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MONOLIGNOL AND DILIGNOL GLYCOSIDES FROM *PINUS CONTORTA* LEAVES

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Key Word Index—*Pinus contorta*; Pinaceae; needles; monolignol glycosides; dilignol glycoside; dihydro-*p*-coumaryl alcohol; dihydroconiferyl alcohol; chavicol.

Abstract—Four monolignol glucosides have been isolated and identified from the needles of *Pinus contorta*. Chavicol 4-*O*-α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranoside, the known 4′-*O*-β-D-glucopyranoside of 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol and shikimic acid were also isolated.

INTRODUCTION

Pinus contorta, a species of economic importance in Ireland, has been subject to periodic attack by the European sawfly (*Neodiprion sertifer*) which has caused defoliation and loss of increment. Preliminary investigation [1] on host-preference research has indicated a variation in susceptibility to pine sawfly between different provenances of *Pinus contorta*. The terpenes present in members of the Pinaceae are known to be the defence agents against predatory attack. It has been clearly demonstrated [2] that the sawfly larvae have breached the defensive mechanism of the pine and use α- and β-pinene and a series of resin acids for their own protection. The larvae are capable of sequestering the defensive chemicals from the ingested plant material.

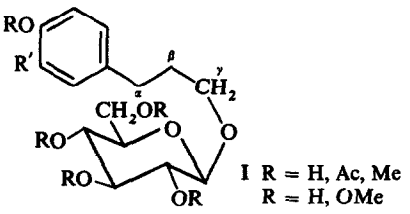
To assist in biological studies on the behaviour pattern of *N. sertifer*, analysis of the water soluble fraction of a MeOH extract of *P. contorta* (Coastal) needles was undertaken. This fraction was thought to contain phenylpropanes, a class reported [3] to exert allelochemic influence in the nutritional and metabolic aspects of insect host-plant specificity. Phenylpropanes have also been shown to function as behavioural, developmental and metabolic allomones and kairomones.

This paper describes the structural determination of 4 monolignol glucosides (I and II, R = H, R′ = H, OMe) and chavicol 4-O-α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranoside which to our knowledge have not previously been reported as natural products, and a dilignol glucoside which has also been recently isolated from *P. sylvestris* needles [4].

RESULTS AND DISCUSSION

The procedure of isolation of the 7 compounds, tentatively named compound 1–7 in order of increasing polarity, is detailed in the experimental section. Compound **1** was obtained as an amorphous hygroscopic powder which had a strong OH absorption in its IR spectrum and which gave, on acid hydrolysis, D-glucose and an aglycone. The PMR spectra of the latter and of its acetate suggested the structure was dihydroconiferyl alcohol, the assignment of which was confirmed by a direct comparison with a synthetic sample. This alcohol has been isolated from the bark of *P. sylvestris*. [5]. It was also prepared, for biosynthetic studies on spruce by Freudenberg [6], by the hydrogenation and hydrolysis of coniferin.

MS of the pentaacetate (I, R = Ac, R′ = OMe) shows ions at *m/e* 554 for the M^+ and *m/e* 331 which arise from a terminal peracetylated hexose residue [7]. Compound **1** is therefore a monoglucoside of dihydroconiferyl alcohol. Based on PMR and MS evidence the glucose



I R = H, Ac, Me
R = H, OMe

moiety was placed on the γ-OH group. The PMR spectrum of the acetate (I, R = Ac, R′ = OMe) shows an aromatic acetoxyl at δ 2.33 and the MS reveals a strong ion at *m/e* 512 ($M^+ - 42$) due to elimination of $.CH_2CO$. Methanolysis of the perMe ether (I, R = Me, R′ = OMe) prepared by the Hakomori method [8], gave the Mepyranoside of 2,3,4,6-tetra-*O*-Me-α- and β-glucose, thus verifying the pyranoside form of the sugar moiety. A β-linkage of the sugar unit was inferred from the anomeric proton signal at δ 4.60 (*d*, *J* = 8 Hz) in the PMR spectrum of the peracetate of **1** indicating that the protons on C-1 and C-2 are *trans*-diaxial [9]. Compound **1** is therefore dihydroconiferyl alcohol γ-*O*-β-D-glucopyranoside.

Acid hydrolysis of compound **2** gave dihydroconiferyl alcohol and D-glucose. MS of the peracetate showed a M^+ at *m/e* 554 and an ion at *m/e* 331 (terminal peracetylated hexose residue) and PMR a triplet (*J* = 6 Hz) at δ

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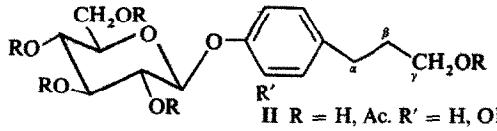
4.16 assigned to the methylene protons of the primary alcohol acetate. Accordingly compound **2** is presumed to have the glucose moiety attached to the phenol group. The pyranoside form of the glucose was evident from PMR of the peracetate and the β-linkage by the presence of one anomeric proton multiplet at δ 4.9 with W(h/2) = 9 Hz in the PMR of **2**. Compound **2** is assigned as dihydroconiferyl alcohol 4-*O*-β-D-glucopyranoside (II, R = H, R′ = OMe) [6].



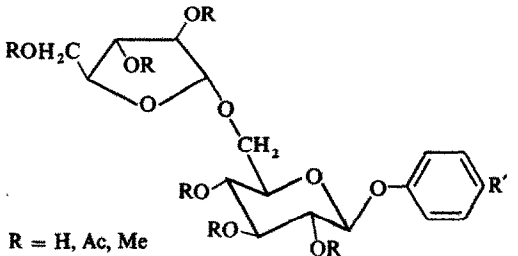
II R = H, Ac. R′ = H, OMe

Compounds **3** and **4**, on hydrolysis, yielded dihydro-*p*-coumaryl alcohol and D-glucose. PMR of the aglycone diacetate supported this assignment and confirmation was obtained by a comparison with an authentic sample of dihydro-*p*-coumaryl alcohol [10]. The MS of the peracetates of **3** and **4** contained peaks due to the M^+ (*m/e* 524) and to terminal peracetylated hexose residues (*m/e* 331). In addition, MS and PMR verified the sites of the glucose linkage, that is in **3** the glucose moiety is on the γ-OH group and in **4** on the phenol group. The pyranoside form of the sugar was supported on evidence from methanolysis of its perMe ether of **3** and from PMR analysis of the acetate of glucoside **4**. The linkage of the glucose unit in both cases is β; this assignment is based on PMR data for the anomeric protons. In the peracetate **3** the signal, a doublet, lies at δ 4.58 (J = 7 Hz) whilst in the glucoside **4** it is a multiplet at δ 4.92 (W(h/2) = 8 Hz). Compounds **3** and **4** are thus considered to be dihydro-*p*-coumaryl alcohol γ-*O*-β-D-glucopyranoside (I R = R′ = H) and 4-*O*-β-D-glucopyranoside (II R = R′ = H) respectively. The latter glucoside has an identical mp to the hydrogenation product of gluco-*p*-coumaryl alcohol [6].

Compound **5**, was hydrolysed with acid and yielded L-arabinose, D-glucose and a series of unidentified aglycones. The MS of the acetylated glycoside (III, R = Ac, R′ = $CH_2CH = CH_2$) showed a M^+ at *m/e* 680 and ions at *m/e* 547 and 259 which were assigned to the residues originating from a terminal peracetylated pentosyl hexose (*m/e* 547) and a terminal peracetylated pentose respectively [11]. The PMR of the peracetate of **5** shows a multiplet δ 6.0 (1H), a broad doublet (2H, J = 14 Hz) at δ 5.1 and a doublet (2H, J = 7 Hz) at δ 3.33 in addition to signals for an A_2B_2 system and those of the acetylated sugar protons. From this data, the aglycone moiety of **5** was considered to be chavicol.

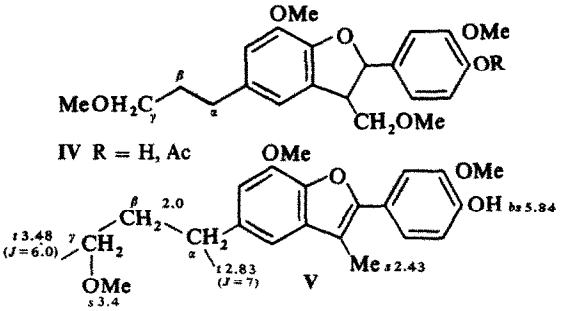
Hydrogenation of glucoside **5** and subsequent acetylation afforded the dihydroacetate which contained a *n*-propane chain as evidenced from the PMR spectrum (δ 0.94 (*t*, J = 7 Hz) δ 1.74 (*m*) and δ 2.60 (*t*, J = 8 Hz)); acid hydrolysis of dihydro-**5** gave dihydrochavicol. Therefore **5** is an L-arabinosyl-D-glucoside of chavicol. The dihydroperMe ether of **5** was subjected to methanolysis, and 4 methylated monosaccharides and an aglycone were isolated. The sugars were identified by TLC as Me 2,3,5-tri-*O*-Me-α- and β-L-arabinopyranosides and Me 2,3,4-tri-*O*-Me-α- and β-D-glucopyranosides. Hydrolysis of this sugar fraction with N HCl gave 2,3,4-tri-*O*-Me-L-arabinose and 2,3,4-tri-*O*-Me-D-glucose. The PMR spectrum of dihydroperMe ether (III, R = Me, R′ = n-C_3H_7) shows anomeric proton signals at δ 4.89 (*m*, W(h/2 = 9 Hz) and δ 5.10 (*s*). By comparison with spectra of arbutin permethylate and Me furanosides of 2,3,5-tri-*O*-Me-α- and β-L-arabinose [12], the sugar configurations in **5** were β-D-glucopyranose and α-L-arabinofuranose.

Consequently the structure chavicol 4-*O*-α-L-arabinofuranosyl-(1 → 6)-β-D-glycopyranside (III, R = H, R′ = $-CH_2.CH = CH_2$) is postulated for compound **5**. A chavicol diglycoside, the rutinoside, has been reported previously [13].



III R = H, Ac, Me
R′ = n-C_3H_7; —$CH_2CH = CH_2$

Compound **6** was obtained as an amorphous powder which on hydrolysis with emulsin gave D-glucose and 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol (identical to an authentic sample kindly supplied by Professor Theander). That only one sugar unit was linked in the glycoside **6** was evident from the PMR spectrum of its peracetate. To confirm the position of the sugar unit (7 or 4′-) the methanolysis products of the permethylate were investigated. Two aglycones and the Me pyranosides of 2,3,4,6-tetra-*O*-Me-α- and β-D-glucose were obtained. One aglycone was dihydrodehydrodiconiferyl alcohol diMe ether (IV, R = H) the acetate of which revealed in its PMR spectrum the chemical shift difference for the aromatic OMe that supported their location in different rings. The structure (**V**) was proposed for the second aglycone as it showed a conjugated vinyl Me group (δ 2.43) in its PMR spectrum.



IV R = H, Ac

Compound **6** is identical with the dilignol glucoside isolated from *P. sylvestris* needles [4].

The major component in the water soluble fraction of the MeOH extract was **7** (0.21% dry needle basis) which was identified as shikimic acid. Shikimic acid has been found previously in species on *Pinus* and several investigators have noted that a change in concentration of the acid occurs with the age of the plant.

EXPERIMENTAL

Mps (uncorr) were determined using a Kofler hot-stage apparatus. PMR spectra were determined at 60 MHz from solns in $CDCl_3$ (TMS as internal reference) unless otherwise specified. TLC of monosaccharides were run on cellulose plates using a double ascending method with *n*-BuOH–C_5H_5N–H_2O

(3:2:1) as solvent and *p*-anisidine hydrochloride (0.1 M MeOH soln) as visualising agent (after heating). Kiselgel 60F-254 (Merck) were used for general TLC. Visualisation of the plates was achieved by spraying with H_2SO_4 (10%) in $FeCl_3$ in EtOH. Si gel column chromatography was carried out with Kisel 60 (70–230 mesh) (Merck) with a sample: adsorbent ratio of 1:30–40 unless otherwise indicated. PLC was with Kiselgel 60 PF (Merck). Sephadex LH-20 was used with a sample: adsorbent ratio of 1:150–200.

Extraction of needles. Air-dried *Pinus contorta* (Coastal variety) needles (2.2 Kg), collected at Avondale, Co. Wicklow, (Spring 1975) were milled and extracted, under reflux, successively with *n*-hexane and MeOH. The residue, from evaporation of the MeOH, was digested with hot H_2O and the aq. soln extracted with C_6H_6 and EtOAc. The solid, from EtOAc extraction was redissolved in MeOH, treated with $Pb(OAc)_2$ and filtered. The MeOH soln was subjected to H_2S and refiltered prior to evaporation. The residue in H_2O was chromatographed on polyamide (100 g, eluent: $H_2O \rightarrow$ MeOH) to yield 5 fractions (I–V). Fraction (I) (91 g) was redissolved in H_2O, extracted with EtOAc–*n*-BuOH (2:1) (A; 13 g), *n*-BuOH (B; 18 g) and then evaporated (C; 52 g). Fractionation of extracts A and B was achieved by chromatography on columns of Si gel using $CHCl_3$–MeOH–H_2O (8:2:0.1 → 7:3:0.2) and subsequently on Sephadex columns using H_2O as solvent. The detailed analysis of the least polar fractions from the extracts A and B is discussed in part II. Extract A gave compound **1**, **3** and **5** in small amounts whilst B yielded compounds **1**, **3** (0.94 g), **5** (0.18 g) and **6** (0.15 g). Extract C was poased through Sephadex (eluent: MeOH). The major fraction, a brown syrup (44 g), was chromatographed on Si gel (1 kg) (eluent: $CHCl_3$–MeOH–H_2O; 8:2:0.2 → 7:3:0.2). 4 fractions C(i–iv) were collected. Fraction C(i) is discussed in Part II and C(ii) and (iv) were passed through Sephadex (eluent: H_2O) to give compound **1** (0.41 g) and compound **7** (4.62 g) respectively. C(iii) was subfractioned on Sephadex (eluent: H_2O) to afford compound **2** (0.04 g) and compound **3** (0.8 g). A middle fraction was acetylated. The crude acetate was chromatographed on Si gel (eluent: *n*-hexane–EtOAc (1:1)) to give compound **2** acetate and compound **4** acetate (0.03 g). Deacetylation of the latter by refluxing with IR-45 (1 g) in MeOH (2 ml) for 5 hr gave compound **4** (0.01 g). Fraction IV (13.3 g) was a yellow powder. An aliquot (2.07 g) was chromatographed on Sephadex (100 g) (eluent: Me_2CO) to afford 3 fractions (i–iii) ((i) = 1.003 g; (ii) = 0.856 g; (iii) = 0.489 g). Fraction (ii) was subjected to PLC (eluent: $CHCl_3$–MeOH–H_2O (7:3:1), lower layer) and the band R_f 0.38 was eluted (0.653 g). A yellow solid crystallized from H_2O when catechin separated as needles (0.457 g, 0.07% dry wt) mp 170–171°; $[\alpha]_D^{22} \pm 0°$ (*c* 0.93, MeOH) $[\alpha]_{578}^{22} + 12.0°$ (*c* 1.13, 50% Me_2CO) (Found: C, 52.10 H, 5.55. Calc. for $C_{15}H_{14}O_6.3H_2O$ C, 52.32; H, 5.86%); λ_{max} (MeOH)nm (log ε) 280 (3.56) (identical NMR, UV (+)-catechin). Fractions III and V have not yet been investigated.

Dihydroconiferyl alcohol γ-O-β-D-glucopyranoside (**1**). The glucopyranoside was isolated as an amorphous hydroscopic powder $[\alpha]_D^{20°} -21.6$ (*c* 0.50 MeOH), ν_{max} (KBr) cm^{-1} 3500 ~ 3300, λ_{max} (MeOH)nm(ε) 2.81 (3187). Acetylation (46 mg) with Ac_2O–Py and chromatography of the product on Si gel (eluent: *n*-hexane–EtOAc (1:1) gave the pentaacetate as needles (28 mg) (MeOH), mp 114–115°, $[\alpha]_D^{20°} - 14.5°$ (*c* 0.55 $CHCl_3$) (Found: C, 56.33; H, 6.31. $C_{26}H_{34}O_{13}$ requirès C, 56.31; H, 6.18%). MS *m/e* 554 (M^+, $C_{26}H_{34}O_{13}^+$), 512 (M^+-42) 331 ($C_{14}H_{19}O_9^+$). PMR: δ 1.9 (2H, *m*, β-CH_2) 2 ~ 2.2 (12H, 4 × OAc) 2.33 (3H, *s*, ϕ-OAc) 2.7 (2H, *J* = 7 Hz, α-CH_2) 3.89 (3H, *s*, OMe) 4.3 (2H, *m*, glucose 6-H) 4.6 (1H, *d*, *J* = 8 Hz anomeric H) 5.0 ~ 5·4 (3H, *m*, glucose, 2,3,4-H) 6.75 ~ 7.2 (3H, *m*, ϕ-H). Compound **1** (300 mg) was hydrolysed with 2N H_2SO_4 (5 ml) for 3 hr. The reaction mixture was diluted with H_2O, extracted with $CHCl_3$ and the $CHCl_3$ layer washed, dried and evaporated. The residue was chromatographed on Si gel (eluent: EtOAc) to give dihydroconiferyl alcohol (100 mg) as an oil. (Found: C, 66.27; H, 7.79. Calc. for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74%). λ_{max} (MeOH)nm(ε) 282 (2509). PMR: δ 1.9 (2H, *m*, β-CH_2—) 2.7 (2H, *t*, *J* = 7 Hz, α-CH_2—) 3.74 (2H, *t*, *J* = 6 Hz, γ-CH_2—) 3.9 (3H, *s*, —OMe) 6.7 ~ 7.1 (3H, *m*, ϕ-H). In the 100 MHz spectrum irradiating the multiplet δ 1.9 caused a collapse of triplets δ 2.70 and δ 3.74 to two singlets (identical NMR, IR and TLC authentic sample of dihydroconiferyl alcohol [5, 6]. Acetylation of the aglycone (70 mg) afforded a crude acetate purified on a Si gel column (eluent: *n*-hexane–EtOAc (2:1)) to give dihydroconiferyl alcohol diacetate (80 mg) as an oil. (Found: C, 63.10; H, 6.92. $C_{14}H_{18}O_5$ requires: C, 63,14; H, 6.81%). PMR: δ 2 (2H, *m*, β-CH_2-) 2.07 (3H, *s*, OAc) 2.32 (3H, *s*, ϕ-OAc), 2.72 (2H, *t*, *J* = 7 Hz, α-CH_2—) 3.87 (3H, *s*, OMe), 4.17 (2H, *t*, *J* = 6 Hz, α-CH_2OAc), 6.8 ~ 7.2 (3H, *m*, ϕ-H). The aq. layer of the hydrolysis mixture was neutralised with Amberlite IR-45, then concd and examined by TLC. Glu was detected. The concentrate was purified on a Si gel column (eluent: $CHCl_3$–MeOH–H_2O; 25:17:2) and subsequently on Sephadex (eluent: MeOH) to yield D-glucose (64 mg) $[\alpha]_D^{20°} + 49.6°$ (*c* 3.0, H_2O). The osazone crystallised from MeOH as yellow needles (18 mg) mp 199–201° (identical mp, $[\alpha]_D$ to glucocazone).

Permethylation of dihydroconiferyl alcohol γ-O-β-D-glucopyranoside (**1**). The glucopyranoside (38 mg) was methylated with NaH, CH_3I and DMSO [8]. The reaction mixture was diluted with H_2O, extracted with $CHCl_3$ and the $CHCl_3$ layer washed, dried and evaporated. The residue was chromatographed on Si gel (eluent: *n*-hexane–EtOAc 1:1) to give the Me ether (I, R=Me, R′=OMe) (17 mg) as an oil, $[\alpha]_D^{24°} - 12.0°$. (*c* 0.50, $CHCl_3$). λ_{max} ($CHCl_3$)nm(ε) 282 (2650), 287 (2236). PMR: δ 2.0 (2H, *m*, β-CH_2—) 2.72 (2H, *t* *J* = 7 Hz, α-CH_2—) 3.43, 3.56, 3.66 (12H, sugar OMe × 4) 3.87, 3.91 (6H, 2 × *s*, OMe) 6.83 (3H, *s*, ϕ-H). Methanolysis of the hexaMe ether (10 mg) by reflux with HCl (8%) in MeOH (2 ml) for 2 hr gave a mixture which was treated with Ag_2CO_3 and filtered. Evaporation of the filtrate and examination of the residue by TLC (C_6H_6–Me_2CO, 4:1) showed the aglycone and the Me pyranosides of 2,3,4,6-tetra-*O*-Me-α- and β-D-glu (identical TLC to 2,3,4,6-tetra-*O*-Me-D-glucopyranoside: α-anomer R_f 0.38, β-anomer R_f 0.5).

Dihydroconiferyl alcohol, 4-O-β-D-glucopyranoside (**2**). Needles from MeOH–Me_2CO, mp 130–132° (lit. [6] mp 143°). (Found: C, 54.34: H, 6.93. Calc. for $C_{16}H_{24}O_8.\frac{1}{2}H_2O$. C, 54.38; H, 7.13%). $[\alpha]_D^{18°} - 52.2°$ (*c* 0.47, MeOH); ν_{max} (KBr) cm^{-1} 3500 ~ 3300 (OH); λ_{max} (MeOH)nm(ε) 276 (1976). PMR: δ ($CDCl_3$ + CD_3OD) 1.9 (2H, *m*, -β-CH_2—) 271 (2H, *t*, *J* = 7 Hz, α-CH_2—) 3.66 (2H, *t*, *J* = 6.5 Hz, γ-CH_2—) 3.93 (3H, *s*, OMe) 4.90 (1H, *m*, W(h/2) = 9 Hz anomeric H of glucose) 6.70 ~ 7.35 (3H, *m*, ϕ-H). Peracetylation of **II** afforded an oil $[\alpha]_D^{20°}$ – 22.5° (*c* 0.21, $CHCl_3$); ν_{max} (Nujol) cm^{-1} 1745; λ_{max} ($CHCl_3$)nm(ε) 276 (2200), 280 (2200). MS *m/e* 554 (M^+. $C_{26}H_{34}O_{13}^+$) 331 ($C_{14}H_{19}O_9^+$). PMR: δ 1.9 (2H, *m*, -β-CH_2—), 2.0 ~ 2.2 (15H, 5 × OAc) 2.7 (2H, *t*, *J* = 8 Hz, α-CH_2—) 3.88 (3H, *s*, OMe) 4.16 (2H, *t*, *J* = 6 Hz, γ-CH_2OAc) 4.32 (2H, *m*, glucose 6-H) 5.0 ~ 5.5 (4H, *m*, glucose 1,2,3,4-H) 6.7 ~ 7.3 (3H, *m*, ϕ-H). Compound **2** (40 mg) was hydrolysed with HCl (1 M) in H_2O for 30 min at 100°. The reaction micture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed, dried and evaporated. The residue was passed through a Si gel column (eluent: EtOAc) to afford dihydroconiferyl alcohol (10 mg) as an oil (identical IR, TLC to authentic sample). The aq. layer was neutralised with Amberlite IR-45 and concd. Purification of the residue on Si gel and Sephadex (as for the hydrolysis of **1** gave D-glucose (13 mg) $[\alpha]_D^{20°} + 44.8°$ (*c* 1.25, H_2O).

*Dihydro-*p*-coumaryl alcohol γ-O-β-D-glucopyranoside* (**3**). Needles from H_2O, mp 164–166° $[\alpha]_D^{20°} - 24.3°$ (*c* 0.47, MeOH; λ_{max} (KBr) cm^{-1}, 3500 ~ 3300 (OH); λ_{max} (MeOH)nm(ε) 279 (3032). It was acetylated (Ac_2O–Py) to give an oil $[\alpha]_D^{20°} - 13.8$ (*c* 0.56, $CHCl_3$). ν_{max} (Nujol) cm^{-1} 1745 MS *m/e* 524 (M^+, $C_{25}H_{32}O_{12}^+$), 482 (M^+-42) 331 ($C_{14}H_{19}O_9^+$). PMR: δ 1.9 (2H, *m*, β-CH_2—) 2 ~ 2.2 (12H, 4 × OAc) 2.3 (3H, *s*, ϕ-OAc) 2.7 (2H, *t*, *J* = 7 Hz, α-CH_2—) 4.3 (2H, *m*, glucose 6H) 4.58 (1H, *d*, *J* = 7 Hz anomeric H) 5 ~ 5.4 (3H, *m*, 2,3,4-H glucose) 7.07 (δ_A) 7.3 (δ_B) (4H, *q*, *J* = 9 Hz A_2B_2 system). Compound **3** (50 mg) was methylated [8] to give the perMe ether as an oil (40 mg) $[\alpha]_D^{24°} - 17.3$ (*c* 0.85, $CHCl_3$). λ_{max} ($CHCl_3$)nm(ε) 278 (1604), 285 (1581). PMR: δ 2 (2H, *m*, β-CH_2) 2.72 (2H, *t*, *J* = 7 Hz, α-CH_2) 3.44, 3.58, 3.66, 3.68 (12H, 4 × OMe) 3.81 (3H, *s*, ϕ-OMe)

4.26 (1H, *d*, J = 7 Hz anomeric H) 6.90 (δ_A), 7.22 (δ_B) (4H, J = 9 Hz A_2B_2 system). Compound **3** (350 mg) was hydrolyzed with H_2SO_4 (2N) in H_2O (5 ml) for 3 hr. The reaction mixture was diluted with H_2O and extracted with EtOAc. The organic layer was washed, dried and evaporated to a residue which was chromatographed on Si gel with EtOAc to give dihydro-*p*-coumaryl alcohol as needles from CH_2Cl_2–*n*-hexane, mp 49–50°. (Found: C, 71.11; H, 8.01. Calc. for $C_9H_{12}O_2$: C, 71.02; H, 7.95%). λ_{max} (MeOH)nm(ε) 279 (1824) (identical mmp, IR, TLC, dihydro-*p*-coumaryl alcohol [6]). Acetylation of the aglycone afforded an oil (Found: C, 65.61; H, 7.31. Calc. for $C_{13}H_{16}O_4$; C, 66.08; H, 6.83%). PMR: δ 1.9 (2H, *m*, β-CH_2—) 2.03 (3H, *s*, -OAc) 2.27 (3H, *s*, φ-OAc) 2.7 (2H, *t*, J = 7 Hz, α-CH_2—) 4.12 (2H, *t*, J = 6 Hz, γ-CH_2OAc) 7.02 (δ_A) 7.24 (δ_B) (4H, *q*, J = 9 Hz A_2B_2 system). The sugar fraction was examined by TLC and purified on Si gel and Sephadex LH-20 columns and D-glu $[\alpha]_D^{20°}$ + 49.5° (*c* 0.80, H_2O) was obtained. An aglycone was obtained by methanolysis together with two methylated sugars identical on TLC with synthetic samples of Me pyranosides of 2,3,4,6-tetra-*O*-Me-α- and β-D-glu.

Dihydro-p-*coumaryl alcohol 4*-O-β-D-*glucopyranoside* (**4**). Needles from Me_2CO mp 132–134° (lit. [6] mp 132–133°) $[\alpha]_D^{20°}$ − 35.1° (*c* 0.65, MeOH), (Found: C, 54.53; H, 6.94. Calc. for $C_{15}H_{22}O_7.H_2O$; C, 54.21; 7.28%). λ_{max} (MeOH)nm(ε) 274 (1121) 280 (971). PMR ($CDCl_3$ + CD_3OD): δ 1.9 (2H, *m*, β-CH_2—) 2.7 (2H, *t*, J = 7 Hz, α-CH_2—) 3.66 (2H, *t*, J = 6.5 Hz, γ-CH_2OH) 4.92 (1H, *m*, W(h/2) = 8 Hz, anomeric H) 7.17 (4H, *m*, φ-H). The glucoside (II, R=R′=H) was hydrolysed with N HCl in H_2O for 1 hr to yield D-glucose and dihydro-*p*-coumaryl alcohol (identical mp, TLC with authentic sample). Acetylation afforded an acetate as an oil. MS *m/e* 524 (M^+, $C_{25}H_{32}O_{12}^+$), 331 ($C_{14}H_{19}O_9^+$). PMR (100 MHz): δ 1.96 (2H, *m*, β-CH_2—) 2.05 (15H, 4 × OAc (sugar) + -CH_2OAc) 2.65 (2H, *t*, J = 7 Hz, α-CH_2—) 3.81 (1H, *m*, glucose 5-H) 4.07 (2H, *t*, J = 7 Hz γ-CH_2OAc) 4.23 (2H, *m*, glucose 6H) 5.0 ~ 5.4 (4H, *m*, glucose 1,2,3,4-H) 6.91 (δ_A) 7.11 (δ_B) (4H, *q*, J = 9 Hz A_2B_2 system).

Chavicol 4-O-α-L-*arabinofuranosyl-(1,6)*-β-D-*glucopyranoside* (**5**). Needles (from H_2O), mp 109–112°. (Found: C, 54.63; H, 6.56. $C_{20}H_{28}O_{10}.\frac{1}{2}H_2O$ requires C, 54.91; H, 6.68%). $[\alpha]_D^{18°}$ − 87.8° (*c* 0.55, MeOH). ν(KBr) cm^{-1} 3500, 3300. λ_{max} (MeOH) nm(ε) 259 (1718) 271 (1562) 279 (1250). Acetylation gave a peracetate which was purified by chromatography on Si gel (eluent: *n*-hexane–EtOAc (1:1) and crystallized as needles (MeOH) mp 104–106° (Found: C, 56.40; H, 6.2. $C_{32}H_{40}O_{16}$ requires: C, 56.46; H, 5.92%). $[\alpha]_D^{20°}$ − 11.0° (*c* 0.40, $CHCl_3$) ν_{max} (Nujol) cm^{-1} 1740. λ_{max} ($CHCl_3$) nm(ε) 267 (1496), 272 (1496) 280 (1275). MS: *m/e* 680 (M^+ $C_{32}H_{40}O_{16}^+$), 547 (peracetylated pentosyl hexose residue $C_{23}H_{31}O_{15}^+$) 259 (terminal peracetylated pentose residue $C_{11}H_{15}O_7^+$). PMR: δ 2 ~ 2.2 (18H, 6 × OAc) 3.4 (2H, *d*, J = 7 Hz, φC$\underline{H}_2$.CH=CH_2) 5.1 (2H, *bd*, J = 14 Hz, CH_2CH=C$\underline{H}_2$) 6 (1H, *m*, CH_2C$\underline{H}$=CH_2) 7.01 (δ_A) 7.23 (δ_B) (4H, *q*, J = 9 Hz, A_2B_2 system). Compound **5** (350 mg) was hydrogenated with Pd–C (100 mg; 10%) in EtOH to give a dihydroderivative (340 mg) as an amorphous powder $[\alpha]_D^{20°}$ − 72.8° (*c* 0.25, MeOH) λ_{max} (MeOH)nm(ε) 274 (1049), 280 (946). Acetylation (Ac_2O–Py) of the dihydroglycoside gave a peracetate which crystallised from MeOH as needles, mp 130–132°. (Found: C, 56.00; H, 6.44. $C_{32}H_{42}O_{16}$ requires C, 56.30; H, 6.28%). $[\alpha]_D^{20°}$ − 4.0° (*c* 0.25, $CHCl_3$), λ_{max} ($CHCl_3$)nm(ε) 267 (1337) 280 (928). PMR: δ 0.94 (3H, *t*, J = 7 Hz, Me) 1.8 (2H, *m*, β-CH_2—) 2 ~ 2.2 (18H, 6 × OAc) 2.6 (2H, *t*, J = 7 Hz, α-C$\underline{H}_2$—) 6.99 (δ_A) 7.21 (δ_B) (4H, *q*, J = 9 Hz, A_2B_2 system). Permethylation of the dihydroglycoside (100 mg) and chromatography of the product on Si gel (eluent: *n*-hexane–EtOAc (1:1)) gave the perMe ether (50 mg) as needles (MeOH), mp 79–80°; $[\alpha]_D^{20°}$ − 85.0° (*c* 0.28. $CHCl_3$). (Found: C, 60.94; H, 8.22. $C_{26}H_{42}O_{10}$ requires C, 60.68; H, 8.23%). λ_{max} ($CHCl_3$)nm(ε) 274 (1176), 281 (972). PMR: δ 0.93 (3H, *t*, J = 7 Hz, Me) 1.56 (2H, *m*, β-CH_2—) 2.58 (2H, *t*, J = 8 Hz α-CH_2—) 3.3, 3.43, 3.47, 3.58, 3.7 (18H, 6 × OMe), 4.89 (1H, *bd*, J = 5 Hz anomeric H glucose) 5.1 (1H, *s*, anomeric-H arabinose) 7.12 (4H, *s*, φ-H). Compound **5** (90 mg) was hydrolysed by heating with N HCl in H_2O for 30 min. The reaction mixture was diluted and extracted with $CHCl_3$. The aq. layer was neutralised with Amberlite IR-45, concd and examined by TLC. Arab and glu were detected. The concentrate was chromatographed on Si gel (eluent: $CHCl_3$–MeOH–H_2O (7:3:0.5 → 25:17:2)) and then Sephadex LH-20 (MeOH) to give two compounds, an oil (8 mg) $[\alpha]_D^{20°}$ + 98.7° (*c* 0.45, H_2O) and an oil (22 mg) $[\alpha]_D^{21°}$ + 44 (*c* 1.0, H_2O) which were identified as L-arabinose and D-glucose respectively. The dihydroglycoside of **5** (90 mg) was hydrolysed as before and the residue from the $CHCl_3$ layer was chromatographed on Si gel (eluent: *n*-hexane–EtOAc (3:1)) to give dihydrochavicol as an oil. (Found: C, 79.77; H, 8.79. Calc. for $C_9H_{12}O$ C, 79.37; H, 8.88%). λ_{max} (MeOH)nm(ε) 279 (2040). PMR: δ 0.91 (3H, *t*, J = 7 Hz, Me) 1.54 (2H, *m*, —C$\underline{H}_2$—) 2·54 (2H, *t*, J = 7 Hz, φ-C$\underline{H}_2$—) 6.02 (1H, *s*, φ-OH) 6.84 (δ_A) 7.12 (δ_B) (4H, *q*, A_2B_2 system) (identical TLC, PMR with a synthetic sample). On TLC the sugar fraction showed two spots, arab and glu. The dihydropermethyl ether of **5** (10 mg) was refluxed with HCl (8%) in MeOH (2 ml) for 2 hr and worked up as described previously. The methylated sugar fraction was examined by TLC. 4 spots were detected and identified as the Me glycosides of 2,3,5-tri-*O*-Mel-α- and β-L-arabinofuranose and 2,3,4-tri-*O*-Me-α- and β-D-glucopyranose by comparison with synthetic samples. (R_f values of Me 2,3,5-tri-*O*-Me-L-arabinofuranoside: α and/or β-anomer, 0.64, 0.51 (solvent A); 0.81, 0.7 (solvent B); Me 2,3,4-tri-*O*-Me-D-glucopyranoside: β- and α-anomer 0.2, 0.13 (solvent A); 0.51, 0.35 (solvent B)). Solvent A = C_6H_6–MeCO (4:1); Solvent B = EtOAc–MeOH (50:1). The methylated sugar fraction was hydrolysed with N HCl in H_2O for 2 hr, the hydrolysate was neutralised with Amberlite IR-45, evaporated and the residue was examined by TLC (EtOAc–MeOH, 25:1). Two spots were detected and identified as 2,3,5-tri-*O*-Me-L-arabinose (R_f 0.63) and 2,3,4-tri-*O*-Me-D-glucose (R_f 0.29) by comparison with synthetic samples. Dihydrochavicol was also found in the hydrolysate.

4′-O-β-D-glucopyranoside of 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranopropanol (**6**). Amorphous powder $[\alpha]_D^{24°}$ − 41.1 (*c* 0.26, MeOH), ν (KBr) cm^{-1}: 3500 3200; λ_{max} (MeOH)nm(ε) 281 (5666). Acetylation (Ac_2O–Py) gave a peracetate as an amorphous powder $[\alpha]_D^{21}$ − 12.0° (*c* 0.25, $CHCl_3$). ν_{max} (Nujol) cm^{-1} 1740. λ_{max} ($CHCl_3$)nm(ε) 281 (2117). PMR: δ 2.1 ~ 2.2 (18H, 6 × OAc) 2.33 (3H, *s*, OAc) 3.9 (3H, *s*, OMe), 2.71 (2H, *t*, J = 7 Hz, α-C$\underline{H}_2$—) 4.17 (2H, *t*, J = 6.0 Hz, γ-CH_2OAc) 4.9 ~ 5.5 (4H, *m*, glu 1,2,3,4-H) 5.65 (1H, *d*, J = 6 Hz, φ-CH— 6.9 ~ 7.4 (5H, *m*, φ-H). Compound **6** (140 mg) in H_2O (5 ml) was incubated with almond emulsin (50 mg) at 37° for 2 hr and the hydrolysate then shaken with EtOAc. The aq. layer was concd and chromatographed on Si gel and Sephadex LH-20 to give D-glucose as an oil $[\alpha]_D^{20°}$ + 51.43° (*c* 0.35, H_2O). The organic layer was concd and chromatographed on Si gel (eluent: EtOAc) to yield an aglycone (62 mg) as an oil $[\alpha]_D^{21°}$ + 5.4° (*c* 0.70, MeOH): λ_{max} (MeOH)nm(ε) 283 (4153). PMR ($CDCl_3$ + CD_3OD): δ 1.9 (2H, *m*, β-CH_2—), 2.66 (2H, *t*, J = 7 Hz, φ-CH_2—) 3.66 (2H, *t*, J = 6 Hz, —CH_2C$\underline{H}_2$OH) 3.92 (3H, *s*, OMe) 5.56 (1H, *d*, J = 6 Hz, φ-CH—) 6.6 ~ 7.2 (5H, *m*, φ-H). The aglycone was identified by direct comparison (TLC cochromatography) as 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol. Acetylation of the aglycone (Ac_2O–Py) gave an oil; λ_{max} ($CHCl_3$)nm(ε) 279 (1871). PMR (100 MHz): δ 1.9 (2H, *m*, β-CH_2) 2.05 (3H, *s*, OAc) 2.07 (3H, *s*, OAc), 2.29 (6H, *s*, 2 × OAc), 2.64 (2H, *t*, J = 7 Hz, φ-CH_2-) 3.7 (1H, *m*, —CH—CH—CH_2), 3.82 (3H, *s*, OMe) 4.08 (2H, *t*, J = 7 Hz, —CH_2C$\underline{H}_2$OAc) 4.28 (1H, *q*, J = 11, 8 Hz, —CH.C$\underline{H}_2$.OAc) 4.46 (1H, *q*, J = 11, 6 Hz, —CH—C$\underline{H}_2$.OAc) 5.56 (1H, *d*, J = 6 Hz, φ-CHO—CH—) 6.8 ~ 7.1 (5H, *m*, φ-H). Irradiation of the multiplet centre at δ 3.7 (C_3-H)caused collapse of the triplet at δ 4.08 and quartet at δ 4.28 to two doublets and the doublet at δ 5.56 to a singlet. Methylation of **6** (70 mg) gave a product which was subjected to PLC (*n*-hexane–EtOAc, 5:6) to afford an oil (72 mg). $[\alpha]_D^{21}$ − 21.8° (*c* 0.45, $CHCl_3$). λ_{max} ($CHCl_3$)nm(ε) 282 (3838). PMR: δ 1.95 (2H, *m*, β-CH_2—), 2.7 (2H, *m*, J = 8 Hz, α-CH_2—) 3.4 (3H, *s*, -CH_2OMe), 3.44 (6H, *s*,

—CH_2OMe and sugar-OMe) 3.6, 3.7, 3.75 (9H, sugar OMe × 3), 3.88 (3H, *s*, ϕ-OMe) 3.95 (3H, *s*, ϕ-OMe), 4.83 (1H, *bd*, J = 7 Hz, aonmeric H of glucose) 5.6 (1H, *m*, W(h/2) = 8 Hz, ϕ-C<u>H</u>—CH—) 6.6 ~ 7.4 (5H, *m*, ϕ-H). Methanolysis of the octaMe ether of **6** (70 mg) with HCl–MeOH (8%) for 1 hr, worked up in the usual manner gave two aglycones and Me 2,3,4,6-tetra-*O*-Me-α- and β-D-glucopyranosides which were detected by TLC. Chromatography of the methanolysis products by PLC (C_6H_6–Me_2CO, 4:1) gave 2,3-dihydro-7-methoxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-methoxymethyl-5-benzofuran-1-methoxypropane (18 mg) (**IV** R = H) and 3-methyl-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-5-benzofuran-1-methoxypropane (13 mg) (**V**). The tetrMe ether (**IV**, R = H) was an oil, $[\alpha]_D^{20}$ + 17.5° (*c* 0.85, $CHCl_3$) λ_{max} ($CHCl_3$) nm(ε) 284 (5821) 310 (1096). PMR: δ 1.9 (2H, *m*, β-CH_2) 2.7 (2H, *t*, J = 8 Hz, α-CH_2—) 3.41, 3.44 (6H, 2 × *s*, —CH_2O<u>Me</u>) 3.46 (2H, *t*, J = 6 Hz. —CH_2<u>CH_2</u>OMe) 3.7 (2H, *m*, —CH_2OMe) 3.91, 3.94 (6H, 2 × *s*, OMe) 5.55 (1H, *m*, W(h/2) = 9 Hz, —<u>C</u>H—CH) 5.74 (1H, *bs*, 4′-OH) 6.6 ~ 7.2 (5H, *m*, ϕ-H). Acetylation of **IV**, R = H (17 mg) gave a monoacetate (16 mg) as an oil $[\alpha]_D^{20°}$ −2.5° (*c* 0.80, $CHCl_3$) λ_{max} ($CHCl_3$) nm(ε) 280 (3924) 290 (2311). PMR: δ 2.31 (3H, *s*, OAc) 3.39, 3.44 (6H, 2 × *s*, —CH_2OCH_3), 3.85, 3.95 (6H, 2 × *s*, OMe). The methanolysis product (**V**) was on oil, λ_{max} ($CHCl_3$)nm(ε) 304 (13829). PMR: δ 2 (2H, *m*, β-CH_2—) 2.43 (3H, *s*, Me) 2.83 (2H, *t*, J = 7 Hz, α-CH_2—) 3.41 (3H, *s*, —CH_2O<u>Me</u>) 3.48 (2H, *t*, J = 6 Hz, γ-CH_2—) 4.02, 4.07 (6H, 2 × *s*, OMe) 5.84 (1H, *bs*, O<u>H</u>) 6.6 ~ 7.6 (5H, *m*, ϕ-H). *Compound* **7**. Needles from MeOH, mp 184–186°. ν_{max} (KBr) cm^{-1} 3480, 3390, 3220, 2870, 1690 (identical mp, TLC, IR to shikimic acid).

Syntheses of authentic samples. Dihydroconiferyl alcohol. Acetylation of ferulic acid (140 mg) with Ac_2O–Py, followed by methylation with CH_2N_2 gave Me-4-acetoxy-3-methoxycinnamate (80 mg) as an oil. PMR: δ 2.34 (3H, *s*, ϕ-OAc) 3.85 (3H, *s*, OMe or CO_2Me), 3.9 (3H, *s*, OMe or CO_2Me), 6.46 (1H, *d*, J = 16 Hz, ϕ-CH=C<u>H</u>.CO.) 6.6 ~ 7.5 (3H, *m*, ϕ-H) 7.76 (1H, *d*, J = 16 Hz, ϕ-<u>C</u>H=CH.CO). The Me ester (80 mg) in dry THF and $LiAlH_4$ was refluxed for 6 hr. Excess reagent was decomposed with H_2O, the reaction mixture evaporated *in vacuo* and extracted with EtOAc. The extracts were dried, evaporated and the residue (25 mg) hydrogenated with Pd–C (10%) in EtOH to give dihydroconiferyl alcohol (7 mg) which was purified on Si gel (eluent: *n*-hexane–EtOAc, 1:1). Dihydro-*p*-coumaryl alcohol: hydrogenation of *p*-coumaryl alcohol (80 mg) with Pd–C (50 mg; 10%) in EtOH followed by PLC purification (EtOAc) gave the dihydro derivative (50 mg). Dihydrochavicol: hydrogenation of anethole with Pd–C (10%) in EtOH gave dihydroanethole as an oil. PMR: δ 0.93 (3H, *t*, J = 7 Hz, Me) 1.57 (2H, *m*, CH_2<u>CH_2</u>CH_3) 2.57 (2H, *t*, J = 7 Hz, ϕ-C<u>H_2</u>—) 3.8 (3H, *s*, OMe) 6.92 (δ_A) 7.22 (δ_B) (4H, *q*, J = 9 Hz, A_2B_2 system). Demethylation by reflux in Ac_2O and HI for 1 hr, followed by treatment with aq. Na_2SO_3 and extraction with $CHCl_3$ gave, on evaporation, a residue. The residue was purified on Si gel (eluent: *n*-hexane–EtOAc 3:1) to yield dihydrochavicol.

Me 2,3,4,6-tetra-O-Me-α- and β-D-glucopyranosides. Methanolysis of arbutin (hydroquinone β-D-glucopyranoside) permethylate provided 2,3,4,6-tetra-*O*-Me ethers.

Me 2,3,4-tri-O-Me-α- and β-D-glucopyranosides. Rutin (quercetin α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside) permethylate was methanolysed to give 2,3,4-tri-*O*-Me ethers together with Me 2,3,4-tri-*O*-Me-L-rhamnopyranoside.

Me 2,3,5-tri-O-Me-α and β-L-arabinofuranosides. Me L-arabinosides which were obtained by the usual methyl glycosidation (HCl–MeOH) were methylated by the Kuhn method (DMF, Ag_2O, CH_3I) to give 2,3,5-tri-*O*-Me ethers together with 2,3,4-tri-*O*-Me ethers.

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