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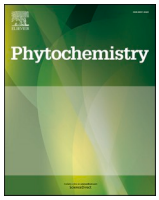

Corrigendum

Corrigendum to "Chemotaxonomic investigation of Apocynaceae for retronecine-type pyrrolizidine alkaloids using HPLC-MS/MS" [Phytochemistry 185 (2021) 112662]

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In this publication, using three precursor (PREC) analyses (low resolution LC-MS/MS), we suggested with low confidence that two compounds, yielding ions of *m/z* 432 and 328 Da, could be retronecine-type pyrrolizidine alkaloids (PAs). The compound exhibiting a mass of *m/z* 432 Da (herein referred to as compound **1**) was detected in leaf samples of multiple *Wrightia* R.Br. species, *Marsdenia tinctoria* R.Br., *M. glabra* Costantin, as well as *Wrightia tinctoria* (Roxb.) R.Br. seeds. The compound exhibiting a mass of 328 Da (herein referred to as compound **2**) was found in *W. tinctoria* seeds. We tentatively suggested that compound **1** could be 2-aminobenzoyl-O-β-D-apiofuranosyl-(1 → 6)- β- D-glucopyranoside (2-aminobenzoyl glycoside (Fig 1) and compound **2** blepharin (Fig 2), based on previous reports of specialized metabolites from *Wrightia antidysenterica* (L.) R.Br. and *Wrightia religiosa* (Teijsm. & Binn.) Benth. ex Kurz (Sahakitpichan et al., 2018; Srinroch et al., 2019; Traxler et al., 2021) and predicted fragmentation patterns.

To clarify the identity of the two ions, a high-resolution LC-MS analysis was performed on two seed samples of *W. tinctoria*. The molecular ions for both compounds and their fragments were identified in both samples (Fig 3 and 4), this time with high resolution, allowing for identification of molecular formula. Additionally, an isolated standard of blepharin from *W. religiosa* (Traxler et al., 2021) was provided by Johann Schinnerl and analyzed via LC-HRMS for comparison of the 328 Da ion (molecular ion and fragments) between the standard and samples. The blepharin standard was determined to be contaminated with compound **1**, the 2-aminobenzoyl glycoside. Identical chromatography and mass spectra were obtained for blepharin and the 2-aminobenzoyl glycoside in the isolated standard, as well as in both samples from the original investigation (Figs. 3 and 4).

Masses of the observed ions were compared to predicted masses through mass accuracy calculations (Tables 1 and 2). Masses of all observed ions were determined to be less than 5 ppm from their predicted structures, providing further evidence compounds **1** and **2** are not PAs. For secondary confirmation a product ion scan with a collision energy (CE) of 35 V, comparable to the CE utilized by Barny et al. (2021) in PREC analyses, was used to fragment the molecular ions of interest (432 & 328 Da). The same fragments for each compound were observed regardless of the collision energy utilized (LC-HRMS & LC-MS/MS Sciex 5600+).

In this supplemental study, we confirm with LC-HRMS and a blepharin standard that the 432 and 328 Da ions previously observed in two *W. tinctoria* seed samples are 2-aminobenzoyl glycoside (Fig. 1) and blepharin (Fig. 2), respectively, not potential PAs as we previously suggested, albeit with low confidence. We further suggest that two of the three ions (300 and 270 Da) that co-eluted with compound **1** (Table 2 in Barny et al., 2021) in PREC analyses are likely in-source fragments of 2-aminobenzoyl glycoside (Fig. 1) and not additional candidate PAs. Currently, we remain unsure of the identity of the third ion (328.5 Da) that co-eluted with compound **1** in our 2021 publication. HRMS allowed us to determine that fragment ions with the same masses as PA fragments at low resolution (120/138 Da) have different molecular masses and thus different molecular formulas (120.0444/138.0550 Da; Tables 1 and 2) from predicted PA fragments (120.0808/138.0913 Da; Fig. 4 in Barny et al., 2021), falsifying our initial hypotheses. This re-investigation, in the context of previous investigations on the

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specialized metabolites of *Wrightia* species (references in Barny et al., 2021), leads us to conclude that it is unlikely that *Wrightia tinctoria* seeds have retronecine-type PAs.

Materials and methods

Samples were analyzed by LC-MS on a SCIEX Exion LC with a SCIEX 5600+ TripleTOF MS. Separation was achieved on an ACE C18 column (3 μm particle, 150 × 4.6 mm) maintained at 40 °C at a flowrate of 0.7 mL/min. A gradient of 90/10 (HPLC grade water/acetonitrile with 0.1% formic acid) was held for 2 minutes then ramped to 0/100 over 18 min, then held at 0/100 for 7 minutes, and returned to 10/90 for re-equilibration for 6 minutes for a 33-min total LC analysis (Table 3).

For both experiments, the MS was calibrated with SCIEX APCI positive calibrant solution (Part 4460131) prior to sample analysis. Samples were analyzed in ESI positive mode with DP = 80 V, GAS1 = GAS2 = 60 psi, curtain gas = 30 psi, ISV = 5500 V, and source temperature of 550 °C. For LC-TOFMS analysis the MS was scanned over 50–800 Da with CE = 10 V. Alternatively, for product scans a CE = 35 V with a CE spread of 5 V was utilized. For the product scan of the 432 and 328 Da ions, the TOF was scanned from 50 to 500 and 50–350 Da, respectively.

Vouchers of tested samples, seeds of *Wrightia tinctoria* (specimen numbers 29370 and 29271), are deposited in the seed collection of the PH herbarium.

Acknowledgements

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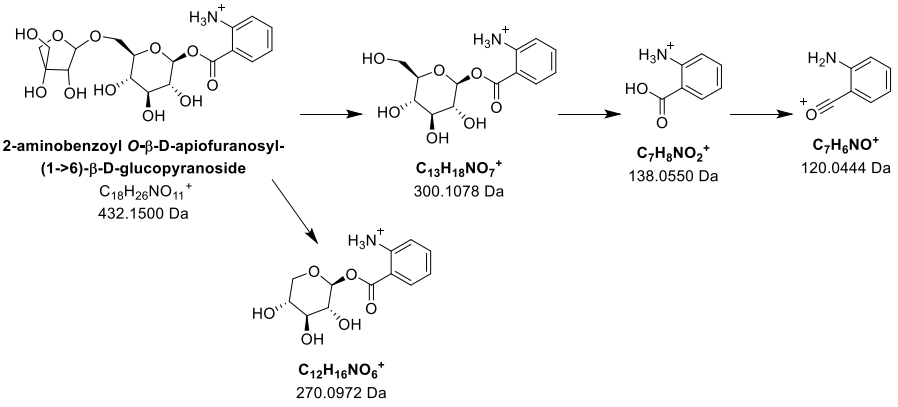

Fig. 1. Proposed fragmentation of compound **1**, 2-aminobenzoyl O-β-D-apiofuranosyl-(1 → 6)- β- D-glucopyranoside (2-aminobenzoyl glycoside).

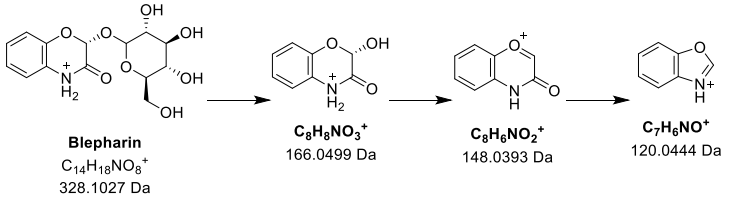

Fig. 2. Proposed fragmentation of compound **2**, blepharin.

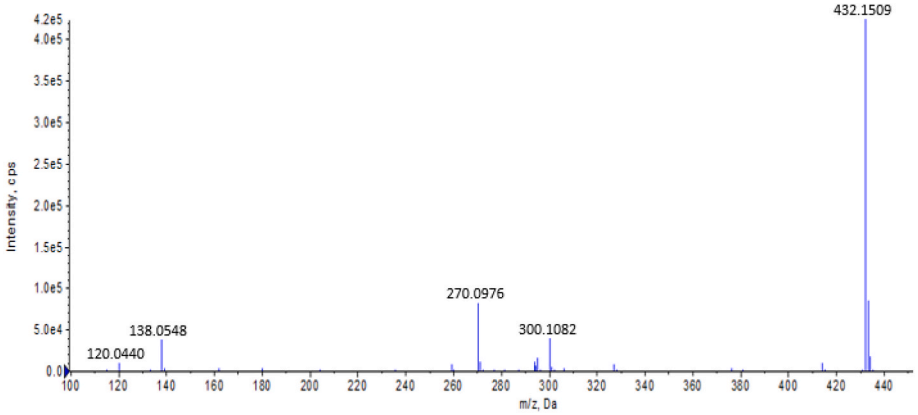

Fig. 3. Mass spectrum of compound **1**, 2-aminobenzoyl glycoside from a *Wrightia tinctoria* seed sample [#29271]; identical spectra were obtained from sample # 29370 and the (contaminated) blepharin standard.

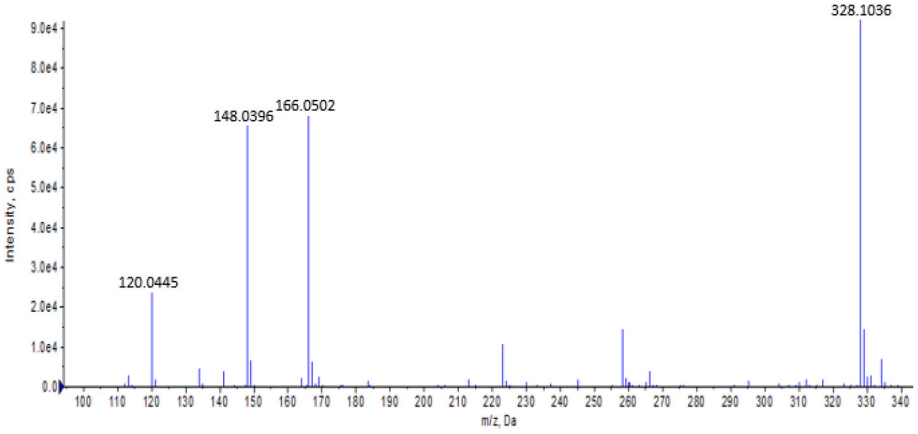

Fig. 4. Mass spectrum of compound **2**, blepharin from a *Wrightia tinctoria* seed sample [#29271]; identical spectra were obtained from sample # 29370 and the blepharin standard.

Table 1. Mass accuracy calculations for 2-aminobenzoyl glycoside from *Wrightia tinctoria* seed sample [#29271]; mass accuracy of the molecular ions and fragments in sample # 29370 were also less than 5 ppm.

Molecular Ion/Fragment	Calculated (Da)	Observed (Da)	Error (ppm)
2-aminobenzoyl glycoside	432.1500	432.1509	2.08
$C_{13}H_{18}NO_7^+$	300.1078	300.1082	1.33
$C_{12}H_{16}NO_6^+$	270.0972	270.0976	1.48
$C_7H_8NO_2^+$	138.0550	138.0548	1.45
$C_7H_6NO^+$	120.0444	120.0440	3.33

Table 2. Mass accuracy calculations for blepharin from *Wrightia tinctoria* seed sample [#29271]; mass accuracy of the molecular ions and fragments in sample # 29370 and standard were also less than 5 ppm.

Molecular Ion/Fragment	Calculated (Da)	Observed (Da)	Error (ppm)
Blepharin	328.1027	328.1036	2.74
$C_8H_8NO_3^+$	166.0499	166.0502	1.81
$C_8H_6NO_2^+$	148.0393	148.0396	2.03
$C_7H_6NO^+$	120.0444	120.0445	0.83

Table 3. LC gradient used for the separation of compounds **1** & **2** in the standard and samples.

Time (mins)	% B (CH_3CN)
0	10
2.00	10
20.00	100
27.00	100
27.10	10
33.00	10