

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

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Mangesh S. Sawant,^a Pradeep J. Nadkarni,^a Umesh R. Desai,^b Rita Katoch,^a Shilpa S. Korde^a and Girish K. Trivedi^{*a}

^a Department of Chemistry, Indian Institute of Technology, Powai, Mumbai-400076, India

^b Department of Medicinal Chemistry, School of Pharmacy, 410N. 12th Street, Richmond, VA 23298-0581, USA

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The configurational and conformational analyses of α -methylene- γ -butyro steroidal spirolactones have been accomplished using 2D COSY, NOESY, ROESY and HETEROCOSY 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 conformational analysis of the 4-en-3-one steroids has been dealt with by Sridharan *et al.*⁹ Marat and co-workers have reported the conformational 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 steroid 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 (HETEROCOSY). The conformations of spiro- α -methylene- γ -butyrolactone steroids 1-17 (Fig. 1) have been deduced. In addition, the con-

formation 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 HETEROCOSY 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.

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

The Reformatsky reaction of 17 β -acetoxyandrostane-3-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 ppm and the two vinylic triplets at 6.22 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 19-H₃ 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 double triplet at 1.92 ppm (*J* = 4.2, 13.8 Hz) and a triplet

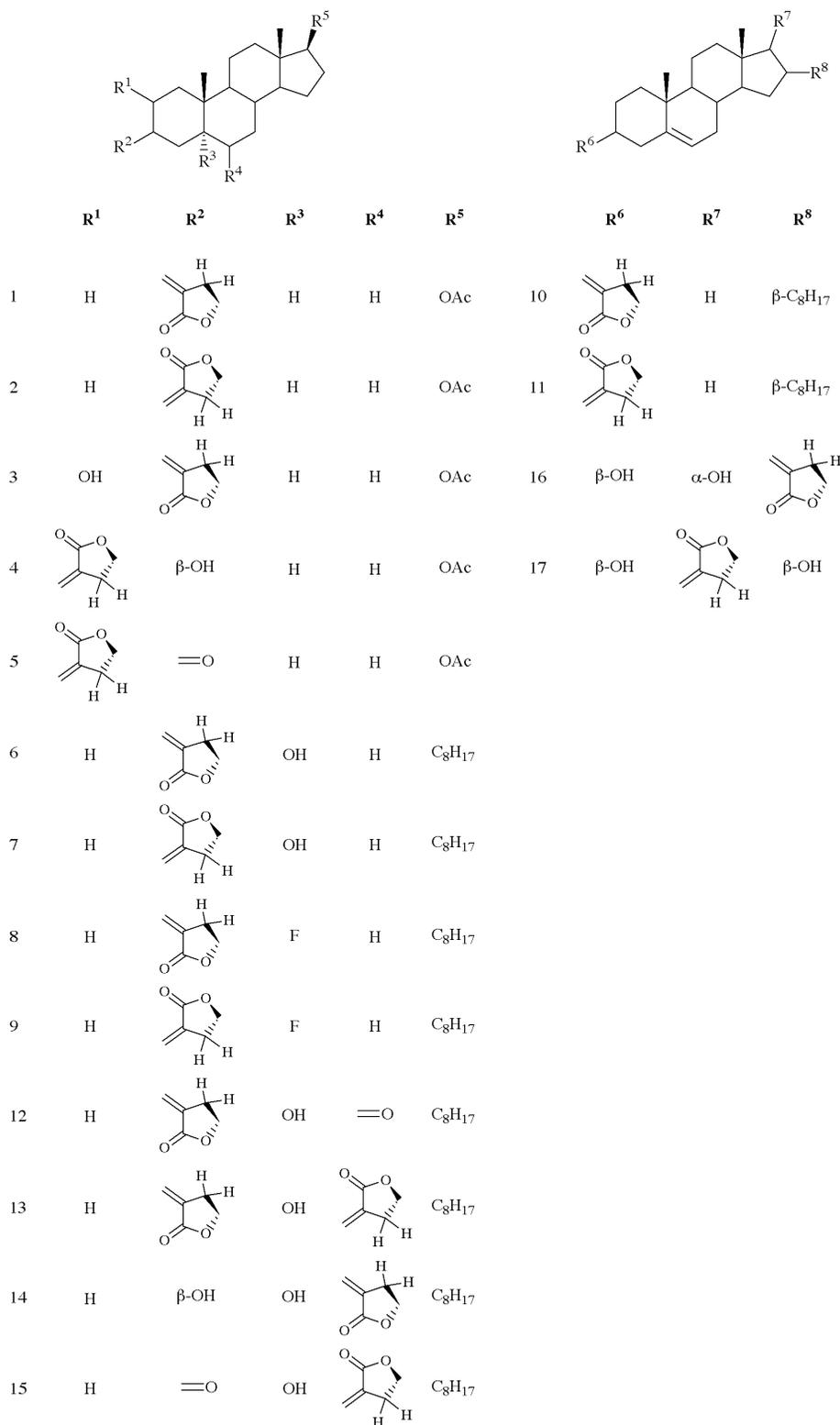


Fig. 1 Structures of spiroactones 1–17 subjected to conformational and configurational analysis.

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 ^1H - ^{13}C HETERO COSY 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 ^1H - ^{13}C HETERO COSY 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 -acetoxyandrostane as against 3α -acetoxyandrostane¹³ 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

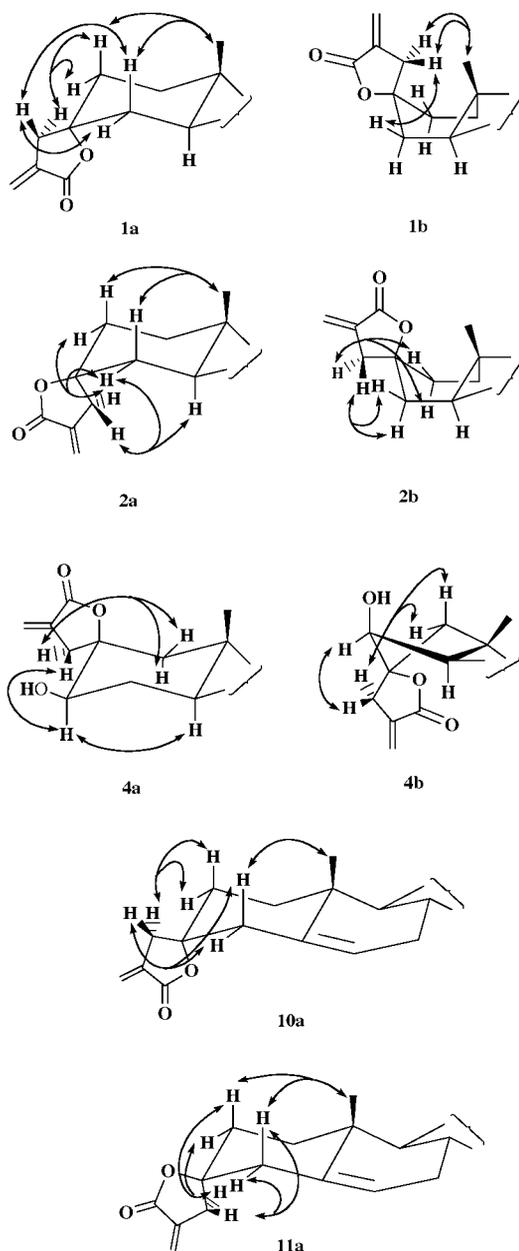


Fig. 2 Putative stereochemical structures of spiro lactones 1, 2, 4, 6, 7, 10 and 11.

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 α and 4 α protons are possible with the stereostructures 1a, 2a and 2b, the cross peaks between the lactone methylene protons and the 1 α and 5 α protons are only possible with structure 2a (Fig. 2). Therefore the minor diastereomer 2 has an equatorial configuration of the spiro lactone ring.

The 5 α -H signal of the major diastereomer was deshielded by 0.61 ppm compared to that of the androstane.¹⁴ The significant deshielding indicates that the oxygen atom of the lactone ring should be in *syn* diaxial position with respect to the 5 α -H. This prediction is supported by the work of Schneider *et al.*¹¹ which has shown that a 1,3-*syn* diaxial interaction between a proton and a hydroxy group of the cyclohexane ring results in a down-field shift of the proton by approximately 0.5 ppm. Deshielding of a similar order was observed for 1 α -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 α , 2 β , 4 α and 4 β protons. The reson-

ance of 4 β -H and 2 β -H showed cross peaks with the 19-H₃ signal. 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 1b is clearly ruled out thus establishing stereochemistry of the major diastereomer 1 as 1a.

(2R)-Spiro[(17 β -acetoxy-3 β -hydroxy-5 α -androstane)-2,2'-(4'-methylene-5'-oxotetrahydrofuran)] (4)

The Reformatsky reaction of 17 β -acetoxy-3 β -hydroxy-5 α -androstane-2-one afforded a single diastereomer 4 of spiro- α -methylene- γ -butyrolactone in 76% yield. The ¹H NMR spectrum of the diastereomer showed vinylic 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 vinylic protons. The triplet of doublets at 3.16 ppm was therefore assigned to the lactone methylene 3'-*pro-R* 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 3 α -H and the doublet observed at 2.04 ppm was assigned to 1 β -H. The 19-H₃ resonated at 0.99 ppm. Analysis of the ¹H-¹³C HETEROCOSY spectrum assisted in the assignments of 1 α , 4 α , 4 β and 5 α protons to 1.20, 1.57, 1.65 and 1.21 ppm, respectively. The deshielding of the 19-H₃ by 0.2 ppm compared to that of the androstane¹³ 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 4 β -H by 0.39 ppm compared to that of the 3 β -hydroxyandrostane¹⁴ augurs well for axial orientation of the oxygen attached to the spirocentre. The ROESY spectrum of compound 4 showed cross peaks between the 3 α and 3'-*pro-S* protons, and between the 1 α , 1 β and 3'-*pro-R* protons. In addition, the 3 α -H signal showed cross peaks with the signals for 1 α -H and 5 α -H while 1 β -H showed a connectivity with 19-H₃.

Study of Dreiding's stereomodels indicates that the ROESY cross peaks between the lactone methylene protons and the 3 α , 1 α and 1 β 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 3 α and 1 α , and the 3 α and 5 α protons can arise only in the chair conformation 4a. In addition, the boat form 4b is expected to cause deshielding of the 5 α -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 spiro lactone 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 4 α , 4 β and 5 α 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 spiro lactone 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 ¹H NMR spectrum of the major spiro lactone obtained from cholest-4-en-3-one exhibited a triplet corresponding to

lactone methylene protons at 2.70 ppm. A double doublet was observed at 2.16 ppm corresponding to 4 α -H and the 4 β -H signal was observed as a multiplet in the range 2.53–2.48 ppm. The 19-H₃ resonated at 1.01 ppm. The ¹H–¹³C HETEROCOSY spectrum of the major spirolactone led to the assignments of 2 α and 2 β protons to the resonance at 1.74 ppm. The ¹H NMR spectrum of the minor 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 4 β -H over the range 2.74–2.68 ppm. The 19-H₃ singlet appeared at 1.05 ppm. The ¹H–¹³C HETEROCOSY spectrum helped in assignments of the protons 1 α , 1 β , 2 α , 2 β , 4 α and 4 β to signals at 1.01, 1.87, 1.64, 2.06, 1.97 and 2.72 ppm, respectively. The deshielding of 19-H₃ 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 4 β -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 2 α -H, 2 β -H, 4 α -H and 4 β -H. The 2 β and 4 β proton resonances further exhibited cross peaks with the 19-H₃ singlet. Therefore the major diastereomer **10** should have an axial configuration of the spirolactone which is further supported by Dreiding's stereostructure **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 2.55 ppm and 1 α -H while a relatively weak cross peak was observed between another lactone methylene proton and 1 α -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 2 α -H signal and the 3'-*pro-S* proton showed a cross peak with the 4 α -H signal. The resonance for 19-H₃ exhibited cross peaks with the 4 β and 2 β proton multiplets. In addition, a cross peak was also detected between the 2 β and 4 β proton resonances. These connectivities confirm the equatorial configuration of the minor spirolactone **11a**.

(3'S,6'S)-Dispiro[(4-methylene-5-oxotetrahydrofuran)-2,3'-(5 α '-hydroxycholestane)-6',2''-(4'-methylene-5''-oxotetrahydrofuran)] (13)

The ¹H NMR spectrum of the dilactone obtained from 5 α '-hydroxycholestane-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-H₃ singlet was observed at 1.15 ppm in the ¹H NMR spectrum and a doublet corresponding to 4 β -H was located at 1.96 ppm. A singlet observed at 3.56 ppm disappeared on D₂O exchange and was assigned to the 5 α -hydroxy proton. The ¹H–¹³C HETERO-COSY spectrum led to the assignments of the 1 α , 4 α , 7 α , 7 β , 8 β

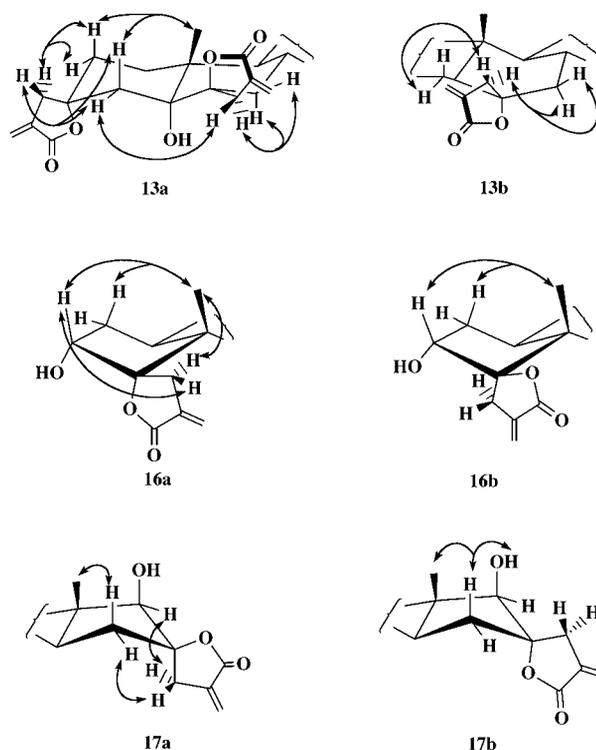


Fig. 3 Putative stereochemical structures of spirolactones **13**, **14**, **15**, **16** and **17**.

and 9 α 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 5 α -hydroxy proton. The lactone methylene protons 3'-*pro-R*- and 3'-*pro-S*- exhibited cross peaks with 2 α , 2 β , 4 α and 4 β protons in the ROESY spectrum of the spirolactone. The 2 β -H and 4 β -H signals showed cross peaks with that of 19-H₃. Thus the axial configuration of the spirolactone at the position 3 was confirmed. The deshielding of the 19-H₃ 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 4 α -H while the 3''-*pro-S* proton showed cross peaks with the 7 α -H and 7 β -H. The 3''-*pro-R* proton, however, did not show connectivity with 4 β -H, and neither the 3''-*pro-R* nor the 3''-*pro-S* proton signals exhibited cross peaks with the 19-H₃ 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 8 β protons compared to that of the spirolactone **6**, however, cannot be explained in the case of the boat conformer. Also, the 9 α -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**).

(6S)-Spiro[(3 β ,5 α -dihydroxycholestane)-6,2'-(4'-methylene-5'-oxotetrahydrofuran)] (14) and (6S)-spiro[(5 α -hydroxy-3-oxocholestane)-6,2'-(4'-methylene-5'-oxotetrahydrofuran)] (15)

Only one diastereomer of spirolactone **14** was obtained from 3 β ,5 α -dihydroxycholestan-6-one in 79% yield. The ¹H NMR spectrum of the spirolactone exhibited a 19-H₃ 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 spiro lactone ring at position 6 of compound **13**. This suggests that the spiro lactone **14** has the axial configuration. The ^1H NMR spectrum of the oxidised product **15** showed a 19- H_3 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 4 β -H was located at 2.72 ppm. The ^1H - ^{13}C HETEROCOSY spectrum of the compound led to the assignments of the 2 β -H, 7 α -H, 7 β -H and 8 β -H to the signals at 2.43, 1.58, 1.72 and 1.84 ppm, respectively. The ROESY spectrum of the spiro lactone **15** revealed cross peaks of 19- H_3 with 2 β -H and 4 β -H. The 3'-*pro-R* proton was connected to 4 α -H while the 3'-*pro-S* proton signal showed cross peaks with the 7 α -H and 7 β -H resonances. These observations can be explained by the configuration of the (6*S*)-spiro lactone depicted in Dreiding's stereostructure **13a** (Fig. 3). Therefore the spiro lactone **15** exists in an axial configuration at the position 6. This also establishes the stereochemistry of the spiro lactone **14**.

(17*S*)-Spiro[(3 β ,16 α -dihydroxyandrost-5-ene)-17,2'-(4'-methyl-ene-5'-oxotetrahydrofuran)] (16)

The ^1H NMR spectrum of the only diastereomer of the spiro lactone obtained from 3 β ,16 α -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 2.95 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 3 α -H whereas another multiplet observed over the range 4.30–4.24 ppm was assigned to 16 β -H. The singlet corresponding to 18- H_3 was located at 0.80 ppm. The ROESY spectrum of the spiro lactone exhibited cross peaks between the 18- H_3 singlet and the resonance for 16 β -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 16 β -H supported the α configuration of the spiro lactone. Studies of Dreiding's stereomodels ruled out the possibility of the methylene protons exhibiting cross peaks with 16 β and 18-methyl protons for the β configuration of the spiro lactone **16b**, thus establishing the stereochemistry of the spiro lactone **16** as **16a** (Fig. 3).

(16*R*)-Spiro[(3 β ,17 β -dihydroxyandrost-5-ene)-16,2'-(4'-methyl-ene-5'-oxotetrahydrofuran)] (17)

The ^1H NMR spectrum of the single spiro lactone obtained from 3 β ,17 β -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 17 α -H while a multiplet appearing over the range 3.56–3.48 ppm corresponded to 3 α -H. The singlet corresponding to 18- H_3 was observed at 0.87 ppm. The ^1H - ^{13}C HETEROCOSY spectrum led to the assignment of the peak at 1.95 ppm to 15 α -H. The NOESY spectrum of the spiro lactone exhibited connectivity between 18-methyl group and 15 β -H. The lactone methylene 3'-*pro-R* proton showed a cross peak with 17 α -H suggesting the β disposition of the oxygen atom attached to the spirocentre. This rules out the stereostructure **17b** wherein the oxygen atom of the spiro lactone is α . The observation of the cross peaks between the 3'-*pro-S*-H resonance and 15 α -H resonance confirmed the β configuration of the spiro lactone at the position 16. The studies of Dreiding's stereo-

models (Fig. 3) were also consistent with the β configuration of the spiro lactone **17a**.

In conclusion, the configurational and conformational analyses indicate that the only or major spiro lactones formed at 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 spiro lactones have equatorial configurations. In the spiro lactones at position 2, although *syn*-axial interaction of the axial substituent of the spiro lactone with 19-methyl group is present, the A ring is in the chair conformation. This is supported by the molecular modelling studies performed on the spiro lactone **4**. The spiro lactones 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 spiro lactones 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 VXR 300S spectrometer with a digital resolution of 0.156 Hz as 10–15 mM solutions in CDCl_3 (a drop of CD_3OD was added for the spiro lactone **16**) at ambient temperatures. TMS was used as the internal standard. The ^1H NMR spectrum of the spiro lactone **2** was recorded on a 500 MHz Bruker AM500 spectrometer under similar conditions. The ^1H - ^1H COSY 90 experiments were carried out with $\pi/2-t_1-\pi/2$ -FID (free induction decay) pulse sequence. The homonuclear ROESY experiments were carried out with $\pi/2-t_1-\pi/2-t_m$ (spin lock)- $\pi/2$ -FID pulse sequence on Varian VXR 300S spectrometer. The spectra were recorded with 256 increments in t_1 values and 1024 data points for the t_2 dimension. The FIDs were Fourier transformed onto a data matrix of $1\text{k} \times 1\text{k}$ with phase shifted sine-bell window functions. The t_m used were in the range of 600–700 ms. The NOESY experiments were performed and processed under similar conditions with a mixing time of 1.0 s. The ^1H - ^{13}C HETEROCOSY experiments were performed on a Varian VXR 300S spectrometer with 256 data points for the f_1 dimension and 512 data points for the f_2 dimension. The FIDs were Fourier transformed onto a data matrix of $1\text{k} \times 2\text{k}$ with sine-bell window functions. Polarisation was optimised for $^1J_{\text{CH}} = 135$ 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 f_1 dimension and 2048 data points for the f_2 dimension. The FIDs were Fourier transformed onto a data matrix of 512×2048 . Only those ^1H resonances and ROESY/NOESY connectivities, which are important for configurational and conformational assignments are discussed. The spiro lactones having chair conformations of ring A or ring B with oxygen atom attached to the spirocentre in the axial disposition are termed as axial spiro lactones and those with equatorial disposition are termed as equatorial spiro lactones.

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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