Synthetic Approach, Regio- and Stereochemical Characterization and Differentiation of New Potential Antioxidant *C*- And *O*-Arylglycosides

Gianluca Giorgi,*[a] Fabio Ponticelli,[b] Laura Salvini,[a] Antoaneta Trendafilova,[b] Massimo Valoti,[c] and Federica Pessina[c]

Keywords: Antioxidants / Bioorganic chemistry / Glycosides / Stereochemistry / Structure elucidation

Two series of *C*- and *O*-arylglycosides with potential antioxidant properties have been synthesized, characterized and structurally differentiated. Reinvestigation of a synthetic approach has provided better insight into the products obtained and their chemical structures. Regio- and stereochemical characterization and differentiation of each compound have been carried out in solution, in the crystal, and in the gas phase. A comparison between the data obtained in solution and the crystal structures suggests closely related features in the two states. Mass spectrometry proved very effective for characterization of and differentiation between *C*- and *O*-isomers, as well as positional isomers. Unimolecular reactions occurring in the gas phase are specific to the chemical structures, and ion abundances can be related to their stabilities. This study has allowed the evaluation of the influences of the different linkages between the two moieties and the positions of the substituents on the chemical properties of the compounds. The *C*-isomers show antioxidant capability, as peroxyl radical scavengers, and inhibit lipid peroxidation.

Introduction

One of the most common classes of compounds occurring in nature is represented by carbohydrates and glycosides.[1−3] Depending on their chemical structures, they show different chemico-physical properties and can play key roles in a wide variety of biological processes.

In the last few years there has been growing interest in the discovery of new glycosides with enhanced, selective and distinctive properties. New synthetic approaches and effective methodologies for the identification and structural characterization of new compounds have been developed.[4−6]

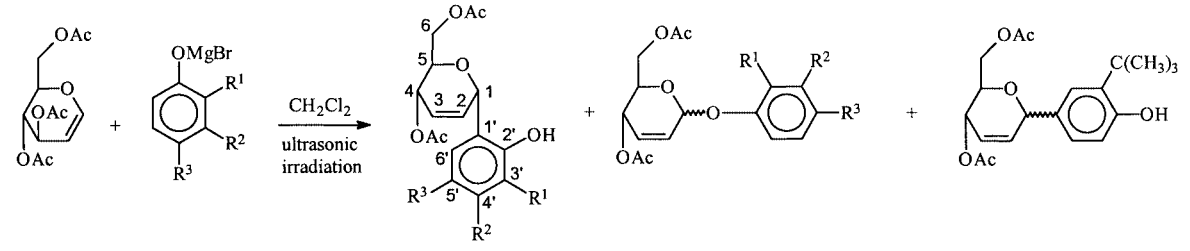
1,4-Disubstituted phenol derivatives, such as 2(3)-*tert*-butyl-4-hydroxyanisole (BHA), are food preservatives[7] and protective agents against oxidative stress processes in vivo.[8,9] Modification of their chemical structures by the introduction of appropriate groups on their skeletons allow their properties, such as their hydrophilic/lipophilic characters, to be enhanced and modulated, consequently affecting-their cell permeability. On the other hand, the electron-donor or -acceptor natures of the substituents modify the oxidation potential of the phenolic derivatives.

In a research project aimed at obtaining new antioxidant agents, we have recently synthesized new *O*-glycopyranosyl arenes.[10,11] As the oxygen bridge between the two moieties represents a reactive and preferential site towards hydrolysis and attack by different reagents, our efforts have more recently been directed towards the use of bromomagnesium salts of hindered phenols for the synthesis of new series of *C*- (**1a**−**d**) and *O*-glycopyranosyl arenes (**2a**−**d**, Scheme 1). These compounds are analogues of either 2(3)-*tert*-butyl-4-hydroxyanisole or 2,5-di-*tert*-butyl-1,4-benzohydroquinone (BHQ), with increased water solubility due to the presence of a sugar moiety, and show stability towards hydrolysis. Each series encompasses positional isomers differing in the substitution pattern on the arene ring. Corresponding compounds in each series (i.e., **1a** and **2a**) are isobaric structural isomers, differing in the linkage between the two rings. Furthermore, stereoisomers differing in the configuration at C-1 are also obtained.

The structural characterization and differentiation of these compounds is important for the evaluation of the role exerted by the position of the substituents at the phenyl ring and of the different linkage between the two moieties on chemico-physical properties of the compounds.

A synthetic approach towards these new compounds has been investigated and is presented here, together with their

[a] Centro Interdipartimentale di Analisi e Determinazioni Strutturali, Università degli Studi di Siena,
via Aldo Moro, 53100 Siena, Italy
Fax: (internat.) + 39-0577/234137
E-mail: gianluca.giorgi@unisi.it
[b] Dipartimento di Chimica, Università degli Studi di Siena
via Aldo Moro, 53100 Siena, Italy
[c] Istituto di Scienze Farmacologiche, Università degli Studi di Siena
via Aldo Moro, 53100 Siena, Italy



4 **5a–d** **1a–d** **2a–d** **3** (only from **5a**)

	R^1	R^2	R^3
a	*t*Bu	H	H
b	H	*t*Bu	H
c	H	H	*t*Bu
d	*t*Bu	H	OMe

Scheme 1

structural and regio- and stereochemical characterization and differentiation in solution, in the solid state and in the gas phase.

Results and Discussion

Synthetic Approach

The difficulties encountered in the preparation of glycosides of diphenols such as *tert*-butylhydroquinone arise from the presence of many sites of attack and, as usual for sugar molecules, from the anomerism at C-1. Consequently, the structures of the reaction products cannot be assigned a priori.

The reaction between bromomagnesium salts of phenols as glycosyl acceptors and acetyl-D-glucals as glycosyl donors has been reported to be a good way to obtain *C*-glycosides.[12] In our hands, however, the reaction showed rather complex behavior.

Under the above conditions we obtained the corresponding pseudoaxial *C*-glycosides **1a**−**d** from the 3,4,6-tri-*O*-acetyl-D-glucal (**4**) and hindered phenols **5a**−**d** as expected,[12] in moderate to good yields (17−77%, Scheme 1).

In addition, however, the *O*-substitution products **2a**−**d** originating from a Ferrier rearrangement[13] by carbon nucleophiles were always produced, although to a lesser extent. The reaction was also highly stereoselective in this case, the C-1 α-anomer generally being obtained. Only when the phenol **5b** was used as a reagent were both the α- and β-anomers of **2b** produced in equal amounts.

In the case of the bromomagnesium salt of 2-*tert*-butylphenol (**5a**), both the α- and β-anomers of the *C*-glycoside **3**, produced by *para*-alkylation (Scheme 1), were also isolated from the reaction mixture.

The *O*-glycosides **2a**−**d** did not give the corresponding *C*-glycosides after prolonged reaction times under the above conditions.

Structural Characterization in Solution

Useful information for the structural characterization of these compounds was obtained from their ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2).

The two series of *C*- and *O*-glycosides can be distinguished by the number of signals and the coupling pattern of the aromatic ring. The presence/absence of the OH signal in the ^{1}H NMR spectrum permits differentiation between *C*-compounds (**1a**−**d**, **3**) and *O*-derivatives (**2a**−**d**).

The C-1 anomeric configuration was assigned to the *O*-glycosides (**2a**−**d**) on the basis of the ^{1}H NMR spectroscopic data, coupling constant values (Table 1) and NOE effects. The α-anomers show the larger values for $J_{4,5}$.[11,14] In the current case, they are in the range of 9.3 (**α-2b**) to 9.5 (**α-2a**) Hz. On the other hand, this value is highly reduced in β-isomers ($J_{4,5}$ = 2.0 Hz for **β-2b**). In addition, the strong connectivity between 1-H and 5-H that exists in β-anomers produces a positive NOE effect, not observed for their corresponding α-anomers.

With regard to the *C*-glycoside series (**1a**−**d**, **3**), the β-configuration at C-1 has been assigned to the more dextrorotatory compound.[12] In addition, the absence/presence of NOE effects between 1-H and 5-H suggests the α- or β-configuration. This is in agreement with the crystal structures of **α-1b** and **α-1d**, in which the mean 1-H···5-H distance is 3.44 Å (see below). This quite long distance does not produce a positive NMR NOE effect between the protons at positions 1 and 5. On the other hand, in analogous β-anomers, in which this NOE effect is present, the 1-H···5-H distance is close to 2.5 Å, as found in the X-ray crystal structure of 1′-(4,6-di-*O*-acetyl-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranosyl)-4′-methoxybenzene.[15]

X-ray Crystal Structures of α-1b, α-1d and α-2c

Single crystals of three representative members of the compounds under investigation − **α-1b**, **α-1d** and **α-2c** − were subjected to X-ray crystallography (Figures 1 and 2). The structure of **α-2c** is particularly interesting, because no

Table 1. ^{1}H NMR spectroscopic data of compounds **1a**−**b**, **2a**, **2b**, **2d** and **3**; δ [ppm] (*J* [Hz])

Proton	**α-1a**	**α-1b**	**α-2a**	**α-2b**	**β-2b**	**α-2d**	**α-3**	**β-3**
1	5.53 br. s	5.47 br. s	5.74 br. s	5.69 br. s	5.80 br. s	5.60 br. s	5.27 br. s	5.14 br
2	6.05 ddd (2.0, 2.4, 10.4)	6.26 ddd (1.5, 2.0, 10.6)	6.05 br. s	6.02 br. s	6.12−6.13 br.	6.01 s	5.97 dt (1.8, 2.5, 10.0)	5.83 dt (1.8, 12.0)
3	6.32 ddd (1.4, 3.4, 10.4)	6.02 dt (2.5, 10.6)	6.05 s	6.02 br. s	6.12−6.13 br.	6.01 s	6.14 dt (1.2, 2.9, 10.0)	5.93 dt (1.4, 1.4, 12.0)
4	5.28 br. d (7.6)	5.25 m	5.43 dd (9.5, 1.0)	5.40 br. d (9.3)	5.17 br. t (2.0)	5.39 brd (9.4)	5.30 m	5.42 brd (8.7)
5	3.90 ddd (4.3, 6.5, 7.6)	3.96 m	4.12−4.40 m	4.10−4.33 m	4.18−4.35 m	4.11−4.38 m	3.88 ddd (3.3, 5.8, 6.7)	3.93 ddd (2.8, 5.5, 8.8)
6	4.15 dd (4.3, 12.0)	4.22 m	4.12−4.40 m	4.10−4.33 m	4.18−4.35 m	4.11−4.38 m	4.29 dd (5.8, 12.0)	4.20 dd (5.5, 12.0)
6′	4.20 dd (6.5, 12.0)	4.22 m	4.12−4.40 m	4.10−4.33 m	4.18−4.35 m	4.11−4.38 m	4.10 dd (3.3, 12.0)	4.31 dd (2.8, 12.0)
Ac	2.07 s	2.08 s	2.00 s	1.98 s	1.85 s	2.02 s	2.07 s	2.08 s
Ac′	2.09 s	2.08 s	2.13 s	2.10 s	2.12 s	2.11 s	2.10 s	2.12 s
*t*Bu	1.44 s	1.29 s	1.39 s	1.31 s	1.31 s	1.36 s	1.42 s	1.40 s
Ar	6.82 t (7.7), 6.96 dd (1.5, 7.7) 7.30 dd (1.5, 7.7)	6.89 dd (8.0, 2.1), 6.98 d (8.0), 6.95 d (2.0)	6.97 td (1.5, 7.4) 7.18 td (1.5, 7.4) 7.30 m (2 H)	6.95 dd (7.5, 2.1), 7.06 d (7.5), 7.08 d (2.1), 7.24 t (7.5)	6.93 dd (7.8, 2.3), 7.05 d (7.8), 7.09 d (2.3), 7.22 t (7.8)	6.67 dd (9.0, 3.1), 6.87 d (3.0), 7.23 d (9.0)	6.65 d (8.0), 7.08 dd (2.0, 8.0), 7.32 d (2.0)	6.64 d (8), 7.03 dd (2.1, 8.0), 7.21 d (2.1)
Other signals		OH: 7.15 s				OMe: 3.77 s	OH: 5.42 s	OH: 4.98 s

Table 2. ^{13}C data of derivatives **1a**, **1b**, **1d**, **2d** and **3**; δ [ppm]

Carbon atom	**α-1a**	**α-1b**	**α-1d**	**α-2d**	**α-3**	**β-3**
1	72.69	72.29	72.59	93.42	73.80	77.68
2	130.65	130.97	130.39	129.82	132.09	133.11
3	125.39	124.71	125.74	127.24	124.38	124.70
4	65.16	64.88	65.10	65.11	65.16	65.65
5	70.16	70.68	70.19	67.88	68.95	74.84
6	62.76	62.10	62.82	62.72	63.02	63.83
1′	124.07	120.63	124.73	140.19	130.01	131.10
2′	155.18	155.52	152.22	137.00	127.31	126.15
3′	137.72	117.03	139.06	110.10	136.34	136.17
4′	119.25	154.01	111.64	154.27	154.67	154.57
5′	127.65	114.39	149.05	113.99	116.13	116.65
6′	126.75	128.17	113.91	116.06	126.58	126.66
C(*C*H$_3$)$_3$	29.61	31.17	29.57	29.89	29.44	29.44
C(CH$_3$)$_3$	34.84	34.58	35.07	34.90	34.61	34.55
CO*C*H$_3$	20.69	20.69	20.93	20.71	20.78	20.86
	20.92	20.93	20.69	20.94	21.04	21.06
*C*OCH$_3$	170.24	170.24	170.74 (2C)	170.27	170.69	170.53
	170.74	170.72			171.11	171.13
O*C*H$_3$			55.73	55.53		

X-ray crystal structures of *O*-aryl glycosides are present in the Cambridge Crystallographic Database.

Each compound crystallizes in a chiral space group, $P2_12_12_1$, suggesting the presence of a pure diastereoisomer. As the starting D-glucal has the (*S*,*R*) configuration at the atoms C(4) and C(5), respectively, and their stereochemistry is not affected by the synthetic procedure, it was possible to determine the configuration of C(1) unambiguously. This proved to be (*R*), corresponding to α, for all three compounds.

While the asymmetric units of **α-1b** and **α-2c** each contain only one molecule, that of **α-1d** is made up of two molecules

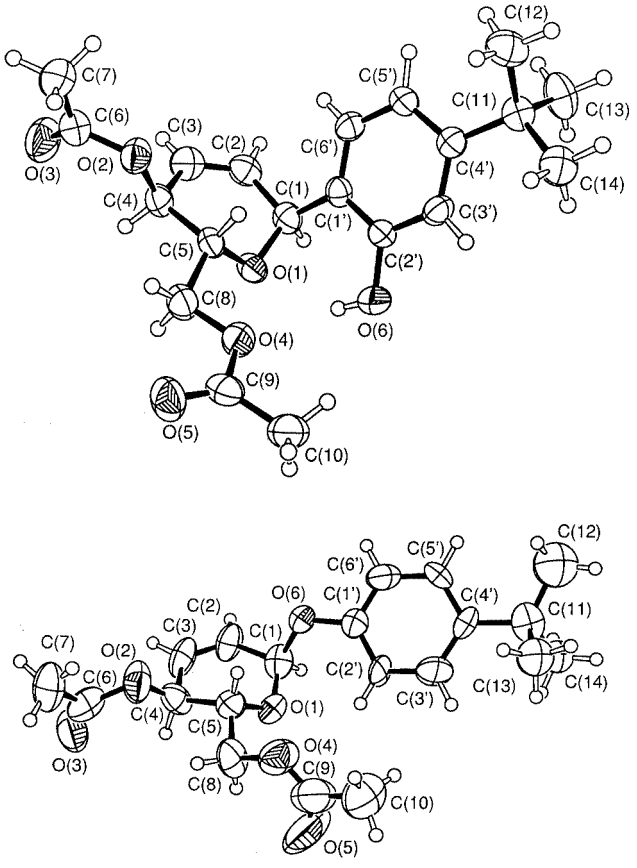


Figure 1. Crystal structures of compounds **α-1b** (top) and **α-2c** (bottom); ellipsoids enclose 50% probability; in compound **α-2c** only C(12), C(13), C(14), with a s.o.f. equal to 0.51(2), are reported

(Figures 1 and 2). Bond lengths and angles (Table 3) prove to be in agreement with those reported for analogous compounds. As an example, the O(1)−C(5) distance is 1.453(4)

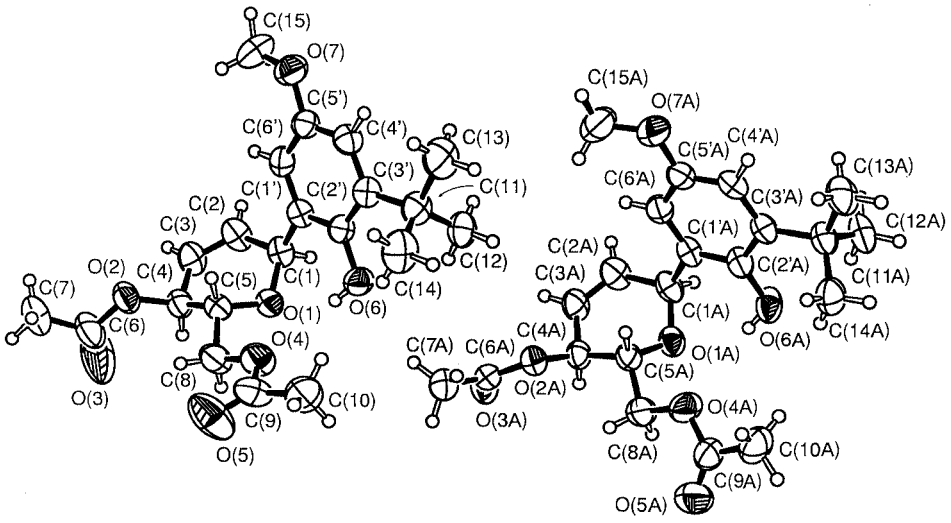


Figure 2. Crystal structure of compound **α-1d**; ellipsoids enclose 50% probability; for O(3) the atomic position with s.o.f. equal to 0.55(3) is reported

Table 3. Selected bond lengths [Å], angles [°] and puckering parameters for **α-1b**, **α-1d** and **α-2c**

	α-1b	**α-1d** (molecule 1)	**α-1d** (molecule 2)	**α-2c**
O(1)−C(1)	1.453(4)	1.451(3)	1.444(3)	1.423(11)
C(5)−O(1)	1.437(4)	1.425(3)	1.425(3)	1.430(11)
O(6)−C(1′)				1.401(9)
C(4)−O(2)	1.450(5)	1.450(3)	1.449(3)	1.435(12)
O(2)−C(6)	1.359(5)	1.298(4)	1.360(4)	1.335(14)
C(6)−O(3)	1.196(5)	1.254(10)[a]	1.192(4)	1.212(13)
C(5)−O(1)−C(1)	111.3(3)	113.8(2)	112.4(2)	112.8(9)
C(4)−O(2)−C(6)	117.3(3)	120.2(3)	116.2(2)	115.4(10)
O(1)−C(1)−C(1′)	110.5(3)	111.7(2)	110.5(2)	
O(1)−C(1)−O(6)				112.1(9)

Enopyranoid puckering parameters	**α-1b**	**α-1d** (molecule 1)	**α-1d** (molecule 2)	α-**2c**	Ref.[15] [b]	Ref.[17] [c]
q_2 [Å]	0.41	0.41	0.41	0.36	0.40	0.42
q_3 [Å]	0.30	0.31	0.32	0.33	0.32	0.32
Θ [°]	54.0	52.8	52.0	47.6	51.2	52.9
Φ [°]	343.85	322.4	327.9	325.4	321.7	318.4
Q [Å]	0.51	0.51	0.52	0.49	0.52	0.52

[a] Site occupation factor for O(3) is 0.55(3). [b] 1′-(4,6-Di-*O*-acetyl-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranosyl)-4′-methoxybenzene. [c] (2,3-Dideoxy-α-D-*erythro*-hex-2-enopyranosyl)benzene.

Å in **α-1b** and 1.430(11) Å in **α-2c**, while it is 1.451(3) and 1.444(3) Å in the two molecules of **α-1d**.

C(1), C(2), C(3) and C(4) in the three compounds are almost coplanar, defining the plane π, while O(1) and C(5) deviate dissymmetrically on both sites of this plane. The deviations in the two molecules of **α-1d** and in **α-2c** are quite similar, with differences of less than 0.2 Å. This suggests that the enopyranosyl ring has a distorted half-chair $^0H_5$ conformation in both these compounds (Table 3). In the case of **α-1b**, on the other hand, C(5) and O(1) deviate from π by 0.209 and −0.542 Å, respectively, showing a deformation towards the E_5 conformation. It is reasonable that crystal field constraints should play a key role in establishing and stabilizing a given conformation of the ring, while both the nature of its substituents and the configuration of the asymmetrical carbon atoms seem to have minor roles.

In **α-1b** and **α-1d**, the α-configuration of C(1) and the orientation of the C(1)−C(7) bond cause the arene ring to be below the enopyranosyl ring in an almost parallel position with respect to the C(1)−C(2) bond. The dihedral angles between the phenyl ring and the plane π are equal to 70.8° in **α-1b** and to 54.8 and 66.5° in the two molecules of **α-1d**. This suggests that the conformation of the arene moiety approaches the energetic minimum of the "axial perpendicular" conformation found in the phenylcyclohexane.[16] In analogous compounds, such as 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*threo*-hexenopyranosylbenzene, their mutual orientation is much closer to perpendicularity [81.7(7)°].[17]

The deviation from perpendicularity observed in these compounds can be related to different factors: the distortion of the enopyranosyl ring, steric hindrance due to the presence of the hydroxy and the *tert*-butyl groups on the phenyl ring, electronic repulsion, and crystal field constraints. In addition, a driving force is represented by the occurrence of a strong intramolecular hydrogen bond between the hydroxy group and O(1).

For derivative **α-2c**, in which the linkage between the two rings is through an oxygen atom, the dihedral angle between the phenyl ring and the plane π is equal to 44.9°. The C(1)···C(1′) distance is equal to 2.43 Å, while the bond angle C(1)−O(6)−C(1′) is equal to 119.1(8)°. The crystal packing is stabilized by van der Waals interactions. In the structures of **α-1b** and **α-1d**, the presence of the hydroxy group in an *ortho* position in the phenyl ring causes extensive intra- and intermolecular hydrogen bonding interactions. A strong intramolecular hydrogen bond exists between O(6)−H···O(1), with H···O distances equal to 2.065(3) Å in **α-1b**, and 1.949(2) and 2.049(2) Å in the two molecules of **α-1d**.

Structural Characterization and Regiochemical Differentiation in the Gas Phase

Different mass spectrometry experiments were carried out for the characterization and regiochemical differentiation of isomeric compounds **1a**−**d**, **2a**−**d** and **3** in the gas phase.

Under electron ionization conditions, both *C*- and *O*-linked derivatives produce molecular ions with very low relative abundances. In *C*-linked derivatives (Figure 3) the mass spectra are generally dominated by ions at $m/z = 229$, that constitute the base peak for compounds **1b**, **1c** and **3**. These species are produced by successive loss of CH_3COOH and $^{\cdot}CH_2OC(O)CH_3$ from the molecular ions. Their high stability is reasonably attributable to an arylpyrylium cation as depicted in Scheme 2. For isomer **1a** the base peak of the mass spectrum is constituted by ions at

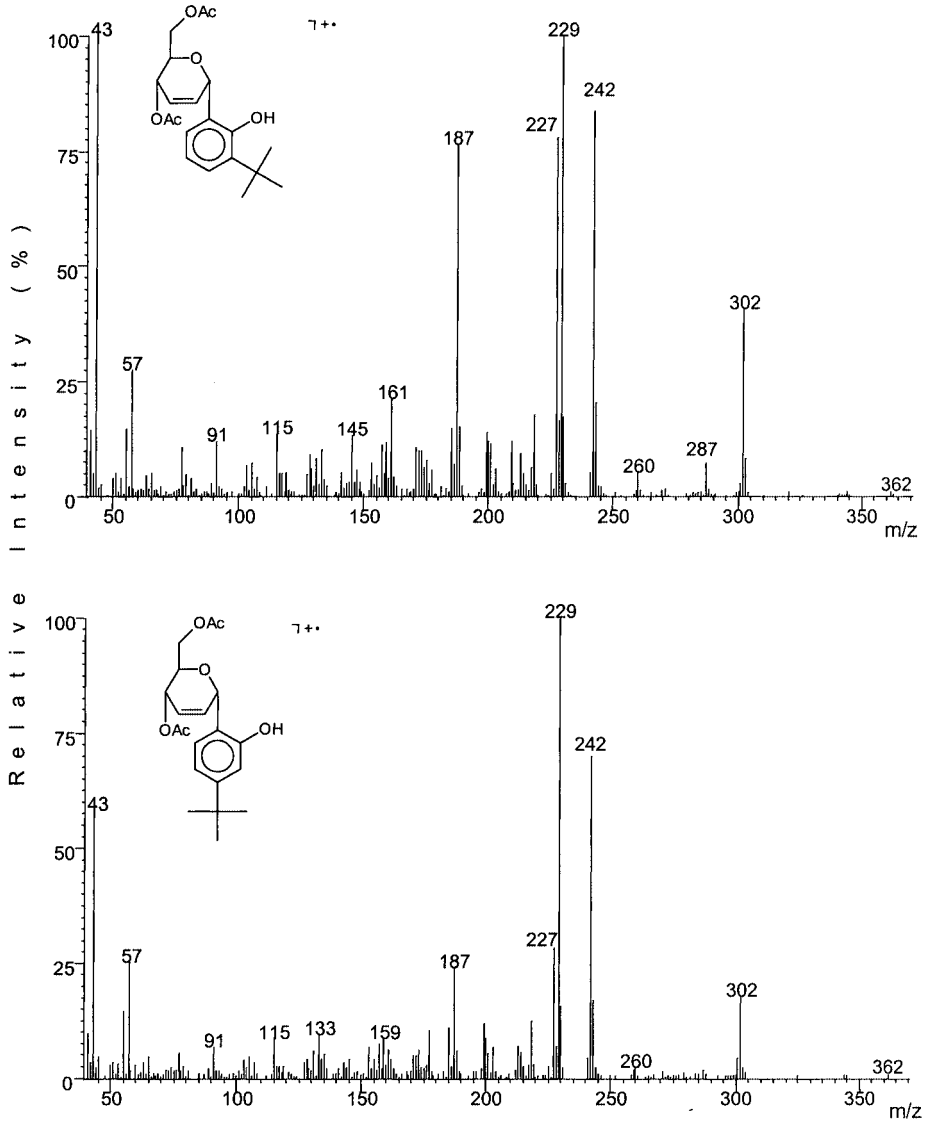
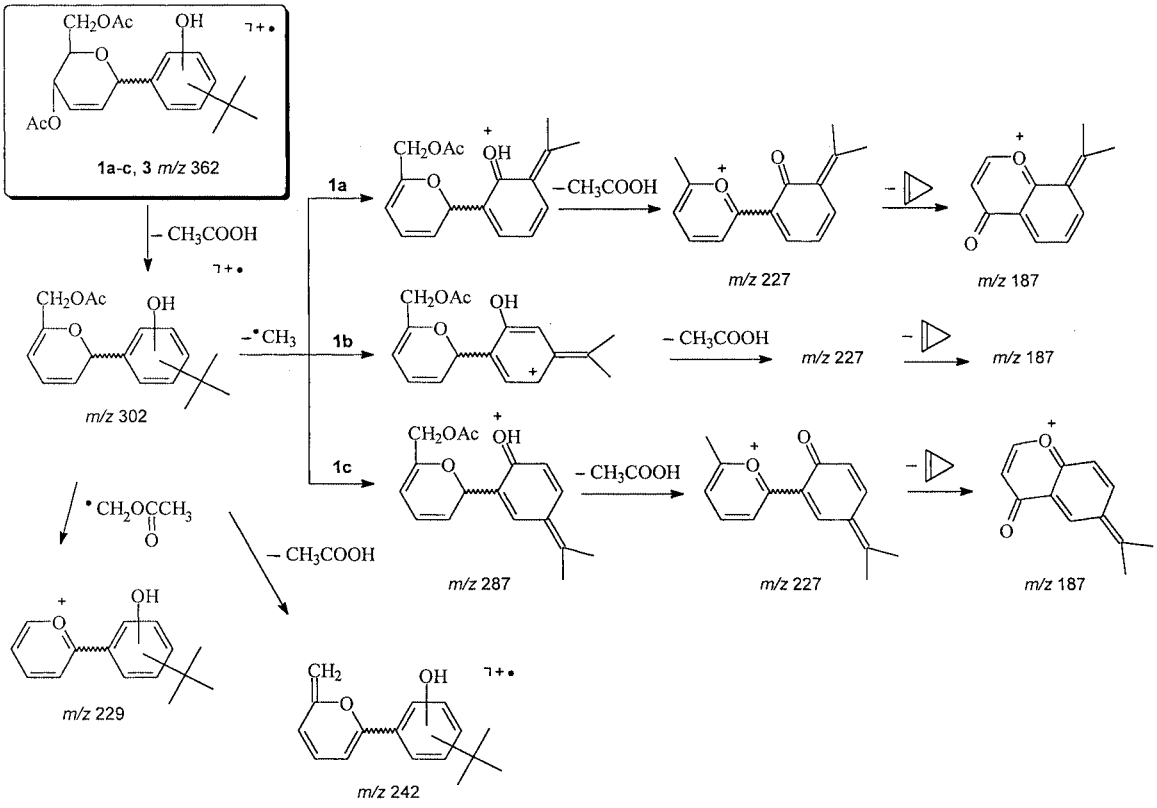


Figure 3. Electron ionization mass spectra of the *C*-linked arylglycoside isomers **α-1a** (top) and **α-1b** (bottom)



Scheme 2

m/*z* = 43, corresponding to CH_3CO^+ (Figure 3, top). Losses of one or two CH_3COOH molecules from the molecular ion of **1a**–**c** and **3** yield ions at *m*/*z* = 302 and 242, respectively (Scheme 2).

The position of the hydroxy group produces significant regiochemical effects, and the mutual position of the *tert*-butyl influences their relative intensities. In fact when the hydroxy group is *ortho* with respect to the linkage with the sugar moiety, as in **1a**–**c**, distinctive ions are produced at *m*/*z* = 287, 227 and 187 (Figure 3). Their relative intensities are high for the isomers with *tert*-butyl in *ortho* (**1a**, Figure 3, top) and *para* isomer (**1c**) with respect to the hydroxy group, while for the *meta* isomer **1b** (Figure 3, bottom) their intensities are much lower. This can be explained by the consideration that resonance structures can greatly stabilize the ions produced by the *ortho* and *para* isomers with respect to those formed by the *meta* derivative. The species at *m*/*z* = 287 is attributable to the loss of $^{\cdot}CH_3$ from the *tert*-butyl substituent from ions at *m*/*z* = 302, producing a stable carbocation (Scheme 2). The species at *m*/*z* = 227 can reasonably be due to the neutral loss of CH_3COOH from the ion at *m*/*z* = 287, while ions at *m*/*z* = 187 are due to the loss of 40 u, reasonably due to cyclopropene, from ions at *m*/*z* = 227 (Scheme 2). This interpretation is supported by collision-induced dissociation experiments carried out on different ions.

The species at *m*/*z* = 287, 227 and 187 are not formed by the isomer **3** in which the hydroxy group is in the *para* position. Its mass spectrum is dominated by two abundant ions at *m*/*z* = 242 (70%) and 229 (100%) (Figure 4, bottom).

The mass spectra of *O*-linked glycosides **2a**–**d** are totally different from those of their *C*-linked analogues. This allows rapid and unequivocal assignment of a *C*- or *O*-linkage between the two rings at the extremely low concentrations typical of mass spectrometry. The main unimolecular fragmentation reaction occurring in the gas phase for *O*-linked glycosides **2a**–**d** consists of the cleavage of the bridge between the two moieties, with charge retention mostly on the sugar moiety (Figure 4, top). This causes the formation of the species at *m*/*z* = 213, which can lose CH_3COOH, thus yielding the species at *m*/*z* = 153. These last ions can in turn eliminate CH_2CO, producing ions at *m*/*z* = 111 (Scheme 3).

Mass spectra and product ion spectra obtained by MS/MS experiments do not allow stereochemical differentiation of the α-and β-anomers of compounds **1a**–**d** and **2a**–**d**. On the other hand, preliminary experiments involving self ionization and chemical reactions occurring in the gas phase can differentiate between these stereoisomers. Further studies are in progress.[18]

Inhibition of Lipid Peroxide Formation

The antioxidant activity of the phenol derivatives **1a**–**d** is given in Table 4. All were able to inhibit peroxide formation in both linoleate and ascorbate systems, with the degree of inhibition being generally greater in the linoleate

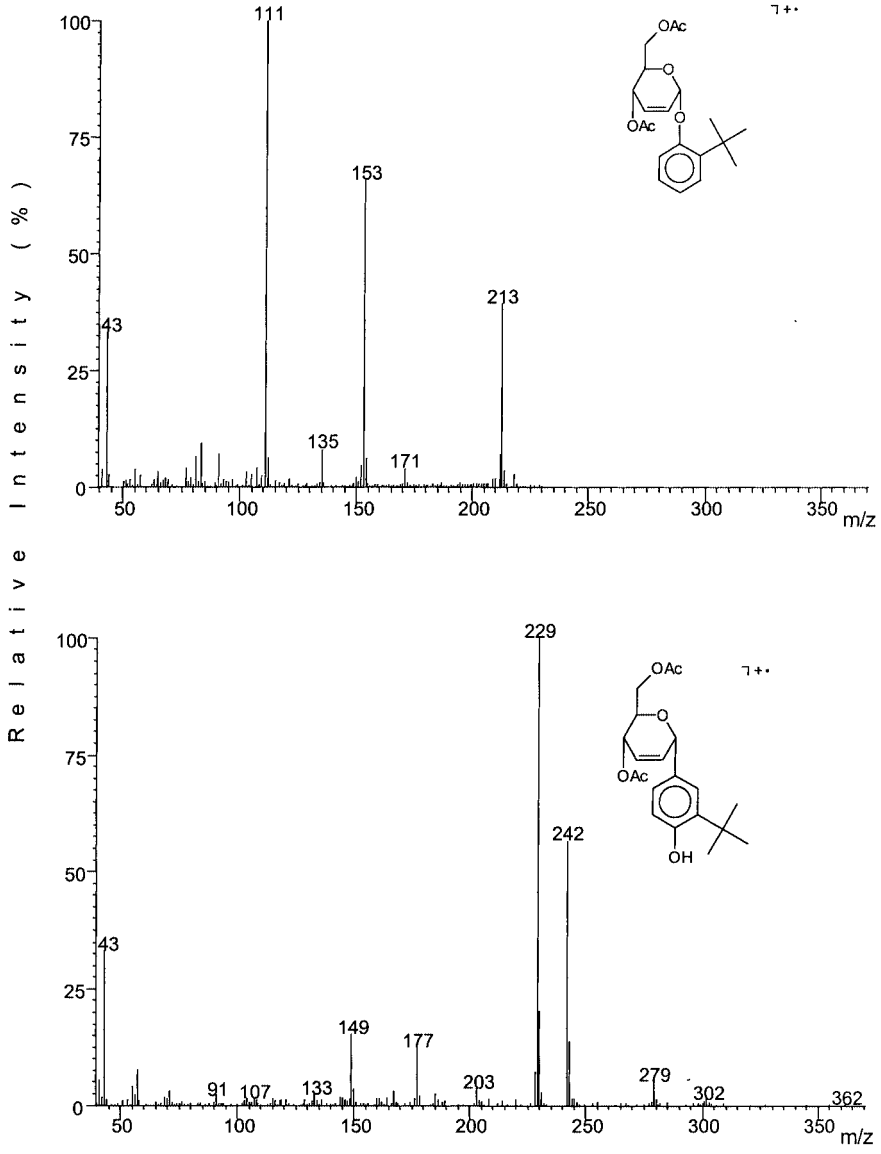
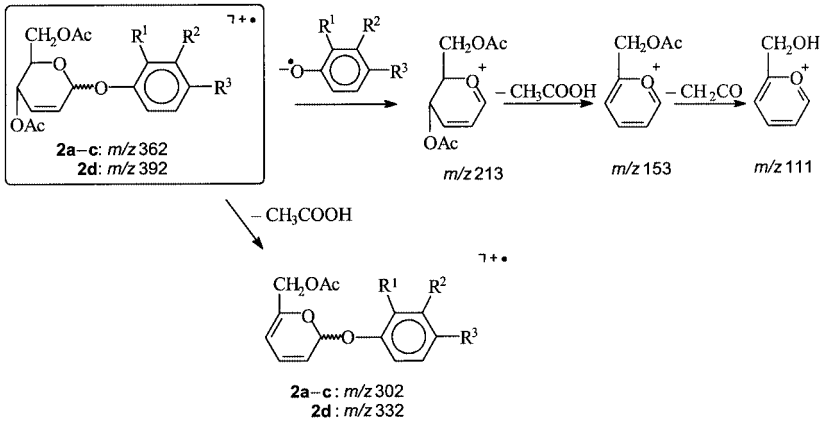


Figure 4. Comparison between the electron ionization mass spectra of *O*- and *C*-linked isomers: **α-2a** (top) and **α-3** (bottom)



Scheme 3

Table 4. Rank order of inhibition of peroxides formation by novel antioxidants; values are presented as means ± S.E.M. of four different experiments, and one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons were performed

Compound	AAPH-linoleic acid IC_{50} [M]	Compound	Fe^{2+}- ascorbic acid-microsomes IC_{50} [M]
1d	$1.04 \pm 0.12 \times 10^{-7}$	BHA	$3.68 \pm 0.42 \times 10^{-6}$
BHA	$4.25 \pm 1.73 \times 10^{-7}$	**1d**	$4.27 \pm 0.05 \times 10^{-6}$
1a	$6.88 \pm 0.89 \times 10^{-7}$	**1a**	$4.45 \pm 0.26 \times 10^{-5}$ [a]
β-3	$9.08 \pm 2.10 \times 10^{-7}$	**β-3**	$3.39 \pm 0.09 \times 10^{-5}$ [a]
α-3	$9.78 \pm 1.03 \times 10^{-7}$	**α-3**	$4.65 \pm 0.17 \times 10^{-5}$ [a]
1c	$2.13 \pm 0.40 \times 10^{-6}$	**1c**	$1.07 \pm 0.21 \times 10^{-4}$ [a]
1b	$1.28 \pm 0.24 \times 10^{-5}$ [b]	**1b**	nd

[a] $p < 0.01$ versus BHA; nd, not detected. [b] $p < 0.05$.

system. Compound **1d** shows an antioxidant activity similar to that of BHA. The activity of the other compounds is characterized by IC_{50} values significantly higher than that of BHA only in the Fe^{2+}−ascorbic acid/microsomes assay. Compound **1b** exhibits the lowest antioxidant activity in the linoleate assay system and zero activity towards peroxidation of microsomes. The *O*-derivatives **2a**−**d** that do not possess a phenol OH group do not inhibit peroxide formation in both systems.

Conclusion

This study has allowed new series of *C*- and *O*-arylglycosides with potential antioxidant properties to be obtained. The compounds are isobaric structural isomers and some of them are also stereoisomers.

A combined approach, involving their regio- and stereochemical characterization and differentiation in solution, in the crystal, and in the gas phase proved very effective for evaluation of the influence exerted on the chemico-physical properties of the compounds by the positions of substituents on the phenyl ring and by the different linkages between the two moieties.

X-ray crystallography allowed the absolute configurations of the C(1) atoms and conformational parameters to be determined. In all the compounds, independent of the linkage between the two rings, the arene moiety exists in an almost perpendicular arrangement with respect to the enopyranosyl ring. Strong intramolecular hydrogen bonds are present in the structures of derivatives **α-1b** and **α-1d**. Comparison between the data obtained in solution and the crystal structures suggests closely related features in the two states.

The gas-phase behavior of isobaric compounds with different linkages between the two rings is totally different. This allows rapid and unequivocal assignment of a *C*- or *O*-linked glycosides at the extremely low concentrations typical of mass spectrometry. In addition, unimolecular fragmentation reactions occurring in the gas phase produce ions that are strictly dependant on the regiochemistry of the different compounds. Difference in relative intensities of common ions can be interpreted on the basis of the stability of the ionic species. Further studies aimed at the stereochemical differentiation of these compounds in the gas phase are in progress.

Experimental Section

General: TLC was performed on precoated silica gel 60 F_{254} plates with detection by UV light or by spraying with 50% H_2SO_4 in CH_3OH, followed by heating at 125 °C. Melting points were determined with a Kofler hot stage apparatus and are uncorrected. [α] values were determined with an optical activity polarimeter. Column chromatography was performed on silica gel (Merck, 0.063−0.2 mm). Dichloromethane was distilled from calcium hydride and stored over 4 Å molecular sieves. Petroleum ether refers to the fraction of b.p. 40−60 °C. NMR spectra were recorded on $CDCl_3$ solutions with a Bruker AC 200 spectrometer, at 200.13 MHz for ^1H and 50.33 MHz for ^{13}C. NOE experiments were carried out by using the NOEDIFF pulse sequence. Mass spectrometric experiments were carried out with a Saturn 2000 (Varian, Walnut Creek, CA, USA) ion trap. The ionization time was 2 ms, reaction time 100 ms, manifold temperature 150 °C and helium buffer pressure 3×10^{-5} Torr. The ion trap was coupled with a Varian 3800 gas chromatograph. A DB-5 column (30m, 0.25 mm ID, Supelco) was used.

Reactions between Bromomagnesium Phenolates and Tri-*O*-acetylglucal: A solution of the appropriate phenol (4 mmol) in diethyl ether (15 mL) was added dropwise, with stirring at room temperature, to a solution of ethylmagnesium bromide (4 mmol, Fluka) in diethyl ether. The diethyl ether was removed under vacuum, and anhydrous CH_2Cl_2 (20 mL) was added. The reaction vessel was placed in an ice-cooled sonication bath and a solution of 3,4,6-tri-*O*-acetyl-D-glucal (1 mmol, Fluka) in CH_2Cl_2 (5 mL) was added dropwise. After 8 to 10 h, the mixture was quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3×20 mL). The combined extracts were dried and concentrated under reduced pressure. The residue was column-chromatographed with petroleum ether/ethyl ether (2:1, v/v) to give first the *O*-glycosides **2a**−**d** and then the *C*-glycosides **1a**−**d**. Order of elution (R_f), yields, physical and spectroscopic properties of the obtained compounds are reported in Table 5.

X-ray Crystal Structure Determinations: Single crystals of **α-1b**, **α-1d** and **α-2c** were obtained by dissolving a few mg in methanol and allowing the solution to concentrate at room temperature. A Siemens P4 four-circle diffractometer with graphite-monochromated Mo-K_α radiation (λ = 0.71069 Å) and the $\omega/2\theta$ scan technique were used for data collection. The structures were solved by direct methods implemented in the SHELXS-97 program.[19] The refine-

Table 5. Yield, physical properties and analytical data of glycosides **1a−d**, **2a−d** and **3**

Compound	Yield%	R_f (PE/EE, 2:1)[a]	$[\alpha]_D^{20}$ ($CHCl_3$)	M.p. [°C] (EE/PE)[a]	Formula	Calcd. (found)
α-1a	17	0.53	+97.22 (c = 0.36)	79−81	$C_{20}H_{26}O_6$	C 66.28 (66.00), H 7.23 (7.06)
α-1b	66	0.29	+39.68 (c = 0.63)	103−105	$C_{20}H_{26}O_6$	C 66.28 (66.59), H 7.23 (7.51)
α-1c	77	known	+56.82 (c = 0.44) (+58)[17]	oil		
α-1d	43	known	+51.02 (c = 0.49) (+53)[17]	75−76 (77−79)[17]		
α-2a	11	0.60	+85.71 (c = 0.35)	oil	$C_{20}H_{26}O_6$	C 66.28 (66.12), H 7.23 (7.44)
α-2b	7	0.51	+109.03 (c = 0.32)	oil	$C_{20}H_{26}O_6$	C 66.28 (65.99), H 7.23 (7.42)
β-2b	5	0.44	+56.18 (c = 0.18)	oil	$C_{20}H_{26}O_6$	C 66.28 (66.50), H 7.23 (7.05)
α-2c	6	known	+125 (c = 0.16) (+129)[17]	114−116 (116−117)[17]		
α-2d	13	0.43	+128.21 (c = 0.62)	oil	$C_{21}H_{28}O_7$	C 64.27 (64.11), H 7.19 (7.35)
α-3	14	0.32	−15.58 (c = 0.32)	105−107	$C_{20}H_{26}O_6$	C, 66.28 (66.49), H 7.23 (7.34)
β-3	14	0.33	+194.17 (c = 0.21)	129−131	$C_{20}H_{26}O_6$	C 66.28 (66.13), H 7.23 (7.38)

[a] EE = diethyl ether; PE = petroleum ether.

Table 6. Crystal data for **α-1b**, **α-1d** and **α-2c**

	α-1b	**α-1d**	**α-2c**
Empirical formula	$C_{20}H_{26}O_6$	$C_{21}H_{28}O_7$	$C_{20}H_{26}O_6$
M	362.41	392.42	362.41
Crystal size [mm]	0.10 × 0.25 × 0.20	0.20 × 0.25 × 0.40	0.10 ×0.15 × 0.30
Crystal system	orthorhombic	orthorhombic	orthorhombic
Space group	$P2_12_12_1$ (no. 19)	$P2_12_12_1$ (no. 19)	$P2_12_12_1$ (no. 19)
a [Å]	5.534(1)	11.702(1)	5.535(1)
b [Å]	11.390(1)	14.780(2)	18.390(3)
c [Å]	30.380(3)	24.439(2)	19.742(4)
V [Å^3]	1914.9(4)	4226.9(3)	2009.5(6)
Temperature [K]	293	293	293
Z	4	8	4
F(000)	776	1680	776
$D_{calcd.}$ [g cm^{-3}]	1.26	1.23	1.20
μ(Mo-K_α) [mm^{-1}]	0.092	0.092	0.088
Scan mode	ω	ω	ω
Scan range [°]	1 ≤ θ ≤ 25	1 ≤ θ ≤ 25	2 ≤ θ ≤ 25
Scan width [°]	0.94	1.02	0.76
Scan speed [° min^{-1}]	2.8	3.0	3.0
Independent reflections	3378 (R_{int} = 0.04)	4887 (R_{int} = 0.02)	2606 (R_{int} = 0.04)
Obsd. reflections [$I > 2\sigma(I)$]	1900	3910	714
No. parameters refined	281	529	236
R_1 [$I > 2\sigma(I)$]	0.06	0.04	0.08
wR_2 [$I > 2\sigma(I)$]	0.09	0.09	0.10

ments were carried out by full-matrix anisotropic least squares on F^2 for all reflections for non-H atoms by use of the SHELXL-97 program.[20] In the structure of **α-1d**, two positions were refined for O(3), with site occupation factors equal to 0.55(3) and 0.45(3). In the structure of **α-2c**, disorder at the *tert*-butyl group was treated by refining two different positions for atoms C(12), C(13) and C(14) with site occupation factors equal to 0.51(2) and 0.49(2). These atoms were refined isotropically. Crystal data are reported in Table 6. CCDC-180252 (**α-2c**) -180253 (**α-1d**) and -180254 (**α-1b**) contain the supplementary crystallographic data for this paper.

These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Inhibition of Lipid Peroxide Formation: This test was performed by use of two model systems, as already described.[21] The first was based on oxidation of linoleic acid, initiated by 2,2′-azobis-2-amidinopropane hydrochloride (AAPH), a thermolabile azo compound that, on decomposition, forms radicals that abstract hydrogen atoms from linoleic acid. AAPH (11 mM) was added to a suspension of linoleic acid (33 mM) in 50 mM Na phosphate buffer (pH = 7.4), in the chamber of an O_2 electrode, thermostatted at 37 °C. O_2 consumption was monitored for approximately 6 min before addition of the antioxidant at different concentrations. O_2 consumption due to AAPH decomposition was determined separately and subtracted from the peroxidation rate of linoleic acid. In the second system, peroxidation of rat liver microsomes initiated by addition of a peroxidising mixture of Fe^{2+}/Fe^{3+}−ascorbic acid (30 mM), was measured as O_2 consumption at 29 °C. Reaction mixtures, in a final volume of 3 mL, contained 0.5 mg of microsomal protein, 14.7 mM Me_2SO as vehicle and 20 mM KH_2PO_4/KOH buffer (pH = 6.0).

[1] A. Varki, *Glycobiology* **1993**, *3*, 97−130.
[2] *Carbohydrate Chemistry* (Ed.: J. F. Kennedy), Oxford University Press, New York, **1988**.
[3] *Naturally Occurring Glycosides* (Ed.: R. Ikan), J. Wiley & Son, New York, **1999**.
[4] K. Suzuki, T. Matsumoto in *Preparative Carbohydrate Chemistry* (Ed.: S. Hanessian), Marcel Dekker, New York, **1997**, p. 527−542.
[5] G. Smith, S. F. Pedersen, J. A. Leary, *J. Org. Chem.* **1997**, *62*, 2152−2154.
[6] S. P. Gaucher, J. A. Leary, *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 269−272.
[7] R. J. Sims, J. A. Fioriti, *CRC Handbook of Food Additives*, 2nd ed. (Ed.: T. E. Furia), CRC Press, Boca Raton, FL, **1980**, vol. II, chapter 1.
[8] G. P. Sgaragli, M. Valoti, B. Gorelli, F. Fusi, M. Palmi, P. Mantovani, *Br. J. Pharmacol.* **1993**, *110*, 369−377.
[9] F. Pessina, R. Kalfin, L. Esposito, F. Fusi, M. Valoti, F. Ponticelli, G.P. Sgaragli, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2001**, *364*, 462−471, and references cited therein.
[10] F. Ponticelli, A. Trendafilova, *International Symposium on Food Antioxidants and Human Health*, Certosa di Pontignano, Siena, Italy, November 8, **1998**, p. 29.
[11] F. Ponticelli, A. Trendafilova, M. Valoti, S. Saponara, G. P. Sgaragli, *Carbohydr. Res.* **2001**, *330*, 459−468.
[12] G. Casiraghi, M. Cornia, G. Rassu, L. Zetta, G. Gasparri Fava, M. F. Belicchi, *Carbohydr. Res.* **1989**, *191*, 243−251.
[13] R. J. Ferrier, N. Prasad, *J. Chem. Soc. C* **1969**, 570−575.
[14] E. Wieczorek, J. Thiem, *J. Carbohydr. Chem.* **1998**, *17*, 785−809.
[15] G. Casiraghi, M. Cornia, L. Colombo, G. Rassu, G. G. Fava, M. F. Belicchi, L. Zetta, *Tetrahedron Lett.* **1988**, *29*, 5549−5552.
[16] N. L. Allinger, M. T. Tribble, *Tetrahedron Lett.* **1971**, 3259−3262.
[17] H. Gillier-Pandraud, R. Brahmi, V. Bellosta-Dechavanne, S. Czernecki, *Can. J. Chem.* **1985**, *63*, 491−494.
[18] M. V. Da Silva, M. C. Perlat, J. C. Tabet, G. Giorgi, L. Salvini, F. Ponticelli, in preparation.
[19] G. M. Sheldrick, *SHELXS-97*, Rel. 97−2, Universität Göttingen, **1997**.
[20] G. M. Sheldrick, *SHELXL-97*, Rel. 97−2, Universität Göttingen, **1997**.
[21] M. Valoti, H. J. Sipe, Jr., G. P. Sgaragli, R. Mason, *Arch. Biochem. Biophys.* **1989**, *269*, 423−432.

Received August 14, 2002
[O02501]