Gas Phase Reactivity of Isomeric Arylglycosides Towards Amines. A Chemical Ionization Mass Spectrometry and Tandem Mass Spectrometry Study

Gianluca Giorgi, Laura Salvini, and Fabio Ponticelli

Dipartimento di Chimica and Centro Interdipartimentale di Analisi e Determinazioni Strutturali, Università di Siena, Siena, Italy

Chemical ionization mass spectrometry (MS) and tandem mass spectrometry (MS/MS) experiments have been performed for the structural characterization and isomeric differentiation of two series of *C*- and *O*-linked arylglycosides with potential antioxidant activity. Different amines have been used for producing gas phase chemical ionization. Depending on their proton affinity and steric hindrance, adduct ions with different stability are formed. The most stable adducts are produced by ethylamine and they have been extensively structurally characterized by experimental and theoretical approaches. Energy resolved chemical ionization tandem mass spectrometric experiments have allowed unambiguous characterization and differentiation of both the anomers differing at the configuration of the glycosidic C(1) atom, and regio- and structural isomers at extremely low concentrations, typical of mass spectrometry. This study has shown that amine chemical ionization mass spectrometry and MS/MS are powerful and versatile tools for the structural characterization of arylglycosides. (J Am Soc Mass Spectrom 2004, 15, 244–252) © 2004 American Society for Mass Spectrometry

Carbohydrates and glycosides play a key role in many biological processes [1–3]. Depending on their chemical structure, they can exist as large classes of isomers differing in the linkage between the different moieties, in their stereochemistry, in branching of the various units, in nature and position of substituents.

A growing interest in the discovery, characterization, and isomeric differentiation of new glycosides having enhanced, selective, and distinctive properties has been developed in the last decade. Owing to their complexity, the set-up of methods characterized by high selectivity, specificity, and sensitivity has a crucial role. Most of these are based on mass spectrometry (MS) and MS/MS approaches [4–9]. Gas-phase stable complexes obtained by chemical ionization (CI) [10] with different reagents have been used for the structural characterization and differentiation of oligosaccharides and glycosides [11–19].

The linkage between a sugar and a substituted phenol produces arylglycosides that can show different antioxidant properties and can be used as protective agents against oxidative stress processes in vivo [20]. Chemical modifications of their skeleton allow enhancement and modulation of their properties and antioxidant activities.

We recently synthesized *C*- and *O*-glycopyranosyl arenes (**1**–**3**, Scheme **1**) as potential antioxidant agents [21]. They are analogous to 2(3)-*tert*-butyl-4-hydroxyanisole with increased water solubility due to the presence of the sugar moiety. These compounds show different types of isomerism. Inside each series, the compounds are positional isomers, differing in the position of the *tert*-butyl group; members of Series **1** and **3**, and Compound **2** are structural isomers. Generally they are α-anomers, while for some of them, i.e., **2** and **3b**, both the α and β anomers are available. All the compounds have the same molecular weight of 362 u.

The study of these compounds in the gas phase is particularly difficult and complex. Under electron ionization only fragment ions are detected and no information on the whole molecule can be obtained. Recently, we have been able to differentiate these compounds by using the self-ionization technique occurring in an ion trap mass spectrometer [22]. On the other hand, this technique is not available on most of the commercial instrumentation.

Aimed at studying the gas phase ion chemistry and reactivity of arylglycosides **1**–**3**, CI experiments have been carried out by using different amines. As the reactivity is affected not only by the difference in the proton affinities between the reagent and the analyte but also steric effects can play an important role, three

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Address reprint requests to Dr. G. Giorgi, Centro Interdipartimentale di Analisi e Determinazioni Strutturali, Università di Siena, via Aldo Moro, 53100 Siena, Italy. E-mail: gianluca.giorgi@unisi.it

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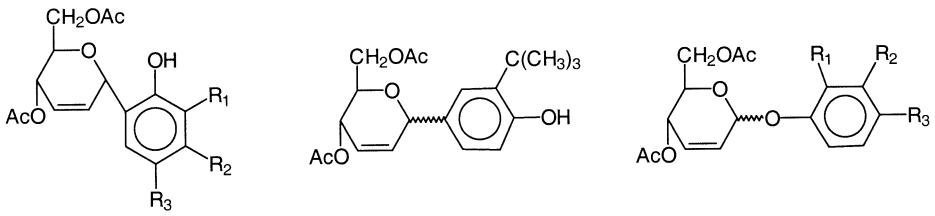


1a-c **2** **3a-c**

	R_1	R_2	R_3
a	*tert*-Bu	H	H
b	H	*tert*-Bu	H
c	H	H	*tert*-Bu

Scheme 1

differently hindered amines have been used. MS/MS and energy resolved mass spectrometric experiments have also been carried out.

In order to rationalize the experimental data, theoretical calculations have been carried out on ion-molecule adducts produced by *C*- and *O*-arylglycosides under chemical ionization conditions.

Experimental

Compounds **1–3** have been prepared according to the synthetic approaches reported in references [21] and [23]. All the reagents (high purity grade) used in the CI experiments were purchased from Fluka (Fluka Chemie GmbH, Buchs, Switzerland) and used without any further purification.

Chemical ionization mass spectrometric experiments were carried out on a Saturn 2000 ion trap coupled with a Varian 3800 gas chromatograph (Varian, Walnut Creek, CA). Aliquots of 0.5 μL of solutions 1.0×10^{-5} M in chloroform have been introduced into the gas chromatograph inlet. A DB-5 column (30 m, 0.25 mm i.d., 0.25 μm film thickness, Supelco, Bellefonte, PA, USA) was used. The oven temperature was programmed from 60 °C (1 min) to 150 °C at 30 °C/min. The temperature was then ramped to 250 °C at 20 °C/min. The transfer line was maintained at 265 °C and the injector at 220 °C. Under CI conditions, the pressure inside of the ion source was $\sim 3 \times 10^{-3}$ torr.

Low energy collision induced dissociations occurring inside the trap were carried out by using the non-resonant method. An excitation time of 20 msec and an amplitude of 55 V were used.

Energy resolved mass spectrometry was used to obtain breakdown curves under CID conditions. The adduct ions $[M + \text{amine} + H]^+$, produced under CI conditions, were selected as precursor ions and the resonant excitation mode was used. The starting tickle voltage has been set to 200 mV and increased in 20 mV increments until the final value of 360 mV was reached. The excitation time was 20 msec. In the case of the α and β anomers at C(1), a mixture 1:1 has been prepared and the two compounds have been analyzed in the same gas chromatographic run.

The reported values of relative intensities in MS/MS spectra are the mean of 5–10 scans. Replicate MS/MS experiments performed in the same day and in different days show variations in the relative intensities of ions within 10%.

Theoretical calculations were performed by using the semiempirical method PM3 [24] implemented in MOPAC v 6.0 [25]. Since all the investigated species are even-electron ions, the restricted Hartree-Fock procedure was chosen. When they were available, the starting geometries used in the calculations were taken from the X-ray structures [23].

Results and Discussion

Chemical Ionization Mass Spectra

Preliminary CI experiments between the arylglycosides **1–3** and low proton affinity (PA) reagents, i.e., methanol and acetonitrile, showed that the protonation reactions are highly exothermal [26]. This causes an extended fragmentation that prevents any stereochemical differentiation and regiochemical characterization of the compounds under investigation. For these reasons, gas-phase reactions using reagents with higher PA values, and in particular amines, have been carried out.

When ethylamine (PA 912.0 kJ/mol [27]) is used as a CI reagent gas, very stable $[M + NH_3Et]^+$ adduct ions (*m/z* 408, relative intensity = 100%), that carry most of the total ion current, are produced (Figure 1), while protonated molecules are absent for all the compounds. The CI mass spectra show fragment ions that evidence

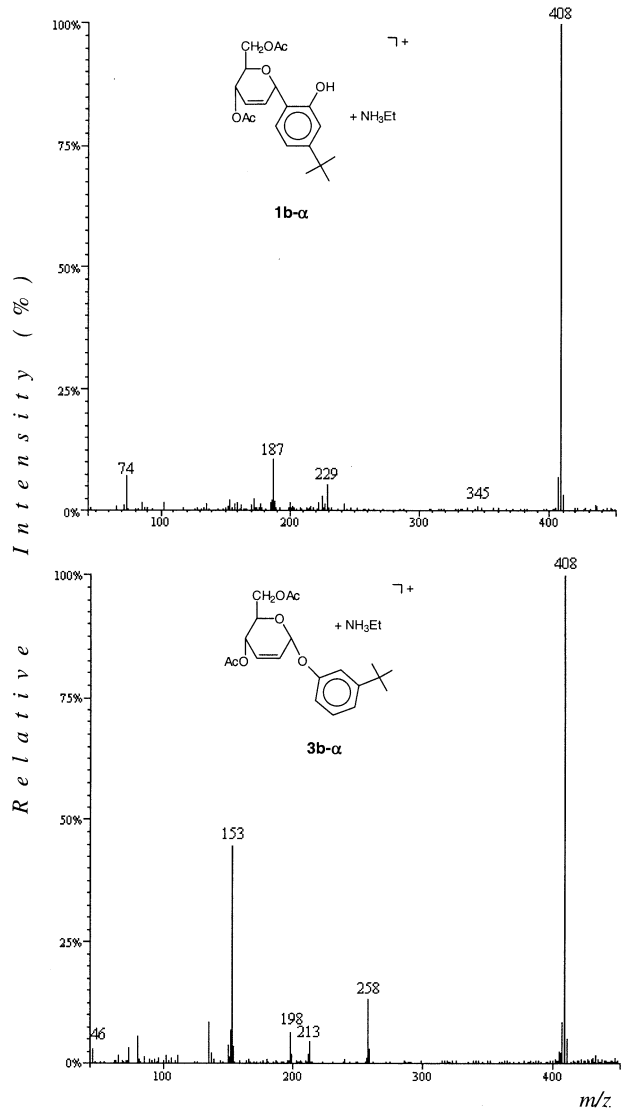


Figure 1. Ethylamine CI mass spectra of **1b**-α (top) and **3b**-α (bottom).

a different gas-phase reactivity for the *C*- and *O*-arylglycosides. Regarding *C*-linked derivatives (**1a–c**, **2**), all the fragment ions contain only the arylglycosidic moiety, thus suggesting a favorable dissociation of the amine from the adduct ions.

Differently, the fragment ions observed in the (NH_2Et)-CI mass spectra of *O*-arylglycosides **3a–c** can be divided into two groups. The first is formed by the ionic species at *m/z* 213 and 153 (Figure 1, bottom), attributable to elimination of the aryl moiety followed by loss of CH_3COOH. As they are also observed under EI conditions [23], it follows that they do not contain the amine moiety. A priori they might have originated by different pathways, and some of them should involve elimination of the amine from the adduct ions. On the other hand, as the MS/MS product ion spectra do not show elimination of the amine from the adduct ions $[M + NH_3Et]^+$ (see below), it results that these ionic species

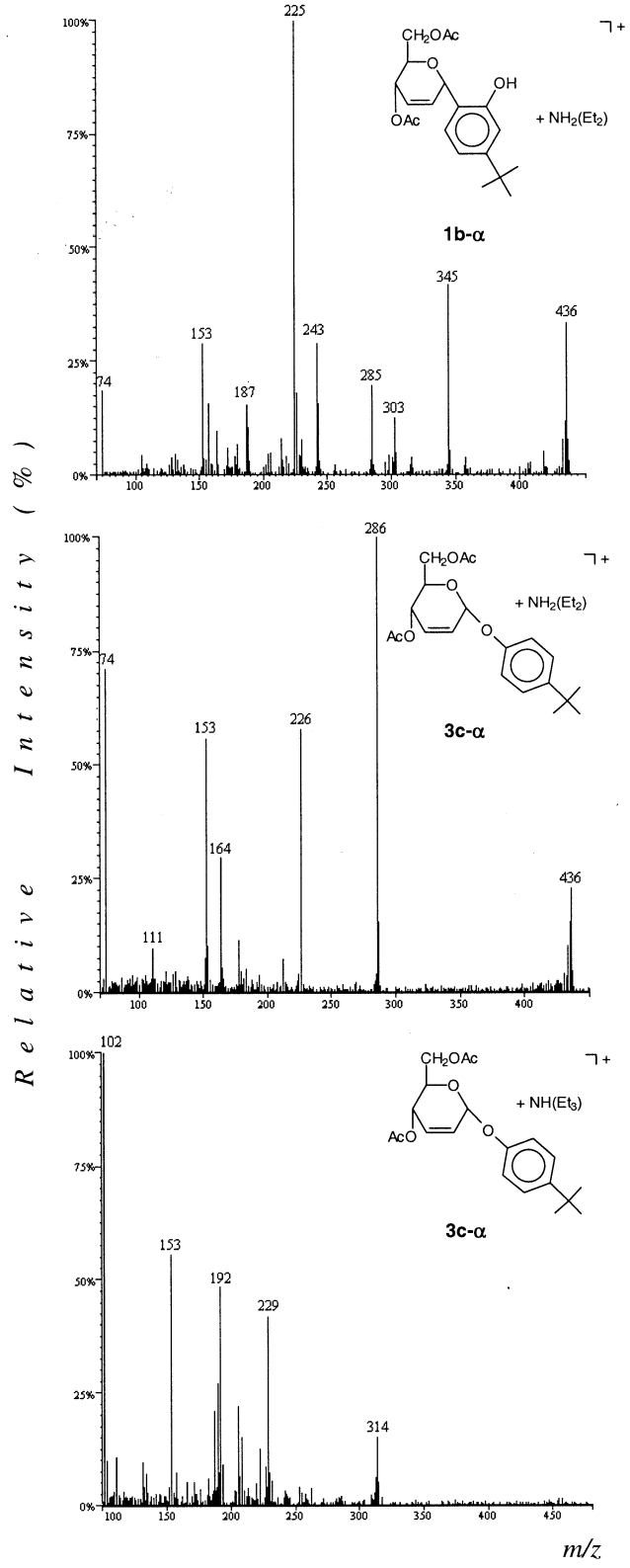


Figure 2. Comparison between amine CI mass spectra: diethylamine: **1b**-α (top), **3c**-α (middle); triethylamine: **3c**-α (bottom).

are yielded by fragmentation of the $[M + H]^+$ ions, whose lifetime is too short to be detectable in the CI mass spectra. Alternatively these ions might be produced by the radical cations $[M]^{+\bullet}$, as it occurs under EI conditions [23].

Table 1. Percentage of the total ion current[a] of significant ions displayed in the CID spectra of the adduct ions at *m/z* 408 produced by isomers **1–3** under ethylamine chemical ionization conditions

Ions (Th)	*C*-linked arylglycosides					*O*-linked arylglycosides			
	1a	**1b**	**1c**	**2-α**	**2-β**	**3a**	**3b-α**	**3b-β**	**3c**
408	60.94	28.87	16.97	4.40	73.14	9.47	14.11	14.62	11.03
345		<1	<1	8.85	1.71				
285	1.74	6.44	3.08	13.10	4.99				
258						32.11	33.20	27.68	35.17
243	<1	1.18		67.74	18.93				
240	35.59	55.08	74.88	3.17	<1	24.21	24.07	25.60	25.00
198	1.30	7.64	4.48	2.73	<1	7.37	6.43	8.30	7.76
225					<1				
180						12.11	9.75	9.38	8.45
187					<1				
153						14.74	12.45	14.43	12.59

[a]Calculated as: 100 × ion intensity/Σ intensity (all sample ions).

The second group of fragment ions produced by isomers **3a–c** is formed by ionic species at *m/z* 258 and 198 (Figure 1, bottom). The former ions contain the glycosidic moiety plus the protonated amine. A successive loss of CH_3COOH yields ions at *m/z* 198. The attribution of these ionic species is confirmed by the mass-to-charge shifts observed when diethyl- and triethylamine are used as reagent gases (see below).

It follows that the formation of fragment ions containing the amine is a distinctive feature of the *O*-linked glycosides **3a–c**. This allows a rapid and unambiguous assignment of an unknown compound to *C*- or *O*-arylglycosides.

As the stabilization of the complex between a protonated amine and the analyte is affected, not only by the difference in the proton affinities of the two species but also by steric effects, other amines differing both in the proton affinity and in the molecular size have been used as CI reagent gas (Figure 2).

Diethylamine (PA 952.4 kJ/mol, [27]) CI mass spectra of **1a–c** show the formation of adduct ions $[M + NH_2(Et)_2]^+$ at *m/z* 436 whose relative intensities are below 50%. The most abundant ions are at *m/z* 225 and correspond to consecutive eliminations of the neutral amine and two CH_3COOH molecules from the adduct ions. On the other hand, when diethylamine CI experiments are performed for *O*-arylglycosides **3a–c** the most abundant ions are at *m/z* 286 due to the elimination of the *tert*-butylphenol moiety (Figure 2, middle).

Inside each series, the regiochemistry also plays an important role in driving the reactivity of the analytes and the decomposition of the adduct ions. For example, the species $[M + NH_2(Et)_2]^+$ produced by **3a** show the lowest relative intensity if compared with the other isomers of the same series. In this case, the *tert*-butyl group in *ortho* position on the arene ring, combined with the large size of the diethyl amine, can play an important role in destabilizing the resulting adduct ions. On the other hand, when triethylamine (PA 981.8 kJ/mol [27]) is used as CI reagent, no stable adducts with the intact molecules are observed. Only fragment ions, together with unreacted ions $[NH(Et)_3]^+$, that constitute the most abundant peak are produced by all the compounds (Figure 2, bottom).

From these data it follows that ethylamine produces the most stable adduct ions with the compounds under investigation. In order to obtain more information on the nature of the interactions between the arylglycosides and the amine, CID product ion mass spectra of the adduct ions $[M + NH_3Et]^+$ have been performed for Compounds **1–3**.

Collision Induced Dissociations of the Adduct ions $[M + NH_3Et]^+$

The MS/MS data obtained by selecting the adducts $[M + NH_3Et]^+$ (*m/z* 408) produced by the arylglycosides **1–3** are reported in Table 1. A comparison between the MS/MS spectra produced by the adducts $[M + NH_3Et]^+$ of **1c** and **2** is reported in Figure 3.

C-linked derivatives **1a–c** show the most abundant product ions at *m/z* 225 due to loss of an acetic acid molecule from the ions at *m/z* 285. The latter ions can be produced by consecutive losses of (NH_2Et, H_2O, CH_3COOH) from the adduct ions at *m/z* 408.

On the contrary, adduct ions $[M + NH_3Et]^+$ produced by **2** (Figure 3, bottom) eliminate the amine and a water molecule producing species at *m/z* 345 that, in turn, can further eliminate CH_3COOH, yielding ions at *m/z* 285. The successive loss of formaldehyde produces ions at *m/z* 243 that have the highest abundance.

To explain this different behavior due to the regiochemistry at the arene ring, it is important to evaluate the possible sites of interactions between the protonated amine and the arylglycoside. They might be the oxygen atoms of the two acetyl groups at positions 4 and 5 of the glycosidic ring, the hydroxy group on the arene ring, and/or the endocyclic oxygen of the sugar. Each of these donor sites could competitively interact with the

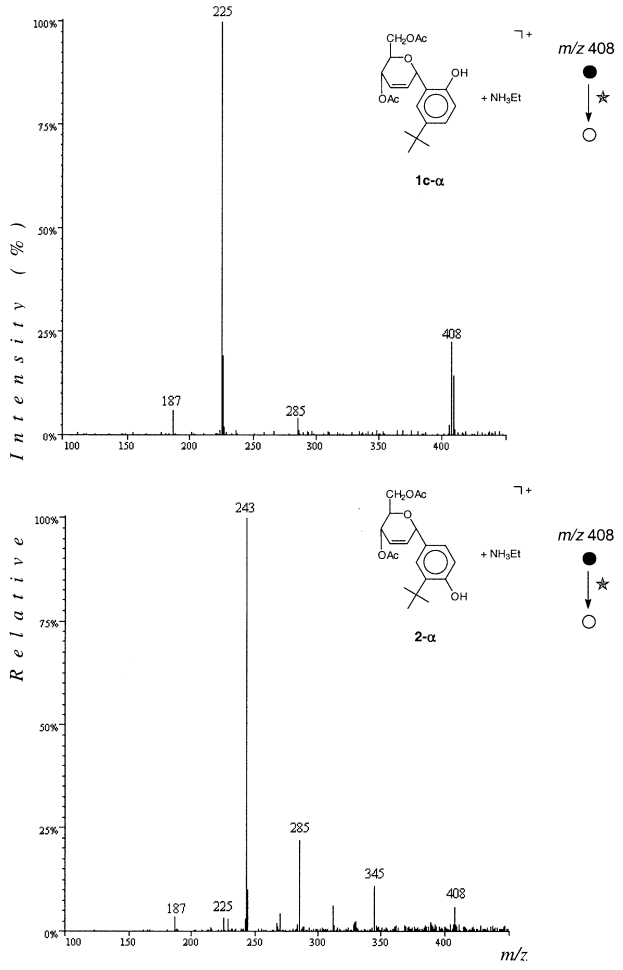


Figure 3. CID mass spectra obtained selecting the $[M + NH_3Et]^+$ ions (*m/z* 408) for *C*-linked arylglycosides **1c**-α (top) and **2**-α (bottom).

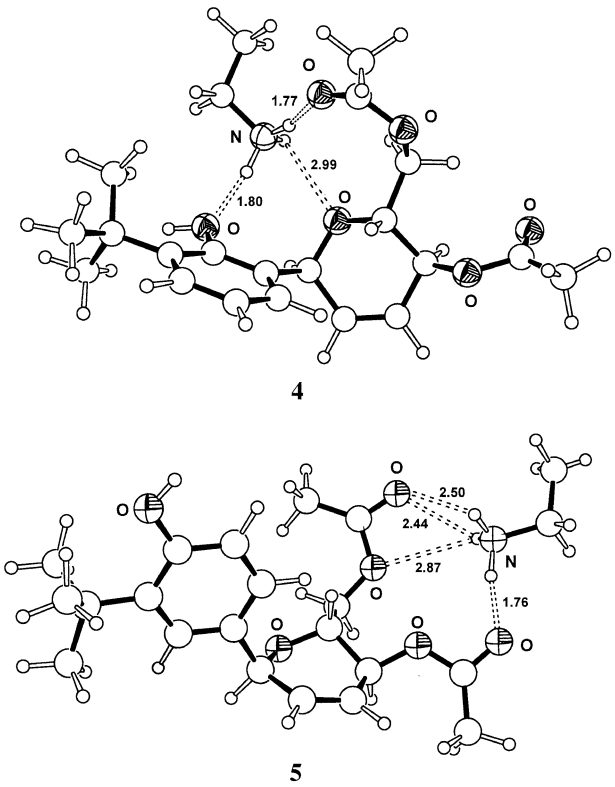


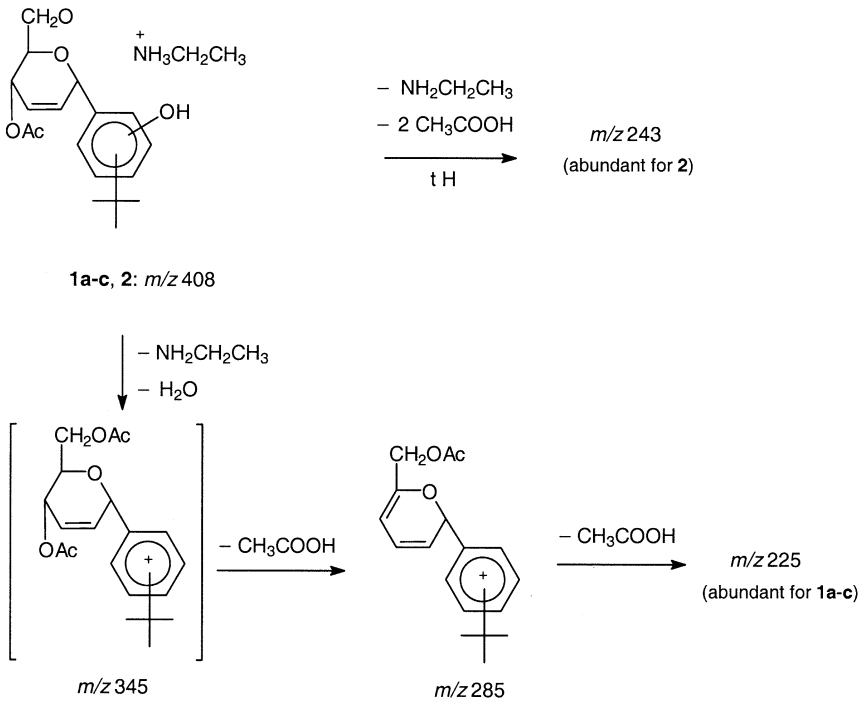
Figure 4. The most stable structures calculated for the adduct ions $[M + NH_3Et]^+$ produced by *C*-arylglycosides **1a**-α (top, **4**) and **2**-α (bottom, **5**). Distances in Å.

protonated amine, and the difference between the relative proton affinity of each site and the reagent gas can affect the stability of the resulting adduct ions. Owing to the stabilization due to hydrogen bonding and electrostatic interactions, conformations of the analyte that allow a large number of interactions are generally the most favored.

Theoretical calculations have shown that in the most stable structure obtained for **1a**-α (Structure **4**, Figure 4, top) the protonated amine interacts with the carbonyl oxygen of the acetyl moiety in position 5, with the hydroxy group at the arene ring, and with the endocyclic oxygens of the sugar moiety. When the protonated amine interacts only with the oxygen atoms of the two acetyl groups at positions 4 and 5, the resulting energy-minimized structure is 71 kJ/mol less stable than **4**.

In the case of Compound **2**, a structure analogous to **4** cannot be produced because the hydroxy group is far from the acetyl moieties. In this case different structures in which the amine solvates the hydroxy group, or alternatively the two acetyl moieties, can be proposed. The most stable structure, i.e., **5** (Figure 4, bottom), shows hydrogen bonding interactions between the protonated amine and the oxygen atoms of the carbonyl oxygen atoms at positions 4 and 5 of the sugar with (N)H. . .O distances equal to 1.76 and 2.44 Å, respectively. A further interaction occurs between the protonated amine and the acetyl oxygen at position 5 (Figure 4, bottom). This structure allows rationalization of the eliminations of NH_2Et and two molecules of acetic acid from the adduct ions as observed under CID conditions. If the protonated amine interacts only with the hydroxy group in *para* on the arene moiety, a less stable structure (ΔE = 71 kJ/mol) is produced. The decomposition pattern proposed for the adducts of Compounds **1a–c** and **2** is depicted in Scheme **2**. The fragmentation cascade begins with the loss of NH_2Et followed by consecutive neutral losses water and/or acetic acid molecules.

The study of collision induced dissociations of the adduct ions formed with ethylamine has also been extended to the *O*-arylglycosides **3a–c** (Table 1). Their adduct ions (*m/z* 408) show decomposition processes due to the neutral loss of the hydroxyarene moiety, yielding the species at *m/z* 258 (Figure 5). As shown by MS/MS measurements carried out by selecting as precursor ions the species at *m/z* 258, the latter can eliminate a molecule of water producing ions at *m/z* 240. The species at *m/z* 198 may be the result of the elimination of CH_3COOH from ions at *m/z* 258 or of CH_2CO from those at *m/z* 240.



Scheme 2

In contrast with the gas-phase behavior of *C*-linked derivatives **1a–c**, **2**, and as already observed for the CI spectra, also under CID conditions all the main fragment ions of the *O*-linked derivatives contain the ethylamine residue. This suggests that the interactions between the protonated amine and the *O*-arylglycosides **3a–c** are strong, and hydrogen bonds could play an important role in stabilizing the resulting adduct ions. Furthermore, the facile loss of the neutral hydroxyarene from the adduct ions suggests the occurrence of strong interactions between the protonated amine and the oxygen atom bridging the two rings. In fact, if it were the case, a proton transfer from the amine to the oxygen should make possible the elimination of the neutral hydroxyarene. And indeed, the most stable structure for the adduct $[\mathbf{3c} + NH_3Et]^+$ shows that the protonated amine interacts with both the oxygen bridging the two rings and with the acetyl moiety at position 5 (Structure **6**, Figure 6). The hydrogen bonds are quite strong with distances (N)H. . .O equal to 1.79 and 1.77 Å, respectively. The presence of both these interactions is of particular importance because it allows explanation of both the elimination of the neutral hydroxyarene moiety and the consecutive loss of CH_3COOH from the adduct ions $[\mathbf{3c} + NH_3Et]^+$, as it is observed experimentally. Another possible structure, in which the protonated amine interacts with the carbonyl oxygen atoms of the acetyl groups in positions 4 and 5 was 33 kJ/mol less stable than **6**. The same trend is observed for the other members of Series **3**, i.e., **3a–b**.

A concerted addition-elimination mechanism might occur in the gas phase, involving the addition of the amine at C(1) and the consequent elimination of the *tert*-butylphenol (Scheme **3**). As a water molecule is eliminated from the ions at *m/z* 258, it can be assumed that the opening of the pyranosidic ring, according to the tautomeric equilibrium as reported in Scheme **3**, should occur.

Energy Resolved Tandem Mass Spectrometry

In order to differentiate regio- and stereoisomers, energy resolved chemical ionization tandem mass spectrometry experiments with different tickle voltages were carried out. This approach consists of the investigation of the fragmentation kinetics of ions as a function of their internal energy. This is possible by measuring the CID spectra versus the variation of collision energy, yielding "CID curves" [28, 29].

The CID curves obtained by monitoring the variations of relative intensities of the adduct ions $[M + NH_3Et]^+$ (*m/z* 408) and of their fragment ions at *m/z* 243 for stereoisomers **2**-α and **2**-β are reported in Figure 7.

An interesting stereochemical effect is shown by the crossing point between the two curves. Compound **2**-α shows a crossing point of 317 mV while for its anomer **2**-β the crossing point is at a higher value, i.e., 352 mV.

In the case of Compounds **3a–c**, the CID curves have been obtained by monitoring the variations of the relative intensities of the adduct ions $[M + NH_3Et]^+$ (*m/z* 408) and of the species at *m/z* 258. For all the α isomers, the curves exhibit similar shapes (Figure 8). This suggests the occurrence of strictly related mechanisms in the elimination of the hydroxyarene from all

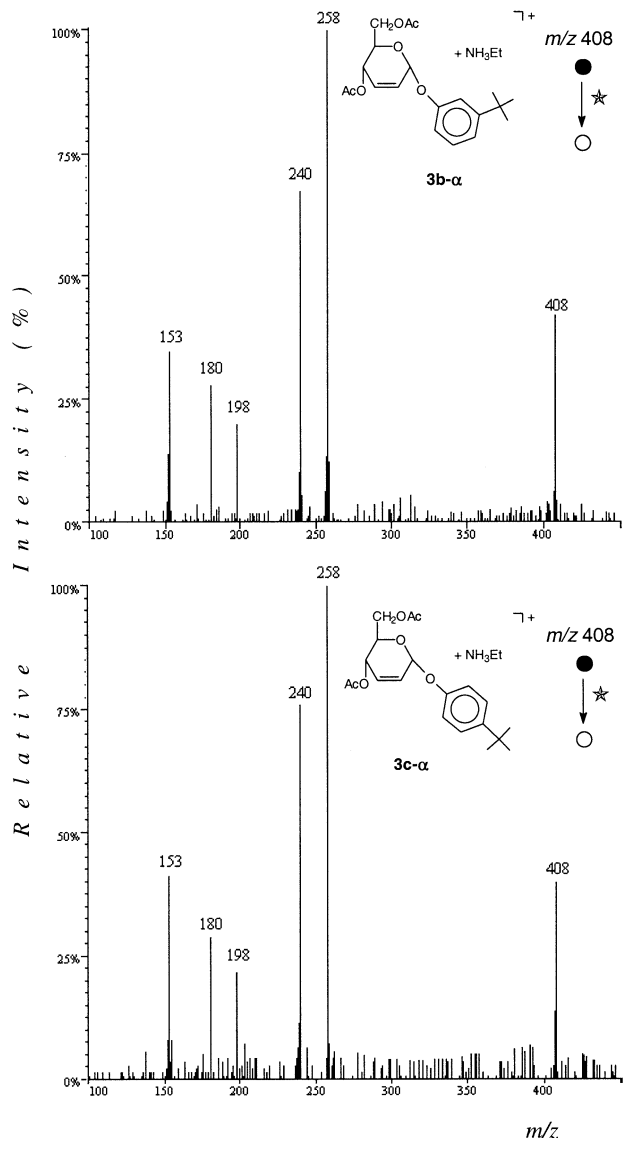


Figure 5. CID mass spectra obtained selecting the $[M + NH_3Et]^+$ ions (*m/z* 408) for *O*-linked arylglycosides **3b**-α (top) and **3c**-α (bottom).

the isomers. As observed for the anomers of **2**, the crossing point between the curves is strictly dependant upon the stereochemistry at C(1). For all the α anomers of **3a–c** the crossing point is at about 315 mV. This can be explained by considering that the only difference between these analytes is due to the different regiochemistry of the hydroxyarene that is lost during the monitored fragmentation.

When the same experiments are carried out on the β anomer of **3b** the crossing point of the two curves shift towards a higher value (340 mV) (Figure 8). It is noteworthy that the same trend is observed for the *C*-linked α and β anomers of **2**.

This suggests that the fragmentation kinetics of the adducts between ethylamine and the α anomers as a function of their internal energy is similar, independently from the linkage between the two rings. Furthermore, the stereochemistry plays a crucial role in fragmentation kinetics of compounds of Series **1–3**. The adduct ions formed by the ethylamine with the β anomer of the Compounds **2** and **3b** are more stable than that produced with the α, independently from the linkage between the two rings or the regiochemistry at the arene moiety.

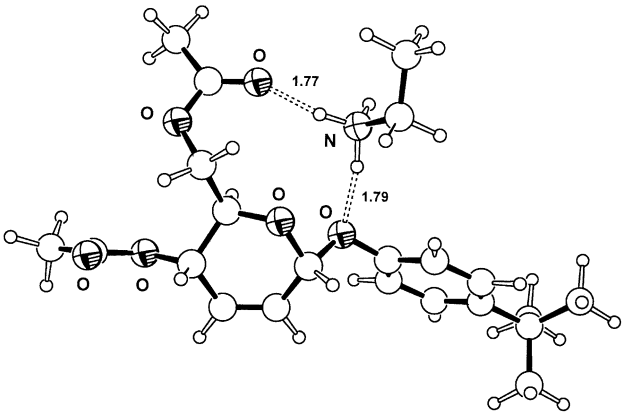


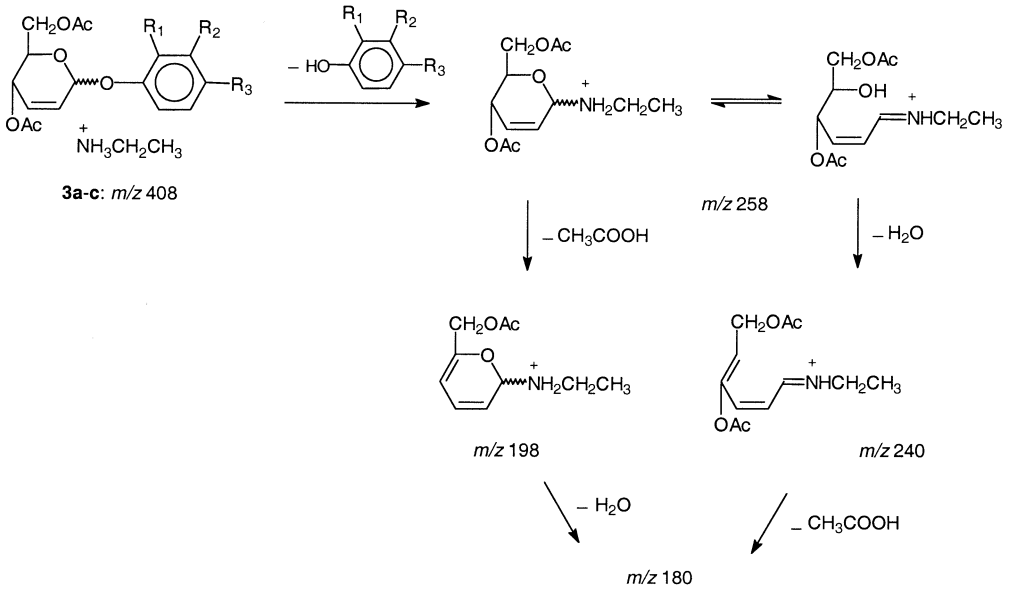
Figure 6. The most stable structure calculated for the adduct ions $[\mathbf{3c}\text{-}\alpha + NH_3Et]^+$ (**6**). Distances in Å.

Conclusions

Chemical ionization mass spectrometry and tandem mass spectrometry have been used for studying the gas-phase reactivities of a series of regio- and stereoisomers of arylglycosides with potential antioxidant activity.

Different amines, i.e., ethyl-, diethyl-, and triethylamine, have been used. Both the proton affinity of the amine and its steric hindrance have important roles in driving the gas-phase reactivity of the arylglycosides under investigation. In particular, when ethylamine is used, very stable $[M + NH_3Et]^+$ adduct ions are observed for all the compounds. Fragment ions detected in the CI spectra are dependent upon the linkage between the sugar and the aryl moiety and upon the position of the substituents. This allows to easily differentiate *C*- and *O*-arylglycosides.

Collision induced dissociation experiments carried out on the adduct ions $[M + NH_3Et]^+$ have further evidenced a different gas-phase behavior depending on the nature of the linkage between the two moieties and on the regiochemistry of the arene ring. Structural characterization of gas phase ions has also been accompanied by theoretical calculations carried out on adduct ions. The energy-minimized structures suggest that hydroxy group at the arene moiety, the endocyclic oxygen atom of the sugar and the acetyl group at position 5 are involved in hydrogen bonding interactions with the protonated ethylamine in the formation of the adduct ions of Compounds **1a–c**. Differently, in the case of **2**, the protonated amine and the neutral



Scheme 3

molecule interact through the oxygen atoms of the acetyl moieties. The calculations suggest that strong gas phase hydrogen bonding interactions occur between the protonated amine, the oxygen of the acetyl group at position 5, and the oxygen bridging the two rings in the adducts produced by the *O*-linked derivatives **3a–c**.

In order to differentiate the α and β anomers of Compounds **1–3**, energy resolved chemical ionization tandem mass spectrometric experiments have been carried out. The CID curves have allowed unambiguous characterization and differentiation of stereoisomers differing for the configuration of the glycosidic C(1)

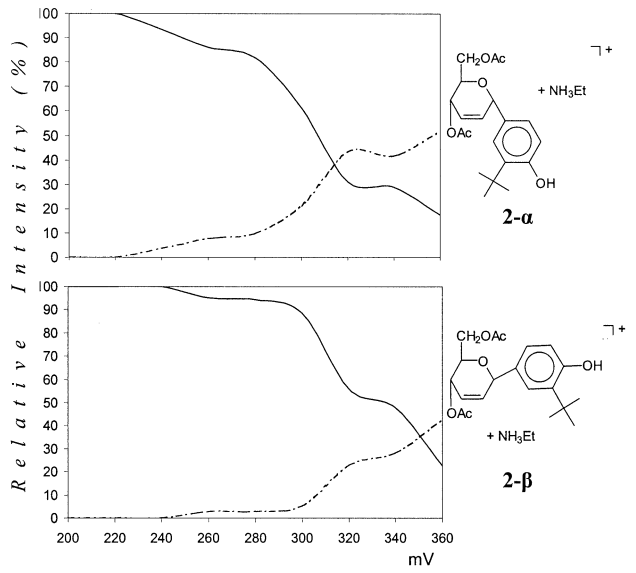


Figure 7. Energy resolved tandem mass spectrometry curves. The relative intensities of ions at *m/z* 408 (straight line) and that of product ions at *m/z* 243 (dot/dash line) against the tickle voltage are reported for isomers **2**-α (top) and **2**-β (bottom).

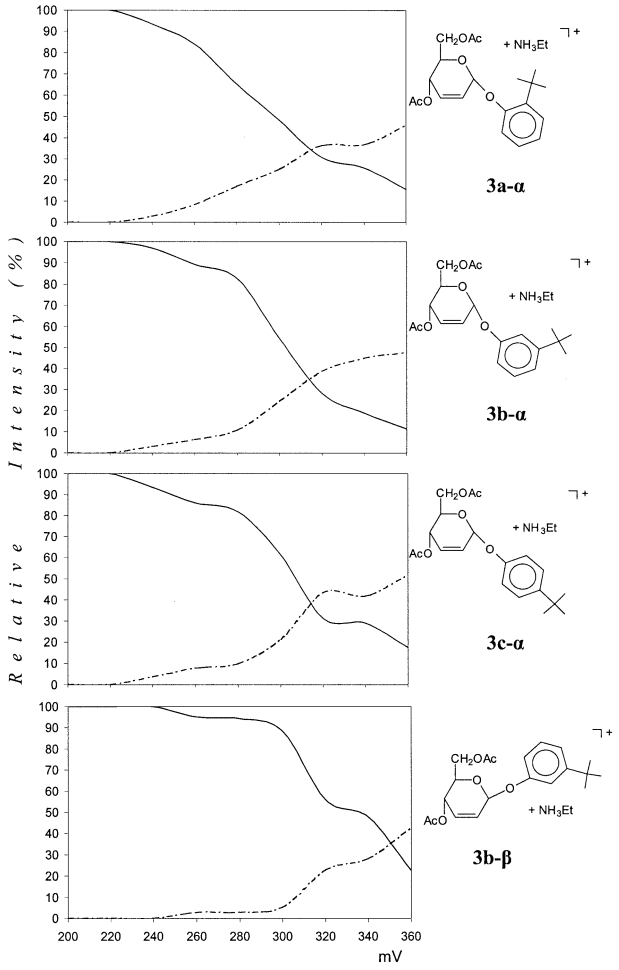


Figure 8. Energy resolved tandem mass spectrometry curves. The relative intensities of ions at *m/z* 408 (straight line) and that of product ions at *m/z* 258 (dot/dash line) against the tickle voltage are reported. From top to bottom: **3a**-α, **3b**-α, **3c**-α, and **3b**-β.

atom. Independently from the linkage between the sugar and the regiochemistry at the arene, the adducts formed by the β anomers are more stable than those produced by the α derivatives.

The approach proposed in this study is a suitable tool for selective and rapid regio- and stereochemical characterization and differentiation of arylglycosides.

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