COMPLETE ASSIGNMENTS OF THE ^{1}H, ^{13}C AND ^{15}N SPECTRA FOR (±)-MONASTROL BY 1D AND 2D HR NMR TECHNIQUES

TERMINĂRI COMPLETE ALE SPECTRELOR ^{1}H, ^{13}C ȘI ^{15}N PENTRU (±)-MONASTROL PRIN TEHNICI RMN HR 1D ȘI 2D

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Elena GORINCIOI,
ORCID: 0000-0002-5087-2777
e-mail: elena.gorincioi@sti.usm.md
Institute of Chemistry, Moldova State University
Natalia CIOBANU,
ORCID: 0000-0002-1321-9277
e-mail: natalia.ciobanu@ichem.md
Institute of Chemistry, Moldova State University

Summary. *The current communication reports on the use of Nuclear Magnetic Resonance (NMR) Spectroscopy for full NMR characterization of monastrol, a well-explored synthetic molecule that has demonstrated significant biological effects of suppressing the motility of the mitotic motor protein kinesin Eg5. Our work has been aimed at complete NMR characterization of the title compound, since no available data in the literature were found, presenting the full assignment of ^{1}H, ^{13}C and ^{15}N nuclei in its structure. The combination of 1D and 2D HETCOR ^{1}H-^{13}C and ^{1}H-^{15}N NMR experiments spectra were employed to provide an unambiguous set of assignments. NMR characteristics for the nitrogen nuclei of N,N'-disubstituted thiourea fragment are presented for the first time.*

Keywords: *assignment, HETCOR, (±)-monastrol, NMR.*

INTRODUCTION

Monastrol (Ethyl 6-methyl-4-(3-hydroxyphenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate) is an important representative of 3,4-dihydropyrimidin-2-(1*H*)-ones (DHPM) and an attractive target molecule for organic chemists due to its remarkable biological properties. The pioneering discovery by T.U. Mayer *et al*. in 1999 of its role as a mitosis blocker by kinesin Eg5 inhibition paved the route for development of the new antitumor drugs on the basis of monastrol itself and other DHPM derivatives [1, 2]. The smartest method that is actually applied for monastrol's synthesis is the Biginelli multicomponent reaction (MCR), discovered in 1891 by Pietro Biginelli [3], which furnishes it as a racemate (Figure 1).

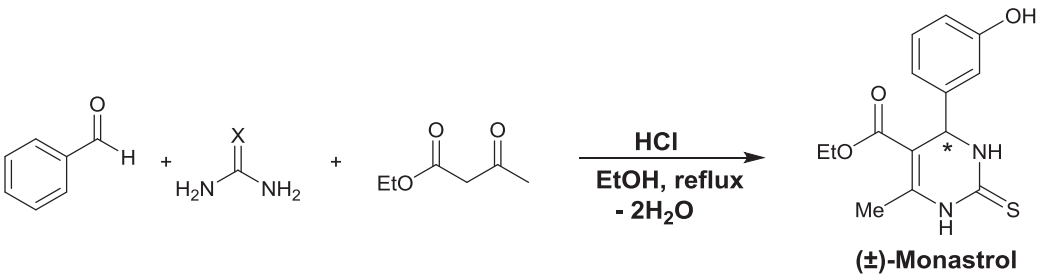

Figure 1. Scheme for preparation of *rac*-monastrol by using the Biginelli reaction.

Following the observation of the *S*-enantiomer's enhanced biological effects [4], efforts have been focused both on synthesizing it [5] and separating the enantiomers of *rac*-monastrol [6].

We have recently presented the results of our sustained effort to advance greener methods to expedite access to (±)-monastrol [7-10]. In continuation of our research line [11], we report herein on the full ^{1}H, ^{13}C and ^{15}N NMR characterization of the title-compound.

The proposed material may offer numerous educational benefits in teaching and learning chemistry, especially for advanced students in the master programs in organic/bioorganic chemistry, for which the necessity is impetuous of handling the NMR data obtained after acquiring the 1D and 2D NMR spectra on ^{1}H, ^{13}C and ^{15}N nuclei of a pure compound.

MATERIALS AND METHODS

NMR spectra were recorded on a Bruker Avance 400 spectrometer equipped with an inverse probe and *z*-gradient accessories and operating at constant magnetic field of 9.4 T. Sample was measured in 5 mm tube at 298 K with dimethyl sulfoxide-d_6 (DMSO-d_6) as a solvent. Chemical shifts (δ) are reported in parts per million (ppm) and are referenced to the residual non-deuterated solvent peak (2.50 ppm for ^{1}H and 40.00 ppm for ^{13}C). The 1D (^{1}H, ^{13}C and DEPT-135) and 2D homo- ($^{1}H/^{1}H$ COSY) and heteronuclear ($^{1}H/^{13}C$ gHSQC, $^{1}H/^{13}C$ HMBC and ($^{1}H/^{15}N$ HMQC) NMR experiments were performed through standard pulse sequences. The ^{15}N NMR chemical shifts are reported relative to liquid NH_3 [12]. Data analysis has been accomplished by using Bruker TOPSPIN software. (±)-Monastrol has been prepared according to a sustainable protocol [10], its physico-chemical characteristics being identical to the reported ones [13].

RESULTS AND DISCUSSION

Detailed NMR characteristics for (±)-monastrol have been obtained on the basis of its 1D (^{1}H, ^{13}C, DEPT-135) and 2D homo- ($^{1}H/^{1}H$ COSY-45) and heteronuclear ($^{1}H/^{13}C$ gHSQC, $^{1}H/^{13}C$ HMBC and ($^{1}H/^{15}N$ HMQC) correlation spectra. Presentation of the che-

mical shifts for the nitrogen nuclei of N,N'-disubstituted thiourea fragment has not been found in the literature, being herewith discussed for the first time. Figure 2 depicts the ^{1}H NMR spectrum of monastrol and the used atom numbering for description of the chemical shifts of the ^{1}H, ^{13}C and ^{15}N nuclei, as well. Experimental ^{1}H NMR data were in accordance with the reported in the literature [13]. In particular, ^{1}H NMR spectrum contains the broad singlet signals of the protons of the tetrahydropyrimidine moiety at the N-1 (10.32) ppm, and N-3 (9.63) ppm position, respectively, the broad singlet proton of phenolic group C-11 (9.50 ppm) and the multiplet signals of the aromatic protons from the positions C-9, C-10, C-8, C-12 (7.12;7.10; 6.66-6.63 ppm) too. The protons of the methyl group C-16 resonated as a singlet at δ 2.28 ppm, whilst protons for ethyl moiety were found as triplet at δ 1.12 ppm (t, J=7.5 Hz, 3H, C-15) and quartet at δ 4.03 ppm, (q, J.7.5 Hz, 2H, C-14).

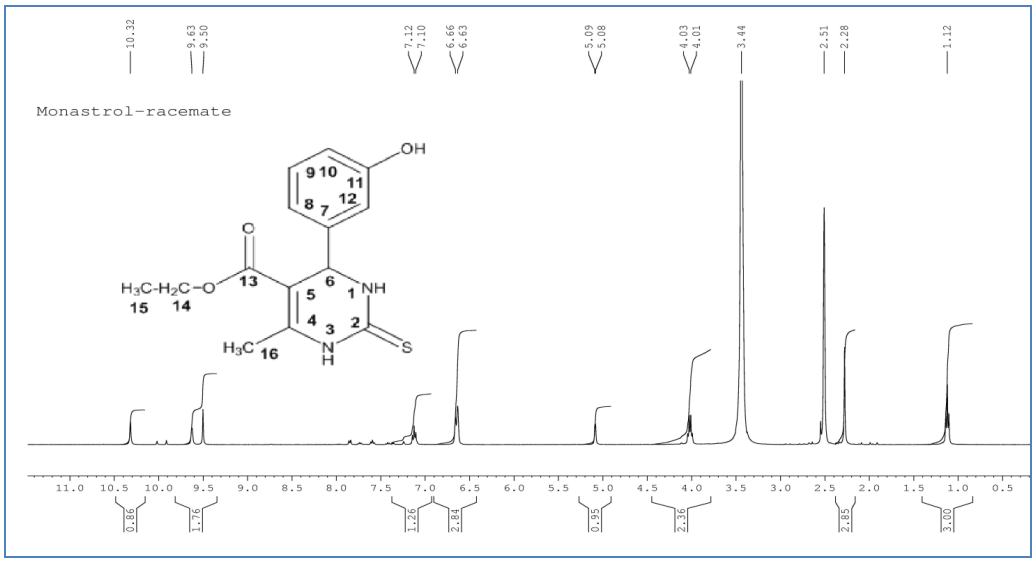

Figure 2. ^{1}H NMR spectrum for *rac*-monastrol

Comparative analysis of ^{13}C and DEPT-135 NMR experiments (Figure 3) allowed the differentiation of quaternary carbon atoms with sp2 hybridization from protonated carbons, with different degrees of protonation, namely: methyls (δ 14.50, C-15 and 17.61 ppm, C-16) and methine ((δ 54.38 ppm, C-6), δ 113.69, 115.08, 117.47 and 129.98 ppm, C-8, C-10, C-12 and C-9)), 7 signals totally, *versus* the only one signal of the methylene group from ethyl fragment (δ 60.09 ppm, C-14).

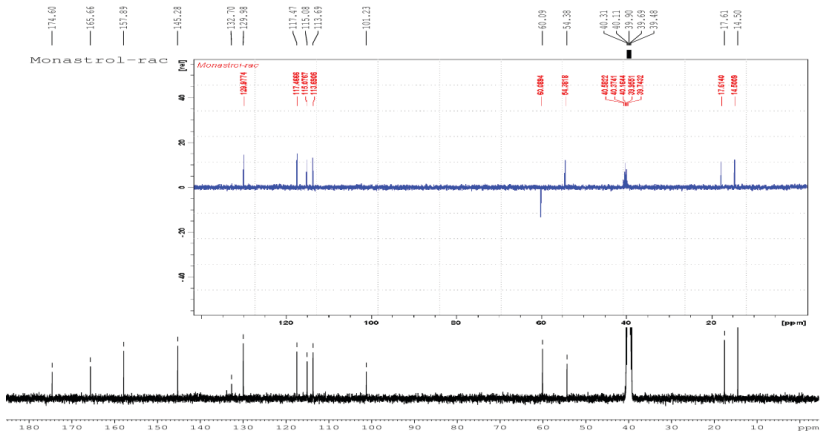

Figure 3. ^{13}C (black) and DEPT (blue) NMR spectra for *rac*-monastrol

In the ^{13}C NMR spectrum (Figure 3) the signals of the aromatic quaternary ring carbons are also present (δ 145.30, 157.89 ppm, C-7 and C-11), as well as signals for C-4 and C-5 (δ 101.23, 145.30 ppm (overlapped with the signal for C-7). The C-2 carbon of thioxo fragment resonated at 174.60 ppm, while the C-13 nucleus has been assigned the 165.66 ppm resonance. Unambiguous assignment for quaternary carbons has been achieved by use of HETCOR ^{1}H/^{13}C HMBC tecknique. All six quaternary carbon atoms showed heterocorrelations with the protons in the structure and thus characteristics of these carbon nuclei do not present any ambiguity. Figure 4 illustrates the ^{1}H/^{13}C HMBC NMR spectrum of monastrol. Some cross-peaks that were particularly relevant for assignments are, for example: δ 10.32/101.23 ppm, δ 2.28./101.23 ppm, δ 5.09/101, 23 ppm, which confirmed the attribution of the δ 101.23 ppm signal to the C-6 carbon atom; δ 2.28/165.66 ppm, δ 4.03/165.66 ppm, δ 5.09/165.66 ppm to the carbon C-13.

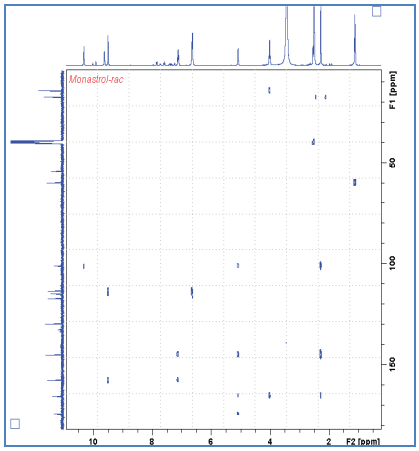

Figure 4. ^{1}H/^{13}C HMBC NMR spectrum of (±)-monastrol

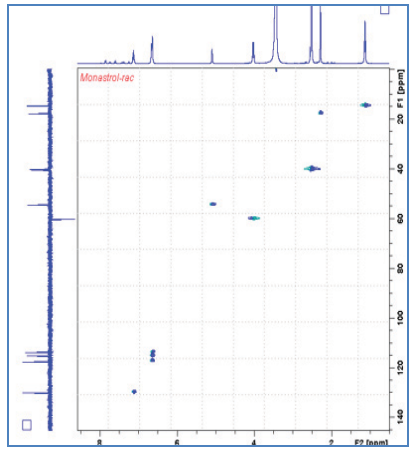

Figure 5. ^{1}H/^{13}C HSQC NMR spectrum of (±)-monastrol

The use of the $^{1}H/^{13}C$ HSQC NMR experiment allowed the distinct assignment of the resonances for all protonated carbon atoms. Specifically, the eight heterocorrelations found in the $^{1}H/^{13}C$ HSQC NMR spectrum: δ 1.12/14.50 ppm, δ 2.28/17.61 ppm, δ 4.03/60.09 ppm, δ 5.09/54.38 ppm, δ 7.12/129.98 ppm, δ 6.63/113.69 ppm, δ 6.66/115.08 ppm, δ 7.10/117.47 ppm (Figure 5) have successfully completed the structural NMR study for the title compound.

Five systems of spin-spin interactions were present in the $^{1}H/^{1}H$ COSY-45 NMR spectrum, (Figure 6), namely: the protons of ethyl group (δ 4.03/δ 1.12 ppm cross-peak), the methine proton at C-6 with the protons of C-16 methyl group (δ 5.09/δ 2.28 ppm weak cross-peak), aromatic C-8(H)-C-9(H)-C-10(H) protons (δ 6.63/δ 6.66/δ 7.12 ppm cross-peak), patterns of the protons of the tetrahydropyrimidine fragment: 3(N)H-C-6-(H) (δ 5.09/δ 9.63 ppm cross-peack), as well as the protons of the disubstituted thiourea fragment (δ 9.63/ δ 10.32 ppm cross peak).

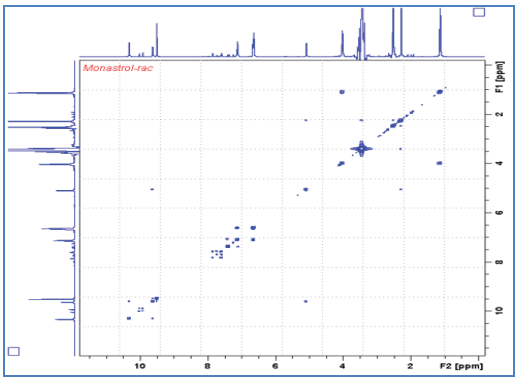

Figure 6. ^{1}H / ^{1}H COSY-45 NMR spectrum of (±)-monastrol

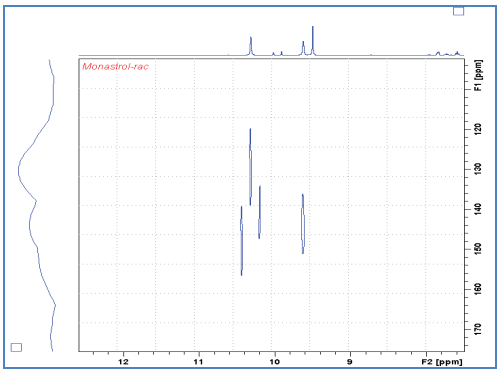

Figure 7. $^{1}H/^{15}N$ HMBC NMR spectrum of (±)-monastrol

It should be mentioned that the characteristics for the N(1) and N(3) nitrogen nuclei of the disubstituted thiourea fragment that is present in the monastrol structure have not been described in the specialized literature. By using the $^{1}H/^{15}N$ HMBC NMR experiment it was possible to detect and characterize these nuclei. Thus, from the long-range $^{1}H/^{15}N$ heterocorrelations at δ 10.32/130.7 ppm and δ 9.63/145.8 ppm, the N(3) and N(1) nuclei, respectively, were identified (Figure7).

CONCLUSIONS

In conclusion, the full ^{1}H, ^{13}C and ^{15}N NMR characterization of *rac*-monastrol has been described. The combination of 1D and 2D HETCOR ^{1}H-^{13}C and ^{1}H-^{15}N NMR techniques allowed providing a doubtless set of assignments for all nuclei. Presentation of the NMR characteristics for the nitrogen nuclei of N,N'-disubstituted thiourea fragment is unprecedented.

The offered herein example of structural characterization of a synthetic bioactive compound is focused on the use of NMR as one of the spectral techniques that it it indispensable for advanced students in organic chemistry. The paper demonstrates that NMR may successfully solve various structural problems, including detailed structural characterization on the basis of correct interpretation of the 1D and 2D NMR data. At the same time, the proposed herein example may be advantageously integrated into a practical course of organic & medicinal/pharmaceutical chemistry, by combination, for example, of experimental work for the synthesis of *rac*-monastrol by using the Biginelli MCR and green chemistry approaches. The associated lectures covering concepts of MCRs, catalysis, green chemistry, HR NMR would connect theoretical principles with their practical experiences. In virtue of the biological significance of monastrol, this molecule is rather appealing for laboratory practice classes with master students, whilst involvement of the adjacent NMR part to the lectures is intended to increase class interest considerably, complementing in a an optimal mode the curriculum.

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