

Article

Identification of Iridoids in Edible Honeysuckle Berries (*Lonicera caerulea* L. var. *kamtschatica* Sevast.) by UPLC-ESI-qTOF-MS/MS

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Abstract: Iridoid profiles of honeysuckle berry were studied. Compounds were identified by ultra-performance liquid chromatography coupled with electrospray ionization mass spectrometry UPLC-ESI-qTOF-MS/MS in positive and negative ions mode. The MS fragmentation pathways of detected iridoid glycosides were also studied in both modes. In the negative ESI mass spectra, iridoids with a methyl ester or lactone structure have preferentially produced adduct $[M + \text{HCOOH} - \text{H}]^-$ ions. However, protonated ions of molecular fragments, which were released by glycosidic bond cleavage and following fragmentation of aglycone rings, were more usable for iridoid structure analysis. In addition, the neutral losses of H_2O , CO , CO_2 , CH_3OH , acetylene, ethenone and cyclopropynone have provided data confirming the presence of functional substituents in the aglycone. Among the 13 iridoids, 11 were identified in honeysuckle berries for the first time: pentosides of loganic acid (two isomers), pentosides of loganin (three isomers), pentosyl sweroside, and additionally 7-*epi*-loganic acid, 7-*epi*-loganin, sweroside, secologanin, and secoxyloganin. The five pentoside derivatives of loganic acid and loganin have not been previously detected in the analyzed species. Honeysuckle berries are a source of iridoids with different structures, compounds that are rarely present in fruits.

Keywords: iridoids; loganin 7-O-pentoside; *epi*-isomers; honeysuckle berries; LC-ESI-qTOF-MS/MS; fragmentation pathway

1. Introduction

Lonicera caerulea L. var. *kamtschatica* Sevast. (Caprifoliaceae) is a fruit bush originating from the Far East of Russia (Kamchatka Peninsula). Edible honeysuckle berries are gaining popularity in many European countries, Japan, China, Canada and others. These berries are good fresh fruit for consumption and good raw material for the production of juices, snacks, dry fruits, and jams [1–3]. Anthocyanins, flavan-3-ols, flavonols, phenolic acids, and vitamin C are the main compositional groups in honeysuckle berries that exhibit antioxidant, antibacterial, antiviral and anti-inflammatory effects [1,3–8]. Honeysuckle berries' health benefits have been known and used for a long time in traditional medicine in Russia and China.

Iridoids are a large group of secondary metabolites found both in a variety of plant and selected animal species. They belong to the monoterpenes with a cyclopentanopyran skeleton and occur in plant materials naturally as glucoside forms. Iridoids, depending on the chemical structure, exhibit different pharmacological properties, such as antibiotic, anti-inflammatory or hypertensive activities [9]. They have varying degrees of bitterness. Especially known in this respect are secoiridoids, which also

exhibit the feature of deterrence of herbivores. The secoiridoids are a subclass of iridoids with an opening of the cyclopentane ring between the carbon atoms C-7 and C-8. A large variety of iridoid structures exists. To identify them, particularly in complex plant extracts, the LC-MS method is the most useful, especially when coupled to a soft ionization source, e.g., electrospray ionization (ESI). Quadrupole time-of-flight (QTOF) MS/MS allows one to measure mass with high accuracy and to track fragmentation of ions, which enables good interpretation of results, contributing to determination of the empirical chemical formulas. Many authors successfully identify iridoids in raw plants by this method. Some authors have analyzed iridoids in negative mode [10–12], others in positive mode [13], the mode used depending on the structure of chemical compounds.

Iridoids, in contrast to polyphenols, are rarely found in fruits, but they are found in green parts of plants. Exceptions are, e.g., cornelian cherry fruits [14,15]. Among the species of the genus *Lonicera*, iridoids have been identified mainly in leaves of *L. caerulea* [16,17] and in flowers, buds, stem, leaves, and caulis of *L. japonica* [12,13,18,19]. There are few reports on iridoid contents in fruits of *Lonicera*. Whitehead and Bowers [20] determined six compounds from this group in non-edible fruits from the species *Lonicera morrowii* A. Gray, *Lonicera tatarica* L., and their hybrid *Lonicera* × *bella* Zabel. In the edible fruits from *L. caerulea*, only one bitter iridoid was identified—7-oxologanin [21]. To our knowledge, there are no studies available on iridoids in berries from the species *L. caerulea* var. *kamtschatica*, except our own previous conference reports [14,22]. Therefore, the aim of this study was to present a complete qualitative determination of iridoids in berries of blue honeysuckle varieties (*L. caerulea* var. *kamtschatica*). This is the first detailed paper about iridoid composition of blue honeysuckle berries.

2. Results and Discussion

The acidified methanolic extract of honeysuckle berries was analyzed by the UPLC-qTOF-MS/MS method. The compounds were identified by their UPLC retention times, elution order, UV-Vis (200–600 nm) and MS spectra, and by comparison with available standards and literature data. The UPLC chromatogram of honeysuckle berry extract, obtained in the UV spectral region (254 nm), is shown in Figure 1. UV-Vis spectra of 13 compounds indicate that they are iridoids. In our previous initial studies, we determined five iridoids (loganic acid, loganin and their three derivatives) from honeysuckle berries [14,22], but we could not completely prove their structures. In this study, 13 iridoids were exactly identified by LC-MS/MS; among these, eight compounds were discovered for the first time.

Iridoids with methyl ester or lactone structure showed a strong tendency for associated product ion formation in negative ionizations; therefore MS data of ions of these and other compounds, in both negative and positive ionization modes, were investigated (Table 1). In the literature, those associated product ions are commonly called adducts [10,11,19]. Depending on the acid applied in the mobile phase, formic acid [11] or acetic acid [19] adducts may appear. Under our analysis conditions, possible associations of loganin, secologanin and sweroside with formic acid connected by hydrogen bonds are shown in Figure 2. However, in the case of secoxyloganin, which possesses a methyl ester and additionally a free carboxyl group, we did not observe a similar phenomenon. The reason for this difference may be mutual influence of the discussed groups resulting in internal weak hydrogen bonds, preventing formation of an association with formic acid.

The results of LC-MS/MS analysis of four compounds (peaks 1, 2, 7, and 10) were compared to the results of the available iridoid standards. The compounds 1 and 2 (t_R 3.73 min, and t_R 4.27 min), and the loganic acid standard have a molecular ion at m/z 375.1276 $[M - H]^-$ and a fragment ion at m/z 213.0769 $[M - 162 - H]^-$ in negative ESI mode, and a pseudomolecular ion at m/z 377.1440 $[M + H]^+$ and a fragment ion at m/z 215.0913 $[M - 162 + H]^+$ in positive ESI mode (Table 1). Compounds 7 and 10 (t_R 5.73 min, t_R 6.54 min) and the loganin standard displayed a formic acid associated ion at m/z 435.1502 $[M + 46 - H]^-$ and a fragment ion at m/z 227.0939 $[M - 162 - H]^-$ in negative ESI mode, and a pseudomolecular ion at m/z 391.1554 $[M + H]^+$ and a fragment ion at m/z 229.1062 $[M - 162 + H]^+$ in positive ESI mode.

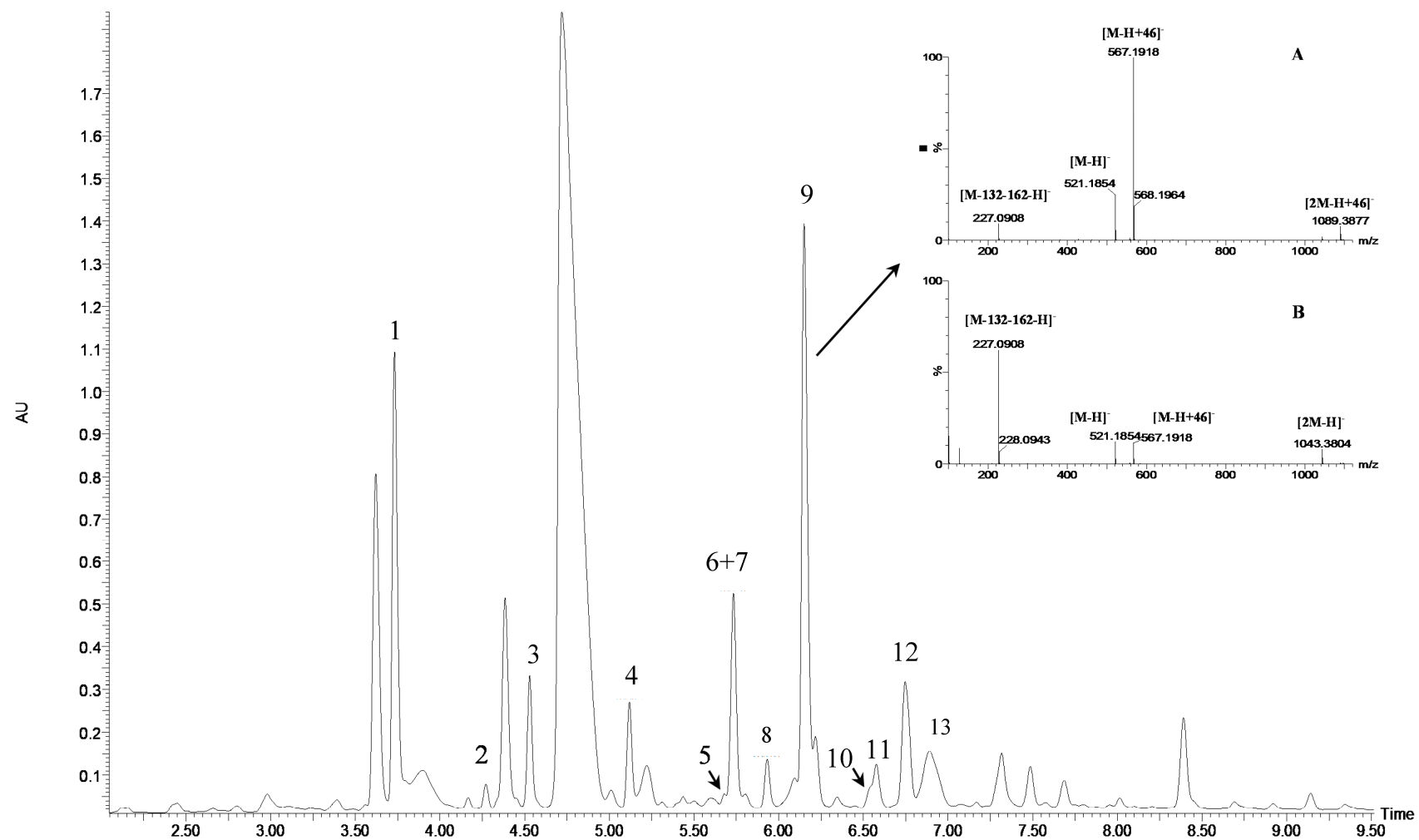


Figure 1. UPLC-DAD chromatogram (254 nm) of compounds of methanolic extract from honeysuckle berries (*Lonicera caerulea* L.) and mass spectra of loganin 7-O-pentoside (peak 9) before (A) and after (B) fragmentations in negative mode. The peak number corresponds to the number in Table 1.

Table 1. MS data for identification of iridoids of honeysuckle berries (*Lonicera caerulea* L.).

Peak No.	t_R (min)	Ion	MS ¹ (m/z)	MS ² (m/z)	Identification
1	3.73	–	375.1276 [M – H] [–] 751.2630 [2M – H] [–] 1127.4083 [3M – H] [–]	213.0769 [M – 162 – H] [–] , 195.0650 [M – 162 – 18 – H] [–] , 169.0855 [M – 162 – 44 – H] [–] , 151.0771 [M – 162 – 18 – 44 – H] [–] , 125.0608 [M – 162 – 18 – 44 – 26 – H] [–] , 101.0232	loganic acid
		+	377.1440 [M + H] ⁺ 753.2834 [2M + H] ⁺	215.0913 [M – 162 + H] ⁺ , 197.0831 [M – 162 – 18 + H] ⁺ , 179.0701 [M – 162 – 18 – 18 + H] ⁺ , 151.0394 [M – 162 – 18 – 18 – 28 + H] ⁺ , 161.0598 [M – 162 – 18 – 18 – 18 + H] ⁺ , 133.0659 [M – 162 – 18 – 18 – 28 – 18 + H] ⁺ , 109.0671 [M – 162 – 18 – 18 – 18 – 52 + H] ⁺	
2	4.27	–	375.1276 [M – H] [–] 751.2630 [2M – H] [–]	213.0769 [M – 162 – H] [–] , 195.0650 [M – 162 – 18 – H] [–] , 169.0855 [M – 162 – 44 – H] [–] , 151.0771 [M – 162 – 44 – 18 – H] [–] , 125.0608 [M – 162 – 18 – 44 – 26 – H] [–] , 01.0232	7- <i>epi</i> -loganic acid
		+	377.1440 [M + H] ⁺ 753.2834 [2M + H] ⁺	215.0913 [M – 162 + H] ⁺ , 197.0831 [M – 162 – 188 + H] ⁺ , 179.0701 [M – 162 – 18 – 18 + H] ⁺ , 151.0394 [M – 162 – 18 – 18 – 28 + H] ⁺ , 161.0598 [M – 162 – 18 – 18 – 18 + H] ⁺ , 133.0659 [M – 162 – 18 – 18 – 28 – 18 + H] ⁺ , 109.0671 [M – 162 – 18 – 18 – 18 – 52 + H] ⁺	
3	4.53	–	507.1746 [M – H] [–] 1015.3484 [2M – H] [–]	375.1356 [M – 132 – H] [–] , 345.0806 [M – 162 – H] [–] , 213.0769 [M – 132 – 162 – H] [–] , 195.0650 [M – 162 – 18 – H] [–] , 169.0855 [M – 132 – 162 – 44 – H] [–] , 151.0746 [M – 132 – 162 – 44 – 18 – H] [–] , 125.0608 [M – 132 – 162 – 18 – 44 – 26 – H] [–] , 101.0232	loganic acid 7- <i>O</i> -pentoside
		+	509.1876 [M + H] ⁺ 1017.3707 [2M + H] ⁺	491.1775 [M – 18 + H] ⁺ , 377.1493 [M – 132 + H] ⁺ , 359.1348 [M – 18 – 132 + H] ⁺ , 347.1344 [M – 162 + H] ⁺ , 329.1228 [M – 18 – 162 + H] ⁺ , 215.0913 [M – 132 – 162 + H] ⁺ , 197.0802 [M – 132 – 162 – 18 + H] ⁺ , 179.0701 [M – 132 – 162 – 18 – 18 + H] ⁺ , 161.0598 [M – 132 – 162 – 18 – 18 – 18 + H] ⁺ , 151.0771 [M – 132 – 162 – 18 – 18 – 28 + H] ⁺ , 133.0659 [M – 132 – 162 – 18 – 18 – 28 – 18 + H] ⁺ , 109.0671 [M – 132 – 162 – 18 – 18 – 18 – 52 + H] ⁺	
4	5.12	–	507.1746 [M – H] [–] 1015.3484 [2M – H] [–]	357.1144 [M – 132 – 18 – H] [–] , 327.1092 [M – 162 – 18 – H] [–] , 195.0650 [M – 132 – 18 – 162 – H] [–] , 177.0549 [M – 132 – 18 – 162 – 18 – H] [–] , 151.0771 [M – 132 – 18 – 162 – 18 – 44 – H] [–] , 133.0654 [M – 132 – 18 – 162 – 18 – 44 – H] [–] , 125.0608 [M – 132 – 18 – 162 – 44 – 26 – H] [–] , 101.0232	7- <i>epi</i> -loganic acid 7- <i>O</i> -pentoside
		+	509.1876 [M + H] ⁺ 1017.3707 [2M + H] ⁺	491.1775 [M – 18 + H] ⁺ , 377.1493 [M – 132 + H] ⁺ , 359.1348 [M – 18 – 132 + H] ⁺ , 347.1344 [M – 162 + H] ⁺ , 329.1228 [M – 18 – 162 + H] ⁺ , 215.0913 [M – 132 – 162 + H] ⁺ , 197.0802 [M – 132 – 162 – 18 + H] ⁺ , 179.0701 [M – 132 – 162 – 18 – 18 + H] ⁺ , 161.0598 [M – 132 – 162 – 18 – 18 – 18 + H] ⁺ , 151.0771 [M – 132 – 162 – 18 – 18 – 28 + H] ⁺ , 133.0659 [M – 132 – 162 – 18 – 18 – 28 – 18 + H] ⁺ , 109.0671 [M – 132 – 162 – 18 – 18 – 18 – 52 + H] ⁺	

Table 1. Cont.

Peak No.	t_R (min)	Ion	MS^1 (m/z)	MS^2 (m/z)	Identification
5	5.68	–	521.1854 [M – H] [–] 567.1918 [M – H + 46] [–] 1089.3577 [2M – H + 46] [–]	389.1423 [M – 132 – H] [–] , 227.0908 [M – 132 – 162 – H] [–] , 209.0805 [M – 132 – 162 – 18 – H] [–] , 127.0748 [M – 132 – 162 – 100 – H] [–] , 101.0232	pentosyl loganin
		+	523.2039 [M + H] ⁺	391.1595 [M – 132 + H] ⁺ , 229.1062 [M – 132 – 162 + H] ⁺ , 211.0961 [M – 132 – 162 – 18 + H] ⁺ , 197.0802 [M – 132 – 162 – 32 + H] ⁺ , 179.0701 [M – 132 – 162 – 18 – 32 + H] ⁺ , 161.0598 [M – 132 – 162 – 18 – 32 – 18 + H] ⁺ , 151.0394 [M – 132 – 162 – 18 – 32 – 28 + H] ⁺ , 133.0659 [M – 132 – 162 – 18 – 32 – 18 – 28 + H] ⁺ , 109.0650 [M – 132 – 162 – 18 – 32 – 18 – 52 + H] ⁺	
6	5.73	–	357.1183 [M – H] [–] 403.1223 [M – H + 46] [–] 761.1247 [2M – H + 46] [–]	195.0650 [M – 162 – H] [–] , 149.0438 [M – 162 – 18 – 28 – H] [–] , 125.0241 [M – 132 – 162 – 70 – H] [–] , 101.0232	sweroside
		+	359.1347 [M + H] ⁺ 717.2608 [2M + H] ⁺	197.0802 [M – 162 + H] ⁺ , 179.0701 [M – 162 – 18 + H] ⁺ , 151.0771 [M – 162 – 18 – 28 + H] ⁺ , 127.0409 [M – 162 – 70 + H] ⁺ , 109.0671 [M – 162 – 18 – 28 – 42 + H] ⁺	
7	5.73	–	389.1342 [M – H] [–] 435.1502 [M – H + 46] [–] 793.2800 [2M – H + 46] [–]	227.0939 [M – 162 – H] [–] , 209.0805 [M – 162 – 18 – H] [–] , 197.0797 [M – 162 – 32 – H] [–] , 149 [M – 162 – 18 – 32 – 28 – H] [–] , 131.0356 [M – 162 – 18 – 32 – 28 – 18 – H] [–] , 101.0232	loganin
		+	391.1554 [M + H] ⁺ 749.2858 [2M + H] ⁺	373.1504 [M – 18 + H] ⁺ , 229.1062 [M – 162 + H] ⁺ , 211.0961 [M – 162 – 18 + H] ⁺ , 179.0701 [M – 162 – 18 – 32 + H] ⁺ , 151.0771 [M – 162 – 18 – 32 – 28 + H] ⁺ , 133.0659 [M – 162 – 18 – 32 – 28 – 18 + H] ⁺	
8	5.93	–	489.1630 [M – H] [–] 535.1502 [M – H + 46] [–] 1025.3314 [2M – H + 46] [–]	195.0650 [M – 132 – 162 – H] [–] , 125.0241 [M – 132 – 162 – 70 – H] [–] , 101.0232	pentosyl sweroside
		+	491.1775 [M + H] ⁺	359.1347 [M – 132 + H] ⁺ , 197.0802 [M – 132 – 162 + H] ⁺ , 179.0701 [M – 132 – 162 – 18 + H] ⁺ , 151.0746 [M – 132 – 162 – 18 – 28 + H] ⁺ , 127.0409 [M – 132 – 162 – 70 + H] ⁺ , 109.0671 [M – 132 – 162 – 18 – 28 – 42 + H] ⁺	
9	6.15	–	521.1854 [M – H] [–] 567.1918 [M – H + 46] [–] 1043.3804 [2M – H] [–] 1089.3577 [2M – H + 46] [–]	389.1342 [M – 132 – H] [–] , 371.0636 [M – 132 – 18 – H] [–] , 227.0939 [M – 132 – 162 – H] [–] , 209.0805 [M – 132 – 162 – 18 – H] [–] , 127.0748 [M – 132 – 162 – 100 – H] [–] , 101.0232	loganin 7-O-pentoside
		+	523.2039 [M + H] ⁺ 1045.4009 [2M + H] ⁺	505.1936 [M – 18 + H] ⁺ , 391.1595 [M – 132 + H] ⁺ , 373.1504 [M – 18 – 132 + H] ⁺ , 361.1494 [M – 162 + H] ⁺ , 343.1367 [M – 18 – 162 + H] ⁺ , 229.1062 [M – 132 – 162 + H] ⁺ , 211.0961 [M – 132 – 162 – 18 + H] ⁺ , 197.0802 [M – 132 – 162 – 32 + H] ⁺ , 179.0701 [M – 132 – 162 – 32 – 18 + H] ⁺ , 151.0394 [M – 132 – 162 – 32 – 18 – 28 + H] ⁺ , 161.0598 [M – 132 – 162 – 32 – 18 – 18 + H] ⁺ , 133.0659 [M – 132 – 162 – 32 – 18 – 18 – 28 + H] ⁺ , 109.0650 [M – 132 – 162 – 32 – 18 – 18 – 52 + H] ⁺	

Table 1. Cont.

Peak No.	t_R (min)	Ion	MS^1 (m/z)	MS^2 (m/z)	Identification
10	6.54	–	<u>435.1502</u> [M – H + 46] [–] 793.2800 [2M – H + 46] [–]	<u>227.0939</u> [M – 162 – H] [–] , 209.0805 [M – 162 – 18 – H] [–] , 197.0797 [M – 162 – 32 – H] [–] , 149 [M – 162 – 18 – 32 – 28 – H] [–] , 131.0356 [M – 162 – 18 – 32 – 28 – 18 – H] [–] , 101.0232	7- <i>epi</i> -loganin
		+	<u>391.1554</u> [M + H] ⁺ 749.2858 [2M + H] ⁺	373.1504 [M – 18 + H] ⁺ , 229.1062 [M – 162 + H] ⁺ , <u>211.0961</u> [M – 162 – 18 + H] ⁺ , 179.0701 [M – 162 – 18 – 32 + H] ⁺ , 151.0771 [M – 162 – 18 – 32 – 28 + H] ⁺ , 133.0659 [M – 162 – 18 – 32 – 28 – 18 + H] ⁺	
11	6.58	–	<u>403.1263</u> [M – H] [–] 807.2567 [2M – H] [–]	<u>371.0952</u> [M – 32 – H] [–] , 333.0848 [M – 42 – 28 – H] [–] , <u>223.0622</u> [M – 162 – 18 – H] [–] , 191.0354 [M – 162 – 18 – 32 – H] [–] , 165.0565 [M – 162 – 18 – 32 – 28 – H] [–] , <u>121.0288</u> [M – 162 – 18 – 32 – 28 – H] [–] , 101.0232	secoxyloganin
		+	405.1383 [M + H] ⁺	373.1504 [M – 32 + H] ⁺ , 345.0262 [M – 32 – 28 + H] ⁺ , 243.0849 [M – 162 + H] ⁺ , 225.0774 [M – 162 – 18 + H] ⁺ , 211.0961 [M – 162 – 32 + H] ⁺ , 193.0862 [M – 162 – 18 – 32 + H] ⁺ , 167.0700 [M – 162 – 18 – 32 – 26 + H] ⁺ , 165.0562 [M – 162 – 18 – 32 – 28 + H] ⁺ , 127.0380 [M – 32 – 28 – 28 – 28 + H] ⁺ , 123.0450 [M – 162 – 18 – 32 – 26 – 44 + H] ⁺	
12	6.78	–	<u>521.1854</u> [M – H] [–] <u>567.1918</u> [M – H + 46] [–] 1043.3804 [2M – H] [–] 1089.3577 [2M – H + 46] [–]	389.1342 [M – 132 – H] [–] , 371.0636 [M – 132 – 18 – H] [–] , 227.0939 [M – 132 – 162 – H] [–] , 209.0805 [M – 132 – 162 – 18 – H] [–] , 101.0232	7- <i>epi</i> -loganin 7- <i>O</i> -pentoside
		+	<u>523.2039</u> [M + H] ⁺ 1045.4009 [2M + H] ⁺	505.1936 [M – 18 + H] ⁺ , 391.1595 [M – 132 + H] ⁺ , 373.1504 [M – 18 – 132 + H] ⁺ , 361.1494 [M – 162 + H] ⁺ , 343.1367 [M – 18 – 162 + H] ⁺ , 229.1062 [M – 132 – 162 + H] ⁺ , <u>211.0961</u> [M – 132 – 162 – 18 + H] ⁺ , 197.0802 [M – 132 – 162 – 32 + H] ⁺ , 179.0701 [M – 132 – 162 – 18 – 32 + H] ⁺ , 151.0394 [M – 132 – 162 – 18 – 32 – 28 + H] ⁺ , 161.0598 [M – 132 – 162 – 18 – 32 – 18 + H] ⁺ , 133.0659 [M – 132 – 162 – 18 – 32 – 18 – 28 + H] ⁺ , 109.0650 [M – 132 – 162 – 18 – 32 – 18 – 52 + H] ⁺	
13	6.89	–	<u>387.1302</u> [M – H] [–] <u>433.1331</u> [M – H + 46] [–]	<u>225.0781</u> [M – 162 – H] [–] , 179.0540 [M – 162 – 18 – 28 – H] [–] , <u>155.0347</u> [M – 162 – 18 – 28 – 24 – H] [–] , 123.0456 [M – 162 – 18 – 28 – 24 – 32 – H] [–] , 101.0232	secologanin
		+	389.1433 [M + H] ⁺	<u>227.0920</u> [M – 162 + H] ⁺ , 209.0799 [M – 162 – 18 + H] ⁺ , 195.0666 [M – 162 – 32 + H] ⁺ , 177.0557 [M – 162 – 18 – 32 + H] ⁺ , <u>165.0562</u> [M – 162 – 18 – 44 + H] ⁺ , 151.0394 [M – 162 – 18 – 32 – 26 + H] ⁺ , 149.0599 [M – 162 – 18 – 32 – 28 + H] ⁺ , 139.0399 [M – 162 – 32 – 28 – 28 + H] ⁺ , 109.0308 [M – 162 – 18 – 32 – 26 – 42 + H] ⁺ , 107.0513 [M – 162 – 18 – 32 – 28 – 42 + H] ⁺	

Main signals are underlined.

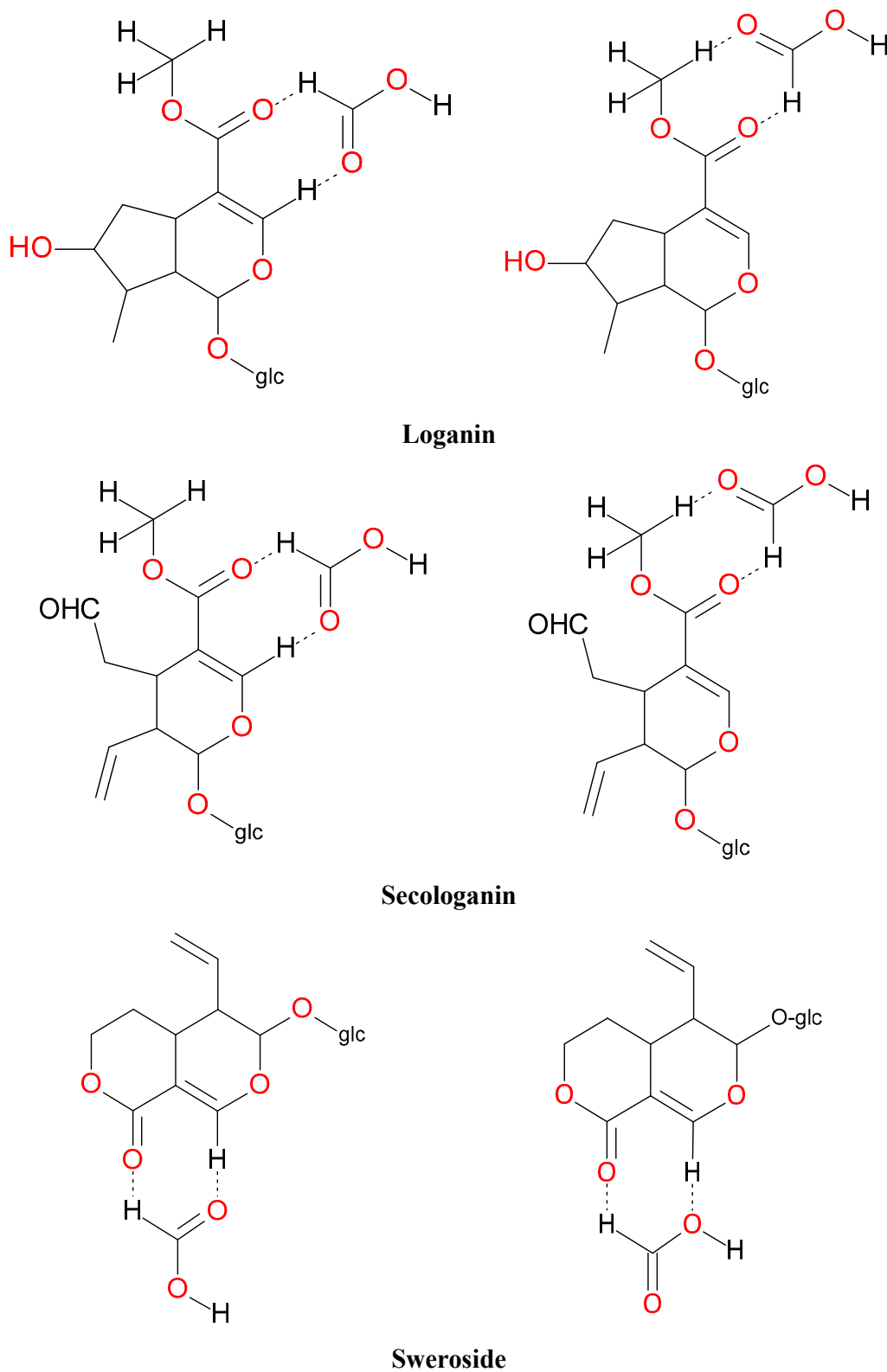


Figure 2. Loganin, secologanin, and sweroside with formic acid possible adducts.

These data, UV-Vis absorption spectra and retention times show that compounds **1** and **7** are loganic acid and loganin, respectively. Identification of these compounds was consistent with our previous studies [14,22]. Compounds **2** and **10** displayed the same pseudomolecular and fragment

ions in negative and positive mode as compounds **1** and **7**, respectively, but they differed in abundance of the major fragment ions $[M - 162 + H]^+$ and $[M - 162 - 18 + H]^+$. In compound **1** and loganic acid standard, higher abundance was observed for ions at m/z 213.0769 $[M - 162 - H]^-$ and 215.0913 $[M - 162 + H]^+$, while in compound **2** higher abundance was observed for ions at m/z 195.0678 $[M - 162 - 18 + H]^+$ and 197.0831 $[M - 162 - 18 + H]^+$ (Figure 3). This indicates that compound **2** is 7-*epi*-loganic acid. Similar differences were observed in the case of compounds **10** and **7**, but only in positive mode. In compound **7** and in loganin standard, higher abundance was detected for the ion at m/z 229.1062 $[M - 162 + H]^+$ and in compound **10** for the ion at m/z 211.0961 $[M - 162 - 18 + H]^+$ (Figure 4). This indicates that compound **10** is 7-*epi*-loganin. Ion $[M - 162 + H]^+$ is more stable when the $-OH$ group in C-7 is above the molecule plane. Conversely, when the $-OH$ group is below the plane, this ion is less stable, or its stabilization is not possible; therefore the ion appears after loss of water (18 Da). Similar observations were made by Madhusudanan et al. [23] for the epimers of loganin after fragmentation in positive mode.

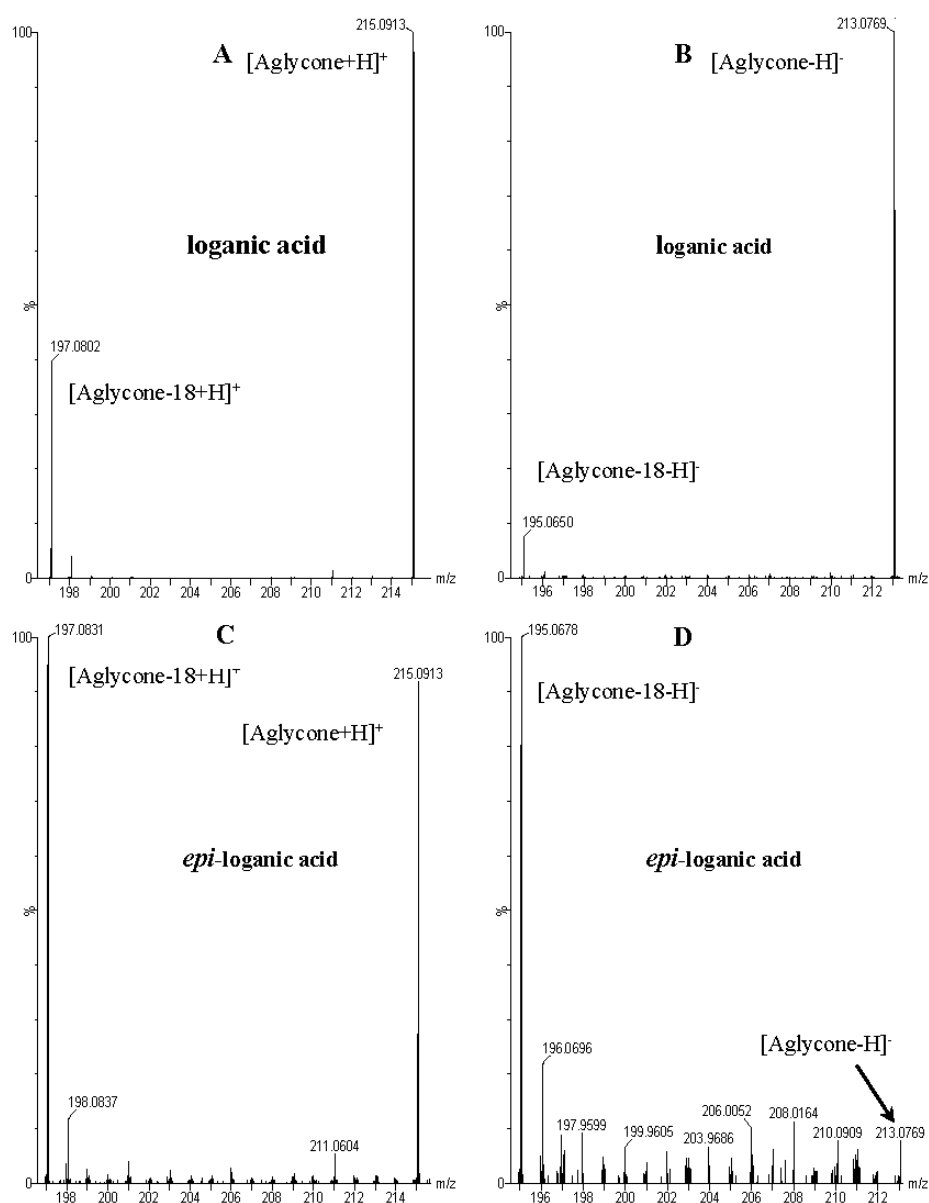


Figure 3. Mass spectra of the aglycone ions of loganic acid (**1**) and *epi*-loganic acid (**2**) after fragmentation in positive (**A,C**) and negative (**B,D**) mode.

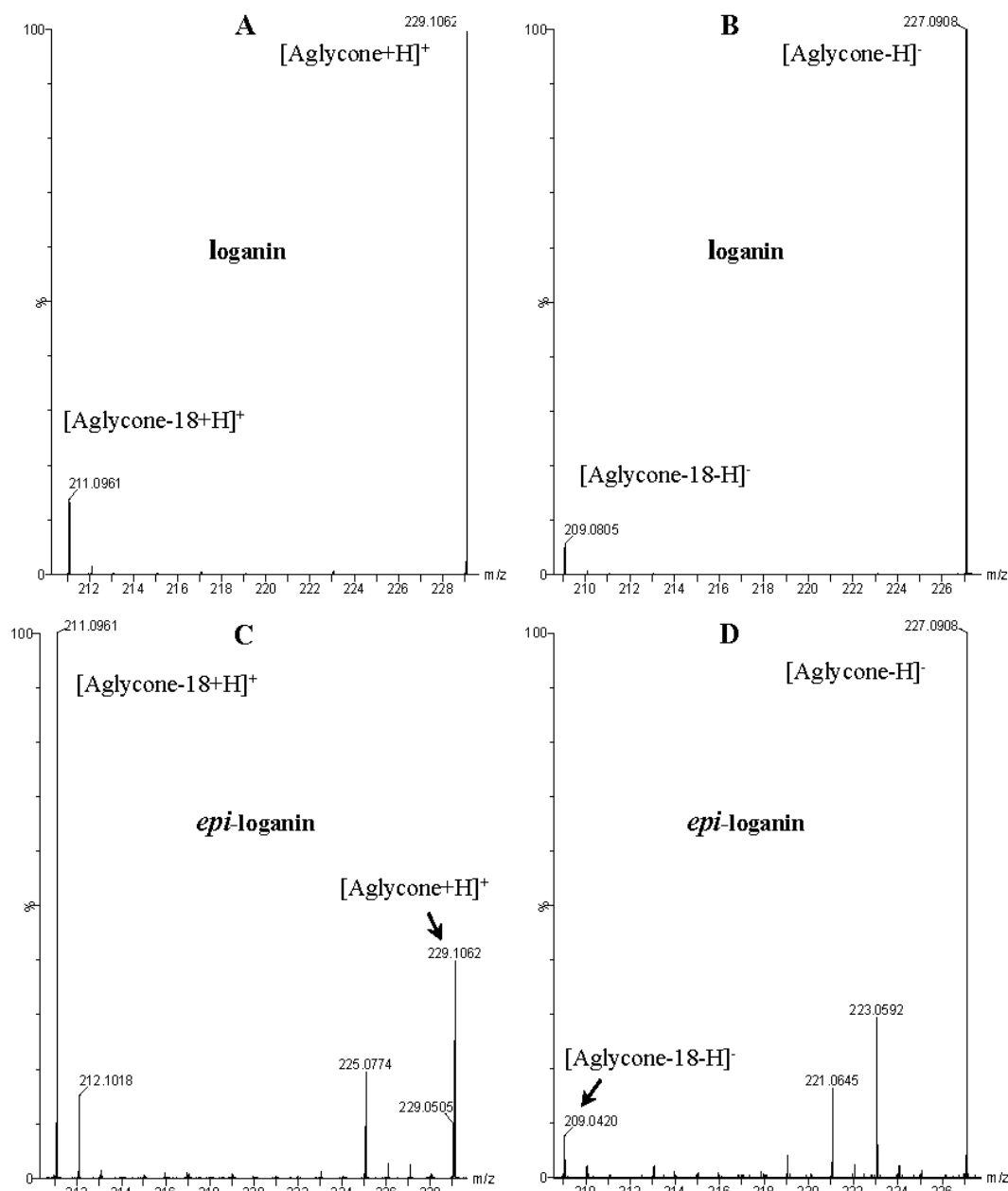


Figure 4. Mass spectra of the aglycone ions of loganin (7) and *epi*-loganin (10) after fragmentation in positive (A,C) and negative (B,D) mode.

Compounds **3** (t_R 4.53 min) and **4** (t_R 5.2 min) also display the same pseudomolecular ions $[\text{M} - \text{H}]^-$ at m/z 507.1746 and $[\text{M} + \text{H}]^+$ at m/z 509.1876, and the same fragment ion $[\text{M} - 132 + \text{H}]^+$ at m/z 377.1493 (Table 1). These ions corresponded to the molecular ion of the loganic acid after loss of 132 Da. The difference between these compounds, in addition to the retention times, is in MS2. Compound **3**, after fragmentation, gave a stable fragment ion $[\text{M} - 132 - 162 - \text{H}]^-$ at m/z 213.0769 in negative mode, and $[\text{M} - 132 - 162 + \text{H}]^+$ at m/z 215.0913 in positive mode, whereas compound **4** gave a stable fragment ion, after loss of water – 18 Da, $[\text{M} - 132 - 162 - 18 - \text{H}]^-$ at m/z 195.0650 in negative mode and $[\text{M} - 132 - 162 - 18 + \text{H}]^+$ at m/z 197.0802 in positive mode, which is comparable to compounds **1** and **2** (Table 1; Figures 3 and 5). These results suggest that compounds **3** and **4** are pentose derivatives of loganic acid and *epi*-loganic acid, respectively. Fragment ions at m/z 345.0806 $[\text{M} - 162 - \text{H}]^-$ and 347.1344 $[\text{M} - 162 + \text{H}]^+$ (compound **3**) and 327.1092 $[\text{M} - 162 - 18 - \text{H}]^-$ and

329.1228 $[M - 162 - 18 + H]^+$ (compound 4) show that the pentose is not attached to glucose, and does not form with it a disaccharide. Loss of glucose, while preserving the glycosidic bond of the pentose, indicates that the only possible position to attach pentose to aglycone is at the $-OH$ group in C-7 (Tables 2 and 3, Figure 5). This proves that compounds 3 and 4 are loganic acid 7-O-pentoside and 7-*epi*-loganic acid 7-O-pentoside, respectively. The fragmentation pathways of loganic acid and its derivatives in positive and negative mode are shown in Figure 5. The molecular ion at m/z 509.1876 had two fragmentation pathways: one through preceding loss of water followed by that of the sugars, and conversely the second primarily through loss of sugars followed by that of water. In addition, the neutral losses of CO, CO₂, acetylene and cyclopropynone have provided data for confirming the presence of functional substituents in the aglycone. All possible secondary structures of monoisotopic ions after negative and positive fragmentations of 7-O-pentoside loganic acid, 7-O-pentoside *epi*-loganic acid, loganic acid, and *epi*-loganic acid are shown in Tables 2 and 3.

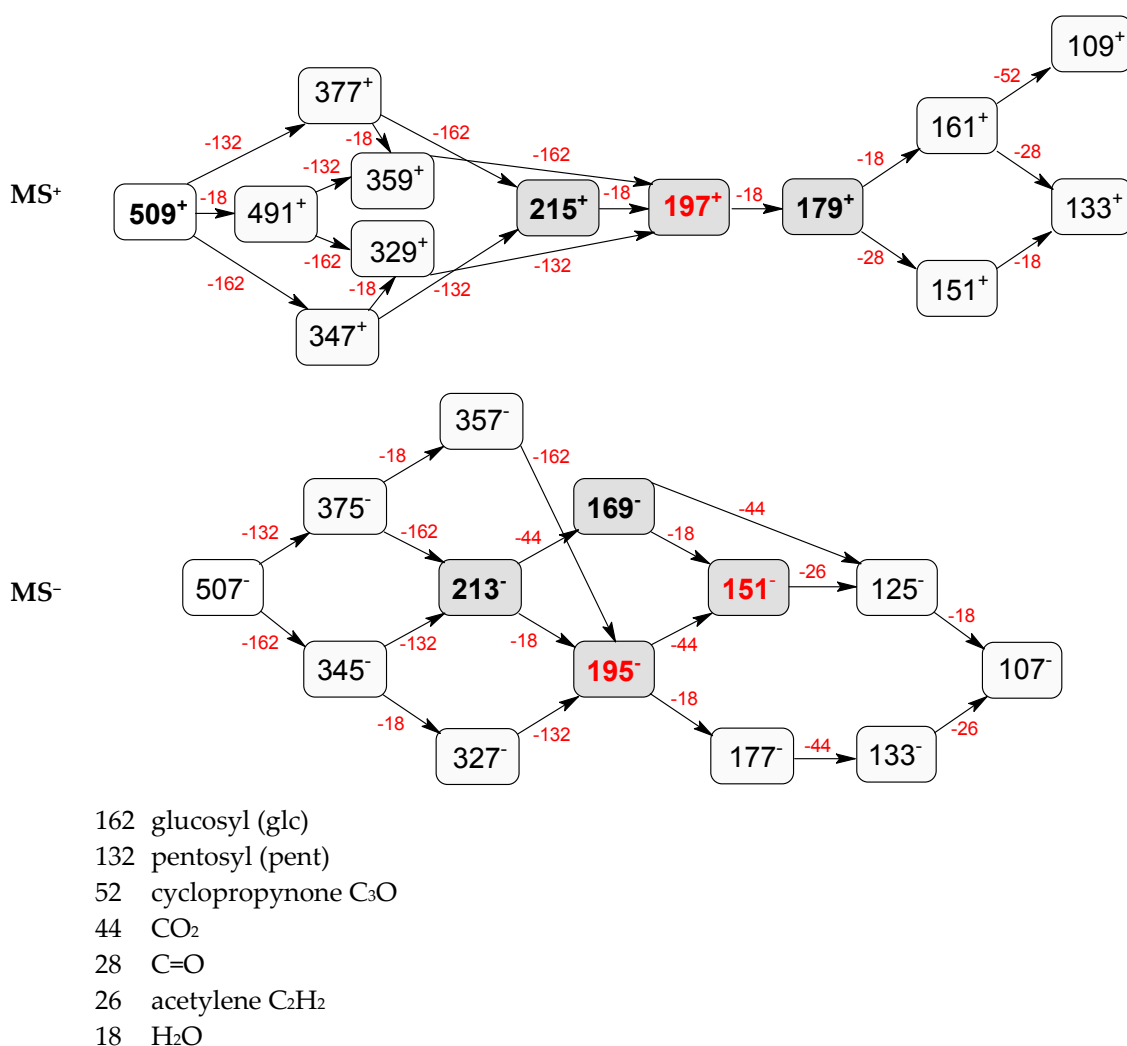


Figure 5. Fragmentation pathway of loganic acid 7-O-pentoside ($509^+ / 507^-$), *epi*-loganic 7-O-pentoside acid ($509^+ / 507^-$), loganic acid ($377^+ / 375^-$), and *epi*-loganic acid ($377^+ / 375^-$) in the positive (MS^+) and negative (MS^-) mode with the dominant ions in form *epi* (red ions).

Table 2. Characteristics of monoisotopic ion in negative mode and chemical structures of loganic acid 7-O-pentoside, *epi*-loganic acid 7-O-pentoside, loganic acid, *epi*-loganic acid, and their possible fragments.

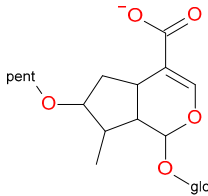
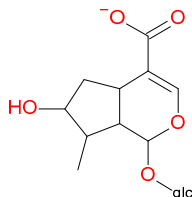
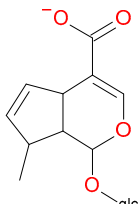
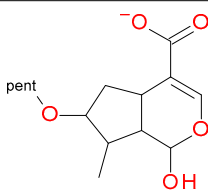
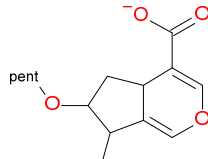
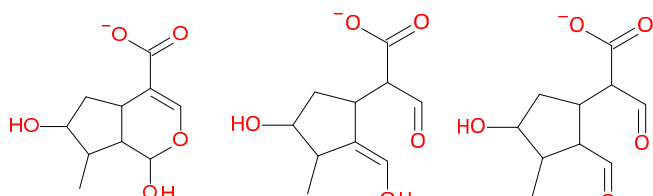
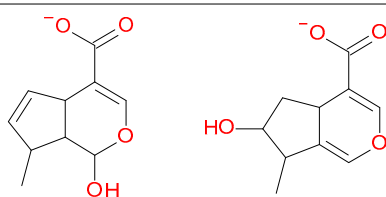
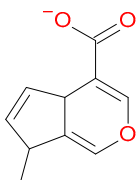
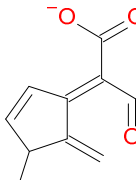
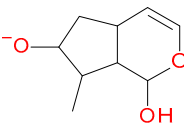
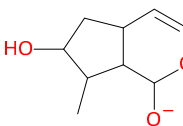
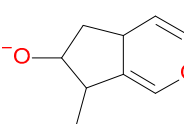
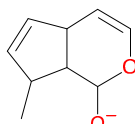
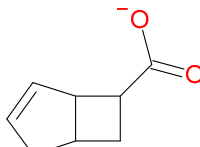
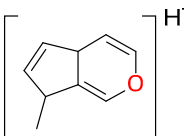
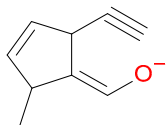
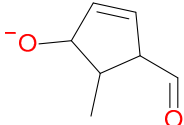
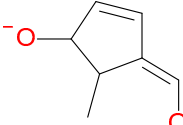
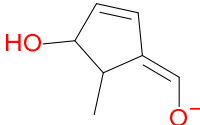
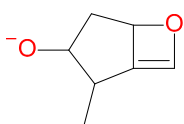
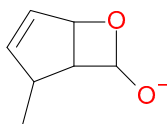
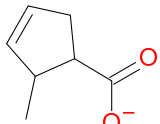
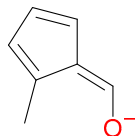
Monoisotopic Ion [M – H] [–] <i>m/z</i>		Plausible Structure	
Found	Formula		
Calculated	Error		
507.1746 507.17137 –0.00323	C ₂₁ H ₃₁ O ₁₄		
375.1276 375.12912 0.00152	C ₁₆ H ₂₃ O ₁₀		
357.1144 357.11855 0.00415	C ₁₆ H ₂₁ O ₉		
345.1122 345.11855 0.00635	C ₁₅ H ₂₁ O ₉		
327.1092 327.10799 –0.00121	C ₁₅ H ₁₉ O ₈		
213.0769 213.07629 –0.00069	Aglycone C ₁₀ H ₁₃ O ₅		
195.0650 195.06573 0.00073	C ₁₀ H ₁₁ O ₄		

Table 2. Cont.

Monoisotopic Ion [M – H] [−] <i>m/z</i>		Plausible Structure		
Found	Formula			
Calculated Error				
177.0549 177.05517 0.00027	C ₁₀ H ₉ O ₃			
169.0855 169.08646 0.00096	C ₉ H ₁₃ O ₃			
151.0771 151.0759 −0.0012	C ₉ H ₁₁ O ₂			
133.0654 133.06534 −0.00006	C ₉ H ₉ O			
125.0608 125.06025 −0.00055	C ₇ H ₉ O ₂			
				
107.0494 107.04969 0.00029	C ₇ H ₇ O			
179.0522 161.0444 143.0344 149.0438 131.0356 119.0341 113.0238 101.0232	Glucose/pentose fragments: 179 (−18) → 161 (−18) → 143 (−18) → 125 149 (−18) → 131 (−18) → 113 119 (−18) → 101			

pent, pentose; glc, glucose.

Table 3. Characteristics of monoisotopic ion in positive mode and chemical structures of loganic acid 7-O-pentosideside, *epi*-loganic acid 7-O-pentoside, loganic acid, *epi*-loganic acid, and their possible fragments.

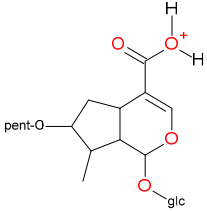
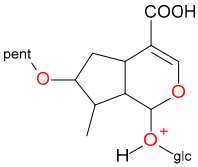
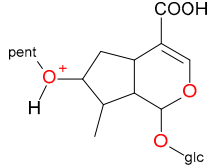
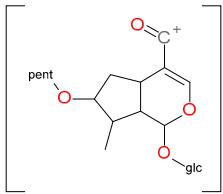
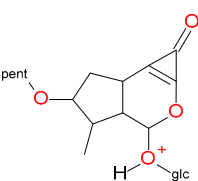
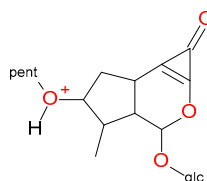
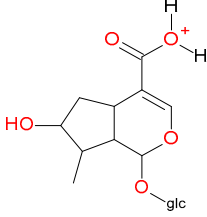
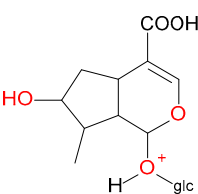
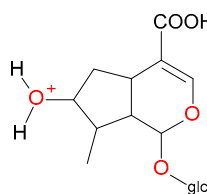
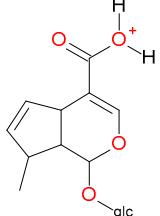
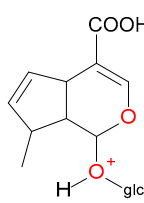
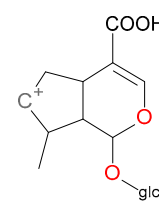
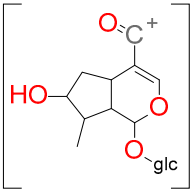
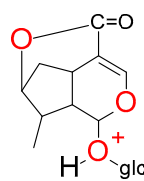
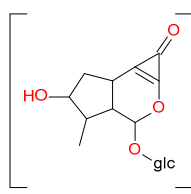
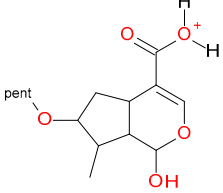
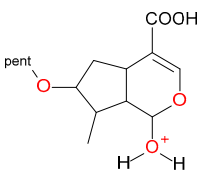
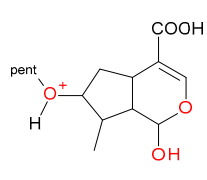
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Found	Formula			
Calculated Error				
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491.1775 491.17646 −0.00104	C ₂₁ H ₃₁ O ₁₃			
377.1493 377.14477 −0.00453	C ₁₆ H ₂₅ O ₁₀			
359.1348 359.1342 −0.0006	C ₁₆ H ₂₃ O ₉			
				
347.1344 347.1342 −0.0002	C ₁₅ H ₂₃ O ₉			

Table 3. Cont.

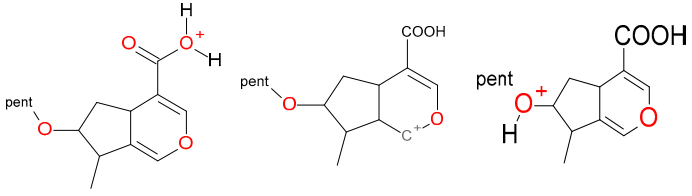
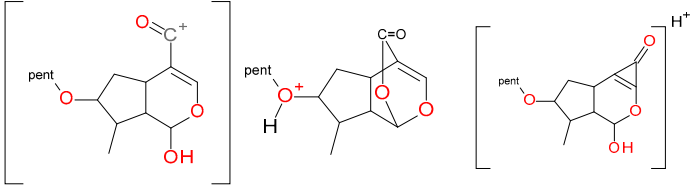
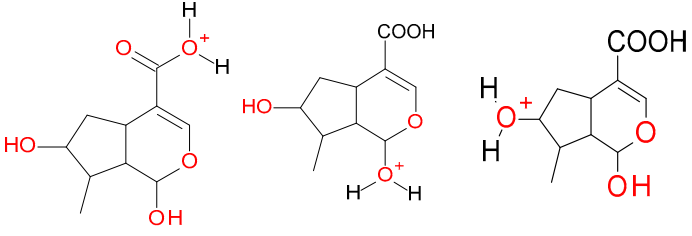
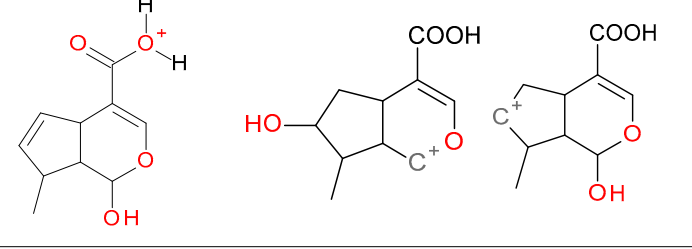
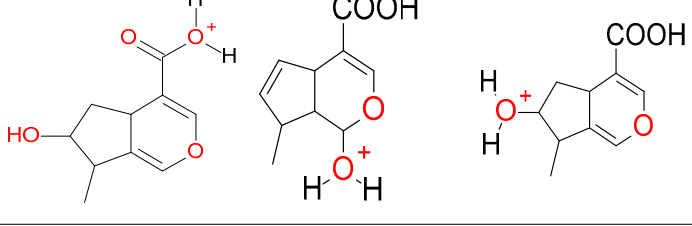
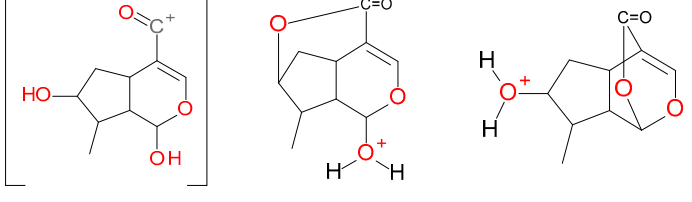
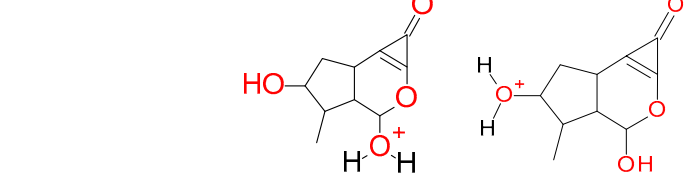
Monoisotopic Ion $[M + H]^+$ m/z		Formula	Plausible Structure
Found	Calculated Error		
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0.00084			
215.0919	215.09194	Aglycone $C_{10}H_{15}O_5$	
0.00004			
			
			
197.0816	197.08138	$C_{10}H_{13}O_4$	
−0.00022			

Table 3. Cont.

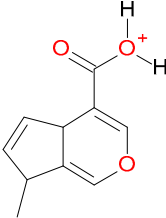
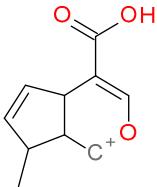
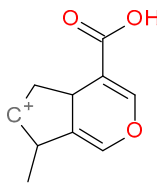
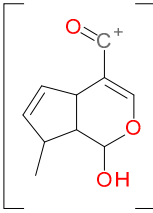
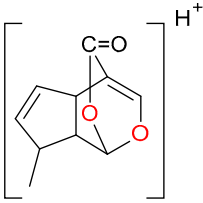
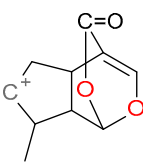
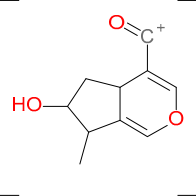
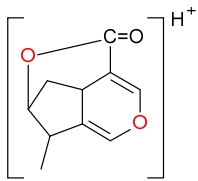
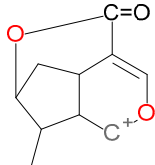
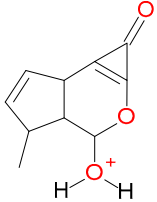
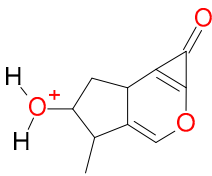
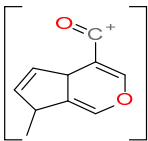
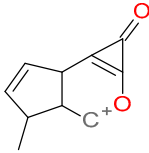
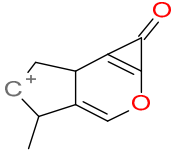
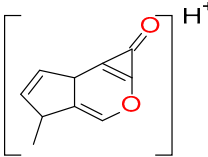
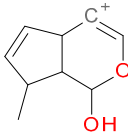
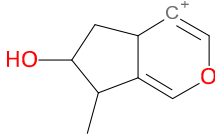
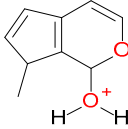
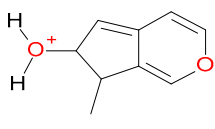
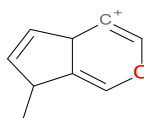
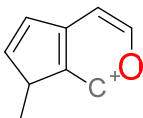
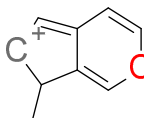
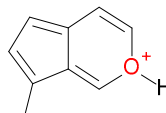
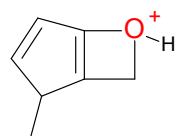
Monoisotopic Ion $[M + H]^+$ m/z		Plausible Structure		
Found	Formula			
Calculated Error				
179.0721 179.07082 −0.00128	$C_{10}H_{11}O_3$			
				
				
				
161.0598 161.06025 0.00045	$C_{10}H_9O_2$			
				
151.0771 151.0759 −0.0012	$C_9H_{11}O_2$			
				

Table 3. Cont.

Monoisotopic Ion [M + H] ⁺ <i>m/z</i>		Plausible Structure		
Found	Formula			
Calculated Error				
133.0659 133.06534 −0.00056	C ₉ H ₉ O			
				
109.0671 109.06534 −0.00176	C ₇ H ₉ O			
pent, pentose; glc, glucose.				

Compounds **5**, **6**, **8**, **9**, **12**, and **13**, like loganin (**7**) and *epi*-loganin (**10**), all displayed the formic acid adduct [M + 46 − H][−] in negative mode, suggesting that their structures may contain for example the methyl ester functional group or lactone [11]. Similarly, a strong tendency for formation of an acetic adduct was observed by Zhang et al. (2015) for iridoids without a carboxyl group at the mobile phase with acetic acid [19]. Compound **5** (*t_R* 5.68 min) showed the same formic acid adduct as compounds **9** (*t_R* 6.15 min) and **12** (*t_R* 6.78 min) at *m/z* 567.1918 [M + 46 − H][−] and the same fragment ion at *m/z* 227.0939 [M − 132 − 162 − H][−] (Table 1; Figure 1). In positive mode, these compounds had a pseudomolecular ion at *m/z* 523.2039 [M + H]⁺ and a fragment ion at *m/z* 229.1062 [M − 132 − 162 + H]⁺. So these were identified tentatively as pentose derivatives of loganin. Compound **9** displayed the same fragmentation ions [M − 162 + H]⁺ and [M − 162 − 18 + H]⁺ as compound **12** at *m/z* 361.1494 and 343.1367, respectively. Compound **5** did not show these ions. According to these data, in compounds **9** and **12**, pentose is attached to aglycone at the −OH group in C-7, whereas in compound **5**, pentose is attached to glucose producing disaccharide (Tables 4 and 5). Compounds **5** and **9** exhibited a stable fragment ion at *m/z* 229.1062 [M − 132 − 162 + H]⁺, whereas compound **12** exhibited a stable ion after loss of water at *m/z* 211.0961 [M − 132 − 162 − 18 + H]⁺, which is comparable to compounds **7** and **10** (Figure 4). Further characteristic protonated fragment ions, which were produced after neutral loss of methanol (32 Da) at *m/z* 197.0802 and 179.0701 were also detected. Thus, they were pentosyl loganin (**5**), loganin 7-*O*-pentoside (**9**), and 7-*epi*-loganin 7-*O*-pentoside (**12**). The pentose derivatives of loganin, as derivatives of loganic acid, had two fragmentation pathways (Figure 6). In negative ion mode, iridoids with the structure of methyl ester showed weaker ionization, and delivered only a few fragmentation ions. In the case of loganin and 7-*epi*-loganin glycosides, relatively high abundance of ions resulted from free aglycone (*m/z* 227.0748) and from two fragments produced as a result of opening the pyran ring from their basic skeleton (*m/z* 127.0748 and 101.0252, Table 4).

The most possible structures of monoisotopic ions after negative and positive fragmentations of pentosyl-loganin, loganin 7-*O*-pentoside, 7-*epi*-loganin 7-*O*-pentoside, loganin, and 7-*epi*-loganin are shown in Tables 4 and 5. The pentose derivatives of both loganic acid and loganin epimers were identified in edible honeysuckle berries for the first time. Additionally, to our knowledge, those compounds were not described in raw materials (plants). In the literature, only apiofuranosyl attached to iridoids, such as sweroside and mussaenosidic acid, has been described [24,25]. Complete elucidation of structures of detected iridoid pentosides, especially glycone (sugar) moieties, will be

possible after further studies, including separation of individual compounds from plant material and their spectroscopic analysis.

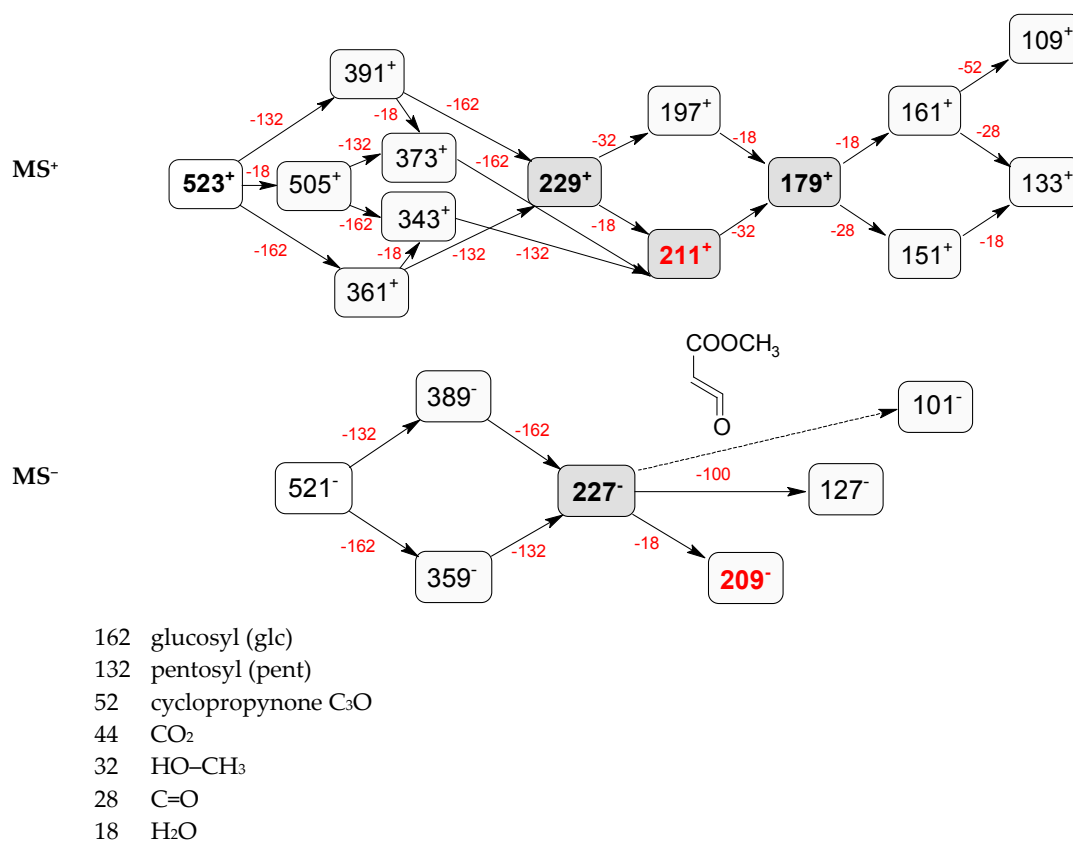


Figure 6. Fragmentation pathway of pentosyl loganin (523⁺/521[−]), loganin 7-*O*-pentoside (523⁺/521[−]), *epi*-loganin 7-*O*-pentoside (523⁺/521[−]), loganin (391⁺/389[−]), and 7-*epi*-loganin (391⁺/389[−]) in the positive (MS⁺) and negative (MS[−]) mode.

Table 4. Characteristics of monoisotopic ion in negative mode and chemical structures of pentosyl loganin, loganin 7-*O*-pentoside, 7-*epi*-loganin 7-*O*-pentoside, loganin, and 7-*epi*-loganin, and their possible fragments.

Monoisotopic Ion [M − H] [−] <i>m/z</i>			Plausible Structure
Found	Formula	Calculated Error	
567.1918	C ₂₂ H ₃₃ O ₁₄ CH ₂ O ₂	[M + FA − H] [−]	
567.1925		0.0007	
521.1854			
521.18702		0.00162	
435.1502	C ₁₇ H ₂₅ O ₁₀ CH ₂ O ₂	[M + FA − H] [−]	
435.15024		0.00004	
389.1423			
389.14477		0.00247	

Table 4. Cont.

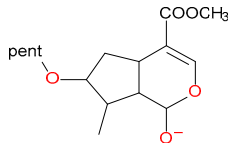
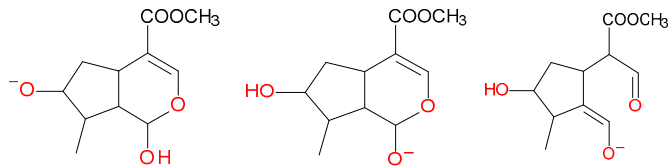
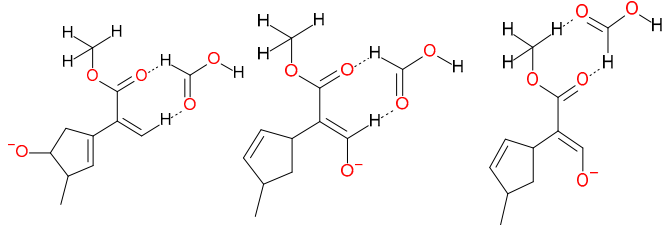
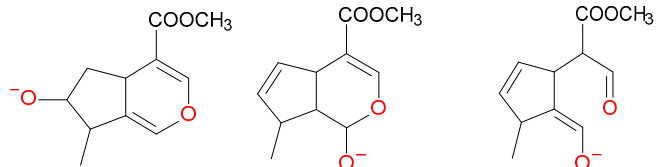
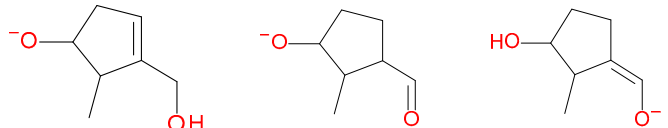
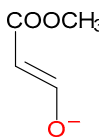
Monoisotopic Ion [M – H] [–] m/z		Plausible Structure			
Found	Calculated Error	Formula			
359.1379	359.1342	C ₁₆ H ₂₃ O ₉			
–0.0037					
227.0908	227.09194	Aglycone C ₁₁ H ₁₅ O ₅			
0.00114					
		C ₁₀ H ₁₃ O ₃ CH ₂ O ₂			
209.0805	209.08138	C ₁₁ H ₁₃ O ₄			
0.00088					
127.0748	127.0759	C ₇ H ₁₁ O ₂			
0.0011					
101.0252	101.02387	C ₄ H ₅ O ₃			
–0.00133					
179.0540	161.0449	Glucose/pentose fragments: 179 (–18) → 161 (–18) → 143 (–18) → 125 149 (–18) → 131 (–18) → 113 119 (–18) → 101			
143.0368					
149.0448					
131.0333					
119.0354					
113.0238					
101.0268					
pent, pentose; glc, glucose.					

Table 5. Characteristics of monoisotopic ion in positive mode and chemical structures of pentosyl loganin, loganin 7-O-pentoside, 7-*epi*-loganin 7-O-pentoside, loganin, and 7-*epi*-loganin, and their possible fragments.

Monoisotopic Ion [M + H] ⁺ <i>m/z</i>		Plausible Structure		
Found	Calculated Error	Formula		
523.2039	523.20267 −0.00123	C ₂₂ H ₃₅ O ₁₄		
505.1936	505.19211 −0.00149	C ₂₂ H ₃₃ O ₁₃		
391.1595	391.16041 0.00091	C ₁₇ H ₂₇ O ₁₀		
373.1504	373.14985 −0.00055	C ₁₇ H ₂₅ O ₉		
361.1494	361.14985 −0.00045	C ₁₆ H ₂₅ O ₉		

Table 5. Cont.

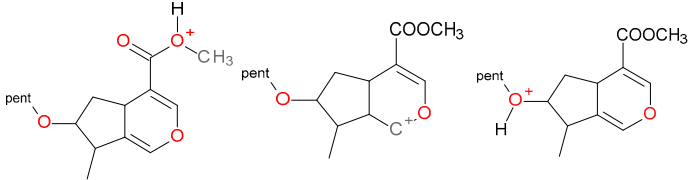
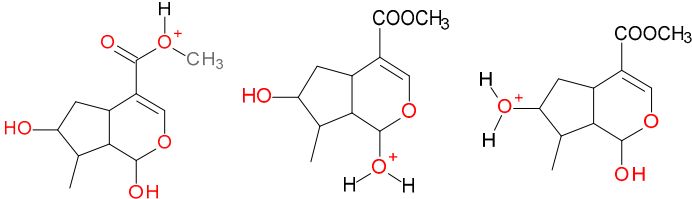
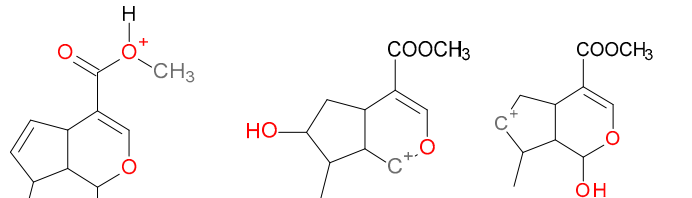
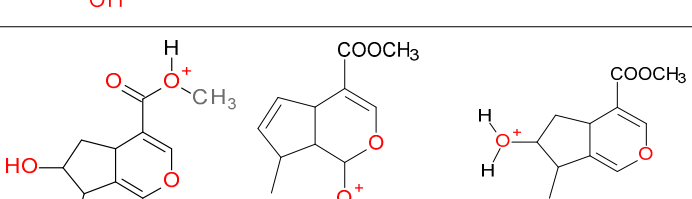
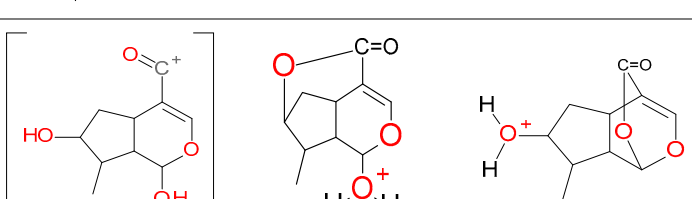
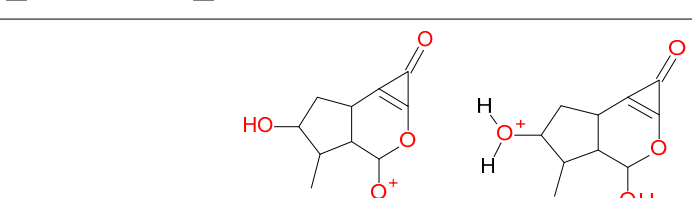
Monoisotopic Ion $[M + H]^+$ m/z		Plausible Structure		
Found	Calculated Error	Formula		
343.1367	343.13929	$C_{16}H_{23}O_8$		
229.1062	229.10759	Aglycone $C_{11}H_{17}O_5$		
211.0961	197.09703	$C_{11}H_{15}O_4$		
197.0802	197.08138	$C_{10}H_{13}O_4$		
	0.000118			
				

Table 5. Cont.

Monoisotopic Ion [M + H] ⁺ m/z		Plausible Structure
Found	Formula	
Calculated Error		
179.0701		
179.07082	C ₁₀ H ₁₁ O ₃	
0.00072		
161.0598		
161.06025	C ₁₀ H ₉ O ₂	
0.00045		
151.0771		See Table 3
151.0759	C ₉ H ₁₁ O ₂	
−0.0012		
133.0659		
133.06534	C ₉ H ₉ O	
−0.00056		
109.0650		
109.06534	C ₇ H ₉ O	
0.00034		

pent, pentose; glc, glucose.

For compound **13** (t_R 6.89 min), the formic acid adduct $[M + 46 - H]^-$ and fragment ion $[M - 162 - H]^-$ were at m/z 433.1331 and 225.0781, respectively. In positive mode this compound gave an $[M + H]^+$ ion at m/z 389.1433 and a fragment ion at m/z 227.0920 (aglycone). Between protonated fragment ions, there were also detected signals deriving from structures after loss of ethenone (42 Da, $CH_2=C=O$), which was specific for the aldehyde group ($-CH_2-CHO$) (Table 1).

According to these data and a previous study [19], this compound was tentatively identified as secologanin. This secoiridoid has been identified not only in *L. japonica* [12] but also in Cornus species [26].

Compound **11** (t_R 6.58 min) exhibited the pseudomolecular ion $[M - H]^-$ at m/z 403.1263 and the fragment ions at m/z 371.0952, 223.0622, 179.0568, and 121.0288, which corresponded to secoxyloganin. These data are consistent with the literature [18]. Identification of the compound as secoxyloganin is also confirmed by data in positive mode (Table 1) and the literature data [13].

Compounds **6** (t_R 5.73 min) and **8** (t_R 5.93 min) exhibited the formic acid adducts $[M + 46 - H]^-$ at m/z 403.1223 and 535.1502, respectively (Table 1). Their pseudomolecular ions $[M - H]^-$ at m/z 357.1183 (**6**) and 489.1342 (**8**) in negative mode, and ions $[M + H]^+$ at m/z 359.1347 (**6**) and 491.1775 (**8**) in positive mode, suggest that the difference between these compounds is 132 Da. The loss of a glucose unit (162 Da) in compound **6** and pentose and glucose units (132 Da and 162 Da, respectively) in compound **8** generated the aglycone ion at m/z 195.0650 and 197.0802 in negative and positive mode, respectively. According to these data and the literature [13,18,19], compound **6** was identified as sweroside, and compound **8** as pentosyl sweroside. 6'-O- β -apiofuranosyl sweroside was identified in the ethanolic extract of the roots of *L. quinquelocularis* by Kumar et al. [24].

3. Materials and Methods

3.1. Chemicals

Acetonitrile for LC-MS was purchased from POCh (Gliwice, Poland). Formic acid was acquired from Sigma-Aldrich (Steinheim, Germany). Loganic acid and loganin were purchased from Extrasynthese (Lyon Nord, France).

3.2. Plant Material

Honeysuckle berries (*Lonicera caerulea* L. var. *kamtschatica* Sevest.) of “Atut” cultivars were used for this study. Fruits were collected from the Research Station for Cultivar Testing in Masłowice and hand-harvested at the stage of consumption during the growing season of May 2014. Before analysis, fruits were frozen and stored at $-20\text{ }^{\circ}\text{C}$.

3.3. Extraction of Compounds for Qualitative Analysis

Frozen fruits of honeysuckle were homogenized and 5 g of the homogenate was extracted with 50 mL of 80% aqueous methanol (*v/v*) acidified with 1% HCl by ultrasonication for 20 min. The extract was centrifuged and diluted (re-distilled water with the ratio 1:1, *v/v*). For UPLC-MS analysis the supernatant was filtered through a Hydrophilic PTFE 0.22 μm membrane (Millex Simplicity Filter, Merck, Darmstadt, Germany) and used for analysis.

3.4. Identification of Iridoids by UPLC-qTOF-MS/MS

The method was described previously [27]. Identification of compounds was performed on the Acquity ultra-performance liquid chromatography (UPLC) system, coupled with a quadrupole-time of flight (q-TOF) MS instrument (UPLC/Synapt q-TOF MS, Waters Corp., Milford, MA, USA), with an electrospray ionization (ESI) source. Separation was achieved on the Acquity TM BEH C18 column (100 mm $\times$ 2.1 mm i.d., 1.7 μm ; Waters). The mobile phase was a mixture of 4.5% aq. formic acid *v/v* (A) and acetonitrile (B). The gradient program was as follows: initial conditions—1% B in A, 12 min—25% B in A, 12.5 min—100% B, 13.5 min—1% B in A. The flow rate was 0.45 mL/min and the injection volume was 5 μL . The column was operated at $30\text{ }^{\circ}\text{C}$. UV-vis absorption spectra were recorded on-line during UPLC analysis, and the spectral measurements were made in the wavelength range of 200–600 nm, in steps of 2 nm. The major operating parameters for the q-TOF MS were set as follows: capillary voltage 2.0 kV, cone voltage 40 V, cone gas flow of 11 L/h, collision energy 28–30 eV, source temperature $100\text{ }^{\circ}\text{C}$, desolvation temperature $250\text{ }^{\circ}\text{C}$, collision gas, argon; desolvation gas (nitrogen) flow rate, 600 L/h; data acquisition range, *m/z* 100–2000 Da; ionization mode, negative and positive. The data were collected with Mass-Lynx V 4.1 software. The runs were monitored at the wavelength of 254 nm.

4. Conclusions

In this study, 13 iridoids in the methanolic extract from honeysuckle berries were characterized using an UPLC-ESI-qTOF-MS/MS method in positive and negative mode, which can be complementary. The study shows that in honeysuckle berries there is a range of different iridoids, which are rarely present in edible fruits. The use of both ionization modes enabled the elucidation of the structure of analyzed iridoid glycosides, especially the position of pentose substitution and the aglycone stereochemistry. The presence of fragment ions deriving from the secondary pentosides, after the cleavage of glucose, confirmed additional glycosylation of aglycone at the C-7 position (at the $-\text{OH}$ group). For diagnostic purposes, more useful data were derived from the MS^+ spectra, which come from the fragment structures formed after the successive loss of monosaccharide residues and neutral molecules as water (18 Da), CO (28 Da), CO_2 (44 Da), acetylene (26 Da), methanol (32 Da), ethenone (42 Da) and cyclopropynone (52 Da). It was also observed that the iridoids with the methyl ester structure poorly ionized in the negative ion current, which makes the identification of their epimer pairs particularly difficult. Among the 13 iridoids, 11 were identified for the first time. The identified compounds included pentoside derivatives of loganic acid (two isomers), loganin (three isomers), and sweroside (one compound) and additionally epimeric pairs of loganic acid and loganin, sweroside, secologanin and secoxyloganin. The five pentoside derivatives of loganic acid and loganin have not previously been detected in the analyzed species.

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Author Contributions: Alicja Z. Kucharska designed the research and performed the experimental work. Alicja Z. Kucharska and Izabela Fecka performed composition and structure analysis, wrote the manuscript, discussed the results, commented on the manuscript, and reviewed and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Sample Availability: Samples of the analyzed frozen fruits are available from the authors.



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