Chem. Pharm. Bull. 41(6) 1173—1176 (1993)

The ^{1}H- and ^{13}C-Nuclear Magnetic Resonance Spectra of Manzamine C and Related Compounds

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Received November 24, 1992

The ^{1}H- and ^{13}C-NMR spectra of manzamine C (1) and its analogs (2—8) have been analyzed by applying HC-COSY (or HMQC) and COLOC (or HMBC) methods. The slow ring-inversion of the piperidine part in 6 was observed at room temperature. The free energy of activation for interconversion of rotamers in the amide analog (8) was evaluated.

Keywords ^{1}H-NMR; ^{13}C-NMR; 2D-NMR; ^{1}H-detected heteronuclear multiple bond connectivity (HMBC); manzamine C; β-carboline

In the preceding paper[1] we described a reexamination of the ^{1}H- and ^{13}C-NMR spectra of harman (**9**) and *N*-methylharman (**10**), which have the basic ring system of β-carboline alkaloids, and unambiguously assigned all the protons and carbons in the β-carboline ring. Recently, we have reported the total synthesis[2b] of the antitumor marine β-carboline alkaloid[3] manzamine C (**1**) and related compounds (**2, 3**). On the basis of the results obtained in the preceding paper,[1] we now present an assignment of the ^{1}H- and ^{13}C-NMR signals of manzamine C (**1**) and manzamine C analogs (**2**—**8**) having various azacycles instead of the azaundecene ring at the 1-position of **1**, which we had prepared for the evaluation of their biological activity[4] (Chart 1).

The ^{1}H- and ^{13}C-NMR spectra of manzamine C (**1**) were examined by applying nuclear Overhauser effect (NOE) difference spectroscopy (DIFNOE), proton detected heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) methods as in our previous paper, and it appeared that the assignments of protons and carbons of the β-carboline part of manzamine C (**1**) were similar to those of harman (**9**).

In our previous paper,[2b] the proton signals at δ 2.92 and 3.33 were assigned to the α- and β-methylenes, respectively, corresponding to the two carbon unit connecting the β-carboline and the azaundecene rings. However, the HMQC measurement of manzamine C (**1**) disclosed that this assignment must be reversed, because correlation peaks were observed between the peaks at δ 2.92 and 52.82 (C-β), and the peaks at δ 3.33 and 34.66 (C-α).

The assignment of protons in the azaundecene ring of manzamine C (**1**) was confirmed by HH-chemical shift correlation spectroscopy (COSY) from the 8′-H signal (δ 5.47), which must be due to the olefinic protons. The ^{1}H-NMR spectra of azacyclic segments in manzamine C analogs (**2**—**8**) were similar to those reported[5] for pyrrolidine, piperidine, perhydroazepine, and perhydroazocine. These ^{1}H-NMR spectral data are summarized in Table I.

However, the ^{1}H-NMR spectra of the piperidine derivative **6** at room temperature showed two broad singlets at δ 2.67 and 1.66 due to the 2′- and the 4′-methylenes, in contrast to simple *N*-alkyl piperidines, which show rapid ring inversion. At 55 °C, the former peak (δ 2.67) changed to a triplet ($J=5.5$ Hz) and the latter peak (δ 1.66) changed to a quintet ($J=5.5$ Hz). These phenomena led us to study the temperature effect on the ^{1}H-NMR spectrum of **6**. The methylene signals of the 2′- and the 4′-position changed to broader peaks at 10 and 0 °C. At -20 °C the peak due to the 2′-position methylene was split into two broad peaks at δ 3.17 and 2.06, while at -40 °C the peak due to the 4′-position methylene was split into two broad peaks at δ

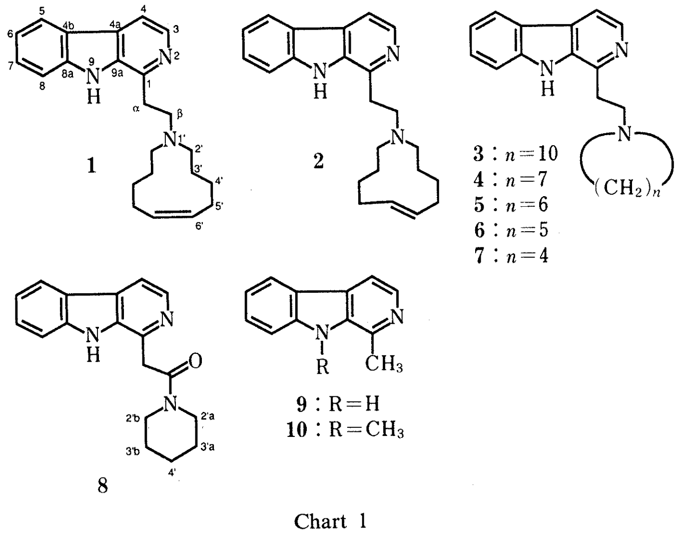


Chart 1

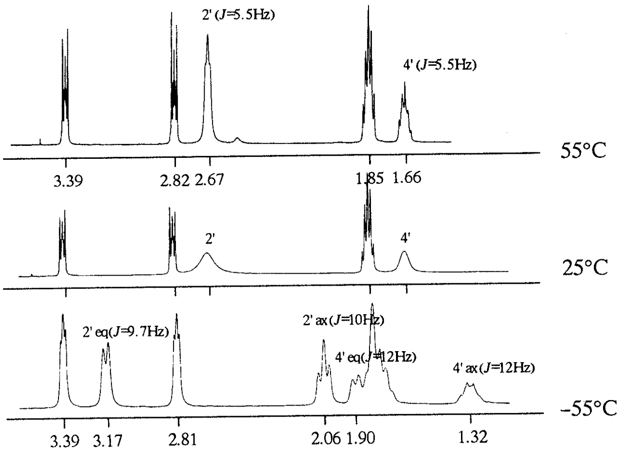
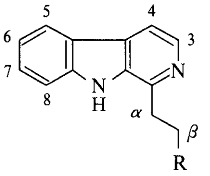
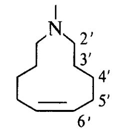
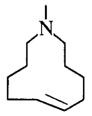
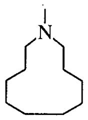
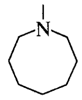
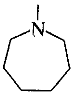
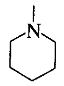
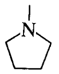


Chart 2. ^{1}H-NMR Spectrum of the Six-Membered Ring Derivative (**6**) in $CDCl_3$

TABLE I. The ^{1}H Assignments of Manzamine C (**1**) and Related Compounds **2**—**7**



R=	 **1**	 **2**	 **3**	 **4**	 **5**	 **6**	 **7**
3	8.27 (d, 5.2)	8.26 (d, 5.5)	8.34 (d, 5.2)	8.28 (d, 5.3)	8.27 (d, 5.3)	8.28 (d, 5.2)	8.29 (d, 5.2)
4	7.81 (d, 5.2)	7.81 (d, 5.5)	7.81 (d, 5.2)	7.84 (d, 5.3)	7.84 (d, 5.5)	7.82 (d, 5.2)	7.83 (d, 5.2)
5	8.12 (d, 8.0)	8.11 (d, 5.5)	8.11 (d, 7.7)	8.12 (d, 5.3)	8.13 (d, 7.9)	8.13 (d, 8.0)	8.11 (d, 7.8)
6	7.23 (m)	7.26 (m)	7.26 (m)	7.26 (m)	7.26 (m)	7.23 (d, 8.0)	7.25 (m)
7	7.50 (m)	7.51 (m)	7.54 (m)	7.53 (m)	7.54 (m)	7.52 (m)	7.46 (m)
8							7.50 (m)
NH	12.74 (s)	12.74 (s)	10.14 (s)	11.99 (s)	12.70 (s)	12.96 (br s)	12.72 (s)
α	3.33 (t, 5.4)	3.33 (t-like, 5.2)	3.32 (t, 6.6)	3.43 (t, 5.5)	3.40 (t, 5.5)	3.39 (t-like)	3.43 (t, 5.3)
β	2.92 (t, 5.4)	2.92 (m)	2.99 (t, 6.6)	3.05 (t, 5.5)	2.99 (t, 5.5)	2.83 (t-like)	3.04 (t, 5.3)
2′	2.85 (t, 7.5)	2.84 (t-like, 8.0)	2.64 (m)	2.92 (m)	2.88 (m)	2.67 (br s)	2.80 (br s)
3′	1.78 (m)	1.68 (s-like)	1.52 (m)	1.81 (m)	1.84 (m)	1.85 (t-like)	2.01 (br s)
4′	1.53 (m)	1.39 (s-like)				1.66 (br s)	
5′	2.32 (q-like, 5.4)	2.19 (s-like)					
6′	5.47 (t-like, 5.1)	5.51 (m)					

Figures in parentheses are coupling constants in hertz (Hz). Sample weight 3—5 mg, dissolved in CDCl$_3$ (0.7 ml).

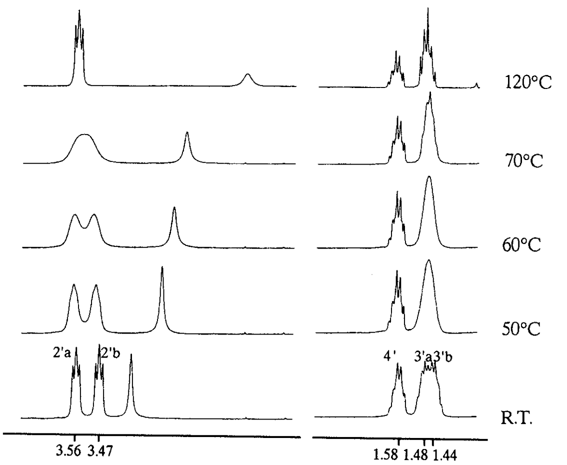


Chart 3. ^{1}H-NMR Spectrum of the Six-Membered Ring Amide Derivative (**8**) in DMSO-d_6

1.90 and 1.32. At −55 °C, the peak at δ 3.17 split into a doublet (J=9.7 Hz) and the peak at δ 2.06 showed a triplet (J=10 Hz). Therefore, the former peak (δ 3.17) was assigned to the 2′-equatorial protons and the latter peak (δ 2.06) to the 2′-axial protons. The peaks at δ 1.90 and 1.32 were assigned to the 4′-equatorial protons and the 4′-axial protons, respectively, as the former peak became a doublet (J=12 Hz) and the latter peak, a broad quartet (J=12 Hz) at −55° C (Chart 2).

We found that the amide **8**, the precursor of **6**, in chloroform-d (CDCl$_3$) exhibits two peaks due to the 2′-protons at δ 3.68 and 3.53, and two peaks due to the 3′-protons at δ 1.28 and 1.41, respectively, at room temperature as a result of restricted rotation of the amide bond. None of the amides corresponding to **1**—**5**, and **7** exhibits this phenomenon. The peak at δ 3.68 may be assigned to the 2′a-protons, which are on the same side as the carbonyl group. The peak at δ 3.53 was assigned to the 2′b-protons located on the opposite side to the carbonyl group. The quintets at δ 1.28, 1.41, and 1.51 were assigned to the 3′a-, 3′b-, and 4′-methylenes, respectively, by means of the spin-decoupling (SD) method. The ^{1}H-NMR spectra of **8** in dimethyl sulfoxide-d_6 (DMSO-d_6) at room temperature showed similar peaks to those seen in CDCl$_3$, except for two peaks due to the 3′-protons that overlapped each other. By the SD method with the 2′a (δ 3.56) and 2′b (δ 3.47) protons, the peak at δ 1.48 was assigned to the 3′a-protons, and the peak at δ 1.44, to the 3′b-protons.

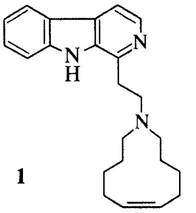
The ^{1}H-NMR spectra of **8** in DMSO-d_6 at 50, 60, 70, and 120 °C are shown in Chart 3.

At 50 °C the peak due to the 3′-protons became a broad singlet, and the peak due to the 2′-protons became a broad singlet at 70 °C. At 120 °C, the peak due to the 2′-protons became a triplet (J=5.5 Hz) at δ 3.53, and a quintet (J=5.5 Hz) was observed for the 3′-protons at δ 1.45.

The ^{13}C-NMR spectra of the piperidine derivative **6** were measured by the same method and all the carbons were assigned. These data are shown in Table II. The chemical shifts of the 2′- and 3′-carbons of **6** were shifted downfield 5 and 3 ppm from those of manzamine C (**1**).

The ^{13}C-NMR spectra of **8** showed slightly different signals from those of **6** due to the presence of the amide carbonyl group at the β-position. The HC-COSY and correlation spectroscopy *via* long-range coupling (COLOC) method allowed clear assignments of all carbons, as

TABLE II. The ^{13}C-Assignments of Manzamine C (**1**) and the Six-Membered Ring Derivative (**6**)



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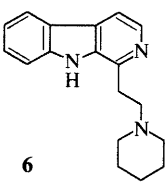

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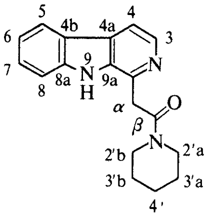
	1 (3 mg/CDCl$_3$)			**6** (38 mg/CDCl$_3$)		
	δ_C	HMBC (10 Hz)	HMBC (5 Hz)	δ_C	COLOC (8 Hz)	COLOC (4 Hz)
1	145.68	—	—	145.87	3H (3), 1′H (2)	1′H (2)
3	137.51	1C (3), 4C (2), 4aC (3)	1C(3), 4C (2), 4aC (3)	137.49	3H (1)	4H (2)
4	113.11	9aC (3)	3C (2), 4bC (3), 9aC (3)	113.14	3H (2)	3H (2)
4a	128.28	—	—	128.31	3H (3), 9H (3)	3H (3)
4b	122.01	—	—	122.03	9H (3)	6H (3)
5	121.72	7C (3), 8aC (3)	8aC (3)	121.77	7H (3)	7H (3)
6	119.16	8C (3)	8C (3)	119.16	8H (3)	8H (3)
7	127.68	5C (3), 8aC (3)	5C (3), 8aC (3)	127.69	Not observed	Not observed
8	111.90	Not observed	6C (3)	111.76	Not observed	6H (3)
8a	140.69	—	—	140.67	7H (3), 9H (2)	Not observed
9a	135.58	Not observed	Not observed	135.65	4H (3), 1′H (3)	4H (3), 1′H (3)
α	34.66	1C (2), 2′C (2)	1C (2), 2′C (2), 9aC (3)	34.21	2′H (2)	2′H (2)
β	52.84	4′C (3)	1C (3), 1′C (2), 4′C (3)	57.53	1′H (2)	1′H (2)
2′	48.99	2′C (3), 4′C (1), 5′C (2), 6′C (3)	2′C (3), 4′C (1)	54.43	Not observed	Not observed
3′	23.31	4′C (2), 6′C (2), 7′C (3)	4′C (2), 7′C (3)	26.33	2′H (3), 6′H (2)	Not observed
4′	24.93	4′C (3), 8′C (3)	4′C (3), 5′C (2), 7′C (2), 8′C (3)	24.35	5′H (2)	Not observed
5′	26.04	5′C (3), 6′C (2), 8′C (2)	5′C (3), 6′C (2), 8′C (2)			
6′	131.02	7′C (2)	7′C (2)			

The value in parentheses is the long-range C–H coupling constant, $^1J_{CH} = 135$ Hz. The number in parentheses denotes the number of bonds involved in the correlation.

TABLE III. The ^{1}H- and ^{13}C-Assignments of the Six-Membered Ring Amide Compound (**8**)



	CDCl$_3$ (30 mg)		DMSO-d_6 (10 mg)		CDCl$_3$ (30 mg)			DMSO-d_6 (30 mg)		
	δ_H	J (Hz)	δ_H	J (Hz)	δ_C		COLOC (8 Hz)	δ_C (RT)	COLOC (8 Hz)	δ_C (150°C)
1	—	—	—	—	138.30	C	1′H (2), 3H (3)	140.61	1′H (2), 3H (3)	140.95
3	8.33	d, 5.4	8.23	d, 5.3	138.48	CH	1′H (4)	137.37	3H (1), 4H (2)	137.93
4	7.85	d, 5.4	7.97	d, 5.3	113.77	CH	3H (2), 4H (1)	113.19	3H (2), 4H (1)	113.04
4a	—	—	—	—	129.01	C	3H (3), 9H (3)	127.43	3H (3), 9H (3)	128.22
4b	—	—	—	—	121.42	C	8H (3), 9H (3)	120.91	4H (3), 8H (3), 9H (3)	121.56
5	8.09	d, 7.9, 0.7	8.20	d, 8.1	121.59	CH	Not observed	121.58	Not observed	121.45
6	7.26	ddd, 7.9, 7.9, 2.0	7.23	d, 7.7, 0.6	119.87	CH	8H (3)	119.14	8H (3)	119.45
7	7.54	m	7.53	d, 8.2, 1.1	128.35	CH	Not observed	127.84	Not observed	127.88
8	7.54	m	7.62	d, 8.2	112.22	CH	Not observed	111.92	8H (1)	112.24
8a	—	—	—	—	140.58	C	7H (3), 9H (2)	140.33	7H (3), 9H (2)	140.68
9	9.84	s	11.43	s	—	—	—	—	—	—
9a	—	—	—	—	135.59	C	4H (3), 9H (2)	134.87	4H (3), 9H (2)	135.36
α	4.30	s	4.20	s	43.45	CH$_2$	1′H (1)	39.48	Not observed	40.44
β	—	—	—	—	168.23	C	1′H (2), 4′aH (3)	167.23	1′H (2), 4′aH (3)	167.87
2′a	3.68	t, 5.6	3.56	t, 5.3	47.81	CH$_2$	4′aH (1)	46.49	4′aH (1)	44.79
2′b	3.53	t, 5.6	3.47	t, 5.3	43.28	CH$_2$	4′bH (1)	42.08	4′bH (1)	
3′a	1.28	q, 5.6	1.48	q, 5.0	26.24	CH$_2$	4′aH (2)	25.24	4′bH (4), 5′bH (3)	25.62
3′b	1.41	q, 5.6	1.44	q, overlap	25.54	CH$_2$	4′bH (2)	25.86	4′aH (4), 5′aH (3)	
4′	1.51	q, 5.6	1.58	q, 5.0	24.23	CH$_2$	4′aH (3), 4′bH (3)	23.95	4′aH (3), 4′bH (3), 5′aH (2), 5′bH (2)	23.97

The value in parentheses is the long-range C–H coupling constant, $^1J_{CH} = 135$ Hz. The sample weight in parentheses was dissolved in the indicated solvent (0.7 ml). RT = room temperature.

summarized in Table III.

As found in the ^{1}H-NMR spectra, the ^{13}C-NMR spectra of **8** showed two peaks due to the 2′-carbon as a result of the restricted rotation of the amide band. The coalescence temperatures for the 2′-proton and the 2′-carbon signals were 70 and 150 °C, respectively. The calculated free energy of activation was 78.6 kJ/mol (18.76 kcal/mol) for the protons and 78.4 kJ/mol (18.71 kcal/mol) for the carbon.

In conclusion, the ^{1}H- and ^{13}C-NMR spectra of manzamine C (**1**) and its analogs (**2**—**8**) were analyzed properly by means of HMQC, HMBC, HH-COSY, HC-COSY, and other measurements.

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. The 1D- and 2D-NMR spectra were measured by the use of JEOL standard pulse sequences, {NON (SGNON), SD (SGHOM), DIFNOE (NOEDIF), HH-COSY (VCOSYNH), HMQC (VHMQCD-BBH), HMBC (VHMBC), HC-COSY (VCHSHF), COLOC (VCOLOC), NOESY (VNOENH), COM (SGBCM), DEPT (DEPTD)}, and accumulated data were treated by using JEOL standard software (1.6 version). $CDCl_3$ (99.8 atom%D, Isotec Inc., U.S.A.), DMSO-d_6 (99.9 atom%D, Aldrich Chemical Co., U.S.A.) were used as solvents. Manzamine C (**1**), colorless prisms (mp 90.0—92.0 °C), manzamine C *trans* isomer (**2**), pale brown prisms (mp 145—145.5 °C), and dihydromanzamine C (**3**), pale yellow prisms (mp 151—153 °C) were prepared as described in a previous paper.[2)] Preparation of other compounds (**4**—**8**) will be reported separately.

References and Notes

1) H. Seki, A. Hashimoto, and T. Hino, *Chem. Pharm. Bull.*, **41**, 1169 (1993).

2) *a*) Y. Torisawa, A. Hashimoto, M. Nakagawa, and T. Hino, *Tetrahedron Lett.*, **30**, 6549 (1989); *b*) Y. Torisawa, A. Hashimoto, M. Nakagawa, H. Seki, R. Hara, and T. Hino, *Tetrahedron*, **47**, 8067 (1991).

3) R. Sakai, S. Kohmoto, T. Higa, C. W. Jefford, and G. Bernardinelli, *Tetrahedron Lett.*, **28**, 5493 (1987).

4) M. Nakagawa, A. Hashimoto, Y. Torisawa, M. Ohkouchi, T. Hino, T. Iimori, M. Nagasawa, T. Tsuruoka, and K. Kawarajoh, Abstracts of Papers, the 112th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, March 1992, Part 2, p. 270.

5) Handbook of Proton-NMR Spectra and Data, ed. by Asahi Research Center Co., Ltd., Academic Press, Tokyo, 1987, Compound No. 396, 601, 4518, 4769.