

Spectral Assignments and Reference Data

Reassignment of the ^1H NMR spectrum of fusidic acid and total assignment of ^1H and ^{13}C NMR spectra of some selected fusidane derivatives

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Reassignment of the ^1H and ^{13}C NMR spectra of fusidic acid and the total assignment of lumifusidic acid and some important di- and tetra-hydrofusidic acid derivatives are reported. Copyright © 2002 John Wiley & Sons, Ltd.

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INTRODUCTION

Fusidic acid (**1**)^{1,2} is an important antibiotic used in the treatment of staphylococcal infections in man. Fusidanes differ from common steroids in the androstane and cholestane families by their unique tetracyclic ring system with a chair–boat–chair conformation and their additional methyl groups.

Although the chemical, biological and medical properties of fusidanes are well described in the literature,³ very few assigned NMR data have been published. Only ^{13}C and crude ^1H NMR data of **1** obtained by selective ^{13}C – ^1H spin decoupling⁴ have previously been published.

In this paper we give the revised total and unambiguous ^1H and ^{13}C assignments fusidic acid in addition to assignments for related saturated analogues of biological importance.⁴

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Table 1. ^1H chemical shifts (ppm) of selected fusidanes^a

H at C number	1	2	3	4	5	6	7	8	9	10	11	12
1	1.51/2.17	1.44/2.30	1.51/2.17	1.39/2.22	1.46/2.16	1.50/2.15	1.40/2.16	1.51/2.23	1.51/2.16	1.50/2.13	1.48/2.19	1.50/2.15
2	1.75/1.86	1.82	1.65/1.85	1.78	1.74/1.80	1.74/1.85	1.77	1.75/1.84	1.74/1.86	1.74/1.87	1.72/1.83	1.74/1.86
3	3.76	3.80	3.76	3.77	3.71	3.75	3.75	3.74	3.75	3.76	3.73	3.74
4	1.58	1.51	1.58	1.49	1.51	1.58	1.51	1.56	1.60	1.61	1.56	1.59
5	2.11	2.16	2.11	2.12	2.06	2.07	2.10	2.11	2.07	2.07	2.09	2.07
6	1.13/1.59	1.13/1.68	1.10/1.61	1.13/1.68	1.10/1.62	1.07/1.57	1.11/1.66	1.13/1.67	1.07/1.58	1.06/1.55	1.10/1.62	1.08/1.58
7	1.12/1.74	1.12/1.61	1.14/1.74	1.06/1.65	1.07/1.71	1.07/1.76	1.08/1.66	1.16/1.65	1.11/1.82	1.13/1.83	1.12/1.74	1.11/1.82
8	—	—	—	—	—	—	—	—	—	—	—	—
9	1.57	1.64	1.57	1.53	1.52	1.54	1.62	1.51	1.49	1.48	1.45	1.49
10	—	—	—	—	—	—	—	—	—	—	—	—
11	4.35	4.28	4.36	4.24	4.30	4.34	4.24	4.38	4.37	4.30	4.28	4.37
12	1.85/2.33	1.54/2.07	1.85/2.29	1.42/1.65	1.41/1.74	1.66/1.92	1.60/1.69	1.83/2.08	1.56/1.66	1.80/1.95	1.51/1.74	1.57/1.65
13	3.06	2.95	3.05	2.01	2.12	2.60	2.51	3.53	2.65	2.66	2.26	2.65
14	—	—	—	—	—	—	—	—	—	—	—	—
15	1.30/2.19	1.36/2.05	1.30/2.18	1.15/2.08	1.18/2.04	1.34/1.96	1.41/1.90	1.22/2.28	1.73/1.88	1.79/1.90	1.78/1.85	1.72/1.87
16	5.88	5.64	5.87	4.91	5.33	5.32	5.39	4.94	5.00	4.99	4.86	5.01
17	—	—	—	2.11	2.13	2.71	2.68*	—	2.70	3.15	2.62	2.68
18	0.91	0.95	0.91	0.95	0.94	1.01	1.01	0.81	0.89	0.90	0.84	0.88
19	0.98	0.98	0.98	0.97	0.96	0.97	0.97	0.97	0.97	0.96	0.95	0.96
20	—	—	—	2.25	2.57	2.71	2.68*	—	2.52	2.79	2.70	2.49
21	—	—	—	—	—	—	—	—	—	—	—	—
22	2.46	2.17	2.39	1.41/1.59	1.37/1.72	1.52/1.85	1.49	2.21	1.47/1.82	1.71/1.86	1.55/1.89	1.41/1.76
23	2.07/2.17	2.10/2.15	1.33/1.48	1.21/1.33	1.32	1.99	1.93/2.01	2.21/2.34	2.00/2.09	2.21/2.29	1.43	1.35
24	5.10	5.05	1.18	1.14	1.17	5.06	5.05	5.11	5.09	5.09	1.21	1.19
25	—	—	1.53	1.49	1.51	—	—	—	—	—	1.55	1.54
26	1.60	1.58	0.86*	0.84*	0.86	1.58	1.57	1.60	1.61	1.63	0.88*	0.87*
27	1.67	1.66	0.86*	0.84*	0.86	1.68	1.66	1.68	1.69	1.70	0.88*	0.87*
28	0.92	0.91	0.92	0.92	0.91	0.91	0.91	0.93	0.92	0.92	0.92	0.92
30	1.38	1.34	1.38	1.35	1.30	1.33	1.31	1.51	1.36	1.34	1.38	1.36
acetyl	1.96	2.03	1.96	2.02	2.04	1.95	2.01	—	—	—	—	—

^a Asterisk indicates interchangeable signals.

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EXPERIMENTAL

Materials

All compounds used were prepared and purified as described previously.^{1,2,5} The purity of all compounds characterized was more than 95%. The Empirical formulae of all the structures described were confirmed by mass spectrometry. Elementary analysis, UV, IR and X-ray^{6,7} single crystal crystallography were used whenever appropriate.

Spectra

All NMR experiments were performed at 300 K in CDCl₃ (Merck UVASOLE) on either a Bruker ARX300 spectrometer equipped with a 5 mm QNP probe or on a Bruker DRX500 equipped with a 5 mm z gradient inverse broad-band probe. The sample concentration used varied between 0.03 and 0.1 mmol ml⁻¹. Tetramethylsilane ($\delta = 0.00$ ppm) was used as internal reference. The 1D experiments used flip angles of 30° and 45° for ¹H and ¹³C respectively, and pulse delays of 5 s and 2 s were used for ¹H and ¹³C respectively.

Standard pulse sequences were taken from the Bruker pulse program library and special parameters were adjusted individually for each molecule investigated.

1D ¹H, proton decoupled (CPD) ¹³C and DEPT-135 spectra were used to obtain the ¹H and ¹³C chemical shifts. The minimum

frequencies per point used during acquisition for 1D spectra were 0.15 Hz (¹H) and 0.33 Hz (¹³C). Zero filling up to four times was used whenever necessary to obtain sufficient digital resolution. Exponential multiplication (0.2 Hz (¹H) and 1–2 Hz (¹³C)) was applied before Fourier transformation.

Proton–proton connectivities were established through COSY and HH-TOCSY experiments (min. eight scans per, *t*₁, (256–512) × 2048 data matrix size).

The corresponding carbon atoms were assigned through HMQC (min. eight scans per *t*₁, 256 × 2048 data matrix size) and CH-TOCSY (32 scans per *t*₁, 512 × 2048 data matrix size) experiments, whereas connectivities to quaternary carbon atoms were obtained from a Z-gradient version of the HMBC (two to eight scans per *t*₁, 256 × 2048 data matrix size) experiment.

The stereochemistry was finally confirmed through a phase-sensitive 2D NOESY experiment and the adiabatic version of T-ROESY using a shaped off-resonance spinlock pulse for mixing.⁸

RESULTS AND DISCUSSION

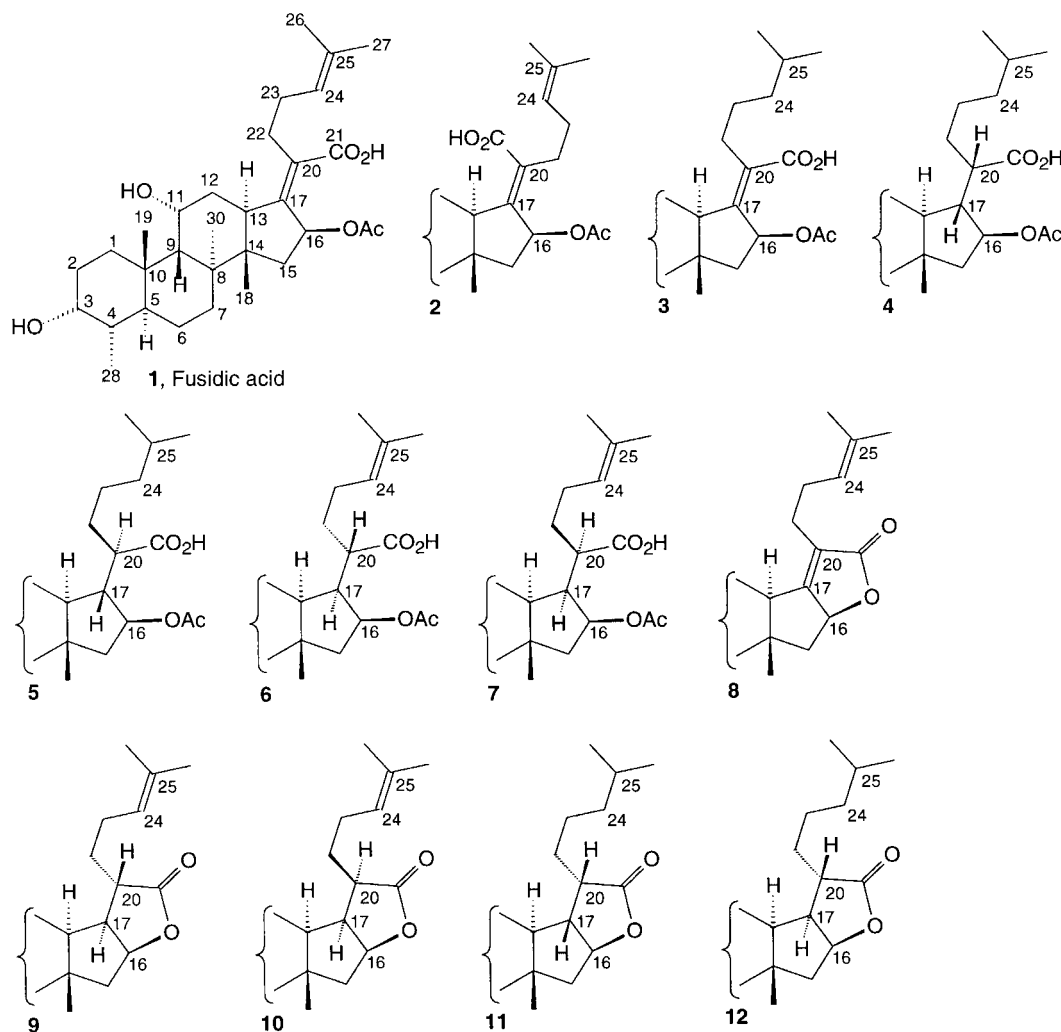
The total assignments of the ¹H and ¹³C NMR spectra of the compounds investigated are given in Tables 1 and 2 respectively.

Table 2. ¹³C chemical shifts (ppm) of selected fusidane^a

C number	1	2	3	4	5	6	7	8	9	10	11	12
1	30.17	29.23*	30.17	29.31	29.62	30.38	29.57	30.09	30.43	30.47	30.09	30.35
2	29.84	29.30*	29.83	29.31	29.71	29.98	29.72	29.86	29.97	30.02	29.95	29.96
3	71.53	72.47	71.53	72.26	71.71	71.47	71.97	71.32	71.35	71.36	71.31	71.32
4	36.38	37.36	36.39	37.60	37.04	36.23	37.00	36.77	36.08	35.75	36.59	36.18
5	36.01	34.86	36.00	34.72	35.60	36.40	35.17	35.84	36.63	36.67	36.06	36.47
6	20.87	21.74	20.88	21.90	21.40	21.00	21.60	21.07	21.02	20.76	21.11	21.07
7	32.14	29.99	32.13	30.16	31.69	32.56	30.61	31.51	33.04	33.42	32.31	32.85
8	39.48	40.06	39.48	40.25	40.39	40.63	40.81	40.83	41.39	41.02	40.12	41.37
9	49.32	49.67	49.33	49.99	50.29	49.40	49.32	50.48	49.63	49.20	50.00	49.65
10	36.95	36.30	36.95	36.24	36.78	37.19	36.45	37.07	37.35	37.34	37.02	37.26
11	68.24	68.47	68.28	68.52	68.55	68.85	68.83	67.86	68.72	68.63	68.42	68.66
12	35.58	33.53	35.47	35.01	34.22	34.38	32.91	31.70	32.89	34.55	35.55	32.98
13	44.29	42.37	44.11	40.59	38.26	38.28	39.29	38.17	38.75	41.39	36.59	38.72
14	48.72	49.67	48.69	49.36	48.63	49.40	49.18	55.20	48.70	48.16	51.32	48.68
15	38.96	39.41	38.96	39.77	40.05	40.63	41.08	34.05	39.98	40.27	39.98	39.95
16	74.47	74.95	74.45	79.65	76.52	76.37	75.96	81.88	84.25	83.50	82.93	84.28
17	150.75	141.84	149.79	48.36	48.23	44.39	45.63*	170.02	46.83	42.90	44.24	46.84
18	17.78*	17.10	17.80	16.10	16.30	17.26	17.29	19.94	17.53	19.60	17.46	17.46
19	22.99	24.32	23.01	24.52	23.70	22.74	23.98	23.42	22.61	22.21	23.21	22.75
20	129.64	132.10	130.22	49.92	45.15	45.23	44.33*	123.47	42.39	44.05	44.09	42.80
21	174.37	173.42	174.50	178.86	178.53	181.44	180.00	176.62	180.99	179.96	178.55	181.16
22	28.77	32.14	28.85	31.58	30.12	32.90	33.97	27.46	33.84	27.68	26.00	33.89
23	28.46	26.89	27.78	25.12	25.82	25.39	25.68	24.04	25.69	27.08	26.29	25.06
24	123.10	123.28	38.74	38.81	38.98	123.32	123.47	123.32	122.91	123.47	38.78	38.63
25	132.58	132.36	27.81	27.94	27.92	132.37	132.08	132.75	132.86	133.16	27.89	27.79
26	17.84*	17.79	22.62*	22.74*	22.69	17.71	17.65	17.79	17.84	17.92	22.69*	22.65*
27	25.71	25.66	22.58*	22.51*	22.69	25.67	25.69	25.68	25.69	25.77	22.51*	22.56*
28	15.92	15.72	15.92	15.79	16.04	16.00	15.82	15.99	15.99	15.98	15.96	16.00
30	23.94	21.74	23.93	21.68	22.57	23.79	22.29	23.02	23.79	24.83	23.26	23.65
CO	170.70	170.51	170.75	170.79	170.81	170.09	170.30					
COCH ₃	20.60	21.29	20.60	21.37	21.40	20.65	21.36					

^a Asterisk indicates interchangeable signals.

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The structural differences between the compounds investigated are generally observed in the ^1H and ^{13}C chemical shifts from the nuclei in rings C and D.

Changing the orientation of the side chain as in going from 1 to 2 results in a 9 ppm upfield shift of C-17, whereas C-20 and C-22 show smaller downfield shifts. C-12 and the two H-12 protons are also affected by the side-chain orientation, but not to the same degree as the protons at C-16 and C-22, which show considerable downfield shifts.

Differences in the ring strain between rings C and D can be observed by comparing 1 and 8: C-12, C-13, C-15 and C-20 show considerable upfield shifts, whereas C-14, C-16 and especially C-17 show large downfield shifts. Also, C-18 is affected by the ring strain. In the ^1H spectra, only the protons at C-13, C-16 and C-22 are affected considerably.

Changing the stereochemistry at C-20 from *S* to *R* on the 17*S* isomers (6 and 7) has very little effect, whereas a similar change on the 17*S* isomers (4 and 5) shows small upfield shifts on C-13, C-16 and C-20, as well as a considerable downfield shift on the protons at C-16 and C-20. Changing the stereochemistry at C-17 from *S* to *R* (4 to 6) results in upfield shifts on C-13, C-16, C-17, C-19, C-20 and C-21, whereas C-18 shows a downfield shift.

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