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CARBON-13 NMR SPECTRA OF 5β-STEROIDAL SAPOGENINS. REASSIGNMENT OF THE F-RING CARBON SIGNALS OF (25S)-SPIROSTANS

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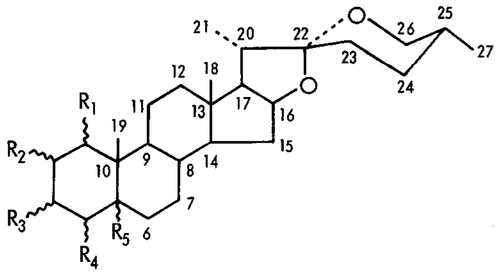
Abstract. All ^{13}C NMR signals of 5β-steroidal sapogenins were assigned; in particular, the F-ring carbon signals were reassigned using deuteriated diotigenin (12) derivatives and the INEPT (insensitive nuclei enhanced by polarization transfer) spectra.

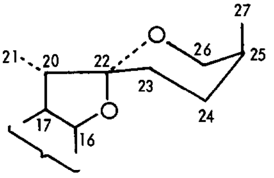
For a successful study of the biosynthesis of natural products using ^{13}C NMR spectroscopy, it is essential to assign unambiguously ^{13}C signals of related compounds. In the course of our study, we have encountered the assignment problem for some 5β-spirostans. Among ^{13}C NMR data for more than 400 steroids,[1] those for about 20 spirostans have been reported thus far.[2-4] However, the F-ring carbon signals of (25S)-spirostans have remained ambiguous (see the TABLE).[2,3] We report here the reassignment of these ^{13}C signals together with the full assignments of those of about ten 5β-spirostans having hydroxy groups on the A-ring isolated from natural sources in this laboratory,[5,6] and the usefulness of the newly developed INEPT (insensitive nuclei enhanced by polarization transfer) spectral technique[7] for discriminating carbon types, particularly CH from CH_2 and CH_3 in the case where their signals are heavily overlapped in ordinary ^{1}H single-frequency off-resonance decoupled (SFORD) ^{13}C spectra.

^{13}C NMR spectra of (25R)-5β- and/or the corresponding (25S)-5β-spirostan derivatives (1-14) were determined in $CDCl_3$ and/or $[^{2}H_5]$pyridine (C_5D_5N) along with two pairs of (25R)-5α- and (25R)-Δ^5-spirostan derivatives and their 25-epimers (15-18) for comparison. The ^{13}C signals were first assigned using known chemical shift rules, literature values,[1-4] SFORD techniques, and chemical shift comparisons from compound to compound.

Assignments of ^{13}C signals of di-, tri-, and tetrahydroxylated A-rings are not easy because hydroxy-substitution shift rules for the steroid skeleton[8] are no longer operative for 1,2-dihydroxylation[2b] and probably for 1,3-di- and 1,2,3-trihydroxylations. However, the ^{1}H signal assignments for protons attached to the OH-bearing carbons of 5-14 were easy in C_5D_5N at 100 MHz. Thus, several ^{1}H selective-decoupling experiments on the 25-MHz ^{13}C spectra were used to determine the carbon signal assignments unambiguously (the TABLE). The signal multiplicities were confirmed by the INEPT techniques in 50-MHz ^{13}C spectra[7c] in the cases where signals were crowded closely together, e.g., the isorhodeasapogenin (5) and its diacetate (5a) cases.

As can be seen from the TABLE, the assignments of the F-ring carbons of the (25R)-series were straightforward according to Eggert and Djerassi.[2a] We also confirmed the assignments as follows. When diotigenin triacetate (12a) was treated with DCl-EtOD according to Marker's "Iso-reaction" condition,[9,10] [20,23,23',25-$^{2}H_4$]isodiotigenin (19) was obtained,[14] m/e 452(M^+);[15] its triacetate 19a showed four singlet methyl ^{1}H NMR signals in $CDCl_3$ at δ_H 0.74





(25R)-Series	R1	R2	R3	R4	R5	(25S)-Series
(25R)-5β-Spirostan (1)	H	H	H	H	β-H	
Epismilagenin (2)	H	H	α-OH	H	β-H	
Smilagenin (3)	H	H	β-OH	H	β-H	Sarsasapogenin (4)
Isorhodeasapogenin (5)	β-OH	H	β-OH	H	β-H	Rhodeasapogenin (6)
Yonogenin (7)	H	β-OH	α-OH	H	β-H	Neoyonogenin (8)
Tokorogenin (9)	β-OH	β-OH	α-OH	H	β-H	Neotokorogenin (10)
Isodiotigenin (11)	H	β-OH	α-OH	β-OH	β-H	Diotigenin (12)
Kogagenin (13)	β-OH	β-OH	α-OH	H	β-OH	
Kitigenin (14)	β-OH	H	β-OH	β-OH	β-OH	
Tigogenin (15)	H	H	β-OH	H	α-H	Neotigogenin (16)
Diosgenin (17)	H	H	β-OH	H	Δ^5	Yamogenin (18)

(H-18), 0.78 (H-27), 0.95 (H-21), and 1.05 (H-19).[6,12a] By comparing the ^{13}C spectrum of 19a with that of the non-labelled compound 11a in $CDCl_3$, we found that the signals at δ_C 30.3 (C-25), 31.4(C-23), and 41.6 (C-20) in 11a disappeared in 19a, and that the signals at δ_C 14.5 (C-21), 17.1 (C-27), 28.8 (C-24), and 62.2 (C-17) in 11a were shifted upfield by -0.2, -0.1, -0.4, and -0.1 ppm, respectively, in 19a by the deuterium isotope effect.[16]

On the other hand, in the spectra of the (25S)-series, the signals in $CDCl_3$ (C_5D_5N) at δ_C 27.1 (27.5) assigned to C-23 by Eggert and Djerassi[2a] and to C-24 by Marquardt[3] were found to be doublets by SFORD or INEPT experiments. Thus, this signal should be due to C-25. The two remaining triplet signals due to C-23 and C-24 at δ_C 26.0 (26.4) and 25.8 (26.2) could be distinguished from each other as follows. When diotigenin (12) was treated by acid-catalyzed exchange reaction under reflux for 72 hours in a mixture of conc. DCl and CD_3OD (3:6.7), [20,23,23'-$^2H_3$]diotigenin (20) was obtained, m/e 451(M^+);[17] δ_H(C_5D_5N) 0.83s (H-18), 1.03s (H-19), 1.14s (H-21), and 1.07d (H-27).[6,18] In the ^{13}C spectrum in C_5D_5N, the signals at δ_C 42.5 (C-20) and 26.4 (C-23 or C-24) in 12 disappeared, and the signals at δ_C 14.9 (C-21), 26.2 (C-24 or C-23), and 62.8 (C-17) in 12 were shifted upfield (-0.2, -0.3, and -0.1 ppm, respectively) by the deuterium isotope effect.[16] If δ_{C-23} were 26.2, the isotope shift of -0.5 ppm for the C-24 signal would be too large because C-25 did not deuteriated. Similar phenomena of the spectral change from 12a to 20a were also observed. Thus, the two signals at δ_C 26.4 and 26.2 in C_5D_5N (26.0 and 25.8 in $CDCl_3$) were assigned to C-23 and C-24, respectively.

All the ^{13}C signals of the (25R)- and (25S)-spirostans were thus assigned and the data are listed in the TABLE. These data should be useful for biosynthetic studies of steroidal sapogenins, some of which are in progress in these laboratories. During this study, the INEPT spectral technique[7] has proved to be a powerful tool for assigning ^{13}C signal multiplicities.

TABLE. ^{13}C Chemical Shifts, δ_C, in $CDCl_3$ and/or C_5D_5N (in parentheses)[a]

Carbon No.	1	2			3, 4			5, 6	7, 8		9, 10
				b		c					
C-1	37.6	35.5	(36.0)	[37.6]	29.9	[29.9]	(30.6)	(73.4)	43.8	(44.7)	(76.6)
2	21.3	30.5	(31.4)	[31.6]	27.8	[27.8]	(28.6)	(32.9)	71.0	(71.3)	(74.2)
3	27.0[e]	71.8	(71.1)	[71.4]	66.9	[67.0]	(66.0)	(68.2)	76.2	(77.0)	(71.2)
4	27.2[e]	36.5	(37.2)	[36.1]	33.5	[33.6]	(34.4)	(34.4)	35.4	(35.5)	(35.3)
5	43.7	42.1	(42.4)	[42.9]	36.5	[36.6]	(37.0)	(31.2)	41.7	(42.3)	(35.9)
6	27.4	27.1	(27.5)	[27.8]	26.6	[26.5]	(27.2)	(26.7)	26.4[e]	(26.9)	(26.5)
7	26.8	26.7	(27.0)	[27.2]	26.6	[26.5]	(26.9)	(26.7)	26.6[e]	(26.9)	(26.5)
8	35.5	35.5	(35.7)	[36.3]	35.3	[35.3]	(35.6)	(35.9)	35.4	(35.7)	(35.6)
9	40.6	40.6	(40.8)	[41.3]	40.3	[40.3]	(40.4)	(42.2)	42.2	(42.3)	(42.0)
10	35.5	34.7	(34.9)	[35.3]	35.3	[35.3]	(35.6)	(40.4)	36.7	(37.2)	(41.2)
11	20.6	20.6	(20.9)	[21.2]	20.9	[20.9]	(21.2)	(21.1)	20.9	(21.1)	(21.2)
12	40.3	40.3	(40.2)	[40.6]	39.9	[39.9]	(40.2)	(40.4)	40.1	(40.2)	(40.1)
13	40.6	40.6	(40.8)	[41.2]	40.7	[40.7]	(41.0)	(40.7)	40.6	(40.8)	(40.6)
14	56.5	56.4	(56.4)	[56.8]	56.5	[56.5]	(56.6)	(56.4)	56.2	(56.3)	(56.3)
15	31.7	31.8	(32.1)	[32.4]	31.7	[31.8]	(32.2)	(32.2)	31.8	(32.1)	(32.1)
16	81.0	80.9	(81.2)	[81.4]	80.9	[80.9]	(81.2)	(81.2)	80.8	(81.2)	(81.1)
17[f]	62.3	62.3	(63.1)	[63.7]	62.3	[62.4]	(63.2)	(63.2)	62.2	(63.1)	(63.1)
18	16.4	16.5	(16.6)	[16.6]	16.4	[16.4]	(16.6)	(16.6)	16.5	(16.6)	(16.6)
19	24.2	23.4	(23.7)	[23.7]	23.9	[23.8]	(24.2)	(19.4)	23.5	(23.6)	(19.1)

Carbon No.	11, 12	13	14	15, 16			17, 18				
					c, g			c, h		b, h	b, i
C-1	(44.2)	(79.2)	(73.6)	37.0	[37.0]	(37.5)	37.3	[37.2]	(37.8)	[37.8]	[37.8]
2	(68.7)	(73.8)	(33.3)	31.4	[31.5]	(32.5)	31.4	[31.6]	(32.5)	[32.5]	[32.5]
3	(83.0)	(69.0)	(71.1)	71.2	[71.2]	(70.6)	71.6	[71.5]	(71.2)	[71.2]	[71.2]
4	(70.8)	(42.5)	(68.0)	38.2	[38.2]	(39.3)	42.3	[42.2]	(43.4)	[43.4]	[43.4]
5	(49.2)	(76.8)	(78.3)	44.9	[44.9]	(45.2)	140.9	[140.8]	(142.0)	[142.0]	[142.0]
6	(21.5)	(35.6)	(30.4)	28.6	[28.6]	(29.1)	121.3	[121.3]	(121.0)	[121.0]	[121.0]
7	(26.6)	(28.8)	(28.5)	32.2	[32.3]	(32.5)	32.0	[32.0]	(32.3)	[31.8][d]	[31.8][d]
8	(35.6)	(34.9)	(35.0)	35.1	[35.2]	(35.4)	31.4	[32.4]	(31.8)	[32.3][d]	[32.3][d]
9	(44.2)	(45.3)	(45.4)	54.4	[54.4]	(54.6)	50.1	[50.1]	(50.4)	[50.5]	[50.5]
10	(36.6)	(44.7)	(45.7)	35.6	[35.6]	(35.9)	36.6	[36.6]	(37.0)	[37.0]	[37.0]
11	(21.2)	(21.6)	(21.4)	21.1	[21.1]	(21.4)	20.9	[20.9]	(21.2)	[21.2]	[21.2]
12	(40.1)	(39.9)	(40.1)	40.1	[40.1]	(40.3)	39.8	[39.8]	(40.0)	[40.0]	[40.0]
13	(40.8)	(40.6)	(40.7)	40.6	[40.6]	(40.8)	40.3	[40.2]	(40.5)	[40.5]	[40.5]
14	(56.3)	(56.1)	(56.3)	56.3	[56.3]	(56.6)	56.5	[56.5]	(56.8)	[56.8]	[56.8]
15	(32.0)	(32.2)	(32.2)	31.8	[31.8]	(32.1)	31.8	[31.8]	(32.3)	[32.3]	[29.3][d]
16	(81.2)	(81.1)	(81.1)	80.8	[80.7]	(81.1)	80.8	[80.7]	(81.1)	[81.1]	[81.1]
17[f]	(63.0)	(63.0)	(63.0)	62.3	[62.2]	(63.1)	62.1	[62.1]	(62.9)	[63.0]	[62.8]
18	(16.6)	(16.5)	(16.6)	16.5	[16.5]	(16.7)	16.3	[16.3]	(16.4)	[16.4]	[15.0][d]
19	(23.8)	(13.6)	(13.8)	12.3	[12.4]	(12.5)	19.4	[19.4]	(19.6)	[19.6]	[19.6]

Carbon No.	(25R)-series[k]					(25S)-series[k]			
				b, j	b, h		c		b, i
C-20	41.6	[41.6]	(42.0)	[42.3]	[42.0]	42.2	[42.1]	(42.5)	[42.5]
21	14.5	[14.4]	(15.0)	[14.9]	[15.0]	14.3	[14.3]	(14.9)	[16.4][d]
22	109.2	[109.1]	(109.2)	[109.3]	[109.2]	109.7	[109.5]	(109.7)	[109.7]
23	31.4	[31.4]	(31.9)	[32.2][d]	[32.6][d]	26.0	[27.1][d]	(26.4)	[26.2][d]
24	28.8	[28.8]	(29.3)	[29.5]	[29.3]	25.8	[25.8[e]][d]	(26.2)	[27.6][d]
25	30.3	[30.3]	(30.6)	[30.8]	[30.6]	27.1	[26.0[e]][d]	(27.5)	[26.4][d]
26	66.8	[66.8]	(66.9)	[67.2]	[66.9]	65.2	[65.0]	(65.1)	[65.1]
27	17.1	[17.1]	(17.3)	[17.2]	[17.3]	16.1	[16.1]	(16.3)	[14.9][d]

TABLE (cont'd)

[a] ^{13}C NMR spectra were recorded on a Varian NV-14 FT NMR (15.087 MHz), an XL-100-12A NMR (25.160 MHz), and/or an XL-200 SCM-FT NMR (50.309 MHz)[7c] spectrometer using 8- or 5-mm spinning tubes at ordinary probe temperature. As an internal standard TMS was used. Accuracies of δ_C were about ±0.1 ppm as acquisition times used in the FT measurements were more than 0.6 s. Concentrations were about 0.1 mmol/mL or less when the sample solubility was limited. ^{1}H NMR spectra were taken with a Varian HA-100 and/or an EM-360L NMR spectrometer. Accuracies of δ_H were about ±0.02 ppm or less. Data on the acetates (2a-18a) will be reported in a full paper.

[b] Data measured in C_5D_5N reported by Marquardt.[3]

[c] Data measured in $CDCl_3$ reported by Eggert and Djerassi.[2a]

[d] Assignments that need to be revised on the basis of the present study.

[e] Assignments may be interchanged in each vertical column.

[f] For the (25S)-series, 0.2 ppm should be subtracted from these figures.

[g-j] Data for 15, 17, 18, and 2, respectively.

[k] These data could be applied only to spirostans having substituent(s) on the A-ring.

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REFERENCES

(1) J.W. Blunt and J.B. Stothers, *Org. Magn. Resonance* **9**, 439 (1977).

(2) (a) H. Eggert and C. Djerassi, *Tetrahedron Lett.* 3635 (1975); (b) C.L. VanAntwerp, H. Eggert, G.D. Meakins, J.O. Miners, and C. Djerassi, *J. Org. Chem.* **42**, 789 (1977).

(3) F.-H. Marquardt, *Chem. Ind. (London)* 94 (1978).

(4) R. Radeglia, G. Adam, and H. Ripperger, *Tetrahedron Lett.* 903 (1977); R. Tschesche and W. Führer, *Chem. Ber.* **111**, 3300 (1978); G.J. Bird, D.J. Collins, F.W. Eastwood, and R.H. Exner, *Austral. J. Chem.* **32**, 797 (1979).

(5) For a review, see K. Takeda, *Progr. Phytochem.* **3**, 287 (1972).

(6) For a compilation of ^{1}H NMR data on spirostans, see K. Tori and K. Aono, *Ann. Rept. Shionogi Res. Lab.* **14**, 136 (1964).

(7) (a) D.P. Burum and R.R. Ernst, *J. Magn. Resonance* **39**, 163 (1980), and references cited therein; (b) D.M. Doddrell and D.T. Pegg, *J. Am. Chem. Soc.* **102**, 6388 (1980); (c) For experimental procedures, see G.A. Gray, *Varian Instruments at Work* No. NMR-14 (1980).

(8) H. Eggert, C.L. VanAntwerp, N.S. Bhacca, and C. Djerassi, *J. Org. Chem.* **41**, 71 (1976).

(9) (a) R.E. Marker and E. Rohrmann, *J. Am. Chem. Soc.* **61**, 846 (1939); (b) R.E. Marker, R.B. Wagner, P.R. Ulshafer, E.L. Wittbecker, D.P.J. Goldsmith, and C.H. Ruof, *Ibid.* **69**, 2167 (1947); (c) L.F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp. (1959).

(10) The isomerization reaction[9a] of (25S)- to (25R)-spirostans has been useful for assigning the stereochemistry at C-25 of steroidal sapogenines.[9c] A reaction mechanism has also been suggested.[11] However, some doubts have been aroused[12] about this reaction by the use of Callow's procedure.[13,14]

(11) R. B. Woodward, F. Sondheimer, and Y. Mazur, *J. Am. Chem. Soc.* **80**, 6693 (1958).

(12) (a) W. H. Faul, A. Failli, and C. Djerassi, *J. Org. Chem.* **35**, 2571 (1970); (b) W. H. Faul and C. Djerassi, *Org. Mass Spectr.* **3**, 1187 (1970).

(13) R. K. Callow, V.H.T. James, O. Kennard, J.E. Page, P.N. Paton, and L.R. di Sanseverino, *J. Chem. Soc. (C)* 288 (1966); R.K. Callow and P.N. Massy-Beresford, *J. Chem. Soc.* 2645 (1958).

(14) "Iso-reaction" converts 12a into the (25R)-epimer with a good yield; see K. Takeda, T. Okanishi, A. Akahori, and F. Yasuda, *Chem. Pharm. Bull.* **16**, 421 (1968). We also obtained 19a from 11a by the same procedure with the treatment of 12a with a good yield. Thus, the substitution of a deuterium atom at C-25 by "Iso-reaction" was confirmed.[15]

(15) The deuterium atom contents estimated for 19a (%): 1[$^2H_0$], 1[$^2H_1$], 1[$^2H_2$], 9[$^2H_3$], 88[$^2H_4$].

(16) For example, see A.P. Tulloch, *Org. Magn. Resonance* **11**, 109 (1978).

(17) The deuterium atom contents (%): 2[$^2H_0$], 3[$^2H_1$], 17[$^2H_2$], 55[$^2H_3$], 22[$^2H_4$], 1[$^2H_5$].

(18) The 200-MHz ^{1}H spectrum showed that epimer 19 was contained by about 10% in this specimen. Thus, "Iso-reaction" was confirmed to occur only slightly by acid hydrolysis in MeOH. See A. Akahori, F. Yasuda, K. Kagawa, and T. Iwao, *Chem. Pharm. Bull.* **21**, 1799 (1973).

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