International Journal of Mass Spectrometry 286 (2009) 43–52

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Gas phase self-association of Eudistomin U controlled by gas phase acidity and origin of its interaction with nucleobases

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ARTICLE INFO

Article history:
Received 17 March 2008
Received in revised form 15 May 2009
Accepted 18 May 2009
Available online 23 May 2009

Keywords:
β-carboline
Nucleobase
Gas phase acidity
Self-association
Stability

ABSTRACT

Electrospray ionization mass spectrometry was used to investigate the intrinsic chemical properties of β-carbolines: Eudistomin U (as EU) and 5-Br-Eudistomin U (as BrEU) yielding self-association in negative ion mode. It was observed that the deprotonated homodimer of EU was more stable in the gas phase than BrEU. Indeed, BrEU presents a higher polarizability that could influence the dimerization energy. On the contrary, the stability energies calculated at B3LYP/6-31+G*//AM1 level showed a small difference for these two dimers. To understand the experimental stability variation, the experimental and theoretical gas phase acidities of these two β-carbolines have been estimated. It has been shown that EU is less acidic than BrEU in the gas phase, which could explain the lower stability of the $[2BrEU-H]^-$ dimer. In addition, the investigation of non-covalent interaction of β-carbolines and nucleobases has been carried out. It was experimentally demonstrated that the deprotonated heterodimer's relative stability scale was $[Gua+M-H]^- \gg [Thy+M-H]^- > [Ade+M-H]^-$, but the Cyt/β-carboline complexes were not observed. However, the absence of Cyt/β-carboline complexes could be explained by the great acidity difference between Cyt and β-carbolines, yielding unstable non-covalent complexes. The studied β-carbolines showed a particular affinity with guanine which suggested a strong interaction, from a structural and reactivity point of view. Calculated reaction pathways rationalize the set of experimental results concerning the heterodimer stabilisation reflected by the $V_{1/2}$ value of breakdown curves of deprotonated heterodimers.

1. Introduction

Eudistomin U is a marine alkaloid isolated from the genus *Lissoclinum fragile* of Caribbean ascidian [1]. This natural alkaloid is structurally similar to β-carbolines. It has been shown that this family of alkaloids is capable to bind DNA, and presents an efficient anti-bacterial activity. The particular property of these β-carbolines toward the interaction with DNA could be a possible reason to rationalize its effect as a drug against cancer [2–5].

It has been shown that DNA-binding drugs interact with DNA duplex in two main ways [6]: either through minor groove binding or major groove intercalation [7,8]. In fact, minor groove binding involves mainly hydrogen-bonding whereas major groove intercalation results mainly from stacking effects. Especially, it is considered that interactions between β-carbolines and DNA could explain the anti-tumor activity of this drug by influencing the cell proliferation [2]. Ten years ago, the first synthesis of β-carboline was performed by Molina et al. [9]. More recently, Wen and coworkers [10] prepared Eudistomin U (noted as EU) and its analog, such as bromide substituted Eudistomin U (noted as BrEU) to explore their possible improved activities as "lead compound" or drug candidate against cancer disease for future pharmacological strategies.

As an efficient analytical approach, mass spectrometry has been used in drug discovery for decades. With the advent of soft desorption/ionization methods such as electrospray ionization (ESI) [11] and matrix-assisted laser desorption ionization (MALDI) [12] techniques, mass spectrometry is considered as a useful analytical tool for studying biomolecules. It has been extended to all stages of drug discovery, including target identification and characterization, structure elucidation of synthetic compounds and early drug metabolism and pharmacokinetics, etc. The ESI mode, under soft desolvation energy condition, is able to preserve non-covalent associations from the solution to the gas phase [13–15]. Furthermore, this ionization mode has been used for instance to study specific intermolecular interaction such as specific self-

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doi:10.1016/j.ijms.2009.05.003

association between DNA and DNA-binding drug with anti-tumor, anti-viral or anti-bacterial activity. As a sensitive and specific technique, ESI-MS can provide binding stoichiometry, binding affinity, specificity, and elucidation of the structure of the complex from relatively small amounts of material. Such an approach allows comparing drug selectivity and specificity toward DNA [15]. The relative binding strengths can be estimated by mass spectrometery in two approaches: (i) changing the skimmer voltage for producing different ion desolvation conditions [16], and (ii) using MS/MS experiments (with collision induced dissociation, CID) of non-covalent DNA/ligand complexes free of solvent [17] under various collision energy conditions. With these methods, it is possible to evaluate not only the binding mode and binding site location of drugs with DNA, but also their structure–activity relationship.

In this work, the study of intrinsic chemical properties of β-carbolines (M being either EU or BrEU) and their non-covalent complexation with nucleobases are described as a preliminary step prior to investigate their interaction strength toward doubly strand DNA. Our goals are to: (i) enlighten the substituent effects on the stability of β-carboline homodimers and self-association, especially on its conformation interaction, and (ii) investigate the DNA binding origin of the studied compounds. As shown recently, several thermochemical parameters such as basicity/acidity of drug [15] as well as of the nucleobases, may influence dsDNA/drug interaction strength and orient gas phase dissociations [7,15,18]. For such purposes, the gas phase stability of the $[2M-H]^-$ homodimers prepared from EU and BrEU were compared by CID experiments under different energy conditions. Based on the Cooks' kinetic method [19] as extended by Fenselau [20], and following the *alternative* treatment of Armentrout [21] their respective gas phase acidity was determined. To investigate the mechanism of non-covalent association, similar CID experiments were performed from non-covalent $[base+M-H]^-$ complex. A binding-affinity order can be experimentally established to obtain an association trend in the case of nucleobases. In addition, the experimental results have been compared with DFT calculations providing β-carbolines' homodimer conformation, estimation of their gas phase acidity as well as the stability of heterodimer $[base+M-H]^-$.

2. Experimental

2.1. Syntheses

From tryptamine and indole-3-aldehyde as starting materials, through condensation, Pictet–Spengler cyclization and dehydrogenation three steps, the alkaloids and its derivatives were prepared [10,22].

2.2. Sample preparation

Synthesized β-carboline derivatives [10] (stock solution 1 mg/mL in $CHCl_3$/MeOH 1:9) were dissolved in MeOH with a final concentration of 20 pmol/μL. β-carbolines were mixed with nucleobase in equimolar proportion for non-covalent interaction study.

2.3. Mass spectrometry

2.3.1. Triple quadrupole instrument

A modified triple quadrupole (Micromass Quattro I, Manchester, England) equipped with an ESI ion source was operated in the negative ion mode. The electropsray high voltage was set to −3000 V and the cone voltage was 30 V. The collision gas was argon (10^{-4} mBar), the collision condition E_{lab} was from 5 eV to 50 eV, syringe pump was used to infuse the sample into the ESI source at a flow rate of 400 μL/h. The presented spectra are the average of 50 scans.

2.3.2. Ion trap instrument

An ion trap mass spectrometer (Esquire 3000, Bruker, Bremen, Germen) equipped with an Agilent orthogonal ESI source was used in negative ion mode. Soft desolvation conditions have been used in order to preserve the non-covalent specie properties. The source parameters are: capillary + 3500 V, capillary exit −55 V, skimmer −15 V, LMCO 48 Th. Nitrogen was used as drying gas using a flow rate of 7 L min^{-1} and a temperature of 250 °C. Sample solutions were infused using a syringe pump into the ESI source at a flow rate of 200 μL h^{-1}. The presented spectra are the average of 50 scans.

Experimental errors (standard deviations) were calculated from experimental deviations and taking into account uncertainties of the reference acidity values based on the Armentrout treatment. More recently, Armentrout considered that errors in the ΔH° values are in the order of ±4 to ±12 kJ mol^{-1} (±9 to ±30 J mol^{-1} K^{-1} for activation entropy differences) whereas Drahos et al. suggested an estimated error of ± 5 kJ mol^{-1} (±10 J mol^{-1} K^{-1} for $\Delta\Delta S^\circ(A_0,A_i)$) [23,24]. These latter error values are considered in this work and in addition experimental relative deviation is provided under brackets.

2.4. Theoretical procedure

2.4.1. Acidity calculations

Acidity calculations have been performed for carbolines EU and BrEU after full geometry optimization at B3LYP/aug-cc-pVTZ//B3LYP/6-31 + G* level using Gaussian 98 package [25] with ZPE corrected energy values [26]. Introduction of the translational enthalpy for the proton at 298 K has been carried out following published procedures [27].

2.4.2. Dimer stabilization and transition state for proton migration

Due to the size of these molecules, energies were calculated from a single point at B3LYP/6-31 + G*//AM1 level. Critical points (reactants, transition-state structures, intermediates, and product ions) were fully characterized as minima or first-order saddle points by diagonalizing the Hessian matrices of the structures at the corresponding level of optimization (AM1). For the various tautomers, the most stable species proposed by Kenttamaa and co-workers for the deprotonated forms of Gua and Ade and of Thy and Cyt have been used [26,28].

3. Results and discussion

The potentiality to preserve non-covalent association preexisting in solution is mainly achieved by using electrospray ionization. However, the binary complexes detected from the gas phase are not necessarily specific as those existing in solution. In fact, during the desolvation of ionic aggregate complexes, multimeric associations can survive and have a sufficiently long life-time to allow their detection as well as the investigation toward their dissociations after collisional ion activation.

3.1. Relative stability of the deprotonated β-carboline dimers formed by self-assembly

The selected β-carbolines analyzed in the negative ion mode by electrospray are characterized by mass spectra which mainly displayed both the deprotonated monomer $[M-H]^-$ and homodimer $[2M-H]^-$ species. To study their dimerization process and relative stability, the gas phase thermochemical properties of the studied compounds were investigated. The CID experiments performed on

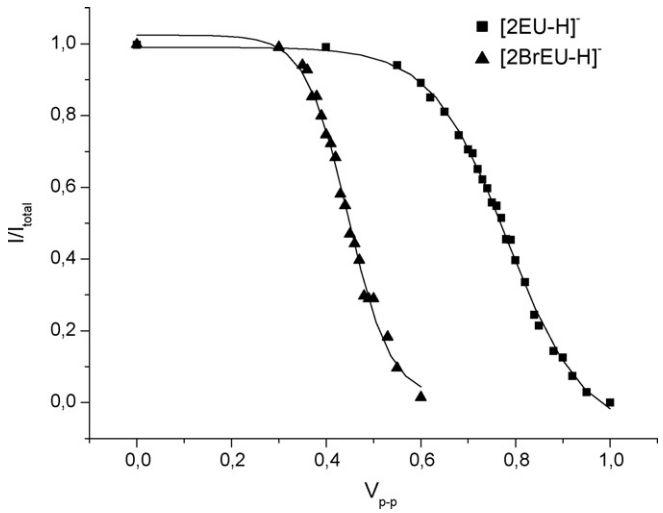


Fig. 1. Breakdown curves of deprotonated [2M−H]⁻ homodimer: *m*/*z* 565 for [2EU-H]⁻ (■) and *m*/*z* 721 for [2BrEU-H]⁻ (▲), obtained under resonant excitation conditions (desolvation conditions: capillary exit offset set to 55 V and skimmer voltage set to 15 V into the ion trap mass spectrometer).

the anionic [2M−H]⁻ homodimers will provide information on the relative stability of the deprotonated dimeric β-carboline species into the ion trap cell. In order to obtain this information, variation of the resonant excitation amplitude (V_{exc}) was carried out. This ion activation yields breakdown curves allowing evaluation of the relative gas phase stability of the studied anionic β-carboline self-assemblies.

The breakdown curves in Fig. 1 have been performed by plotting the relative abundance of the surviving [2M−H]⁻ precursor ions as a function of the resonant ion excitation amplitude.

The gas phase stabilities of the deprotonated β-carboline dimers were ranked in relative term by their half-wave resonant excitation amplitude (noted as $V_{1/2}$) corresponding to the voltage required to decompose 50% of the deprotonated homodimer precursor. It appears that the gas phase stability of the deprotonated EU dimer is higher than that of the BrEU dimer. As a result, the $V_{1/2}$ difference between both the studied β-carbolines reflects the variation of the activation energy required to produce naked deprotonated drugs from the corresponding homodimers. It should to be noted that these two molecules differ only by the presence of a bromine atom. BrEU presents a higher polarizability than EU which is expected to influence the dimer stabilization. Furthermore, the presence of the bromine substituent could cause a steric hindrance, leading to a destabilization of the deprotonated BrEU dimer. Certainly, their respective gas phase acidity could also affect the stability of deprotonated dimers. Indeed, the higher ΔH°_{acid} value (i.e., lower gas phase acidity), the higher the dimer stability (as shown for small systems) [29,30] (*vide infra*).

From the breakdown curves of Fig. 1, another important parameter that can be considered is the slope value at $V_{1/2}$. This parameter can provide relative indications on the transition state properties (loose vs. tight configurations) [31,32] reached during symmetrical dissociations of the β-carboline deprotonated homodimers. The slope values do not strongly differ which may reflect similar transition states for both the deprotonated homodimers during the dissociation processes. In this particular case, it seems that steric effect due to bromine substituent does not play a major role on properties of the transition state yielding deprotonated [M−H]⁻ compound.

In order to rationalize the origin of the stability variation of the deprotonated homodimers, calculation have been carried out (Fig. 2). Surprisingly, it was not possible to evidence a significant difference between the stabilization energies of the anionic [2EU-H]⁻ and [2BrEU-H]⁻ homodimers (Fig. 2), these values being differentiated by only 5 kJ mol^{-1} which is not really significant. It should be noted that the monomeric [EU-H]⁻ and [BrEU-H]⁻ anions presented a planar geometry. This contrasts with the corresponding dimeric [2M−H]⁻ structure which presented a dihedral angle for both the aromatic systems of 31.4 and 32.6°, respectively. It is considered that the weak stability difference between these deprotonated homodimers could be explained by the variation of the torsional energies required when anionic species interact with its neutral precursor. These calculations appear to be in contradiction with the experimental results which indicating that [2EU-H]⁻ is significantly more stable than [2BrEU-H]⁻.

In order to estimate this difference, the energy of each anionic part (keeping the same geometry as in the dimer) has been calculated and this value was substrated to the energy value of the corresponding neutral. But again, the calculated difference is only 8 kJ mol^{-1}. Therefore, the bromine atom does not influence the

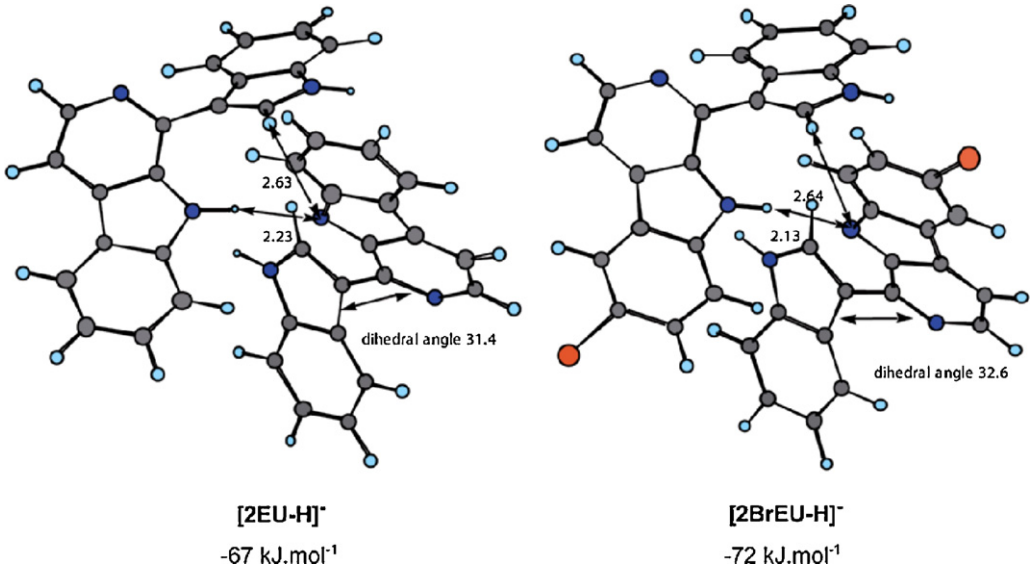


Fig. 2. Geometries of deprotonated [2EU-H]⁻ and [2BrEU-H]⁻ homodimers explored at AM1 level (distance in Å, dihedral angle in degree); stabilization energies (single point) calculated at B3LYP/6-31 + G*//AM1 level (not corrected from ZPE).

dimerization process since it is localized in the opposite side of the hydrogen bond linkage and thus, the dimer gas phase stability change could not be explained by the bromine atom polarizability. However, the small theoretical energy difference seems to be consistent with the weak variation of the experimental breakdown curves slopes [31] which is slightly more "vertical" for [2BrEU-H]$^-$ compared with that for [2EU-H]$^-$ (Fig. 1). Therefore both the [2EU-H]$^-$ and [2BrEU-H]$^-$ homodimers seem to present similar conformation in their respective state of dissociation (Fig. 2). And thus, the corresponding state densities must be comparable. On the other hand, a similar entropic effect must accompany the neutral release since the naked deprotonated monomer keeps a common quasi-planar conformation. However, in the case of [2BrEU-H]$^-$, the conformation of the transition state must be rigidified due to the oriented dipole created by the C-Br bond of each partner of the deprotonated homodimer. In contrast [2EU-H]$^-$ does not present such a group that can polarize the ion/neutral complex. Therefore, several inter-convertible structures more or less stable could coexist in the [2EU-H]$^-$ anion and the less stable forms can dissociate promptly, which explain the variation on sigmoid shape of breakdown curves.

3.2. Gas phase acidity measurements of EU and BrEU

In order to evaluate the role of the "binding proton" in the deprotonated homodimer self-association, the relative acidity of each β-carboline has been investigated. Furthermore, the variation of entropy for proton removing has been compared for a better rationalization of conformation influence.

3.2.1. Experimental acidity determination

Measurements of the relative acidity of studied β-carbolines can be achieved according to the Cook's method [19] by studying the relative abundances of [M−H]$^-$ and [A$_i$–H]$^-$ anions produced from the competitive dissociations of the [M+A$_i$–H]$^-$ deprotonated heterodimer where the A$_i$ compounds are acidic references and M is the β-carboline (Eq. A).

$$[M+A_i\text{-}H]^- \xrightarrow{k_M} [M\text{-}H]^- + A_i \qquad (A.1)$$

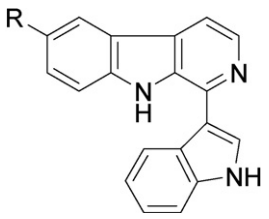
$$[M+A_i\text{-}H]^- \xrightarrow{k_{Ai}} [A_i\text{-}H]^- + M \qquad (A.2)$$

From the RRKM theory description of dissociation rate constant and based on several assumptions, e.g., same T_{eff} for each transition state, similar frequency factor for the cleaved (M−H)$^-$…A$_i$ and (A$_i$–H)$^-$….M bonds (i.e., similar $Q_i^{\#}$ and $Q_M^{\#}$ partition functions characterizing each described transition state), the k_i/k_M ratio can be expressed as in Eq. (B):

$$\ln \frac{k_i}{k_M} = \ln \frac{Q_i^{\#}}{Q_M^{\#}} + \frac{\Delta H^{\circ}_{acid}(M) - \Delta H^{\circ}_{acid}(A_i)}{RT_{eff}} \qquad (B)$$

Based on our approximations, it can be considered that the $Q_i^{\#}/Q_M^{\#}$ ratio is equal to one [20].

This latter assumption requires that both the partners constituting the complex present the same chemical functions and that the frequencies of the cleaved hydrogen bonds are very similar. Unfortunately, it is not possible to find such required references for the β-carboline family and consequently, it is necessary to find another reference compound series. However, for a homogeneous chemical reference series compared to M, the $Q_i^{\#}/Q_M^{\#}$ ratio can be considered as constant [20] (or close to a constant value). For this purpose, substituted benzoic acids family were used as reference compounds because they allow formation of stable non-covalent complexes with the studied β-carbolines (Scheme 1) (Table 1) [33,34].



Scheme 1. Structure of the studied β-carbolines: EU (R = H) and BrEU (R = Br).

Table 1
Acidities (ΔH°_{acid} given in kJ mol^{-1}) of substituted benzoic acids [33,34].

Reference A$_i$		ΔH°_{acid}(kJ mol^{-1})
p-Cl benzoic acid	(**1**)	1404
m-F benzoic acid	(**2**)	1406
p-F benzoic acid	(**3**)	1410
o-F benzoic acid	(**4**)	1415
p-NO_2 benzoic acid	(**5**)	1368
p-CN benzoic acid	(**6**)	1372
p-CF_3 benzoic acid	(**7**)	1390
p-CHO benzoic acid	(**8**)	1392

Nevertheless, the presence of two acidic sites (Scheme 2) into β-carbolines complicates the description of binding sites as well as the use of previous equations used for the Cooks' treatment. Interestingly, they are not interconvertible by intra molecular proton transfer because of the large distance between the considered acidic sites.

Due to the existence of these two deprotonation positions, it is difficult to determine if one form predominates or if the **a** and **b** forms coexist. For this reason, Eq. (B) cannot be simplified but may be modified into Eq. (C):

$$\ln \frac{k_i}{k_M} \approx -\frac{\Delta H^{\circ}_{acid}(A_i)}{RT_{eff}} + \frac{\Delta H^{\circ}_{acid}(M) - T_{eff}[\Delta S^{\circ}_{acid}(M) - \Delta S^{\circ}_{acid}(A_i)]}{RT_{eff}} \qquad (C)$$

which, by introducing the apparent acidities [i.e., $GA^{app}(M, A_i)_{T_{eff}}$] by analogy to the $GB^{app}(M)$ term introduced by Wesdemiotis and co-workers [35] was transformed into Eq. (D).

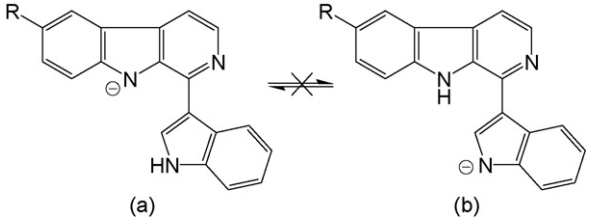
$$\ln \frac{I_i}{I_M} \approx \ln \frac{k_i}{k_M} \approx \frac{GA^{app}(M, A_i)_{T_{eff}} - \Delta H^{\circ}_{acid}(A_i)}{RT_{eff}} \qquad (D)$$

with

$$GA^{app}(M, A_i)_{T_{eff}} = \Delta H^{\circ}_{acid}(M) - T_{eff}[\Delta S^{\circ}_{acid}(M) - \Delta S^{\circ}_{acid}(A_i)] \qquad (E)$$

These relations can be obtained only by considering that both the isomeric [M,(A$_i$–H)]$^-$ and [(M−H),A$_i$]$^-$ forms are in quasi thermal equilibrium which is another acceptable approximation in condition that the intrinsic barrier related to the cross between both the non-covalent forms is sufficiently low.

The determination of the $GA^{app}(M, A_i)_{T_{eff}}$ values have been carried out by studying [M+A$_i$–H]$^-$ heterodimer dissociations under



Scheme 2. The two possible non-convertible structures of deprotonated β-carboline monomer [M−H]$^-$ anion (with R = H, and Br).

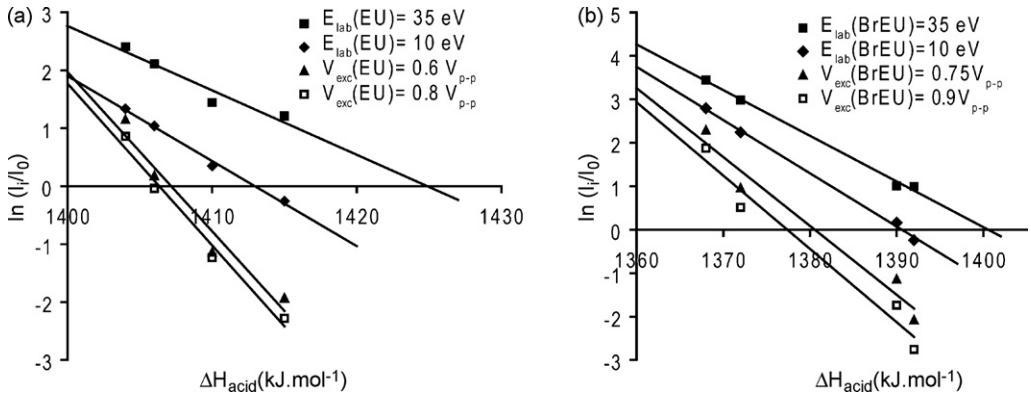


Fig. 3. Plots of $\ln(I_i/I_M)$ ratio vs. $\Delta H^\circ_{acid}(A_i)$ for competitive dissociations of $[A_M + A_i - H]^-$ deprotonated heterodimer constituted by substituted benzoic acids as references (A_i) in interaction with a) EU (m/z 282), and b) BrEU (m/z 360), at different activation energies (V_{exc} for ion trap instrument, shown as ▲ and □; E_{lab} for triple quadrupole instrument, shown as ■ and ♦; respectively).

different collision energy conditions using both the competitive ion trap and triple quadrupole instruments. Following the Eq. (D), $\ln(I_i/I_M)$ vs. $\Delta H^\circ_{acid}(A_i)$ has been plotted as shown in Fig. 3 (all data was not shown here). The x-intercept correspond to $GA^{app}(M, A_i)_{T_{eff}}$ value provided for each different T_{eff}.

In order to compare the collisional activation conditions of the selected deprotonated heterodimers in both the used instruments, the excitation energy (i.e., resonant excitation amplitude V_{exc} for the ion trap and laboratory kinetic energy E_{lab} for the triple quadrupole) ranges have been chosen as: $V_{exc.}$ equal to 0.6–0.8 V_{p-p} and 0.75–0.9 V_{p-p} and E_{lab} equal to 5–50 eV for EU and BrEU, respectively (Fig. 3).

Three observations can be made from Fig. 3: (i) with weaker collision energies (V_{exc} or E_{lab}), $GA^{app}(M, A_i)_{T_{eff}}$ value is lower, (ii) $GA^{app}(M, A_i)_{T_{eff}}$ values obtained from ion trap mass spectrometer are always lower than those provided using triple quadrupole instrument, and (iii) the variation of the $GA^{app}(M, A_i)_{T_{eff}}$ is lower from the ion trap measurements than those obtained in the triple quadrupole. This behavior is expected as the effective temperature is related to the collision energy as well as the instrument physical properties that is used for dissociation of the precursor ions [36,37]. As the apparent gas phase acidity is related to the effective temperature and thus; $GA^{app}(M, A_i)_{T_{eff}}$ [Eq. (E)] rises when the activation energy increases. Because of the differences associated with the instrument geometry and relative kinetic shift, the precursor ions activated in the triple quadrupole cell acquire more excitation energy than those studied in the ion trap cell. Consequently, the observed $GA^{app}(M, A_i)_{T_{eff}}$ is significantly higher in the triple quadrupole if $\Delta\Delta S < 0$ and is not negligible. In this case a little change of activation energy in the collision cell can cause a relatively important $GA^{app}(M, A_i)_{T_{eff}}$ variation. In the ion trap, the multiple collision processes with helium could induce a "*cooling effect*", so T_{eff} for small size ions is almost independent on the excitation amplitude [38–42]. That is why the $GA^{app}(M, A_i)_{T_{eff}}$ variation is weak under resonant excitation conditions from ion trap instruments. The dependence of the $GA^{app}(M, A_i)_{T_{eff}}$ values upon of the effective temperature indicates that the entropic effect cannot be neglected using substituted benzoic acids as references which differ to β-carbolines in terms of chemical natures. Therefore, the frequency of proton transfer between the partners into the deprotonated heterodimers should not be the same and consequently, the non-thermal point called as "isothermal point" is not located at the x-axis [43].

It has been proposed by Vekey [37] that more accurate measurements can be achieved by the combination of experimental data from both the ion trap and triple quadrupole mass spectrometers. This concept was recently successfully applied to the improved measurements of modified proline basicity [38]. This can be reached by providing ion activation under slow heating and single collision conditions, with the ion trap and triple quadrupole instruments, respectively.

To determine experimentally with accuracy the gas phase acidity i.e., $[\Delta H^\circ_{acid}(M)]$ and the difference of entropy variations $[\Delta\Delta S^\circ_{acid}(M,A_i)]$ of proton exchange between A_i and M, Armentrout's *alternative* method [21] was used. This approach is based upon the standard Cooks' kinetic method [19]. The different $GA^{app}(M, A_i)_{T_{eff}}$ values have been plotted as a function of the T_{eff} values according to Eq. (E) (Fig. 4). A weak T_{eff} variation was observed although the effective temperature range obtained with the ion trap measurements is significantly shifted toward the lowest temperature values compared to that obtained using the triple quadrupole instrument. Combination of data provided from both the resonant excitation and ion beam collision modes allows enhancing the ΔH°_{acid} value accuracy in spite of the significant experimental deviations.

The y-intercept of this curve correspond to the $\Delta H^\circ_{acid}(M)$ value. The gas phase acidity values of the studied β-carbolines are $\Delta H^\circ_{acid}(EU) = 1399.8 \pm 5$ (0.6) kJ mol^{-1} and $\Delta H^\circ_{acid}(BrEU) = 1346.7 \pm 5$ (1.5) kJ mol^{-1}. So BrEU is significantly more acidic in the gas phase than EU. This result is consistent with

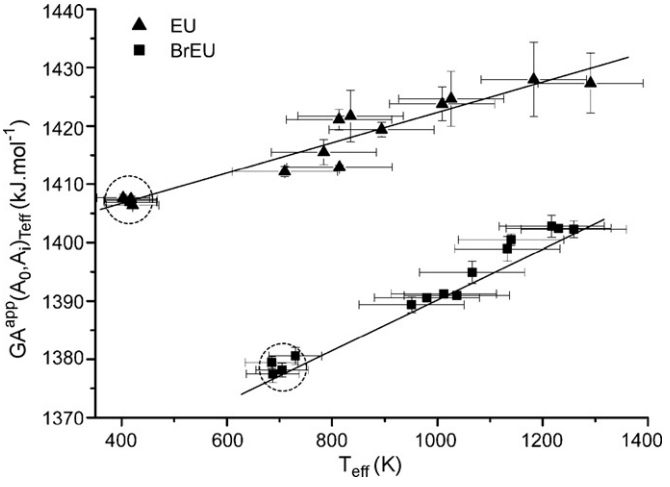


Fig. 4. Evolution of $GA^{app}(M, A_i)_{T_{eff}}$ vs. T_{eff} recorded for (a) EU (♦) and (b) BrEU (■). The data pairs $GA^{app}(M, A_i)_{T_{eff}}$ reported have been obtained by using both the ion trap and triple quadrupole instruments. The activation energies, from resonant excitation (in the circle) and from ion beam collision, evaluate from 0.6 V_{p-p} to 0.8 V_{p-p} for EU (and from 0.75 V_{p-p} to 0.9 V_{p-p} for BrEU) in ion trap, and from 5 eV to 50 eV for EU and BrEU in collision cell of triple quadrupole.

the found higher gas phase stability of $[2EU\text{-}H]^-$ compared to $[2BrEU\text{-}H]^-$ (Fig. 1) [29]. Furthermore, the slope of the best-fit line characterizing the $GA^{app}(M, A_i)_{T_{eff}}$ vs. T_{eff} evolution corresponds to the $\Delta\Delta S^\circ_{acid}(M,A_i)$ values of proton exchange between the A_i and M partners. The experimental $\Delta\Delta S^\circ_{acid}(M,A_i)$ values obtained from the positive slope [Eq. (D)] are -17 ± 10 (1) J mol^{-1} K^{-1} and -44 ± 10 (1.5) J mol^{-1} K^{-1} for EU and BrEU, respectively (Fig. 4). The entropy variation for BrEU is relatively large [40] which could be due to a significant energy barrier related to a conformational change occurring during the non-covalent complex dissociation. This can yield significant errors for $\Delta\Delta S^\circ_{acid}(M,A_i)$ values as well as $[\Delta H^\circ_{acid}(M)]$ determination [24].

3.2.2. Theoretical acidity determinations by DFT calculations

Gas phase acidity has been calculated after full optimization at B3LYP/6-31 + G* level, associated energies being corrected from ZPE, for both the neutral and anionic species. For each β-carboline, it is possible to calculate two acidity values related to the deprotonation site as previously noted in Scheme 2. For EU(**a**) and EU(**b**), the ΔH°_{acid} are 1394.5 kJ mol^{-1} and 1400.8 kJ mol^{-1}, respectively. Concerning the BrEU(**a**) and BrEU(**b**) forms, the ΔH°_{acid} values are 1372.8.2 kJ mol^{-1} and 1390.1 kJ mol^{-1}, respectively. It can be concluded from these acidity values that the deprotonation sites (Scheme 2) do not differ strongly.

In this context, is it difficult to have an interconversion of forms **b** → **a** (for EU or BrEU) by proton transfer? Starting from an isolated "**b**" form, the distance between two nitrogen atoms being too great (≈4.2 Å), a very distorted geometry in the TS conformation is required. The calculation indicates that a barrier of 251 kJ mol^{-1} exists for such isomerization (results not reported herein). However, from the deprotonated homodimer structure, an intermolecular proton transfer seems to be more favored. Indeed, it has been possible to find a transition state (Fig. 5) associated with energy barriers of 48 kJ mol^{-1} and 44 kJ mol^{-1} for the EU(**b**) → EU(**a**) and BrEU(**b**) → BrEU(**a**) proton transfer, respectively.

From these calculations, it is shown that the isomeric deprotonated $[2EU\text{-}H]^-$ and $[2BrEU\text{-}H]^-$ dimers in the **b** form, differ in energy by approximately 12 kJ mol^{-1} (in favor to the $[2EU\text{-}H]^-$ homodimer). This difference was only of 5 kJ mol^{-1}, in the precedent calculation considering the **a** form (Fig. 2). Therefore in both cases, the $[2BrEU\text{-}H]^-$ homodimer presents a higher stability although the stability difference is reinforced with the **b** form.

Overall, the theoretical data and the experimental results are in relatively good agreement: $\Delta H^\circ_{acid}(EU) > \Delta H^\circ_{acid}(BrEU)$. However, a higher ΔH°_{acid} difference is observed experimentally between EU and BrEU [$\Delta\Delta H^\circ_{acid}$(EU, BrEU)]. This could be due to higher entropy variation values related to a conformational change during the competitive dimer dissociations. It should be noted that, from EU and BrEU, the $\Delta\Delta H^\circ_{acid}$(**b**,**a**) values are 7.3 kJ mol^{-1} and 19.3 kJ mol^{-1}, respectively. In addition the intermolecular proton transfer energies are relatively low in the deprotonated homodimers compared to the direct internal proton transfer from $[EU\text{-}H]^-$ and $[BrEU\text{-}H]^-$. Thus, it can be assumed that it is possible to produce both the **a** and **b** conformations during the dimerization. As from EU, the $\Delta\Delta H^\circ_{acid}$(**b**,**a**) value is very small, both the conformations could be generated in similar proportion in ESI. During the competitive dissociation processes, the two forms can evolute in similar ways. In the case of BrEU, the $\Delta\Delta H^\circ_{acid}$(**b**,**a**) value is 19.3 kJ mol^{-1}, this difference could be great enough to favor the production of the preferred conformation. In this case, the deprotonated homodimer's decompositions could be different, the entropy effects are enhanced. That is likely why the entropy variation determined experimentally was lower for EU than for BrEU, although it is was a relatively high value [19].

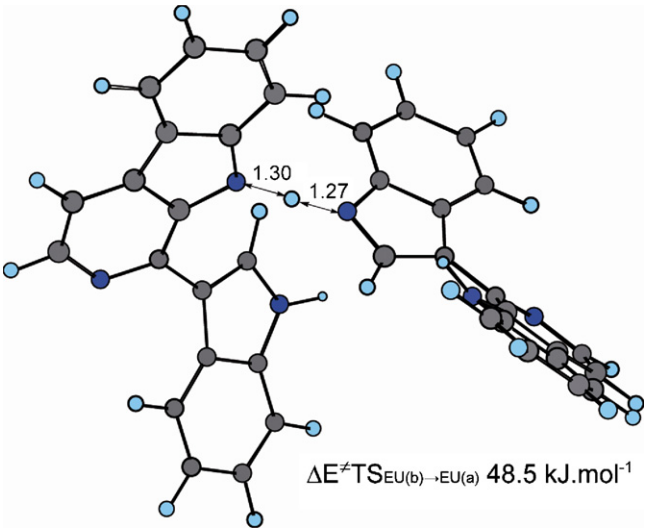


Fig. 5. Transition state geometry (distance in Å) for proton transfer in the $[2EU\text{-}H]^-$ dimer, an associated barrier calculated at the AM1 level.

3.3. Non-covalent interaction between nucleobases and studied β-carbolines

Based upon the previous considerations, in order to get information on the interactions between nucleobases and β-carbolines, the stability of several deprotonated non-covalent complexes has

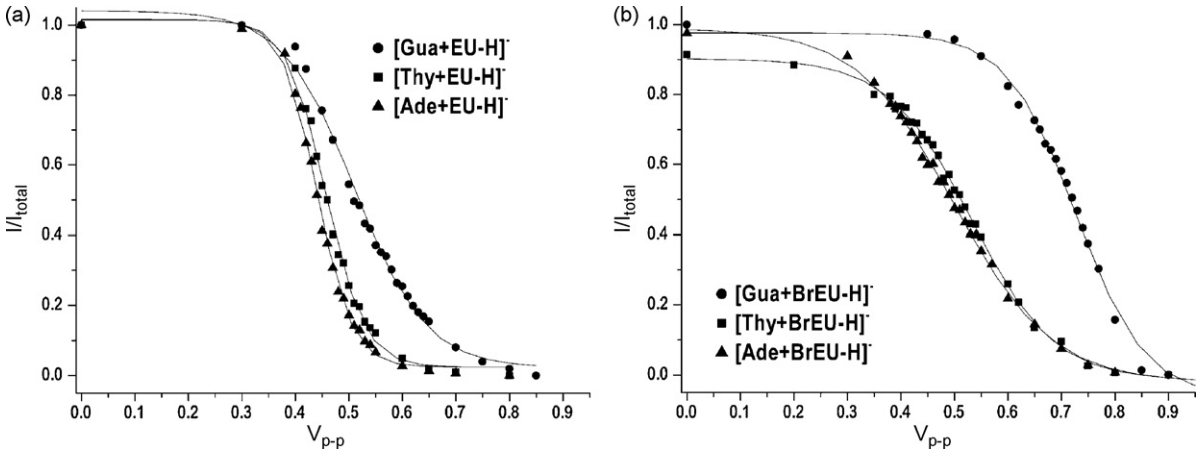


Fig. 6. Breakdown curves of deprotonated heterodimers of β-carbolines and nucleobases recorded on the ion trap instrument (capillary exit offset of 40 V and skimmer 1 of 15 V), (a) [base + EU-H]$^-$ at *m/z* 433, *m/z* 417, *m/z* 408; and (b) [base + BrEU-H]$^-$ at *m/z* 511, *m/z* 495, *m/z* 486; for the M interaction with Gua, Ade and Thy, respectively.

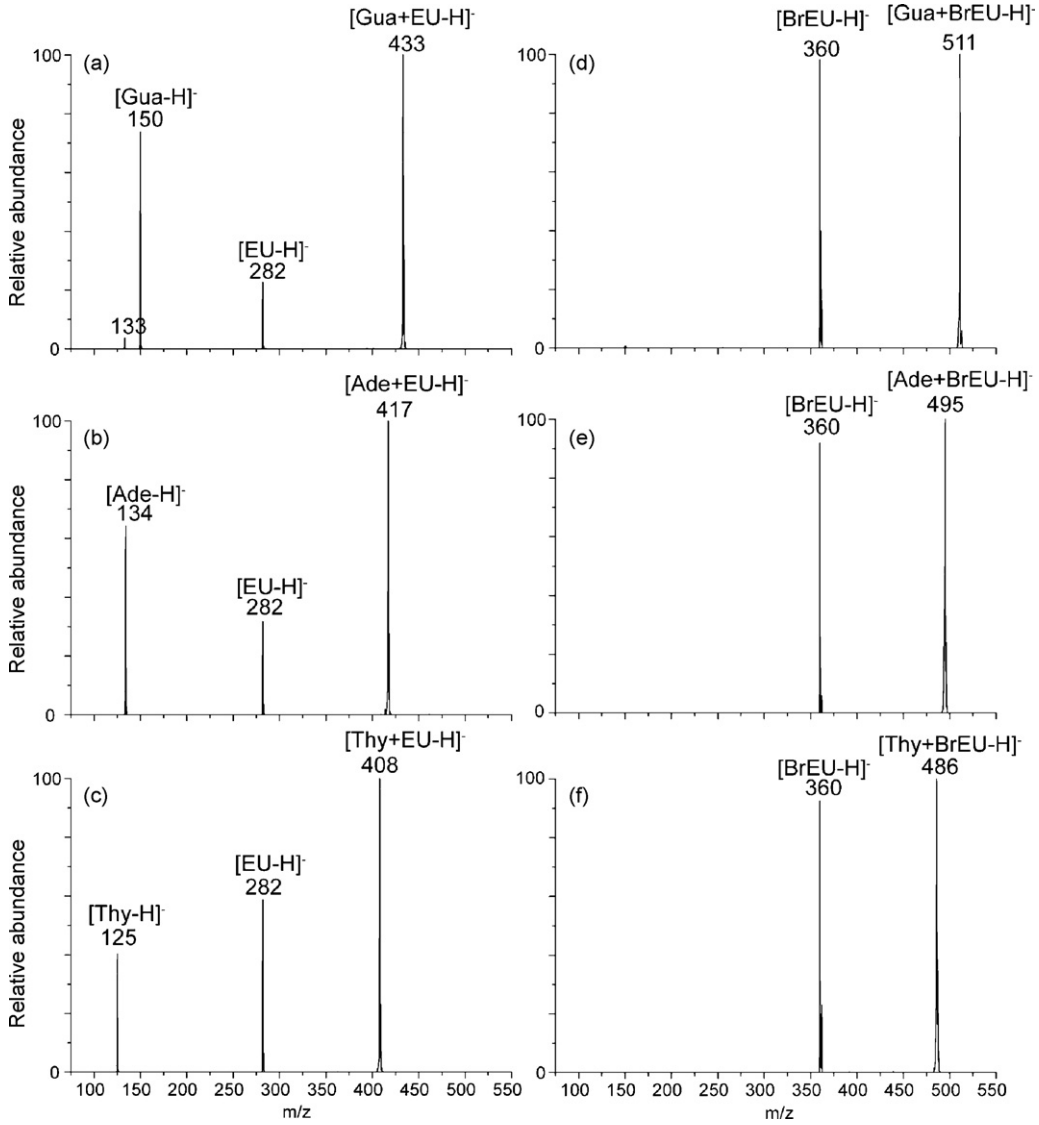


Fig. 7. CID spectra of deprotonated heterodimers: (a) [Gua + EU-H]$^-$ (m/z 433), (b) [Ade + EU-H]$^-$ (m/z 417), (c) [Thy + EU-H]$^-$ (m/z 408), (d) [Gua + BrEU-H]$^-$ (m/z 511), (e) [Ade + BrEU-H]$^-$ (m/z 495), and (f) [Thy + BrEU-H]$^-$ (m/z 486) recorded in the ion trap instrument with excitation amplitude close to the $V_{1/2}$ value.

been investigated under variable resonant excitation conditions in the ion trap cell. By this mean, it is possible to compare the relative gas phase affinity of each β-carboline (M) toward nucleobases that is supposed to be related to the stability of the deprotonated [base + M−H]$^-$ heterodimer. To compare the stability of such complexes, it is required to perform a series of CID experiments on the deprotonated heterodimers at various activation energies by using different resonant excitation amplitude (V_{exc}). The variation of the relative abundance ratios of the surviving precursor ion over the total ion current yields an experimental sigmoid from which the $V_{1/2}$ value can be determined (Fig. 6). It is considered as a characteristic of the deprotonated heterodimer stability. The sigmoids reported in Fig. 6 yield the following deprotonated heterodimers stability order: [Gua + M−H]$^-$ » [Thy + M−H]$^-$ ≥ [Ade + M−H]$^-$ within the experimental deviations. However, the deprotonated [Cyt + M−H]$^-$ dimer has never been produced as stable species under the used experimental conditions in both the ion trap and triple quadrupole instruments. This behavior suggests that under the experimental conditions, this particular dimer is either very unstable in the gas phase after desolvation or not preformed in solution or in droplet.

The Fig. 7 presents the CID spectra obtained from the different dimers at $V_{1/2}$ excitation amplitude. From the relative abundance of the product ions observed in Fig. 7a, b and c, it can be concluded that the acidity of the nucleobase follows the order: guanine > adenine > thymine. This order is in agreement with that of the nucleobase acidity proposed recently from the GA^{app} measurements using the standard Cooks kinetic method i.e., guanine > adenine > thymine > cytosine [44]. However, Beauchamp et al. have suggested that the relative gas phase acidity of the nucleobases was the following order: adenine > thymine > guanine > cytosine [45]. Theoretical calculation values differ somewhat since the order is thymine > guanine > adenine > cytosine [17,46]. If these acidity scales seem contradictory, one trend can be enlightened: the relative acidity values of the guanine, adenine and thymine are relatively close [16,47] whereas cytosine presents an acidity value significantly lower. The strong acidity difference between Cyt and β-carboline may explain the weak stability of the [Cyt + M−H]$^-$ heterodimer and thus, its absence in the ESI mass spectra.

It should be noted that the stability of [Gua + M−H]$^-$ is higher in the case of BrEU. The presence of bromine substituent seems to reinforce the interaction between the heterocyclic systems by its

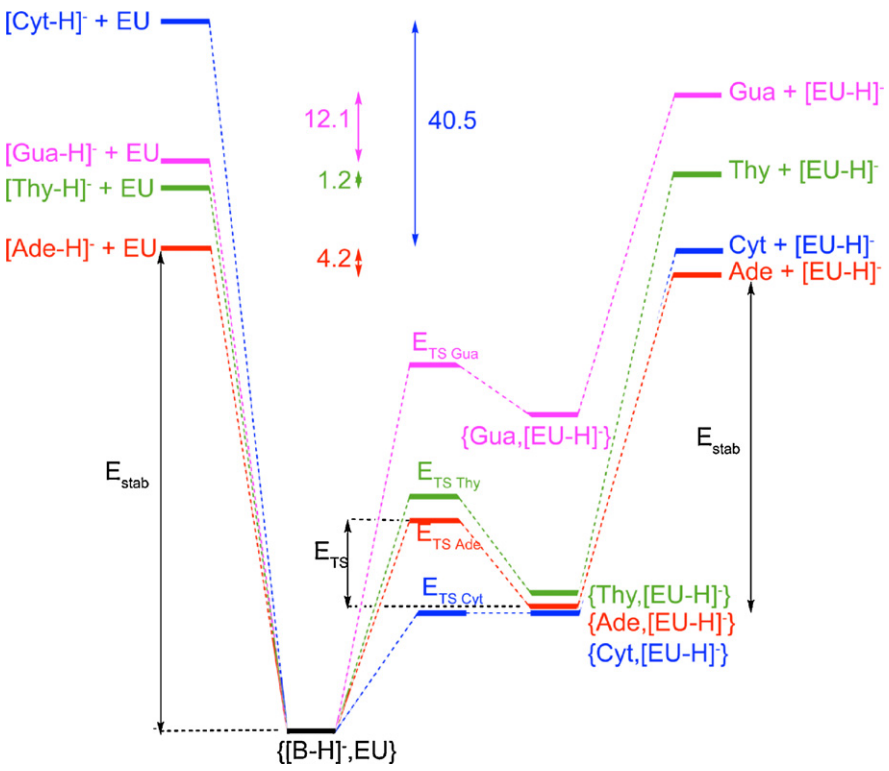


Fig. 8. Potential energy surface corresponding to the competitive decompositions of deprotonated nucleobase/EU complexes calculated using the B3LYP/6-31 + G*//AM1 level.

greater polarizability or its electron withdrawing effect. For both the studied β-carbolines it appears that the [base + M−H]⁻ complexes present the highest stability with guanine. It is interesting to note that this is consistent with their intercalation binding mode [2,4], the β-carbolines associate preferentially with G–C base pairs of DNA double strand.

From this discussion, it is difficult based on the acidity of the nucleobase to rationalize the higher stability of the complexes involving β-carbolines with the Gua base. In order to understand the provided experimental results, calculations have been carried out on these heterodimeric species.

Fig. 8 represents the potential energy surface for the competitive cleavages of the deprotonated EU/nucleobase complexes and the corresponding values are reported in Table 2. The {[B-H]⁻,EU} associations are systematically more stable than those corresponding to the {B,[EU-H]⁻} isomers (Fig. 9). It is shown that the energy barriers required to obtain the {[B-H]⁻,EU} complex from the less stable {B,[EU-H]⁻} isomeric complex are not as expected costly. Therefore because of this weak intrinsic barrier, it is assumed that both the solvated {B,[EU-H]⁻} and {[B-H]⁻,EU} isomeric complexes are in equilibrium. The energy difference ΔE between the {B,[EU-H]⁻} and {[B-H]⁻,EU} isomeric complexes is larger for the complex containing guanine (55.2 kJ mol^{-1}) than those containing adenine and thymine (21.3 and 23.8 kJ mol^{-1} respectively). On the other hand, the weak energy barrier of 10.0 kJ mol^{-1} involved in the reversible internal proton transfer {Gua,[EU-H]⁻} ⇆ {[Gua-H]⁻,EU}, as well as the large difference of ΔE (i.e., 55.2 kJ mol^{-1}) leads to a strong equilibrium shift in favor of [(Gua-H) + EU]⁻. This behavior is very less pronounced from complexes containing Ade and Thy as nucleobase, since their corresponding ΔE energy difference is 21.3 and 23.8 kJ mol^{-1}, respectively. And in this case, the less stable adduct isomer [B + (EU-H)]⁻ should exist in a higher extent.

Interestingly, the theoretical calculations show that the energy level difference between the {B,[EU-H]⁻} and {[B-H]⁻,EU} final states are 1.2, 4.2 and 12.1 kJ mol^{-1} for thymine, adenine and guanine nucleic bases, respectively. These variations are consistent with the experimental results (Fig. 7). Indeed, the CID spectra of each [B + EU-H]⁻ heterodimer presents both the [EU-H]⁻ and [B-H]⁻ product ion abundances relatively close for B = Thy and B = Ade for which the energy level difference is less or equal to 17.5 kJ mol^{-1}. This contrasts with the [(Gua + EU)-H]⁻ complex that yields mainly the [Gua-H]⁻ product ion. Furthermore, it should be noted that the variation of excitation energy does not affected significantly the {[B-H]⁻}/{[EU-H]⁻} ratio, although the precursor ion abundance decreases, by raising the resonant excitations amplitude. This suggested that the dissociation of each {B, [EU-H]⁻} and {[B-H]⁻, EU} dimer are very similar in term of transition state properties and present close frequency factor.

Table 2
Energy levels for the nucleobase/M complexes.

Complex	E_{for} (in hartree)	ΔE (kJ mol^{-1})	E_{stab} (kJ mol^{-1})	ΔE_{stab} (kJ mol^{-1})[a]	E_{TS} (kJ mol^{-1})
{[Ade-H]⁻,EU}	−1362,976125	21.3	83.6	25.9	16.7
{Ade,[EU-H]⁻}	−1362,967973		57.7		
{[Gua-H]⁻,EU}	−1438.213598	55.2	99.1	43.9	10.0
{Gua,[EU-H]⁻}	−1438.201531		55.2		
{[Thy-H]⁻,EU}	−1349.803818	23.8	94.0	22.1	17.5
{Thy,[EU-H]⁻}	−1349.794723		71.9		

[a] {B,[EU-H]⁻} → {[B-H]⁻,EU}.

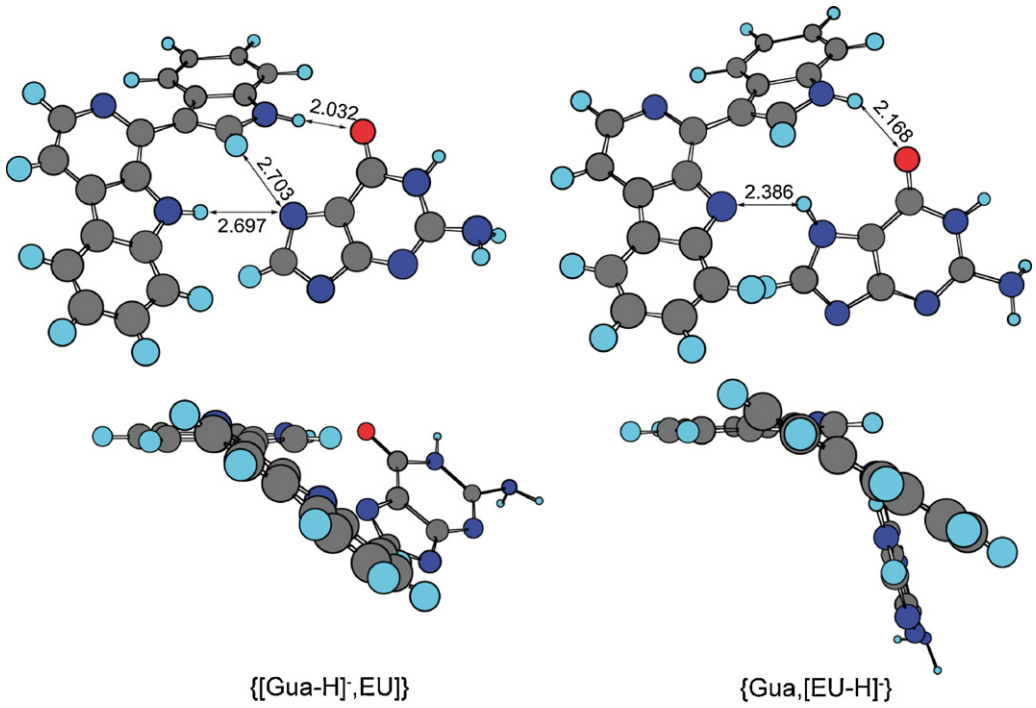


Fig. 9. Calculated conformation of the {[Gua-H]⁻,EU} and {Gua,[EU-H]⁻} deprotonated dimers (distance in Å) using the B3LYP/6-31 + G*//AM1 level.

Finally, it is possible to clarify why the {[Cyt-H]⁻,EU} and {[Cyt-H]⁻,BrEU} complexes have not been detected which imply that they have not been formed or presented a very short life-time. Indeed, as previously mentioned, the difference of acidities of EU (or BrEU) and nucleobases is large which should produce complexes with relatively low stability. In other words, the energetic diagram presented Fig. 8 shows a difference of 40.5 kJ mol^{-1} between the two possible channels of heterodimer fragmentations. It is considered that the gain in stabilization energy during the {[B-H],EU}⁻ associations remain entirely on the complexes ion without dissipation because it is an isolated system in the gas phase during its transfer into ion trap cell. In this case, as soon as the {[Cyt-H],EU}⁻ complex is formed it decomposes with a large rate constant yielding exclusively the [EU-H]⁻ ion. On the other hand, because the spatial orientation of the nucleobases relatively to the drug rings, the stacking effect cannot be significant in contrast with the previous assertion and therefore, the hydrogen bonding should be the main interaction for stabilizing complexes. Concerning BrEU, the same trend was observed although the differences of stability between complexes containing nucleobases seem less accentuated.

4. Conclusion

In this work, the self-association of Eudistomin U was investigated by electrospray-mass spectrometry. The thermochemical properties i.e., acidity of β-carbolines (EU and BrEU) were investigated using different activation condition (yielding different T_{eff}) with triple quadrupole and ion trap instruments. It has been shown that EU ($\Delta H^{\circ}_{acid} = 1399.8 \pm 5\ (0.6)$ kJ mol^{-1}) is less acid than BrEU ($\Delta H^{\circ}_{acid} = 1346.7 \pm 5\ (1.5)$ kJ mol^{-1}) which is due to the presence of bromine atom that enhances the gas phase acidity value for BrEU. In addition, as demonstrated by Meot-Ner, a lower acidity will lead to more stable homodimeric species [29], which explains the higher stability of the [2EU-H]⁻ dimer observed experimentally. This explains why the deprotonated homodimers present a stronger stability difference experimentally whereas the calculated stabilization energy difference is relatively low. In the same way, the non-covalent β-carboline/nucleobase heterodimers have been investigated under low energy CID conditions. From these results it appeared that the two β-carboline/guanine complexes presented a relatively higher stability. Interestingly, this is consistent with the known DNA binding properties of these molecules that bind to DNA double strand with G–C pairs. Bromine seems to play an important role that reinforces the interaction by its greater polarizability or its electron withdrawing effect. The existence of a relatively high isomerization barrier with guanine evidenced by the theoretical calculations allowed to explain the higher stability of the deprotonated guanine/β-carbolines complex. In addition the strong acidity difference between Cyt and the β-carbolines allows to explain the absence of the Cyt/β-carbolines heterodimer in the ESI mass spectra.

Acknowledgments

The authors gratefully acknowledge funding provided by CNRS, University Pierre & Marie Curie and Fudan University that supported this work.

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