Phytochemistry, Vol. 28, No. 8, pp. 2071–2078, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00

STRUCTURES AND ACCUMULATION PATTERNS OF SOLUBLE AND INSOLUBLE PHENOLICS FROM NORWAY SPRUCE NEEDLES

DIETER STRACK,*† JÜRGEN HEILEMANN, VICTOR WRAY‡ and HERBERT DIRKS‡

Botanisches Institut der Universität zu Köln, Gyrhofstrasse 15, D-5000 Köln 41, F.R.G.; *Institut für Pharmazeutische Biologie der Technischen Universität Braunschweig, Mendelssohnstrasse 1, D-3300 Braunschweig; F.R.G.; ‡Gesellschaft für Biotechnologische Forschung (GBF), Mascheroder Weg 1, D-3300 Braunschweig, F.R.G.

(*Received* 5 *January* 1989)

Key Word Index—*Picea abies*; Pinaceae; Norway spruce; phenolics; identification; seasonal accumulation pattern; turnover; translocation; cell wall localization; flavonol glucosyltransferase.

Abstract—Twenty-two soluble phenolics have been isolated from Norway spruce needles and their structures elucidated on the basis of chromatographic (TLC, HPLC), chemical (hydrolysis), enzymic and spectroscopic (UV, NMR, MS) techniques. These phenolics have been quantified by HPLC during the first year of needle development from a forest near Bad Münstereifel (F.R.G.) and showed a differential accumulation pattern. Kaempferol 3-*O*-glucoside showed an interesting metabolism, indicating rapid turnover and/or translocation from a soluble to an insoluble (cell wall bound) pool. The enzyme involved in the formation of this flavonoid, UDP-glucose:flavonol glucosyltransferase, showed a marked transient increase in activity that correlated with the possible kaempferol 3-*O*-glucoside translocation.

INTRODUCTION

Although a large number of phenylpropanoid phenolics with many different types of structure are found in members of the Coniferae (e.g. [1–3]), little is known about the metabolism of these secondary compounds. Dittrich and Kandler [1] identified a variety of phenolic compounds in Norway spruce (*Picea abies* [L.] Karst.) needles and showed by $^{14}CO_2$ pulse chase experiments that there is not only a differential biosynthesis of these compounds during needle development, but also a rapid turnover of some of them.

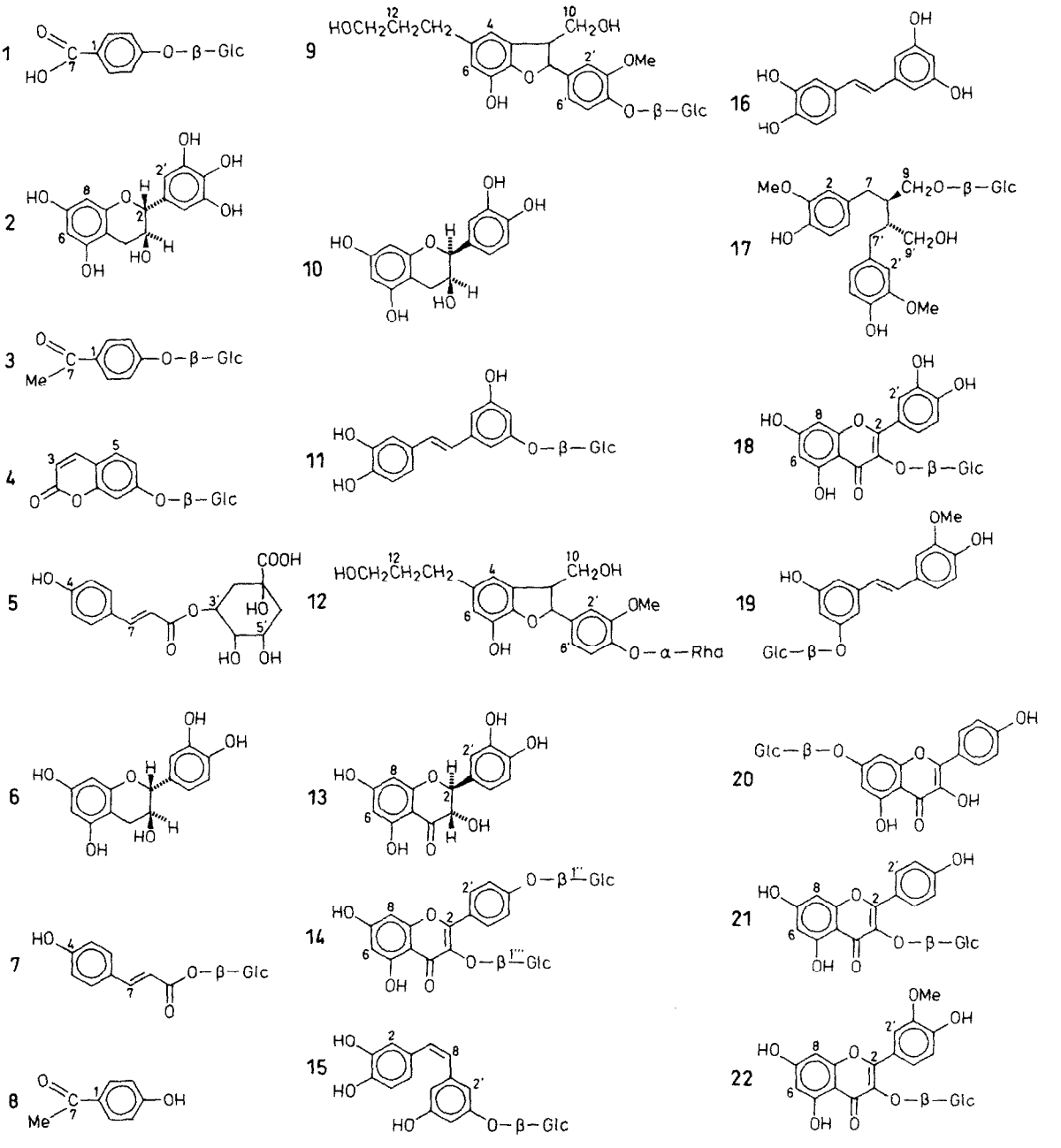
In needles of *Picea* species phenylpropanoids as diverse as acetophenones [4, 5], hydroxybenzoic acids [6], lignans [7], neolignans [8], hydroxycinnamic acids [1], coumarins [9, 10], stilbenes [11] and flavonoids [11] have been described. This affords the opportunity to study within one plant the biosynthesis of these compounds and the regulation of the diverse pathways leading to the different phenylpropanoid skeletons. It has been shown that some of these compounds have important biological functions such as toxicity against fungi [12–14] and inhibition of development of phytophagous insects by interaction of the phenolics with protein utilization [15, 16]. In this paper, we describe the structures of 22 phenolics which have been quantified by HPLC during the first year of needle development. In addition, the accumulation of cell wall-bound phenolics [17, 18] in these needles has also been measured. Finally we describe the metabolism of one of the Norway spruce phenolics, kaempferol 3-glucoside, and the time course of a flavonol glucosyltransferase activity involved in this metabolism.

† Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

Identification

Twenty-two soluble phenolics (**1–22**) have been isolated from methanol–chloroform–7 M formic acid (MCF)-extracts of Norway spruce needles on a semi-preparative scale by TLC on microcrystalline cellulose and open column chromatography on polyamide (perlon) and Sephadex LH-20. Their structures have been identified by direct chromatographic (TLC, HPLC) comparison with reference compounds, i.e. catechin (**6**), *E*-piceatannol 3′-*O*-β-glucopyranoside (**11**), *E*-piceatannol (**16**), isorhapontin (**19**), kaempferol 7-*O*-β-glucopyranoside (**20**), and/or by spectroscopic methods (UV/Vis-, NMR spectroscopy and mass spectrometry), i.e. 4-hydroxybenzoic acid 4-*O*-β-glucopyranoside (**1**), gallocatechin (**2**), picein (4-hydroxyacetophenone 4-*O*-β-glucopyranoside, **3**), skimmin (umbelliferone 7-*O*-β-glucopyranoside, **4**), 3′-*O*-(4-coumaroyl)-quinic acid (**5**), 1′-*O*-(4-coumaroyl)-β-glucopyranoside (**7**), 4-hydroxyacetophenone (**8**), 3-hydroxymethyl-5-(γ-hydroxy-*n*-propyl)-2-(3′-methoxy-4′-*O*-β-glucopyranosylphenyl)-2,3-dihydrobenzofuran (**9**), 3-hydroxymethyl-5-(γ-hydroxy-*n*-propyl)-2-(3′-methoxy-4′-*O*-α-rhamnopyranosyl)-2,3-dihydrobenzofuran (**12**), taxifolin (**13**), kaempferol 3,4′-di-*O*-β-glucopyranoside (**14**), *Z*-piceatannol 3′-*O*-β-glucopyranoside (**15**), *seco*-isolariciresinol 9-*O*-β-glucopyranoside (**17**), quercetin 3-*O*-β-glucopyranoside (**18**), kaempferol 3-*O*-β-glucopyranoside (**21**) and isorhamnetin 3-*O*-β-glucopyranoside (**22**). These compounds belong to different classes of phenolics: the acetophenones (**3**, **8**), hydroxybenzoic acids (**1**), hydroxycinnamic acids (**5**, **7**), coumarins (**4**), stilbenes (**11**, **15**, **16**, **19**), lignans (**17**), neolignans (**9**, **12**) and flavonoids (**2**, **6**, **10**, **13**, **14**, **18**, **20–22**).



In the majority of spectroscopic analyses the structures were readily deduced from the ^{1}H NMR data. Thus characteristic ^{1}H spectra were found for compounds **1**–**5**, **7**, **8**, **9**, **12**–**15**, **17**, **18**, **21** and **22**. In most cases where a sugar moiety was present the H-1 signal identified the configuration of the glycosidic linkage, while signals for H-2 and H-5 appeared as multiplets from which the exact identity of the sugar could not be determined. The identity of glucose was determined in each case by co-chromatography (TLC in S2 of the hydrolysis products and reference material) after acid hydrolysis. In addition, compounds **3**, **4**, **18**, **21** and **22** were synthesized enzymatically from UDP-glucose and the respective aglycones by a protein preparation from Norway spruce needles (data not shown, except for **21**).

For compound **14** the position of the second glucose unit at C-4′ was determined from the low field shift of H-3′ and H-5′ compared to compound **21** and the observation of a nuclear Overhauser enhancement of these protons upon irradiation of the glycosidic proton, H-1″. The characteristic coupling constant and shifts of H-3 and H-4 unambiguously indicated the coumarin residue of compound **4**, while the comparison of the shifts of H-5 to H-8 with those of coumarin [19] identified the substituent position as C-7.

Comparison of the ^{1}H and ^{13}C NMR data of compounds **9** and **12** showed that the only difference was the exchange of an α-rhamnose for a β-glucose moiety. The natures of the sugar and the various fragments in the molecule were found directly from the ^{1}H 2D COSY and 1D spectra in both

CD_3OD and DMSO-d_6. In the latter, the identification of the multiplicities and correlation of the hydroxyl protons immediately determined their positions in the molecule. Nuclear Overhauser effects established the relative positions of both the sugar and methoxyl groups in the second aromatic ring system and those of the hydroxypropyl and hydroxyl in the dihydrobenzofuran ring. These results confirmed the substituent positions obtained from the observation of long-range coupling between the aromatic protons and the various substituents. This information, together with the M_rs from FABMS, affords two alternative structures for the dihydrobenzofuran ring, one in which the hydroxyl group is adjacent to the oxygen of the dihydrofuran ring and a second in which it is adjacent to the carbon of this ring system. The absence of a high field quaternary carbon in the ^{13}C NMR spectrum (at *ca* 110 ppm) rules out the latter possibility.

The pseudo symmetry of compound **17** was easily seen from both the ^1H and ^{13}CNMR data. ^1H 2D COSY spectra established the fragments in the molecule and their shifts indicated the positions of the various substituents. The larger shift difference for the methylene protons of one of the oxygen-bearing methylene groups, caused by the proximity of the glucose moiety, allowed assigment of these protons.

Compounds **1–3**, **6**, **10**, **11**, **18–21** [1] and **8** [20], have been previously identified from Norway spruce needles by chemical methods. The occurrence of a coumaroylglucose has also been described [1], although this was 1′-*O*-(2-coumaroyl)-glucose and not 1′-*O*-(4-coumaroyl)-glucose (**7**). Some others have been spectroscopically investigated, i.e. **9**, **12** and **17** [7]. Compound **13** has been identified in the bark of Norway spruce [21] and is a characteristic constituent of the genus *Larix* [22]. Its glucoside has been found in *Pinus sylvestris* needles [23]. The remaining six compounds have been identified for the first time in Norway spruce needles. Although the tentative occurrence of skimmin (**4**) in *Picea abies* needles [10] has been described and an ester of quinic acid with 4-coumaric acid, which could possibly have the structure of **5**, was found in the cambium of *Tsuga heterophylla* [24]. **14** was found in the seed coat of *Ophipogon jaburan* [25] and **22** in *Cedrus atlantica* needles [26].

Metabolism

Representative HPLC traces of all the compounds identified from Norway spruce needles at the end of first year needle development are shown in Fig. 1. Detection at two different wavelengths (280 and 320 nm) showed that compounds **3**, **5**, **6**, **8** and **11** were the major components in these needles, reaching amounts of 10 to 150 nmol per needle, i.e. concentrations of *ca* 2–30 μmol/g needle fresh weight. These and nine of the minor components have been quantified during the first year of needle development (Table 1). By comparison with other commonly used extraction media, such as 80% aq. methanol, MCF gave the highest yield and the more complex pattern of extractable phenolics under the conditions described. The yields of the β-glucosides were identical in both media, excluding possible artificial hydrolytic reactions.

Whereas skimmin (**4**), 4-hydroxybenzoic acid 4-*O*-glucoside (**1**) and the 4-coumaric acid esters (**5**, **7**) accumulate slowly from May/June onwards, most of the others showed an increase in concentration during the later phases of needle differentiation, e.g. picein (**3**), catechin (**6**), and piceatannol 3′-*O*-glucoside (**11**). These results agree with some of those described earlier [1].

The ratio of 4-hydroxyacetophenone (**8**): picein (**3**) showed marked seasonal variations. It has also been found that there are individual (tree dependent) differences [5]. In addition, we found habitat-dependent aglycone:glucoside ratios (not shown).

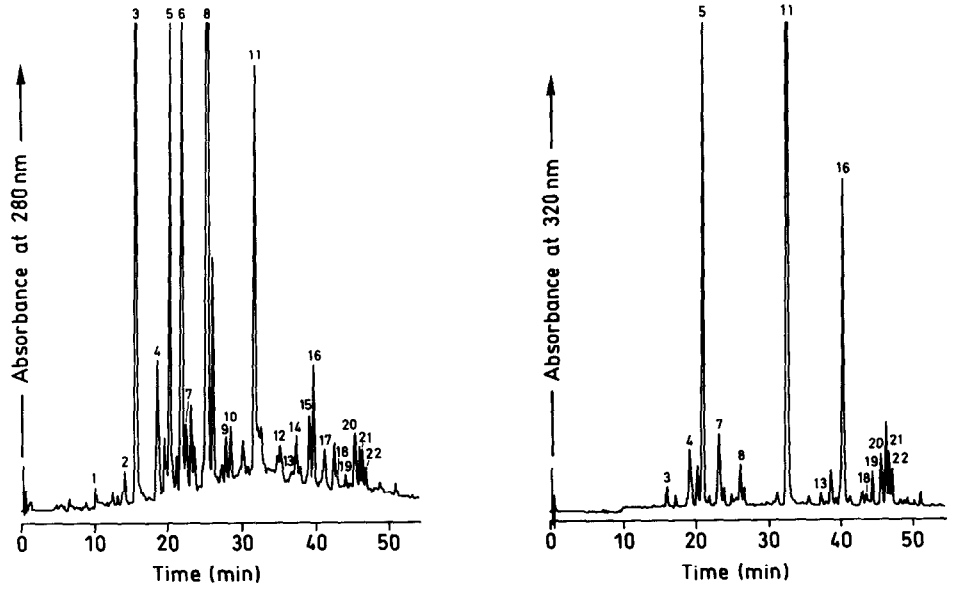


Fig. 1. Traces of HPLC analyses of soluble phenolics (**1–22**) from Norway spruce needles at the end of first year needle development. Nucleosil C_{18} column (4 μm, 250 × 4 mm i.d.; Macherey–Nagel, Düren, F.R.G.) at a flow rate of 1.5 ml/min with a two-step linear gradient: 40 min from solvent A (1.5% H_3PO_4 in H_2O) to 40% solvent B (H_2O–MeOH–MeCN, 1:1:1) in A and then to solvent B in 30 min; detection wavelength of 280 nm (left trace) or 320 nm (right trace) at 0.128 absorbance unit full scale.

Table 1. Amounts of soluble and insoluble phenolics (nmol/needle) in Norway spruce needles in May to December 1987 and January to April 1988

	Soluble phenolics														Insoluble phenolics		
Month	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**11**	**15**	**16**	**21**	**22**	**21**	Coum*	Fer*
May	0.1	—†	—	—	—	14	—	—	—	—	—	—	18	1	—	—	0.6
June	0.8	—	0.2	0.5	2	14	2	0.3	—	—	0.5	—	15	1	0.1	0.7	1.5
July	2	3	6	2.5	2	27	3	7	12	3	1	1	3	1	4.5	12	0.4
August	3	3	25	1.5	5.5	52	3	1	16	4	3	1	3	2	6	13	0.4
September	4	4	20	2	4	40	3	26	12	16	2	3	3	2	7	13	0.6
October	4	3	40	2	7	74	4	14	13	20	4	4	2	2	8	13	1
November	5	6	35	2.5	6	71	4	10	20	15	3	3	2	2	8	14	0.9
December	5	7	37	2.5	8	86	4	11	18	37	4	4	3	1	8	16.5	1.5
January	8	6	29	3	9	83	4	(70)‡	18	63	3	13	2	1	5	12	0.6
February	5	3	32	1	4	64	4	5	12	23	3	4	1	2	5	11	0.6
March	5	6	33	1	5	71	4	12	11	36	3	4	2	2	7	11	0.6
April	7	6	46	3	6	78	4	22	16	33	3	8	2	2	6	11	0.6

* Coum = 4-coumaric acid, Fer = ferulic acid.

† Not detected.

‡ Possible bad point.

One of the flavonol glycosides showed a remarkable and particularly interesting quantitative pattern. Kaempferol 3-*O*-glucoside (**21**) had its highest concentration in the youngest needles in May (18 nmol per needle) and thereafter declined rapidly to 3 nmol by July (Fig. 2). This shows, in conjunction with the results from Dittrich and Kandler [1] from $^{14}CO_2$ pulse chase experiments, that this compound undergoes a very active metabolism, e.g. degradation and/or further modification or conjugation. At least 40% is possibly translocated from the soluble pool into an ester-bound insoluble pool (Fig. 2), which probably represents a carbohydrate-lignin complex of a needle cell wall fraction [17]. Although there is no artificial degradation during liberation by the saponification procedure, which was tested with reference material (not shown), it has to be borne in mind, that it is probably impossible to liberate all of the compound from the site of its cell wall location. Studies on the linking structure, possibly an aliphatic dicarboxylic acid, commonly found in acylated flavonol glycosides [27], are in progress.

It is interesting to note the occurrence of hydroxycinnamic acid-acylated kaempferol glycosides in some other *Picea* species, e.g. in *P. obovata* [28] or *P. koraiensis* [29]. Similar structures might possibly be among the very minor unidentified components (see minor peaks in Fig. 1 which are not marked) in the *P. abies* needles investigated in the present study. Considering the interesting metabolism of the kaempferol 3-*O*-glucoside (**21**) connected with the cell walls of these needles, it is important to look for such acylated flavonoids, paying special attention to acylation with aliphatic dicarboxylic acids. It is, however, also likely that a part of the soluble kaempferol 3-*O*-glucoside (**21**) pool is being removed by oxidative polymerization reactions or degradative routes [30]. This problem awaits further investigation.

An enzymatic study on the biosynthesis of the β-glucosides of the phenolics in Norway spruce needles is in progress in our laboratories and will be published elsewhere. One of the first results which is included in the present work is the time course of a UDP-glucose:flavonol glucosyltransferase activity involved in the formation of kaempferol 3-*O*-glucoside (**21**). This flavonol glucosyltransferase activity is well known [31] but to the best of our knowledge this is the first time that this enzyme has been measured in Coniferae needles. It showed maximal activity near pH 8.4 in bicine buffer. The K_m values for kaempferol and UDP-glucose were near 100 and 285 μM, respectively. Besides kaempferol, quercetin and isorhamnetin were also efficiently glucosylated. This was investigated with the apparently homogenous enzyme as judged by gel electrophoresis and FPLC (not documented).

As expected, the enzyme was already present in the youngest needles at the beginning of May, showed a rapid activity increase from *ca* 250 to 725 pkat per mg protein up to the middle of June and rapidly decreased thereafter reaching 235 pkat at the beginning of July and *ca* 200 pkat in the following months. The same type of activity development is obtained when the activity per needle (highest activity at *ca* 2 pkat) or per needle fresh weight (not shown) are plotted. This transient activity increase during the rapid decrease of kaempferol 3-*O*-glucose (**21**), in contrast to other examples of correlations between accumulating phenolics and enzymes exhibiting their highest activities in stages of highest product accumulation rates (e.g. [32]), indicates that the soluble pool of this compound undergoes a rapid turnover and/or translocation to a cell wall compartment.

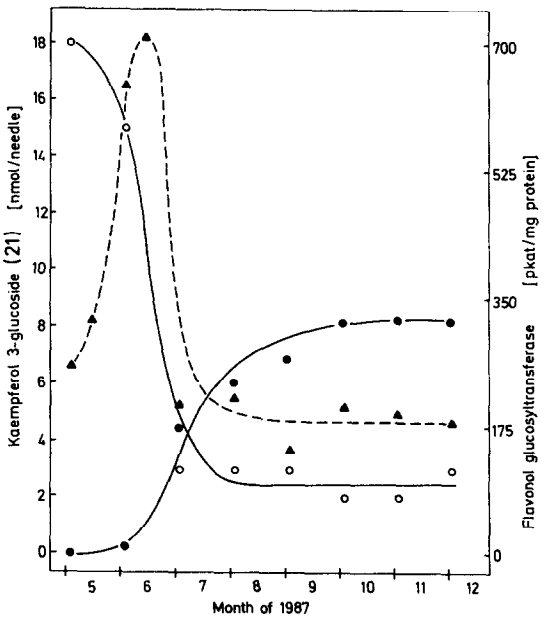


Fig. 2. Quantitative patterns of soluble (○—○) and insoluble (cell wall bound) (●—●) kaempferol 3-*O*-glucoside (**21**) as well as of the UDP-glucose:flavonol 3-*O*-glucosyltransferase (flavonol glucosyltransferase) activity (▲—▲) during the first year of Norway spruce needle development.

EXPERIMENTAL

Plant material. Needles from Norway spruce (*Picea abies* L. Karst.) were collected in 1987 and 1988 from a forest near Bad Münstereifel, F.R.G. The trees were *ca* 35-year-old and showed neither visual infections nor signs of forest decline. The needles were harvested from branches at a height of *ca* 5 m and were immediately frozen with liq. N_2. Once in the laboratory, they were stored at −20 or −70° for metabolite and enzyme extractions, respectively.

Extraction of soluble phenolics. Analytical. Needles (1 g fr. wt) were homogenized in a mortar in the presence of liq. N_2 and quartz sand. The resulting powder was suspended in 10 ml MeOH–$CHCl_3$–7 M HCO_2H (12:5:3; MCF) [33], allowed to stand for 1 hr with continuous stirring and centrifuged. The pellet was re-extracted (× 2) with 5 ml each of the same solvent. The combined extracts were taken to dryness (*in vacuo*) and the residue redissolved in 5 ml MCF from which 100 μl were mixed with 100 μl MCF and 200 μl H_2O and vigorously stirred. The aq. upper phase (300 μl) was used for HPLC analysis. Alternatively extractions were performed with 80% aq. MeOH. Needles harvested from four trees were combined and two extracts analysed separately from which the mean values (HPLC) were calculated.

Semipreparative. Needles (50 g fr. wt) were homogenized in the presence of liq. N_2 in a centrifugal mill (Retsch, type ZM 1). The resulting fine powder was suspended in 500 ml MCF and allowed to stand for 3 hr with continuous stirring. After filtration

the insoluble residue was re-extracted twice with the same solvent. The combined extracts (*ca* 1 l) were taken to dryness (*in vacuo*) and the residue redissolved in 20 ml MCF to which 30 ml $CHCl_3$–H_2O (1:2) was added. This was vigorously stirred, centrifuged and the H_2O phase removed, taken to dryness (*in vacuo*) and the residue redissolved in 5 ml H_2O.

Cell wall preparation and isolation of insoluble phenolics. This was done essentially as described previously [18]. The pellets from the analytical preparation of soluble phenolics were consecutively treated with the following solvents (20 ml) each for 15 min stirring followed by centrifugation: (i) × 1 MeOH; (ii) × 3 H_2O; (iii) × 5 MeOH; (iv) × 2 Me_2CO; (v) × 2 Et_2O. The remaining insoluble material was dried at 60° (*ca* 1 hr). Then it was suspended in 8 ml hot (80°) 1 M aq. NaOH and allowed to stand for *ca* 17 hr at room temp. with continuous stirring. The hydrolysates were centrifuged and aliquots (1 ml) of the supernatants were acidified with 100 μl H_3PO_4. After centrifugation 20 μl of the clear supernatants were analysed by HPLC for the presence of phenolics.

Isolation and purification of soluble phenolics. For isolation of phenolics for spectroscopic (NMR, MS) analyses the preparative extract was fractionated on a polyamide column (perlon, CC 6, 70 × 3.5 cm i.d.; Macherey–Nagel, Düren) using H_2O (elution of compounds **1**, **3**, **4**, **7**–**9**, **12**, **17**), 40% aq. MeOH (compounds **11**, **14**, **15**), 80% aq. MeOH (compounds **2**, **13**, **16**, **18**, **21**, **22**), 100% MeOH and 0.02% NH_4OH in MeOH (compound **5**). These fractions were taken to dryness (*in vacuo*) and the residues redissolved in 2 ml 50% aq. MeOH. The compounds were separated on TLC in Sl: **1**, R_f 0.31 (UV at 254 nm, absorbance), **2**, R_f 0.01 (UV at 254 nm, absorbance), **3**, R_f 0.56 (UV at 254 nm, absorbance), **4**, R_f 0.50 (UV at 350 nm, absorbance), **5**, R_f 0.32 (UV at 350 nm, absorbance; $+NH_3$, blue fluorescence), **7**, R_f 0.42 (UV at 350 nm, absorbance; $+NH_3$, blue fluorescence), **8**, R_f 0.93 (UV at 254 nm, absorbance), **9**, R_f 0.40 (UV at 350 nm, absorbance), **11**, R_f 0.01 (UV at 350 nm, bright blue fluorescence), **12**, R_f 0.57 (UV at 350 nm, absorbance), **13**, R_f 0.28 (UV at 350 nm, absorbance), **14**, R_f 0.16 (UV at 350 nm, absorbance), **15**, R_f 0.16 (UV at 350 nm, bright blue fluorescence), **16**, R_f 0.10 (UV at 350 nm, violet fluorescence), **17**, R_f 0.63 (UV at 350 nm, absorbance), **18**, R_f 0.12 (UV at 350 nm, absorbance; $+NH_3$, yellow fluorescence), **21**, R_f 0.27 (UV at 350 nm, absorbance; $+NH_3$, yellow-green fluorescence) and **22**, R_f 0.32 (UV at 350 nm, absorbance; $+NH_3$, yellow fluorescence). The TLC bands were scraped off and eluted from the cellulose with 50% aq. MeOH (60 min). Final purifications were achieved on a Sephadex LH-20 (102 × 2.5 cm i.d.; Pharmacia, Uppsala, Sweden) elution with 50% aq. MeOH.

Acid hydrolysis and product identification. 60 μl of a soln of the respective glycoside was mixed with 20 μl 2 M HCl and kept for 20–60 min at 100°. The aglycones were separated from the liberated sugars by repeated extraction with Et_2O. TLC of the sugars in S2 [34] gave rhamnose from compound **12** and glucose in the other cases.

TLC. Microcrystalline cellulose ('Avicel', Macherey–Nagel, Düren) plates (preparative: 20 cm × 40 cm; analytical: 20 cm × 20 cm) with S1 ($CHCl_3$–HOAc, 3:2, H_2O satd) and silica gel (impregnated with 0.5 M NaH_2PO_4 in H_2O–MeOH, 3:1) with S2 (*iso*-PrOH–Me_2CO–0.1 M lactic acid, 2:2:1) according to Hansen [34].

HPLC. The liquid chromatograph (two-pump system) and the data processor (Chromatopac C-R3A) for quantitative analyses were from Pharmacia LKB (Freiburg) and Shimadzu (Kyoto, Japan), respectively. Injections were performed via a Rheodyne rotary valve (Cotati, CA, U.S.A.) with a 20 μl loop. For details of the chromatographic conditions see Fig. 1. The following authentic compounds were used as ext. std for quantification and/or identification: 4-hydroxyacetophenone, umbelliferone, 4-coumaric acid, epicatechin and kaempferol 3-*O*-rutinoside from Roth, Karlsruhe; isorhapontin, produced by UV (254 nm) irradiation of rhapontin from Aldrich, Steinheim; and catechin from Serva, Heidelberg; *E*-picetannol as a hydrolysis product from compound **11**; kaempferol 7-*O*-glucoside from a collection E. Wollenweber (Darmstadt).

UV/Vis spectroscopy. UV/Vis spectra were recorded in MeOH. Analyses of the flavonol glycosides gave results (± shift reagents) essentially identical to those described in ref. [35].

Protein preparation and determination of UDP-glucose:flavonol 3-O-glucosyltransferase (flavonol glucosyltransferase). Needles (1 g fr. wt) were ground in a mortar in the presence of liq. N_2, 0.5 g quartz sand, 2 g insoluble Polyclar AT (equilibrated with the extraction buffer) in 15 ml H_3BO_3 buffer (25 mM, pH 9.5) containing 1.5% Triton X-100 (Merck, Darmstadt). The homogenate was allowed to stand for 30 min with continuous stirring and was then passed through Miracloth (Calbiochem, Frankfurt) and the filtrate centrifuged for 20 min at 48,000 g. The enzyme activity was pptd by $(NH_4)_2SO_4$ (35–80% saturation) and the pellet redissolved in 2.5 ml Tris–HCl (25 mM, pH 8.0) which was filtered through Sephadex G-25 (Pharmacia PD-10 column). The eluate (3.5 ml) was used as a source of enzyme activity. The remaining Triton X-100 was removed by treatment for 12 hr of the eluate with 50 mg Bio Bead SM-2 (BioRad, München) [36]. Protein content was determined by the method of ref. [37] using bovine serum albumin as standard.

The reaction mixture (total vol of 100 μl) contained 0.25 mM kaempferol (Roth, Karlsruhe; dissolved in 25 μl ethyleneglycolmonomethylether; Merck, Darmstadt), 0.5 mM UDP-glucose (Sigma, Deisenhofen; dissolved in H_2O), 25 mM bicine buffer (pH 8.0) and 25 μl enzyme preparation. The reaction was started by the addition of enzyme and after incubation for 45 min was terminated by cooling the mixture in liq. N_2. Enzyme activity was determined by HPLC analysis of the reaction mixture. Chromatographic conditions: flow rate 1 ml/min, linear gradient elution in 8 min from 50% solvent B (1.5% H_3PO_4, 20% HOAc and 25% MeCN in H_2O) in A (1.5% H_3PO_4 in H_2O) to solvent B, followed by 6 min at 100% B; detection 350 nm.

NMR and MS. The ^1H and ^{13}C NMR spectra were recorded at ambient temp. on Bruker WM 400 (^1H: 400 MHz, ^{13}C: 100 MHz) and AM 300 (^1H: 300 MHz, ^{13}C: 75 MHz) NMR spectrometers locked to the major deuterium resonance of the solvent. The 2D COSY ^1H spectra were recorded with a 90°-t_1-45°-FID (t_2) pulse sequence. In all cases the data were multiplied by sinebell-functions and one level of zero-filling was used for both t_1 and t_2. All 1D (normal and ^1H NOE difference) and 2D spectra were recorded using the standard Bruker software package. Chemical shifts are given in ppm relative to TMS and coupling constants in Hz. The abbreviation "*d*" implies the signal is one half of an AA'BB' spin system, and *d(complex)* is the signal of a glycosidic proton (H-1) showing second order characteristics from coupling with a second order spin system, i.e. H-2 and H-3 have almost identical chemical shifts.

Negative ion fast atom bombardment (FAB) mass spectra were recorded on a Kratos MS 50 mass spectrometer equipped with a Kratos FAB source. Glycerol was used as matrix.

4-Hydroxybenzoic acid 4-O-*β-glucopyranoside* (**1**). ^1H NMR (CD_3OD): δ 7.990 ["*d*", H-2, H-6, $J(2–3)+(2–5)=8.9$], 7.151 ["*d*", H-3, H-5], 5.036 [*d*, H-1', $J(1'–2')=7.5$], 3.940 [*d*, *d*, H-6'A, $J(6'A-5)=2.0$, $J(6'A–6'B)=12.0$], 3.744 [*d*, *d*, H-6'B, $J(6'B-5)=5.4$], 3.57–3.22 (*m*, H-2', H-3', H-4', H-5'). Irradiation at H-1' gave an NOE for H-3/H-5, H-3' and H-5'.

Gallocatechin (**2**). ^1H NMR (CD_3OD): δ 6.439 [*d*, H-2', H-6', $J(2'–2)=0.5$], 5.958 [*d*, H-6, $J(6–8)=2.3$], 5.898 [*d*, H-8], 4.564

[*d*, *d*, H-2, *J*(2–3) = 7.1], 4.002 [*d*, *d*, *d*, H-3, *J*(3-4a) = 7.8, *J*(3-4e) = 5.3], 2.849 [*d*, *d*, H-4e, *J*(4e-4a) = 16.1], 2.539 [*d*, *d*, H-4a]. FABMS: *m/z* 305 $[M-H]^-$.

Picein (4-O-*β*-*glucopyranosylacetophenone*) (**3**). ^{1}H NMR (CD_3OD): *δ* 8.019 ["*d*", H-2, H-6, *J*(2–3) + (2–5) = 8.9], 7.209 ["*d*", H-3, H-5], 5.072 [*d*(*complex*), H-1′, *J*(1′–2′) = 7.6], 3.944 [*d*, *d*, H-6′A, *J*(6′A-5) = 2.2, *J*(6′A-6′B) = 12.0], 3.744 [*d*, *d*, H-6′B, *J*(6′B-5′) = 5.6], 3.56 (*m*, H-5′), 3.51 (*m*, H-2′), 3.4–3.6 (*m*, H-3′, H-4′), 2.601 (*s*, H-8). ^{13}C NMR (CD_3OD): *δ* 199.46 (*s*, C-7), 163.08 (*s*, C-4), 132.81 (*s*, C-1), 131.60 (*d*, C-2, C-6), 117.37 (*d*, C-3, C-5), 101.73 (*d*, C-1′), 78.35, 78.04 (*d* × 2, C-3′, C-5′), 74.87 (*d*, C-2′), 71.41 (*d*, C-4′), 62.57 (*t*, C-6′), 26.39 (*q*, C-8). FABMS: *m/z* 297 $[M-H]^-$, 135 $[M-C_6H_{11}O_5]^-$.

Skimmin (*umbelliferone* 7-O-*β*-*glucopyranoside*) (**4**). ^{1}H NMR (CD_3OD): *δ* 7.944 [*d*, H-4, *J*(4–3) = 9.8], 7.610 [*d*, H-5, *J*(5–6) = 9.1], 7.15–7.12 (*m*, H-6, H-8), 6.331 (*d*, H-3), 5.078 [*d*(*complex*), H-1′, *J*(1′–2′) = 7.4], 3.952 [*d*, *d*, H-6′A, *J*(6′A-6′B) = 12.6], 3.744 [*d*, *d*, H-6′B, *J*(6′B-5′) = 5.7], 3.60–3.18 (*m*, H-2′, H-3′, H-4′, H-5′).

3′-O-(E-4-*coumaroyl*)-*Quinic acid* (**5**). ^{1}H NMR (CD_3OD): *δ* 7.694 [*d*, H-7, *J*(7–8) = 15.9], 7.501 ["*d*", H-2, H-6, *J*(2–3) + *J*(2–5) = 8.7], 6.842 ("*d*", H-3, H-5), 6.415 (*d*, H-8), 5.43 (*m*, H-3′), 4.11 (*m*, H-5′), 3.77 (*m*, H-4′), 2.17–2.11 (*m*, H-2′a,e), 2.17–2.08 (*m*, H-6′e), 2.01–1.93 (*m*, H-6′a). FABMS: *m/z* 337 $[M-H]^-$, 191 $[M-C_9H_7O_2]^-$, 163 $[C_9H_7O_3]^-$.

1′-O-(E-4-*Coumaroyl*)-*β*-*glucopyranose* (**7**). ^{1}H NMR (CD_3OD): *δ* 7.768 [*d*, H-7, *J*(7–8) = 15.7], 7.523 ["*d*", H-2, H-6, *J*(2–3) + *J*(2–5) = 8.6], 6.853 ("*d*", H-3, H-5), 6.411 (*d*, H-8), 5.584 [*d*, H-1′, *J*(1′–2′) = 8.0], 3.91 (*m*, H-6′A), 3.74 (*m*, H-6′B), 3.52–3.26 (*m*, H-2′, H-3′, H-4′, H-5′). FABMS: *m/z* 325 $[M-H]^-$.

4-*Hydroxyacetophenone* (**8**). ^{1}H NMR (CD_3OD): *δ* 7.926 ["*d*", H-2, H-6, *J*(2–3) + *J*(2–5) = 8.9], 6.876 ("*d*", H-3, H-5), 2.564 (*s*, 1-COMe).

3-*Hydroxymethyl*-5-(*γ*-*hydroxy*-n-*propyl*)-2-(3′-*methoxy*-4′-O-*β*-*glucopyranosylphenyl*)-2,3-*dihydrobenzofuran* (**9**). ^{1}H NMR (DMSO-d_6): *δ* 9.107 (*br s*, 7-OH), 7.061 [*d*, H-5′, *J*(5′–6′) = 8.4], 6.987 [*d*, H-2′, *J*(2′–6′) = 1.7], 6.870 (*d*, *d*, H-5′), 6.524 (*br s*, H-4), 6.488 (*br s*, H-6), 5.443 [*d*, H-2, *J*(2–3) = 6.4], 5.24, 5.09 (*br d* × 2, 2″-OH, 3″-OH), 5.03 (*t*, 10-OH), 5.01 (*d*, 4″-OH), 4.889 [*d*, H-1″, *J*(1″–2″) = 7.5], 4.51 [*t*, 6″-OH, *J*(6″OH-5″) = ~5], 4.43 [*t*, 13-OH, *J*(13-OH-13) = ~5], 3.747 (*s*, 3′-OMe), 3.75–3.54 (*m*, H-10), 3.63 (*m*, H-6″A), 3.40 (*m*, H-6″B), 3.38 (*m*, H-13), 3.37 (*m*, H-3), 3.30–3.22 (*m*, H-2″, H-3″, H-5″), 3.13 (*m*, H-4″), 2.445 ["*t*", H-11, *J*(11 – 12) = ~7], 1.638 [*t*, *d*, H-12, *J*(12 – 13) = ~7]. Irradiation of H-11 gave NOE's at H-4, H-6 and H-12, of H-13 NOE's at H-12, of H-1″ NOE's at H-3″ and H-5″, of H-4 NOE at H-11, and of H-6 NOE at H-11. Long-range coupling was observed in the 2D COSY spectrum between H-2 and H-2′, H-2 and H-6′, H-3 and H-4, H-3 and H-6, H-4 and H-11, H-6 and H-11, H-2′ and 3′-OMe, and H-5′ and H-1″. ^{13}C NMR (CD_3OD): *δ* 151.17 (*s*, C-9), 147.66, 146.51 (*s* × 2, C-3′, C-4′), 141.92, 138.87, 136.97 (*s* × 3, C-5, C-7, C-8), 129.60 (*s*, C-1′), 119.51 (*d*, C-6′), 118.53 (*d*, C-6), 117.20 (*d*, C-5′), 116.72 (*d*, C-4), 111.69 (*d*, C-2′), 103.14 (*d*, C-1″), 88.27 (*d*, C-2), 78.25, 77.97 (*d* × 2, C-3″, C-5″), 75.04 (*d*, C-2″), 71.54 (*d*, C-4″), 65.32 (*t*, C-10), 62.69 (*t*, C-6″), 62.39 (*t*, C-13), 56.94 (*q*, 3′-OMe), 55.92 (*d*, C-3), 35.69 (*t*, C-12), 32.71 (*t*, C-11). FABMS: *m/z* 1015 $[2M-H]^-$, 599 $[M+\text{glycerol}-H]^-$, 507 $[M-H]^-$.

3-*Hydroxymethyl*-5-(*γ*-*hydroxy*-n-*propyl*)-2-(3′-*methoxy*-4′-O-*α*-*rhamnopyranosylphenyl*)-2,3-*dihydrobenzofuran* (**12**). ^{1}H NMR (CD_3OD): *δ* 7.121 [*d*, H-5′, *J*(5′–6′) = 8.4], 7.103 [*d*, H-2′, *J*(2′–6′) = 2.0], 6.978 (*d*, *d*, H-6′), 6.638 (*br s*, H-4), 6.614 (*br s*, H-6), 5.597 [*d*, H-2, *J*(2–3) = 5.8], 5.375 [*d*, H-1″, *J*(1″–2″) = 1.7], 4.098 [*d*, *d*, H-2″ *J*(2″–3″) = 3.4], 3.911 [*d*, *d*, H-3″, *J*(3″–4″) = 9.5], 3.877 [*d*, *d*, H-10A, *J*(10A-3) = 5.8, *J*(10A-10B) = 11.0], 3.849 (*s*, 3′-OMe), 3.84 (*m*, H-5″), 3.786 [*d*, *d*, H-10B, *J*(10B-3) = 7.6], 3.596 [*t*, H-13, *J*(13–12) = 6.5], 3.485 [*t*, H-4″, *J*(4″–5″) = 9.5], 3.485 (*d*, *d*,*d*, H-3), 2.603 [*d*, *d*, H-11, *J*(11–12) = 7.4, 8.0], 1.830 (*m*, H-12), 1.256 [*d*, H-6″, *J*(6″–5″) = 6.2]. ^{13}C NMR (CD_3OD): *δ* 151.99 (*s*, C-9), 146.59, 146.52 (*s* × 2, C-3′, C-4′), 141.92, 139.19, 136.97 (*s* × 3, C-5, C-7, C-8), 129.64 (*s*, C-1′), 119.78 (*d*, C-6′), 119.30 (*d*, C-6), 117.21 (*d*, C-5′), 116.73 (*d*, C-4), 111.76 (*d*, C-2′), 101.63 (*d*, C-1″), 88.36 (*d*, C-2), 73.97, 72.40, 72.12, 70.87 (*d* × 4, C-2″, C-3″, C-4″, C-5″), 65.35 (*t*, C-10), 62.40 (*t*, C-13), 56.68 (*q*, 3′-OMe), 55.90 (*d*, C-3), 35.71 (*t*, C-12), 32.72 (*t*, C-11), 17.91 (*q*, C-6″). FABMS: *m/z* 583 $[M+\text{glycerol}-H]^-$, 491 $[M-H]^-$.

Taxifolin (**13**). ^{1}H NMR (CD_3OD): *δ* 6.999 [*d*, H-2′, *J*(2′–6′) = 1.9], 6.885 [*d*, *d*, H-6′, *J*(6′–5′) = 8.0], 6.834 (*d*, H-5′), 5.923, 5.886 [*d* × 2, H-6, H-8, *J*(6–8) = 2.1], 4.94 (H-2 from COSY), 4.521 [*d*, H-3, *J*(3–2) = 11.4]. FABMS: *m/z* 303 $[M-H]^-$.

Kaempferol 3,4′-*di*-O-*β*-*glucopyranoside* (**14**). ^{1}H NMR (CD_3OD): *δ* 8.180 ["*d*", H-2′, H-6′, *J*(2′–3′) + *J*(2′–5′) = 9.1], 7.295 ("*d*", H-3′, H-5′), 6.454 [*d*, H-8, *J*(8–6) = 2.1], 6.257 (*d*, H-6), 5.325 [*d*(*complex*), H-1‴, *J*(1‴–2‴) = 7.7], 5.081 [*d*(*complex*), H-1″, *J*(1″–2″) = 7.6], 4.02–3.95 (*m*, H-6″A, H-6‴A), 3.80–3.70 (*m*, H-6″B, H-6‴B), 3.60–3.20 (*m*, H-2″, H-2‴, H-3″, H-3‴, H-4″, H-4‴, H-5″, H-5‴]. Irradiation of H-1″ gave NOE's at H-3′, H-5′. FABMS: *m/z* 701 $[M+\text{glycerol}-H]^-$, 609 $[M-H]^-$.

Z-*Piceatannol* 3′-O-*β*-*glucopyranoside* (**15**). ^{1}H NMR (CD_3OD): *δ* 6.789 [*d*, H-2, *J*(2–6) = 1.9], 6.689 [*d*, H-5, *J*(5–6) = 8.1], 6.637 [*d*, *d*, *d*, H-6, *J*(6–7) = 0.4], 6.599 [*d*, *d*, H-2′, *J*(2′–4′) = 1.5, *J*(2′–6′) = 1.5], 6.465 [*d*, *d*, H-7, *J*(7–8) = 12.2], 6.436 (*d*, H-6′), 6.411 (*d*, H-4′), 6.352 (*d*, H-8), 4.703 [*d*, H-1″, *J*(1″–2″) = 7.2], 3.776 [*d*, *d*, H-6″A, *J*(6″A-5″) = 2.6, *J*(6″A-6″B) = 12.2], 3.728 [*d*, *d*, H-6″B, *J*(6″B-5″) = 4.4], 3.5–3.4 (*m*, H-2″, H-3″, H-4″), 3.265 (*m*, H-5″). Irradiation at H-1″ gave NOE's for H-2′ and H-4′. ^{13}C NMR (CD_3OD): *δ* 160.07, 159.26 (*s* × 2, C-3′, C-5′), 145.85 (*s*, C-3, C-4), 141.01 (*s*, C-1′), 131.63, 129.15 (*d* × 2, C-7, C-8), 130.65 (*s*, C-1), 122.25 (*d*, C-6), 117.07, 116.25 (*d* × 2, C-2, C-5), 111.43, 109.33 (*d* × 2, C-2′, C-6′), 104.21 (*d*, C-4′), 102.54 (*d*, C-1″), 78.01, 77.77 (*d* × 2, C-3″, C-5″), 74.90 (*d*, C-2″), 71.13 (*d*, C-4″), 62.27 (*t*, C-6″). FABMS: *m/z* 405 $[M-H]^-$, 243 $[M-C_6H_{11}O_5]^-$.

Secoisolariciresinol 9-O-*β*-*glucopyranoside* (**17**). ^{1}H NMR (CD_3OD): *δ* 6.707, 6.701 [*d* × 2, H-5, H-5′, *J*(5–6) = 8.0, *J*(5′–6′) = 8.0], 6.685, 6.660 [*d*, *d*, (× 2) H-2, H-2′, *J*(2–6) = 1.9, *J*(2′–6′) = 1.9], 6.616, 6.608 (*d*, *d*, (× 2) H-6, H-6′), 4.233 [*d*, H-1″, *J*(1″–2″) = 7.7], 3.93 (*m*, H-9A), 3.92 (*m*, H-6″A), 3.791 (*s*, 3 –, 3′-OMe), 3.71 (*m*, H-6″B), 3.71 (*m*, H-9′A), 3.61 (*m*, H-9′B), 3.58 (*m*, H-9B), 3.42–3.29 (*m*, H-3″, H-4″), 3.32 (*m*, H-5″), 3.246 [*d*, *d*, H-2″, *J*(2″–3″) = 9.1], 2.747 [*d*, *d*, H-7A, *J*(7A-8) = 7.7, *J*(7A-7B) = 13.7], 2.659 (*m*, H-7′AB), 2.636 [*d*, *d*, H-7B, *J*(7B-8) = ~8], 2.12 (*m*, H-8), 2.03 (*m*, H-8′). ^{13}C NMR (CD_3OD): *δ* = 148.90, 148.86 (*s* × 2, C-3, C-3′), 145.60 (*s*, C-4, C-4′), 134.14, 134.08 (*s* × 2, C-1, C-1′), 122.90, 122.85 (*d* × 2, C-6, C-6′), 115.91 (*d*, C-5, C-5′), 113.98, 113.86 (*d* × 2, C-2, C-2′), 104.70 (*d*, C-1″), 78.32, 77.99 (*d* × 2, C-2″, C-3″), 75.32 (*d*, C-5″), 71.88 (*d*, C-4″), 70.61 (*t*, C-9), 62.96 (*t*, C-6″, C-9′), 44.25 (*d*, C-8′), 41.86 (*d*, C-8), 35.77, 35.70 (*t* × 2, C-7, C-7′). FABMS: *m/z* 523 $[M-H]^-$, 361 $[M-C_6H_{11}O_5]^-$.

Quercetin 3-O-*β*-*glucopyranoside* (**18**). ^{1}H NMR (CD_3OD): *δ* 7.751 [*d*, H-2′, *J*(2′–6′) = 2.1], 7.632 [*d*, *d*, H-6′, *J*(6′–5′) = 8.4], 6.908 (*d*, H-5′), 6.416 [*d*, H-8, *J*(8–6) = 2.1], 6.230 (*d*, H-6), 5.285 [*d*, H-1″, *J*(1″–2″) = 7.5], 3.755 [*d*, *d*, H-6″A, *J*(6″A-5″) = 2.4, *J*(6″A-6″B) = 11.8], 3.615 [*d*, *d*, H-6″B, *J*(6″B-5″) = 5.4], 3.54–3.19 (*m*, H-2″, H-3″, H-4″, H-5″).

Kaempferol 3-O-*β*-*glucopyranoside* (**21**). ^{1}H NMR (CD_3OD): *δ* 8.100 ["*d*", H-2′, H-6′, *J*(2′–3′) + *J*(2′–5′) = 9.0], 6.928 ("*d*", H-3′, H-5′), 6.425 [*d*, H-8, *J*(8–6) = 2.1], 6.232 (*d*, H-6), 5.287 [*d*(*complex*), H-1″, *J*(1″–2″) = 7.6], 3.734 [*d*, *d*, H-6″A, *J*(6″A-5″) = 2.4, *J*(6″A-6″B) = 11.9], 3.569 [*d*, *d*, H-6″B, *J*(6″B-5″) = 5.3], 3.50–3.20 (*m*, H-2‴, H-3″, H-4″, H-5″).

Isorhamnetin 3-O-*β*-*glucopyranoside* (**22**). ^{1}H NMR (CD_3OD): *δ* 7.977 [*d*, H-2′, *J*(2′–6′) = 2.0], 7.632 [*d*, *d*, H-6′, *J*(6′–5′) = 8.5], 6.952 (*d*, H-5′), 6.452 [*d*, H-8, *J*(8–6) = 2.1], 6.251 (*d*, H-6), 5.463 [*d*(*complex*), H-1″, *J*(1″–2″) = 7.6], 3.988 (*s*, 4′-OMe), 3.777 [*d*, *d*,

H-6″A, J(6″A-5″] = 2.1, J(6″A-6″B) = 11.9], 3.600 [*d*, *d*, H-6″B, J(6″B-5) = 5.3], 3.44–3.19 (*m*, H-2″, H-3″, H-4″, H-5″).

Acknowledgements—Financial support to D.S. by the Deutsche Forschungsgemeinschaft (Schwerpunktsprogramm "Physiologie der Bäume"), the Minister für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen (Forschungsprogramm "Luftverunreinigungen und Waldschäden") and the Fonds der Chemischen Industrie is gratefully acknowledged. We thank E. Wollenweber (Darmstadt) for a supply of reference kaempferol 7-*O*-glucoside, Regina Bungartz, Eva Klinkott, Martina Mömken and Ruth Nowak for help in the isolation of some of the Norway spruce phenolics as well as Ute Engel for dependable technical assistance. Some of the MS spectra were recorded by the late Dr L. Grotjahn.

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