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Experimental isolation and spectroscopic characterization of squamocin acetogenin combining FT-IR, FT-Raman and UV–Vis spectra with DFT calculations

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A B S T R A C T
Squamocin, an annonaceous acetogenin has been experimentally isolated and characterized in the solid state using the FT-IR and FT-Raman spectra and in methanol solution by UV–visible spectrum. The main bands observed were assigned combining the IR and Raman spectra with hybrid functional B3LYP/6-31G* calculations. Structural, electronic and topological properties were predicted at the same level of theory for the most stable conformer of squamocin in gas phase and methanol solution. A corrected solvation energy value of $-147.54 \text{ kJ/mol}$ was predicted for squamocin in methanol while the atomic population natural (NPA) charges evidence higher values on O atoms of R2 and R3 rings, as compared with the corresponding to lactone ring. Mapped MEP surfaces suggest that nucleophilic regions are located on the O atoms of three rings and of OH bonds belonging to side chain, in agreement with the higher charges values evidenced on these O atoms while electrophilic regions are predicted on the H atoms of OH groups. High stabilities of squamocin in both media was revealed by AIM studies while only in methanol solution by NBO calculations. The expansion of volume and the higher dipole moment in methanol suggest a clear solvation of squamocin by solvent molecules. Gap values have evidenced that squamocin is most reactive in methanol while that its large aliphatic chain produces an increases the reactivity of this $\gamma$-lactone, as compared with ascorbic acid lactone. Reasonable concordances among the predicted UV–visible and IR, Raman spectra with the corresponding experimental ones were found.

1. Introduction
Squamocin is an annonaceous acetogenin (ACG) in whose structure there are a large side chain of CH₂ groups with bis-tetrahydrofuranic (bis-THF) rings [1–11]. Isolation of these ACGs was described by Barrachina et al. [2] from the methanolic extract of Annona cherimolia seeds. Antimicrobial and cytotoxic activities were reported for squamocin [1,2,4,5,8–10] while other ACGs were estimated by structure-activity relationships against human tumor cells [3,7,11]. On the other hand, motrilin, an acetogenin similar to squamocin, have been evaluated as corrosion inhibitors for carbon steel in acidic solutions [12] and its structural, electronic and topological properties were recently studied together with its vibrational and ultraviolet–visible spectra [13]. In this work, we have studied the properties of other ACG isolated from Annona cherimolia structurally similar to motrilin, named squamocin, however, they differ in the position of the OH group linked to the sides chains, being $-(\text{CH}_2)_5-\text{CH}_3$ in squamocin and $-(\text{CH}_2)_4-\text{CH}_3$ in motrilin. In this work, the experimental FT-IR and FT-Raman of squamocin in the solid state and its ultraviolet–visible spectra in methanol solution were reported for first time together with the structural, electronic, topological and vibrational properties. Hence,
the aims of this work are the optimizations of squamocin in gas phase and in methanol solution by using hybrid functional B3LYP/6-31G* method [14,15]. After that, the atomic charges, molecular electrostatic potentials, bond orders, donor-acceptor interaction energies and, topological properties are predicted at the same level of theory. Later, the main bands observed in infrared and Raman spectra are assigned by comparison between the corresponding predicted at the same level of theory with the corresponding experimental ones. Besides, the predictions of reactivities and behaviors of squamocin in the two media by using the frontier orbitals and some global descriptors are the great interest taking into account the antimicrobial and cytotoxic activities that present these ACGs [13,16–21]. Finally, the properties obtained for squamocin are compared with those reported for motrillin and for other molecules containing similar groups [13,16–21]. These studies were carried out with the hybrid B3LYP/6-31G* method due to that the squamocin structure presents 109 atoms and, for these reasons, the assignments of main vibrational normal modes of squamocin were performed by comparisons with assignments reported for species containing similar groups [13,18–22]. Predicted ultraviolet–visible spectrum was compared with the corresponding experimental ones in methanol solution, recorded in the same medium at room temperature. The predicted UV-V, FT-IR and FT-Raman spectra have showed good correlations when they are compared with experimental ones.

2. Experimental

2.1. Isolation

Squamocin, an ACG with adjacent bis-THF with OH groups flanking the THF, was isolated by column chromatography on silica gel 60H (5–40 μm, 7336 Merck). The evolution of column chromatography was monitored by thin layer chromatography (TLC). To perform this procedure, Merck F254 chromatofolios were used [10]. Semi preparative HPLC was carried out on a LiChroCartR 100 RP-18 column (25 × 1 cm i. d., 10 μm particle size), flow rate 1.8 mL/min, using MeOH–H2O 10%.

2.2. Characterization techniques

FT-IR, FT-Raman and UV-V spectroscopies were used to characterize squamocin. A PerkinElmer GX equipment provided with a DTGS detector purged with dry air was employed to record the FT-IR spectrum between 4000 and 400 cm−1 with a total of 256 scans and a resolution of 1 cm−1. The FT-Raman spectrum was recorded at room temperature in a 3500-50 cm−1 range with a resolution of 4 cm−1 and 300 scans by using a Thermo Scientific, DRX Raman Microscope equipped with a laser (excitation line of 1532 nm, 10 mW of laser power). The UV–visible spectrum of the sample was recorded in a 1 mm path length quartz cuvette with methanol at a concentration of 0.1–0.3 mg/mL using a Shimadzu UV–Vis160 A spectrometer.

3. Computational details

The GaussView program [22] was employed to model squamocin while its optimizations in gas phase and in methanol solution were performed with the Revision A.02 of Gaussian 09 program [23] by using the hybrid functional B3LYP/6-31G* [14,15]. The 6-31G* basis set was employed in all calculations due to the large aliphatic side chain that presents this molecule. At this level of calculation, only three stable structures for squamocin were observed in the potential energy surface (PES) and only one of them presents global minimum. The most stable structure of squamocin in methanol solution was optimized with the integral equation formalism variant polarised continuum method (IEFPCM) while the solvation energy in the same medium was predicted by using the universal solvation model [24–26]. The solvation energy was corrected by zero point vibrational energy (ZPVE) in addition to non-electrostatic terms. Properties only for some atoms belonging to lactone and furan rings and OH groups of the most stable conformation of squamocin were studied. Besides, the atomic Merz-Singh-Kollman scheme (MK) [27] together with the versions 3.1 and 2000 of NBO and AIM programs [28–30], respectively were employed to calculate natural population charges (NPA), molecular electrostatic potentials (MEP), mains donor-acceptor energy interactions and topological properties in gas phase and in methanol solution. Then, the GaussView program [22] was used as an important aid to perform the assignments of the mains bands observed in the vibrational spectra of squamocin. Time-dependent DFT calculations (TD-DFT) by using NSstates = 10 at the 6-31G* level of theory with the Revision A.02 of Gaussian 09 program were performed in order to predict the ultraviolet–visible spectrum of squamocin in aqueous solution [23]. Moreover, the frontier orbitals were calculated to obtain the gap values [16,17] and with these parameters the chemical potential (μ), electronegativity (χ), global hardness (η), global softness (S), global electrophilicity index (ω) and nucleophilicity indexes (E) descriptors were computed to predict the reactivities and behaviour of squamocin [18–21]. Then, comparisons of properties predicted for squamocin with those reported for motrillin ACG and other similar species were performed [13,18–21,31,32].

4. Results and discussion

4.1. Structural studies in gas phase and methanol solution

In Fig. 1 is presented the PES as function of the dihedral O45–C44–C37–O34 angle between the two R2 and R3 rings while in Fig. 2 can be seen the most stable structure of squamocin together with the identifications of dihedral O45–C44–C37–O34 angle, its three rings and the atoms numberings only for some simplified fragments indicated by red circles. Clearly, the lactone ring is identified as R1 and the other two as R2 and R3, respectively. In Table 1 are summarized the results of hybrid B3LYP/6-31G* calculations for squamocin ACG in gas phase and in methanol solution.
solution [14,15]. Hence, calculated total energy corrected and uncorrected by zero point vibrational energy (ZPVE), dipolar moment ($\mu$) and volume ($V$) values for squamocin acetogenin in gas phase and methanol solution by using B3LYP/6-31G* method. Corrected by ZPVE and by total non-electrostatic terms ($\Delta G_c/ZPVE$) and uncorrected by ZPVE solvation energies ($\Delta G_e$) are also presented.

### Table 1

| Medium                     | $E$ (Hartrees) | $E_{ZPVE}$ (Hartrees) | $\mu$ (D) | $V$ (Å$^3$) |
|----------------------------|----------------|-----------------------|-----------|-------------|
| GAS                        | $-1975.6402$   | $-1974.6556$          | $6.77$    | $741.8$     |
| PCM                        | $-1975.7044$   | $-1974.7208$          | $9.22$    | $752.7$     |
| Methanol solution (kJ/mol) | $-168.40$      | $-145.33$             | $-147.95$ | $10.9$      |

$\Delta G_{un}$ — uncorrected solvation energy, $\Delta G_{ne}$ — total non electrostatic terms, $\Delta G_c$ — corrected solvation energies.

| Medium                     | $\Delta G_{un}$ | $\Delta G_{ne}$ | $\Delta G_c$ | $\Delta G_{c/ZPVE}$ | $\Delta V$ (Å$^3$) |
|----------------------------|------------------|------------------|--------------|----------------------|---------------------|
| Methanol solution (kJ/mol) | $-23.07$         | $-145.33$        | $-147.95$    |                      | $10.9$              |

$^a$ This work.

Fig. 2. Theoretical molecular structure of the most stable conformer of squamocin and atoms numbering.

### 4.2. Atomic charges and molecular electrostatic potentials (MEP) studies

The presence in squamocin of H bonds donors (three OH groups) and acceptors (seven O atoms) confers at it interesting potential biological and pharmacological properties and, therefore, are of the ascorbic acid (AA) [36,37], as can be seen in Table 2. This comparison is possible because the R1 ring of squamocin ACG is similar to lactone ring of ascorbic acid. Calculated root-mean-square deviation (RMSD) values were used to compare both structures and the results are also presented in Table 2. Then, the reasonably low RMSD values calculated, which are between 0.053 and 0.048 Å for bond lengths and in 1.6° for bond angles, show very good correlations in the geometrical parameters predicted for squamocin in both media. Now, when the predicted bond C3−O1−C6 angles that belong to lactone ring of squamocin in both media are compared with the corresponding to ring of ascorbic acid (109.1°) we observed a slight underestimation in both values (107.4°). In this squamocin acetogenin, it is observed same signs for the dihedral O34−C37−C44−O45 angle in both media, a resulted different from motrillin acetogenin which evidence a notable change of sign, from positive in gas phase to negative in methanol solution [13].
Table 2
Calculated geometrical parameters for squamocin acetogenin in gas phase and methanol solution compared with the experimental ones for ascorbic acid.

| Parameters                   | Gas                | PCM                | Exp\textsuperscript{b} |
|------------------------------|--------------------|--------------------|------------------------|
| Bond lengths (\text{Å})      |                    |                    |                        |
| R1                          |                    |                    |                        |
| C6–O2                       | 1.213              | 1.225              | 1.216                  |
| C3–O1                       | 1.357              | 1.370              | 1.355                  |
| C6–O1                       | 1.424              | 1.409              | 1.444                  |
| R2                          |                    |                    |                        |
| C33–O34                     | 1.448              | 1.453              | 1.355                  |
| C37–O34                     | 1.435              | 1.445              | 1.444                  |
| C44–C37                     | 1.524              | 1.525              |                        |
| R3                          |                    |                    |                        |
| C48–O45                     | 1.436              | 1.449              | 1.355                  |
| C44–O45                     | 1.433              | 1.442              | 1.444                  |
| C31–O91                     | 1.421              | 1.434              |                        |
| C55–O57                     | 1.426              | 1.437              |                        |
| C68–O89                     | 1.433              | 1.442              |                        |
| RMSD                        | 0.048              | 0.053              |                        |
| Bond angles (°)             |                    |                    |                        |
| C1–O1–C6                    | 107.4              | 107.4              | 109.1                  |
| C33–O34–C37                 | 110.8              | 110.9              |                        |
| C44–O45–C48                 | 111.0              | 110.8              |                        |
| RMSD                        | 1.6                | 1.6                |                        |
| Dihedral angles (°)         |                    |                    |                        |
| O34–C37–C44–O45            | 179.6              | 179.5              |                        |

\textsuperscript{a} This work.

\textsuperscript{b} From Refs \[33,34\].

interest as possible drug candidate, as suggested by Veber and Lipinski \[38,39\]. Hence, the atomic Merz-Kollman (MK) and natural population atomic (NPA) charges and the molecular electrostatic potential (MEP) in this acetogenin were studied in gas phase and in methanol solution only for the atoms belonging to the three R1, R2 and R3 rings and to the OH groups. Fig. 2 shows the atoms labelling involved in the three considered moieties of squamocin which are indicated in the figure by circles while in Table 3 are presented the calculated MK and NPA charges and MEP values by using the hybrid B3LYP/6-31G* method. The variations of both MK and NPA charges on the three rings are easily observed in Fig. 3. Analyzing the MK charges on the O and C atoms of ring R1 from Fig. 3a we observed that practically present the same values in both media, with exception of charge on C5 which has positive sign and a null value in gas phase. The NPA charges present different values and in general higher than the MK ones and show the same behaviours in both media. Lower NPA values are observed on the O1, O2, C3 and C5 atoms while these charges on the C4 and C6 atoms increase in methanol so-
in the lactone ring (R1). Hence, the total energy favours to squamocin in methanol solution with a value of 799.27 kJ/mol while in gas phase the value decrease to 745.71 kJ/mol. These values are different from those observed in motrilin acetogenin where the total energy value of 786.63 kJ/mol in methanol solution and of 745.71 kJ/mol in gas phase [13]. In both acetogenins, the high stabilities observed in methanol solution probably can be justified by a higher solute-solvent association due to the H bonds formation in methanol solution and to the low permittivity of solvent (e = 32.613).

The Bader’s theory of atoms in molecules (AIM) through calculations of topological properties allows to analyze different intra- or inter-molecular interactions [29]. Accordingly, the electron density distribution, ρ(r) and the Laplacian values, ∇^2 ρ(r), the eigenvalues (λ1, λ2, λ3) of the Hessian matrix and the λ1/λ3 ratio in the bond critical points (BCPs) and in the ring critical points (RCPs) were calculated with the version 2000 of AIM program [30]. Here, the results for squamocin in both media are given in Table 5. In both media, only a H bond was observed whose properties are λ1/λ3 < 1 and ∇^2 ρ(r) > 0. Molecular models of squamocin in methanol solution are presented in two graphics in Fig. S3 and these correspond to two parts of its structure because it present a large aliphatic chain. In both cases, the new H bond created correspond to O91–H92···O34 interaction formed between the groups corresponding to ring R2. It interaction form a new RCP named RCPN1 while the other R1, R2 and R3 rings only present the RCP1, RCP2 and RCP3. Here, RCP2 and RCP3 can be seen in the upper graphic while the RCP1 in the inferior graphic of Fig. S3. The properties presented in Table 5 show that the distance between the involved O34 and H92 atoms in the new H bond formed is higher in methanol solution (2.1128 Å) than the value in gas phase (2.0640 Å), indicating that this interaction is stronger in gas phase than in solution. Hence, the densities values justify these latter observations. Another important observation is the decreasing in the density value of RCP1 in solution which belong to lactone ring, as compared with the value in gas phase. This fact was also observed in the motrilin acetogenin [13] and, in that case, the decreasing was attributed to the solvation of O atoms with solvent molecules increasing the electron density of R1 ring. Then, the intensity of band associated to C–O stretching mode in methanol solution increase, as was also observed in squamocin.

### 4.4. Frontier orbitals and global descriptors

The mapped MEP surfaces have evidenced that in the two media the nucleophilic regions (seven acceptors atoms) are more extense than the electrophilic one (three donors OH groups) and while the NBO study support the high stability of squamocin in methanol solution. On the other hand, the acceptors (O) and donors (O–H) groups present in the squamocin structure probably support the antimicrobial and cytotoxic properties revealed for this γ-lactone [13,16–21]. For the above reasons, frontier orbitals [16,17] and some global descriptors should be investigated for squamocin in both media [18–21]. First, the gap values for squamocin were calculated in both media with the values of frontier orbitals by using the B3LYP/6–31G* method and, then, with these gap values the
chemical potential ($\mu$), electronegativity ($\chi$), global hardness ($\eta$), global softness ($S$), global electrophilicity index ($\omega$) and nucleophilicity indexes ($E$) descriptors were computed by using known equations [18–21]. Thus, in Table 6 are summarized the calculated HOMO and LUMO, energy band gaps and those mentioned descriptors for squamocin in gas phase and methanol solution by using the hybrid B3LYP/6-31G* method. The equations used to calculate the descriptors are presented in the same Table together with frontier orbitals, gap values and descriptors reported for motrilin, 6-nitro-1,3-benzothiazole-2(3H)-thione and niclosamide species with different biological activities [13,31,32]. Motrilin is an acetogenin as squamocin while the benzothiazol-thione derivative has potential antimicrobial activity and, niclosamide was suggested as potential antiviral to treatment of COVID-19 [13,31,32,40]. When the gap values for squamocin are compared in both media it is observed that the value observed in methanol solution (3.7195 eV) is slightly lower than the observed in gas phase (3.8069 eV). Hence, squamocin is most reactive in methanol solution, as also was predicted for motrilin. This latter acetogenin is most reactive in both media than squamocin because their gap values have lower values. On the other hand, ascorbic acid is less reactive than the two acetogenins (5.5071 eV) while the benzothiazole derivative is the most reactive than all compared compounds, with a gap value of 3.6525 eV. Here, a very important resulted is observed in the two global electrophilicity index ($\omega$) and nucleophilicity ($\pi$) indexes, that is, when the gap values are between 3.6 and 3.8 eV the electrophilicity index ($\omega$) are 5.7/5.9 eV while the nucleophilicity indexes ($\pi$) are $-8.4/-8.9$ eV. On the contrary, the ascorbic acid has a value of $2.1673$ eV while the value of $\pi$ is $-9.5128$ eV. Another important resulted obtained here is that the large aliphatic chains in both acetogenins produce decreasing in the gap values, as compared with ascorbic acid, indicating this way that the large...
aliphatic chains increase the reactivities in the two acetogenins. This latter result was also observed when the length of their side chain increases from cidofiovir up to brincidofiovir [41].

4.5. Vibrational analyses

Squamocin is an acetogenin with 109 atoms and, for this reason, the assignments of main vibrational normal modes of squamocin were performed with the aid of GaussView program [22] and by comparisons with assignments reported for species containing similar groups [13,18–22]. Hence, the harmonic force fields of squamocin in both media were not calculated with the scaled quantum mechanical force field (SQMFF) methodology [42] and due to the 109 atoms present in its structure 321 vibration normal modes are expected for squamocin, where only 129 vibration modes were assigned. The experimental FT-IR and FT-Raman spectra of squamocin in the solid phase are presented in Figs. 4 and 5 compared with the corresponding predicted in the gas phase and methanol solution by using the B3LYP/6-31G* method. To a better correlation between experimental and theoretical basis set [42]. These modes in ascorbic acid were assigned between 1 region for the

Table 6
Calculated HOMO and LUMO, energy band gap, chemical potential (μ), electronegativity (χ), global hardness (η), global softness (S), global electrophilicity index (μ) and global nucleophilicity index (E) for squamocin acetogenin in gas phase and methanol solution by using the hybrid B3LYP/6-31G* method.

| Frontier orbitals (eV) | B3LYP/6-31G* method | Ascorbic acidb |
|------------------------|----------------------|-----------------|
|                        | Squamocin*a          | Ascorbic acidb  |
|                        | Gas phase            | Methanol solution | Methanol solution |
| HOMO                   | −6.5716              | −6.5179         | −6.2083          |
| LUMO                   | −2.7647              | −2.7984         | −0.7012          |
| [GAP]                  | 3.8069               | 3.7195          | 5.5071           |
| Descriptors (eV)       |                      |                 |                 |
| χ                      | −1.9035              | −1.8598         | −2.7536          |
| μ                      | −4.6682              | −4.6582         | −3.4548          |
| η                      | 1.9015               | 1.8598          | 2.7536           |
| S                      | 0.2627               | 0.2689          | 0.1816           |
| ω                      | 5.7242               | 5.8337          | 2.1673           |
| E                      | −8.8856              | −8.683          | −9.5128          |

| Frontier orbitals (eV) | B3LYP/6-31G* method | Ascorbic acidb |
|------------------------|----------------------|-----------------|
|                        | Motrilineb           | Thionec          |
|                        | Gas phase            | Methanol solution | Methanol solution |
| HOMO                   | −6.5335              | −6.5004         | −6.4443          |
| LUMO                   | −2.7620              | −2.8012         | −2.7918          |
| [GAP]                  | 3.7715               | 3.6992          | 3.6525           |
| Descriptors (eV)       |                      |                 |                 |
| χ                      | −1.8585              | −1.8496         | −1.8263          |
| μ                      | −4.6478              | −4.6508         | −4.61805         |
| η                      | 1.8858               | 1.8496          | 1.8263           |
| S                      | 0.2651               | 0.2793          | 0.2738           |
| ω                      | 5.7276               | 5.8472          | 5.8388           |
| E                      | −8.7645              | −8.6021         | −8.4337          |

χ = −[E(LUMO) − E(HOMO)]/2; μ = [E(LUMO) + E(HOMO)]/2; η = [E(LUMO) − E(HOMO)]/2; S = ½(η); ω = μ/2; E = μ + η

a From Ref [13].
b From Ref [31].
c From Ref [32].
d From Niclosamide in ethanol Ref [32].
2998 cm\(^{-1}\) and, with the scaling these modes appear at 2775 cm\(^{-1}\). For this reason, the Raman band at 2725 cm\(^{-1}\) can be assigned to these vibration modes. In squamocin there are two CH\(_3\) groups where one of them belong to the lactone ring and the other one is located at end of the long side chain, as observed in Fig. 2. This ACG has twenty-four CH\(_2\) groups of which twenty belong to the side chain and the remaining four to the R2 and R3 rings, where, the antisymmetric and symmetric modes of those groups are predicted by calculations coupled among them. For these reasons, and due to elevated number of bands only some of these are assigned to those modes, as observed in Table 7. Those antisymmetric CH\(_3\) and CH\(_2\) stretching modes are predicted at higher wavenumbers than the corresponding symmetric ones and, hence, the IR and Raman bands between 3008 and 2844 cm\(^{-1}\) are assigned to those stretching modes. The corresponding symmetric stretching modes are assigned to the IR and Raman bands between 2932 and 2844 cm\(^{-1}\). Note that the intense Raman bands are assigned to symmetric stretching modes, as expected.

4.5.1.2.2000–1000 cm\(^{-1}\) region. The characteristics vibrations expected in this region are the C=O, C=C, C–O and C=C stretching modes, OH and CH in-plane deformation modes and, also the deformation, wagging and rocking modes of CH\(_3\) and CH\(_2\) groups. The C=O and C=C stretching modes of lactone ring are predicted respectively at 1773 and 1550 cm\(^{-1}\) and are easily assigned to the IR and Raman bands located respectively at 1744 and 1649 cm\(^{-1}\). In ascorbic acid those two stretching modes were assigned at 1753 and 1672 cm\(^{-1}\) [21]. On the other hand, the antisymmetric and symmetric deformation modes of CH\(_3\) and CH\(_2\) groups are predicted by calculations between 1543 and 1441 cm\(^{-1}\), hence, according to the calculations they are assigned as in similar species containing these groups [13,18,34,35,41]. The CH in-plane deformation modes (\(\nu\text{C-H}\)) are assigned to the IR and Raman bands between 1499 and 1300 cm\(^{-1}\), as predicted by calculations while for the OH groups these modes are assigned between 1281 and 1136 cm\(^{-1}\). The bands between 1409 and 1265 cm\(^{-1}\) can be assigned to CH\(_2\) wagging modes while the located between 1342 and 1192 cm\(^{-1}\) are assigned to rocking of CH\(_2\) groups. The CH\(_3\) rocking modes of groups are predicted and assigned to the bands between 1057 and 904 cm\(^{-1}\). The group of IR and Raman bands between 1171 and 792 cm\(^{-1}\) can be assigned to C–C and C–O stretching modes because the B3LYP/6-31G* calculations predict clearly these modes in that region [13,34,35,41].

4.5.1.3.1000–20 cm\(^{-1}\) region. The out-of-plane OH and C–H deformation modes, of twisting modes of CH\(_2\) and CH\(_3\) groups, skeletal modes such as, in-plane \(\gamma\text{C} = \text{O}\) and \(\gamma\text{C}-\text{C}\) deformation or out-of-plane \(\gamma\text{C} = \text{O}\), \(\gamma\text{C}-\text{C}\) deformation modes and of deformation rings (\(\beta\text{R}_1, \beta\text{R}_2\)) and torsion rings (\(\tau\text{R}_1, \tau\text{R}_2\)), modes of five members R1, R2 and R3 rings are expected in this region. Those vibration modes are strongly coupled among them due to great quantity of observed bands in the same positions. The out-of-plane OH
Table 7
Observed and calculated wavenumbers (cm\(^{-1}\)) and assignments for squamocin acetogenin in gas phase by using the hybrid B3LYP/6-31G* method.

| Squamocina | Experimental | B3LYP/6-31G* | Assignment* |
|------------|--------------|--------------|-------------|
| IR         | Ra SQM       |              |             |
| 3427 m     | 3415         | r(O-H)       |
| 3427 m     | 3410         | r(O-H)       |
| 3388 m     | 3398         | r(O-H)       |
| 3083sh     | 3079w        | s(C-H)       |
|            | 3008w        | s(C-H)       |
|            | 2983w        | s(C-H)       |
|            | 2983w        | s(C-H)       |
| 2971sh     | 2970w        | s(C-H)       |
| 2953sh     | 2950w        | s(C-H)       |
|            | 2932s        | s(C-H)       |
| 2923vs     | 2904s        | s(C-H)       |
|            | 2877vs       | s(C-H)       |
| 2852s      | 2844s        | s(C-H)       |
|            | 2844s        | s(C-H)       |
|            | 2725w        | s(C-H)       |
| 1744s      | 1740w        | s(C – O)     |
| 1649w      | 1649w        | C–C          |
| 1649w      | 1649w        | C–C          |
| 1649w      | 1649w        | C–C          |
| 1508w      | 1503         | s(C-H)       |
| 1489w      | 1499         | s(C-H)       |
| 1473sh     | 1476sh       | s(C-H)       |
| 1465 m     | 1485         | s(C-H)       |
| 1459 m     | 1455sh       | s(C-H)       |
| 1450w      | 1449         | s(C-H)       |
| 1439w      | 1441 m       | s(C-H)       |
| 1418w      | 1421w        | s(C-H)       |
| 1397w      | 1397w        | wag(CH\(_2\))|
| 1397w      | 1394         | wag(CH\(_2\))|
| 1389w      | 1388         | s(C-H)       |
| 1374w      | 1368w        | s(C-H)       |
| 1374w      | 1366         | s(C-H)       |
| 1363w      | 1361         | wag(CH\(_2\))|
| 1355w      | 1352w        | wag(CH\(_2\))|
| 1355w      | 1342         | s(CH\(_2\))  |
| 1337sh     | 1337         | s(CH\(_2\))  |
| 1319 m     | 1319         | s(CH\(_2\))  |
| 1319 m     | 1319w        | s(CH\(_2\))  |
| 1319 m     | 1311         | wag(CH\(_2\))|
| 1304sh     | 1301sh       | s(CH\(_2\))  |
| 1287w      | 1293w        | s(CH\(_2\))  |
| 1273w      | 1270w        | s(CH\(_2\))  |
| 1253w      | 1252w        | s(CH\(_2\))  |
| 1230w      | 1227w        | s(CH\(_2\))  |
| 1205w      | 1208w        | s(CH\(_2\))  |
|            | 1201w        | s(CH\(_2\))  |
|            | 1188w        | s(CH\(_2\))  |
|            | 1172w        | s(CH\(_2\))  |
| 1172w      | 1171         | s(CH\(_2\))  |
| 1146w      | 1144w        | s(CH\(_2\))  |
| 1146w      | 1144w        | s(CH\(_2\))  |
| 1128sh     | 1130w        | s(CH\(_2\))  |
| 1121 m     | 1112w        | s(CH\(_2\))  |
| 1089sh     | 1088w        | s(CH\(_2\))  |
| 1076s      | 1075w        | s(CH\(_2\))  |
| 1071sh     | 1061 m       | s(CH\(_2\))  |
| 1053 m     |              | s(CH\(_2\))  |
| 1027 m     |              | s(CH\(_2\))  |
| 1000w      | 996w         | s(CH\(_3\))  |
| 980w       | 979w         | s(CH\(_3\))  |
| 960w       | 960w         | s(CH\(_3\))  |
| 953w       | 945w         | s(CH\(_3\))  |
| 928w       | 921sh        | s(CH\(_3\))  |
| 911w       | 908w         | s(CH\(_3\))  |

(continued on next page)
deformation modes or torsion modes are predicted and assigned at 515, 506, 326, 312 and 248 cm\(^{-1}\) while the out-of-plane deformation mode for aromatic C\(\equiv\)H group of lactone ring (\(\gamma\)C-H) is assigned to the weak IR and Raman bands respectively at 840 and 839 cm\(^{-1}\). The calculations predict the twisting modes of two CH\(_3\) groups with weak intensities at 249, 143 and 116 cm\(^{-1}\), hence, these modes can be associated to the Raman bands and the shoulder in the same spectrum at 248, 140 and 104 cm\(^{-1}\). The CH\(_2\) twisting modes are normally assigned between 1041 and 558 cm\(^{-1}\) \([13,18,34,35,41]\), thus, in this ACG these modes are assigned to the bands between 878 and 590 cm\(^{-1}\). The assignments of other skeletal modes are performed according to the calculations and by comparisons with species with similar groups \([13,18\text{–}21,32,34,35,41]\), can be seen in Table 7.

### 5. Ultraviolet–visible spectrum

The experimental UV–visible spectrum of squamocin in
methanol solution is shown in Fig. 6 compared with the corresponding predicted in the same medium by using hybrid B3LYP/6-31G* method. The electronic spectrum of this γ-lactone was predicted with TD-DFT calculations incorporated in the Gaussian09 program [23]. The experimental spectrum shows a very strong band at 213 nm while in the predicted spectrum are observed two intense bands in c. a. 160 and 230 nm and, other weak in c. a. 288 nm. The experimental spectrum was recorded from 200 up to 400 nm and, for this reason, the band predicted between 150 and 200 nm was not experimentally observed. In the experimental UV–Vis spectrum of motrilin ACG in methanol solution the intense band is observed at 220 nm [13]. The NBO calculations predict π→π* and n→π* transitions and, hence, those predicted bands can be assigned combining the IR and Raman spectra with hybrid functional B3LYP/6-31G* calculations. A corrected solvation energy value of −147.54 kJ/mol was predicted for squamocin in methanol while the atomic NPA charges evidence higher values on O atoms of R2 and R3 rings, as compared with the corresponding to lactone ring. Mapped MEP surfaces suggest that nucleophilic regions are located on the O atoms of three rings and of OH bonds belonging to side chain, in agreement with the higher charges values evidenced on these O atoms while electrophilic regions are predicted on the H atoms of OH groups. AIM studies have revealed high stabilities of squamocin in both media while the NBO calculations show higher stability only in methanol solution. The expansion of volume and the higher dipole moment in methanol suggest a clear solvation of squamocin by solvent molecules. The gap values have evidenced that squamocin is most reactive in methanol while that its large aliphatic chain produces an increase in the reactivity of this γ-lactone, as compared with ascorbic acid lactone. Reasonable concordances among the predicted IR, Raman and UV–visible spectra with the corresponding experimental ones were found.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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