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PHENOLS FROM *FAGUS SYLVATICA* AND THEIR ROLE IN DEFENCE AGAINST *CRYPTOCOCCUS FAGISUGA*

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Key Word Index—*Fagus sylvatica*; *Fagaceae*; European beech; *Cryptococcus fagisuga*; beech scale; flavanone glycosides; monolignol glucosides; beech bark disease; defence mechanism.

Abstract—In extracts of inner and outer bark of *Fagus sylvatica*, qualitative dependence of the phenolic composition on infection with *Cryptococcus fagisuga* feeding in the parenchyma tissue was observed. The seven major compounds were isolated and completely assigned, mainly by two-dimensional NMR techniques. Highest concentrations of (2*R*,3*R*)-(+)-glucodistylin, (2*S*,3*S*)-(−)-glucodistylin and 3-*O*-(β-D-xylopyranosyl)taxifolin occur in European beeches strongly infested with beech scale The concentration of *cis*-coniferin was lowered after attack, while the concentrations of catechin, *cis*-isoconiferin and *cis*-syringin remained unaffected. The changes are discussed as a defence reaction.

INTRODUCTION

The beech bark disease is a complex disease of noticeable economic importance for *Fagus sylvatica* in Europe and *Fagus grandifolia* in North America. It is triggered by the combined attack of the beech bark by beech scale (*Cryptococcus fagisuga*) feeding in the bark parenchyma and the bark cancer fungus *Nectria coccinea*. Neither the infestation by scale [1] nor the attack by the fungus [2, 3] alone, can kill the tree. Expression and seriousness of the disease [4] are steered predominantly by the degree of resistance of the individual tree [5], and by stand and weather conditions [6]. Phenolic compounds in the inner and outer bark of the beech play an important role in combating infections. The preferential attack by the beech scale occurs at the trunk, which contains only about 25% of the content of polyphenols (indicated by vanillin/hydrochloric acid) in the roots [7].

The infestation is countered by increasing concentrations of polyphenols in inner and outer bark [6, 8–11]. The differences between attacked and unattacked trees are greater in the inner bark (functional and non-functional phloem without hardbast elements) than in the outer bark (periderm and non-functional phloem with hardbast elements). With the increase of the scale infestation level the probability of an infection by spores of *N. coccinea* rises noticeably. This fungus presumably initiates the long continuous necrosis by which susceptible beeches answer the attack. Equally important goals of this investigation are the analysis of the influence of the attack by *C. fagisuga* on the phenolic metabolism of beech under field concentrations and the identification of the compounds affected by this interaction.

RESULTS

Physiological results

Clear differences in the concentration levels of phenols became obvious, both with respect to the tissue type (inner and outer bark, respectively) and season (Fig. 1). More or less distinct maxima occurred at the time of highest synthetic activity (midsummer). The infestation of beech trees by the beech scale caused an equally directed regulation of the concentrations of the phenolic compounds in both tissues, the differences between unattacked and attacked trees being greater in the inner than in the outer bark. The concentrations of **1**, **3** and **4** were influenced only slightly by the infestation. However, in infested inner and outer bark tissue, the concentrations of **5**, **6** and **7** increased markedly; the concentration of *cis*-coniferin (**2**) was constantly decreased.

Chemical analysis

The seven major phenols (**1**–**7**), which showed a change in concentration in infested beech bark (Fig. 2)

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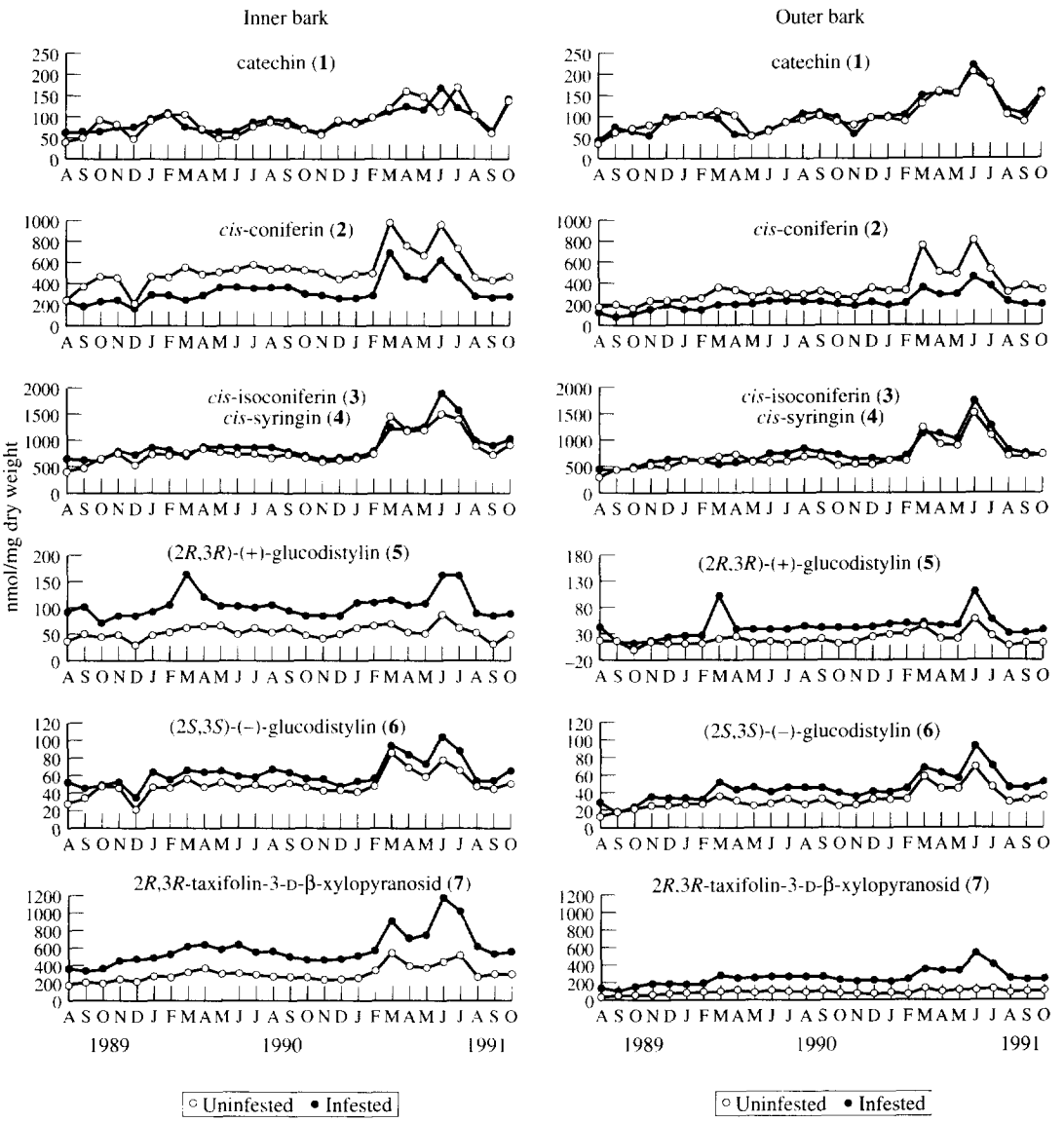


Fig. 1. Seasonal concentration profiles of phenolic compounds in the inner and outer bark of a 150-year-old beech in a natural stand without and after infestation by *Cryptococcus fagisuga*.

were isolated from the methanolic extracts by column chromatography on polyamide, silica gel and Sephadex LH-20. The elucidation of their chemical structures was based mainly on a full analysis of their NMR spectra (^1H and ^{13}C), which was aided by using several two-dimensional techniques (COSY, HMQC, HMBC) and was confirmed by DCI-MS, IR, UV and CD data. Compound **1** was identified as catechin (**1**/ent-**1**) by NMR data, the *trans* diaxial coupling constant of 2-H/3-H ($J = 8$ Hz) and the optical rotation value [12]. The results of ^1H, ^{13}C COSY experiments enabled the ^{13}C NMR signals to be assigned unambiguously.

The most conspicuous features of **2**–**7** were the presence of one sugar unit, which could be assigned by means of the vicinal $^3J_{H,H}$ coupling constants from the ^1H NMR spectra and the ^{13}C NMR data. Except for **7**, a β-D-xylopyranoside, the sugar units contain a β-D-glucopyranosyl residue. The ^1H NMR data for **2**–**4** in all cases indicated one phenolic unit with a 2-propenol-3-yl residue in the *cis* configuration ($J = 12$ Hz). From the coupling pattern of the aromatic protons and two-dimensional NMR data, **2** was identified as *cis*-coniferin [13]. The *O*-β-glucopyranosyl residue is in a different place in **3** compared to **2**, as deduced from the NMR data. Thus **3** could be assigned as *cis*-isoconiferin [13] or faguside [14]. The ^1H NMR data of **4** clearly indicated a symmetrical aromatic substitution with methoxy groups in the 3- and 5-positions. The total assignment could be made by means of two-dimensional NMR experiments (HMBC), leading to *cis*-syringin (**4**) [14].

The NMR data of **5**–**7** are similar to each other and to those of (+)-catechin (**1**). In accordance with their DCI-MS data, the typical IR absorption for OH-chelated arylketones (1623–1643 cm^{-1}) and their chemical behaviour, it could be shown that they are

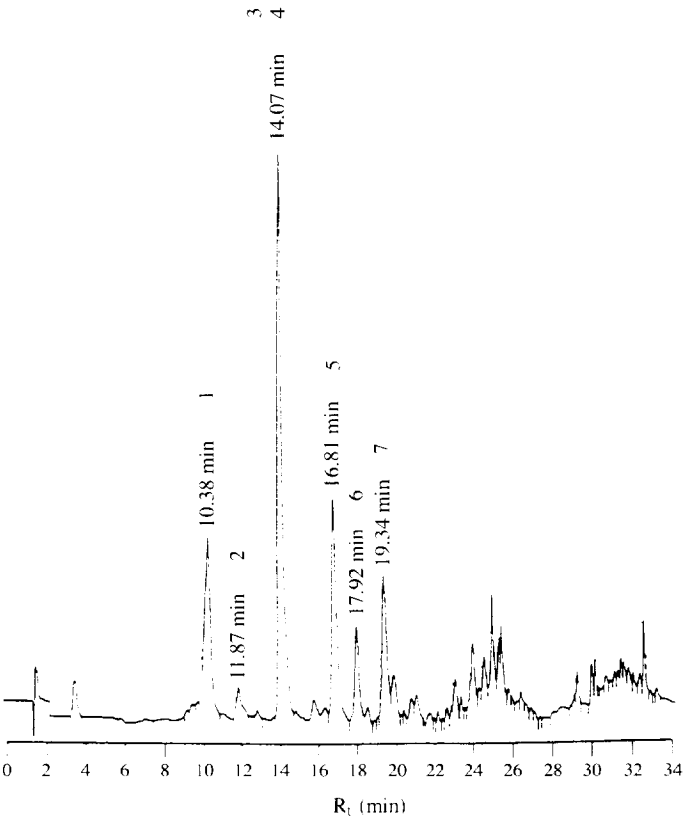
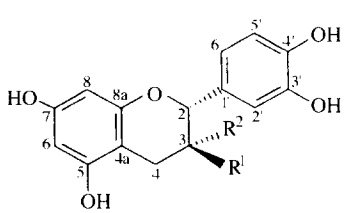
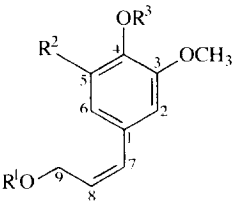


Fig. 2. HPLC of methanolic extracts from *Fagus sylvatica* (Spherisorb ODS-2; H_2O–MeOH gradient).



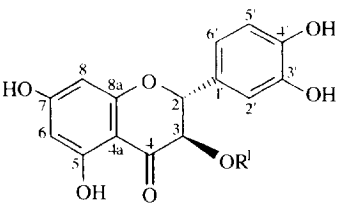
1 $R^1 = OH$ $R^2 = H$



2 $R^1 = H$ $R^2 = H$ $R^3 = Glc$

3 $R^1 = Glc$ $R^2 = H$ $R^3 = H$

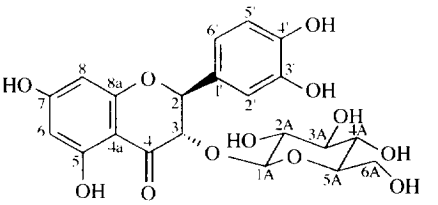
4 $R^1 = H$ $R^2 = OCH_3$ $R^3 = Glc$



5 $R^1 = Glc$

7 $R^1 = Xyl$

8 $R^1 = H$



6

Table 1. R_f values and retention time (HPLC, see Fig. 2)

Compound	R_f		Retention time (min)
	EtOAc/EtOH/HAc (70 : 28 : 2)	$CHCl_3$/EtOH (90 : 10)	
1/*ent*-**1**	0.92	0.42	10.38
2	0.52	0.13	11.87
3	0.64	0.25	14.07
4	0.43	0.25	14.07
5	0.66	0.11	16.81
6	0.73	0.13	17.92
7	0.83	0.20	19.34

flavanone glycosides. In the ^{1}H NMR spectrum of **5** the doublets at δ 5.22 and 4.91 ($J = 9$ Hz) clearly indicated the *trans* position of 2-H/3-H. Thus the aglycone moiety of **5** corresponded to (2*R*,3*R*)-(+)-taxifolin (**8**) [15]. The β-D-glucopyranosyl residue is attached at 3-OH of **8**. This can be seen by the striking high-field shift of the anomeric proton in **5** (δ_H 3.85, *d*, $J = 7.5$ Hz) and the $^3J_{C,H}$ coupling of 1A-H/C-3 ($\delta_H = 3.85$, $\delta_C = 77.25$) and of 3-H/C-1A ($\delta_H = 4.91$, $\delta_C = 102.6$) derived from the HMBC NMR experiment. Thus **5** was identified as (2*R*,3*R*)-(+)-glucodistylin, which has been isolated previously from the leaves of *Chamaecyparis obtusa* [16]. Slight differences in the spectra of **6** suggested that this compound has the same structure type as **5** but with an different stereochemistry. The most remarkable differences can be seen for the anomeric C-1A of the glucopyranosyl residue (**5**: $\delta_H = 3.85$, $\delta_C = 102.6$. **6**: $\delta_H = 4.58$, $\delta_C = 104.6$), particularly the low-field shift of 1A-H (0.73 ppm). As shown for **5**, the position of glucose moiety in **6** definitely followed from the HMBC NMR experiment. Thus **6** was assigned as (2*S*,3*S*)-(−)-glucodistylin, a diastereomer of **5**, which has been isolated previously from *Agrimonia pilosa* [17]. The spectra of **7** were very similar to those of **5** and **6**. However, one significant difference was seen in the DCI-MS spectra (**5**: *m/z* 484. **6**: *m/z*: 484. **7**: *m/z* 454 [M + H + NH_3]$^+$). The less polar behaviour ($R_t = 19.34$ min, Fig. 2, Table 1) of **7** compared with **5** and **6** is in accordance with the fact that the sugar residue must be a pentose, which was characterized on the basis of the ^{13}C NMR signals (δ 65.9 (C-5A), 70.1 (C-4A), 76.3 (C-3A) and 104.1 (C-1A)) as the β-D-xylopyranoside. Two-dimensional NMR experiments supported the glucosidic linkage between C-1A and C-3. The anomeric 1A-H ($\delta_H = 3.72$, *d*, $J = 6$ Hz) showed a high-field shift, as in **5**; thus **7** was identified as (2*R*,3*R*)-taxifolin-3-β-D-xylopyranoside, which has recently been described as a constituent of the leaves of *Thujopsis dolabrata* [18].

Although the compounds isolated are all known, **1**, **3** and **5**–**7** were isolated from *F. sylvatica* for the first time, and their ^{13}C NMR signals could be assigned unambiguously. For some of these compounds, biological activity has been mentioned in earlier studies [15, 19–27].

DISCUSSION

The compounds identified can be divided into three groups with different modes of action in defence reactions: the lignification of attacked tissue, the disturbance of the metabolization of nutrients, and the killing of invading organisms. The glucosides *cis*-coniferin (**2**), *cis*-isoconiferin (**3**) and *cis*-syringin (**4**) are precursors of the lignin biosynthesis after isomerization to the *trans* form [28]. A falling concentration of a monolignol, the aglycone of **2**, may indicate an increased production of lignin and necrotic tissue. Because the stylet is only 1.5 mm long, the scale will not be able to penetrate thicker parts of the bark. Punctures inflicted by *C. fagisuga* must be closed with necrotic material in order to avoid infection of the tree by spores of *N. coccinea*. Compounds **1**, **2** and **4** and the taxifolins, the aglycones of **5**–**7**, are precursors of procyanidin biosynthesis [19, 29–31].

Procyanidins or proanthocyanidins play an important role in the defence and resistance mechanisms of plants [32]. They are able to complex proteins of plant origin, that become accessible during the feeding process, and enzymes of animal digestive systems [33], such as protease, lipase and glucosidase [34], which are necessary for the survival of phytophages [35]. Another possible reaction is the polymerization of procyanidins to hard and insoluble polymers, which inhibit the food uptake of insects [36]. Insecticidal activity of *cis*-isoconiferin (**3**) has been recorded against the wheat pests *Trogonerma granorium*, *Tribolium confusum* and *Sitophilus granarius* [26]. The aglycone of **2** and the syringic acid derived from **4** are fungicides [19]. It has not been ascertained whether they are also effective active against *N. coccinea*. The aglycone of **5** and **7**, taxifolin, is described as an effective fungicide against decay [15, 37], which could protect the tree from infections by wood-destroying fungi following infestation by beech scale.

EXPERIMENTAL

General. TLC was carried out on silica gel 60 F-254 aluminum sheets (Merck) and the compounds were visualized by spraying with vanillin–sulphuric acid (solvent, see Table 1). CC was done on Sephadex LH-

20 (Pharmacia) and silica gel 60 (Macherey & Nagel). The seasonal concentration profiles and the purification were analysed by HPLC on a Biotronic model equipped with a Spherisorb ODS-2 column (3 μm, 20 × 4.6 mm) and a H_2O/MeOH gradient at 254 nm. The concentration changes were followed in both bark tissues monthly from August 1989 to October 1991, in 6 pair comparisons [38] each of an unattacked and attacked tree of a 150-year-old natural beech stand in the Solling hills (Lower Saxony).

Extraction and isolation. The fresh beech bark, collected from the trunks of beeches (*F. sylvatica*) was immediately frozen (solid CO_2) in the field. The bark was brushed clean and separated into inner and outer bark. For preparative isolation, only the bark from unattacked beeches was used. The material was ground using a mortar mill and freeze-dried. The powdered bark (40 g) was extracted with MeOH (5 × 300 ml, rt). The solvent was removed on a rotary evaporator and the crude product (5.5 g) was diluted with H_2O (300 ml, pH 2). The aqueous suspension was first defatted with petrol (6 × 150 ml) and then extracted with EtOAc (5 × 300 ml). The resulting solution was brought to dryness under reduced pressure to yield 2.5 g of solid substance. The aqueous solution was freeze-dried to give a brown powder (3.0 g).

The water-soluble compounds (3.0 g) were applied to a silica gel column (5 × 70 cm) and eluted with EtOAc–EtOH–HOAc (50 : 5 : 1). The fractions were controlled by TLC (see Table 1) and pooled according to the spot pattern. The evaporation residues obtained showed, as major constituents, the compounds *cis*-coniferin (**2**), *cis*-isoconiferin (**3**), and a mixture of *cis*-coniferin (**2**) and small amounts of *cis*-syringin (**4**). The crude glucosides **2** and **3** were purified further on silica gel columns. Compound **3** (69 mg) was eluted with EtOAc–EtOH–HOAc (8 : 2 : 1) and **2** (20 mg) was eluted with $CHCl_3$–EtOH (4 : 1).

The crude EtOAc extract (2.5 g) was applied to a polyamide column (5 × 70 cm) and eluted with MeOH to give three major fractions. The first contained **2**–**4** (990 mg), the second contained **5**–**7** (370 mg) and the third contained crude **1** (187.5 mg). Compounds **2**–**4** were separated on silica gel ($CHCl_3$–EtOH, (4 : 1) and, finally, purified using silica gel EtOAc–EtOH–HOAc (70 : 28 : 2), yielding 200 mg of **3**, 26 mg of **4** and a mixture of **2** and **3** (30 mg).

The mixture of **5**–**7** was fractionated on silica gel with EtOAc–EtOH–HOAc (50 : 5 : 1). The selected fractions were purified further on Sephadex LH-20 or silica gel columns. Compound **7** was eluted with MeOH (Sephadex LH-20) and EtOAc (silica gel, 13 mg), and **6** (22 mg) and **5** (40 mg) with EtOAc–EtOH (90 : 10, silica gel).

Crude **1** was applied on silica gel ($CHCl_3$–EtOH, 4 : 1) to provide 82 mg of pure **1**.

Catechin (**1**/*ent*-**1**). ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ 2.50 (*dd*, J_1 = 15 Hz, J_2 = 2 Hz, 1H, H-4b), 2.89 (*dd*, J_1 = 15 Hz, J_2 = 5 Hz, 1H, H-4a), 3.97 (*ddd*, $J_1 = J_2$ = 8 Hz, J_3 = 5 Hz, 1H, H-3), 4.53 (*d*, *J* = 8 Hz, 1H, H-2), 5.85 (*d*, *J* = 2.5 Hz, 1H, H-6), 6.00 (*d*, *J* = 2.5 Hz, 1H, H-5), 6.73 (*dd*, J_1 = 8 Hz, J_2 = 2 Hz, 1H, H-6′), 6.78 (*d*, *J* = 8 Hz, 1H, H-5′), 6.88 (*d*, *J* = 2 Hz, 1H, H-2′), 8.15 (*s br*, 3H, OH). ^{13}C NMR (125.5 MHz, $(CD_3)_2CO$): δ 28.7 (C-4), 68.2 (C-3), 82.6 (C-2), 95.2 (C-6), 96.1 (C-8), 100.5 (C-4a), 115.2 (C-2′), 115.6 (C-5′), 119.9 (C-6′), 132.0 (C-1′), 145.6 (C-4′), 145.6 (C-3′), 156.7 (C-8a), 157.1 (C-5), 157.6 (C-7).

cis-*Coniferin* (**2**). Mp 185–186 °. DCI-MS *m/z*: 360.2 $[M + H + NH_3]^+$. ^{1}H NMR (300 MHz, CD_3OD): δ 3.40 (*m*, 2H, H-3A and H-4A), 3.49 (m, 2H, H-2A and H-5A), 3.68 (*dd*, J_1 = 12 Hz, J_2 = 6 Hz, 1H, H-6A), 3.85 (*s*, 3H, 3-OCH_3), 3.86 (*m*, 1H, H-6A), 4.33 (*dd*, J_1 = 6 Hz, J_2 = 2 Hz, 2H, H-9), 4.88 (*d*, *J* = 6 Hz, 1H, H-1A), 5.56 (*dt*, J_1 = 12 Hz, J_2 = 6 Hz, 1H, H-8), 6.47 (*d*, *J* = 12 Hz, 1H, H-7), 6.76 (*dd*, J_1 = 8.5 Hz, J_2 = 2 Hz, 1H, H-6), 6.88 (*d*, *J* = 2 Hz, 1H, H-5), 7.13 (*d*, *J* = 8.5 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CD_3OD): δ 56.7 (3-OCH_3), 59.9 (C-9), 62.5 (C-6A), 71.3 (C-4A), 74.9 (C-2A), 77.8 (C-5A), 78.2 (C-3A), 102.7 (C-1A), 114.4 (C-5), 117.6 (C-2), 122.9 (C-6), 131.0 (C-7), 131.9 (C-8), 133.2 (C-1), 147.2 (C-4), 150.5 (C-3).

cis-*Isoconiferin* (**3**). DCI-MS *m/z* 360.2 $[M + H + NH_3]^+$. ^{1}H NMR (300 MHz, CD_3OD): δ 3.20 (*m*, 2H, H-3A and H-5A), 3.42 (*m*, 2H, H-2A and H-4A), 3.55 (*dd*, J_1 = 12 Hz, J_2 = 5 Hz, 1H, H-6A), 3.74 (*dd*, J_1 = 12 Hz, J_2 = 2 Hz, 1H, H-6A), 3.78 (*s*, 3H, 3-OCH_3), 4.25 (*d*, *J* = 8 Hz, 1H, H-1A), 4.33 (*ddd*, J_1 = 12 Hz, J_2 = 6 Hz, J_3 = 2 Hz, 1H, H-9), 4.57 (*ddd*, J_1 = 12 Hz, J_2 = 6 Hz, J_3 = 2 Hz, 1H, H-9), 5.65 (*dt*, J_1 = 12 Hz, J_2 = 6Hz, 1H, H-8), 6.43 (*d*, *J* = 12 Hz, 1H, H-7), 6.61 (*dd*, J_1 = 8.5 Hz, J_2 = 2 Hz, 1H, H-6), 6.68 (*d*, *J* = 8.5 Hz, 1H, H-5), 6.75 (*d*, *J* = 2 Hz, 1H, H-2). ^{13}C NMR (100 MHz, CD_3OD): δ 56.5 (3-OCH_3), 62.7 (C-6A), 67.3 (C-9), 71.6 (C-4A), 75.1 (C-2A), 77.9 (C-5A), 78.1 (C-3A), 103.8 (C-1A), 113.7 (C-2), 116.0 (C-5), 123.1 (C-6), 127.0 (C-8), 129.9 (C-1), 133.0 (C-7), 147.2 (C-4), 148.7 (C-3).

cis-*Syringin* (**4**). DCI-MS *m/z* 390.2 $[M + H + NH_3]^+$. ^{1}H NMR (300 MHz, CD_3OD): δ 3.2 (*m*, 1H, H-5A), 3.39 (*m*, 1H, H-2A) 3.41 (*m*, 1H, H-4A), 3.69 (*dd*, J_1 = 12 Hz, J_2 = 5 Hz, 1H, H-6A), 3.79 (*dd*, J_1 = 12 Hz, J_2 = 4 Hz, 1H, H-6A), 3.85 (*s*, 6H, 2 OCH_3), 4.35 (*dd*, J_1 = 6 Hz, J_2 = 2 Hz, 2H, H-9), 4.89 (*d*, *J* = 8 Hz, 1H, H-1A), 5.80 (*dt*, J_1 = 12 Hz, J_2 = 6 Hz, 1H, H-8), 6.50 (*d*, *J* = 12 Hz, 1H, H-7), 6.54 (*s*, 2H, 2-H and H-6). ^{13}C NMR (125.7 MHz, CD_3OD): δ 57.1 (3-OCH_3, 5-OCH_3), 59.8 (C-9), 62.6 (C-6A), 71.4 (C-4A), 75.7 (C-2A), 77.9 (C-5A), 78.4 (C-3A), 105.3 (C-1A), 108.2 (C-2, C-6), 131.5 (C-7), 132.5 (C-8), 134.8 (C-1, C-4), 154.1 (C-3, C-5).

(2R,3R)-(+)-*Glucodistylin* (**5**). Mp 169–171 °. $[\alpha]_D^{20}$ + 36 (MeOH; *c* 0.2). CD $[\theta]_{328}$ +9339, $[\theta]_{310}$ 0, $[\theta]_{294}$ −21616. DCI-MS 484.2 $[M + H + NH_3]^+$. IR ν_{max}^{KBr} cm^{-1}: 3419 (br), 2928 (s), 1643, 1513 (s), 1462 (s), 1361 (s), 1281 (sh), 1165, 1078 (sh), 810 (s), 759 (s), 585 (s). UV λ_{max}^{MeOH} nm (ε): 206 (47 896), 271 (49 581). ^{1}H NMR (300 MHz, CD_3OD): δ 2.99 (*m*, 1H, J_1 = 9 Hz, J_2 = 6

Hz, J_3 = 2 Hz, 1H, H-3A), 3.10 (*m*, 1H, H-5A), 3.19 (*m*, 1H, H-2A), 3.25 (*m*, 1H, H-4A), 3.60 (*dd*, J_1 = 12 Hz, J_2 = 6 Hz, 1H, H-6A), 3.77 (*dd*, J_1 = 12 Hz, J_2 = 2 Hz, 1H, H-6A), 3.85 (*d*, J = 7.5 Hz, 1H, H-1A), 4.91 (*d*, J = 9 Hz, 1H, H-3), 5.22 (*d*, J = 9 Hz, 1H, H-2), 5.88 (*d*, J = 2 Hz, 1H, H-8), 5.89 (*d*, J = 2 Hz, 1H, H-6), 6.76 (*d*, J = 8 Hz, 1H, H-5′), 6.84 (*dd*, J_1 = 8 Hz, J_2 = 2 Hz, 1H, H-6′), 6.95 (*d*, J = 2 Hz, 1H, H-2′). ^{13}C NMR (100 MHz, CD_3OD): δ 62.6 (C-6A), 71.3 (C-4A), 74.7 (C-2A), 77.2 (C-3), 77.6 (C-5A), 78.2 (C-3A), 83.6 (C-2), 96.4 (C-6), 97.4 (C-8), 102.5 (C-4a), 102.6 (C-1A), 115.9 (C-2′), 116.5 (C-5′), 121.0 (C-6′), 129.0 (C-1′), 146.4 (C-4′), 147.3 (C-3′), 164.1 (C-8a), 165.5 (C-5), 169.5 (C-7), 195.7 (C-4).

(2S,3S)-(−)-*Glucodistylin* (**6**). Mp 180–190 °. $[\alpha]_D^{20}$ −20° (MeOH; *c* 0.3). CD $[\theta]_{323}$ −10497, $[\theta]_{305}$ 0, $[\theta]_{291}$ +4341. DCI-MS *m/z*: 484.2 $[M + H + NH_3]^+$. IR ν_{max}^{KBr} cm^{-1}: 3415 (br), 1637, 1458 (sh), 1368 (s), 1279, 1159, 1077 (sh), 816 (s), 778 (s). UV λ_{max}^{MeOH} nm (ε) = 269 (54 967). ^{1}H NMR (300 MHz, CD_3OD): δ 3.10 (*m*, 1H, H-2A), 3.14 (*m*, 2H, H-3A, H-4A), 3.20 (*m*, 1H, H-5A), 3.55 (*m*, 1H, H-6A), 3.76 (*m*, 1H, H-6A), 4.58 (*d*, J = 7.5 Hz, 1H, H-1A), 4.89 (*d*, J = 9 Hz, 1H, H-3), 5.22 (*d*, J = 9 Hz, 1H, H-2), 5.85 (*m*, 2H, 6-H and H-8), 6.73 (*m*, 2H, H-5′ and H-6′), 6.95 (*d*, J = 2 Hz, 1H, H-2′). ^{13}C NMR (50.3 MHz, CD_3OD): δ 62.8 (C-6A), 71.5 (C-4A), 75.4 (C-2A), 77.7 (C-3), 77.9 (C-5A), 78.0 (C-3A), 83.4 (C-2), 96.3 (C-6), 97.2 (C-8), 102.0 (C-4a), 104.6 (C-1A), 115.9 (C-2′), 116.2 (C-5′), 121.1 (C-6′), 128.8 (C-1′), 146.0 (C-4′), 146.9 (C-3′), 164.0 (C-8a), 165.6 (C-5), 169.1 (C-7), 196.1 (C-4).

*Taxifolin-3-β-*D*-xylopyranoside* (**7**). Mp 190–195 °. $[\alpha]_D^{20}$ + 8.5° (MeOH; *c* 0.3). CD $[\theta]_{332}$ +2914, $[\theta]_{314}$ 0, $[\theta]_{295}$ −10763. DCI-MS *m/z*: 454.2 $[M + H + NH_3]^+$. IR ν_{max}^{KBr}: 3430 (br), 2919 (s), 1623, 1377 (sh), 1042 (s). UV λ_{max}^{MeOH} nm (ε): 269 (90 002). ^{1}H NMR (300 MHz, CD_3OD): δ 2.91 (*dd*, J_1 = 12 Hz, J_2 = 9 Hz, 1H, H-5A), 3.08 (*dd*, *br*, J_1 = 6 Hz, J_2 = 2.5 Hz, 2H, H-2A and H-3A), 3.35 (*m*, 1H, H-4A), 3.72 (*d*, J = 6 Hz, 1H, H-1A), 3.79 (*dd*, J_1 = 12 Hz, J_2 = 5 Hz, 1H, H-5A), 4.60 (*d*, J = 10 Hz, 1H, H-3), 5.06 (*d*, J = 10 Hz, 1H, H-2), 5.75 (*d*, J = 2 Hz, 1H, H-6), 5.77 (*d*, J = 2 Hz, 1H, H-8), 6.65 (*m*, 2H, H-5′ and H-6′), 6.80 (*d*, J = 2 Hz, 1H, H-2). ^{13}C-NMR (100 MHz, CD_3OD): δ 65.9 (C-5A), 70.8 (C-4A), 73.4 (C-2A), 75.7 (C-3A), 77.4 (C-3), 83.6 (C-2), 96.5 (C-6), 97.5 (C-8), 102.4 (C-4a), 103.0 (C-1A), 115.6 (C-2′), 116.2 (C-5′), 120.8 (C-6′), 129.0 (C-1′), 146.5 (C-3′), 147.3 (C-4′), 164.1 (C-8a), 165.5 (C-5), 169.5 (C-7), 195.4 (C-4).

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