Configurational and conformational analyses of α-methylene-γ-butyro steroidal spirolactones

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The configurational and conformational analyses of α-methylene-γ-butyro steroidal spirolactones have been accomplished using 2D COSY, NOE and HETERO-COSY in conjunction with 1D 1H NMR. The analyses indicate that the major or the only spirolactones formed at positions 2, 3 and 6 of the steroids have the steroidal ring bearing the spirolactone in a chair conformation with an axial disposition of the oxygen atom attached to the spirocentre. The corresponding minor spirolactones have equatorial configurations. Steroids with spirolactones at positions 16 and 17 have β and α dispositions, respectively, of the oxygen atom attached to the spirocentre.

The α-methylene-γ-butyrolactone moiety is known to be responsible for various biological activities such as antitumour, phytotoxic and antibacterial.1–3 In our attempts to synthesise such spirolactones, we observed that the introduction of an α-hydroxy substituent provides higher reactivity as well as diastereoselectivity in the organometallic addition to chiral ketones. We chose to employ the Reformatsky reaction on α-hydroxy substituted steroidal ketones for the synthesis of spiro-α-methylene-γ-butyrolactones.4 The stereochemistry of the reaction can be explained through a chelated transition state.5 To understand the orientation of the transition state, the conformational and configurational analysis of the steroidal spirolactones becomes imperative. A large number of reports discussing the configurational and conformational aspects of steroids have been published.6–8 The configurational analysis of the 4-en-3-one steroids has been dealt with by Sridharan et al.9 Marat and co-workers have reported the configurational analysis of the ring A of 2-methyl- and 2,2-dimethyl-3-ketosteroids.10 In an article discussing the complete strategy of total assignment and conformational analysis of steroids, Schneider et al. have exploited NOE difference experiments along with 2D-experiments such as 1H–13C heteronuclear shift correlated spectroscopy and 1H–1H homonuclear J-correlated spectroscopy.11

Although a large number of spiro-α-methylene-γ-butyrolactones have been synthesised using the Reformatsky reaction, the stereochemistry at the spirocentre has been difficult to assign. This can be attributed to the lack of availability of a reliable method to predict 1H and 13C chemical shifts and the lack of through-spin connectivity between the steroidal nucleus and the spirolactone. Benezra and co-workers used 1H NMR assignments in the configurational analysis of the spirolactones.12 Assignments, however, were carried out in both the above cases by comparison of chemical shift values of the corresponding proton signals for the pair of diastereomers. In the event of formation of a single spirolactone, unambiguous stereochemical assignments were not possible.

In the present case, the configurational assignments of the spiro-α-methylene-γ-butyrolactone derivatives have been carried out with extensive use of 2D-NMR experiments like rotational frame Overhauser effect spectroscopy (ROESY) and nuclear Overhauser effect spectroscopy (NOESY) in conjunction with other experiments like correlation spectroscopy (COSY) and heteronuclear correlation spectroscopy (HETERO-COSY). The conformations of spiro-α-methylene-γ-butyrolactone steroids 1–17 (Fig. 1) have been deduced. In addition, the conformation of the spirolactone 4 has also been analysed by molecular modelling.

The configurational assignments of the spiro-α-methylene-γ-butyrolactone ring cannot be carried out using vicinal 1H–1H coupling constants as its spin system is isolated from the rest of the molecule. Hence the following strategy was employed.

1) Analysis of the Dreiding's stereomodels to obtain an idea of relative through space distances between the methylene protons of the spirolactones and the protons of the steroid in the proximity of the spirolactone ring.

2) Analysis of the 1H–1H COSY and 1H–13C HETERO-COSY experiments to determine the chemical shifts of these protons.

3) Analysis of the ROESY and NOESY spectra for information on the relative spatial distances between the protons essential for the configurational assignment.

In the steroids 1–17, spirolactone centres are situated at the 2, 3, 6, 16 and 17 positions. Although each of the seventeen steroids was subjected to the above stated modus operandi to arrive at their stereochemistry, the detailed procedures for the analysis of one steroid for each spirolactone location are discussed below.

(3R)-Spiro[17β-acetoxy-5α-androstane]-3,2′-(4′-methylene-5′-oxotetrahydrofuran)] (1) and (3S)-spiro[17β-acetoxy-5α-androstane]-3,2′-(4′-methylene-5′-oxotetrahydrofuran)] (2)

The Reformatsky reaction of 17β-acetoxyandrost-1-one provided two diastereomers in the ratio 70:30 which could be isolated in pure form. The 1H NMR spectra of both the isomers exhibited two triplets each at 6.22 and 5.60 ppm corresponding to the vinylic protons of the spirolactone moiety. The downfield signals were assigned to the 6′Z protons, and the upfield ones to 6′E protons. A triplet at 2.67 ppm integrating to two protons was observed for the major diastereomer. The COSY plot of this diastereomer showed cross peaks between the proton signals at 2.67 and 5.60 ppm. This correlation indicates that the signal at 2.67 ppm is due to the methylene protons of the lactone ring. Similarly, the methylene protons of the lactone ring of the minor diastereomer was assigned to the signals in the range of 2.79–2.77 ppm. The 19H-h singlet of the major diastereomer resonated at 0.81 ppm whereas that of the minor diastereomer was observed at 0.86 ppm. The 1H NMR of the minor diastereomer also exhibited a doublet triplet at 1.92 ppm (J = 4.2, 13.8 Hz) and a triplet
Fig. 1 Structures of spirolactones 1–17 subjected to conformational and configurational analysis.

at 1.82 ppm ($J = 13.1$ Hz); the coupling pattern and the chemical shifts led to assignments of these peaks to 2β-H and 4β-H, respectively. The $^1$H–$^{13}$C HETEROCOSY spectrum of the minor diastereomer led to assignments of 1α, 2α, 4α and 5α protons to signals at 1.02, 1.59, 1.35 and 1.16 ppm, respectively. Similarly, the $^1$H–$^{13}$C HETEROCOSY spectrum of the major diastereomer revealed chemical shifts of the 4α and 4β protons at 1.46 ppm and the 2α and 2β protons at 1.65 ppm. The 5α-H was located at 1.67 ppm.

The magnitudes of the vicinal coupling constants of the minor diastereomer 2, 13.8 Hz between 2β-H and 1α-H and 13.1 Hz between 4β-H and 5α-H, are in the range expected for a vicinal trans-coupling. This suggests a chair conformation for the minor diastereomer. The downfield shift of the 19-H$_3$ signal of the minor diastereomer compared to that of the major diastereomer falls in line with the greater deshielding observed for 19-H$_3$ of 3β-acetoxyandrostan-3 one against 3α-acetoxyandrostanone which suggests a β disposition for the oxygen atom attached to the spiro carbon in the minor diastereomer. In the ROESY spectrum of the minor diastereomer, the lactone methylene protons exhibited connectivities with 1α-H, 2α-H, 4α-H and 5α-H suggesting spatial proximities of these protons...
with the lactone methylene protons. Studies of Dreiding’s stereomodels showed that although cross peaks between the methylene protons of the lactone and the \( ^2 \) and \( ^4 \) protons are possible with the stereostructures 1a, 2a and 2b, the cross peaks between the lactone methylene protons and the \( ^1 \)a and \( ^5 \) protons are only possible with structure 2a (Fig. 2). Therefore the minor diastereomer 2 has an equatorial configuration of the spirolactone ring.

The \( ^5 \)-H signal of the major diastereomer was deshielded by 0.61 ppm compared to that of the androstane. The significant deshielding indicates that the oxygen atom of the lactone ring should be in syn di axial position with respect to the \( ^5 \)-H. This prediction is supported by the work of Schneider et al.\(^ {13} \) which has shown that a 1,3-syn di axial interaction between a proton and a hydroxy group of the cyclohexane ring results in a downfield shift of the proton by approximately 0.5 ppm. Deshielding of a similar order was observed for \( ^1 \)-H which resonated at 1.37 ppm. These shielding effects are possible only when ring A adopts a chair conformation with the appended lactone moiety axially locked. The ROESY spectrum of the major diastereomer exhibited cross peaks between methylene protons of the lactone ring and \( ^2 \)a, \( ^2 \)-H and \( ^4 \)-H showed cross peaks with the 19-H signals. The appearance of these cross peaks is in accordance with the Dreiding’s stereomodel 1a, corresponding to the chair conformation of the ring A with axial disposition of the oxygen atom attached to the spirocentre, while the stereostructure 2b is clearly ruled out thus establishing stereochemistry of the major diastereomer 1 as 1a.

\[(2R)-Spiro[(1β-acetoxy-3β-hydroxy-5α-androstan-2-one)-2.2'- (4'-methylene-5'-oxotetrahydrofuran)] (4)\]

The Reformatsky reaction of 17β-acetoxy-3β-hydroxy-5α- androstan-2-one afforded a single diastereomer 4 of spiro-α-methylene-γ-butyrolactone in 76% yield. The \( ^1 \)-H NMR spectrum of the diastereomer showed vinlylic triplets at 6.19 and 5.58 ppm and two triple doublets integrating to one proton each at 3.16 and 2.50 ppm. The COSY spectrum revealed connectivity between these triplets of doublets and the vinlylic protons. The triplet of doublets at 3.16 ppm was therefore assigned to the lactone methylene \( 3^-\)pro-S proton and that resonating at 2.50 ppm assigned to the \( 3^-\)pro-S proton. A doublet of doublets at 3.43 ppm was assigned to the \( 5^-\)-H and the doublet observed at 2.04 ppm was assigned to \( 1^-\)-H. The 19-H, resonated at 0.99 ppm. Analysis of the \( ^1 \)-H,\(^ {13} \)C HETEROCOSY spectrum assisted in the assignments of \( ^1 \)-a, \( ^4 \)-b and \( ^5 \) protons to 1.20, 1.57, 1.65 and 1.21 ppm, respectively. The deshielding of the 19-H by 0.2 ppm compared to that of the androstane\(^ {15} \) suggests the possibility of a syn-axial disposition of the oxygen atom attached to the spirocentre and the 19-methyl group. Also the deshielding of the 4β-H by 0.39 ppm compared to that of the 3β-hydroxyandrostane\(^ {14} \) augurs well for axial orientation of the oxygen attached to the spirocentre. The ROESY spectrum of compound 4 showed cross peaks between the 3α and 3'-pro-S protons, and between the 1α, 1β and 3'-pro-R protons. In addition, the \( 3^-\)-H signal showed cross peaks with the signals for \( 1^-\)-H and \( 5^-\)-H while \( 1^-\)-H showed a connectivity with 19-H.

Study of Dreiding’s stereomodels indicates that the ROESY cross peaks between the lactone methylene protons and the 3α, 1α and 1β protons can be explained by both a chair conformation having the β-orientation 4a and a boat conformation having the α-orientation 4b of the oxygen atom attached to the spirocentre (Fig. 2). However, the cross peaks between the 3α and 1a, and the 3α and 5α protons can arise only in the chair conformation 4a. In addition, the boat form 4b is expected to cause deshielding of the 5α-H due to the proximity of the oxygen atom of the lactone ring. In contrast, no significant deshielding was observed. These results suggest that the spirolactone 4 should have ring A in the chair conformation with axial disposition of the oxygen atom attached to the spirocentre 4a. A more rigorous method of using the vicinal proton–proton coupling constants for the determination of the A ring conformation was not possible. The 4α, 4β and 5α proton signals were difficult to resolve, even in 2D-experiments, from the spin system of the steroidal backbone. Hence it was difficult to extract the coupling information between these protons. We, therefore, resorted to molecular modelling to verify the deductions based on the ROESY experiment. The energies calculated, 35 kcal mol\(^{-1} \) and 52 kcal mol\(^{-1} \) for structures 4a and 4b, respectively, suggest clearly that the structure 4a is favoured over structure 4b. Thus, the molecular modelling studies agreed well with the NMR studies. The spirolactone 4 is proposed to have ring A in the chair conformation with an axial disposition of the oxygen atom attached to the spirocentre (4a).

\[(3R)-Spiro[(cholest-5-ene)-3,2'- (4'-methylene-5'-oxotetrahydrofuran)] (10) and (3S)-spiro[(cholest-5-ene)-3,2'-(4'-methylene-5'-oxotetrahydrofuran)] (11)\]

The \( ^1 \)-H NMR spectrum of the major spirolactone obtained from cholest-4-en-3-one exhibited a triplet corresponding to
lactone methylene protons at 2.70 ppm. A doublet doublet was observed at 2.16 ppm corresponding to 4a-H and the 4β-H signal was observed as a multiplet in the range 2.53–2.48 ppm. The 19-H, β-13a-H and 1α-H singlet appeared at 1.05 ppm. The 1H NMR spectrum of the major spirolactone showed a triplet of doublets at 2.55 ppm corresponding to one of the protons of the lactone methylene group. The other proton was observed along with 4β-H over the range 2.74–2.68 ppm. The 19-H singlet appeared at 1.05 ppm. The 1H-13C HETERO COSY spectrum helped in assignments of the protons 1α, 1β, 2α, 2β, 4a and 4β to signals at 1.01, 1.87, 1.64, 2.06, 1.97 and 2.72 ppm, respectively. The deshielding of 19-H of the minor spirolactone by 0.04 ppm compared to that of the major spirolactone is in accordance with the trend observed for pairs of spirolactones 1, 2 and 8, 9. Thus the axial and the equatorial configurations of the major and the minor spirolactones, respectively are suggested. This is supported by the downfield shift of the 4b-H signal of the minor spirolactone compared to that of the major spirolactone. Furthermore, the change from a single triplet corresponding to the two protons of the lactone methylene of the major spirolactone to two distinct triplets of doublets corresponding to the two lactone methylene protons of the minor spirolactone agreed well with the earlier prediction.

The ROESY spectrum of the major spirolactone showed cross peaks between the lactone methylene protons and 2α-H, 2β-H, 4α-H and 4β-H. The 2β and 4β proton resonances further exhibited cross peaks with the 19-H singlet. Therefore the major diastereomer 10 should have an axial configuration of the spirolactone which is further supported by Dreiding’s stereostucture 10a (Fig. 2). The minor diastereomer 11 is consequently expected to have an equatorial configuration of the spirolactone. The ROESY spectrum of the minor spirolactone revealed a strong cross peak between the methylene protons at 2.54 ppm and 1α-H while a relatively weak cross peak was observed between another lactone methylene proton and 1α-H. The former lactone methylene proton was, therefore, assigned as 3′-pro-R-H and the latter one was assigned as 3′-pro-S-H. Furthermore the 3′-pro-R proton exhibited a cross peak with the 2α-H signal and the 3′-pro-S proton showed a cross peak with the 4α-H signal. The resonance for 19-H, exhibited cross peaks with the 4β and 2β proton multiplets. In addition, a cross peak was also detected between the 2β and 4β proton resonances. These connectivities confirm the equatorial configuration of the minor spirolactone 11a. (3′S,6′S)-Dispiro[(4-methylene-5-oxotetrahydrofuran)-2,3′- (5α-hydroxysterolane)-6,2′-(4-methylene-5′-oxotetrahydrofuran)] (13)

The 1H NMR spectrum of the dilactone obtained from 5α′-hydroxycholenane-3,6-dione showed four vinylic triplets at 6.28, 6.20, 5.68 and 5.61 ppm. Two triplets of doublets integrating to one proton each were observed at 3.28 and 2.44 ppm. A multiplet in the range 2.88–2.76 ppm integrating to two protons was also observed. The vinylic protons resonating at 6.28 and 5.68 ppm showed cross peaks with the multiplet over the range 2.82–2.76 ppm in the COSY spectrum. Hence these signals were assigned to the 6′Z and 6′E protons, respectively. The two triple doublets at 3.28 and 2.44 ppm exhibited cross peaks with the remaining vinylic protons and, hence were assigned to the 3′-pro-R and 3′-pro-S protons, respectively. The vinylic triplet at 6.20 ppm was assigned to the 6′Z proton and the one at 5.61 ppm was assigned to the 6′E proton. The 19-H, singlet was observed at 1.15 ppm in the 1H NMR spectrum and a doublet corresponding to 4β-H was located at 1.96 ppm. A singlet observed at 3.56 ppm disappeared on D2O exchange and was assigned to the 5α-hydroxy proton. The 1H-13C HETERO COSY spectrum led to the assignments of the 1α, 4a, 7α, 7β, 8β and 9a protons to 1.45, 1.62, 1.58, 1.72, 1.72 and 1.59 ppm, respectively.

The chemical shift and the multiplet pattern observed for 3′-H suggest the axial configuration of the spirolactone at position 3. This is further supported by the considerable deshielding of the 5α-hydroxy proton. The lactone methylene protons 3′-pro-R- and 3′-pro-S- exhibited cross peaks with 2α, 2β, 4α and 4β protons in the ROESY spectrum of the spirolactone. The 2β-H and 4β-H signals showed cross peaks with that of 19-H. Therefore the axial configuration of the spirolactone at the position 3 was confirmed. The deshielding of the 19-H, singlet by 0.19 ppm compared to that in the spirolactone 6 suggested axial configuration of the spirolactone at position 6. The 3′-pro-R-H showed connectivity with 4α-H while the 3′-pro-S proton showed cross peaks with the 7α-H and 7β-H. The 3′-pro-R proton, however, did not show connectivity with 4β-H, and neither the 3′-pro-R nor the 3′-pro-S proton signals exhibited cross peaks with the 19-H signal. This ruled out the possibility of an equatorial spirolactone at position 6. Dreiding’s stereomodels (Fig. 3) showed that the observed ROESY cross peaks can be explained by a β disposition of the oxygen atom at position 6 with a chair conformation of ring B (13a) or an α disposition of the oxygen atom at position 6 with a boat conformation of ring B (13b). The deshielding of the 19-methyl and 8β protons compared to that of the spirolactone 6, however, cannot be explained in the case of the boat conformer. Also, the 9α-H signal of the spirolactone did not show significant deshielding compared to that of the spirolactone 6, which would be expected for the boat conformer. The spirolactone at the position 6 should therefore have an axial disposition of the oxygen atom with a chair conformation of ring B (13a).

(6′S)-Spiro[(3′β,5α-dihydroxycholestan)-6,2′-(4′-methylene-5′-oxotetrahydrofuran)] (14) and (6′S)-Spiro[(5α-hydroxy-3′-oxocholestan)-6,2′-(4′-methylene-5′-oxotetrahydrofuran)] (15)

Only one diastereomer of spirolactone 14 was obtained from 3′β,5α-dihydroxycholestan-6-one in 79% yield. The 1H NMR spectrum of the spirolactone exhibited a 19-H singlet at 1.23 ppm. The triplets of doublets corresponding to the 3′-pro-R
and 3'-pro-S protons were observed at 3.31 and 2.40 ppm, respectively. The chemical shifts of these lactone methylene protons agree well with those of the spirolactone ring at position 6 of compound 13. This suggests that the spirolactone 14 has the axial configuration. The 1H NMR spectrum of the oxidised product 15 showed a 19-H1 singlet at 1.41 ppm indicating the presence of a keto group at position 6. The triplet of doublets corresponding to the 3'-pro-R proton was observed at 3.24 ppm, whereas the 3'-pro-S proton resonated in the range 2.49–2.40 ppm. A doublet corresponding to 4β-H was located at 2.72 ppm. The 1H-13C HETEROCOSY spectrum of the compound led to the assignments of the 3β-H, 7α-H, 7β-H and 8β-H to the signals at 2.43, 1.58, 1.72 and 1.84 ppm, respectively. The ROESY spectrum of the spirolactone 15 revealed cross peaks of 19-H1 with 2β-H and 4β-H. The 3'-pro-R proton was connected to 4α-H while the 3'-pro-S proton showed cross peaks with the 7α-H and 7β-H resonances. These observations can be explained by the configuration of the (6S)-spirolactone depicted in Diederich’s stereostructure 13a (Fig. 3). Therefore the spirolactone 15 exists in an axial configuration at the position 6. This also establishes the stereochemistry of the spirolactone 14.

(17S)-Spiro[(3β,16a-dihydroxyandrost-5-ene)-17,2'-4-(methylene-5′-oxotetrahydronafuran)] (16)

The 1H NMR spectrum of the only diastereomer of the spirolactone obtained from 3β,16a-dihydroxyandrost-5-en-17-one showed vinylic triplets at 6.20 and 5.63 ppm assignable to the 6′Z and 6′E protons, respectively. The triplets of doublets observed at 2.93 and 2.88 ppm were assigned to the 3′-pro-S and 3′-pro-R protons of the lactone methylene group. A multiplet in the range 3.58–3.49 ppm corresponded to 3α-H whereas another multiplet observed over the range 4.30–4.24 ppm was assigned to 16β-H. The singlet corresponding to 18-H1 was located at 0.80 ppm. The ROESY spectrum of the spirolactone exhibited cross peaks between the 18-H1 singlet and the resonance for 16β-H and also to the resonances of the lactone methylene group suggesting an α orientation of the oxygen atom attached to the spirocentre. The observation of the cross peak between the lactone methylene proton signals and the 16β-H supported the α configuration of the spirolactone. Studies of Diederich’s stereomodels ruled out the possibility of the methylene protons exhibiting cross peaks with 16β and 18-methyl protons for the β configuration of the spirolactone 16b, thus establishing the stereochemistry of the spirolactone 16 as 16a (Fig. 3).

(16R)-Spiro[(3β,17b-dihydroxyandrost-5-ene)-16,2'-4-(methylene-5′-oxotetrahydronafuran)] (17)

The 1H NMR spectrum of the single spirolactone obtained from 3β,17b-dihydroxyandrost-5-en-17-one at the position 16 showed vinylic triplets at 6.21 and 5.63 ppm which were assigned to the 6′Z and 6′E protons, respectively. The triplets of doublets observed at 3.07 and 2.90 ppm corresponded to the 3′-pro-S and 3′-pro-R protons, respectively, of the lactone methylene group. A singlet observed at 3.28 ppm was assigned to 17α-H while a multiplet appearing over the range 3.56–3.48 ppm corresponded to 3α-H. The singlet corresponding to 18-H1 was observed at 0.87 ppm. The 1H-13C HETEROCOSY spectrum led to the assignment of the peak at 1.95 ppm to 15α-H. The NOEY spectrum of the spirolactone exhibited connectivity between 18-methyl group and 15β-H. The lactone methylene 3′-pro-R proton showed a cross peak with 17α-H suggesting the β disposition of the oxygen atom at compound 13. This rules out the stereochemistry of 17b wherein the oxygen atom of the spirolactone is α. The observation of the cross peaks between the 3′-pro-R proton and 15α-H resonance and 15α-H resonance confirmed the β configuration of the spirolactone at the position 16. The studies of Diederich’s stereochemically models (Fig. 3) were also consistent with the β configuration of the spirolactone 17a.

In conclusion, the configurational and conformational analyses indicate that the spirolactone 16 at ambient temperature the 3 position of steroids have an axial disposition of the oxygen atom attached to the spirocentre with ring A in the chair conformation. The corresponding minor spirolactones have equatorial configurations. In the spirolactones at position 2, although syn-axial interaction of the axial substituent of the spirolactone with 19-methyl group is present, the A ring is in the chair conformation. This is supported by the molecular modeling studies performed on the spirolactone 4. The spirolactones at position 6 indicate a chair conformation of the ring B with β-disposition of the oxygen atom attached to the spirocentre in spite of the possibility of a 1,3 syn-axial interaction with the 19-methyl group. The spirolactones at the 16 and 17 positions of steroids are indicated to have β- and α-dispositions, respectively, of the oxygen atoms attached to the spirocentre.

Experimental

The 1H NMR spectra of the compounds were recorded at 300 MHz on a Varian VX3 300S spectrometer with a digital resolution of 0.156 Hz as 10–15 mM solutions in CDCl3 (a drop of CD3OD was added for the spirolactone 16) at ambient temperatures. TMS was used as the internal standard. The 1H NMR spectrum of the spirolactone 2 was recorded on a 500 MHz Bruker AM500 spectrometer under similar conditions. The 1H–1H COSY 90 experiments were carried out with π/2–t1–π/2-FID (free induction decay) pulse sequence. The homonuclear ROESY experiments were carried out with π/2–t1–π/2–τs (spin lock)–π/2-FID pulse sequence on Varian VX3 300S spectrometer. The spectra were recorded with 256 increments in t1 values and 1024 data points for the t2 dimension. The FIDs were Fourier transformed onto a data matrix of 1K × 1K with phase shifted sine-bell window functions. The τs used were in the range of 600–700 ms. The NOEY experiments were performed and processed under similar conditions with a mixing time of 1.0 s. The 1H–13C HETEROCOSY experiments were performed on a Varian VX3 300S spectrometer with 256 data points for the f1 dimension and 512 data points for the f2 dimension. The FIDs were Fourier transformed onto a data matrix of 1K × 2K with sine-bell window functions. Polarisation was optimised for 1JCH = 135 Hz. Decoupling was used during acquisition. A relaxation delay of 1.5 s was used. HMQC experiments were also performed on a Bruker AMX 500 spectrometer with 400 data points for the f1 dimension and 2048 data points for the f2 dimension. The FIDs were Fourier transformed onto a data matrix of 512 × 2048. Only those 1H resonances and ROEY/NOEY connectivities, which are important for configurational and conformational assignments are discussed. The spirolactones having chair conformations of ring A or ring B with oxygen atom attached to the spirocentre in the axial disposition are termed as axial spirolactones and those with equatorial disposition are termed as equatorial spirolactones.

Molecular modelling was done on an SG/4D-20 work station using Biosym Insight software. The Discover program was used for energy minimisation studies. The minimisation involved 600 steps of steepest descent followed by 200 steps of conjugate gradient force field. The resulting minimised structures were further subjected to 1000 steps of molecular dynamics.

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