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GLUCOSIDES AND GLUCOSE ESTERS OF HYDROXYBENZOIC ACIDS IN PLANTS

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Key Word Index—Pinaceae; Brassicaceae; Solanaceae; Lamiaceae; hydroxybenzoic acid glucosides; hydroxybenzoylglucoses; glucose derivatives.

Abstract—Six hydroxybenzoic acid glucosides and three hydroxybenzoylglucoses were synthesized as reference substances and their content in plant extracts was determined by means of capillary GC and HPLC. The results suggest a wide distribution for the glucosides, whereas the glucose esters occur less frequently.

INTRODUCTION

Derivatives of the five common hydroxybenzoic acids salicylic (2-OH), 4-hydroxybenzoic, vanillic (3-OMe, 4-OH), protocatechuic (3,4-diOH), and syringic acids (3,5-diOMe, 4-OH) are widespread in the plant kingdom [1–7], even if their concentration is usually low compared with the ubiquitous hydroxycinnamic acid derivatives. This is based on the hydrolysis of the plant extracts and subsequent investigation of the hydroxybenzoic and hydroxycinnamic acids in the hydrolysate. Hydroxycinnamic acids mainly form esters in living plants [8, 9], especially with quinic acid or glucose, and in most of the cases the amounts of glucosides are much lower than the corresponding esters [10, 11].

In contrast, hydroxybenzoic acid derivatives have been found only in a few plant species [8]. Isolation, structure elucidation and comparison with synthetic reference substances are rare. Salicylic and vanillic acid glucosides and the glucose esters of vanillic and syringic acids have never been isolated and completely characterized from plant material. Isolation and structure elucidation of syringic acid 4-β-D-glucoside has been successful from *Anodendron affine* [12] and *Eukianthus nudipes* [13]. Protocatechuic acid 3-β-D-glucoside was found in *Pyrus* [14]. Birkofer *et al.* isolated 4-hydroxybenzoylglucose from the flowers of *Catalpa bignonioides* and characterized it by spectroscopic methods [15, 16]. Glucosides of 4-hydroxybenzoic, vanillic and protocatechuic acids have been found in needles of conifers, i.e. in species of *Larix* [17–19], *Abies* [17, 20], and *Pinus* [20–22], and vanilloylglucose in needles of *Larix decidua* [23]; none of these compounds has been characterized completely. Recently, 4-hydroxybenzoic acid glucoside was found in pollen of *Pinus densiflora* and was well characterized [24]. Cooper-Driver *et al.* [25] showed that several plant species synthesized both glucosides and glucose esters, when hydroxybenzoic acids were fed to them.

Due to the behaviour of hydroxybenzoic acid derivatives on hydrolysis, it has been assumed that these acids mainly occur as the glucosides rather than as the glucose esters [26–29]. Gallic acid is different from the other hydroxybenzoic acids, in being esterified to glucose in the form of hydrolysable tannin [8].

We synthesized the six glucosides named above (including gallic acid 4-β-D-glucoside) and 1-*O*-β-D-glucose esters of 4-hydroxybenzoic, vanillic and syringic acids as reference substances. The identity was confirmed by enzymatic and spectroscopic methods (UV, IR, ^{1}H NMR, ^{13}C NMR, CIMS, FABMS). We also developed methods for qualitative and quantitative determination of these substances, even when present in trace amounts, by means of capillary GC and HPLC [30–32].

Members of the Apiaceae were investigated to determine the content of 4-hydroxybenzoic acid glucoside [30, 33], and soft fruits were examined for the presence of 4-hydroxybenzoic, protocatechuic and gallic acid glucosides [31, 34]. In order to acquire further information about the forms, in which hydroxybenzoic acids occur in plants, we have now investigated a greater number of species from different plant families.

RESULTS AND DISCUSSION

The hydroxybenzoic acid derivatives of four Lamiaceae, *Capsicum annuum*, two Brassicaceae and two Pinaceae are listed in Tables 1 and 2. The results of both methods employed (see Experimental) were in close agreement with each other. In some cases no quantitative results could be obtained with one of the methods due to interference with other substances, but normally the second method sufficed for the determination. For salicylic acid glucoside in *Ocimum basilicum* and *Majorana hortensis*, exact determinations were not possible. The occurrence of 4-hydroxybenzoic acid and vanillic acid glucosides in Pinaceae needles could be verified, but protocatechuic acid glucoside and vanilloylglucose were not detectable in these samples.

Glucosides of hydroxybenzoic acids, with the exception of gallic acid, are obviously widely distributed in higher plants. All samples, obtained from 24 plant species and investigated by us, contained one or more hydroxybenzoic acid glucosides, except some tropical spices, such

Table 1. Contents (ppm, fr. wt) of hydroxybenzoic acid glucosides by GC and HPLC

Plant species	SaliGluc		*p*-HBGluc		ProtGluc		VaniGluc		SyriGluc	
	GC	HPLC	GC	HPLC	GC	HPLC	GC	HPLC	GC	HPLC
Picea abies (needles)	—	—	26	31	—	—	*	26	—	—
Larix decidua (needles)	—	—	108	130	—	—	*	33	—	—
Sinapis alba (seed)	—	—	9	9	—	—	5	4	—	—
Lepidium sativum (leaves)	+	—	2	3	—	—	2	2	+	2
Capsicum annuum sample 1 (powder)	—	—	*	6	—	—	5	5	—	—
Capsicum annuum sample 2 (powder)	—	—	6	7	—	—	5	5	—	—
Thymus vulgaris (1985) (leaves)	12	11	29	28	3	4	*	43	24	22
Thymus vulgaris (1986) (leaves)	22	*	17	17	3	2	41	45	13	16
Rosmarinus officinalis sample 1 (leaves)	32	35	69	65	5	3	81	77	*	16
Rosmarinus officinalis sample 2 (leaves)	8	*	16	16	3	1	111	125	26	27
Ocimum basilicum (dried leaves)	*ca* 145	*	195	*	—	—	57	*	29	*
Majorana hortensis (dried leaves)	*ca* 90	*	11	*	—	—	26	*	11	14

+, Trace; —, not detectable; *, detectable, but no quantitative determination because of interference with other substances.
SaliGluc, salicylic acid 2-β-D-glucoside; *p*-HBGluc, hydroxybenzoic acid 4-β-D-glucoside; ProtGluc, protocatechuic acid 4-β-D-glucoside; VaniGluc, vanillic acid 4-β-D-glucoside; SyriGluc, syringic acid 4-β-D-glucoside. Gallic acid glucoside was not found in any of the 12 samples.

Table 2. Contents (ppm, fr. wt) of 1-O-hydroxybenzoylglucoses by two methods (A: RP-HPLC, B: normal phase HPLC, benzoyl derivatives)

Plant species	4-Hydroxybenzoyl glucose		Vanilloyl glucose		Syringoyl glucose	
	HPLC-A	HPLC-B	HPLC-A	HPLC-B	HPLC-A	HPLC-B
Picea abies (needles)	—	—	—	—	—	—
Larix decidua (needles)	—	—	—	—	—	—
Sinapis alba (seed)	3	3	—	—	—	—
Lepidium sativum (leaves)	3	3	43	45	6	4
Capsicum annuum sample 1 (powder)	—	—	28	27	—	—
Capsicum annuum sample 2 (powder)	—	—	44	*	—	—
Thymus vulgaris (1985) (leaves)	12	11	—	—	72	70
Thymus vulgaris (1986) (leaves)	4	4	—	—	45	42
Rosmarinus officinalis sample 1 (leaves)	7	9	—	—	—	—
Rosmarinus officinalis sample 2 (leaves)	2	2	—	—	—	—
Ocimum basilicum (dried leaves)	—	—	—	—	28	28
Majorana hortensis (dried leaves)	—	—	—	—	—	—

—, not detectable; *, detectable, but no quantitative determination because of interference with other substances.

as black and white pepper, clove and allspice. In these samples large amounts of free hydroxybenzoic acids were detected. The fresh spices probably undergo fermentation during processing after harvest, resulting in enzymatic cleavage of the conjugates.

With the exception of cinnamon, 4-hydroxybenzoic acid glucoside could be detected in all species, and vanillic acid glucoside was present in all plants investigated. Salicylic acid and syringic acid glucosides occurred only in a few plants. Similarly protocatechuic acid glucoside was not detectable in all samples and occurred in relatively lower amounts than the other glucosides.

Gallic acid glucoside was not found in any sample investigated, and even strawberries, raspberries and blackberries, belonging to the Rosaceae, a typical 'tannin family', had only small amounts of glucosides of gallic and protocatechuic acids [31].

In contrast to the glucosides which occurred in 21 species, glucose esters were present in only six of 24 species. Three of these plants were Lamiaceae, one belonged to the Solanaceae, and two were Brassicaceae. Only one sample (*Lepidium sativum*) contained all three esters together. The results can be compared with the content of hydroxybenzoic acids after hydrolysis, obtained from the work of Schulz and Herrmann [35]. For example, thyme is a plant with a remarkably high content of syringic acid after hydrolysis. This is a consequence of the presence of both glucoside and glucose ester of syringic acid in thyme leaves, together with other conjugates as yet unidentified.

The results of our work indicate that glucosides are an important form, in which hydroxybenzoic acids occur in living plant tissues. Glucose esters represent another class of natural compounds of hydroxybenzoic acids, but they are probably much less widely distributed than the corresponding glucosides. Hydroxybenzoylglucoses were

relatively frequent in the Lamiaceae, Solanaceae, and Brassicaceae.

EXPERIMENTAL

Quantitative analyses. The analytical procedure for the determination of glucosides and glucose esters of hydroxybenzoic acids, including sample clean-up and GC and HPLC analysis is described in detail in an earlier report [32].

Synthesis of glucosides. The hydroxybenzoic acid glucosides were synthesized by the Koenigs–Knorr reaction of methyl esters in the presence of Ag_2O and quinoline via reported methods [34, 36, 37] and subsequent saponification of the ester groups.

Synthesis of 1-O-*glucose esters.* For synthesis of hydroxybenzoylglucoses a modified method already described [38] was employed. Hence the 4-benzyloxybenzoic acid chlorides react with 4,6-benzylidene-glucose-sodium to yield esters; reductive elimination of the protection groups with H_2/Pd gives the corresponding glucose esters.

General. Mps: uncorr; ^{1}H NMR (300 MHz): TMS as int. standard; ^{13}C NMR (75.5 MHz): CD_3OD as int. standard (δ49.0); FABMS: glycerol was used as a matrix; TLC: silica gel 60 F_{254}, 0.2 mm (Merck), spots were visualized under UV irradiation.

Prep. HPLC. HPLC system: LCX PU (Philips), injection valve: Rheodyne 7125 with 2 ml sample loop, column: 250 × 16 mm, LiChrosorb RP-18, 10μm (Gynkotek), detection: UV 255 nm (4-hydroxybenzoyl-), 270 nm (vanilloyl-), 282 nm (syringoyl-); isocratic systems, solvent: I, 8% MeOH in 1% aq. HOAc (4-hydroxybenzoyl-, vanilloyl-), II, 12% MeOH in 1% aq. HOAc, flow: 10 ml/min, collected fractions were freeze dried.

Preparation of 4,6-*benzylidene-α-*D-*glucose-sodium* **(1).** This reagent was synthesized by the Zervas method [39] by the action of benzaldehyde and glucose in the presence of $ZnCl_2$ and preparation of the sodium salt with NaOH.

Preparation of 4-*benzyloxy-benzoyl chlorides* (4-*hydroxybenzoyl* **2**, *vanilloyl* **3**, *syringoyl* **4**). These compounds were made by a method described for syringic acid [40] by reaction of the hydroxybenzoic acids with benzyl chloride in the presence of KOH and subsequent formation of the acid chlorides with PCl_5.

Preparation of 1-O-(4-*benzyloxy-benzoyl*)-4′,6′-O-*benzylidene-β-*D-*glucopyranoses* (4-*hydroxybenzoyl* **5**, *vanilloyl* **6**, *syringoyl* **7**): The acid chlorides **2–4** were dissolved in dry $CHCl_3$, a small excess of the sodium salt **1** was added and the mixture stirred at room temp. TLC [mobile phases: EtOH–EtOAc (9:1) for **5**, Et_2O–HOAc (100:2) for **6** and **7**] was used to indicate the end of the reaction after 90 hr (**5** and **6**) or 24 hr (**7**). $CHCl_3$ was removed *in vacuo*, the residues dissolved in EtOAc, centrifugated, washed with H_2O (2 ×), and the solns dried over Na_2SO_4. The crude products were used for hydration without further purification.

Preparation of 1-O-(4-*hydroxybenzoyl*)-*β-*D-*glucoses* (4-*hydroxybenzoyl* **8**, *vanilloyl* **9**, *syringoyl* **10**). Hydration was performed in EtOH–EtOAc (4:1) containing 0,7–0,8% of HOAc and 0,3% of 10% Pd/C at normal pressure and room temp. (40° for **8**). The reaction was followed by TLC (mobile phase: EtOH–EtOAc (9:1) for **8**, Et_2O–HOAc (50:1) for **9** and **10**) and required 4 hr (**8**), 3 hr (**9**) and 2 hr (**10**). After filtration and addition of H_2O the solutions were conc *in vacuo* and the aq. residues used for prep. HPLC.

1-O-(4-*Hydroxybenzoyl*)-*β-*D-*glucose* **(8)**. Mp 217–219°; IR ν^{KBr} cm^{-1}: 1710, 1295; UV: λ_{max} nm(log ε) 211(4,22), 260 (4,26); ^{1}H NMR (CD_3OD): δ 3,45–3,54 (*m*, 4H, 2-H,3-H,4-H,5-H), 3,74 (*dd*, 1H, $J_{6b,6a}$ = 12,1, $J_{6b,5}$ = 4,5, 6b-H), 3,90 (*d*, 1H, $J_{6a,5}$ = 1,7, 6a-H), 5,71 (*d*, 1H, $J_{1,2}$ = 7,9, 1-H), 6,88 (*d*, 2H, $J_{3',5',2',6'}$ = 8,9,3′-H,5′-H), 8,00 (*d*, 2H, 2′-H,6′-H); ^{13}C NMR (MHz, CD_3OD): δ62,4 (6-C), 71,2 (4-C), 74,1 (2-C), 78,2 (5-C), 78,9 (3-C), 96,1 (1-C), 116.2 (3′-C,5′-C), 133.3 (2′-C,6′-C)); FABMS: *m/z* 299 $[M-H]^-$.

1-O-*Vanilloyl-β-*D-*glucose* **(9)**. Mp 193–194°; IR (KBr) cm^{-1}: 1710, 1285; UV λ_{max} nm(log ε) 226(4,71), 266 (4,19), 294(3,89); ^{1}H NMR (CD_3OD): δ 3,44–3,57 (*m*, 4H, 2-H,3-H,4-H,5-H), 3,74 (*dd*, 1H, $J_{6b,6a}$ = 12,2, $J_{6b,5}$ = 4,4, 6b-H), 3,90 (*dd*, 1H, $J_{6a,5}$ = 1,6, 6a-H), 3,94 (*s*, 3H, OMe), 5,73 (*d*, 1H, $J_{1,2}$ = 7,9, 1-H), 6,90 (*d*, 1H, $J_{5',6'}$ = 8,2, 5′-H), 7,65–7,70 (*m*, 2H, 2′-H,6′-H); ^{13}C NMR (CD_3OD): δ 56,6 (OMe), 62,4 (6-C), 71,2 (4-C), 74,1 (2-C), 78,1 (5-C), 78,7 (3-C), 96,1 (1-C), 114.1 (2′-C) 116,0 (5′-C), 121,9 (1′-C), 125,7 (6′-C), 148,7 (3′-C), 153,3 (4′-C), 166,8 (C = O); FABMS: *m/z* 329 $[M-H]^-$.

1-O-*Syringoyl-β-*D-*glucose* **(10)**: Mp 197°; IR ν^{KBr} cm^{-1}: 1705, 1080; UV: λ_{max} nm(log ε) 218(4,42), 281(4,11); ^{1}H NMR (CD_3OD): δ3,45–3,57 (m, 4H, 2-H,3-H,4-H,5-H), 3,75 (*dd*, 1H, $J_{6b,6a}$ = 12,0, $J_{6b,5}$ = 4,6, 6b-H), 3,91 (*br d*, 1H, 6a-H), 3,93 (*s*, 6H, OMe), 5,74 (*d*, 1H, $J_{1,2}$ = 8,0, 1-H), 7,44 (*s*, 2H, 2′-H,6′-H); ^{13}C NMR (CD_3OD): δ 57,0 (OMe), 62,4 (6-C), 71,2 (4-C), 74,1 (2-C), 78,1 (5-C), 78,8 (3-C), 96,3 (1-C), 108,8 (2′-C,6′-C), 120,7 (1′-C), 142,7 (4′-C), 149,0 (3′-C, 5′-C), 166,8 (C = O); FABMS: *m/z* 359 $[M-H]^-$.

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