http://dx.doi.org/10.21577/0103-5053.20180037

J. Braz. Chem. Soc., Vol. 29, No. 5, 1162-1166, 2018
Printed in Brazil - ©2018 Sociedade Brasileira de Química

Short Report

Mass Spectrometry Analysis of Protonated Marine Natural Product Seriniquinone

Rodrigo M. da Silva,[a] Thais Guaratini,[b] Paula C. Jimenez,[c] William Fenical,[d] Letícia V. Costa-Lotufo,[e] Ricardo Vessecchi[f] and Norberto P. Lopes[*,a]*

[a]*Núcleo de Pesquisas de Produtos Naturais e Sintéticos (NPPNS), Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, 14040-903 Ribeirão Preto-SP, Brazil*

[b]*Lychnoflora Pesquisa e Desenvolvimento em Produtos Naturais LTDA, Campus USP, 14030-090 Ribeirão Preto-SP, Brazil*

[c]*Departamento de Ciências do Mar, Universidade Federal de São Paulo, 11070-100 Santos-SP, Brazil*

[d]*CMBB, Scripps Institution of Oceanography, UC San Diego, 9500 Gilman Drive No. 0204, 92093-0204 La Jolla, CA, USA*

[e]*Departamento de Farmacologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, 05508-900 São Paulo-SP, Brazil*

[f]*Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo,14040-901 Ribeirão Preto-SP, Brazil*

Seriniquinone is a natural quinone isolated from a rare marine bacterium of the genus *Serinicoccus*. This secondary metabolite has been shown to have anticancer properties, which has raised attention of the scientific community. In this short report, we present the first investigation of the gas-phase chemistry fragmentation reactions of seriniquinone in electrospray ionization tandem mass spectrometry (ESI-MS/MS), to be further applied in pharmacokinetics and metabolism studies. All the proposals herein were supported by computational chemistry.

Keywords: *Serinicoccus*, seriniquinone, electrospray ionization mass spectrometry, quinones, computational chemistry

Introduction

The research regarding marine natural products have been responsible for the discovery of promising compounds for the treatment of several diseases, including good prototypes for cancer therapy.[1] Seriniquinone (SQ, Figure 1), a metabolite isolated from a rare marine bacterium of the genus *Serinicoccus*, was recently characterized and evaluated for its cytotoxic activity.[2] SQ belongs to a class of quinones, which is widely present in nature and is considered a target scaffold in medicinal chemistry as a gifted class of anticancer drugs.[2] *In vitro* screening indicated that SQ is highly cytotoxic and selective towards eight out of the nine NCI 60 melanoma cells lines.[2] Biochemical and pharmacological assessments demonstrated that SQ specifically targets a skin protective antimicrobial peptide, dermcidin,[2,3] which has also been described as a pro-survival protein in cancer cell lines.[4] SQ is the only known natural product to bind and modulate such protein, and recent studies have reported dermcidin to be over expressed in some cancers types and linked to a poor prognosis of such disease.[5,6] In addition, studies have also shown that SQ induces autophagocytosis, further leading to an efficient elimination of cancer cells through apoptotic death.[2,7]

The advance of new bioactive molecules from natural sources to drug development requires preclinical evaluations, such as pharmacokinetic profile and metabolism studies, to answer basic safety questions concerning biomedical use of such compounds.[8-10] Serving such a purpose, liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) is considered to be an important analytical technique for identification and quantification of the compounds of interest and their

*e-mail: npelopes@gmail.com
This paper is part of the PubliSBQ Special Issue "IUPAC-2017" (http://publi.sbq.org.br/).

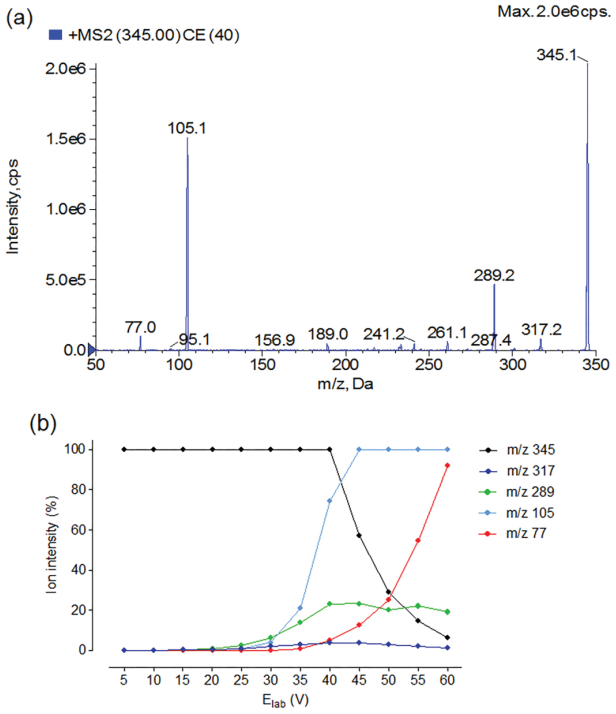


Figure 1. (a) Mass spectrum for seriniquinone and (b) E_{lab} experiments obtained at QqQ analyzer.

metabolites.[11] Besides the high sensitivity for analysis of small amount of substance in complex matrices, the combination of ESI and tandem or sequential mass spectrometry (MS/MS or MS^n) allows the reaction of a precursor ion with a non-reactive collision gas to obtain a fragmentation profile for this ion. ESI combined to collision-induced dissociation (CID) has already been applied for the rapid analysis of quinones, such as lapachol and naphthoquinone derivatives.[12,13]

For an improved application of ESI-MS/MS technique, it is important to define the gas phase reactions involved and to perform the rationalization of a fragmentation pathway of the studied compound.[14] Taking into account the relevant biological activity and such novel mode of action observed for SQ, the goal of this work is to report on the fragmentation pathways of SQ. Previous knowledge of the fragmentation mechanisms is fundamental to determine chemical structures of the biological metabolites of SQ and to support better understanding of experimental results from *in vitro* and *in vivo* preclinical assays.

Experimental

Seriniquinone (SQ) was synthesized as previously described,[2] affording 10 mg for all ESI-MS/MS analysis. Due to a very limited solubility, stock solutions of SQ were prepared in DMSO (99.7%, Sigma-Aldrich, Brazil). Working solutions of 1 μg mL^{-1} were prepared by dilution in methanol/water (95:5), allowing a final DMSO concentration of 0.1%, which has no effect on the ionization process.

The experiments were carried out using two Applied Biosystems/Sciex instruments: an API 3200 triple quadrupole mass spectrometry and a quadrupole ion trap mass spectrometry (AB Sciex, Concord, Canada) equipped with an electrospray source (TurboIonSpray Ion Source). Single compounds were infused at a flow rate of 5.0 μL min^{-1} for tuning compound dependent on MS parameters using a model 11 PLUS syringe pump (Harvard Apparatus, Holliston, USA) directly connected to the interface. Entrance potential (EP) was fixed at 10 V. Specific compound dependent MS parameters were optimized as declustering potential (DP = 70 V), curtain gas (CUR = 10 psi), ionspray voltage (IS = 4500 V) and nebulizer gas (GS1 = 14 psi). The MS was operated in the positive ion and product ion mode.

ESI-MS/MS analyses were performed by CID using nitrogen as the collision gas. The protonated molecule was selected and fragmented with laboratory-frame energy (E_{lab}) in the range of 5 to 60 eV for the precursor ion to achieve the energy-resolved fragmentations curves. Data were acquired and evaluated using Analyst software.[15]

Computational quantum calculations were performed in order to aid the fragmentation on the quinone derivative. Protonated quinonoid compounds exhibit consecutive CO eliminations as the major fragmentation pathways, mainly when proton takes place at the carbonyl.[12,14] Here, the gas-phase basicity for protonation on quinone derivative was performed using the B3LYP/6-31G(d)[16-18] model in Gaussian 03 program.[19]

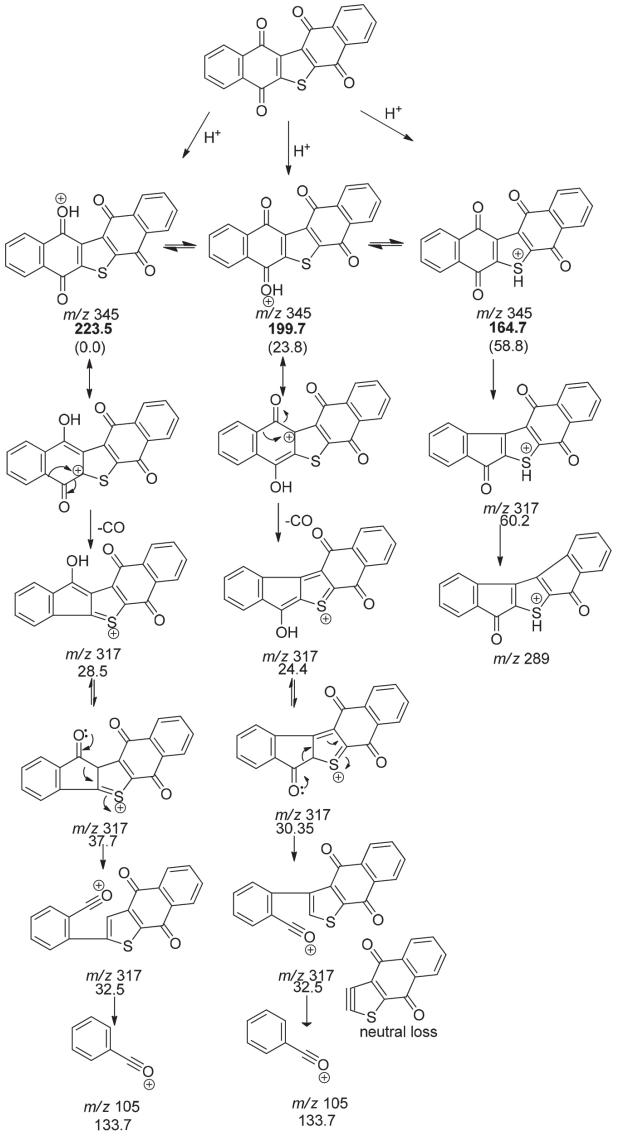
Fragmentation mechanisms were proposed based on the energetic profile for pathways suggested by MS/MS studies. All computed energies were obtained in the B3LYP model[16] and minimal energy surface was obtained by analyzing positive values for vibrational frequencies.

Results and Discussion

The ESI-MS/MS spectrum of protonated SQ displayed product ions of *m/z* 345, 317, 289, 105 and 77. At normal collision energies (around 20 to 25 eV), no fragmentation was observed. This initial analysis of the fragmentation mechanism of SQ showed a high stability for the protonated compound, suggesting high collision energies at the CID process (Figure 1a). The major fragmentation occurs after 35 eV, as observed at E_{lab} plot (Figure 1b).

For the first rationalization, we looked for the most favorable protonation site using computational chemistry. The gas-phase basicities calculated with the

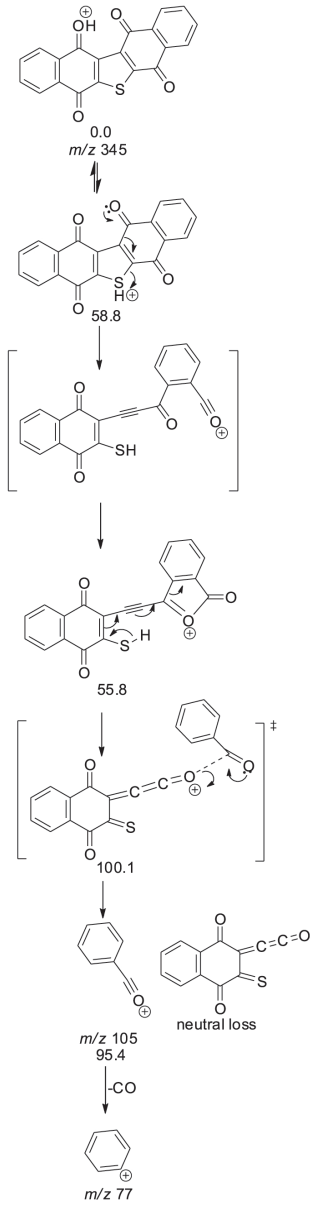
B3LYP/6-31G(d) model[16-18] showed the carbonyl to be the most stable protonation site. Protonation at the oxygen atom is more stable at 58.8 kcal mol^{-1}, when compared to protonation at the sulfur. However, these three possibilities for protonation were explored in our studies, as depicted in Scheme 1.



Scheme 1. Fragmentation mechanisms for protonated seriniquinone. All values are relative Gibbs energies, in kcal mol^{-1}, for fragmentation obtained at B3LYP/6-31G(d). Bold values are gas-phase basicity in kcal mol^{-1}.

The major fragments observed in MS spectra after 35 eV were *m/z* 105 and 77. Thus, the pathways were suggested on the basis of formation of such ions.

From these results, fragmentation pathways were suggested, as displayed on Schemes 1 and 2, considering the possibility of the most stable (protonation at the oxygen atom) or the most reactive ions (protonated at the sulfur atom) to initiate the fragmentation pathways. Formation of the ions at *m/z* 317 and 289 will occur following ring contraction (Scheme 1), after collisional activation of *m/z* 345. The major fragment ions at *m/z* 105 and 77 will also depend on hydrogen migration (Scheme 2). In this case, the critical energy for this migration and that to eject the acylium fragment ion is lower than proposed in Scheme 1.



Scheme 2. Fragmentation mechanisms for protonated seriniquinone. All values are relative Gibbs energies, in kcal mol^{-1}, for fragmentation obtained at B3LYP/6-31G(d).

Detailed analysis of the complete mechanism showed the sequential neutral elimination of CO based on ring contractions, as previously observed for other natural heterocyclic natural compounds produced by microorganism.[20,21] For the four protonation possibilities at the oxygen atoms, an initial tautomeric equilibrium

is necessary before the ring contraction releases CO (Scheme 1). In the case of S protonation, the ring contraction can occur without any previous steps.[14] However, for all possibilities, the formation of the most stable ions at *m/z* 105 and 77 requires an unusual five-member ring with a triple bond. As shown in Scheme 1, the formation of *m/z* 105 requires 133.7 kcal mol^{-1} to occur. Regarding Scheme 2, the relative energy for formation of *m/z* 105 and the transition state were calculated, confirming the probability of this reaction. In this case, an alternative mechanism of fewer steps must be involved, as suggested in Scheme 2. The protonation occurs at the most stable carbonyl group and an anchimeric assistance (from the oxygen lone in opposition of sulfur atom) will afford the intermediated ring opening.[14] The alkyne moiety obtained in this process can have a long range of hydrogen migration, affording a cumulene structure with a terminal carbonyl group. Calculation of the transition state was in agreement with the proposed pathway (Scheme 2) and, after an anchimeric assistance, the oxygen lone pair afforded the most stable ions at *m/z* 105 and 77 (after CO loss).

Conclusions

ESI-MS studies on SQ showed a high stability of the protonated compound when submitted to CID conditions. ESI-MS/MS analyses of protonated SQ have shown that the relative intensity and number of product ions are obtained by two competitive pathways, and computational results corroborated the ESI-MS/MS. These results will aid in the identification of possible SQ products when submitted to metabolism studies. Ultimately, data generated in the present study has shown the higher stability of the SQ moiety. Still, other protocols applying higher collision dissociation energies should be employed in further LC-MS/MS investigations, including pharmacokinetics investigations.

Acknowledgments

The authors gratefully acknowledge the financial support received from FAPESP (2014/50265-3), CAPES and CNPq.

References

1. Newman, D. J.; Cragg, G. M.; *J. Nat. Prod.* **2016**, *79*, 629.
2. Trzoss, L.; Fukuda, T.; Costa-Lotufo, L. V.; Jimenez, P.; La Clair, J. J.; Fenical, W.; *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 14687.
3. Schittek, B.; Hipfel, R.; Sauer, B.; Bauer, J.; Kalbacher, H.; Stevanovic, S.; Schirle, M.; Schroeder, K.; Blin, N.; Meier, F.; Rassner, G.; Garbe, C.; *Nat. Immunol.* **2001**, 1133.
4. Stewart, G. D.; Skipworth, R. J.; Ross, J. A.; Fearon, K. C.; Baracos, V. E.; *Curr. Opin. Clin. Nutr. Metab. Care* **2008**, *11*, 208.
5. Ortega-Martínez, I.; Gardeazabal, J.; Erramuzpe, A.; Sanchez-Diez, A.; Cortés, J.; García-Vázquez, M. D.; Pérez-Yarza, G.; Izu, R.; Díaz-Ramón, J. L.; Fuente, I. M.; Asumendi, A.; Boyano, M. D.; *Int. J. Cancer* **2016**, *139*, 1598.
6. Brauer, H. A.; D'arcy, M.; Libby, T. E.; Thompson, H. J.; Yasui, Y. Y.; Hamajima, N.; Li, C. I.; Troester, M. A.; Lampe, P. D.; *Breast Cancer Res. Treat.* **2014**, 299.
7. La Clair, J. J.; Fenical, W.; Costa-Lotufo, L. V.; *J. Braz. Chem. Soc.* **2016**, *27*, 1346.
8. Singh, S. S.; *Curr. Drug Metab.* **2006**, *7*, 165.
9. Zhang, D.; Luo, G.; Ding, X.; Lu, C.; *Acta Pharm. Sin. B* **2012**, *2*, 549.
10. Moreira, F. L.; Marques, L. M. M.; Lopes, N. P.; de Oliveira, A. R. M.; *Phytochem. Rev.* **2017**, *16*, 355.
11. Ernest, M.; Silva, D. B.; Silva, R. R.; Vêncio, R. Z. N.; Lopes, N. P.; *Nat. Prod. Rep.* **2014**, *31*, 784.
12. Vessecchi, R.; Emery, F. S.; Galembeck, S. E.; Lopes, N. P.; *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2101.
13. Vessecchi, R.; Lopes, J. N. C.; Lopes, N. P.; Galembeck, S. E.; *J. Phys. Chem.* **2011**, *115*, 12780.
14. Demarque, D. P.; Crotti, A. E. M.; Vessecchi, R.; Lopes, J. N. C.; Lopes, N. P.; *Nat. Prod. Rep.* **2016**, *33*, 432.
15. AB Sciex; *Analyst*, version 1.6.2; AB Sciex, Concord, Ontario, Canada, 2012.
16. Lee, C.; Yang, W.; Parr, R. G.; *Phys. Rev. B* **1988**, *37*, 785.
17. Rassolov, V. A.; Pople, J. A.; Ratner, M. A.; Windus, T. L.; *J. Chem. Phys.* **1998**, *109*, 1223.
18. Becke, A. D.; *J. Chem. Phys.* **1993**, *98*, 5648.
19. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, Jr., T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.;

Pople, J. A.; *Gaussian 03*, Gaussian, Inc.: Wallingford, CT, USA, 2004.

20. Furtado, N. A.; Vessecchi, R.; Tomaz, J. C.; Galembeck, S. E.; Bastos, J. K.; Lopes, N. P.; Crotti, A. E.; *J. Mass Spectrom.* **2007**, *42*, 1279.
21. Simon, H.; Hoffman, G.; Hübner, F.; Humpf, H. U.; Karst, U.; *Anal. Bioanal. Chem.* **2016**, *408*, 2471.

Submitted: January 17, 2018
Published online: March 5, 2018