Pergamon

PII: S0031-9422(97)00481-0

Phytochemistry, Vol. 47, No. 2, pp. 255–257, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

DEPENDENCE OF THE ^{1}H NMR CHEMICAL SHIFTS OF RING F RESONANCES ON THE ORIENTATION OF THE 27-METHYL GROUP OF SPIROSTANE-TYPE STEROIDAL SAPOGENINS*

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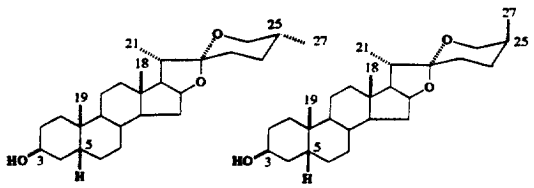
(*Received in revised form* 10 *April* 1997)

Key Word Index—Steroidal sapogenin; 2D NMR, 25*R*/25*S* stereochemical assignments.

Abstract—A relationship between the ^{1}H NMR chemical shifts of the ring F resonances and orientation of the H_3-27 group has been derived for the establishment of 25*R*- and 25*S*-stereochemistry in spirostane type of steroidal sapogenins. © 1997 Elsevier Science Ltd

INTRODUCTION

The steroidal sapogenins and their glycosides (steroidal saponins), widely distributed in various plant families [2], are attracting attention, as some have shown biological activities as inhibition to platelet aggregation [3], reduction of blood glucose level [4], local antitussive effect for dry cough [5], molluscicidal activities [6] and inhibition of the proliferation of various kinds of human malignant tumor cells *in vitro* [7]. The variation in spirostane structure generally arises from the configuration at C-5, i.e. A/B ring junction, and the configuration of the methyl group at C-25. Depending upon the equatorial or axial orientation of the 27-methyl group, the spirostanoids have been grouped in to 25*R* or 25*S* types, respectively. The question of the establishment of 25*R*/25*S* stereochemistry has been earlier addressed by the consideration of IR absorption bands [8, 9], 27-methyl group ^{1}H NMR chemical shift [10] and ^{13}C NMR chemical shifts [11, 12]. Although, the ^{13}C NMR approach has been widely employed for the stereochemical establishment of these compounds [13] the relative insensitivity of ^{13}C NMR as compared with ^{1}H NMR encourages the search for a relationship between the 25*R*/25*S*-stereochemistry and ^{1}H NMR data.



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RESULTS AND DISCUSSION

To derive a primary set of ^{1}H NMR data, a pair of epimeric 5β-spirostanes: smilagenin (25*R*-5β-spirostan-3β-ol (**1**)) and sarsasapogenin (25*S*-5β-spirostan-3β-ol (**2**)) were investigated by two-dimensional techniques [14]. ^{1}H-^{1}H COSY and TOCSY led to the partial assignment of the ^{1}H NMR resonances, which were correlated with the corresponding one-bond coupled ^{13}C signals using HMQC. A combined application of HMQC-TOCSY, HSQC-RELAY and HMBC experiments further led to prove ^{1}H NMR identification of the remaining ^{1}H assignments as well as of the quaternary carbons. The ^{13}C assignments of **2** were verified by INADEQUATE experiments. The ^{1}H and ^{13}C NMR assignments for **1** and **2** are listed in Table 1.

Since spectral data for both compounds were measured under the same experimental conditions, any alteration in the ^{1}H NMR shielding pattern must be due to the difference in H_3-27 stereochemistry. A comparison of the ^{1}H NMR data for **1** and **2** (Table 1) reveals that the ^{1}H NMR resonances exhibit very similar ^{1}H NMR chemical shifts for all of the rings except

* Part 46 in the series, 'NMR spectral Investigations.' For part 45 see ref. [1].

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Table 1. ^{13}C and ^{1}H NMR chemical shift assignments of smilagenin **1** and sarsasapogenin **2**

Carbon	1		2	
	^{13}C	^{1}H	^{13}C	^{1}H
1	29.95	1.40, 1.52	29.95	1.39, 1.50
2	27.82	1.40, 1.58	27.79	1.40, 1.50
3	67.12	4.11	67.12	4.11
4	33.54	1.33, 1.98	33.51	1.32, 1.97
5	36.58	1.72	36.51	1.72
6	26.56	1.16, 1.91	26.56	1.15, 1.90
7	26.54	1.07, 1.58	26.54	1.04, 1.39
8	35.28	1.58	35.27	1.58
9	39.85	1.33	39.85	1.32
10	35.28	—	35.28	—
11	20.90	1.25, 1.40	20.90	1.25, 1.39
12	40.30	1.16, 1.72	40.31	1.15, 1.72
13	40.70	—	40.70	—
14	56.48	1.16	56.47	1.15
15	31.80	1.25, 1.98	31.74	1.25, 1.97
16	80.93	4.40	81.02	4.40
17	62.27	1.77	62.09	1.75
18	16.49	0.76	16.50	0.76
19	23.92	0.98	23.92	0.97
20	41.61	1.86	42.12	1.81
21	14.51	0.97	14.34	0.99
22	109.26	—	109.74	—
23	341.39	1.58, 1.67	25.94	1.39, 1.87
24	28.80	1.46, 1.64	25.77	2.02, 1.39
25	30.31	1.64	27.08	1.69
26	66.86	3.48, 3.38	66.13	3.95, 3.28
27	17.14	0.79	16.05	1.08

ring F. The most noteworthy feature is that the equatorial protons of H_2-23, H_2-24 and H_2-26 resonate 0.2 to 0.5 ppm to lower field in **1** than in **2**, the axial protons resonate 0.07 to 0.19 ppm to higher field (Fig. 1). This implies that an axial orientation of the 27-Me group is reflected not only in its own appearance at 0.29 ppm to lower field, but also in the dispersion of the ^{1}H NMR chemical shifts of the methylene protons occupying β and γ-positions [Fig. 1(a)]. In general, the difference between the chemical shifts for geminal protons at positions C-24 and C-26 was three to four times higher in 25*S*-compounds than in 25*R*-epimers.

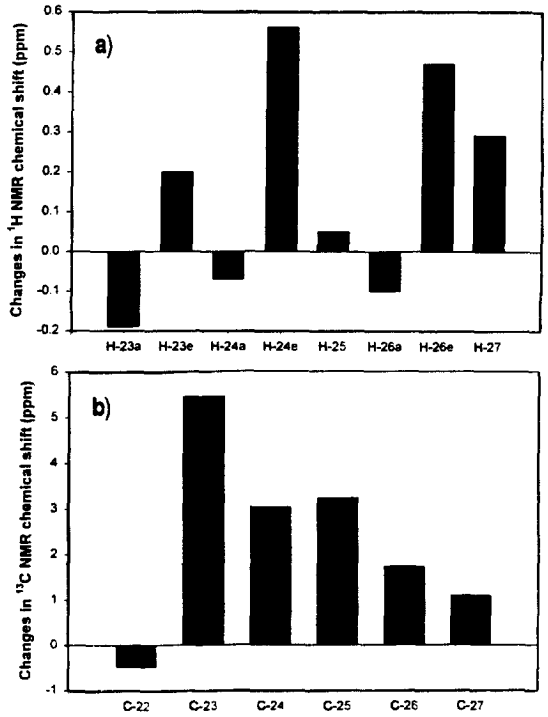

Fig. 1. Changes in the (a) ^{1}H and (b) ^{13}C NMR chemical shifts for ring-F resonances. The bars represents the difference in chemical shifts ($\Delta\delta = \delta_1$-δ_2).

Table 2. ^{1}H NMR chemical shift data for ring F resonances for some steroidal sapogenins and steroidal saponins[+]

	A^{+} [18]	**B**$^{+}$ [19]	**C**$^{+}$ [20]	**D**$^{+}$ [21]	**E**$^{+}$ [22]	**F**$^{+}$ [23]	**G**$^{+}$ [15]	**H**$^{+}$ [16]	**I**$^{+}$ [17]	**J**$^{+}$ [17]	**K**$^{+}$ [24]	**L**$^{+}$ [25]
H_2-23						1.60	1.58	1.58				1.96
						1.72	1.68	1.68				1.39
H_2-24						1.55	1.56	1.64				2.07
						1.55	1.42	1.43				1.46
H-25						1.56	1.62	1.61				1.72
H_2-26	3.58	3.59	3.56	3.59	3.52	3.57	3.35	3.38	4.07	4.05	4.04	3.97
	3.50	3.50	3.47	3.52	3.52	3.46	3.42	3.36	3.38	3.37	3.35	3.31
H_3-27	0.70	0.70	0.70	0.70	0.69	0.71	0.80	0.76	1.08	1.08	1.06	1.13

[+]**A** (25*R*)-5α-spirostane-3β-ol (Tigogenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside}; **B** (25*R*)-5α-spirostane-2α,3β-diol (gitogenin); **C** (25*R*)5α-spirostane-2α,3β-diol (gitogenin)-3-*O*-β-D-galactopyranoside; **D** (25*R*)-5α-spirostane-1β,3β-diol(Brisbagenin); **E** (25*R*)-5α-spirostane-3β, 17-diol (Penogenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-galactopyranosyl-(1 → 3)-β-D-glucopyranoside}; **F** (25*R*)-2α,3β-dihydroxy-5α-spirost-9(11)-en-12-one-3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[*O*-α-L-rhamnopyranosyl-(1 → 4)β-D-glucopyranosyl-(1 → 3)-]*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-galactopyranoside}; **G** (25*R*)-3β-acetoxy-5α-spirost-12-one (Hecogenin acetate); **H** (25*R*) spiros-5-ene-3β-ol (Diosgenin); **I** (25*S*)-5α-spirostane-3β-ol (Neotigogenin); **J** (25*S*)-spirost-5-ene-2α,3β-diol (Neogitogenin); **K** (25*S*) spirost-5-ene-1β,3β-diol(25*S*)-ruscogenin-1-*O*-{*O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-glucopyranoside; **L** (25*S*)spirost-5-ene-1β,3β-diol (25*S*)-ruscogenin-3-*O*-{*O*-β-D-fucopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranoside.

A literature search [13, 15–25] showed that the chemical shifts for H_2-24 have not been frequently reported but the reported values for $\Delta\delta_{H26e-H26a}$ were consistent with the above observations (Table 2). A similar comparison of the ^{13}C NMR chemical shifts for **1** and **2** [Fig. 1(b)] showed that all the carbon resonances except C-22 occur at lower field in **1** than in **2**.

It is worthwhile to mention that substitution in rings A–D does not usually affect the chemical shifts of ring F. However, substitution in ring F will modify the ^{1}H NMR shielding pattern, so the ^{1}H NMR shielding behaviour described will be of general applicability for ring-F unsubstituted 22α-spirostanoids.

EXPERIMENTAL

The 1D and 2D NMR experiments were carried out in $CDCl_3$ in 5 mm tube at ambient temp. (20°) with an indirect detection probe on a Varian Unity 500 MHz NMR spectrometer. The chemical shifts are expressed on the δ scale.

Acknowledgements—PKA is thankful to Prof. Sushil Kumar, Director (CIMAP) and Dr R. P. Sharma, (Head, Phytochemical Technology Division) for constant encouragement.

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