The ^{1}H- and ^{13}C-Nuclear Magnetic Resonance Spectra of Harman. Reinvestigation of the Assignments by One- and Two-Dimensional Methods

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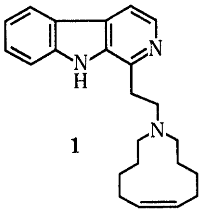
The ^{1}H- and ^{13}C-NMR spectra of harman (2) and *N*-methylharman (3) have been reinvestigated, and definitive assignments of all the protons and carbons of 2 in $CDCl_3$, DMSO-d_6, and CD_3OD were obtained by the use of DEPT, HC-COSY (or HMQC), COLOC (or HMBC), and CNOEDIF techniques.

Keywords harman; ^{1}H-NMR; ^{13}C-NMR; 2D-NMR; ^{13}C-difference NOE; ^{1}H-detected heteronuclear multiple bond connectivity (HMBC)

We have reported a total synthesis of manzamine C (**1**), an antitumor *β*-carboline alkaloid isolated from an Okinawan sponge, and related compounds.[1)] During our studies[2)] on the synthesis of manzamine C (**1**), we encountered difficulty in interpreting the ^{13}C-NMR spectrum of harman (**2**), particularly regarding the signal assignments for C-1, C-4, C-4a, C-4b, C-5, C-6, C-7, C-8a, C-9a, 1′-Me. Some of the earlier data had been measured in different solvents. Harman (**2**) is the fundamental ring system of *β*-carboline alkaloids, and therefore we reinvestigated the ^{1}H- and ^{13}C-NMR spectra of harman (**2**), as well as *N*-methylharman (**3**) (Chart 1).

The ^{13}C-NMR spectrum of harman (**2**) was first reported by Wagner and his co-workers[3)] in 1978; they assigned the protons and carbons of harman (**2**) using its hydrochloride in chloroform-*d* ($CDCl_3$) at 56 °C. They determined the chemical shifts of the methyl and methine carbons by the off-resonance (OFR) and the selective decoupling (SEL) methods, while the quaternary carbons (C-1, C-4a, C-4b, C-8a, and C-9a) were assigned by comparison of the NMR spectra with those of *N*-methylcarbazole and methylindoles. Coune and his co-workers [4)] reported the ^{13}C-NMR spectrum of harman (**2**) free base in dimethyl sulfoxide-d_6 (DMSO-d_6) and assigned the methyl and methine carbons by similar methods, though the method for the assignment of quaternary carbons was not specified.



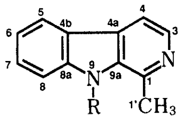

2 : R=H
3 : R=CH_3

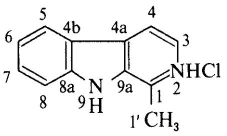
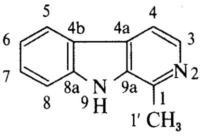
Chart 1

In 1984 Borris and his co-workers[5)] reported the ^{13}C-NMR spectrum of harman (**2**) in methyl alcohol-d_4 (CD_3OD) without describing the method used for the assignment. The reported chemical shifts for the 4- and 7-methines and the 1-, 4a-, 4b-, 8a-, and 9a-quaternary carbons were different from those reported by Coune, although different solvents were used by the two groups.

In the same year, Ohmoto and his co-workers[6)] reported the ^{13}C-NMR spectrum of harman (**2**) in DMSO-d_6 as reference data for *β*-carboline alkaloids, and obtained similar results to those of Wagner's group.

In 1985 Welti[7)] reported the ^{13}C-NMR spectrum of harman (**2**) in DMSO-d_6, and pointed out previous miss-assignments of quaternary carbons at the 4a-, 4b-, 8a-, and 9a-positions in previous reports,[4,6)] applying 1D- and 2D-

TABLE I. The ^{13}C-Assignments of Harman in Previous Papers[3–7)]

					
	Wagner *et al.*,[3)] 1987 $CDCl_3$, δ_C	Coune *et al.*,[4)] 1980 DMSO-d_6, δ_C	Borris *et al.*,[5)] 1984 CD_3OD, δ_C	Ohmoto *et al.*,[6)] 1984 DMSO-d_6, δ_C	Welti,[7)] 1985 DMSO-d_6, δ_C
1	142.1	142.0	140.3	142.23	142.28 (s)
3	137.3	137.3	137.4	137.56	137.63 (d)
4	112.4	112.2	127.5	112.64	112.64 (d)
4a	121.2	121.1	126.8	121.19	127.06 (s)
4b	127.2	127.2	121.1	126.97	121.28 (s)
5	121.3	121.2	119.0	121.69	121.69 (d)
6	127.6	119.0	112.3	127.82	119.24 (d)
7	119.1	127.5	121.4	119.19	127.83 (d)
8	111.9	111.5	111.8	111.98	112.03 (d)
8a	140.6	134.6	134.4	140.49	140.57 (s)
9a	134.7	140.5	141.9	134.64	134.72 (s)
1′	20.3	18.4	20.4	63.52	20.56 (q)

incredible natural abundance double quantum transfer experiment (INADEQUATE) methods to confirm ^{13}C–^{13}C linkages. These data are summarized in Table I.

However, Joseph–Nathan *et al.*[8] cited only Welti's report on harman (**2**) in their review article on the ^{13}C-NMR spectra of indole derivatives, while Erra–Balsells[9] referred to Coune's values for harman (**2**) as reference data for ^{13}C-NNR spectra of azacarbazoles.

In order to confirm the assignments of the ^{13}C-NMR signals of the *β*-carboline moiety of manzamine C (**1**), we first measured the ^{1}H-NMR spectra of harman (**2**) and *N*-methylharman (**3**) in various solvents such as DMSO-d_6, $CDCl_3$, and CD_3OD. The proton signal assignments were confirmed by the HH-chemical shift correlation spectroscopy (COSY), the proton-homo spin decoupling (SD), the nuclear Overhauser enhancement and exchange spectroscopy (NOESY), and the nuclear Overhauser effect (NOE) difference spectroscopy (DIFNOE) methods. The results are summarized in Table II.

These data are essentially in agreement with those previously reported,[3,4] but are better defined. We next measured the ^{13}C-NMR spectrum of harman (**2**) and that of *N*-methylharman (**3**) in various solvents. The peaks corresponding to the methyl, methine, and quaternary carbons have been unambiguously assigned by the proton complete decoupling (COM) and the distortionless enhancement by polarization transfer (DEPT) methods. The assignments of the methyl and methine carbons were confirmed by the HC-COSY or the proton detected heteronuclear multiple quantum coherence (HMQC) measurements utilizing correlation peaks with protons whose positions were established by the ^{1}H-NMR spectra.

TABLE II. ^{1}H-NMR Chemical Shift Data in DMSO-d_6, $CDCl_3$, and CD_3OD

	Harman (**2**) $\delta_H(J)$						*N*-Methylharman (**3**) $\delta_H(J)$	
	DMSO-d_6			$CDCl_3$		CD_3OD	DMSO-d_6	$CDCl_3$
	Ours (10 mg)	Coune *et al.*[4]	Borris *et al.*[5]	Ours (1 mg)	Wagner *et al.*[3]	Ours (5 mg)	Ours (30 mg)	Ours (5 mg)
3	8.20 d (5.2)	8.38 (6)	8.27 (5)	8.37 d (5.2)	—	8.15 d (5.5)	8.20 d (5.2)	8.31 d (5.3)
4	7.92 d (5.2)	7.83 (6)	7.93 (5)	7.83 d (5.5)	—	7.90 d (4.5)	7.95 d (5.2)	7.82 dd (5.3, 0.5)
5	8.19 d (7.5)	8.13 (8)	8.22 (8)	8.12 d (8.0)	8.19	8.13 dd (8.2, 0.74)	8.20 d (7.5)	8.11 td (7.5, 0.5)
6	7.22 td (7.1, 1.1)	7.29 (?)	7.28 m	7.29 td (6.9, 2.2)	7.31	7.24 td (7.4, 1.1)	7.25 td (7.5, 1.1)	7.29 td (7.5, 1.1)
7	7.53 td (7.9, 1.1)	7.5 (?)	7.65 m	7.54 m	7.51	7.53 td (6.9, 1.1)	7.59 td (8.2, 1.1)	7.59 td (7.5, 1.1)
8	7.59 d (7.1)	7.51 (?)		7.54 m	7.56	7.58 dd (8.2, 0.7)	7.66 d (8.2)	7.44 d (8.3)
9	11.55 br s	—	11.60 s	8.62 br s	—	Not observed	4.11[a] s	4.14[a] s
1′	2.76 s	2.83	2.85 s	2.84 s	—	2.80 s	3.02 s	3.10 s

δ values in ppm and coupling constants in Hz. Sample weight in parentheses, dissolved in the indicated solvent (0.7 ml). Analyses of ^{1}H spectra of each compound with the aid of HH-COSY, SD, NOESY, and DIFNOE led to assignment of all ^{1}H signals. *a*) Methyl signals.

TABLE III. Comparison of COLOC and HMBC

Numbering	Harman (**2**)				*N*-Methylharman (**3**)	
	COLOC (8 Hz) DMSO-d_6 (50 mg)	HMBC (10 Hz) $CDCl_3$ (1 mg)	COLOC (8 Hz) CD_3OD (20 mg)	COLOC (4 Hz) DMSO-d_6 (50 mg)	COLOC (10 Hz) DMSO-d_6 (30 mg)	COLOC (5 Hz) DMSO-d_6 (30 mg)
1	3-H(3), 1′-Me(2)	—	3-H(3), 1′-Me(2)	1′-Me(2)	3-H(3), 1′-Me(2)	Not observed
3	3-H(1), 4-H(2)	1-C(3), 4-C(2), 4a-C(3)	3-H(1), 4-H(2)	4-H(2), 1′-Me(4)	3-H(1), 4-H(2)	3-H(1), 4-H(2)
4	4-H(1), 3-H(2)	3-C(2), 4b-C(3), 9a-C(3)	4-H(1), 3-H(2)	4-H(1), 3-H(2)	3-H(2), 4-H(1)	3-H(2), 4-H(1)
4a	3-H(3), 9-H(3)	—	3-H(3)	3-H(3), 9-Me(3)	3-H(3), 9-Me(3)	3-H(3), 6-H(4)
4b	4-H(3), 8-H(3), 9-H(3)	—	6-H(3), 8-H(3)	6-H(3), 8-H(3)	4-H(3), 8-H(3), 9-H(3)	6-H(3), 8-H(3)
5	7-H(3)	7-C(3), 8a-C(3)	7-H(3)	7-H(3)	7-H(3)	7-H(3), 8-H(4)
6	8-H(3)	4b-C(3), 8-C(3)	8-H(3)	8-H(3)	8-H(3)	7-H(2), 8-H(3)
7	5-H(3)	5-C(3), 8a-C(3)	5-H(3)	Not observed	5-H(3)	5-H(3)
8	8-H(1)	4b-C(3), 6-C(3)	Not observed	6-H(3), 8-H(1)	8-H(1)	5-H(4), 6-H(3), 8-H(1)
8a	7-H(3), 9-H(2)	—	7-H(3)	6-H(4)	7-H(3), 9-Me(2)	Not observed
9	—	Not observed	—	—	Not observed	9-Me(1)
9a	4-H(3), 9-H(2), 1′-Me(3)	—	4-H(3), 1′-Me(3)	4-H(3), 9-H(2), 1′-Me(3)	4-H(3), 9-Me(3), 1-Me(3)	Not observed
1′	Not observed	1-C(2), 9a-C(3)	Not observed	Not observed	1′-Me(1)	1′-Me(1)

Number of Hz in parentheses is long-range C–H coupling constant. $^{1}J_{CH} = 135$ Hz. Sample weight in parentheses, dissolved in the indicated solvent (0.7 ml). The number in parentheses indicates the number of bonds involved in the correlation.

The methine peaks of harman (**2**) at δ 137.52, 127.74, 121.65, 119.14, 112.57, and 111.92 showed correlation peaks with the protons at δ 8.20 (3-H), 7.53 (7-H), 8.19 (5-H), 7.22 (6-H), 7.92 (4-H), and 7.59 (8-H), respectively. Therefore, these methine peaks were assigned to the C-3, C-7, C-5, C-6, C-4, and C-8 carbons (Table III).

The assignments of quaternary carbons at the 1-, 4a-, 4b-, 8a-, and 9a-positions were established by the correlation spectroscopy *via* long-range coupling (COLOC) or HMBC method (Table III). Two signals at δ 142.12 and 126.86 each showed a long-range correlation peak with 3-H (δ 8.20). The former peak (δ 142.12) was assigned to C-1, as the chemical shift was lowest and a further long-range correlation peak (^2J) with 1′-Me was observed. On the other hand, the peak at δ 126.86 was assigned to C-4a due to the presence of a further long-range correlation peak (^3J) with 9-H (δ 11.55). The quaternary carbon peaks at δ 134.52 and 121.11 each showed a long-range correlation peak with 4-H (δ 7.92). The former peak (δ 134.52) was assigned to the C-9a carbon due to the presence of a further long-range correlation peak (^3J) with 1′-Me, while the latter peak (δ 121.11) was assigned to C-4b due to the presence of a further long-range correlation peak (^3J) with 8-H (δ 7.59). The peak at δ 140.38 was assigned to C-8a due to the presence of a long-range correlation peak (^3J) with 7-H (δ 7.53). The chemical shifts of these quarternary carbons were essentially independent of the solvent or N_a-methylation.

For the further confirmation of these assignments, carbon NOE difference with proton decoupling (CNOEDIF) measurements were carried out. The irradiation at 1′-Me protons (δ 2.76) caused NOE at C-1, C-9a, and C-1′ carbons, while NOE was observed at the C-1, C-8a, and C-9a carbons on irradiation at 9-H (δ 11.55). Furthermore, irradiation at 4-H (δ 7.92) induced NOE at C-3, C-4, C-4a, and C-5 carbons, and NOE was observed at C-4, C-4b, and C-5 on irradiation at 5-H (δ 8.19). These assignments of carbon signals of harman (**2**) and *N*-methylharman (**3**) are summarized in Table IV.

To obtain proper *J*-parameters for the COLOC and HMBC measurements, the $^1J_{CH}$, $^2J_{CCH}$, and $^3J_{CCCH}$ values were also measured by the gate decoupling method in 1D ^{13}C-NMR. These *J*-values are listed in Table V. They were confirmed by heteronuclear *J*-resolution measurement in the 2D spectrum.

These assignments of the ^{13}C-NMR signals of harman (**2**) coincide with those described by Welti, and the assignments by Coune *et al.* at C-4a and C-4b and at C-8a and C-9a, and the assignments by Wagner *et al.* and Ohmoto *et al.* at C-4a and C-4b, and at C-6 and C-7 must be reversed (Chart 2).

Although the present *J*-resolution results are similar to those of Ohmoto *et al.*,[6] we have found that the coupling

Table IV. ^{13}C-NMR Chemical Shift Data

	Harman (**2**) δ_C			*N*-Methylharman (**3**) δ_C
	DMSO-d_6 (50 mg)	$CDCl_3$ (1 mg)	CD_3OD (20 mg)	DMSO-d_6 (30 mg)
1	142.13	141.76	142.98	141.84
3	137.52	138.77	137.74	137.36
4	112.57	112.91	114.05	112.27
4a	126.86	128.33	129.72	127.49
4b	121.11	122.10	122.63[a]	120.24
5	121.65	121.84	122.63[a]	121.27
6	119.14	120.16	120.72	119.17
7	127.74	128.23	129.34	127.88
8	111.92	111.55	112.87	109.99
8a	140.38	140.09	142.52	141.49
9	—	—	—	31.86
9a	134.52	134.58	136.23	135.14
1′	20.46	20.35	19.70	23.21

δ values in ppm. Sample weight in parentheses, dissolved in the indicated solvent (0.7 ml). Analysis of ^{13}C spectra of each compound, with the aid of COM, DEPT, HC-COSY, COLOC, HMQC, and HMBC led to assignments of all ^{13}C signals. *a*) The peaks overlapped each other, but were differentiated by the *J*-resolution measurement.

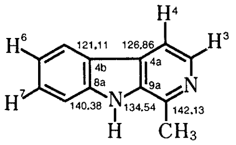


Chart 2

Table V. ^{13}C–^{1}H Coupling Constants (Hz)

Numbering	Harman (**2**)						*N*-Methylharman (**3**)		
	DMSO-d_6 (50 mg)			CD_3OD (20 mg)			DMSO-d_6 (30 mg)		
	^1J	^2J	^3J	^1J	^2J	^3J	^1J	^2J	^3J
1	six d	6.4	11.6, 1.1	six	5.6	16.8	six d	5.6	16.8
3	176.6	2.7	—	177.2	2.8	—	176.9	3.0	—
4	163.0	8.7	—	163.5	7.6	—	163.1	8.9	—
4a	br t	*ca.* 2	5.6, 6.0, 6.9	dd	*ca.* 2	7.6	t d	*ca.* 1.5	8.0
4b	br q		*ca.* 6	br dt		*ca.* 6		m	
5	160.1	—	8.2	159.2	—	7.6	161.9	—	8.0
6	159.9	1.47	7.34	161.0	1.9	6.6	159.5	1.5	7.2
7	159.1	2.2	7.9	158.8	2.4	8.1	159.3	2.0	8.0
8	162.1	—	7.8	152.6	—	7.6	162.4	—	7.8
8a	ddt	3.8	8.8	t		8.8		m	
9							140.0 139.2	— —	— —
9a	ddt	^2J 2.0, ^3J 6.0, ^4J 5.0		br q		2.8	hepta		2.7
1′	126.9	—	—	127.0	—	—	127.2	—	—

^1J, ^2J, and ^3J indicate the proton couplings with carbon through one, two and three bonds, respectively.

with NH was also observed in DMSO-d_6, and this was confirmed by the measurement in CD_3OD and by the long-range selective proton decoupling (LSPD) method. This effect was clearly observed at the quaternary carbon at the 1-position. Furthermore, 8 Hz was found to be a proper J-value in the COLOC and HMBC measurements for β-carbolines. However, it is desirable to analyze the spectra carefully, as the coupling constant (^2J) is close to that of 8 Hz between the proton next to nitrogen and carbons.

Experimental

^{1}H- and ^{13}C-NMR spectra were taken on JEOL JNM-GSX 500 and 400 FT NMR spectrometers with tetramethylsilane (TMS) as an internal standard; chemical shifts are recorded in δ values in ppm and coupling constants in Hz. 1D- and 2D-NMR spectra were measured by the use of JEOL standard pulse sequences (1.6 version), {NON (SGNON), SD (SGHOM), HH-COSY (VCOSYNH), NOESY (VNOENH), DIFNOE (NOEDIF), COM (SGBCM), DEPT (DEFTD), HC-COSY (VCHSHF), COLOC (VCOLOC), 1D-JRES (SGNOE), 2D-JRES (VCJRES), SEL (SGSEL), LSPD (SGSEL), CNOE (CNOEDIF), HMQC (VHMQCD-BBH), HMBC (VHMBC)}, and accumulated data were treated by using JEOL standard software. DMSO-d_6 (99.9 atom%D, Aldrich Chemical Co., U.S.A.), $CDCl_3$ (99.8 atom%D, Isotec Inc., U.S.A.), and CD_3OD (99.8 atom%D, Merck, U.S.A.) were used as solvents. Harman (**2**) (mp 137—138 °C) were purchased by Nacalai Tesque Co., Japan.

***N*-Methylharman (3)** *n*-BuLi (1.6 M solution, 0.34 ml, 1.0 eq) was added slowly to a solution of harman (100 mg, 0.55 mmol) in tetrahydrofuran (THF) (5 ml) at −78 °C with stirring. Methyl iodide (0.034 ml, 0.55 mmol) in THF (1 ml) was then added to the solution with stirring. The mixture was stirred for 1 h at room temperature, then quenched with H_2O and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue (136 mg) was purified by column chromatography (SiO_2, Merck, 10 g, AcOEt) to give *N*-methylharman (90 mg, 83.5%). Recrystallization from Et_2O–pentane gave colorless needles (35 mg), mp 104 °C (reported mp$^{10)}$ 102—104 °C).

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