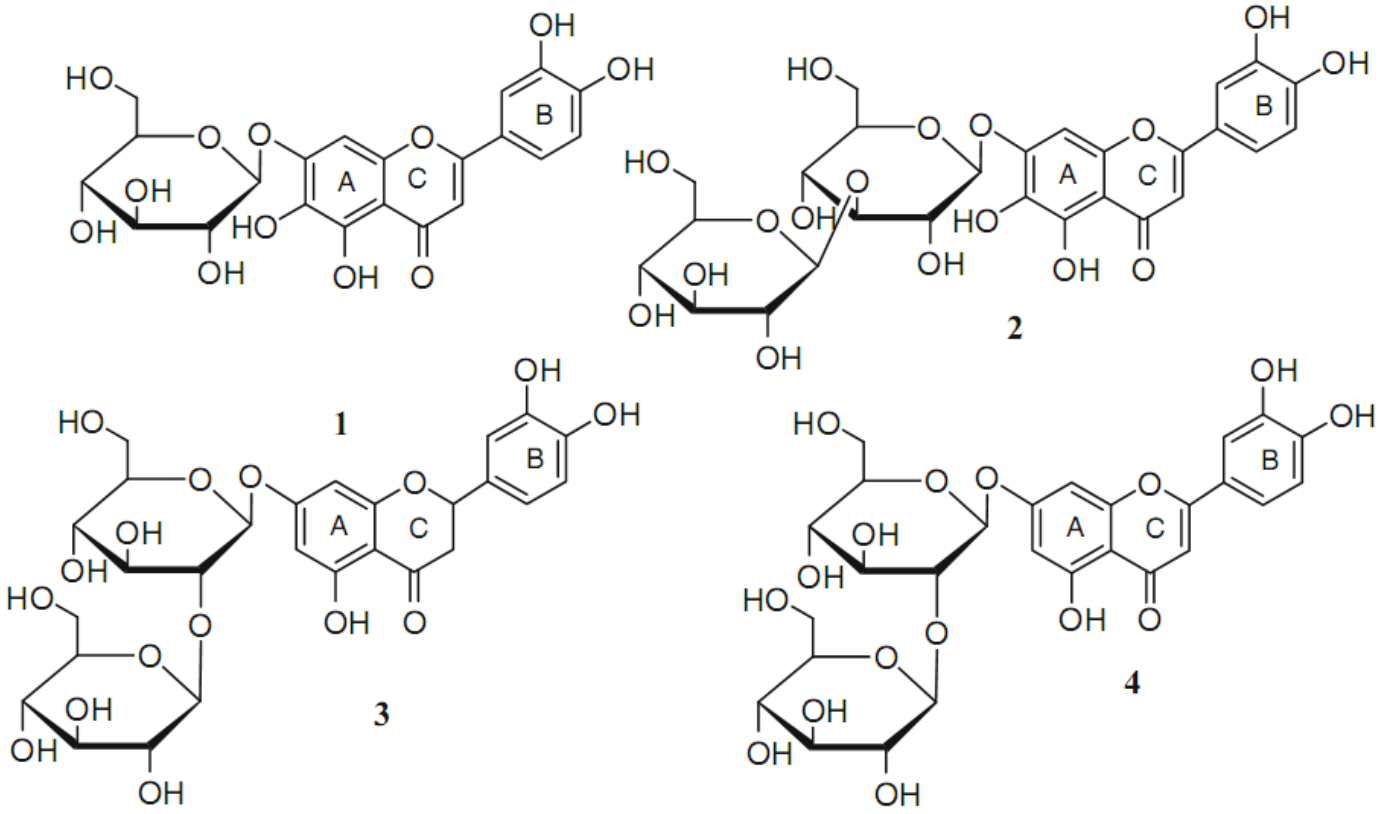
Mass Spectra of Natural Compounds

Flavonoids are polyphenolic compounds which are important bioactive constituents. The basic structure of a flavonoid consists of a 15-carbon (C6–C3–C6) skeleton containing one oxygenated (C) and two aromatic rings (A and B). The most common flavonoids being flavones, flavonols, isoflavones, flavanones, anthocyanins and chalcones. Flavonoids can exist as free aglycones but most of them commonly occur as C- or O-glycosides.



The major diagnostic CID-MS/MS product ions for flavonoid identification are those involving the cleavage of two C–C bonds of the C-ring. These product ions provide information on the number and type of substituent's in A- and B-rings.

These product ions are usually designated according to the nomenclature previously proposed by Ma et al.[28] For free aglycone, the ^{i,j}A and ^{i,j}B labels refer to the product ions containing intact A- and B-rings, respectively, in which the superscripts i and j indicate the C-ring bonds that have been broken. For the flavonoid glycosides, the classical nomenclature proposed by Domon and Costello[56] was adopted.

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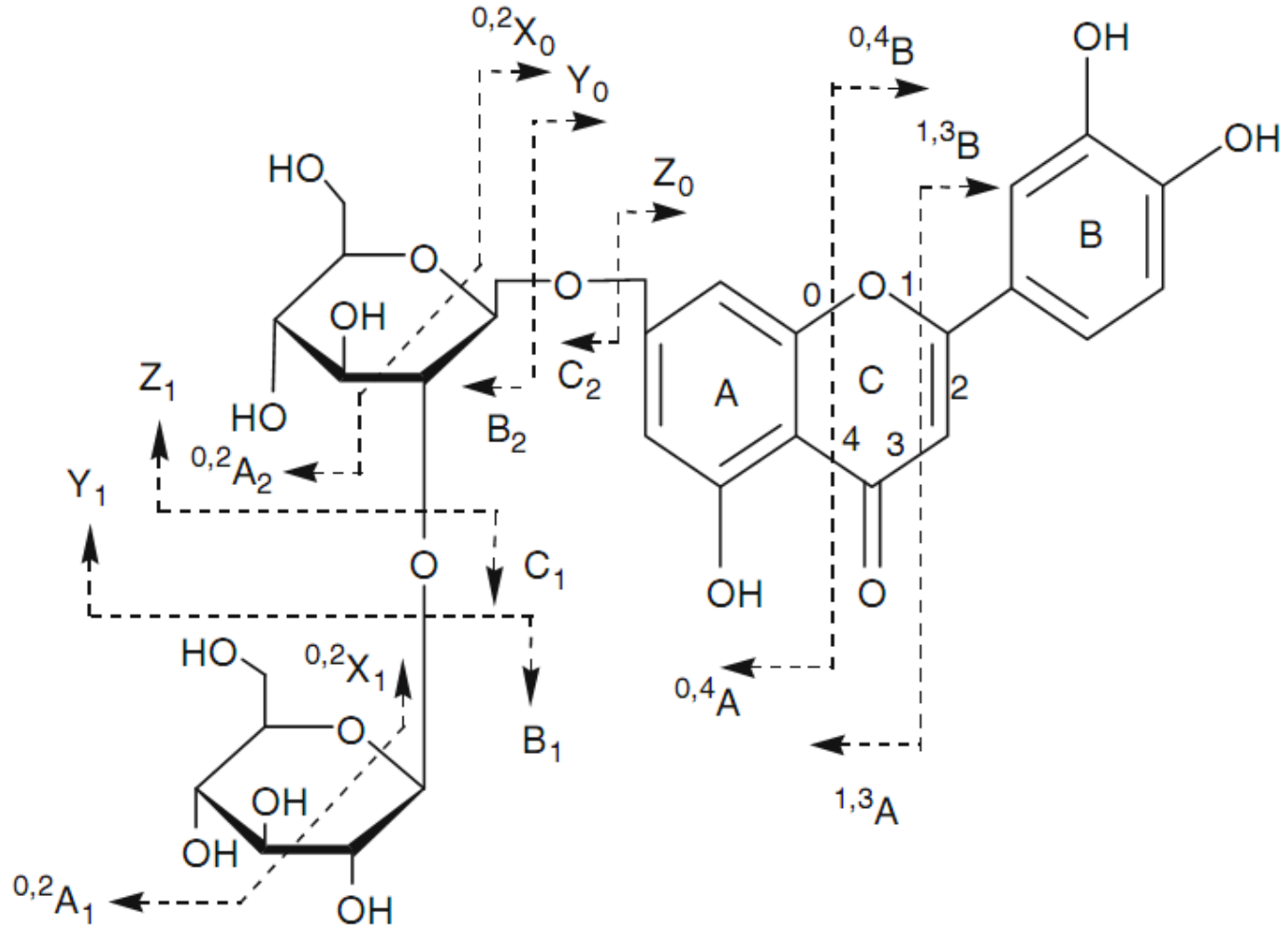


Positive ESI-MS of each studied flavonoid showed the protonated and cationized molecules in addition to fragment ions corresponding to the aglycone ions (Y_0^+). This was also observed when the analyses were conducted in the negative ion mode where the deprotonated molecules and the Y_0^- ions were observed.

Mass spectrometric data for the favonoids 1–4 obtained using positive and negative ESI-MS analyses.

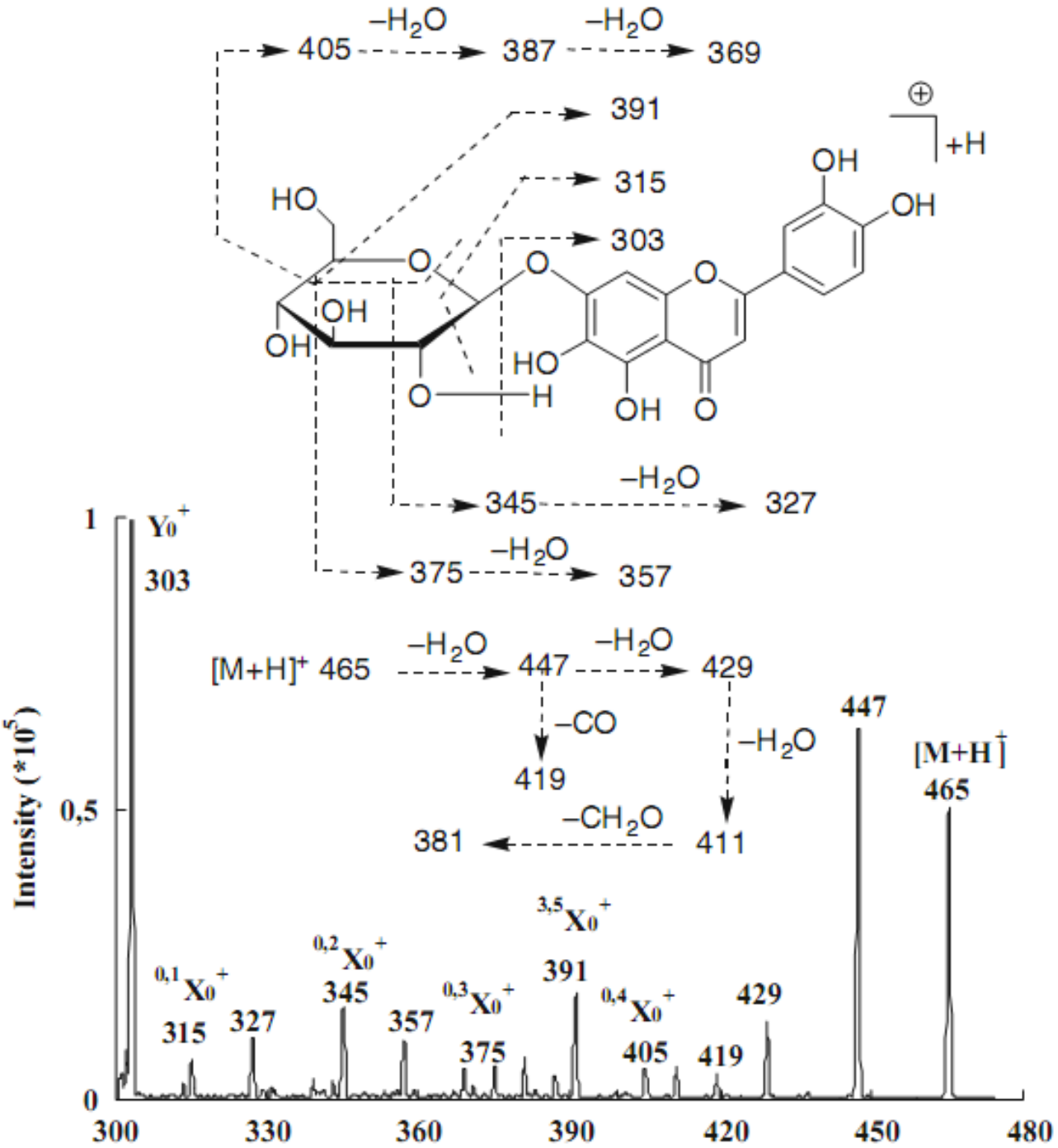
		1	2	3	4
+Mode	$[M + H]^+$	465	627	613	611
	Product ions	303	465, 447, 303	451, 289	449, 287
–Mode	$[M - H]^-$	463	625	611	609
	Product ions	301	463, 445, 301	475, 287	437, 285
Aglycone (Da)		302	302	288	286
Mol. wt. (Da)		464	626	612	610

Compound 1

The CID-MS/MS the protonated molecule $[M + H]^+$ m/z 465 of flavonoid 1 was recorded in the follow.

The product ion Y_0^+ at m/z 303 corresponded to the aglycone moiety. The mass difference of m/z 162 Da between the $[M + H]^+$ peak and the aglycone was in agreement with the glucoside structure form of compound 1.

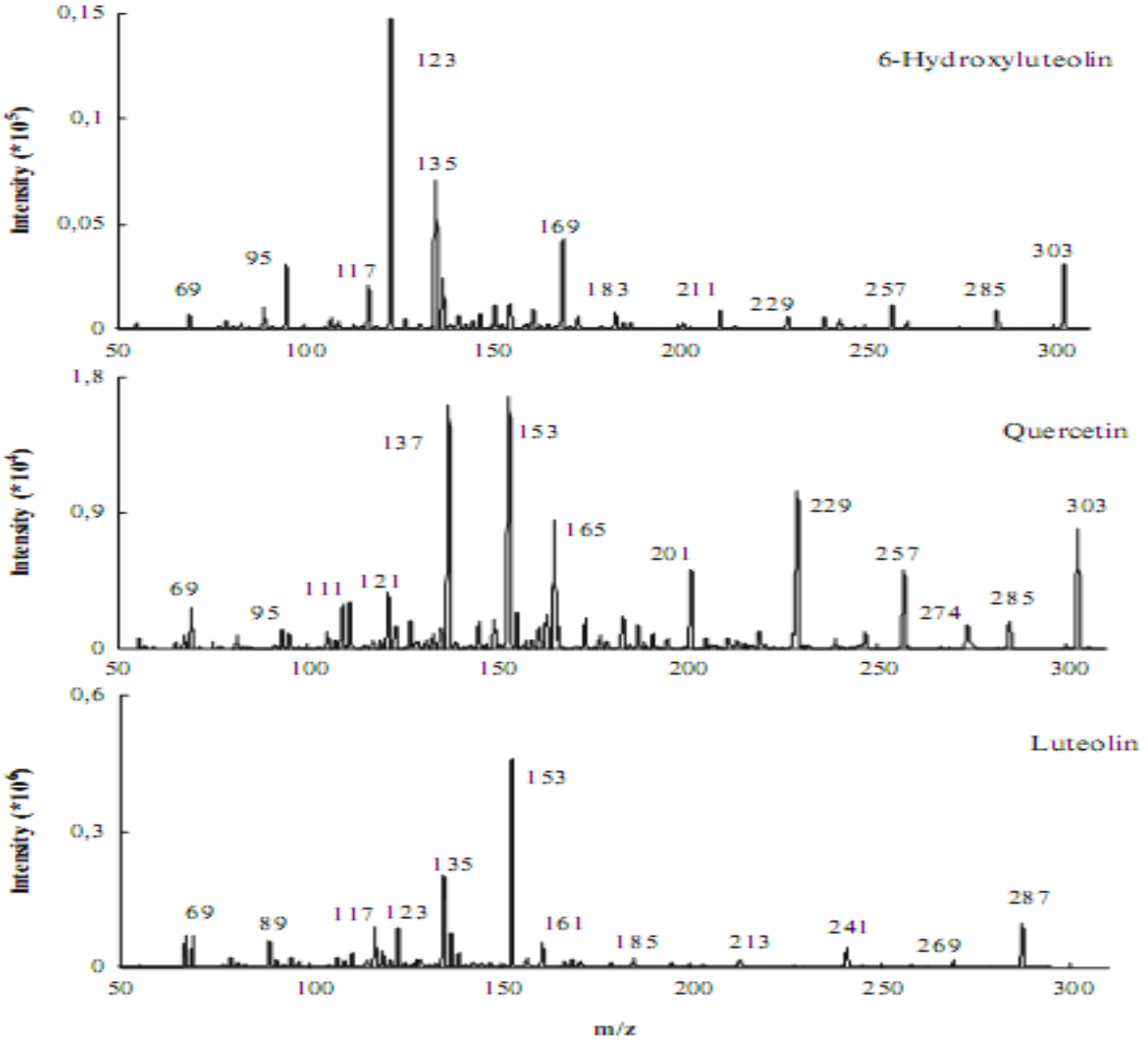
In addition other product ions were observed: $[M + H - H2O]^+$ at m/z 447, $[^{0,4}X_0^+]$ at m/z 405 $[^{0,3}X_0^+]$, at m/z 375, $[^{0,3}X0+ - H_2O]$ at m/z 357, $[^{0,2}X_0^+]$ at m/z 345 $[^{0,2}X_0^+]$ and$[^{0,1}X_0^+]$ at m/z 315.


–H2O
405
387
–H2O
369
391
315
303
+H
HO
OH
OH
O
O
O
OH
OH
HO
O
H
OH
O
–H2O
345
327
1
Y0+
303
375
–H2O
357
[M+H]+ 465
–H2O
447
–H2O
429
447
–CO
–H2O
[M+H]+
465
419
–CH2O
381
411
Intensity (*10^5)
0,5
3,5X0+
391
0,2X0+
345
0,1X0+
315
327
357
0,3X0+
375
0,4X0+
405
419
429
0
300
330
360
390
420
450
480

CID-MS/MS of the sodiated $[M+Na]^+$ ion (m/z 487) afforded the product ions:

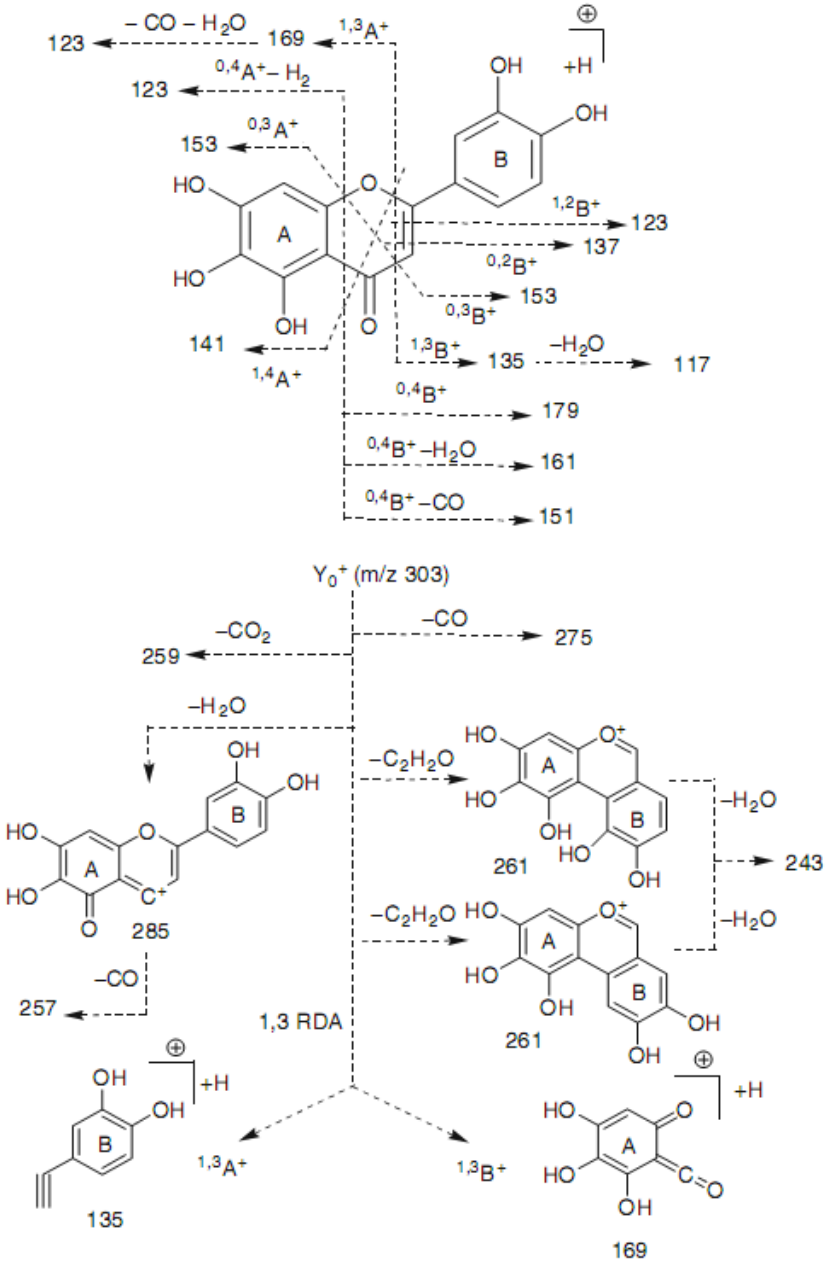
$[M + Na - H_2O]^+$ at m/z 469, $[M + Na - CO]^+$ at m/z 459, $[M + Na - 2H_2O]^+$ at m/z 451 and the following respective product ions at m/z 427 ($^{0,4}X_0^+$), m/z 397 ($^{0,3}X_0^+$), m/z 371 ($^{0,3}X_0^+$-CO), m/z 367 ($^{0,2}X_0^+$)= (344+23) , m/z 353 ($^{1,5}X_0^+$), m/z 325 (Y_0^+), m/z 307 (Z_0^+), m/z 297 ($Y_0^+ - CO$), m/z 269 ($Y_0^+ - 2CO$), and m/z 185 (B_1^+).

Although both aglycones product ions (6-hydroxyluteolin and quercetin) have the same m/z 303 value, their CID-MS/MS spectra under similar low-energy conditions are significantly different



In particular, the product ion (base peak) at m/z 153 for quercetin and corresponding to the $^{1,3}A^+$ product ion was not observed in the case of compound 1 where the $^{1,3}A^+$ product ions at m/z 169 and the complementary product ion was observed at m/z 135 ($^{1,3}B^+$). This indicated that the A ring was substituted with three OH groups. It has been reported that the $^{1,3}A^+$ observed for all flavonoid groups, is generally the most readily formed product ion and constitutes the most abundant product ion. The $^{1,3}A^+$ product ion is most often found at m/z 153 for compounds with a 5,7-dihydroxyl group. The absence of such product ion in the CID spectra of compound 1, in addition to the presence of the product ion at m/z 169, clearly demonstrates the presence of a,trihydroxylated A ring in agreement with the proposed structure.

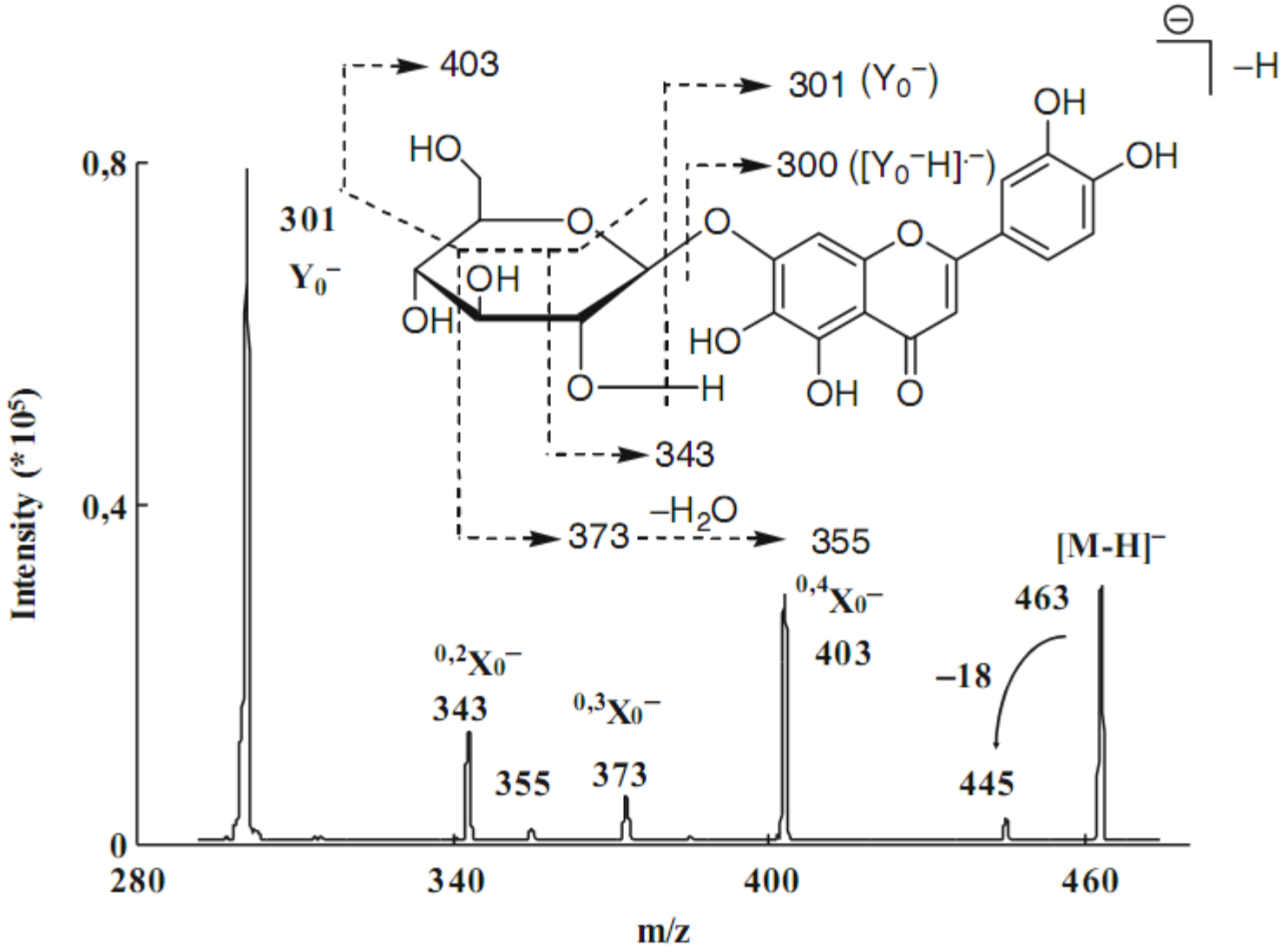
The cleavage of the C-1 and C-3 bonds afforded the product ion $^{1,3}A^+$ at m/z 169 and the product ion $^{1,3}B^+$ at m/z 135. The presence of this pair of product ions clearly provides the substitution pattern in the A (3 OH) and B (2 OH) rings. The obtained product ions can also undergo further fragmentation to create other product ions by successive losses of H2O and CO. The product ion at m/z 123 (base peak) was formed from the product ion at m/z 169, whereas the product ion at m/z 117 was formed form the product ion m/z 135 by loss of water. An RDA-type (Retro Diels Alder) product ion, corresponding to the cleavage of the C-0 and C-4 bond, produced the product ions $^{0,4}A^+$ at m/z 125 and $^{0,4}B^+$ at m/z 179. These latter afforded the product ions: [$4B^+$ − H2O] at m/z 161 and [$^{0,4}B^+$ − CO] at m/z 151 and [$^{0,4}A+ - H_2$] at m/z 123. Cleavage of the C-0 and C-2 bonds afforded the product ion $^{0,2}B+$ at m/z 137. Cleavage of the C-1 and C-2 bond produced the product ions $^{1,2}B+$ and $^{1,2}A^+$ +2H at m/z 123 and 183 respectively. Finally cleavage of the C-0 and C-3 bonds afforded the product ions $^{0,3}B+$ and $^{0,3}A+$ at m/z 153 (low relative abundance). Thus, the following product ions were observed at m/z 285 (−H2O), 275 (−CO), 261 (−C2H2O) and 259 (−CO2) mass units. In addition, we observed other product ions formed from the precursor ion at m/z 303 such as the product ions at m/z 257 (−CO − H2O) and 243 (−C2H2O − H2O).



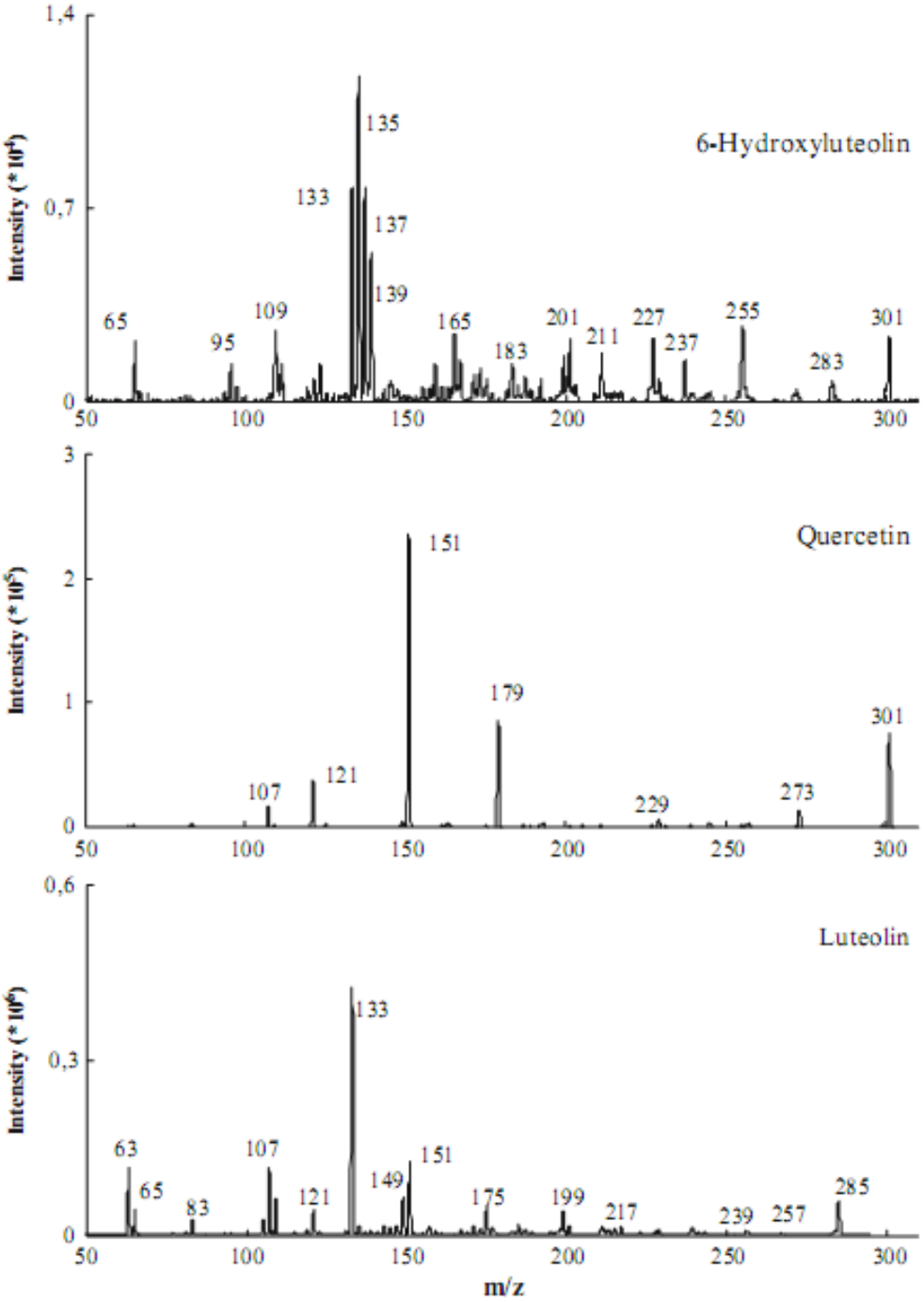
Scheme (2).CID-MS/MS data and proposed fragmentation pattern of the protonated aglycone 1

“Negative Ions”

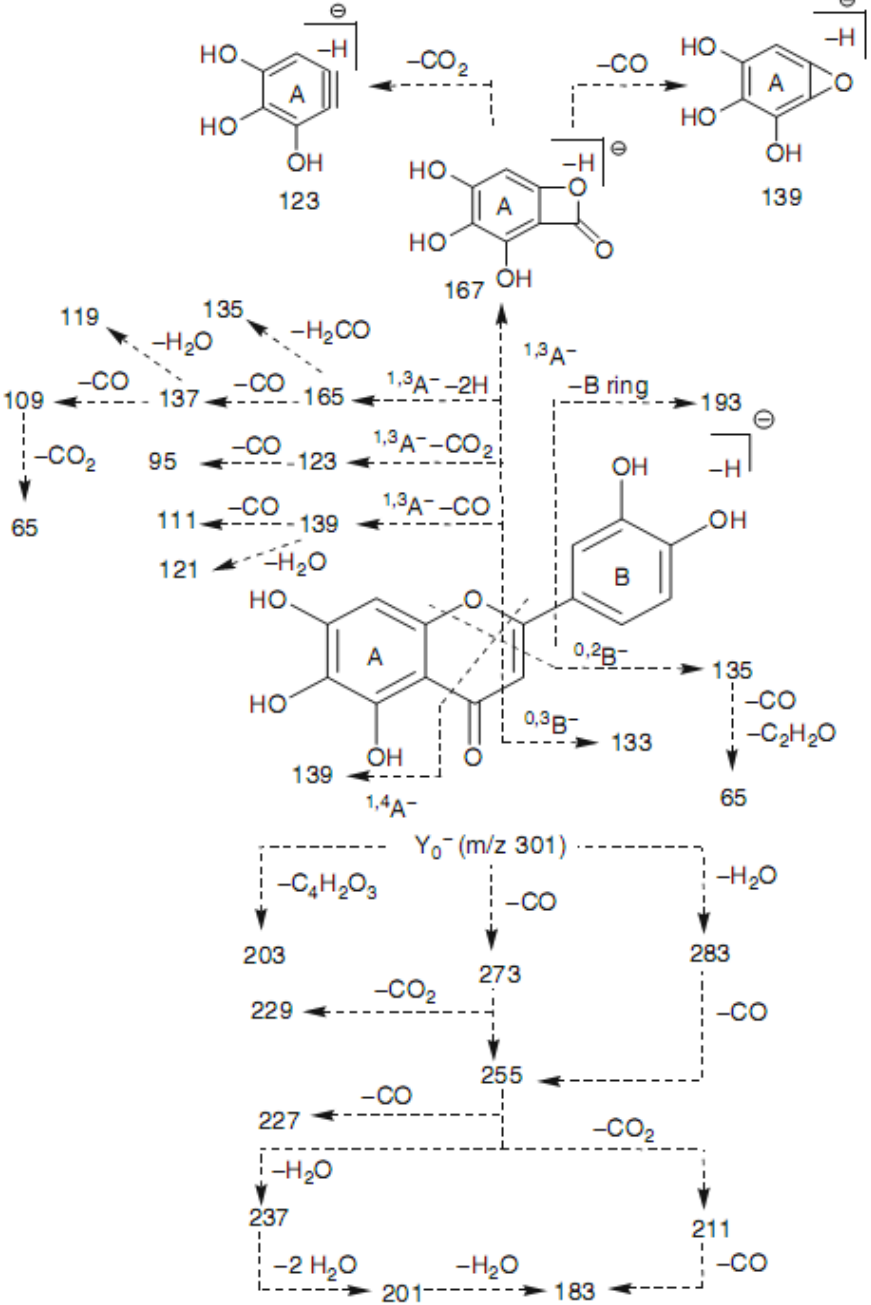
The ESI-MS compound 1 (− ion mode) afforded the deprotonated molecule [M − H]$^-$ at m/z 463. The CID-MS/MS analysis of this latter ion afforded the diagnostic product ion at m/z 301, corresponding to the deprotonated aglycone moiety obtained by the loss of 162 Da in agreement with the monoglucoside structure form (Fig. 4). Other product ions were also observed [M − H − H2O]$^-$ at m/z 445, $^{0,4}X_0^-$ at m/z 403, $^{0,3}X_0^-$ at m/z 373, [^{0,3}X−-H2O] at m/z 355 and ^{0,2}X0−] at m/z 343 which were formed by common fragmentation route. The presence of a product ion at m/z 300 corresponds to the radical aglycone product ion [Y^0 − H]$^-$. The formation of this radical product ion depended on the structure of the flavonoid glycoside and the nature and position of the sugar substitution.



CID-MS/MS of the deprotonated 6-hydroxyluteolin 7-O-glucoside at m/z 463 and the proposed gas-phase fragmentation routes



CID-MS/MS of the deprotonated molecules $[M - H]^-$ of 6-hydroxyluteolin, quercetin and luteolin



CID-MS/MS and proposed fragmentation routes of the deprotonated molecule $[M-H]^-$ of 6-hydroxyluteolin

Figure 5 shows the second-generation CID-MS/MS analysis of aglycone 2 compared to that of the aglycone of the known quercetin and luteolin. The gas-phase fragmentation route is shown in Scheme 3. The obtained results indicated that 6-hydroxyluteolin and quercetin, have the same m/z 301 value albeit their negative CID-MS/MS analyses are different. In particular, the presence of the product ion(base peak) at m/z 151 for quercetin and the observed 1,3A− product ion at m/z 167, which was not observed in the case of the aglycone of compound 1. In addition, the product ion at m/z 135 (base peak) for the 6-hydroxyluteolin was observed and the product ions 1,3A−, 1,3B−at m/z 167, 133 are in agreement with a substitution of the
A ring by three OH groups.

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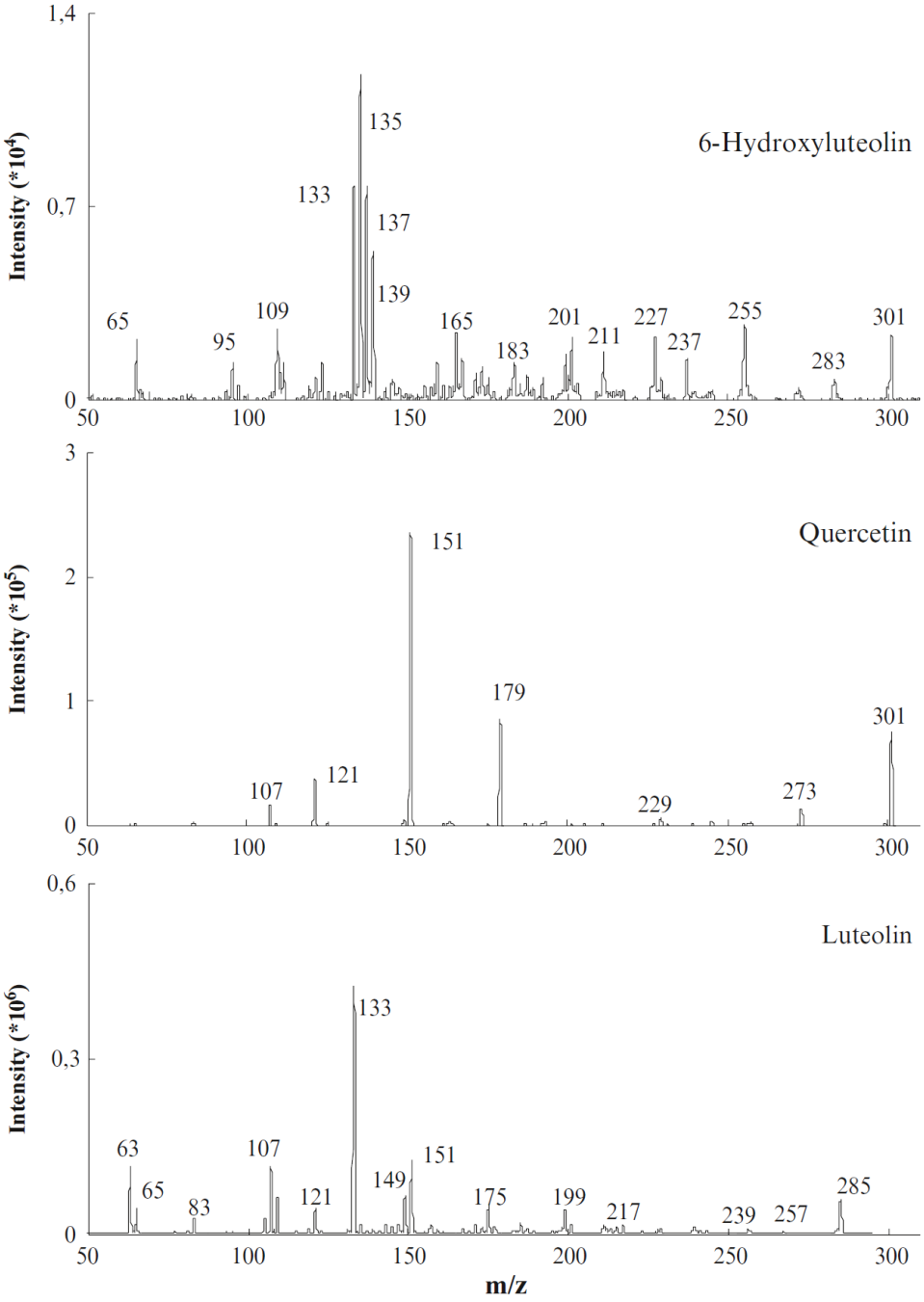
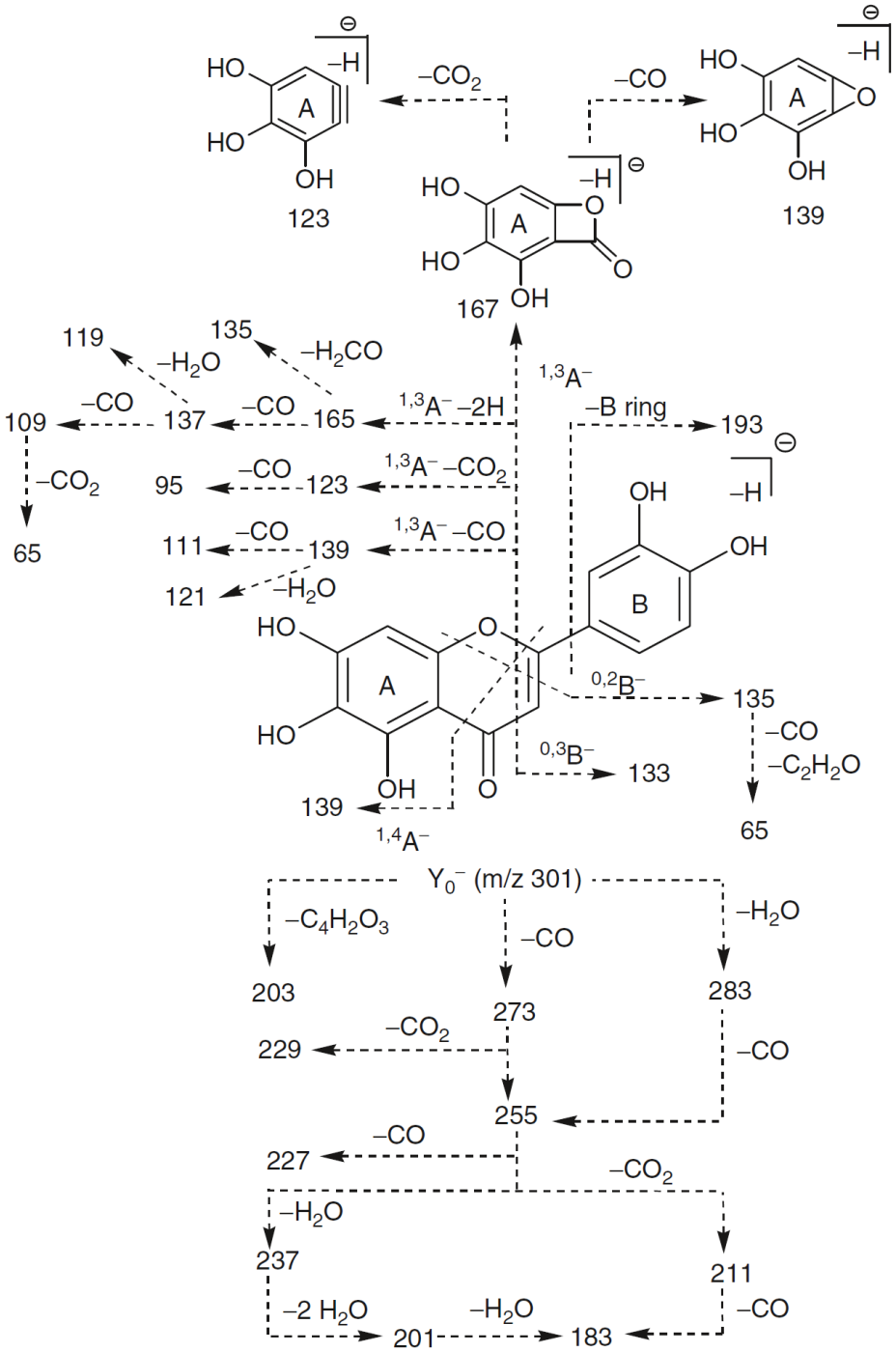
The product ion ^{1,3}A− ion further eliminated of CO and CO2 to form the product ions at m/z 139 and 123 in agreement with literature data concerning the luteolin product ions. We also observed the product ion [^{1,3}A− − 2H] at m/z 165 (Scheme 3). This product ion can further lose CO to afford the product ions at m/z 137 and 121. The product ion observed at m/z 135 was formed either from the ^{0,2}B− product ion or from the product ion at m/z 165 by loss of H2CO. The latter being more probable, due to the fact that this was not observed in the case of luteolin. Further product ions at m/z 139 and 137 yield the ions at m/z 111 and 109 by elimination of H2O. The latter gave, by loss of CO2,the ion observed at m/z 65. This product ion could also arise from the ion at m/z 135 by successive losses of CO and a ketene moiety C2H2O.



Fog. 5. CID-MS/MS of the deprotonated molecules $[M - H]^-$ of 6-hydroxyluteolin, quercetin and luteolin



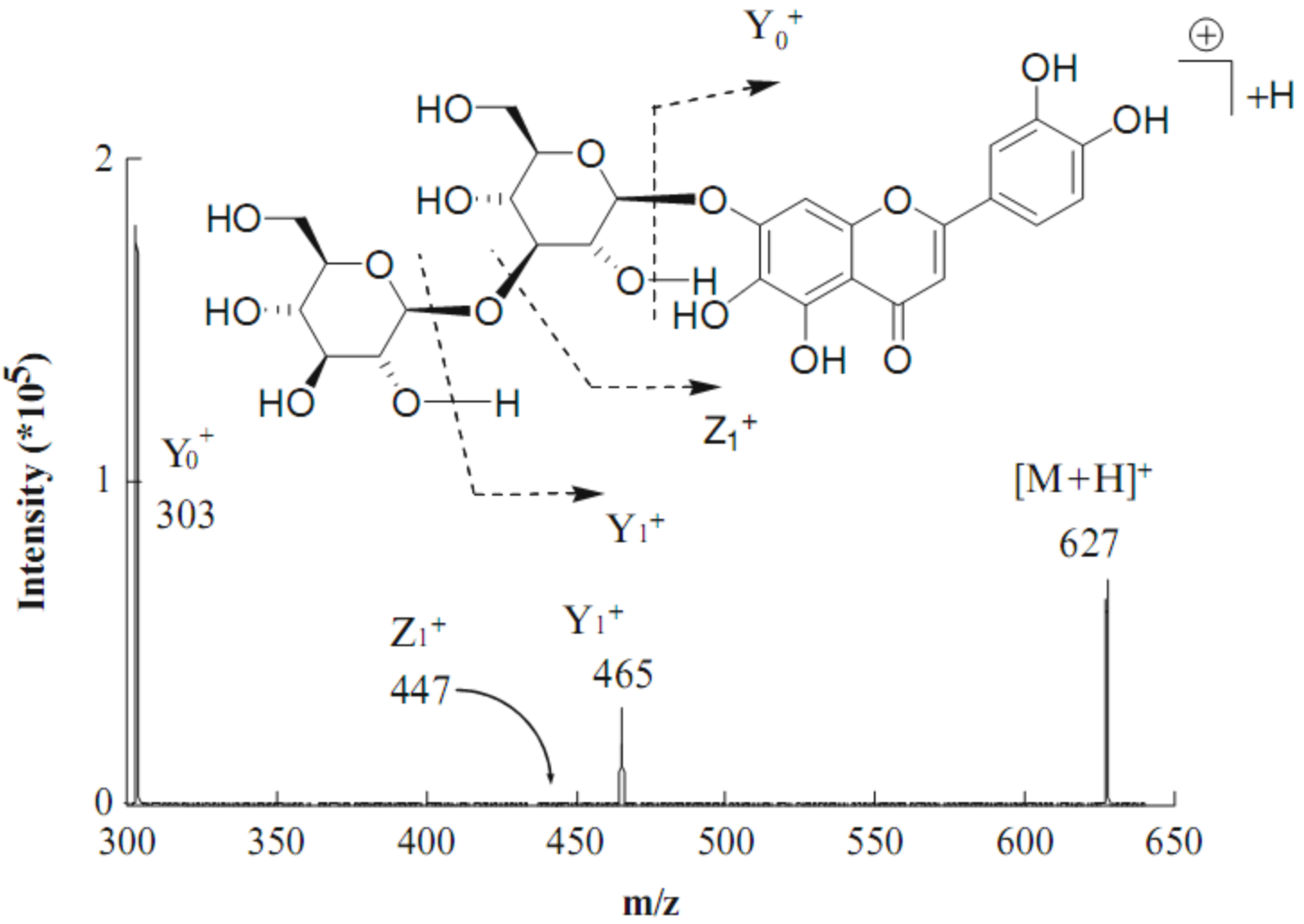
Scheme 3 CID-MS/MS and proposed fragmentation routes of the deprotonated molecule $[M-H]^-$ of 6-hydroxyluteolin

In addition, the second generation CID-MS/MS analysis of the precursor ion at m/z 301, produced from the deprotonated aglycone 2, gave the following diagnostic product ions at m/z 283, 273, 255, 237, 229, 227, 211, 201, 183 and 173 (Scheme 3). The product ion at m/z 283 resulted from the loss of H2O. The product ion at m/z 273 was formed by the loss of CO. The product ion at m/z 273 lost CO2 to yield the product ion at m/z 229. Both the product ions at m/z 283 and 273 yielded the product ion at m/z 255, as shown in Scheme 3; the former eliminated a CO while the latter lost a H2O. The product ion at m/z 255 created the product ions at m/z 237, 227 and 211 by elimination of H2O, CO and CO2 respectively.

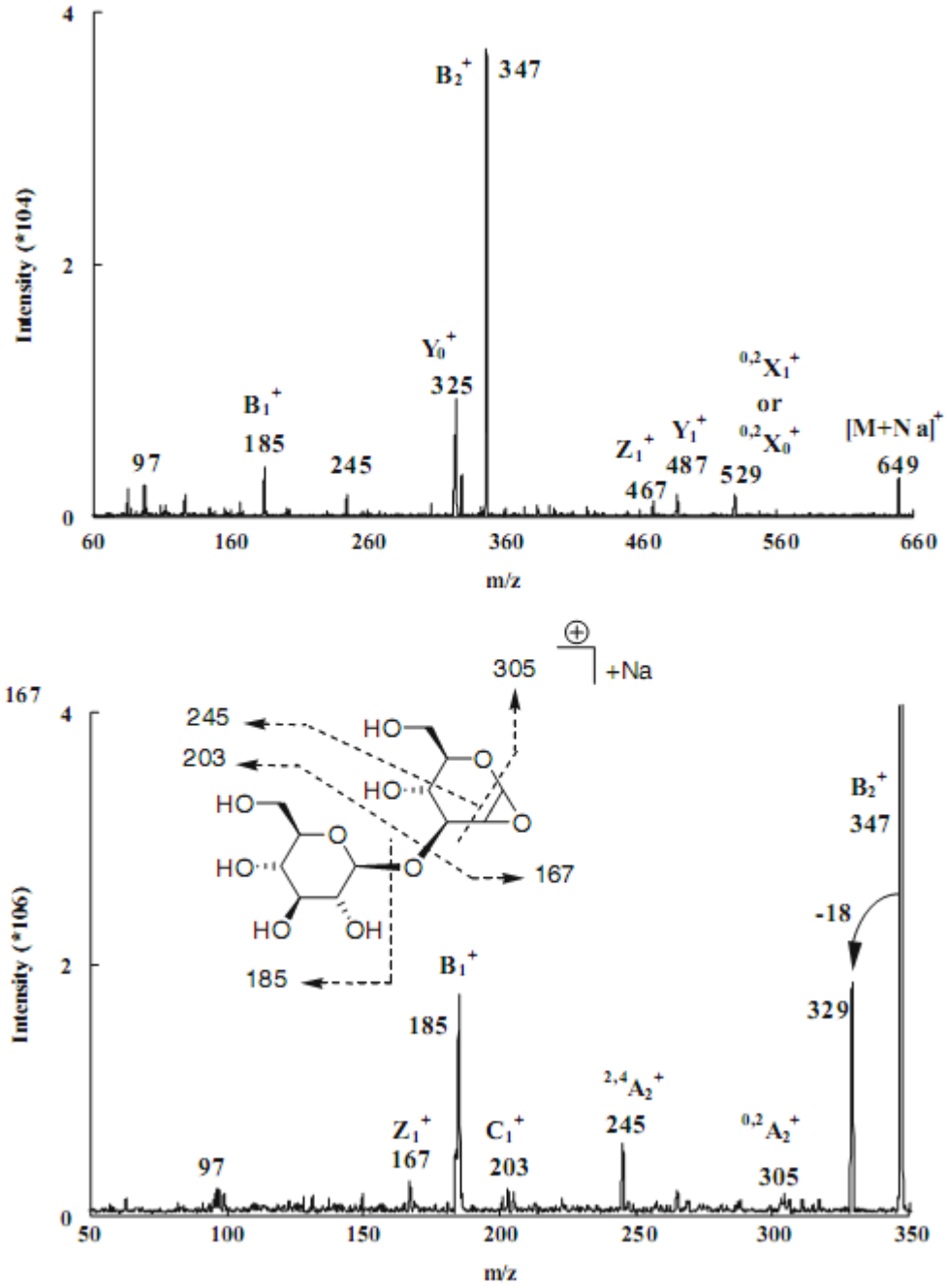
The product ion at m/z 237 afforded the product ion at m/z 201 by loss of two water molecules. Both the product ions at m/z 211 and 201 afforded the product ion at m/z 183, as shown in Scheme 3. The former eliminated a CO, while the latter lost a water molecule to produce the ion at m/z 183. Another product ion was observed at m/z 203 by the loss of a 98 Da corresponding to the C4H2O3 unit. This loss of 98 Da was reported to be characteristic of compound containing trihydroxyl groups on the A ring in agreement of compound 1 structure. The [M-B-ring]− prodcut ion at m/z 193 resulted from the direct cleavage of the bond between the B- and C-rings.

Compound 2

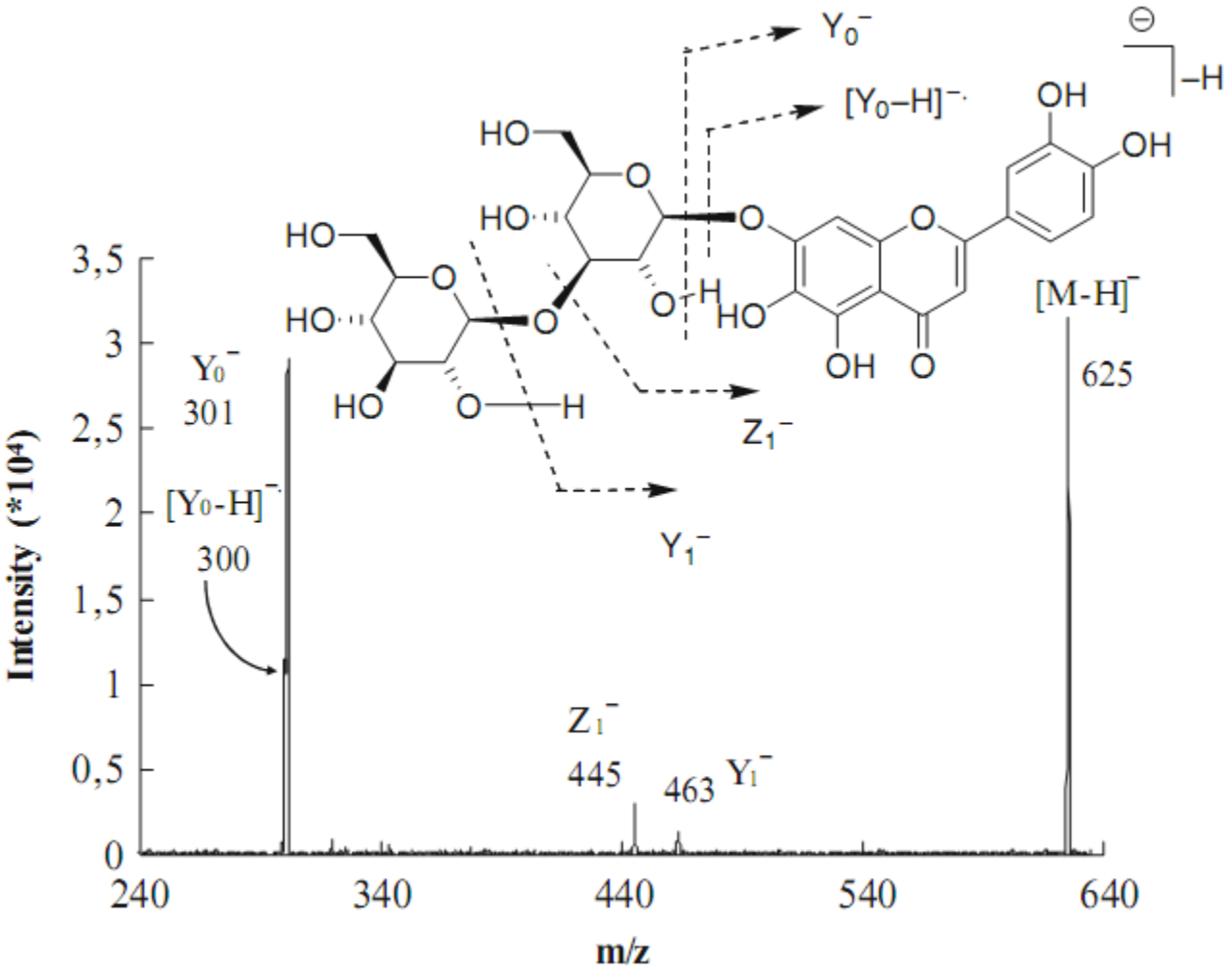
The most intense fragment-ion was observed at m/z 303, corresponding to the protonated aglycone moiety formed by loss of 324 Da (two hexose residues). The CID-MS/MS analysis of the precursor ion extracted from the aglycone of compound 2 indicating that it had the same 6-hydroxyluteolin aglycone skeleton. Another less abundant product ion was observed at m/z 465 formed by loss of the external glucose unit (Y series of fragmentation in polysaccharide chains), while the complementary B type ion was absent.



CID-MS/MS of the protonated molecule of 6-hydroxyluteolin 7-O-laminaribioside at m/z 627 and the proposed gas-phase fragmentation routes



CID mass spectra of the sodiated 6-hydroxyluteolin 7-O-laminaribioside (m/z 649) and of the sodiated diglycosyl moiety (m/z 347)

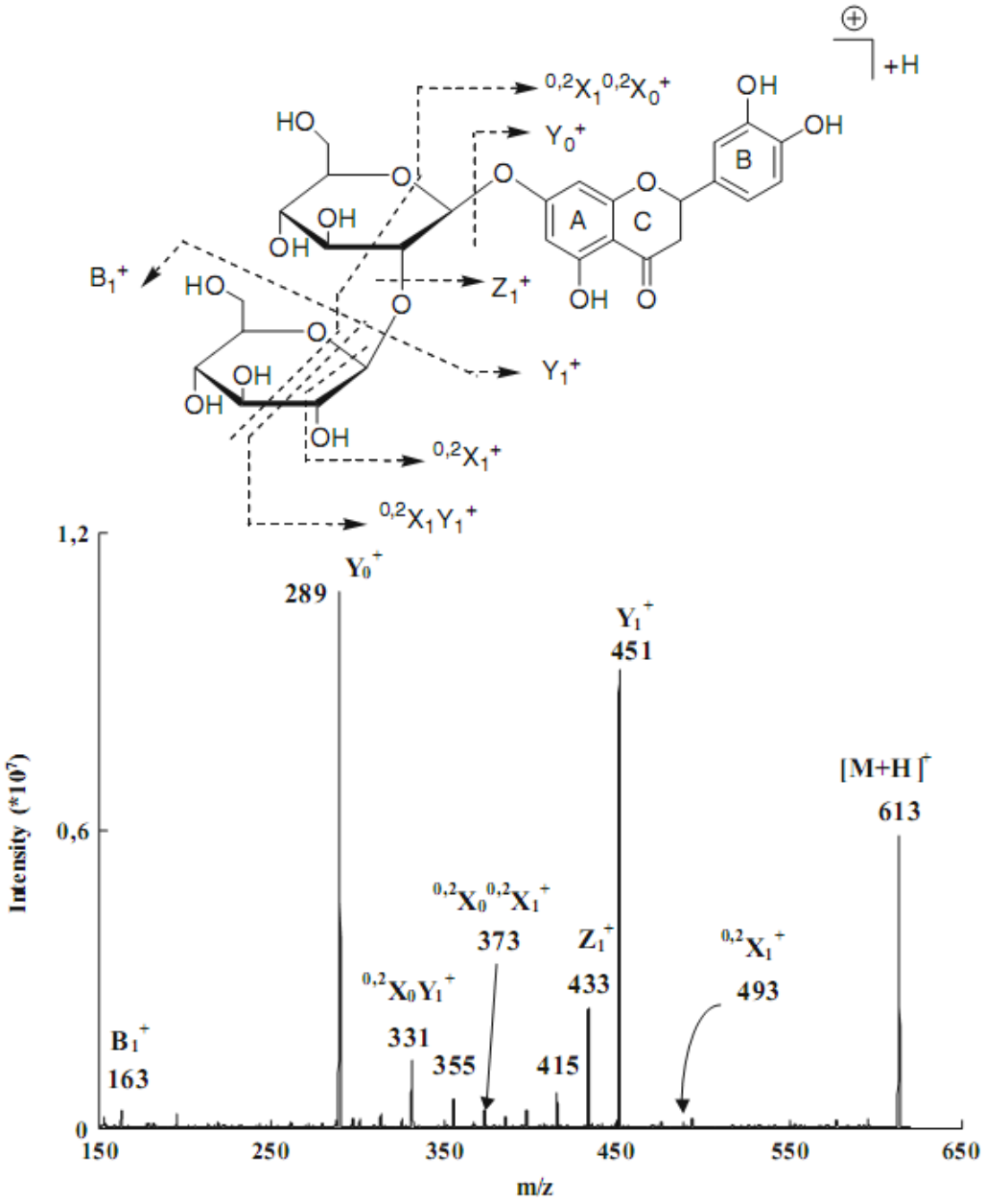


CID-MS/MS of the deprotonated 6-hydroxyluteolin 7-O-laminaribioside (m/z 627) and its proposed gas-phase fragmentaion pattern routes

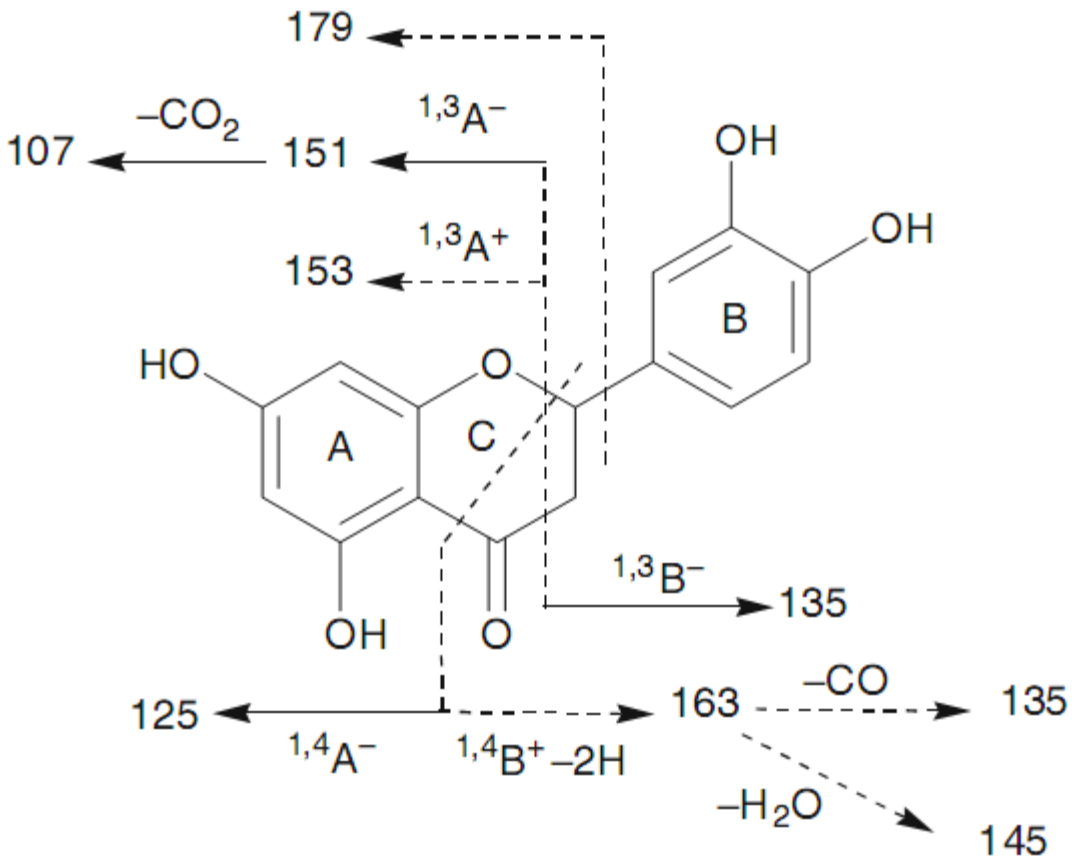
Compound 3

The molecular mass of compound 3 was confirmed as 612 Da by recording the ESI-MS (+ion mode) which showed the protonated molecule $[M + H]^+$ at m/z 613.

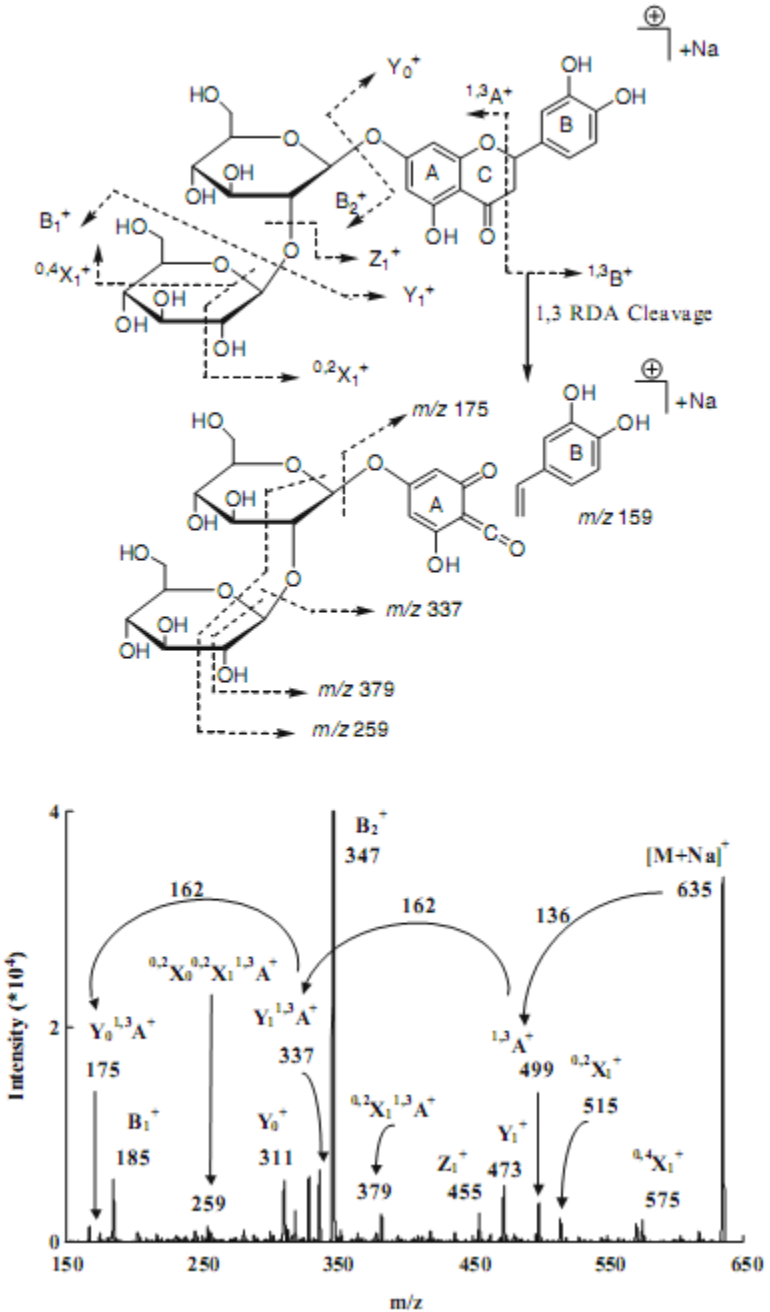
A loss of 324 Da was also observed indicating the presence of a two hexose residues. This results in the formation of the product-ion at m/z 289, suggesting that compound 3 was a flavanone-based compound.



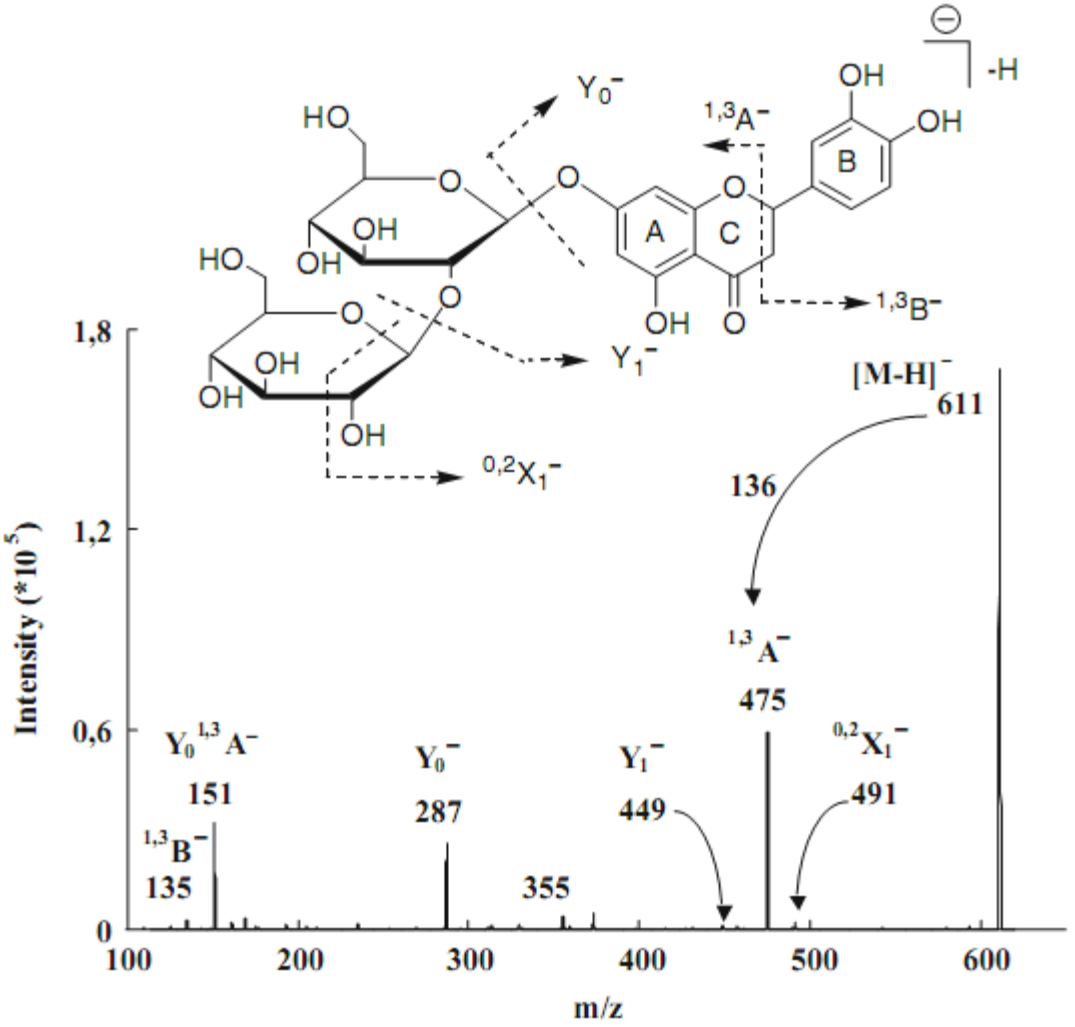
CID-MS/MS analyses of the protonated eriodictyol 7-O-sophoroside 3 at m/ 613 and its proposed gas-phase fragmentation route



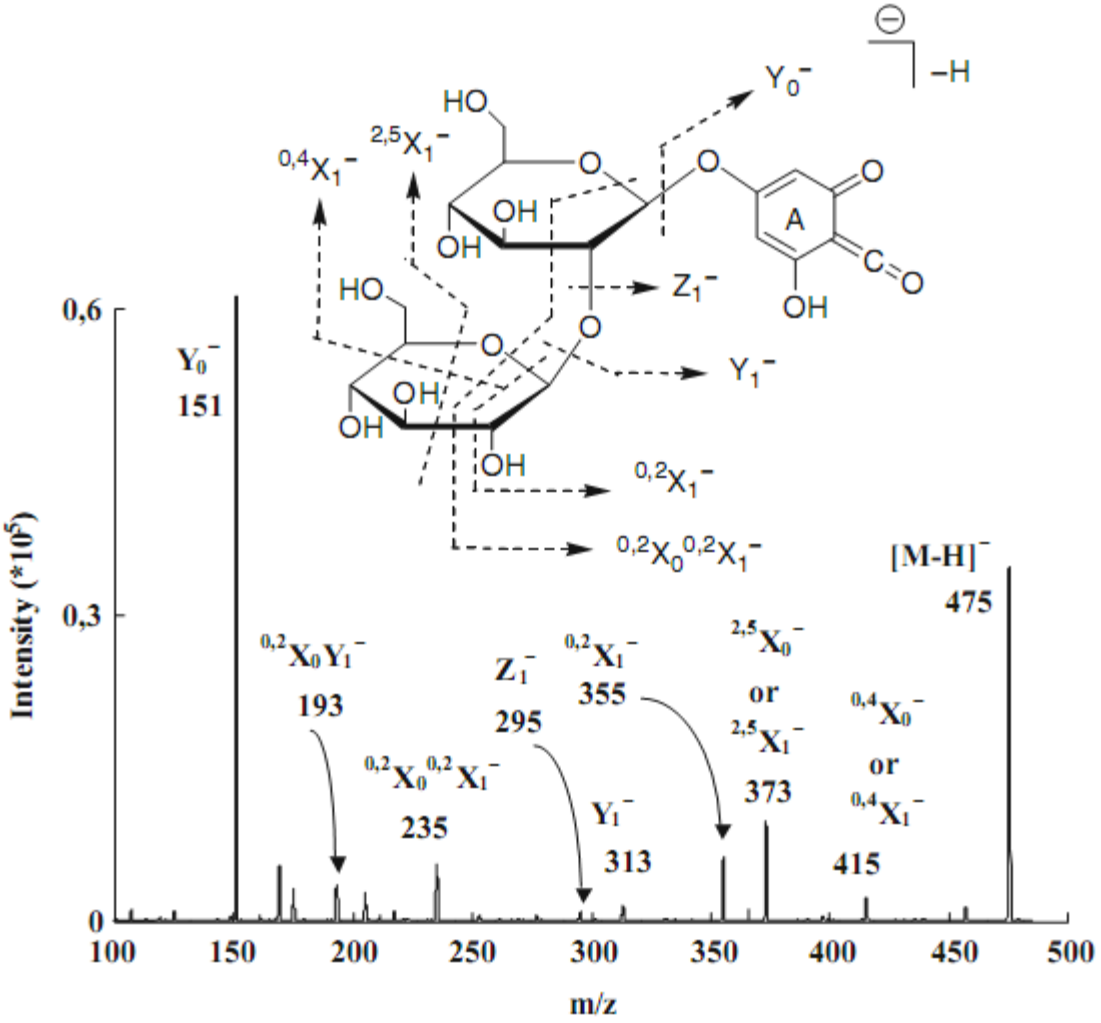
CID-MS/MS analyses of the protonated (dashed arrows) and deprotonated (full arrows) molecules of the aglycone of eriodictyol



CID-MS/MS of the sodiated eriodictyol 7-O-sophoroside 3 molecule at m/z 635 and its proposed gas-phase fragmentation routes



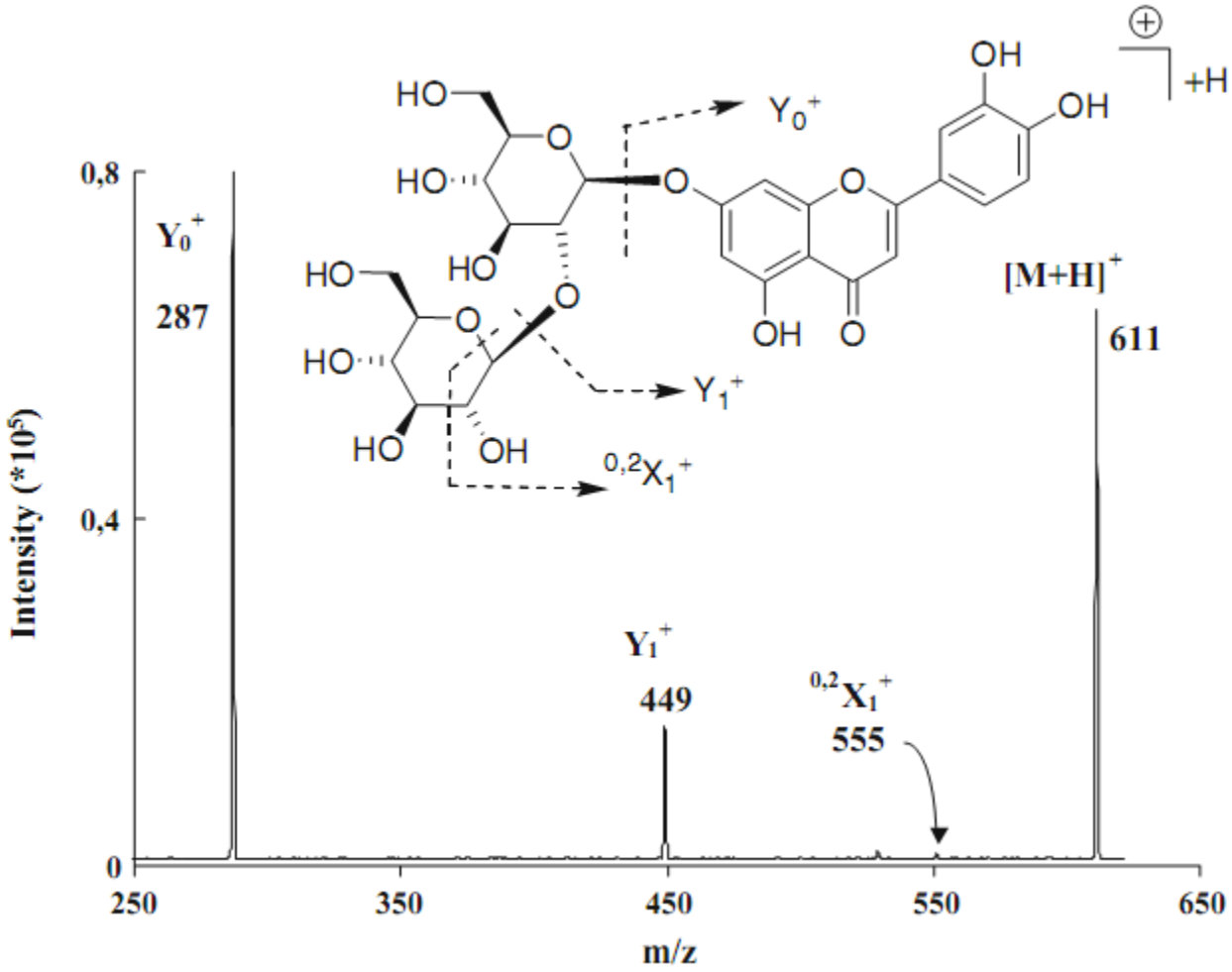
CID-MS/MS of deprotonated eriodictyol 7-O-sophoroside 3 at m/z 611 and its proposed gas-phase fragmentation routes



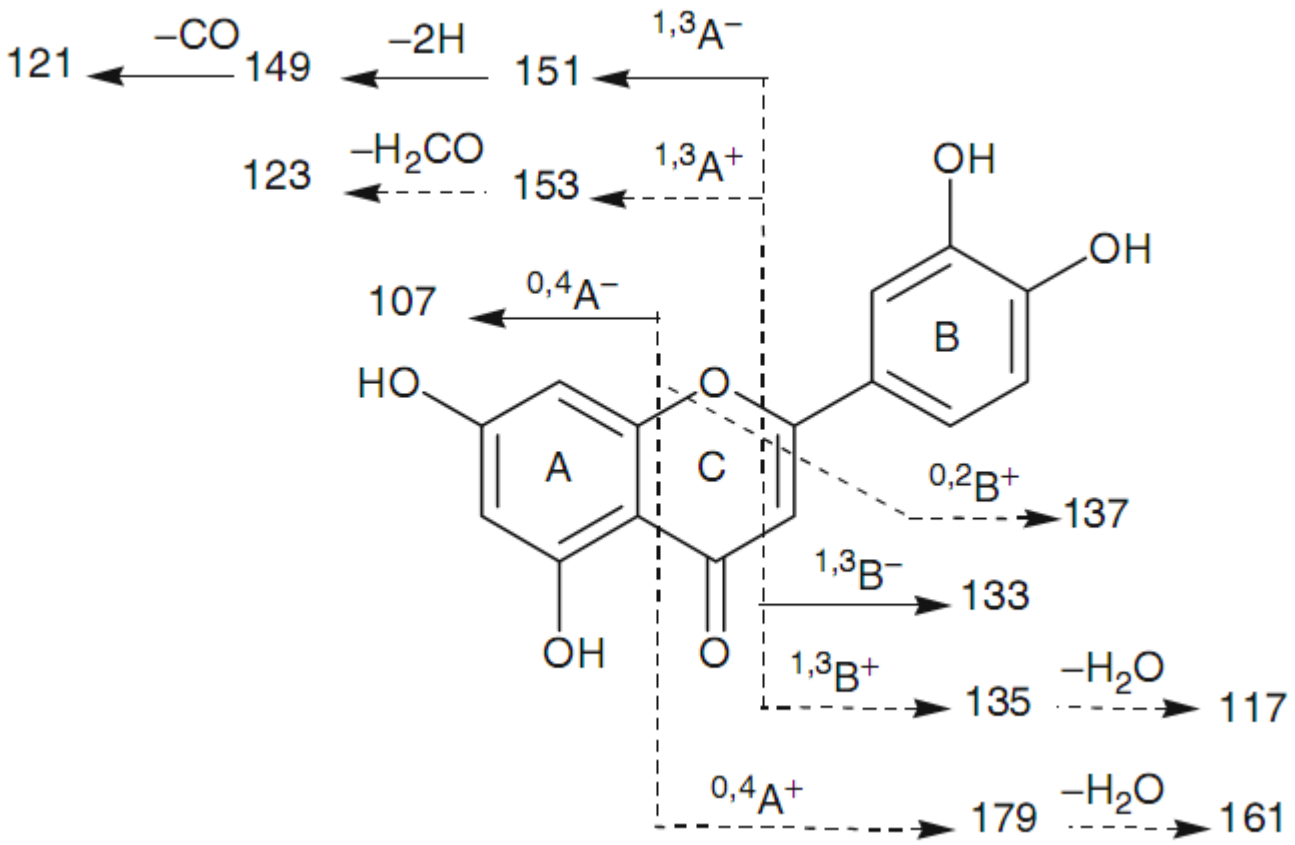
Second-generation CID-MS/MS of the $^{1,2}A^-$ at m/z 475 extracted from compound 3 and its proposed gas-phase frgamentation routes

Compound 4

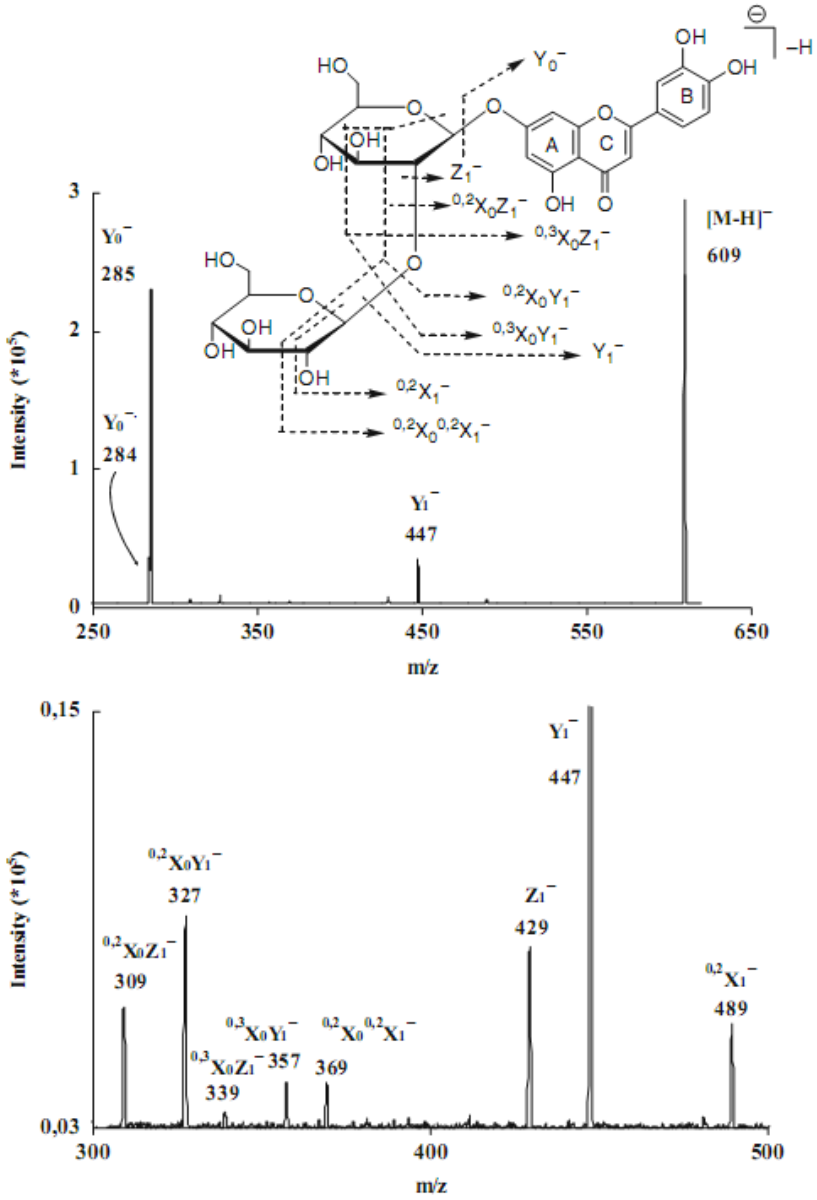
The CID-MS/MS of protonated molecule $[M + H]^+$ of compound 4 afforded characteristic product ions of the flavonoid O-diglycosides13, giving two major product ions Y0+ and Y1 at m/z 287 and 449 respectively. The Y_0^+ at m/z 287 was the base peak, while the Y_1^+ product ion at m/z 449 was observed (low abundance) suggesting the existence of a diglycoside moiety, as it is known that compounds which exhibit a high abundance of m/z 449 have two glucose units attached to the different positions of the aglycone.



CID-MS/MS of the protonated luteolin 7-O-sophoroside 4 (m/z 611) and its proposed gas-phase fragmentation routes



Main product ionss observed in CID-MS/MS of the $[M + H]^+$ (dashed arrows) and $[M - H]^-$(full arrows) ions of luteolin



CID-MS/MS of the deprotonated luteolin 7-O-sophoroside 4 at m/z 609 and its proposed gas-phase fragmentation routes