4-O-β-D-Glucosides of Hydroxybenzoic and Hydroxycinnamic Acids – Their Synthesis and Determination in Berry Fruit and Vegetable

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4-O-β-D-Glucosides of Hydroxybenzoic Acids, 4-O-β-D-Glucosides of Hydroxycinnamic Acids, Synthesis, Quantitative Determination, Berry Fruit, Vegetable

Protocatechuic acid-4-, gallic acid-4-, caffeic acid-4-, ferulic acid- and *p*-coumaric acid-O-β-D-glucoside were synthesized. These substances were characterized by UV, ^{1}H NMR, ^{13}C NMR and FAB-MS. Their proportions in berry fruit and vegetable were determined by means of HPLC and capillary GC.

Introduction

The derivatives of hydroxybenzoic and hydroxycinnamic acids are widespread in the plant kingdom. A considerable number of these derivatives are well known [1]. Nevertheless there is only little existing information about the glucosides of these compounds and their distribution.

Syringic acid-O-β-D-glucoside was detected in *Anodendron affine* [2], as well as *p*-hydroxybenzoic acid-O-β-D-glucoside in a great number of Apiaceae [3]. Protocatechuic acid-4-O-β-D-glucoside was found in cockroaches of *Periplaneta americana* and *Blatta orientalis* [4].

The glucosides of hydroxycinnamic acids also could be detected in plants. The O-β-D-glucosides of *p*-coumaric and ferulic acid were found in the needles of Conifers besides the glucosides of *p*-hydroxybenzoic, protocatechuic and vanillic acid [5–8]. But these compounds were not completely characterized.

Furthermore the glucosides of caffeic, *p*-coumaric and ferulic acid were detected in tobacco [9] and in tomatoes [10–12].

To acquine further information about the distribution of the 4-O-β-D-glucosides of the hydroxybenzoic and hydroxycinnamic acids we synthesized these compounds. The substances were characterized by UV, ^{1}H NMR, ^{13}C NMR and FAB-MS. This process was necessary to obtain pure and authentic reference substances for HPLC and GC. Additionally the paper reports on the quantitative determination of these glucosides in fruit and vegetable.

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Materials and Methods

Synthesis of protocatechuic- and gallic acid-4-O-β-D-glucoside

Scheme 1 shows the course of synthesis of both glucosides.

3-O-acetyl-protocatechuic acid

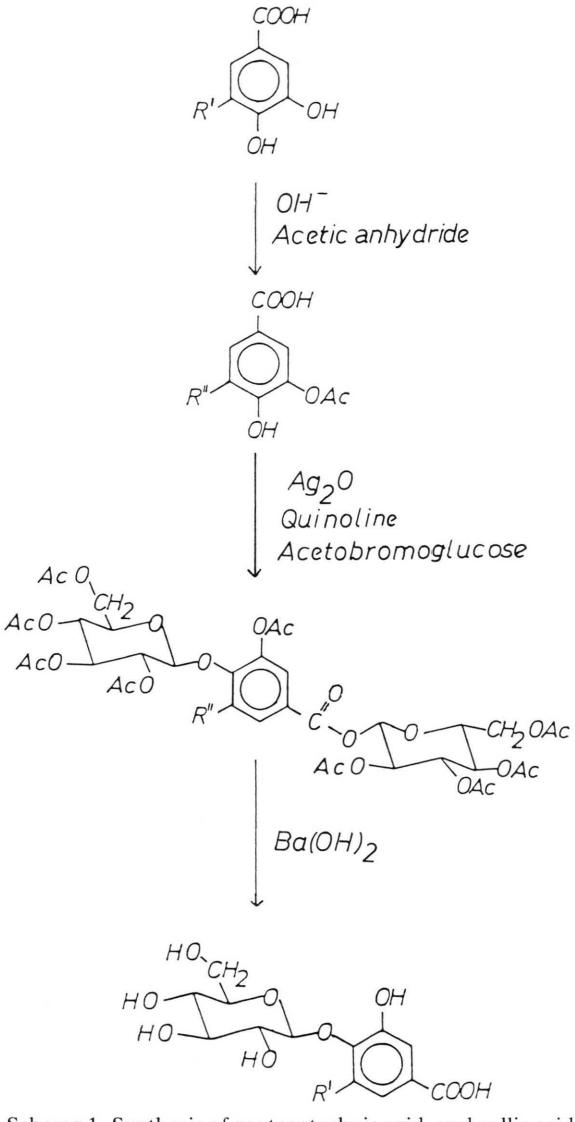
By the method of Lesser and Gad [13] the 3-O-acetyl-protocatechuic acid was prepared for use as a protected start material.

Protocatechuic acid (4.5 g) was dissolved in 2 N NaOH (15 ml). After cooling with ice, 2 N NaOH (15 ml) and acetic acid anhydride (3 g) were added simultaneously while stirring the solution. After one hour and acidifying with HCl the resulting 3-O-acetyl-protocatechuic acid was filtered off. This crude product was used for the following reactions.

Acetylated 1-O-protocatechoyl-(4-O-β-D-glucosyl)-β-D-glucose

The received derivative was coupled with α-acetobromoglucose (2,3,4,6-tetra-O-acetyl-D-glucosyl-α-bromide) to an acetylated 1-O-protocatechoyl-(4-O-β-D-glucosyl)-β-D-glucose in presence of quinoline and silver oxide [14].

3-O-acetyl-protocatechuic acid (3 g) was dissolved in quinoline (7 ml). This solution was treated with α-acetobromoglucose (5 g) and silver oxide (1.4 g). While stirring intensively the reactants became warm. Afterwards the mixture was allowed to stand for 2–3 hours in a vacuum desiccator. Subsequently it was extracted with acetic acid (10 ml). Then the filtered extract was poured into iced water (100 ml). The resulting product being insoluble and crude was purified by repeated recrystallization from methanol.



Scheme 1. Synthesis of protocatechuic acid- and gallic acid-4-O-β-D-glucoside. R′ = H, protocatechuic acid; R′ = OH, gallic acid; R″ = H, 3-acetylprotocatechuic acid; R″ = OAc, 3,5-diacetylgallic acid.

Protocatechuic acid-4-O-β-D-glucoside

Protocatechuic acid-4-O-β-D-glucoside was prepared by alkaline hydrolysis [15].

The acetylated 1-O-protocatechoyl-(4-O-β-D-glucosyl)-β-D-glucose (1.5 g) was dissolved in methanol (10–15 ml) during warming. Using nitrogen atmosphere a saturated solution of barium hydroxide (10 ml) was added.

This solution was kept in a boiling water bath for 5 min. After cooling to room temperature the mixture was neutralized with sulphuric acid. Afterwards the precipitated barium sulphate was filtered off. Using preparative HPLC the crude product was purified.

3,5-Diacetyl-gallic acid

This compound was synthesized using the method of Lesser and Gad [13].

Gallic acid (5 g) was dissolved in 2 N NaOH (15 ml) and cooled with ice. While stirring 2 N NaOH (30 ml) and acetic acid anhydride (6 g) were added simultaneously. After one hour the mixture was acidified with HCl. The precipitating 3,5-diacetyl-gallic acid was filtered off.

Gallic acid-4-O-β-D-glucoside

This glucoside was prepared as described above for protocatechuic acid-4-O-β-D-glucoside.

Synthesis of caffeic acid-4- and p-coumaric acid-O-β-D-glucoside

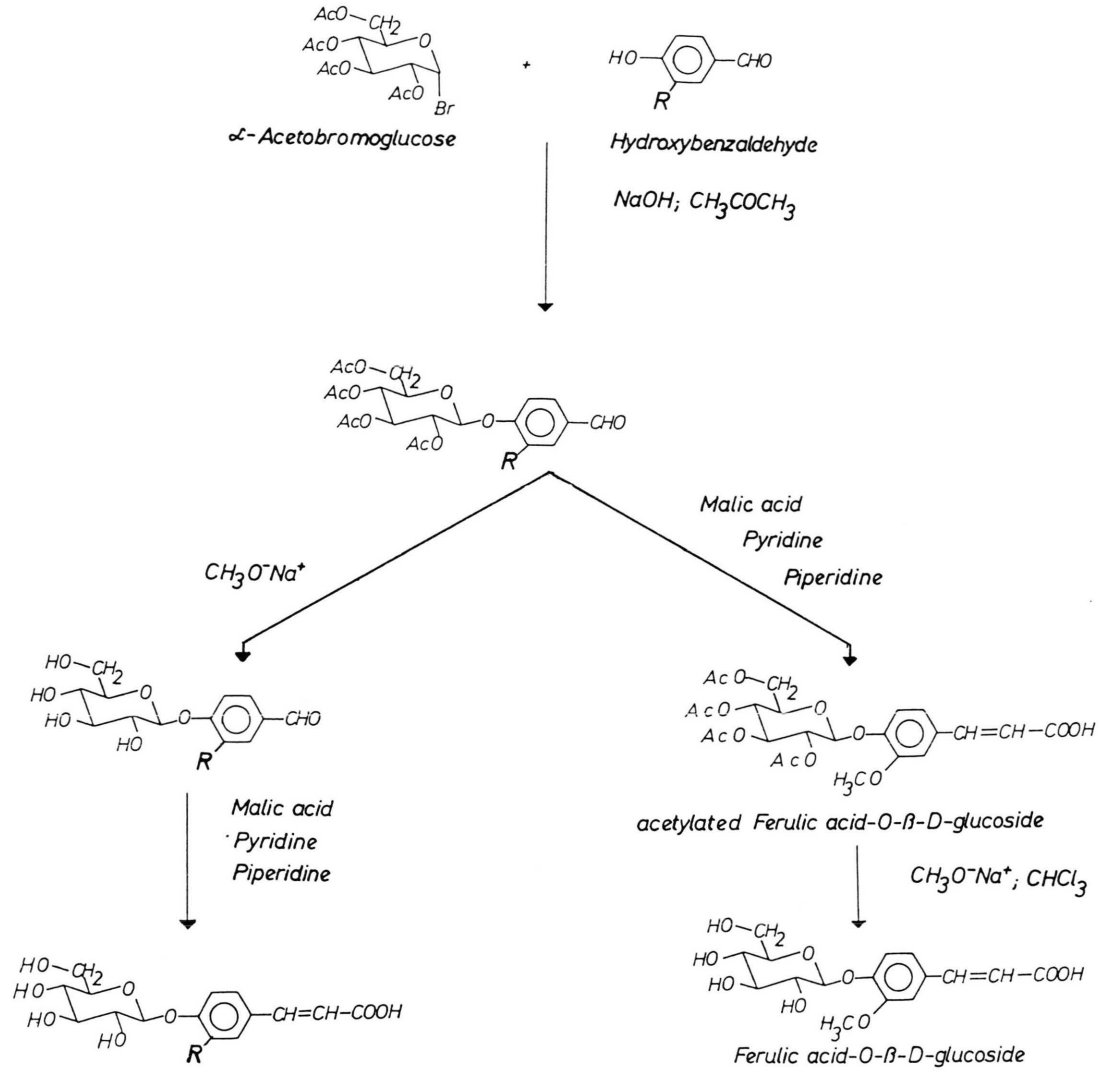
Forming the glucosides of the corresponding hydroxybenzaldehydes (*p*-hydroxybenzaldehyde and protocatechualdehyde) using α-acetobromoglucose, the resulting glucosides were obtained by deacetylation and a final Knoevenagel-reaction [16–18]. The process of synthesis will be described using *p*-coumaric acid-O-β-D-glucoside as an example (Scheme 2).

Acetylated 4-O-β-D-glucosyl-benzaldehyde

p-Hydroxybenzaldehyde (6.1 g) and α-acetobromoglucose (8.0 g) were dissolved in acetone (40 ml). After addition of 7% NaOH (10 ml) the solution was stirred intensively and was allowed to react for 24 h. After this time the solution was poured into iced water and stirred for 60 min. In the mean-time crystallization started slowly. The crystalls were filtered off and became recrystallized from 50% methanol.

4-O-β-D-glucosyl-benzaldehyde

After the desolving of the acetylated product, using methanol (20 ml) as solvent, 0.1 N methanolic sodium methylate (0.5 ml) as reactant was added. This mixture was allowed to saponify for 20 min in a water bath at 80 °C under reflux. The still warm solution was treated with petroleum ether until it became turbid. Colourless crystalls precipitated slowly,



Scheme 2. Synthesis of *p*-coumaric acid-, caffeic acid- and ferulic acid-O-β-D-glucoside. R = H, *p*-hydroxybenzaldehyde resp. *p*-coumaric acid; R = OH, protocatechualdehyde resp. caffeic acid; R = OCH_3, vanillin.

which were recrystallized twice from methanol for purification.

p-Coumaric acid-O-β-D-glucoside

Malonic acid (0.6 g) and the O-β-D-glucoside of *p*-hydroxybenzaldehyde (0.5 g) were dissolved in pyridine (2 ml). Piperidine (0.03 ml) was added as a catalytic agent. This mixture was heated up to 78–80 °C for 12–14 h. The start of the reaction can be observed by nascent bubbles of carbondioxide. The solution was poured into ether, which contained 5% acetic acid. With stirring precipitation started.

The ether was decanted and the rest of it was blown off. The residue was dissolved in ethanol and precipitated again with ether. After the ether has been drained off the glucoside was recrystallized from water.

Caffeic acid-4-O-β-D-glucoside

This glucoside was prepared as described above for *p*-coumaric acid-O-β-D-glucoside.

Synthesis of ferulic acid-O-β-D-glucoside

Vanillin as start material was treated with α-acetobromoglucose by the method of Reichel and Schickle [19]. The resulting acetylated glucoside of vanillin was caused to react with malonic acid to acetylated ferulic acid-O-β-D-glucoside by the method of Hann [20]. Subsequently to the Knoevenagel-reaction the product was saponified (Scheme 2).

Acetylated vanillin-O-β-D-glucoside

Vanillin (6.0 g) and α-acetobromoglucose were dissolved with acetone (25 ml). After addition of a 10% solution of NaOH (10 ml) the mixture was stirred. Afterwards the mixture was allowed to react for six hours. Subsequently iced water was added until turbidity occurred. At first the product precipitated oily, but solidified to a crystalline mass soon after stirring carefully. The precipitate was recrystallized twice from water.

Acetylated ferulic acid-O-β-D-glucoside

The received compound (2.5 g) and malonic acid (1.4 g) were dissolved in pyridine (5 ml) and piperidine (0.03 ml). This solution was allowed to react for 60 min in a boiling water bath. After cooling down to room temperature water (50 ml) was added causing a yellow oily precipitate. The oily mass was stirred intensively with water and crystallization slowly started. The fluid was drained off and the residue was recrystallized from ethanol.

Ferulic acid-O-β-D-glucoside

The acetylated glucoside (1.0 g) was dissolved in dry chloroform. This mixture was cooled down to 0 °C in a refrigerator. Rapidly 1 N methanolic sodium methylate (5.5 ml) was added causing a white precipitation. After a while (30–60 min) an equivalent amount of sulphuric acid was poured in. The solution was brought to dryness using a rotation evaporator. The dry residue was washed out with ethanol repeatedly. The ethanolic extracts were dried out again. Recrystallization from water and finally from ethanol followed.

Chemical identification

Alkaline hydrolysis: The glucoside (1–2 mg), dissolved in 2 N NaOH (2 ml) was allowed to react for four hours at room temperature under nitrogen atmosphere. After neutralization with 2 N HCl this solution can be used directly for HPLC and for GC after derivatisation (see enzymatical hydrolysis).

Acidic hydrolysis: The glucoside (1–2 mg), dissolved in 2 N HCl (2 ml), was heated under reflux up to 90 °C in a water bath for 90 min. After neutralization with 2 N NaOH the solution was analysed by HPLC.

Enzymatical hydrolysis: The glucoside (1–2 mg) was added to a solution of 1 mg β-glucosidase in bidistilled water (1 mg). This mixture was kept at 36 °C for 24 h. Simultaneously a control test with maltose was carried out using the conditions described above. After drying the residue was trimethylsylated with a solution (0.5 ml) consisting of N,O-bistrimethylsilylacetamide (BSA) and trimethylchlorsilane (TMCS) (20:1). The reaction mixture was kept at 70 °C for 60 min.

Apparatus: GC (Carlo Erba); capillary column: SE-30 (self-made), 30 m, 0.3 mm i.d.; carrier: nitrogen; detector: FID; detector- and injector temperature: 300 °C; temperature gradient: 120 °C–8 °C/min–270 °C.

UV-Spectroscopy

UV-spectra were recorded with a SP-8-500 (Philips) and a Hewlett-Packard UV-Vis spectrophotometer. Methanol was used as a solvent for all of the spectra.

NMR-Spectra

The ^{1}H NMR-spectra of gallic acid-4- and the acetylated ferulic acid-O-β-D-glucoside were recorded with a Bruker WM-400 NMR-spectrometer. The spectrum of protocatechuic acid-4-O-β-D-glucoside was taken on a Bruker WH-90, FT-spectrometer, 90 MHz. The spectra of the acetylated glucosides of caffeic and *p*-coumaric acid were re-

corded on a 60 MHz Bruker NMR-spectrometer. A Bruker WH 80 was used for the ^{13}C NMR spectra. Deuterated chloroform and methanol were used as solvents.

FAB-MS

The negative ion mass spectra of the hydroxycinnamic acid glucosides were recorded with a Kratos MS 50 spectrometer equipped with a Kratos FAB source. The Xenon gun was set at 9 kV. Glycerol was used as matrix.

Plant material

All the fruits and vegetables investigated were harvested fully ripe. Until sample preparation and determination, the products were kept deep frozen. The sample preparation, clean-up and the selective separation of hydroxycinnamic acid derivatives on polyamide were carried out as described elsewhere [12, 22].

Quantitative determination

The quantitative determination was carried out by means of HPLC and/or capillary GC.

GC-system: Carlo Erba; capillary column: OV-1701 (self-made), 30 m, 0.28 mm i.d.; carrier: nitrogen; detector: FID; detector- and injector temperature: 300 °C; temperature gradient: 220 °C−4 °C/min−270 °C, then isotherm.

HPLC-system: Pye Unicam (Philips); injection valve: Rheodyne 7125 with a 0.01 ml sample loop; column: 250 × 4.6 mm i.d., Shandon ODS-Hypersil, 5 μm; flow: 1.0 ml/min; detection: single wavelength detection for quantitative results: a) 251 nm for hydroxybenzoic acid glucosides and 320 nm for hydroxycinnamic acid glucosides and b) multi-wavelength detection for identification and purity tests with a diode-array-detector 1040A (Hewlett-Packard); solvents: 1. 2% acetic acid (isocratic) for hydroxybenzoic acid glucosides and 2. gradient elution for hydroxycinnamic acid glucosides: (A) = 10% methanol in 2% acetic acid, (B) = methanol; gradient program: 0% (B) to 29% (B) in (A) within 50 min (linear), subsequently for 5 min kept at 100% (B) (isocratic); integrator: 3390A (Hewlett-Packard): autosampler LCX (Pye Unicam).

Results and Discussion

Hydroxybenzoic acid glucosides

In contrast to previous synthesis [15] we used protocatechuic acid and gallic acid instead of their methyl-esters. This economizes one step of the synthesis. In the presence of quinoline the carboxylic group as well as the phenolate group attack the C-1 of the α-acetobromoglucose nucleophilicly. Acetylated 1-O-galloyl-(4-O-β-D-glucosyl)-β-D-glucose and 1-O-protocatechoyl-(4-O-β-D-glucosyl)-β-D-glucose resulted as intermediates respectively. Subse-

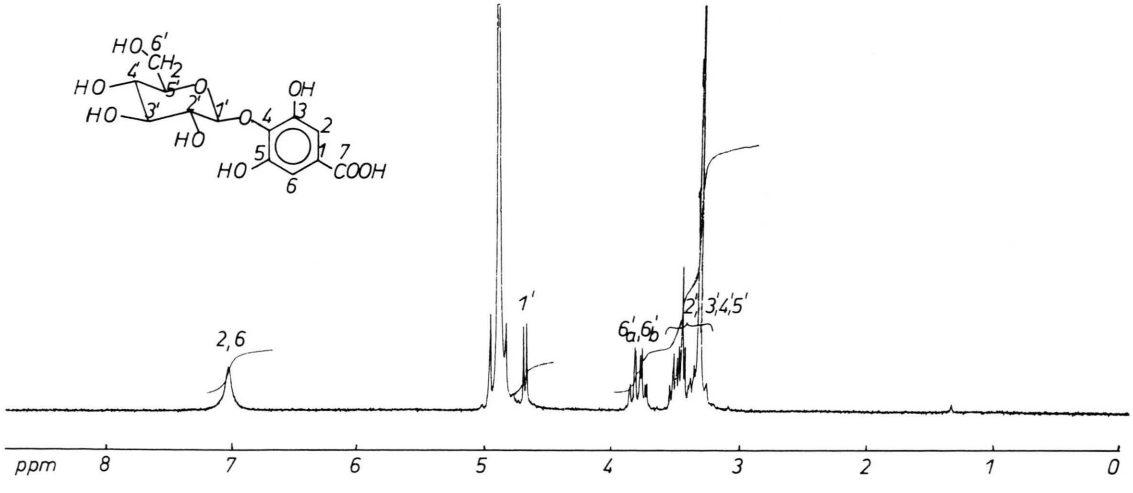


Fig. 1. ^1H NMR spectrum of gallic acid-4-O-β-D-glucoside (solvent: CD_3OD).

quently the ester bonds were saponified by alkaline hydrolysis with a saturated aqueous solution of $Ba(OH)_2$. The resulting glucosides were purified by preparative HPLC.

Enzymatical hydrolysis with β-glucosidase confirmed the structure of the two glucosides. Both could be hydrolyzed completely. The hydroxybenzoic acids and the glucose could be detected in about equimolar ratio in each case.

The UV-spectra showed the typical absorptions for glucosidic linkages. A hypsochromic shift resulted in contrast to the free acids. The maximum of protocatechuic acid-4-O-β-D-glucoside was 250 nm with a shoulder at 293 nm. For gallic acid-4-O-β-D-glucoside we received a maximum at 255 nm with a shoulder at 296 nm. The position of the glucosidic linkage was readily confirmed from their ^{1}H- and ^{13}C NMR spectra. The broad proton signal (7.1 ppm) of the gallic acid-4-O-β-D-glucoside (Fig. 1) conforms with the two aromatic protons. The aromatic residue has a plane of symmetry and hence is coupled to the sugar moiety through the C-4 oxygen of the aromatic residue. In the region between 7 and 8 ppm the ^{1}H NMR spectrum of protocatechuic acid-4-O-β-D-glucoside was typical for 1,3,4-substituted aromatic substances. The position of the glucosidic linkage can not be determined from this spectrum. In the ^{13}C NMR spectrum the shift to the higher field of the C-4 and the shift to the deeper field of the C-1 of the two glucosides allow the conformation of the position of the glucosidic linkage through C-4 oxygen. The signals of the C-3, C-5, C-2 and C-6 respectively of the spectrum of the gallic acid-4-O-β-D-glucoside give only a common signal and show the symmetry of the aromatic residue. Signal assignments for both glucosides are given in Table I and Table II and confirm the β-glucosidic linkage too.

Hydroxycinnamic acid glucosides

Normally the well known Koenigs-Knorr-reaction will be used for such reactions forming glucosidic linkages (see hydroxybenzoic acid glucosides).

Table I. ^{1}H NMR-data of protocatechuic acid-4-O-β-D-glucoside (A) and gallic acid-4-O-β-D-glucoside (B) in CD_3OD (ppm).

	C-2	C-5	C-6	C-1′	C-2′–C-6′
A	7.5	7.2	7.55	4.9	3.2–4.0
B	7.1	–	7.1	4.7	3.3–4.0

Table II. ^{13}C NMR-data of protocatechuic acid-4-O-β-D-glucoside (A) and gallic acid-4-O-β-D-glucoside (B) in CD_3OD (ppm).

	C-1	C-2	C-3	C-4	C-5	C-6	C-7
A	125.0	118.1	145.7	149.7	116.0	124.1	170.3
B	–	111.3	150.2	137.0	150.2	111.3	–
	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′	
A	102.2	73.6	77.0	70.2	76.2	61.4	
B	104.8	74.2	77.2	70.0	76.4	61.8	

For the synthesis of hydroxycinnamic acid glucosides we preferred a reaction within an aqueous alkaline milieu as it has been introduced by Michael in 1879 for the synthesis of the phenol-β-D-glucosides [23]. Synthesis of hydroxycinnamic acid glucosides via Koenigs-Knorr-reaction has been described by Durkee and Siddiqui [24]. Relative to the course of the synthesis of β-glucosides starting from α-halogenoses it will be referred to the corresponding publications [23, 25].

Intermediates and final products of all the synthesis have been investigated by HPLC, using several wavelengths, with regards to the purity of the compounds. The purity of the glucosides amounted to 97–99%.

The melting points of the synthesized glucosides were congrous with these cited in literature [16–20].

The results of several methods of hydrolysis distinctly showed the creation of hydroxycinnamic acid

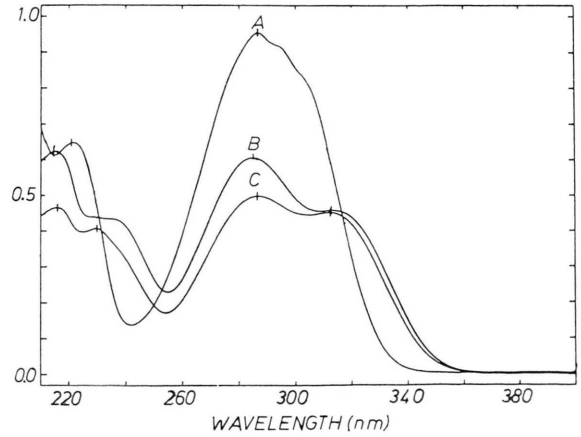


Fig. 2. UV-spectra of *p*-coumaric acid-, caffeic acid-4- and ferulic acid-O-β-D-glucoside (solvent: methanol). A = *p*-coumaric acid-O-β-D-glucoside, B = caffeic acid-4-O-β-D-glucoside, C = ferulic acid-O-β-D-glucoside.

structure from the corresponding hydroxybenzaldehydes as well as the glucosidic linkage. So the alkaline hydrolysis showed no breaking up of the glucosides, while the acidic one could split the glucosides. As well, the enzymatical hydrolysis showed complete division. The pure β-glucosidic effect of the used β-glucosidase was controlled by a control test with maltose which was not broken up. The corresponding hydroxycinnamic acids and the glucose were indicated in equimolar ratio by GC.

The UV-spectra of the glucosides showed a distinct shift of the absorption maximum to lower wavelength in contrast to the free hydroxycinnamic acids (Fig. 2). The maximum of *p*-coumaric acid-O-β-D-glucoside lay at 287 nm, that of caffeic acid-4-O-β-D-glucoside lay at 285 nm and that of ferulic acid-O-β-D-glucoside at 286 nm. This will be confirmed by datas of literature [9, 26]. Also, the ^{1}H NMR data showed definitely the phenyl-propenyl-structure. Both of the vinyl-protons at 6.3 and 7.7 ppm were split into doubletts.

Additionally the constant coupling of $J = 16$ Hz pointed to a *trans* position of the vinyl-protons (*cis:* $J = 12$ Hz). The signal of the carboxyl group could not be detected distinctly in all of the NMR-spectra. At the same time there were a strong signal of the deuterated chloroform in all of the spectra. Therefore strong replacement effects of the carboxylic proton with the solvent could not be excluded.

The chemical shift of the sugar protons of all of the synthesized glucosides have been in the usual range, between 3.8 and 4.2 ppm as well as between 5.2 and 5.3 ppm. The splitting of the single sugar protons could only be studied at the spectrum of the acetylated ferulic acid-O-β-D-glucoside (400 MHz). The acetal proton was shifted to the higher field and coincided with the neighboring CH-proton. This behaviour can be explained by the neighboring position of an aromat. Additionally the constant coupling of $J = 7.5$ Hz directed to a β-glucosidic linkage (α-glucosidic: $J = 4$ Hz) (Table III).

The received results were confirmed by the negative ion MS spectrum. With this method labile linkages, *i.e.* glucosidic ones, can be characterized more effective, as it enables the detection of the mole-peak (M-H). Other methods like EI or CI will damage this compounds immediately without recording a mole-peak.

Fig. 3 shows the neg. FAB-MS of ferulic acid-O-β-D-glucoside. We determined the M-H peak at 325 for *p*-coumaric acid-O-β-D-glucoside, for caffeic acid-4-O-β-D-glucoside at 341 and for ferulic acid-O-β-D-glucoside 355 (mass). *p*-Coumaric acid (163), caffeic acid (179) and ferulic acid (193) could be detected as fragments. The masses 181/183, 255, 275, 309 and 367 are glycerol adduct ions and have to be interpreted as matrix ions (Fig. 3).

Our investigations show extensive distribution of phenolic glucosides in berry fruit (Table IV, Fig. 4). The fruits of Saxifragaceae like currants and gooseberries contained 2–10 ppm of all phenolic acid glucosides investigated. High amounts of glucosides were detected in high bush blueberries. In raspberries and blackberries the β-glucosides of *p*-coumaric and protocatechuic acids occur regularly.

According to our hitherto investigations the glucosides of hydroxycinnamic acids are less distributed in vegetables. The highest amount of these compounds could be detected in tomatoes (Fig. 5, Table V). No glucosides could be found in other vegetables of the Solanaceae like bell-peppers or aubergines (egg-plants). Lower concentrations of glucosides were detectable in kale and pods or leaves of broad beans. Peas only showed caffeic acid-4-O-β-D-glucoside. Ferulic acid-O-β-D-glucoside could be detected in carrot tops.

Table III. ^{1}H NMR-data of the hydroxycinnamic acid glucosides. A: *p*-coumaric acid-O-β-D-glucoside, B = caffeic acid-4-O-β-D-glucoside, C = ferulic acid-O-β-D-glucoside.

	Vinyl-H		Aromat-H		Methoxy-H	Acetyl-H	Glucose-H		Acetyl-H (Glucose)
	a	b	c	d					
A	6.4	7.8	7.0	7.6	–	–	4.2–4.3;	5.1–5.3	2.1
B	6.3	7.7	7.0–7.3	–	–	2.3	4.2;	5.1–5.3	2.0–2.1
C	6.35	7.7	7.1	–	3.9	–	3.8; 5.0–5.3;	4.2–4.3	2.0–2.1

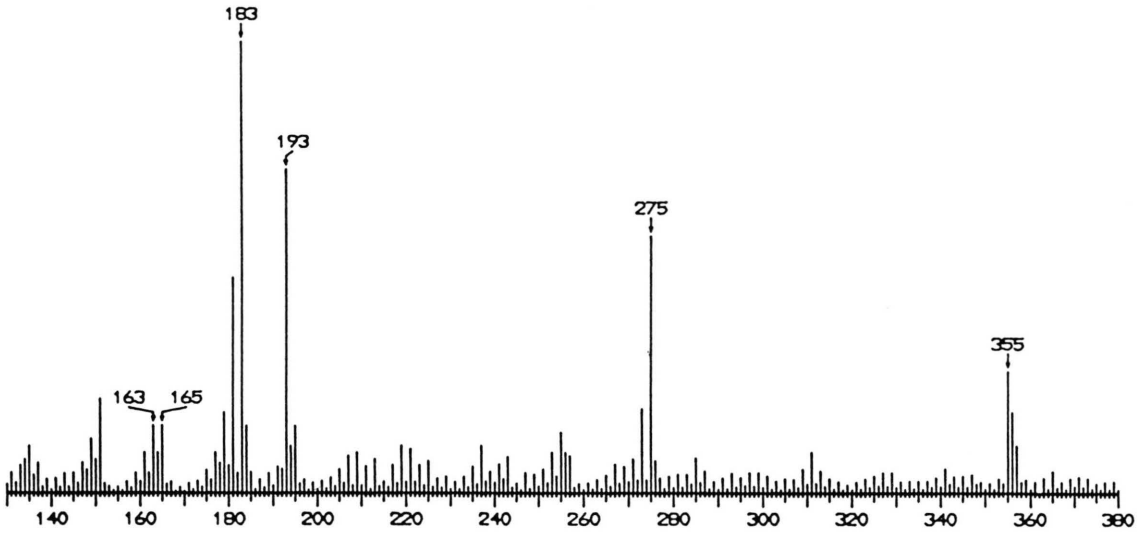


Fig. 3. Neg. FAB-MS of ferulic acid-O-β-D-glucoside (matrix: glycerol).

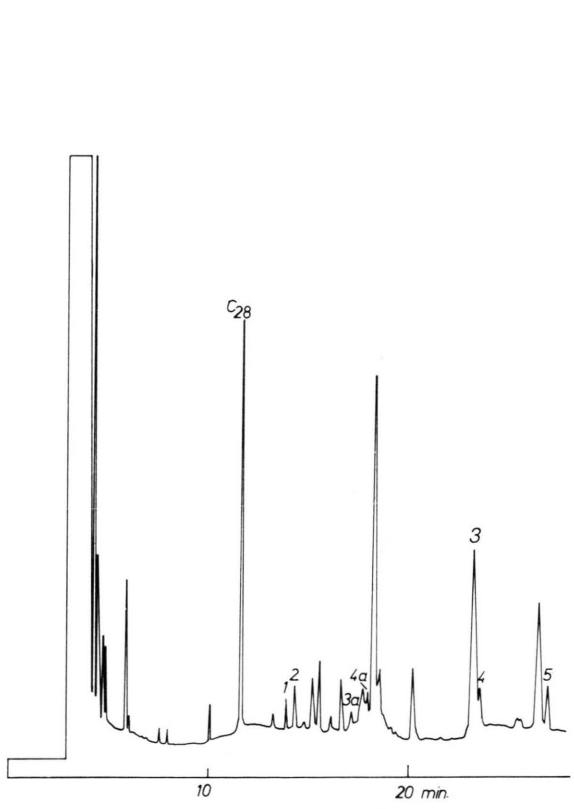


Fig. 4. GC-chromatogram of high bush blueberries. 1: gallic acid-4-O-β-D-glucoside, 2: protocatechuic acid-4-O-β-D-glucoside, 3: *p*-coumaric acid-O-β-D-glucoside, 4 = caffeic acid-4-O-β-D-glucoside, 5 = ferulic acid-O-β-D-glucoside, "a" indicates the *cis*-isomer of the corresponding trans glucoside.

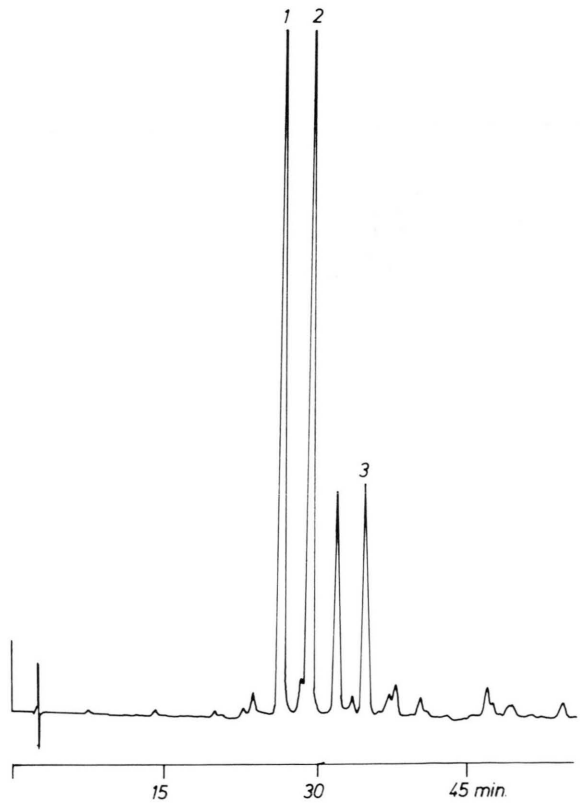


Fig. 5. HPLC-chromatogram of tomatoes (methanolic PA-SC-extract [12]). 1: *p*-coumaric acid-O-β-D-glucoside, 2 = caffeic acid-4-O-β-D-glucoside, 3 = ferulic acid-O-β-D-glucoside.

Table IV. Contents of hydroxybenzoic acid and hydroxycinnamic acid glucosides in berry fruit received by HPLC (ppm). CafG: caffeic acid-4-O-β-D-glucoside, pCoumG: *p*-coumaric acid-O-β-D-glucoside, FerG: ferulic acid-O-β-D-glucoside, ProG: protocatechuic acid-4-O-β-D-glucoside, GalG: gallic acid-4-O-β-D-glucoside; "+" = trace (below 2 ppm), "−" = not detectable.

	CafG	pCoumG	FerG	ProG	GalG
Strawberries					
Tago	−	+	−	−	−
Litessa	−	+	−	−	−
Raspberries					
Glen Glova	−	9	2	+	−
Maling Promise	−	6	−	+	−
Blackberries					
Thornless Evergreen	−	4	−	6	−
Black Thornfree	−	2	−	2	−
Red Currants					
Red Lake	2	10	−	2	−
Fays Fruchtbare	2	13	+	+	−
Black Currants					
Silmu	2	5	2	−	−
Ni 76	2	7	3	2	3
Gooseberries					
Gelbe Triumph	2	4	2	2	+
Mauks frühe Rote	2	6	4	7	+
Blueberries					
Bluecrop	3	4	5	4	7
Heerma	3	15	6	4	3

Table V. Contents of hydroxycinnamic acid glucosides in vegetable received by HPLC (ppm). Assignment see Table IV.

	pCoumG	CafG	FerG
Tomato			
Baroso	28	25	8
Dombo	25	25	8
Gold. Königin	62	30	11
VSB 3212	68	48	13
Ostona	41	39	12
Bellina	42	37	15
Ronald	19	29	10
Hellfrucht	20	21	9
Haubners Vollendung	28	29	12
Pea			
Leaves	−	7	−
Pods Wunder v. Kelvedon	−	2	−
Noblesse	−	5	−
Broad Bean			
Pods Hedosa	+	+	3
Osna grün	+	+	2
Leaves Hedosa	+	3	5
Osna grün	+	3	+
Carrot Top			
Pariska	+	+	+
St. Carausch	+	+	+
Rubin	+	+	7
Kale			
Stalk	2	2	+
Leaves	2	3	−

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