Short communication

Drug Testing and Analysis

Received: 12 October 2010 Revised: 11 December 2010 Accepted: 12 December 2010 Published online in Wiley Online Library: 2 February 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.257

Spectral data analyses and structure elucidation of metoprolol tartrate

Jun-qin Qiao,[a] Ya-qin Wang,[b] Chen-chen Liu,[a] Wei Zhu,[a] Hong-zhen Lian[a]* and Xin Ge[a]*

The infrared spectrum, ultraviolet spectrum, mass spectra, and nuclear magnetic resonance spectra of metoprolol tartrate, an antiarrhythmic medicine, were reported and interpreted comprehensively. The vibrations of functional groups in infrared spectrum, electron transitions in ultraviolet spectrum, and main characteristic fragmentations in mass spectra of metoprolol tartrate were discussed. All the ^{1}H and ^{13}C NMR chemical shifts were assigned by means of distoritionless enhancement by polarization transfer-135 and -90, ^{1}H-^{1}H correlation spectroscopy, and ^{13}C-^{1}H correlation spectroscopy via short- and long-range coupling.

Keywords: metoprolol tartrate (MT); infrared spectrum (IR); ultraviolet spectrum (UV); mass spectra (MS); nuclear magnetic resonance (NMR)

Introduction

Metoprolol tartrate (MT), 1-(isopropylamino)-3-(p-(2-methoxyethyl)phenoxy)-2-propano (±)-2-propano tartrate (Figure 1), is a cardioselective □β_1-adrenoceptor antagonist which has weak membrane stabilizing effect but without intrinsic sympathomimetic activity. It is approved to control arterial hypertension,[1] and to treat angina pectoris,[2] chronic heart failure,[3–4] myocardial infarct, hypertrophic, arrhythmia, hyperthyroidism, and cardiac neurosis.[5–6]

Many research studies have reported on clinical pharmacology and pharmacokinetics of MT,[7–9] and several analytical methods have been developed for the determination of MT including high performance liquid chromatography (HPLC),[10–11] flow-injection chemiluminescence,[12] and spectroscopy[13–14] in much of the literature. Some works studied the spectral properties of MT,[15–19] but these data were scattered in different publications and not exhaustive. The complete literature reporting the spectral data of this compound is not available up-to-date. This present paper provides full spectral data for the structure identification of MT. The suitable measurements on MT have been carried out to get the infrared spectrum (IR), ultraviolet spectrum (UV), mass spectra (MS), ^{1}H and ^{13}C nuclear magnetic resonance spectra (NMR), distoritionless enhancement by polarization transfer-135 (DEPT-135) and -90 (DEPT-90), ^{1}H-^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC). The main characteristic of MS fragmentation, IR vibrations of functional groups, and UV electron transitions were explained. Uppermost, ^{1}H NMR and ^{13}C NMR spectral features were described in detail and all the chemical shifts were assigned by using DEPT-135 and DEPT-90, ^{1}H-^{1}H COSY, HSQC, and HMBC. The reported data are fundamental not only to structurally identify and characterize MT drug substances and formulated drug products, but also to analyze and determine MT in various biological fluids via online techniques, such as liquid chromatography and capillary electrophoresis combined with UV, MS, IR, and NMR, which have become more and more popular recently for the purpose of pharmaceutical investigation.

Experimental

Materials and reagents

MT used for spectral measurements was kindly supplied by Jiangsu Tianhe Medical Institute Co. Ltd. (Changzhou, Jiangsu, PRC). The purity of this compound was checked by HPLC (99.22%) according to the chromatographic condition developed in Aqil *et al*[20] meeting the requirement of structure identification. Wahaha water (Wahaha Group Ltd, Hangzhou, China) was filtered by an acetyl cellulose membrane (0.45 μm) before use.

Apparatus and conditions

The IR spectrum was recorded by using a NEXUS 870 Fourier transform infrared (FT-IR) spectrometer (Nicolet, Madison, WI, USA) with a resolution of 4 cm^{-1}. The solid sample was measured in the range of 4000 to 400 cm^{-1} as KBr pellet.

* *Correspondence to: Hong-zhen Lian, Center of Materials Analysis, Key Laboratory of Analytical Chemistry for Life Science (Ministry of Education of China), School of Chemistry & Chemical Engineering, Nanjing University, 22 Hankou Road, Nanjing 210093, China. E-mail: hzlian@nju.edu.cn*

Xin Ge, Center of Materials Analysis, Key Laboratory of Analytical Chemistry for Life Science (Ministry of Education of China), School of Chemistry & Chemical Engineering, Nanjing University, 22 Hankou Road, Nanjing 210093, China. E-mail: gexin@nju.edu.cn

a *Center of Materials Analysis, Key Laboratory of Analytical Chemistry for Life Science (Ministry of Education of China), School of Chemistry & Chemical Engineering, Nanjing University, 22 Hankou Road, Nanjing 210093, China*

b *School of Materials and Chemical Engineering, Anhui University of Architecture, 856 South Jinzhai Road, Hefei 230022, China*

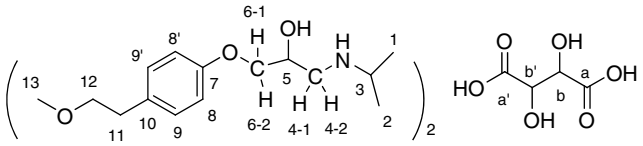

Figure 1. Structure of MT.

The UV spectrum was achieved on a Shimadzu UV-VIS 2401 spectrophotometer (Shimadzu, Kyoto, Japan). The absorptivity of MT at a 2.90×10^{-5} mol/L solution of water was examined in the wavelength range of 200–500 nm.

MS measurement was primarily performed with an Agilent 1290 Infinity HPLC coupled to an Agilent 6460 triple quadrupole mass spectrometer (Agilent Scientific, Santa Clara, CA, USA) equipped with a jet stream electrospray ionization (ESI) probe. Direct injection was used in the analysis with methanol as mobile phase at a flow rate of 0.2 ml/min. The source parameters were as follows: nitrogen gas as drying and sheath gas at flow rates of 10 and 8 L/min, respectively; drying and sheath gas temperatures, 300 and 250 °C, respectively; nebulizer pressure, 45 psi; capillary voltage, 3500 V. Full mass scans (100–1000 *m/z*) were carried out in positive mode, followed by product ion detection of the selected precursor ion *m/z* = 268.1. High purity nitrogen (>99.999%) was used as collision gas with collision energy of 20 eV. Then MS measurement was performed with Micromass TOF mass spectrometer (Micromass, Manchester, UK) operated in the electron impact ionization (EI) mode. The electron energy was set to 70 eV and the resolution was set to 7000. The source manifold temperature was maintained at 250 °C.

^{1}H and ^{13}C NMR spectra were taken on an AVANCE AV-500 spectrometer (Bruker, Fällandon, Switzerland) operating at 500.13 and 125.77 MHz, respectively, using a 5-mm sample tube at 303 K. Dimethyl sulfoxide-D_6 (DMSO-D_6) was used as the solvent, and tetramethylsilane (TMS) and DMSO-D_6 were used as the references for the measurement of H and C chemical shifts, respectively.

Results and discussion

IR spectrum of MT

The obtained IR spectrum of MT (Figure 2) was similar to that in Slegers *et al*.[18] The wavenumbers (cm^{-1}) of bands observed were assigned as shown in Table 1.

Interestingly, there were no N-H stretching vibration of $-NH_2$ and O-H stretching vibration of –COOH groups above 3000 cm^{-1} in the IR spectrum. Inversely, the N-H stretching vibration displayed blue shift to the region of 3000.0–2700.0 cm^{-1}. All of these phenomena indicate that MT exists as NH_3^+ and COO^- ions when it is in solid state.

UV spectrum of MT

MT in water showed a maximal absorption at the wavelength of 220.5 nm, which was assigned to K (Konjugierte) bands. The molar absorption coefficient at the maximum was 2.46×10^4 $(mol/L)^{-1}$ cm^{-1}. There was characteristic absorption at 274.5 nm, which corresponded to E (Ethylenic) bands. The molar absorption coefficient of this peak was 5.96×10^3 $(mol/L)^{-1}$ cm^{-1}.

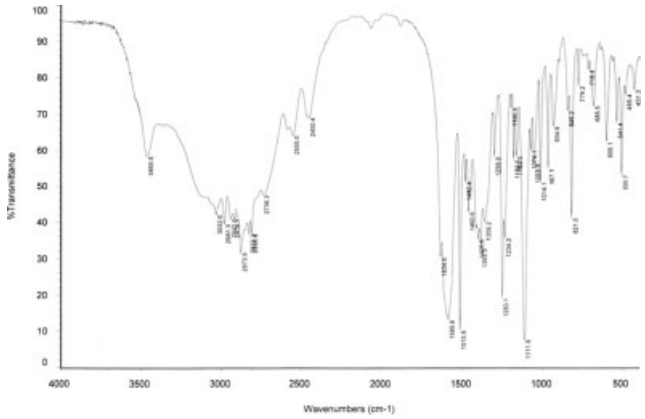

Figure 2. IR spectrum of MT.

Table 1. Assignments of IR signals of MT

Wavelength of absorption (cm^{-1})	Intensity of band	Attribution of vibration
3460.8	(middle, m)	νO-H
3032.6	m	ν=C-H
3000.0 ~ 2700.0	m	νN-H
2981.3, 2942.0, 2918.7	m	ν_{as}C-H
2873.9	m	ν_{s}C-H
1585.6	(strong, s)	νC-O
1513.8	s	νC=C
1480.4, 1383.3	m	δ_{s}C-H
1460.6	m	δ_{as}C-H
1299.8	(weak, w)	νC-C or C-N
1250.1, 1234.2, 1111.9	s, m, s	ν_{as}C-O
821.0	m	δ=C-H

Mass spectra of MT

In ESI-MS of positive ion mode (Figure 3), a base peak of m/z 268.1, i.e. the monoprotonated quasi-molecular ion of metoprolol $[M + H]^+$, was observed predominately. The MS/MS spectrum of precursor ion *m/z* 268.1 was in agreement with that in previous work.[18–19] No molecular peak of MT was observed in both positive and negative ESI ionization modes, even in atmospheric pressure chemical ionization (APCI), due to the weak binding force between metoprolol and tartrate, as reported in Thevis *et al*.[19] EI-MS gave the fragment ion signals of MT at *m/z* (relative abundance/%) 252.1 (0.60), 249.1 (0.34), 223.1 (1.86), 152.0 (2.23), 116.1 (1.24), 107.0 (50.19), 98.1 (5.99), 72.0 (100), 56.0 (18.13), and 45.0 (8.37) (Figure 4) as listed in NIST mass spectra library. The base peak *m/z* 72.0 corresponded to $H_2C{=}\overset{+}{N}HCH(CH_3)_2$, which was produced through α split induced by the free radical centre of atom nitrogen. The next high abundant fragment ion at *m/z* 107.0 was assigned to $\overset{+}{C}H_2$-Ph-OH, generated through the loss of CH_3-O-CH_2 from the fragment ion of *m/z* 152.0. The other fragment ion signals were weak and could be explained in the fragmentation pattern given in Figure 5, which was consistent with the expected structure of MT.

NMR spectra of MT

The structure of MT was elucidated further, and C and H atoms were assigned by using ^{1}H NMR, full decoupling ^{13}C NMR, DEPT-135 and -90, ^{1}H-^{1}H COSY, HSQC, and HMBC.

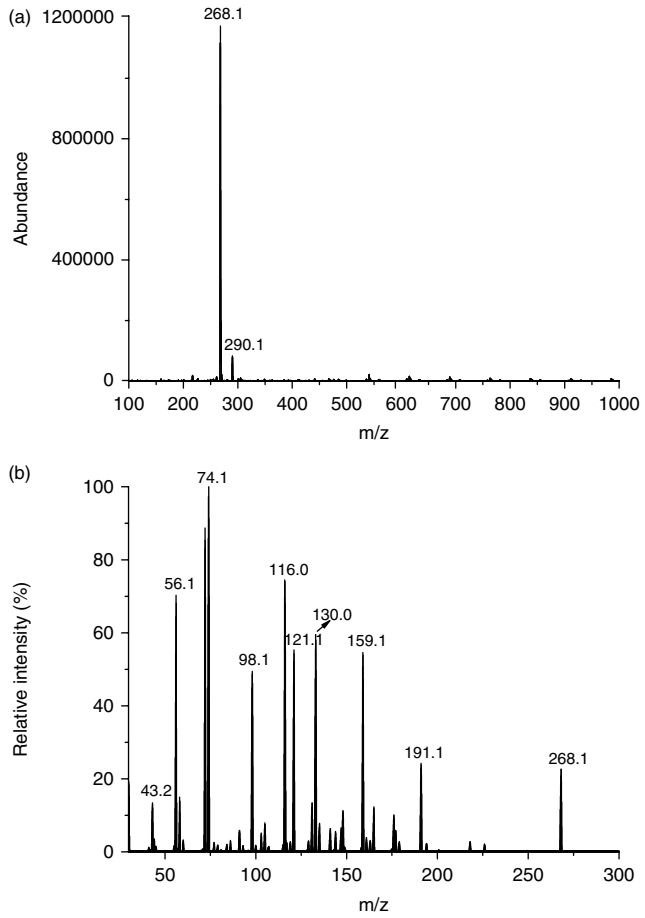

Figure 3. ESI-MS spectra of MT (a) and corresponding product ion (b) in positive mode.

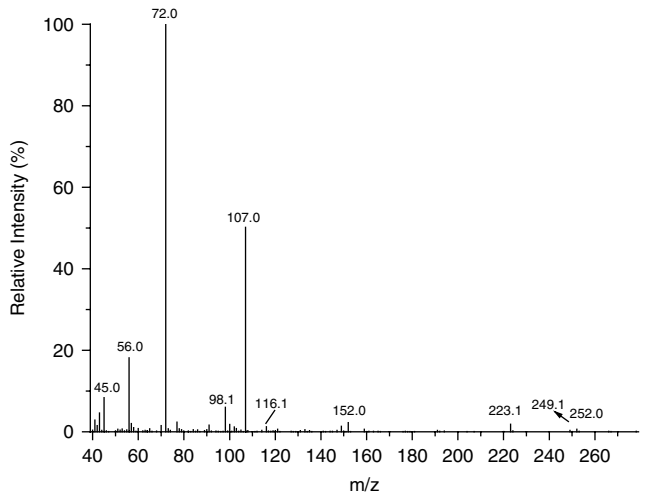

Figure 4. EI-MS spectrum of MT.

The active H protons did not appear due to the use of DMSO-D_6 as solvent, and there are 24 H proton signals in ^{1}H NMR spectrum (Figure 6). Fifteen C signals are shown in ^{13}C NMR (Figure 7), among which three primary carbon, four secondary carbon, five tertiary carbon, and three quaternary carbon atoms were obtained through the comparison among the spectra of DEPT-90, DEPT-135, and full decoupling ^{13}C NMR. For the symmetry of structure, a-C and a′-C, b-C and b′-C, 8-C and 8′-C, and 9-C and 9′-C had same chemical shift (δ), respectively, so there were 17 C signals in ^{13}C NMR in fact. The given NMR information of C and H numbers accords with that of MT.

In ^{13}C NMR spectrum, the signals at δ 18.82 ppm and δ 19.25 ppm in the highest field were assigned to 1-C and 2-C. The signal at δ 176.72 ppm in the lowest field, a typical chemical shift of carboxyl carbon, was explained as a-C and a′-C. In the interval between 130 and 160 ppm, there were signals of quaternary carbon on benzene, and obviously 7-C was in a lower field compared with 10-C for the deshielding effect of oxygen atom. Therefore, the chemical shift of 7-C was 156.88 ppm and that of 10-C was 131.19 ppm. And what's more, 10-C could be confirmed further by its coupling with 11-H in HSQC. On aromatic ring, 8-C and 9-C are in the ortho- and meta-position of –OR, respectively, and thus lay in the higher field and lower field with the chemical shifts of 114.37 and 129.73 ppm. The signal at δ 49.33 ppm corresponded to 3-C, which could be deduced on the basis of 3-H (δ 3.20–3.24 ppm, 1H) through HSQC. 3-H was verified by its coupling with 1-H and 2-H, whose chemical shifts were in the range of 1.21–1.22 ppm. In DEPT-90, there were only two tertiary carbon signals, i.e. 5-C and b-C left unassigned. Because 5-C is connected to electron donating group –OH while b-C is connected to not only electron donating group –OH but also to electron drawing group –COOH, the chemical shift of 5-C (δ 65.55 ppm) was in the higher field compared with that of b-C (δ 72.91 ppm). As for secondary carbon, 4-C, 6-C and 12-C are all connected to electron drawing groups while 11-C not, so the carbon atom located at the highest field 49.33 ppm was assigned to 11-C. In that case, 11-H (δ 2.73 ppm) was confirmed by HSQC. The signal arose in δ 73.04 ppm exhibited a long coupling with 11-H in HMBC, which must be 12-C. 6-C (δ 70.15 ppm) was in a lower field than 4-C (δ 47.51 ppm) due to the higher deshielding effect of oxygen than nitrogen. The assignments of all ^{13}C NMR chemical shifts are summarized in Table 2.

The ^{1}H NMR signals were assigned unambiguously on the basis of two-dimensional HSQC, and they could be confirmed together by ^{1}H-^{1}H COSY and HMBC (Table 3). The results were consistent with those in Jung *et al.*[16] except a few differences as follows. The obtained signals in the range of δ 1.20–1.22 ppm corresponding to 1-H and 2-H were triplet, which seems disagreeing with general split law. A possible reason was that the protons of 1-H and 2-H have different coupling with 3-H due to their different chemical environments. 1-H and 2-H produced individual doublets. However, one peak from 1-H overlapped with one peak from 2-H. Therefore, 3-H (δ 3.21–3.24 ppm) gave mutiplet. In addition, b-H and b′-H are chemical equivalent, corresponding to the same single peak at 3.99 ppm.

Acknowledgements

This work was supported by National Natural Science Foundation of China (90913012, J0830307), National Basic Research Program of China (973 program, 2009CB421601, 2011CB911003), National Science Funds for Creative Research Groups (20821063), Analysis & Test Fund, and Starting Fund for Young Teachers of Center of Materials Analysis, Nanjing University. We are grateful to Prof. Zeper Abliz, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, for his help in mass spectral interpretation.

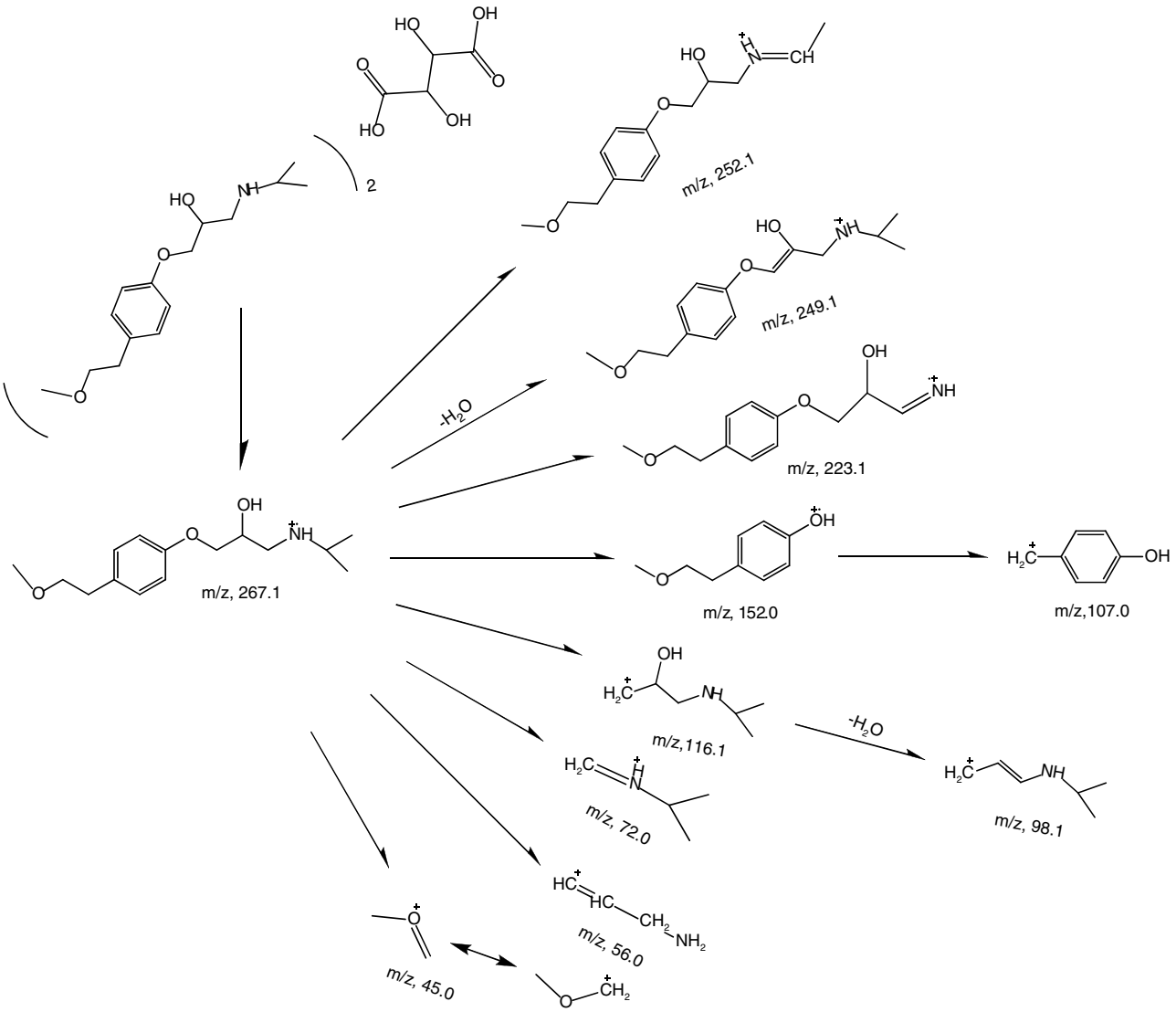

Figure 5. EI-MS fragmentation pattern of MT.

Table 2. Assignments of the ^{13}C NMR signals of MT

^{13}C Chemical shifts δ_C ($\times 10^{-6}$) ppm	Carbon type	Assignment	HSQC δ_H ($\times 10^{-6}$) ppm	HMBC δ_H ($\times 10^{-6}$) ppm
176.72	OCO	a-C, a′-C	–	3.99
156.88	C	7-C	–	7.13, 6.86
131.19	C	10-C	–	7.13, 6.86
129.73	CH	9-C, 9′-C	7.13	6.86, 2.73
114.37	CH	8-C, 8′-C	6.86	7.13
73.04	CH_2	12-C	3.47	2.73
72.91	CH	b-C, b′-C	3.99	–
70.15	CH_2	6-C	3.94, 3.91	4.17, 3.05, 2.89
65.55	CH	5-C	4.17	3.94, 3.91, 3.05, 2.89
57.74	CH_3	13-C	3.19	3.47
49.33	CH	3-C	3.24-3.21	1.22, 1.21
47.51	CH_2	4-C	3.05, 2.89	4.17, 3.94, 3.91
34.45	CH_2	11-C	2.73	7.13, 3.47
19.25	CH_3	2-C	1.22	3.24-3.21
18.82	CH_3	1-C	1.21	3.24-3.21

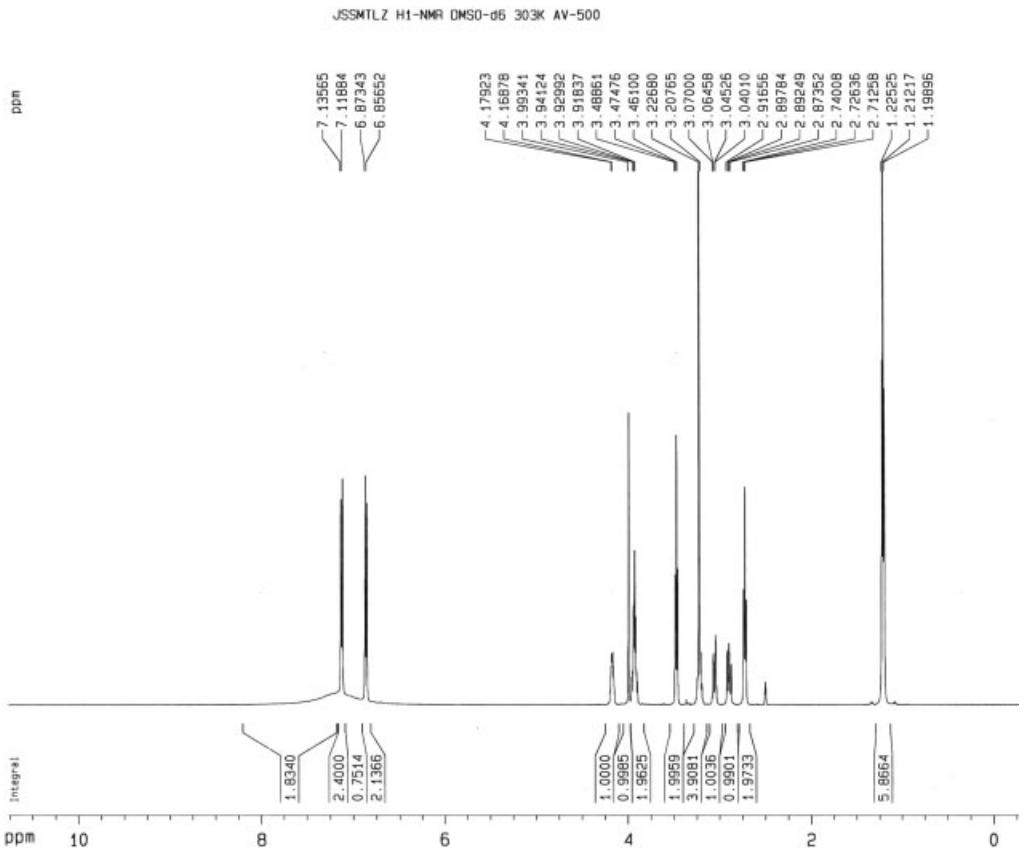

Figure 6. ^{1}H NMR spectrum of MT.

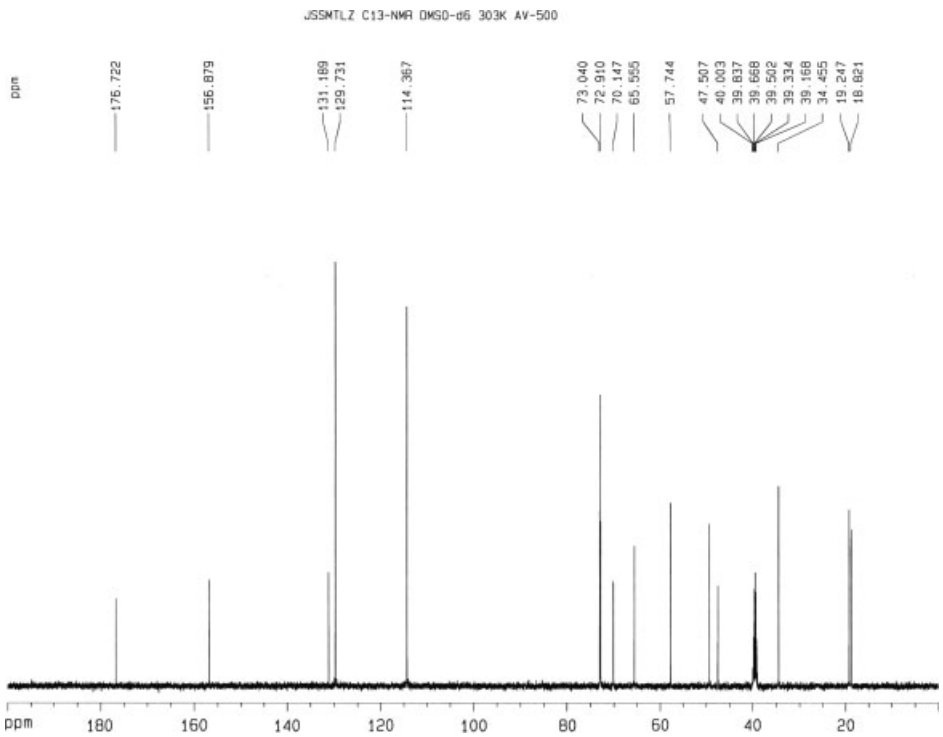

Figure 7. Full decoupling ^{13}C NMR spectrum of MT.

Table 3. Assignments of ^{1}H NMR signals of MT

^{1}H Chemical shifts δ_H ($\times 10^{-6}$) ppm	Multiplicity	Proton number	Coupling constant (Hz)	Assignment	^{1}H-^{1}H COSY δ_H ($\times 10^{-6}$) ppm
7.13	d	2	8.50	9-H, 9′-H	6.86
6.86	d	2	8.50	8-H, 8′-H	7.13
4.17	m	1	5.63, 5.62, 2.64, 2.42	5-H	3.94, 3.91, 3.05, 2.89
3.99	s	2	–	b-H, b′-H	–
3.91	dd	1	10.00, 5.62	6-1-H	4.17, 3.94
3.94	dd	1	10.00, 5.63	6-2-H	4.17, 3.91
3.47	t	2	6.85	12-H	2.73
3.24-3.21	m	1	6.50, 7.00	3-H	1.22, 1.21
3.19	s	1	–	13-H	–
3.05	dd	1	2.64, 12.00	4-1-H	4.17, 2.89
2.89	dd	1	9.42, 12.00	4-2-H	4.17, 3.05
2.73	t	2	6.85	11-H	3.47
1.22	d	3	6.50	2-H	3.24-3.21
1.21	d	3	7.00	1-H	3.24-3.21

References

[1] M. Remiszewska, Z. Jastrzebski, H. Czyzewska-Szafran, M. Wutkiewicz, *Eur. J. Pharmacol.* **1993**, *250*, 355.
[2] R. C. Ahuja, N. Sinha, R. Ravi Kumar, R. K. Saran, *Int. J. Cardiol.* **1993**, *41*, 191.
[3] MERIT-HF Study Group. *Lancet* **1999**, *353*, 2001.
[4] B. A. Groenning, J. C. Nilsson, L. Sondergaard, T. Fritz-Hansen, H. B. W. Larsson, P. R. Hildebrandt, *J. Am. Coll. Cardiol.* **2000**, *36*, 2072.
[5] *The United States Pharmacopoeia Convention, USP30-NF25*, United States Pharmacopeial: Rockville, MD, p. 2649.
[6] V. M. Karapandzic, B. D. Vujisic-Tesic, P. M. Pesko, B. M. Nenadic, D. D. Babic, *J. Clin. Anesth.* **2008**, *20*, 284.
[7] A. S. Go, J. R. Yang, J. H. Gurwitz, J. Hsu, K. Lane, R. Platt, *Arch. Intern. Med.* **2008**, *168*, 2415.
[8] M. Aqil, A. Ali, Y. Sultana, N. Saha. *Clin. Drug Invest.* **2007**, *27*, 833.
[9] M. Metra, L. D. Cas, J. G. F. Cleland, *J. Card. Fail.* **2006**, *12*, 177.
[10] M. Jasińska, B. Karwowski, D. Orszulak-Michalak, U. Kurczewska, *Acta Pol. Pharm.* **2009**, *66*, 697.
[11] K. V. Gowda, U. Mandal, P. S. Selvan, W. D. Sam Solomon, A. Ghosha, A. K. Sarkar, S. Agarwal, T. N. Raob, T. K. Pal, *J. Chromatogr. B* **2007**, *858*, 13.
[12] H. Y. Liu, J. J. Ren, Y. H. Hao, P. G. He, Y. Z. Fang, *J. Pharmaceut. Biomed.* **2006**, *42*, 384.
[13] M. Bharkatiya, R. K. Nema, A. Goyal, *Ind. Pharm.* **2009**, *8*, 79, 83.
[14] B. Magdalena, B. Dan, C. Ioana, I. Mihaela, L. B. Daniela, *Farmacia* **2008**, *66*, 363.
[15] G. P. Moloney, D. J. Craik, M. N. Iskander, T. L. Nero, *J. Chem. Soc., Perkin Trans. 2* **1998**, *2*, 199.
[16] S. H. Jung, P. T. Linh, H. K. Lim, H. J. Kim, K. H. Kim, J. S. Kang, *Arch. Pharm. Res.* **2000**, *23*, 226.
[17] M. S. Tehrani, M. T. Vardini, P. A. Azar, S. W. Husain, *Chin. J. Chem.* **2010**, *28*, 647.
[18] C. Slegers, A. Maquille, W. Deridder, E. Sonveaux, J. L. H. Jiwan, B. Tilquin. *Radiat. Phys. Chem.* **2006**, *75*, 977.
[19] M. Thevis, G. Opfermann, W. Schänzer, *Biomed. Chromatogr.* **2001**, *15*, 393.
[20] M. Aqil, A. Ali, A. Ahad, Y. Sultana, A. K. Najmi, N. Saha, *Acta Chromatogr.* **2007**, *19*, 130.