H_{20}-C_{20}-$C_{22}-H_{22}$.

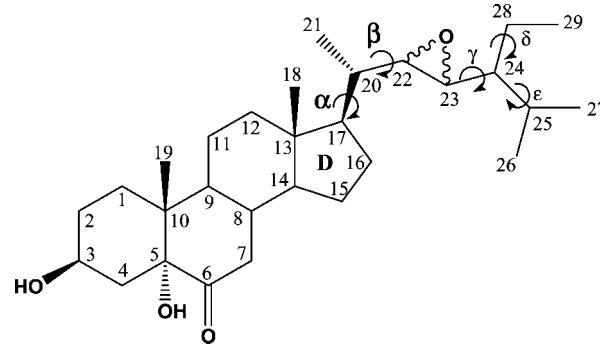

Figure 1. 6-Keto-22,23-epoxistigmasterol-3β,5-diol. Dihedrals $C_{16}-C_{17}-C_{20}-C_{21}$, $C_{17}-C_{20}-C_{22}-C_{23}$, $C_{22}-C_{23}-C_{24}-C_{25}$, $C_{23}-C_{24}-C_{28}-C_{29}$ and $C_{23}-C_{24}-C_{25}-C_{26}$ are defined as α, β, γ, ϵ and δ, respectively.

In this work, we provide a detailed analysis of the sources of ^{13}C chemical shift differences in the C_{22},C_{23}-epoxides, which correctly explains the origin of the chemical shift differences observed in these compounds. In our approach, we performed a conformational search by means of molecular dynamics (MD) simulations and experimental NMR data (NOEs), to later explain the observed chemical shift differences on the basis of γ effects. Whereas approaches using molecular modeling have been reported,[16−18] MD of epoxides in lateral chains, have not, to our knowledge, been previously carried out.

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TABLE 1: Comparison of ^{13}C Chemical Shifts of Interest in (22*S*,23*S*)- and (22*R*,23*R*)-3β-Acetoxystigmast-22,23-epoxy-5,6β-diol[12,13] ($\pm$0.1 ppm)[12,13]

nucleus	*RR*	*SS*
C_{13}	43.0	43.1
C_{14}	55.4	55.4
C_{15}	24.4	24.4
C_{16}	27.9	27.1
C_{17}	53.4	56.0
C_{18}	12.1	12.3
C_{20}	38.7	38.7
C_{21}	16.2	16.3
C_{22}	62.2	63.0
C_{23}	62.1	58.6
C_{24}	48.3	48.6
C_{25}	20.9	21.0
C_{26}	19.4	20.2
C_{27}	19.6	20.9
C_{28}	19.6	19.4
C_{29}	20.2	19.5

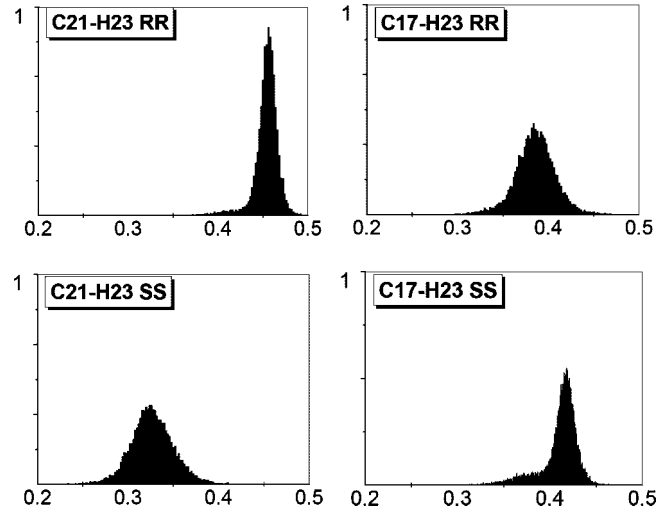


Figure 3. Histogram of distances (nm) from H23 to C21/C17.

As a model compound for our study, we choose two BR analogs from stigmasterol, (22*S*,23*S*)- and (22*R*,23*R*)-3β-acetoxystigmast-22,23-epoxy-5,6β-diol[12,13] (Figure 1). These compounds display the typical chemical shift pattern of epoxide derivatives of stigmasterol and were selected due to the existence of complete ^1H and ^{13}C resonance assignments.[12,13] Some chemical shifts of interest are shown in Table 1.

Experimental Section

NMR Experiments. All NMR experiments were carried out in $CDCl_3$ at 300 K with TMS as an internal reference. The ^{13}C and NOEDIFF spectra were collected with a Bruker ACF-250 spectrometer, operating at 250.13 MHz proton resonance frequency. The NOEDIFF experiments were performed by selective irradiation during 1.5 s.

The 1D ^1H and 2D $^1H/^1H$-ROESY spectra were recorded with an Avance Bruker spectrometer operating at 400.13 MHz. A spin-lock time of 800 ms was used.

Molecular Dynamics. The simulations were performed using the program GROMOS96.[19] The epoxide system is not included in the standard GROMOS force field.[20] A parametrization for this moiety was previously reported.[21]

The simulated system consists of a single solute molecule and 108 chloroform molecules as solvent, in a truncated octahedral periodic box of length 28 Å. The starting geometry was optimized by molecular mechanics using the steepest descent method. The MD simulations were performed at constant temperature (300 K, bath relaxation time 0.1 ps) and pressure (1 atm, bath relaxation time 0.5 ps), using a time step of 0.002 ps and a cutoff radius of 8 and 14 Å (the later updated every five time steps). The first 100 ps of the run were considered to be the equilibration time. After that, the geometric parameters were recorded every 250 steps. The simulation time was 10 ns.

Results and Discussion

Conformational Analysis of the Side Chains of the *SS* and *RR* Isomers. To clarify the origin of the chemical shift differences between the *SS* and *RR* isomers, we considered the interactions of C_{17} and C_{23} with its γ neighbors. MD simulations were performed to estimate the most populated conformations, with the focus on the spirostanic side chain connected to the D-ring of the steroidal moiety (Figure 1). The conformation of this side-chain is determined by the dihedral angles $C_{16}-C_{17}-C_{20}-C_{22}$ (α), $C_{17}-C_{20}-C_{22}-C_{23}$ (β), $C_{22}-C_{23}-C_{24}-C_{25}$ (γ), $C_{23}-C_{24}-C_{25}-C_{26}$ (δ) and $C_{23}-C_{24}-C_{28}-C_{29}$ (ϵ). The differences in the angles γ, δ, and ϵ, however, are small and do not lead to significant differences in the distributions of the distances $C_{23}-C_{26}$, $C_{23}-C_{27}$ or $C_{23}-C_{29}$ between the *SS* and *RR* isomers (data not shown) and are therefore not further considered, as they unlikely cause significant differences in the C_{17} or C_{23} chemical shifts.

On the other hand, the conformations defined by the dihedral angles α and β are different in both isomers (Figure 2). The MD simulations show that in *SS* β adopt values around 200°, which is in good agreement with the observed $^3J_{H_{20}-H_{22}}$ value

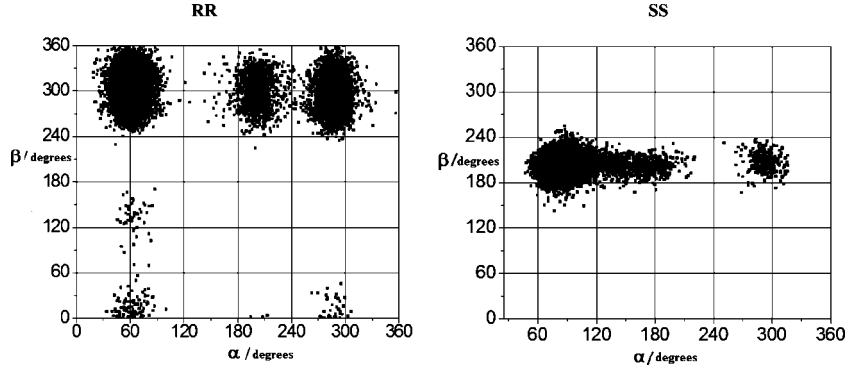


Figure 2. MD conformations obtained for the isomers *RR* (left) and *SS* (right) around the torsion angles $\alpha(C_{16}-C_{17}-C_{20}-C_{21})$ and $\beta(C_{17}-C_{20}-C_{22}-C_{23})$.

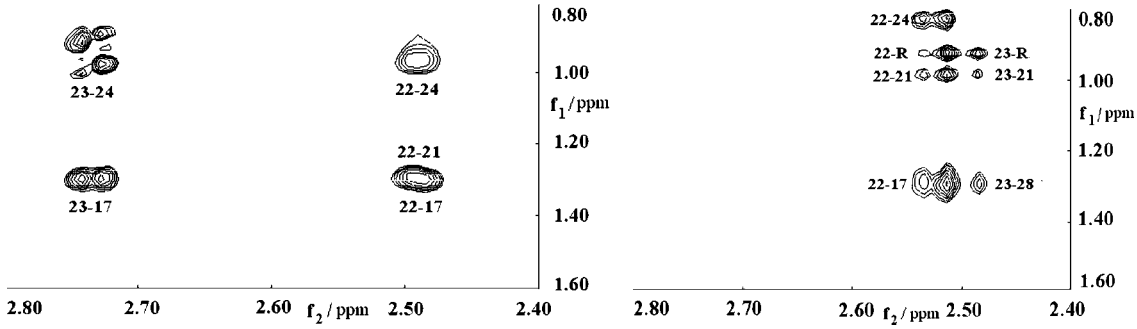
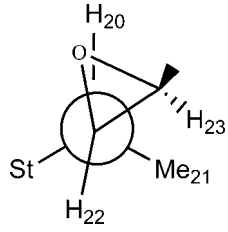


Figure 4. Partial ROESY spectra of 22*R*,23*R* and 22*S*,23*S* compounds.

SCHEME 1: Relative Positions of Methyl 21, C_{23} and H_{23} That Favors Shielding of C_{23} in *SS*



of 9.6 Hz, reflecting a predominantly *trans* arrangement between H_{20} and H_{22}, whereas the α angle displays a major conformation at ~90°, with minor conformations at approximately 180° and 300°. The prevalence of the (t, +g) (i.e., (200°, 90°)) conformation is also in good agreement with the observed ROE cross peak between $H_{22}-H_{17}$ and, taking into account the relative orientation of H_{24} (i.e., adopting a *trans* arrangement with H_{23} as indicated by $^3J_{H_{23}-H_{24}} > 10$ Hz), also the ROE cross peak between $H_{22}-H_{24}$.

In the case of *RR*, β adopts values around 300° in most of the simulation, and α is distributed between three conformations: two almost equally populated conformations with maxima at 60° and 290°, and a minor conformation at 200°. In one of these predominant conformations, i.e., (−g, −g) which corresponds to (300°, 300°), the proton H_{17} is spatially closer to the epoxide group than in any other conformation. This is in good agreement with the intense ROE cross peak observed between $H_{17}-H_{23}$. Moreover, the observation of a ROE cross peak between $H_{17}-H_{22}$ can be explained only by the presence of a significant population of the (−g, +g) conformation (300°, 60°). All this confirms the presence of an equilibrium between (−g, −g) and (−g, +g) conformations in the *RR* isomer.

Analysis of the Chemical Shift Differences. The differences in chemical shifts of C_{23} and C_{17} in the *RR* and *SS* epoxides probably arise from conformational differences between these isomers, originating variations in their pattern of steric interactions with γ neighbors, which are known to affect the shielding. For example, in *SS* the obtained (t, +g) conformation place methyl 21 closer to H_{23} as in the two predominant conformations of the *RR* conformer, which explains a shielding of C_{23} in *SS* via γ effect. A more detailed analysis of the chemical shift differences is presented below.

Chemical Shift Differences at C_{23}. As already mentioned above, the dihedral angles γ, δ, and ϵ displayed no significant variation between both isomers. Therefore, their shielding effects on C_{23} are not further taken into account, leaving C_{17} and C_{21} as the only γ neighbors that can cause differences in C_{23}. The relative positions of these atoms are determined by the dihedral angle β, which adopts different values in each isomer (approximately 300° in *RR* and 180° in *SS*, Figure 2). This results in a much shorter $C_{21}-H_{23}$ distance in the 22*S*,23*S* isomer than in the 22*R*,23*R* (Figure 3), as confirmed by the presence in the ROESY spectrum of the *SS* isomer of a strong cross-peak connecting H_{23} and H_{21} (Figure 4). Such a cross-peak is missing in the *RR* isomer as the result of much longer $H_{23}-H_{21}$ distances (Figure 3). These experimental facts sustain the results of the MD simulations and suggest that the greater shielding of C23 in the *SS* isomer is due to steric interaction with C_{21} (see Scheme 1).

Chemical Shift Differences at C_{17}. For the analysis of C_{17} we take into account only the neighbors in γ position belonging to the lateral chain (C_{23} and the epoxide oxygen). The interactions of C_{17} with this atoms are the ones changing from one isomer to the other and depend on the torsion angles α and β (Figure 1).

The MD results show that there are three populated conformations in *RR* isomer but there is only one predominant conformation and two others scarcely populated in the *SS* compound (Figure 2). In the *RR* isomer, the distances from H_{17} to its γ neighbors are considerably shorter in the (−g, −g) conformation than in any other conformer of both isomers (Figure 5). The H_{17} is pointing to the oxirane ring, this fact favor a shielding effect over C_{17} (see Scheme 2). The presence of this conformation could origin the differences in statistically weighed chemical shifts of C_{17} due to the close vicinity of their

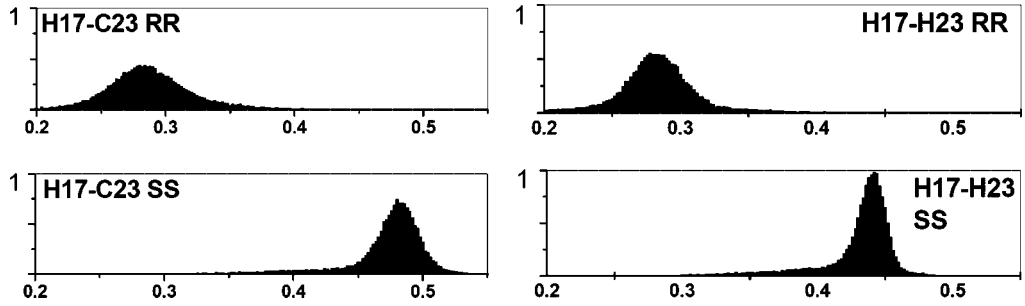
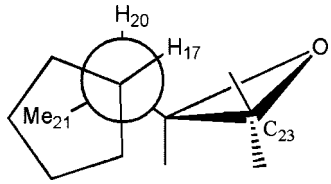


Figure 5. Histograms of distances relevant to H_{17} from MD simulations.

SCHEME 2: Relative Positions of Epoxide Ring, C_{17} and H_{17} That Favors Shielding of C_{17} in *RR*



γ neighbors C_{23} and O. The moderate population of this conformation is in agreement with the fact that the $\Delta\delta$ in C_{17} is less pronounced than in C_{23}.

The presence of $H_{17}-H_{23}$ and $H_{17}-H_{22}$ cross peaks in the ROESY spectrum of the *RR* isomer (Figure 4) establish the close vicinity between these protons, only compatible with simultaneously populated (−g, −g) and (−g, +g) conformations. The detection of NOE on H_{17} in a NOEDIFF spectrum by irradiation of H_{23} confirms the previous result.

Conclusions

The differences found in the ^{13}C spectra of 22*R*,23*R*- and 22*S*,23*S*-epoxides of stigmasterols can be interpreted as due to the existence of different conformations. A rigorous analysis is reported in this work.

It has been demonstrated that the differences in the local conformations generate differences on the shieldings of C_{23} and C_{17} that can be rationalized by steric γ effects.

The C_{23} in *SS* isomer is shifted to higher fields with respect to *RR* due to a shielding effect of methyl 21 not present in the *RR* isomer.

The C_{17} is shifted to higher fields in the *RR* isomer with respect to *SS* due to a shielding effect exerted by its γ neighbors in the oxirane ring in a relatively populated conformation adopted only by this isomer.

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