STRUCTURAL INVESTIGATION OF DEXTROMETHORPHAN USING MASS SPECTROMETRY AND THERMAL ANALYSES COMBINED WITH MO CALCULATIONS

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ABSTRACT

(C₁₈H₂₅NO, mole mass = 271) was investigated using thermal analyses (TA) measurements (TG/DTG and DSC) compared with EI mass spectral (MS) electron impact fragmentation at 70 eV of electron energy. Semi-empirical MO calculations using the PM-3 procedure were performed with dextromethorphan (DMP) as a neutral molecule and the corresponding positively charged ion. These included molecular geometry (bond length), bond order, bond strain, charge distribution on different atoms, heat of formation, and ionisation energy. The mass spectral fragmentation pathways and thermal analyses decomposition were proposed and compared with each other to select the most suitable scheme. In electron ionisation (EI) mass spectral fragmentation, the initial rup-

ture is due to C₃H₇N (bridge) loss followed by C₉H₁₁O. In TA, the primary loss is due to loss of C₃H₇N Bridge + HBr (after H₂O loss of crystallisation). TA revealed a high response of the drug to the temperature variation with very fast rate. It decomposed in several sequential steps in temperature range from 100°C to 600°C. The initial thermal decomposition was similar to that obtained using MS. So, it is possible to select the proper pathway using both techniques. This comparison was successfully confirmed by MO calculation. The structural-reactivity effect on metabolism and the biological effect compared with codeine are discussed.

Keywords: Dextromethorphan structure, Mass Spectrometry, Thermal analyses, MO calculation

INTRODUCTION

Dextromethorphan (DMP, methoxy-methyl morphinan) is an antitussive agent used in many nonprescription cough and cold medications [1]. DM has an IUPAC name (+)-3-methoxy-17-methyl-(9α, 13α, 14α)- morphinan (C₁₈H₂₅NO, m/z=271) and is a synthetic product that is chemically related to codeine [2]. The drug is a safe, oral antitussive that is widely available [3 – 6]. The structural formula and numbering system of the drug is given in Fig. 1.

A number of methods have been reported to measure DM in biological fluids, including high-performance liquid chromatography (HPLC) [7, 8], gas chromatography (GC) [9, 10] and gas chromatography–mass spectrometry (GC–MS) [11–14, 1]. The latter technique is highly sensitive [5].

Rapid advances in biological sciences have led to an increased demand for chemical and structural information from biological systems. Mass spectrometry plays a pivotal role in the structural characterisation of biological molecules [15]. The technique is important because it provides a substantial amount of structural information using

a small amount of sample. Moreover, the techniques offer comparative advantages in speed and productivity for pharmaceutical analysis [16]. In contrast, thermal analysis delivers extremely sensitive measurements of heat change, which can be applied on a broad scale with pharmaceutical development. These methods provide unique information relating to thermodynamic data of the system studied [17]. The increasing use of the combined techniques in TA could provide more specific information and thus facilities a more rapid interpretation of the curves obtained [17].

In the electron ionisation (EI) mass spectra, the fragmentation consists of competitive and consecutive unimolecular fragmentation [18]. The fragmentation of the ionised molecule depends mainly on their internal energy [19]. The thermo-gravimetric TG/DTG analysis provides quantitative information on weight losses due to decomposition and/or evaporation of low molecular weight fragments as a function of time and temperature. In conjunction with mass spectrometric analysis [20–22], the nature of the released fragments

Dextromethorphan drug (DMP)

Figure 1. Structure and numbering system of DMP for, C, N and O skelton.

may be deduced, thus greatly facilitating the interpretation of thermal degradation processes. In contrast, the computational quantum chemistry could provide additional information about the atoms and bonds, which could be used successfully in an interpretation of experimental results [23]. The application of the computational quantum chemistry in addition to experimental results (MS and TA) gives valuable information about the atoms and bonds, which helps in the description and prediction of the primary fragmentation site of cleavage and subsequent ones [24–28].

Although the literature is wealthy in information related to the biological activities of DMP and its metabolites *in vivo* and *in vitro* [3–6], there seems to be a lack of any correlation between chemical behaviour and its electronic structure.

The main aim of this work was to perform an experimental and theoretical investigation of DM using thermal analyses (TA) and EI mass spectral (MS) fragmentation at 70 eV. In addition, MO calculations were performed using the PM3 procedure on the neutral molecule and charged molecule to investigate geometrical parameters (bond length, bond order, bond strain, heats of formation ionisation energy) and charge distribution.

The calculations correlated the experimental results [TA and MS] to obtained information regarding the stability of the drug and the prediction of the site of primary fragmentation and subsequent ones. This study could be helpful in establishing a quantitative and qualitative structure–activity relationship for the drug, which is the main objective of this work. It is worth mentioning that there is scant literature concerning such a comparative study.

Moreover, the authors tried to correlate and discuss the structure reactivity and relationship between DM and Codeine [26], which are chemically related to each other [2] and which affect the O and OH on MS and TA and MO parameters. In addition, they attempted to find the metabolite structures in the mass spectrum and correlated the biological behaviour of the two drugs.

EXPERIMENTAL

Mass spectrometry (MS)

The electron ionisation (EI) mass spectrum of DMP was obtained using a Shimadzu GC-MS-Qp 1000 PX quadruple mass spectrometer with an electron multiplier detector equipped with the GC-MS data system. The direct probe for solid material was used in this study. The sample was put into a glass sample micro vial by a needle ($\approx 1 \mu\text{g}$ max). The vial was installed on the tip of the DP containing the heating cable and was inserted into the evacuated ion source. The sample was ionised by an electron beam emitted from the filament with the generated ions being effectively introduced into the analyser by the focusing and extractor lens systems. The MS was continuously scanned, and the spectra obtained were stored. The electron ionisation mass spectra were obtained at an ionising energy value of 70 eV, ionisation current of 60 μA and a vacuum greater than 10^{-6} torr.

Thermal analysis (TA)

The thermal analyses of DMP were made using a conventional thermal analyser (Shimadzu system of DTA-50 and 30 series TG-50). The mass loss for 5 mg of sample and the heat response to changes in the sample were measured from room temperature up to 600°C. The heating rate in an inert argon atmosphere was 10°C min⁻¹. These instruments were calibrated using indium metal as a thermal stable material. The reproducibility of the instrument reading was determined by repeating each experiment more than twice.

Quantum chemical calculations

The MO calculations were performed using a semi-empirical molecular orbital calculation. The method used in these computations was the parametric PM-3 method described by Stewart [29]. The geometry of all stable species studied was completely optimised with respect to all geometrical variables using the Eigen vector following (EF) routine [30]. The program was run under the molecular orbital calculation package MOPAC2000 by Stewart [31] for microcomputers.

RESULTS AND DISCUSSION

The chemistry and reactivity of pharmaceutical drugs are of great interest because of their importance in treating various diseases. Knowledge of the thermal decomposition mechanism of the drug is very important to understand the chemical processes in biological systems. It is difficult to establish the exact major fragmentation pathway in EI using conventional MS. However, the combination of experimental techniques (TA and MS) and MO calculation is very important to understand the following topics:

- 1 -The primary site fragmentation process and its major fragmentation pathways in both techniques.
- 2 -The stability of the drug as a neutral molecule in the solid-state phase and molecular ion in the gas phase.
- 3 -Selection of the most probable decomposition pathway in both TA and MS.

Thermal analysis (TA)

The TG/DTA curves of DMP (Fig. 2) were displayed between 25°C and 600°C. The drug has the general formula $C_{18}H_{25}NO.HBr.H_2O$ in solid-state phase with a molecular weight (MW) of 370.3 and melting point (m.p.) $\cong 125^\circ\text{C}$.

It is clear from the thermal survey of the drug that there are four mass losses in the TGA (Table 1). The first one appeared at 100-150°C with a mass loss % of 4.1, which may be attributed to the loss of water present in the drug (cal. mass loss % = 4.8). The second mass loss was approximately 32.5% and occurred at 150-350°C. The third one was 54.57% and occurred at 350-450°C. The final one was 5.68% and occurred at 450-600°C, which may be attributed to the loss of the remainder. These mass losses were observed at the three main peaks at values: 120.8°C, 287.2°C and 378.6°C in DTG, respectively. These mass losses also appeared as two endothermic peaks in the DTA. The first one appeared at 110.77°C, requiring an energy $E_1 = +242.6$ J/g, and the second one appeared at 362°C, requiring an energy $E_2 = +356.75$ J/g. The fragmentation of this compound and its chemical changes occurring after this thermal degradation appeared at 555.56°C as a very strong exothermic peak requiring an energy $E_3 = 513.5$ J/g.

Mass spectral behaviour of dextromethorphan (MS)

The electron impact (EI) mass spectrum for DMP drug at 70 eV was recorded and investigated. A typical mass spectrum (barograph) of the drug is shown in Fig. 3.

Scheme 1 shows the proposed principal fragmentation pathways of DMP following electron impact at 70 eV. In addition, prominent ions and their relative intensities with some common ions formed from codeine [26] are listed

Fig.2a : TGA curve of Dextromethorphan Hydrobromide

Fig.2b : Dr.TGA of Dextromethorphan Hydrobromide

Fig 2 C: DTA of Dextromethorphan Hydrobromide

Table 1: Structure identification of Dextromethorphan Hydrobromide

TGA		DTA		
Wt. loss%	Temp.range °C	Peak temp. °C	Description	E J/g
4.10	100-150	118.77	Endothermic	+242.6
32.50	150-350	367.6	endothermic	+365.75
54.57	350-450	555.56	St.exothermic peak	-613.5
5.68 Σ Wt.loss %=96.85	450-600			

*Dr. TGA gives three main peaks temperatures at 120.58, 287.23 and 378.62 oC.

Scheme (1): Mass spectral fragmentation pathways of Dextromethorphan

Fig. (3): Mass spectrum of DMP at 70 eV.

Dextromethorphan				Codeine		
m/z	R.I.	Structure	Process	m/z	Process	R.I.
271	31.6	$C_{18}H_{25}NO^+$	M^+	299	M^+	100%
270	20.3	$C_{18}H_{24}NO^+$	$[M - 1]^+$	298	$[M - 1]^+$	14.3
214	26.6	$C_{15}H_{18}O^+$	$[M - C_3H_7N]^+$	242	$[M - C_3H_7N]^+$	9.3
150	83.0	$C_9H_{12}NO^+$	$[M - C_9H_{13}]^+$			
82	12.5	$C_6H_{10}^+$	$[M - C_3H_7N - C_9H_8O]^+$			
59	100%	$C_3H_9N^+$	$[M - C_{15}H_{16}O]^+$			

Table 2: The prominent ions in the mass spectrum of DMP drug in comparison with codeine drug [26].

in Table 2. The mass spectrum of DMP showed that the molecular ion ($C_{18}H_{25}NO^+$, $m/z = 271$) signal represented approximately 30% of the base peak (= 100%) at $m/z = 59$ [$C_3H_9N^+$], indicating a moderate stability of the molecular ion in gas phase compared with codeine [26].

The main fragmentation pathway for DMP after ionisation at 8.83 eV consists of three principal pathways (paths 1-3) as rationalised in scheme 1. The formation of the fragment ion at signal $m/z = 214$ (path 1, scheme 1) with R.I. = 26.6% is mainly due to the formation of $[M-C_3H_7N]^+$ as a loss of the C_3H_7N bridge by disruption of the C_2-C_{18} and $C_{10}-C_{17}$ bonds. It is worth mentioning that this fragment ion, $C_{15}H_{18}O^+$ ($M-C_3H_7$), was formed directly from the molecular ion. This formation was confirmed by the full scan positive ion mass spectrum using tandem mass spectrometry (MS/MS) [6] as a prominent peak. Subsequent cleavage of the C_7-C_2 and C_1-C_{10} bonds (ring B) occurred forming the $C_6H_{10}^+$ fragment ion ($m/z = 82$, R.I. = 12.5%) (path 1 scheme 1).

Two important possible modes of fragmentation (paths 2 and 3 scheme 1) are due to the cleavage of the two bonds β to the nitrogen atom (i.e., cleavage of C_1-C_{10} and C_9-C_{10} competitively forming two important fragment ions in the mass spectrum.) First, cleavage of the C_1-C_{10} bond and formation of the most prominent fragment (the base peak) at $m/z = 59$ (path 2) of formula C_3H_9N . Also, cleavage of the C_9-C_{10} bond formed the second prominent peak at $m/z = 150$ (R.I. = 83.0%) with the structure $C_9H_{12}NO$ (path 3).

Structural determinations for the compounds of unknown structure are generally based upon information obtained through the use of a variety of modern instruments. The instrumental methods usually include mass spectrometric studies, and the techniques are well known to organic chemists and scientists [32].

It is known that DMP is chemically related to codeine [2] but that it differs in biological effect. DMP is an antitussive agent used in many nonprescription cough and cold medications [1]. Codeine has an analgesic effect [26]. Biotransformation (metabolites) *in vivo* and *in vitro* of the two drugs has been investigated by many investigators [3–6]. Metabolism studies often lead to compounds with unknown structures that are related to the material under study. The chemical structure and metabolic pathway of DMP were rationalised [5] in Fig. 3.

The metabolism of DMP is primarily by *o*-demethylation to dextrophan. DMP is also metabolised to 3-methoxymorphinan and 3-hydroxymorphinan.

It was noted that from mass spectrum of DMP, the signal due to C_2H_4 loss was observed with minor abundance $m/z = 243$, R.I. = 1.21, which may be due to the formation of hydroxymorphinan.

Comparing the fragmentation pathway of DMP to codeine [26], one can notice that the two drugs are similar in the initial processes (i.e., loss of the bridge C_3H_7N and the ring opening of the two bonds β to the nitrogen atom). The subsequent fragmentation differed because of the presence of different substituent groups (O and OH).

Computation

Molecular orbital calculations gave valuable information regarding the structure and reactivity of the molecules and molecular ions. The computational data supports the experimental data. The parameters calculated using the MO calculation include geometry, bond order, bond strain, charge distribution, heat of formation and ionisation energy.

The investigation of the molecular structure of DMP with the common formula $C_{18}H_{25}NO$ was of interest in this work, and this investigation aimed to predict the weakest bond cleavage and the stability of the neutral molecule by TA and the charged molecular by MS.

Table 3 shows the comparison of computed bond length, bond order and bond strain using the PM3 method for neutral and molecular cationic forms. Table 4 shows a comparison of computed partial charges of neutral and charged species. The computations reveal some important results.

- 1 The charge density localised on the nitrogen atom increased from -0.076 to 0.489 from a neutral to charged atom, which reveals that the electron disruption upon ionisation at 8.83 eV occurs in the nitrogen atom. No appreciable change in the charge on the other atom was due to ionisation of the molecule.
- 2 C_2-C_{18} is the lowest bond order for neutral molecule (0.964) and charged cationic form (0.967).

Correlation of TA, MS and MO-calculations.

DMP is an essential cough and cold medication [1]. The drug is chemically related to codeine [2]. As indicated previously [32], a determination of initial bond cleavage is an important first step.

The scope of this investigation was restricted to a search or prediction and discerned the features of initial bond disruption during the course of fragmentation of DMP. Empirical observations indicate that the course of subsequent fragmentation is determined to a large extent by the initial bond disruption of the molecular ion in MS [34]. It is quite acceptable to say that the computational quantum chemistry can provide additional data, which can be used successfully to interpret both TA and MS experimental results. These theoretical data are particularly valuable for mass spectral scientists. These scientists study gas-phase species, which can be handled much more easily by quantum chemistry [33].

The mass spectrum of DMP in gas-phase ion revealed three competitive processes (1 – 3, scheme 1).

PM3 calculation (Table 3) revealed that $C_{10}-N_{17}$ was the weakest bond in the charged system (weakest bond order at 0.961, large bond length = 1.484 Å and bond strain). Moreover, the weakness of this bond ($C_{10}-N_{17}$) was due to the presence of a lone pair on the nitrogen. Table 3 shows that the electron disruption upon ionisation occurred from the nitrogen, which increased the weak-

Bond	Bond length (Å)		Bond order		Bond strain (k Cal/mol)	
	Neutral	Ionic	Neutral	Ionic	Neutral	Ionic
C1-C2	1.541	1.542	0.965	0.970	0.206	0.189
C1-C6	1.525	1.531	0.984	0.983	0.049	0.208
C1-C10	1.533	1.542	0.973	0.962	0.049	0.101
C2-C3	1.548	1.547	0.969	0.971	0.457	0.639
C2-C7	1.518	1.520	0.969	0.974	0.252	0.290
C3-C4	1.519	1.519	0.993	0.996	0.034	0.071
C4-C5	1.518	1.515	0.992	0.997	0.107	0.004
C5-C6	1.526	1.522	0.987	0.992	0.174	0.095
C7-C8	1.403	1.405	1.376	1.365	0.082	0.140
C7-C14	1.395	1.393	1.424	1.442	0.084	0.097
C8-C9	1.493	1.491	0.990	0.997	0.035	0.058
C8-C11	1.396	1.397	1.409	1.401	0.044	0.047
C9-C10	1.533	1.535	0.971	0.959	0.073	0.115
C11-C12	1.388	1.387	1.429	1.444	0.007	0.003
C12-C13	1.389	1.401	1.373	1.360	0.015	0.010
C13-C14	1.400	1.405	1.371	1.351	0.012	0.015
C13-C15	1.382	1.372	1.029	1.067	0.038	0.038
C15-C16	1.406	1.409	0.987	0.980	0.008	0.009
C10-N17	1.503	1.484	0.972	0.961	0.226	0.192
C2-C18	1.545	1.547	0.964	0.967	0.118	0.178
C18-C19	1.524	1.525	0.990	0.980	0.075	0.031
N17-C20	1.480	1.450	0.997	1.013	0.057	0.063
C19-N17	1.492	1.460	0.980	0.994	0.092	0.059

Table 3: Comparison of computed bond length, bond order and bond strain (k Cal mol⁻¹) using PM3 method for neutral and molecular cation.

ness of the bond. After an easy disruption of this bond, the subsequent bond is C₂-C₁₈ (bond order = 0.967, bond length = 1.547 Å and bond strain = 0.178 K cal mol⁻¹). Disruption of these two bonds (C10-N₁₇ and C₂-C₁₈) formed a fragment ion at m/z = 214 by loss C₃H₇N (bridge) from the molecular ion. The subsequent loss of C₉H₁₁O (rupture of the B ring) formed the fragment ion C₆H₁₀ at m/z = 82, which can lead to a loss of H₂ to form the fragment ion C₆H₈ at m/z = 80 (process 1, scheme 1). One can conclude that process 1 is the principal one for DMP, which can help to interpret TA decomposition.

The primary TA decomposition of DMP (wt loss = 4.1%) between 100 °C and 150°C was mainly due to loss of water. It was followed by a mass loss of 32.5% between 150 °C and 350°C. On the basis of the MO calculation (Table 2), C₂-C₁₈ was the first bond cleavage of neutral system having the lowest bond order at 0.964 with a long bond length (1.545 Å) and large bond strain (0.118 k cal mol⁻¹) followed by C₁₀-N₁₇ (bond order = 0.972, bond length = 1.503 Å and bond strain = 0.226 k cal mol⁻¹). As a result of the disruption of these two bonds, C₂-C18 and C₁₀-N₁₇ lost the bridge C₃H₇N. The wt loss of 32.5%

resulted from the bridge and HBr molecule (C₃H₇N + HBr). The subsequent third mass loss of 54.57% occurred at 350-450°C and may be attributed to the loss of OCH₃C₆H₄CH₂CH₂ (i.e., C₉H₁₁O) via the cleavage of the B-ring (C₂-C₇ and C₁-C₁₀).

The final mass loss of 5.68%, which occurred at temperature range 450-600°C, may be attributed to the loss of the remainder of the drug molecule. It is possible to rationalise the thermal degradation scheme 2 as follows.

The dextromethorphan (DMP-HBr) neutral compound is stable up to 150°C before being fragmented by the loss of the bridge C₃H₇N + HBr, whereas codeine fragments at 160°C [26]. In contrast, the molecular ion of DMP is RI = 30%, while the molecular ion of codeine is considered the base peak (RI = 100%). This high stability of codeine may be due to the presence of OH and O atoms in its skeleton.

This work provides further insights into utility of experimental TA and MS techniques and a theoretical investigation with MO calculation using the semi-empirical PM3 procedure on DMP. From the practical and theoretical techniques, we concluded that the primary fragmentation of DMP is initiated by the loss of C₃H₇N + HBr in

Scheme (2) : Thermal Decomposition of Dextromethorphan Hydrobromide Drug

Atom	Partial Charge	
	Neutral	cation
C1	- 0.073	- 0.072
C2	0.013	0.014
C3	- 0.107	- 0.106
C4	- 0.093	- 0.097
C5	- 0.091	- 0.096
C6	- 0.098	- 0.094
C7	- 0.019	- 0.047
C8	- 0.102	- 0.174
C9	- 0.080	- 0.050
C10	- 0.048	- 0.144
C11	- 0.073	- 0.042
C12	- 0.181	- 0.164
C13	0.087	0.124
C14	- 0.150	- 0.142
O15	- 0.189	- 0.177
C16	0.051	0.047
N17	- 0.076	0.489
C18	- 0.095	- 0.116
C19	- 0.077	- 0.169
C20	- 0.094	- 0.198

Table 4: Comparison of computed partial charge using PM3 procedure for neutral and molecular ion for DMP drug.

TA and C_3H_7N in MS. MO-calculation confirmed these processes. Subsequent cleavage in both techniques is due to the cleavage of the B ring and loss of the neutral fragment $C_9H_{11}O$. DMP can be completely dissociated between 150 and 600°C in TA, while in MS, the molecule

can be fragmented after a few electron volts above the ionisation energy of 8.83 eV. Theoretical calculation can help to select the most probable fragmentation pathway in both techniques.

Signal at $m/z = 243$ and R.I. = 1.2% may have the structure $C_{16}H_{21}NO$ (hydroxymorphinan structure), which is the metabolite of DMP by the loss of $CH_2 + CH_2$.

Compared with codeine, we noticed the following occurrences due to the presence of O and OH atoms.

- 1 -DMP is less stable than codeine in TA (decomposes $\cong 10^\circ C$ before codeine).
- 2 -The stability of the molecular ion of DMP in gas phase (MS) is about 30% compared with codeine (100%).
- 3 -The biological behaviour of DMP is significantly different from codeine due to the presence of two excess O-atoms in the skeleton of the latter, which may help its transport to the brain and its differential effect.

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