5α/5β Stereochemistry of spirostanol and furostanol saponins

Steroidal saponins constitute an important class of plant secondary metabolites and are mostly found in monocotyledonous angiosperms. These compounds are known to possess a vast array of bioactivities, including anticancer, adjuvant, immunostimulant, anti-inflammatory, antimicrobial, hypocholesterolaemic, antimicrobial and antioxidant[1,2]. Structurally, steroidal saponins are classified as spirostanol and furostanol glycosides[3]. A third and relatively less common class of steroidal saponins is furospirostanols. The saponins of this class generally have a polyoxygenated A-ring and/or one or more double bonds present in the rings A and B[4].

On the basis of their structural features, both spirostanol and furostanol saponins may be further classified as 5α, 5β or Δ^5 compounds. 5α and 5β compounds originate as a result of A/B ring fusion of the steroidal nucleus which is *trans* in the case of the former and *cis* for the latter (Figure 1)[5]. The structure and stereochemistry of these compounds are established on the basis of their spectral behaviour. Most importantly, NMR techniques have been used to study the parent skeleton, substitution patterns, monosaccharide units and their linkages in the sapogenin and/or saponin molecules[5–9].

The chemical shifts of C-5, C-7, C-9 and C-19 are generally employed for determining the type of ring fusion, and hence 5α/5β stereochemistry in the steroidal saponins. These carbons appear deshielded in the case of 5α compounds compared to the other classes[5]. However, no empirical rules have been laid to distinguish the saponins of 5α/5β series with analogous substitution patterns. We present a method based on ^{1}H and ^{13}C NMR chemical shifts for the assignment of 5α/5β stereochemistry in the spirostanol and furostanol saponins.

A literature search of the ^{1}H and ^{13}C NMR data of spirostanol and furostanol saponins was conducted to derive a relation between A/B ring stereochemistry and the chemical shifts of C/H-3, C/H$_2$-4, C/H-5, C/H$_2$-6, C/H$_2$-7, C/H-8, C/H-9 and C/H$_3$-19.

Chemical shifts of the ring A/B carbons and protons have been used to determine the type of fusion between these two rings and consequently the stereochemistry of H-5. The most important indicators of the stereochemistry in this case are the NMR resonances of C/H-3, C/H-5, C/H-9 and C/H-19. In the case of *trans* fusion and H-5α, the methine H-3 resonates between δ 3.5 and 4.0 when C_5D_5N is used as a solvent and C-3 appears at δ 75.0–77.9. These chemical shifts were noted to be independent of the skeleton type (spirostanol or furostanol) as well as the stereochemistry at C-25 (*R/S*). A deshielded H-3 (δ 4.24–4.39) and a shielded C-3 (δ 76.2–74.0) indicate *cis*-fusion (Tables 1 and 2)[10–24].

Similarly, an α-oriented H-5 appears at δ 0.58–0.87; H-5α of the furostanols being more shielded. However, in both spirostanol as well as furostanol, it resonates upfield to 1 ppm. On the contrary, a β-oriented H-5 always appears at a higher frequency (δ 1.77–2.22). The average chemical shift of C-5 in a *trans*-A/B system is δ 44.6 compared to δ 36.6 in a *cis*-system. A significant difference in the chemical shifts of C/H-9 in *trans*- and *cis*-fused A/B rings was noted. The average chemical shift of C/H-9 in the

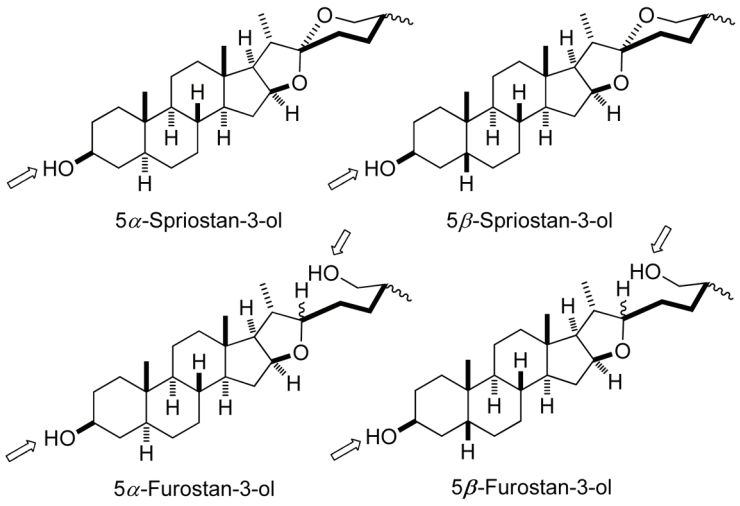

Figure 1. Structures of 5α/5β-spirostan-3-ol and 5α/5β-furostan-3-ol. Arrows depict the typical glycosylation positions.

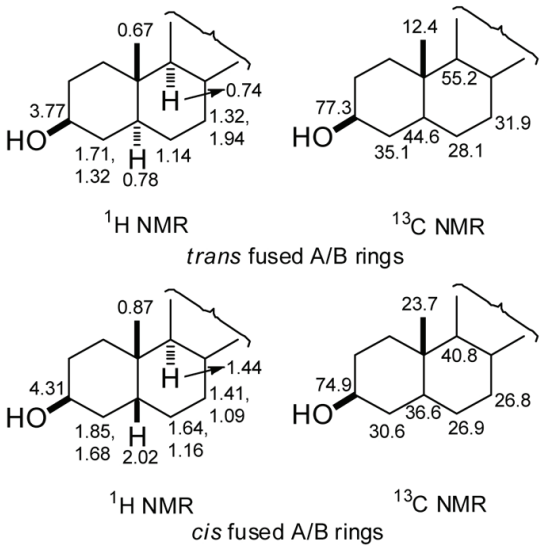

Figure 2. The average ^{1}H and ^{13}C NMR chemical shifts of *trans*- and *cis*-fused A/B rings of steroidal saponins.

Table 1. ^{1}H and ^{13}C NMR chemical shifts of 5α-spirostanol and 5α-furostanol saponins[a]

Compound	Class	Solvent	H-3	C-3	H-4	C-4	H-5	C-5	H-6	C-6	H-7	C-7	H-8	C-8	H-9	C-9	H-19	C-19	Reference
1	Spirostane	C_5D_5N	3.89	77.3	1.76, 1.22	34.7	0.84	44.6	1.11	28.7	1.56, 2.10	31.5	1.76	34.4	0.88	55.7	0.65	11.8	10
2	Spirostane	C_5D_5N	3.93	77.5	1.77, 1.33	34.9	0.87	44.7	1.10, 1.03	29.0	1.50, 0.77	32.5	1.38	35.3	0.48	54.5	0.63	12.4	10
3	Spirostane	C_5D_5N	3.87	77.1	1.77, 1.33	34.6	0.82	44.5	1.07	28.6	1.55, 2.08	31.5	1.74	34.3	0.87	55.5	0.66	11.7	11
4	Spirostane	C_5D_5N	4.00	75.0	1.77, 1.30	34.3	0.84	44.1	1.09	28.2	1.60, 2.09	31.1	1.72	34.0	1.34	55.6	0.62	11.4	12
5	Spirostane	C_5D_5N	3.83	77.6	1.83, 1.56	34.8	0.84	44.6	1.10	28.9	1.93, 1.32	32.4	1.33	35.2	0.45	54.4	0.64	12.3	13
6	Furostane	DMSO-d6	3.43	77.6	1.54, 1.27	38.2	0.58	44.0	1.28, 1.26	21.7	1.95, 1.22	31.7	1.50	35.1	0.60	53.6	0.69	16.2	14
7	Furostane	$CDCl_3$ + CD_3OD	3.43	77.6	1.54, 1.27	38.2	0.58	46.5	1.28, 1.26	21.7	1.95, 1.22	31.7	1.50	35.1	0.60	55.8	0.77	16.9	14
8	Spirostane	C_5D_5N	3.87	77.1		34.6		44.4		28.5		31.4		34.3		55.5	0.65	11.7	15
9	Spirostane	C_5D_5N	3.89	77.3		34.7	0.88	44.5		28.8		32.1		35.2		54.4	0.82	12.3	16
10	Spirostane	C_5D_5N	3.93	77.0		34.7		44.6		28.3		31.5		34.4		55.6	0.68	11.8	17
11	Spirostane	C_5D_5N	3.87	77.1		34.7		44.5		28.6		31.5		34.5		55.7	0.67	11.8	17
12	Spirostane	C_5D_5N	3.88	77.4		34.8		44.6		28.7		31.6		34.5		55.5	0.67	11.9	17
13	Spirostane	C_5D_5N	3.85	77.5		34.8		44.7		28.8		31.9		34.6		55.8	0.70	12.0	17
14	Spirostane	C_5D_5N	3.77	77.4		34.8		44.6		28.7		31.9		34.5		55.7	0.66	11.8	17
15	Spirostane	C_5D_5N	3.82	77.3		34.7		44.5		28.7		31.9		34.4		55.6	0.63	11.8	17
16	Spirostane	C_5D_5N	3.58	77.9		35.0		44.8		29.1		32.6		35.4		54.6	0.63	12.5	18
17	Spirostane	C_5D_5N	3.58	77.5		34.9		44.7		29.0		32.5		35.5		54.5	0.62	12.4	18
18	Furostane	C_5D_5N	3.77	77.8		34.8		44.6		28.7		31.9		34.5		55.8	0.65/1.11*	11.9	18
19	Furostane	C_5D_5N	3.77	77.8		34.7		44.6		28.7		31.8		34.3		55.7	0.63/1.11*	11.8	18
20	Furostane	C_5D_5N	3.77	77.8		34.9		44.8		29.1		32.6		35.4		54.6	0.61/0.86*	12.4	18
21	Furostane	C_5D_5N	3.78	77.8		34.9		44.8		29.1		32.5		35.4		54.6	0.63/0.86*	12.4	18

[a]**1**, Hecogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-xylopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl(1 → 4)-α-D-galactopyranoside; **2**, Neotigogenin3-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 2)[β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside; **3**, (3β,5α, 25*R*)-12-Oxospirostan-3-yl 6-deoxy-α-L-mannopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (sisalasaponin D); **4**, Hecogenin-3-O-β-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside; **5**, (25*S*)-5α-Spirostan-3β,27-diol-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside (smilscobinoside B); **6**, (25*S*)-5α-Furost-3β,22α,26-triol 3-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-α-L-rhamnopyranoside (longipetaloside A); **7**, (25*S*)-5α-Furostan-3β,22α,26-triol-3-*O*-β-D-glucopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glactopyranosyl]-(1 → 2)-β-D-xylopyranosyl-(1 → 3)-β-D-xylopyranoside (longipetaloside B); **8**, (25R)-3β-[(*O*-β-D-Glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranosyl)-oxy]-5α-spirostan-12-one; **9**, 3-[*O*-β-D-Glucopyranosyl-(1 → 2)-*O*-[*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[*O*-β-D-glucopyranosyl-(1 → 6)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D galactopyranosyl)-oxy]-(3β,5α,25R)-spirostane; **10**, Hecogenin-3-*O*-β-D-galactopyranoside (agaveside A); **11**, Hecogenin-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (agaveside B); **12**, Hecogenin 3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside; **13**, Hecogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (agamenoside D); **14**, Hecogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (agamenoside E); **15**, Hecogenin 3-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranosy-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (agamenoside F); **16**, Tigogenin 3-O-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (polianthoside B); **17**, Tigogenin 3-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranosy-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (polianthoside C); **18**, 26-*O*-β-D-Glucopyranosyl-(25*R*)-5α-furost-3β,22α,26-triol-12-one 3-*O*-β-D-glucopyranosyl-(1 → 2)[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (polianthoside D); **19**, 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furost-3β,22α,26-triol-12-one 3-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (polianthoside E); **20**, 26-*O*-β-D-Glucopyranosyl-(25*R*)-5α-furost-3β,22α,26-triol 3-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside (polianthoside F); **21**, 26-*O*-β-D-Glucopyranosyl-(25*R*)-5α-furost-3β,22α,26-triol 3-*O*-β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside; *Values of H-19/H-18.

Table 2. ^{1}H and ^{13}C NMR chemical shifts of 5β-spirostanol and 5β-furostanol saponins[b]

Compound	Class	Solvent	H-3	C-3	H-4	C-4	H-5	C-5	H-6	C-6	H-7	C-7	H-8	C-8	H-9	C-9	H-19	C-19	Reference
22	Spirostane	C_5D_5N	4.28	76.2	1.85, 1.75	31.0	2.15	37.1	1.77, 1.14	27.3	1.19, 0.91	27.1	1.46	35.6	1.24	40.6	0.96	24.3	19
23	Spirostane	DMSO-d6	4.30	75.5	1.79, 1.58	30.6	2.15	37.0	1.34, 1.26	26.7	1.30, 0.94	27.0	1.60	35.7	1.24	40.2	0.98	24.4	20
24	Spirostane	C_5D_5N	4.31	75.3	1.78, 1.58	30.1	2.16	36.7	1.39, 1.23	26.9	1.31, 1.07	27.1	1.67	35.4	1.23	40.4	0.95	24.2	20
25	Spirostane	C_5D_5N	4.24	75.3		30.5	2.22	36.5		26.4	1.17, 0.90	26.5	1.83	34.7	1.68	41.9	0.96	23.2	21
26	Furostane	C_5D_5N	4.33	74.3	1.25	30.1	1.79	34.8	1.86, 1.10	26.9	1.75, 1.38	26.8	2.08	36.6	1.72	42.0	0.83	23.1	22
27	Furostane	C_5D_5N	4.31	74.1	1.25	30.1	1.77	34.8	1.84, 1.10	26.9	1.77, 1.39	26.8	2.06	36.6	1.72	42.0	0.82	23.1	22
28	Furostane	C_5D_5N	4.39	75.6	1.97, 1.81	30.6	1.94	37.0	1.26	26.8	0.94	26.8	1.50	35.4	1.29	40.3	0.82	23.9	23
29	Spirostane	C_5D_5N	4.31	75.6		32.5		37.4		27.3		27.1		35.6		40.6	0.85	24.2	19
30	Spirostane	C_5D_5N	4.32	75.4		30.4		37.4		27.3		27.1		35.9		40.7	0.82	24.2	19
31	Spirostane	C_5D_5N	4.31	74.7		30.6		36.9		27.0		26.7		35.5		40.2	0.82	23.8	24
32	Spirostane	C_5D_5N	4.37	74.4		30.5		36.9		27.0		26.8		35.6		40.2	0.85	23.9	24
33	Spirostane	C_5D_5N	4.32	74.7		30.7		36.9		27.0		26.7		35.5		40.2	0.87	23.9	24
34	Spirostane	C_5D_5N	4.27	74.3		30.6		36.5		26.7		26.3		34.7		41.9	0.83	23.0	24
35	Spirostane	C_5D_5N	4.31	74.0		30.6		36.5		26.8		26.4		34.7		41.9	0.85	23.0	24
36	Furostane	C_5D_5N	4.31	74.7		30.6		36.9		27.0		26.7		35.5		40.2	0.83	23.8	24
37	Furostane	C_5D_5N	4.32	74.7		30.6		36.9		27.0		26.7		35.5		40.2	0.87	23.8	24
38	Furostane	C_5D_5N	4.31	74.8		30.7		36.9		27.0		26.7		35.5		40.2	0.87	23.9	24

[b]**22**, (25*S*)-5β-Spirostan-3β-ol 3-*O*-β-D-glucopyranosyl-(1 → 6)-[α-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (racemoside A); **23**, (25*S*)-5β-Spirostan-3β-ol 3-*O*-[α-L-arabinopyranosyl-(1 → 2)]-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranoside (shatavaroside A); **24**, (25*S*)-5β-Spirostan-3β-ol 3-*O*-[β-D-glucopyranosyl(1 → 2)]-[β-D-xylopyranosyl-(1 → 4)]-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranoside (shatavaroside B); **25**, (3β,5β,25*R*)-3-[(2-*O*-β-D-Glucopyranosyl-β-D-galactopyranosyl)oxy]spirostan-12-one (elephanoside H); **26**, (25*S*)-26-*O*-β-D-Glucopyranosyl-5β-furostan-3β, 22α, 26-triol-12-one 3-*O*-β-D-glucopyranoside; **27**, (25*S*)-26-*O*-β-D-Glucopyranosyl-22α-methoxy-5β-furostan-3β, 26-diol-12-one 3-*O*-β-D-glucopyranoside; **28**, (25*S*)-26-*O*-β-D-Glucopyranosyl-22-hydroxy-5β-furostan-3β,26-diol 3-*O*-β-D-galactopyranoside; **29**, (25*S*)-5β-Spirostan-3β-ol 3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside (racemoside B); **30**, (25*S*)-5β-Spirostan-3β-ol 3-*O*-α-L-rhamnopyranosyl-(1 → 6)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside (racemoside C); **31**, (25*R*)-5β-Spirostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside; **32**, (25*R*)-5β-Spirostan-3β-yl *O*-β-D-glucopyranosyl(1 → 3)-β-D-galactopyranoside; **33**, (25*R*)-5β-Spirostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranosyl)oxy]5β-spirostan-12-one; **34**, (25*R*)-3β-[(*O*-β-D-Glucopyranosyl-(1 → 4)-β-D-galactopyranosyl)oxy]5β-spirostan-12-one; **35**, (25*R*)-3β-[(*O*-β-D-Glucopyranosyl-(1 → 3)-β-D-galactopyranosyl)oxy]-5β-spirostan-12-one; **36**, (25*R*)-26-[(β-D-Glucopyranosyl)oxy]-22α-methoxy-5β-furostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside; **37**, (25*R*)-26-[(β-D-Glucopyranosyl)oxy]-22α-methoxy-5β-furostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside; **38**, (25*R*)-26[(*O*-β-D-Glucopyranosyl-(1 → 6)-β-D-glucopyranosyl)oxy]-22α-methoxy-5β-furostan-3β-yl *O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

trans-fusion was 55.2/0.74, which is different from the δ values of C/H-9 in *cis*-fusion (40.8/1.44).

Another important determinant of the ring fusion type is the 19-CH_3 group which is present at the A/B junction and is always β-oriented. The methyl protons appear at δ 0.62–0.82 in the *trans*- and δ 0.82–0.98 in the *cis*-fused A/B rings. A clearer indication is provided by the ^{13}C resonances of 19-CH_3 where *trans* stereochemistry is shown by chemical shift of δ 11.4–16.9 and *cis* is shown by higher-frequency signals present between δ 23.0 and 24.4. In the case of saponins with *trans*-fused A/B rings, the ^1H NMR chemicals shifts for 19-CH_3 groups showed adequate closeness (δ 0.62–0.82) in all cases, except in the case of those reported by Jin *et al.*[18] for compounds **18–21**. These authors have reported chemical shifts higher than δ 0.82 for 19-CH_3 groups of compounds **18** (δ 1.11), **19** (δ 1.11), **20** (δ 0.86) and **21** (δ 0.86), which are contrasting to the values of majority of the compounds. It is noteworthy that the chemical shifts assigned to 18-CH_3 group in compounds **18–21** ranged from δ 0.61 to 0.65 (shielded compared to 19-CH_3 in all four cases)[18]. However, the literature suggests that 19-CH_3 appears at a lower frequency than 18-CH_3 group. Therefore, it may be inferred that 18- and 19-CH_3 resonances are oppositely assigned in the compounds **18–21**, and should be reinvestigated.

Complete ^1H NMR data have not been reported in all cases and the chemical shifts for methyls and signals downfield than 3 ppm are given. However, the reported ^1H NMR chemical shifts provide sufficient evidence for deriving the correlation between A/B ring fusion type and NMR chemical shifts. Overall, the A/B ring junction stereochemistry considerably influenced the chemical shifts of C/H-3 to C/H-7, C/H-9 and C/H-19 whereas the chemical shifts of C/H-8 were independent of the type of ring fusion. Figure 2 shows the average chemical shifts of *trans*- and *cis*-fused A/B rings.

Thus, a correlation between A/B ring junction stereochemistry and NMR resonances of spirostanol/furostanol saponins has been established. This can be utilized for ascertaining the 5α/5β stereochemistry of saponins.

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Occurrence of the new invasive pest, fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in the maize fields of Karnataka, India

We report here the occurrence of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in India, which is a devastating pest in American continent on several crops[1]. *S. frugiperda* is a polyphagous pest that causes significant losses to agricultural crops. The caterpillars feed on leaves, stems and reproductive parts of more than 100 plant species[2] that include maize, rice, sorghum, sugarcane, cabbage, beet, peanut, soybean, alfalfa, onion, tomato, potato and cotton[2,3]. In Brazil, *S. frugiperda* causes up to 34% reduction in maize grain yield[4] that amounts to an annual loss of US$ 400 million[5]. The pest accounts for annual crop losses in excess of US$ 500 million