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Thioglycerol matrix interactions in the positive ion fast atom bombardment mass spectrometry of several Hantzsch and Biginelli ester derivatives of boronic acids

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RATIONALE: Fast atom bombardment (FAB) ionization is a valuable tool for analyzing non-volatile or thermally labile compounds when harder ionization methods (EI or CI) have been unsuccessful. Unexpected interactions between matrix and analyte may, however, occur in FAB ionization leading to the formation of artifacts whose structures have to be determined.

METHODS: We used esters synthesized by our research group following green chemistry protocols. Artifacts relating to the target molecules were produced in the solvent cage using positive ion FAB using thioglycerol as the matrix and analyzed by accurate mass measurement.

RESULTS: Through interactions between thioglycerol and the boron atom, various adducts associated with boroncontaining Hantzsch and Biginelli ester derivatives appeared in the FAB mass spectra. These interactions must arise in a solvent cage involving a Lewis acid-base bond. Accurate mass determinations of these adducts confirmed the presence of the target molecules.

CONCLUSIONS: Interactions between the thioglycerol matrix and the eleven boron-containing Hantzsch and Biginelli ester derivatives in addition to the three reagents were seen in their FAB⁺ spectra. These interactions, together with their accurate mass determinations, allowed us to determine the structures of the new molecules. Copyright © 2013 John Wiley & Sons, Ltd.

Soft mass spectrometry (MS) ionization modes are particularly useful for analyzing non-volatile or thermolabile compounds. In the Fast Atom Bombardment (FAB) ionization mode the sample is suspended in a non-volatile liquid matrix,^[1] and, in positive ion FAB, an [M+H]⁺ ion is usually produced, allowing the molecular mass of the target molecule to be determined. However, anomalous artifact ions sometimes appear in the FAB spectrum: derived from processes such as solvent condensation, ligand exchange, dehalogenation, hydride abstraction, and adducts due to abrupt changes in surface tension in the region of bombardment, as well as from convective and diffusive processes.^[2,3] The presence of these artifact ions can lead to confusion in the determination of the molecular mass of the target molecule.

On the other hand, Hantzsch esters (1,4-dihydropyridines) and Biginelli esters (3,4-dihydropirimidinones) are important classes of calcium-channel blockers that have emerged as valuable drugs for treating cardiovascular diseases, including the reduction of hypertension.^[4–7]

The increasing importance of boronic acids has led to the development of new mild and efficient methods for a large number of boron-containing compounds (BCC). These molecules have long been used as antiseptics, antibiotics, cosmetics and insecticides. In addition, BCC have been used to target biomolecules involved in cancer therapy, including through non-specific applications (such as boron neutron capture therapy) and direct selective action on some cancer targets.^[8] As a part of our ongoing research program, we are interested in developing green chemistry protocols for the production of novel hybrid heterocyclic molecules with possible pharmacological activity, mainly in the absence of solvent and using microwave or infrared irradiation to activate the reactions.^[9–14] These synthesized molecules are characterized by mass spectrometry.^[11,15–19]

The aim of this work was to study the novel thioglycerol matrix interactions observed during the FAB⁺ analysis of various BCC obtained by green chemistry. The BCCs studied were the reagents *o-*, *m-*, and *p*-boronic acids (1a-c), the boronic derivatives of the Hantzsch ester (5a-c, 6a-c, and 7b-c) and the Biginelli ester (9a-c) (labeled BC–Hantzsch and BC–Biginelli, respectively), the syntheses of which are shown in Schemes 1 and 2. When using thioglycerol as the FAB matrix, however, we did not obtain the expected [M+H]⁺ ions, but several artifact and other ions were found, and analyzed using

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Scheme 1. Synthesis of BC-Hantzsch esters.



Scheme 2. Synthesis of BC-Biginelli esters.

high-resolution mass spectrometry (HRMS). We obtained the artifact ion accurate masses and, consequently, their unequivocal elemental compositions.

EXPERIMENTAL

Chemicals

Formylphenylboronic acids (*o-*, *m-* and *p-*), ethyl and methyl acetoacetate, 1,3-cyclohexanedione, ammonium acetate and thioglycerol were purchased from Sigma-Aldrich Chemistry (St. Louis, MO, USA). Urea was obtained from Merck (Naucalpan, México) reagent grade ethanol, *n*-hexane, and ethyl acetate were acquired from Merck. All of them were used without further purification.

Mass spectrometry

Positive ion FAB and HRMS-FAB spectra were obtained using a JMS-700 MStation mass spectrometer (JEOL, Japan). The measurements were performed at 25 °C, using a FAB energy of 6 keV, an emission current of 10 mA, an acceleration voltage of 10 kV, with Xe as the ionization gas and thioglycerol as the matrix. The HRMS experiments were carried out with thioglycerol as the matrix and PEG (poly(ethylene glycol)) ions from *m*/*z* 200 to 800 as internal mass references at 10 000, 20 000, 30 000, and 45 000 resolution. Accurate masses were calculated as the mean of the data obtained from 5–6 scans, determined from the top and centroid mass of the ion signals. Elemental compositions were calculated within a mass range of \pm 10 ppm from the measured mass.

Preparation of BC-Hantzsch and BC-Biginelli esters

The studied molecules were obtained,^[19] using Schemes 1 and 2, by green synthesis. These compounds were characterized by their ¹H, ¹³C and ¹¹B NMR data together with their IR spectra.

RESULTS AND DISCUSSION

The expected molecular or $[M+H]^+$ ions of the target molecules **5a-c**, **6a-c**, **7b-c** and **9a-c** were not obtained using electron ionization, chemical ionization, or FAB⁺ (with glycerol, *m*-nitrobenzyl alcohol, or *o*-octyloxynitrobenzene matrices). Therefore, as it is known that the thioglycerol (TG) matrix affords interesting interactions,^[1,3] it was considered that the sulphur atom, by means of Lewis interaction inside the matrix with the empty orbital of the target BCC, could produce FAB adducts related to the target molecules.

Study of the boron-containing reagents

The boron-containing reagents **1a–c** were analyzed using the same FAB conditions as employed for the analysis of the target molecules. These all have molecular masses of 150 Da.

An ion was observed at m/z 205 for the *ortho*-isomer reagent and it is suggested that this is the oxathiaborinanic cation, $o-[MTG]^+$ (Table 1, Fig. 1(a)), with calculated and experimental masses of m/z 205.0489 and 205.0495 (-2.5 ppm error), respectively, corresponding to $C_{10}H_{10}O_2S_1^{11}B_1$. To validate this structure, the $o-[MTG-1]^+$ ion at m/z 204 was also

Table 1. HRMS of the adducts of formylphenylboronic acids and Hantzsch and Biginelli ester derivatives

				[MTG-1] ⁺			
Reagent or product	Adduct*	$[MTG]^+$ Calc/Exp (<i>m</i> /z)	Mass error (ppm)	$[MTG^{10}B]^+$ Calc/Exp (<i>m</i> /z)	Mass error (ppm)	$[MTG-H]^+$ Calc/Exp (<i>m</i> / <i>z</i>)	Mass error (ppm)
1a 1b-c 5a-c 6a-c 7b-c 9a-c	o-[MTG] ⁺ m,p-[MTG] ⁺ o,m,p-[MTG] ⁺ o,m,p-[MTG] ⁺ o,m,p-[MTG] ⁺	205.0489/205.0495 223.0595/223.0600 416.1334/416.1339 444.1647/444.1652 410.1592/410.1597 395.1443/395.1448	-2.5 +5.2 -0.5 +0.5 +4.6 -4.3	204.0527/204.0531 222.0635/222.0637 415.1349/415.1375 443.1685/443.1688 409.1626/409.1634 394.1487/394.1484	-1.8 -0.6 -6.4 -0.9 -2.0 +0.8	204.0411/204.0416 222.0516/222.0522 415.1255/415.1261 443.1568/443.1574 409.1514/409.1519 394.1364/394.1370	-5.3 -2.3 -6.4 -2.3 -1.3 +7.0
*TG = thioglycerol Calc = calculated; Exp = experimental							



Figure 1. FAB⁺ mass spectra and the corresponding $[MTG]^+$ of reagents: *ortho* (a) and *meta* (b).

evaluated, either as the ¹⁰B species, *o*-[MTG¹⁰B]⁺, or as the ion formed by hydrogen loss, *o*-[MTG–H]⁺, from *m/z* 205 (see Table 1). The accurate masses for both ions in the doublet at *m/z* 204 confirmed the proposed structure, the ¹⁰B ion, $C_{10}H_{10}O_2S_1^{10}B_1$, having a calculated mass of *m/z* 204.0527 and an experimental mass of 204.0531 (–1.8 ppm error), and the $C_{10}H_9O_2S_1^{11}B_1$ (hydrogen loss) ion having a calculated mass of *m/z* 204.0411 and an experimental mass of 204.0416 (–5.3 ppm error). The base peak at *m/z* 133 could be formed by loss of water from the [M+H]⁺ ion, and its expected formula was verified as $C_7H_6O_2^{-11}B$ by HRMS (calculated mass *m/z* 133.0455, experimental mass 133.0461 (–5.9 ppm error).

The *meta-* and *para-*regioisomeric reagent thioglycerol adducts, *m*-[MTG]⁺ and *p*-[MTG]⁺, both appeared as peaks at *m*/*z* 223, with relative abundances of 93% and 9%, respectively, and these were assigned as hydroxyallylborothioate ions (Table 1, Fig. 1(b)). These assignments were supported by their elemental compositions, $C_{10}H_{12}O_3S_1^{11}B_1$, determined from their accurate masses: calculated mass *m*/*z* 223.0595,

experimental mass 223.0600 (+5.2 ppm error). To validate the structures of m,p-[MTG]⁺, the respective m,p-[MTG-1]⁺ ions were examined for their isotopic contributions m, p-[MTG¹⁰B]⁺ and hydrogen losses m,p-[MTG-H]⁺ (Table 1). Both accurate masses were obtained and m,p-[MTG¹⁰B]⁺ was assigned to C₁₀H₁₂O₃S₁¹⁰B₁ from a calculated mass of m/z 222.0635 and an experimental mass of 222.0637 (-0.6 ppm error), with m,p-[MTG-H]⁺ assigned to C₁₀H₁₁O₃S₁¹¹B₁ from a calculated mass of m/z 222.0516 and an experimental mass of 222.0522 (-2.3 ppm error).

BC-Hantzsch esters

Target compounds 5a-c exhibited *o*,*m*,*p*-[MTG]⁺ FAB⁺ adducts at *m*/*z* 416, corresponding to a 2-propynylborothioate moiety (Table 1, Fig. 2(a)), with relative abundances of 17%, 10%, and 7%, for the *ortho*, *meta* and *para* structures, respectively. These structural assignments were derived from the elemental composition, $C_{20}H_{23}^{11}B_1N_1O_6S_1$, determined from the HRMS



Figure 2. FAB⁺ mass spectra of Hantzsch esters and the corresponding [MTG]⁺: *ortho* **5a** (a), *ortho* **6a** (b), and *para* **7c** (c).

accurate masses for each ion. The ion derived from **5a** had a calculated mass of m/z 416.1334 and a measured mass of 416.1339 (-0.5 ppm error). These proposed structure were supported by HRMS data obtained for the ions at m/z 415, both

for the isotopic contribution o-[MTG¹⁰B]⁺ and for a hydrogen atom loss o-[MTG–H]⁺ (Table 1), yielding the elemental compositions $C_{20}H_{23}^{10}B_1N_1O_6S_1$, calculated mass m/z 415.1349 and experimental mass 415.1375 (–6.4 ppm error), and $C_{20}H_{22}^{11}B_1N_1O_6S_1$, calculated mass m/z 415.1255, experimental mass 415.1261 (-6.4 ppm error). Two interesting and complementary fragment ions from **5a** were: m/z 284, which was assigned to an *ortho* effect (*ii*, Fig. 2(a)), and validated by HRMS ($C_{16}H_{14}N_1O_4$, calculated mass m/z 284.0917, experimental mass 284.0923 (-5.4 ppm error); and the base peak, m/z 224, which appeared to be formed by loss of the aryl substituent at position 4 (*iii*, Fig. 2(a)). This assignment was validated by HRMS results ($C_{11}H_{14}N_1O_4$, calculated mass m/z 224.0917, experimental mass 224.0917 (-2.6 ppm error)).

Analogous behavior was found for target compounds 6a-c, and the corresponding $o,m,p-[MTG]^+$ adducts, at m/z 444 (8%, 12%, and 13%, respectively), were also assigned to a 2-propynylborothioate moiety (Table 1, Fig. 2(b)), and validated by HRMS data (C22H2711B1N1O6S1, calculated mass m/z 444.1647, experimental mass 444.1652 (+0.5 ppm error)). These assignments were also confirmed by obtaining accurate masses for the isotopic contribution ($C_{22}H_{27}^{10}B_1N_1O_6S_1$, calculated mass m/z 443.1685 and experimental mass 443.1688 (-0.9 ppm error)), and for a hydrogen atom loss $(C_{22}H_{26}^{11}B_1N_1O_6S_1, \text{ calculated mass } m/z 443.1568, \text{ experimental})$ mass 443.1574 (-2.3 ppm error)). An ortho effect was also detected (*iv*, Fig. 2(b)) in the formation of the ion at m/z 284, which was again validated by HRMS ($C_{16}H_{14}N_1O_4$, calculated mass m/z 284.0917, experimental mass 284.0923 (+1.5 ppm error)). The base peak, at m/z 252, was also formed by loss of the aryl substituent at C-4 (v, Fig. 2(b)), with this assignment being supported by HRMS data (C13H18N1O4, calculated mass *m/z* 252.1230, experimental mass 252.1236 (+0.5 ppm error)).

The FAB⁺ spectra of compounds **7b**–**c** (**7a** was not obtained because of steric hindrance) exhibited m,p-[MTG]⁺ adducts at m/z 410, which were assigned as hydroxyallylborothioate ions (Table 1, Fig. 2(c)), with relative abundances of 13% for the *meta* and 7% for the *para* isomers. The structural assignments were in agreement with the elemental composition C₂₂H₂₅¹¹B₁N₁O₄S₁, and the accurate mass data (calculated mass m/z 410.1592, experimental mass 410.1597 (+4.6 ppm error)) supported this assignment. The corresponding accurate masses for the isotopic contribution of ¹⁰B and the loss of a hydrogen atom were also determined to validate these assignments, as in the previous examples (Table 1). The elemental compositions again supported the assignments, with the C₂₂H₂₅¹⁰B₁N₁O₄S₁ ion

having a calculated mass of m/z 409.1626 and an experimental mass of 409.1634 (-2.0 ppm error) and the $C_{22}H_{24}^{11}B_1N_1O_4S_1$ ion having a calculated mass of m/z 409.1514 and an experimental mass of 409.1519 (+4.3 ppm error). The base peaks, at m/z 216, corresponded to the loss of an arylboronic moiety from m/z 410, to provide a 3,4,6, 7-tetrahydroacridine-1,8-dionic cation (vi, Fig. 2(c)). This structural assignment was based on the elemental composition, $C_{13}H_{14}N_1O_2$, obtained from the experimental mass of m/z 216.1025, with a calculated mass of m/z 216.1019 (+1.6 ppm error).

BC-Biginelli esters

The FAB⁺ spectra of compounds 9a-c contained $o_{,m,p}$ -[MTG]⁺ adduct peaks at m/z 395, with relative abundances of 1%, 11%, and 6%, corresponding to dihydroxypropylborothioate ions (Table 1, Fig. 3). These structural assignments were based on their accurate masses (calculated mass m/z 395.1443, experimental mass 395.1448 (-4.3 ppm error)) and fitted the elemental composition $C_{17}H_{24}^{11}B_1N_2O_6S_1$. Validation of these assignments was provided by study of the m-[MTG-1]⁺ ions by HRMS, for the isotopic contribution m-[MTG¹⁰B]⁺ and hydrogen loss m-[MTG-H]⁺ (Table 1). The two ions gave the formulae $C_{17}H_{24}^{10}B_1N_2O_6S_1$ (calculated mass m/z 394.1487, experimental mass 394.1484 (+0.8 ppm error)) and $C_{17}H_{23}^{11}B_1N_2O_6S_1$ (calculated mass m/z 394.1364 experimental mass 394.1370 (+7.0 ppm error)). Another ion, at *m/z* 217, with relative abundances of 2%, 14%, and 9%, for the ortho, meta, and para species, respectively, was assigned to the 4-cyclobutenyl-3,4,7,8-tetrahydroquinazolinic cation (vii, Fig. 3), and this was confirmed by the accurate mass data $(C_{12}H_{13}N_2O_2)$ calculated mass m/z 217.0971, experimental mass 217.0977 (+3.3 ppm error)).

Finally, a fragment ion at m/z 165, with relative abundances of 3%, 8%, and 9%, for the *ortho*, *meta*, and *para* species, respectively, was assigned as the 3,4,7,8-tetrahydroquinazolinic cation (*viii*, Fig. 3), and this assignment was confirmed by HRMS which indicated a formula of C₈H₉N₂O₂ (calculated mass m/z 165.0658, experimental mass 165.0664 (+3.1 ppm error)).



Figure 3. FAB⁺ mass spectrum of Biginelli ester and the corresponding [MTG]⁺: *meta* **9b**.



CONCLUSIONS

Interesting interactions between a thioglycerol matrix and fourteen boron-containing molecules related to Hantzsch and Biginelli esters were seen in their positive ion FAB spectra. The reactions seemed to occur in the solvent cage and involve Lewis acid-base interactions, in which particular thioglycerol fragment ions acted as chelating agents, through the sulfur atom, with the boron atom of the target compounds. In general, the accurate mass determination of these adducts allowed us to verify the molecular formulae and confirm the structures of the esters studied.

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