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

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*

Received (in Cambridge) 25th March 1999, Accepted 14th July 1999

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 ^{1}H 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-ketosteriods.[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 ^{1}H–^{13}C heteronuclear shift correlated spectroscopy and ^{1}H–^{1}H 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 ^{1}H and ^{13}C chemical shifts and the lack of through-spin connectivity between the steroid nucleus and the spirolactone. Benezra and co-workers used ^{1}H 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 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 ^{1}H–^{1}H 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 ^{1}H–^{1}H COSY and ^{1}H–^{13}C 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[(17β-acetoxy-5α-androstane)-3,2′-(4′-methylene-5′-oxotetrahydrofuran)] (1) and (3*S*)-spiro[(17β-acetoxy-5α-androstane)-3,2′-(4′-methylene-5′-oxotetrahydrofuran)] (2)

The Reformatsky reaction of 17β-acetoxyandrostan-3-one provided two diastereomers in the ratio 70 : 30 which could be isolated in pure form. The ^{1}H 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-H_3 singlet of the major diastereomer resonated at 0.81 ppm whereas that of the minor diastereomer was observed at 0.86 ppm. The ^{1}H NMR of the minor diastereomer also exhibited a double triplet at 1.92 ppm (J = 4.2, 13.8 Hz) and a triplet

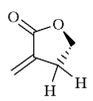
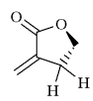
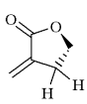
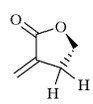
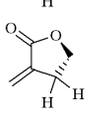
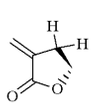
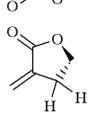
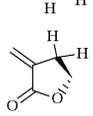
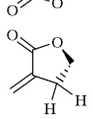
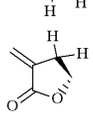
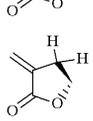
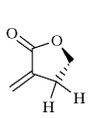
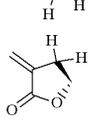
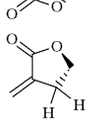
	R^1	R^2	R^3	R^4	R^5		R^6	R^7	R^8
1	H		H	H	OAc	10		H	β-C_8H_{17}
2	H		H	H	OAc	11		H	β-C_8H_{17}
3	OH		H	H	OAc	16	β-OH	α-OH	
4		β-OH	H	H	OAc	17	β-OH		β-OH
5		=O	H	H	OAc				
6	H		OH	H	C_8H_{17}				
7	H		OH	H	C_8H_{17}				
8	H		F	H	C_8H_{17}				
9	H		F	H	C_8H_{17}				
12	H		OH	=O	C_8H_{17}				
13	H		OH		C_8H_{17}				
14	H	β-OH	OH		C_8H_{17}				
15	H	=O	OH		C_8H_{17}				

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 ^1H–^{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 ^1H–^{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-acetoxyandrostane as against 3α-acetoxyandrostane[13] 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

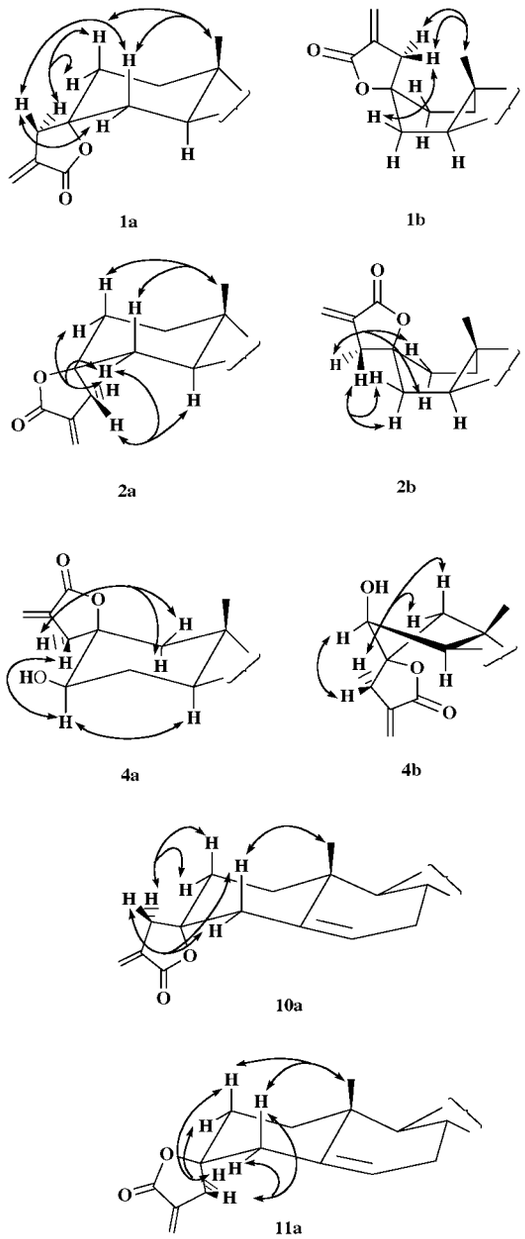


Fig. 2 Putative stereochemical structures of spirolactones **1**, **2**, **4**, **6**, **7**, **10** and **11**.

with the lactone methylene protons. Studies of Dreiding's stereomodels showed that although cross peaks between the methylene protons of the lactone and the 2α and 4α protons are possible with the stereostructures **1a**, **2a** and **2b**, the cross peaks between the lactone methyelene protons and the 1α and 5α protons are only possible with structure **2a** (Fig. 2). Therefore the minor diastereomer **2** has an equatorial configuration of the spirolactone ring.

The 5α-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 5α-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 downfield shift of the proton by approximately 0.5 ppm. Deshielding of a similar order was observed for 1α-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 2α, 2β, 4α and 4β protons. The resonance of 4β-H and 2β-H showed cross peaks with the 19-H_3 signal. The appearance of these cross peaks is in accordance with the Drieding'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**.

(2*R*)-Spiro[(17β-acetoxy-3β-hydroxy-5α-androstane)-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 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 3α-H and the doublet observed at 2.04 ppm was assigned to 1β-H. The 19-H_3 resonated at 0.99 ppm. Analysis of the ^{1}H–^{13}C HETEROCOSY spectrum assisted in the assignments of 1α, 4α, 4β and 5α protons to 1.20, 1.57, 1.65 and 1.21 ppm, respectively. The desheilding of the 19-H_3 by 0.2 ppm compared to that of the androstane[13] 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 3α-H signal showed cross peaks with the signals for 1α-H and 5α-H while 1β-H showed a connectivity with 19-H_3.

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 1α, 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**).

(3*R*)-Spiro[(cholest-5-ene)-3,2′-(4′-methylene-5′-oxotetrahydrofuran)] (10) and (3*S*)-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 double doublet was observed at 2.16 ppm corresponding to 4α-H and the 4β-H signal was observed as a multiplet in the range 2.53–2.48 ppm. The 19-H_3 resonated at 1.01 ppm. The ^1H–^{13}C HETEROCOSY spectrum of the major spirolactone led to the assignments of 2α and 2β 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 4β-H over the range 2.74–2.68 ppm. The 19-H_3 singlet appeared at 1.05 ppm. The ^1H–^{13}C HETEROCOSY spectrum helped in assignments of the protons 1α, 1β, 2α, 2β, 4α 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_3 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 4β-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_3 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 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_3 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′-hydroxycholestane)-6′,2″-(4″-methylene-5″-oxotetrahydrofuran)] (13)

The ^1H NMR spectrum of the dilactone obtained from 5α′-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-H_3 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 D_2O exchange and was assigned to the 5α-hydroxy proton. The ^1H–^{13}C HETEROCOSY spectrum led to the assignments of the 1α, 4α, 7α, 7β, 8β and 9α protons to 1.45, 1.62, 1.58, 1.72, 1.72 and 1.59 ppm, respectively.

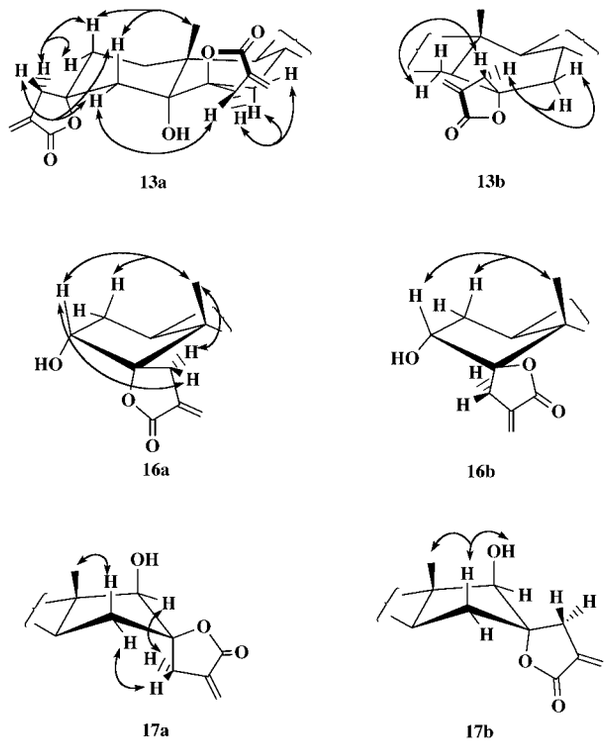


Fig. 3 Putative stereochemical structures of spirolactones **13**, **14**, **15**, **16** and **17**.

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_3. Thus the axial configuration of the spirolactone at the position 3 was confirmed. The deshielding of the 19-H_3 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_3 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[(3β,5α-dihydroxycholestane)-6,2′-(4′-methylene-5′-oxotetrahydrofuran)] (14) and (6*S*)-spiro[(5α-hydroxy-3-oxocholestane)-6,2′-(4′-methylene-5′-oxotetrahydrofuran)] (15)

Only one diastereomer of spirolactone **14** was obtained from 3β,5α-dihydroxycholestan-6-one in 79% yield. The ^1H NMR spectrum of the spirolactone exhibited a 19-H_3 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 ^{1}H 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 4β-H was located at 2.72 ppm. The ^{1}H–^{13}C HETEROCOSY spectrum of the compound led to the assignments of the 2β-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-H_3 with 2β-H and 4β-H. The 3′-*pro*-*R* proton was connected to 4α-H while the 3′-*pro*-*S* proton signal showed cross peaks with the 7α-H and 7β-H resonances. These observations can be explained by the configuration of the (6*S*)-spirolactone depicted in Dreiding'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**.

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

The ^{1}H NMR spectrum of the only diastereomer of the spirolactone obtained from 3β,16α-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 3α-H whereas another multiplet observed over the range 4.30–4.24 ppm was assigned to 16β-H. The singlet corresponding to 18-H_3 was located at 0.80 ppm. The ROESY spectrum of the spirolactone exhibited cross peaks between the 18-H_3 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 Dreiding'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).

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

The ^{1}H NMR spectrum of the single spirolactone obtained from 3β,17β-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-H_3 was observed at 0.87 ppm. The ^{1}H–^{13}C HETEROCOSY spectrum led to the assignment of the peak at 1.95 ppm to 15α-H. The NOESY 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 attached to the spirocentre. This rules out the stereostructure **17b** wherein the oxygen atom of the spirolactone is α. The observation of the cross peaks between the 3′-*pro*-*S*-H resonance and 15α-H resonance confirmed the β configuration of the spirolactone at the position 16. The studies of Dreiding's stereomodels (Fig. 3) were also consistent with the β configuration of the spirolactone **17a**.

In conclusion, the configurational and conformational analyses indicate that the only or major spirolactones formed at the 3 position of steriods 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 modelling 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 steriods are indicated to have β- and α-dispositions, respectively, of the oxygen atoms attached to the spirocentre.

Experimental

The ^{1}H 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 spirolactone **16**) at ambient temperatures. TMS was used as the internal standard. The ^{1}H NMR spectrum of the spirolactone **2** was recorded on a 500 MHz Bruker AM500 spectrometer under similar conditions. The ^{1}H–^{1}H COSY 90 experiments were carried out with π/2–t_1–π/2-FID (free induction decay) pulse sequence. The homonuclear ROESY experiments were carried out with π/2–t_1–π/2–t_m(spin lock)–π/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 1k × 1k 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 ^{1}H–^{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 1k × 2k with sine-bell window functions. Polarisation was optimised for $^1J_{CH}$ = 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 ^{1}H resonances and ROESY/NOESY 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.

Acknowledgements

We thank CSIR, New Delhi, BRNS, Mumbai and DST, New Delhi for financial assistance. Spectral facilities provided by RSIC, IIT, Mumbai (300 MHz NMR) and the National Facility for high field NMR, TIFR, Mumbai (500 MHz NMR) are gratefully acknowledged. The authors also wish to

place on record their gratitude towards Professor A. K. Lala, Department of Chemistry, IIT, Mumbai for the molecular modelling studies.

References

1 M. Ogura, G. A. Cordell and N. R. Fransworth, *Phytochemistry*, 1978, **17**, 957.
2 E. Rodriguez, G. H. N. Towers and J. C. Mitchell, *Phytochemistry*, 1976, **15**, 1573.
3 O. Spring, K. Albert and W. Gradmann, *Phytochemistry*, 1981, **20**, 1883.
4 M. S. Sawant, R. Katoch, G. K. Trivedi and U. R. Desai, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 843.
5 X. Chen, E. R. Hotelano, E. L. Eliel and S. V. Frye, *J. Am. Chem. Soc.*, 1992, **114**, 1778.
6 D. J. Collins, J. J. Hobbs and S. Sternhell, *Aust. J. Chem.*, 1963, **16**, 1030.
7 L. D. Hall and J. K. M. Sanders, *J. Chem. Soc.*, *Chem. Commun.*, 1980, 366.
8 K. Marat, J. F. Templeton and V. P. Shashikumar, *Magn. Reson. Chem.*, 1987, **25**, 730.
9 R. Sridharan, U. R. Desai, R. M. Rao and G. K. Trivedi, *Steroids*, 1993, **58**, 170.
10 K. Marat, J. F. Templeton and V. P. Shashikumar, *Magn. Reson. Chem.*, 1987, **25**, 25.
11 H. Schneider, U. Buchheit, N. Becker, G. Schmidt and U. Siel, *J. Am. Chem. Soc.*, 1985, **107**, 7027.
12 G. Schlewer, J. L. Stampf and C. Benezra, *J. Med. Chem.*, 1980, **23**, 1031.
13 R. F. Zurchur, *Helv. Chim. Acta*, 1963, **46**, 2054.
14 D. N. Kirk, H. C. Thomas, C. Douglas, K. A. White, K. E. Smith, S. Latif and R. W. P. Hubbard, *J. Chem. Soc.*, *Perkin Trans. 2*, 1990, 1567.

Paper 9/02415H