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Synthesis of aryl glycosides as *vir* gene inducers of *Agrobacterium tumefaciens**

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ABSTRACT

Aryl β-glycopyranosides have been synthesized by coupling syringaldehyde (3-methoxyvanillin) with L-fucose, D-galactose, and maltose; acetosyringone (4-hydroxy-3,5-dimethoxyacetophenone) with L-fucose and maltose; acetovanillone (4-hydroxy-3-methoxyacetophenone) with L-fucose; and syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid) with D-galactose. The procedure using peracetylated glycosyl halides and sodium phenolates in aqueous acetone afforded the acetylated β-glycosides, which were deacetylated.

INTRODUCTION

A variety of simple plant phenolic compounds have been shown to trigger expression of virulence in the soil phytopathogen, *Agrobacterium tumefaciens* (for reviews, see Nester and Gordon[1], and Zambryski *et al.*[2]). Acetosyringone and α-hydroxyacetosyringone were identified as agents of *vir* induction in exudates of tobacco cells[3], and also released by wounded, metabolically active plant cells. Components of lignin or its precursors, such as coniferyl alcohol and sinapyl alcohol, also act as signal molecules[3,4]. On the one hand, plant cell cultures are able to convert exogenous phenolic compounds into their corresponding D-glucosides[5] and on the other hand, a large number of phenolic D-glucosides have been isolated from the bark and leaves of trees of the family Salicaceae and identified[6–8]. More recently, a new inducer, the coniferin [4-(3-hydroxy-1-propenyl)-2-methoxyphenyl β-D-glucopyranoside] has been isolated from *Pseudotsuga menziesii* (Douglas fir) and showed a strong inducing activity for transformation of gymnosperms by *Agrobacterium tumefaciens* strains[9]. The same derivative has been previously characterized from extracts of root cultures of *Linum flavum*[10].

Agrobacterium tumefaciens is a useful tool for plant genetic engineering. But some species, even among dicotyledonous plants, are recalcitrant to transformation, perhaps because of an absence of a specific gene inducer. For this purpose, we undertook to develop the chemical synthesis of aryl glycosides as potential gene inducers[11]. We describe herein the synthesis of some β-L-fucopyranosides, β-D-galactopyranosides, and

* Dedicated to Professor Jean Montreuil on the occasion of his 70th birthday.

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TABLE I

^{1}H-N.m.r. data[a] of compounds **2, 4, 6, 9,** and **11**[b]; and **3, 5, 7, 10,** and **12**[c]

Atoms	**2**	**3**	**4**	**5**	**6**	**7**	**9**	**10**	**11**	**12**
H-2	7.50	7.61	7.19	7.32	7.11	7.33	7.10	7.31	7.33	7.42
	m	d (2)								
H-5	7.14	7.23								
	d (8)	d (8.5)								
H-6	7.50	7.70	7.19	7.32	7.11	7.33	7.10	7.31	7.33	7.42
	m	dd (2,8.5)								
H-7					9.86	9.81	9.86	9.79		
H-8	2.55	2.65	2.56	2.65						
OCH_3	3.86	3.96	3.88	3.93	3.89	3.94	3.89	3.93	3.86	3.94
H-1′	4.99	5.20	5.04	5.12	5.08	5.15	5.06	5.16	5.04	5.14
	d (8)	d (7.5)	d (8)	d (7.5)	d (8)	d (7.5)	d (8)	d (7.5)	d (8)	d (7.5)
H-2′	5.50	3.89	5.48	3.80	5.48	3.80	5.51	3.83	5.51	3.84
	dd (8,10.5)	dd (7,10)	dd (8,10.5)	dd (7.5,10)	dd (8,10.5)	dd (7.5,10)	dd (8,10.5)	dd (7.5,10)	dd (8,10.5)	dd (7.5,9.5)
H-3′	5.09	3.82	5.05	3.71	5.05	3.71	5.06	3.70	5.06	3.74–3.70
	dd (3.5,10.5)	dd (3,10)	dd (3.5,10.5)	dd (3.5,10)	dd (3.5,10.5)	dd (3.5,10)	dd (3,11)	m	dd (3.5,10.5)	m
H-4′	5.28	3.87	5.22	3.78–3.73	5.22	3.76	5.38	3.94	5.37	3.97
	d (3.5)	dd (1,3)	dd (1,3.5)	m	dd (1.5,3.5)	dd (1,3.5)	dd (1,3.5)	d (3)	dd (1,3.5)	d (3.5)
H-5′	3.91	4.06	3.76	3.78–3.73	3.77	3.74	3.87	3.62	3.86	3.64
	q (6.5)	dq (1,6.5)	dq (1,6.5)	m	dq (1,6.5)	dq (1,6.5)	dt (1,6.5)	t (6)	m	t (6)
H-6′a	1.26	1.32	1.20	1.21	1.19	1.21	4.16	3.70	4.16	3.74–3.70
	d (6.5)	d (6.5)	d (6.5)	d (6.5)	d (6.5)	d (6.5)	dd (6.5,11)	m	dd (6.5,11)	m
H-6′b							4.10	3.70	4.11	3.74 3.70
							dd (7,11)	m	dd (7,11)	m
$COCH_3$	2.18,2.04		2.19,1. 98		2.19,1.98		2.17,2.00		2.16,2.00	
	1.99		1.97		1.97		1.98,1.97		1.98,1.97	

[a] δ Values, J and J' values are noted in parentheses. [b] For a solution in $CDCl_3$, [c] For a solution in D_2O.

β-maltosides of acetovanillone (**27**) (4-hydroxy-3-methoxyacetophenone), acetosyringone (**28**) (4-hydroxy-3,5-dimethoxyacetophenone), syringaldehyde (**29**) (3-methoxyvanillin), and syringic acid (**30**) (4-hydroxy-3,5-dimethoxybenzoic acid).

RESULTS AND DISCUSSION

In Mauthner's original procedure[12], previously used for the syntheses of various aryl glycosides[11], the 1:1 molar ratio between the phenolate and the glycosyl halide gave poor yields for the preparation of aryl β-L-fucosides (26%). A 3:1 molar ratio afforded a better yield (40%) for the synthesis of **4** and **6**. All the isolated compounds had the β configuration, as determined by ^{1}H-n.m.r. spectroscopy (Table I). The *O*-deacetylated glycosides were homogeneous in liquid chromatography (h.p.l.c.) on a RP-18 column using an isocratic mobile phase (acetonitrile–acetic acid–water)[13]. For the same aglycon, the acetosyringyl group (**b**), the elution times were increased in the following order: β-D-galactopyranoside < β-maltoside < β-D-glucopyranoside < β-L-fucopyranoside, and finally the free acetosyringone (Table II). The less hydrophilic β-L-fucopyranosides were strongly retarded under the chromatographic conditions used.

Acetosyringonyl β-L-fucopyranoside was the most active monoglycoside tested that induced the expression of *Agrobacterium tumefaciens* virulence (*vir*) genes[14].

TABLE II

H.p.l.c.[a] data of deacetylated glycosides **3, 5, 7, 10, 12–18, 22, 24,** and **25**, and related phenols **27–30**

	Compounds			
	Syringic acid	*Syringaldehyde*	*Acetovanillone*	*Acetosyringone*
Free phenyl derivative	7.43 (**30**)	12.85 (**29**)	14.51 (**27**)	15.63 (**28**)
Related aryl β-glycosides				
L-Fucosyl		9.44 (**7**)	8.41 (**3**)	11.24 (**5**)
D-Glucosyl	3.07 (**16**)[b]	4.48 (**15**)[b]	4.47 (**13**)[b]	5.30 (**14**)[b]
Maltosyl		4.32 (**24**)	4.14 (**25**)[b]	4.92 (**22**)
D-Galactosyl	2.77 (**12**)	4.07 (**10**)	4.07 (**17**)[b]	4.81 (**18**)[b]

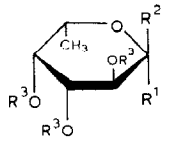
[a] In the isocratic, mobile phase 7:1:42 acetonitrile–acetic acid–water with a flow of 1 mL·min^{-1} and detection at A_{270}. The sample (1 mg) was dissolved in bidistilled water (1 mL) and 10 μL of the solution were injected. [b] Previously described (see ref. 11).

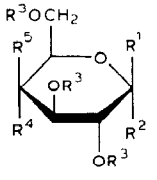
EXPERIMENTAL

General methods. — Melting points were determined with a Leitz hot-plate microscope and are uncorrected. Optical rotations were measured with a Perkin–Elmer spectropolarimeter model 141. I.r. spectra were recorded with a Perkin–Elmer 257 spectrometer for KBr pellets. ^{1}H-N.m.r. spectra were recorded with an AM 300 WB Bruker spectrometer, m.s. data with a Nermag R-10-10C spectrometer in the chemical-

ionization mode using ammonia to generate ions, and u.v. and visible absorption spectra with an Uvikon 860 spectrophotometer. T.l.c. was performed on Silica Gel 60 F_{254} (Merck) and spots were detected by fluorescence and the phosphomolybdic–sulfuric acid $\{H_7[P(Mo_2O_7)_6]$–$H_2SO_4\}$ reagent. H.p.l.c. was performed with a Spectra–Physics high performance liquid chromatograph, equipped with a SP 8700 ternary-solvent-delivery system, a SP 8440 spectrophotometric detector attached to a SP 4270 integrator, an ODS Spheri-5 analytical column (23 cm; Spectra–Physics) and a RP-18 guard column (7μm × 2 cm) (Spectra–Physics). 2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl chloride (**1**), 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**8**), and 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-maltosyl bromide (**20**) were purchased from Sigma Chem. Co. (La Verpillière, France). Acetovanillone (**27**), acetosyringone (**28**), syringaldehyde (**29**), and syringic acid (**30**) were from Aldrich (Strasbourg, France). Silica Gel 60 (0.04–0.06 mm, 230–400 mesh) and other chemical reagents were purchased from Merck (Nogent-sur-Marne, France).

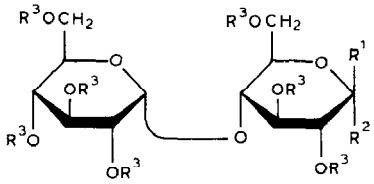
General procedure for glycosylation. — A stirred solution of the phenol (2.05 mmol) in 0.286M NaOH (7 mL, 2 mmol) was cooled to 14° and treated with a solution of per-*O*-acetylglycosyl halide (1.95 mmol) in acetone (7 mL), added dropwise. The solution was stirred at room temperature for 3–5 h in the dark, and then concentrated to dryness. Examination of the syrupy residue by t.l.c. on silica gel revealed the presence of the desired glycoside as the major component, some excess phenolic compound, and some partially deacetylated glycosyl derivatives. The syrup was evaporated under vacuum and acetylated with acetic anhydride (2 mL) in pyridine (2 mL) for 50 min at 90°. The solution was then concentrated to dryness under reduced pressure.



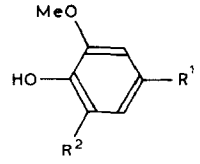


1 R^1 = H, R^2 = Cl, R^3 = Ac
2 R^1 = α, R^2 = H, R^3 = Ac
3 R^1 = α, R^2 = R^3 = H
4 R^1 = b, R^2 = H, R^3 = Ac
5 R^1 = b, R^2 = R^3 = H
6 R^1 = c, R^2 = H, R^3 = Ac
7 R^1 = c, R^2 = R^3 = H

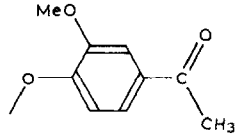
	R^1	R^2	R^3	R^4	R^5
8	H	Br	Ac	H	OAc
9	c	H	Ac	H	OAc
10	c	H	H	H	OH
11	d	H	Ac	H	OAc
12	d	H	H	H	OH
13	α	H	H	OH	H
14	b	H	H	OH	H
15	c	H	H	OH	H
16	d	H	H	OH	H
17	α	H	H	H	OH
18	b	H	H	H	OH
19	H	OH	Ac	OAc	H



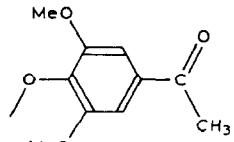
20 $R^1 = H, R^2 = Br, R^3 = Ac$
21 $R^1 = b, R^2 = H, R^3 = Ac$
22 $R^1 = b, R^2 = R^3 = H$
23 $R^1 = c, R^2 = H, R^3 = Ac$
24 $R^1 = c, R^2 = R^3 = H$
25 $R^1 = a, R^2 = R^3 = H$
26 $R^1 = OH, R^2 = H, R^3 = Ac$



27 $R^1 = Ac, R^2 = H$
28 $R^1 = Ac, R^2 = OMe$
29 $R^1 = CHO, R^2 = OMe$
30 $R^1 = CO_2H, R^2 = OMe$



a



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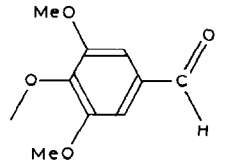
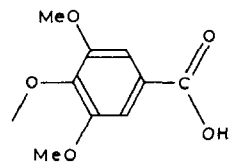


c



d

Purification was achieved by chromatography on a silica gel column using 1:1 chloroform–ethyl acetate as solvent.

General procedure for deacetylation. — To a solution of phenyl per-*O*-acetylglycoside (0.5 mmol) in anhydrous methanol (4–8 mL) was added dropwise a solution of M sodium methoxide in methanol (25 μL, 25 μmol). After being kept for 6 h at room temperature, the solution was rendered neutral with acetic acid, and the solvent removed by evaporation. The solid residue crystallized as described.

3-Methoxy-4-(2,3,4-tri-O-*acetyl-β*-L-*fucopyranosyloxy)acetophenone* (**2**). — This compound was obtained by the general procedure for glycosylation from acetovanillone (**27**; 340 mg, 2.05 mmol) and 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl chloride (**1**; 602 mg, 1.95 mmol). After acetylation and concentration to dryness, t.l.c. in 2:1 chloroform–ethyl acetate showed **2** (R_F 0.52), some 4-acetoxy-3-methoxyacetophenone (R_F 0.77), and some 1,2,3,4-tetra-*O*-acetyl-L-fucopyranose (R_F 0.69). By chromatography on a silica gel column (2.2 × 75 cm) using 2:1 chloroform–ethyl acetate as solvent, compound **2** was isolated as a colorless foam (273 mg, 32%), $[\alpha]_D^{25}$ + 7° (*c* 1, chloroform), $[\alpha]_{546}^{25}$ + 9° (*c* 1, chloroform); ν_{max}^{KBr} 1735 (OAc), 1670 (CO ketone), and 800 cm^{-1} (CH arom.); m.s.: *m/z* (%) 456 (9, MNH_4^+), 439 (3, MH^+), 273 (100, MH^+ − **27**), 213 (13, 273 − CH_3CO_2H), 167 (5, **27**·H^+), and 153 (23, 273 − 2 CH_3CO_2H).

Anal. Calc. for $C_{21}H_{26}O_{10}$·0.5 H_2O: C, 56.38; H, 6.04. Found: C, 56.71; H, 6.06.

4-β-L-Fucopyranosyloxy-3-methoxyacetophenone (**3**). — This compound was obtained from **2** by the general procedure for deacetylation. The progress of the reaction was monitored by t.l.c. on silica gel in 4:1 chloroform–methanol [R_F 0.97 (**2**), 0.61 (**3**)]. The deacetylation was quantitative and **3** crystallized from ethanol, m.p. 192–194°, $[\alpha]_D^{25}$ +24° (*c* 1, *N,N*-dimethylformamide), $[\alpha]_{546}^{25}$ +30° (*c* 1, *N,N*-dimethylformamide); λ_{max} 301 nm (log ε 3.78), λ_{max} 267 nm (log ε 4.04); ν_{max}^{KBr} 3360 (OH), 1655 (CO ketone), 1270 (CO), 1215 (CO methoxy), 1070 (CO sugar), and 810 cm^{-1} (CH arom.); m.s.: *m/z* (%) 330 (3, MNH_4^+), 313 (3, MH^+), 167 (100, **27**·H^+), and 151 (20, **27**$^+$ − CH_3).

Anal. Calc. for $C_{15}H_{20}O_7 \cdot H_2O$: C, 54.55; H, 6.67. Found: C, 54.11; H, 6.78.

*3,5-Dimethoxy-4-(2,3,4-tri-*O*-acetyl-β-L-fucopyranosyloxy)acetophenone* (**4**). — 4-Hydroxy-3,5-dimethoxyacetophenone (**28**, acetosyringone; 1.145 g, 5.85 mmol) was dissolved in 0.446M NaOH (13 mL, 5.80 mmol) and subjected to glycosidation in the usual manner with a solution of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl chloride (**1**; 602 mg, 1.95 mmol) in acetone (13 mL). After acetylation and concentration to dryness, t.l.c. on silica gel in 2:1 chloroform–ethyl acetate showed **4** (R_F 0.43), some 4-acetoxy-3,5-dimethoxyacetophenone (R_F 0.71), and some 1,2,3,4-tetra-*O*-acetyl-L-fucopyranose (R_F 0.69). Compound **4** was purified by chromatography on a silica gel column (2.2 × 75 cm) with 2:1 chloroform–ethyl acetate as solvent (338 mg, 37%). It crystallized from methanol, m.p. 170–171°, $[\alpha]_D^{25}$ −8° (*c* 1, chloroform), $[\alpha]_{546}^{25}$ −10° (*c* 1, chloroform); ν_{max}^{KBr} 1740 (OAc), 1680 (CO ketone), 1220 (CO methoxy and sugar), 860 and 830 cm^{-1} (CH arom.); m.s.: *m/z* (%) 486 (100, MNH_4^+), 273 (84, MH^+ − **28**), 213 (11, 273 − CH_3CO_2H), 197 (70, **28**·H^+), 181 (15, **28**$^+$ − CH_3), and 153 (22, 273 − 2 CH_3CO_2H).

Anal. Calc. for $C_{22}H_{28}O_{11}$: C, 56.41; H, 5.98. Found: C, 56.35; H, 5.70.

4-β-L-Fucopyranosyloxy-3,5-dimethoxyacetophenone (**5**). — This compound was obtained from **4** by deacetylation in the usual manner; t.l.c. (4:1 chloroform–methanol) R_F 0.60. It crystallized from ethanol as needles, m.p. 187–188°, $[\alpha]_D^{25}$ +16° (*c* 1, *N,N*-dimethylformamide), $[\alpha]_{546}^{25}$ +17° (*c* 1, *N,N*-dimethylformamide); λ_{max} 277 nm (log ε 4.00); ν_{max}^{KBr} 3400 (OH), 1670 (CO ketone), 1230 (CO methoxy), 1060 (CO sugar), and 830 cm^{-1} (CH arom.); m.s.: *m/z* (%) 360 (2, MNH_4^+), 197 (100, **28**·H^+), 181 (16, **28**$^+$ − CH_3), and 164 (5, MNH_4^+ − **28**).

Anal. Calc. for $C_{16}H_{22}O_8$: C, 56.14; H, 6.43. Found: C, 56.07; H, 6.66.

*3,5-Dimethoxy-4-(2,3,4-tri-*O*-acetyl-β-L-fucopyranosyloxy)benzaldehyde* (**6**). — 3-Methoxyvanillin (**29**, syringaldehyde; 1.065 g, 5.85 mmol) was dissolved in 0.446 M NaOH (13 mL, 5.80 mmol) and subjected to glycosidation in the usual manner with a solution of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl chloride (**1**; 602 mg, 1.95 mmol) in acetone (13 mL). After acetylation and concentration to dryness, t.l.c. on silica gel in 2:1 chloroform–ethyl acetate showed **6** (R_F 0.47), some 4-acetoxy-3-methoxyvanillin (R_F 0.74), and some 1,2,3,4-tetra-*O*-acetyl-L-fucopyranose (R_F 0.69). Compound **6** was purified by chromatography on a silica gel column (2.2 × 75 cm) with 2:1 chloroform–ethyl acetate as solvent (354 mg, 40%). It crystallized from methanol, m.p. 146–148°, $[\alpha]_D^{25}$ −8° (*c* 1, chloroform), $[\alpha]_{546}^{25}$ −12° (*c* 1, chloroform); ν_{max}^{KBr} 1725 (OAc), 1675 (CO formyl), and 1210 cm^{-1} (CO methoxy and sugar); m.s.: *m/z* (%) 472 (70, MNH_4^+), 273

(100, MH^+ – **29**), 213 (5, 273 – CH_3CO_2H), 153 (5, 273 – 2 CH_3CO_2H), and 43 (5, CH_3CO^+).

Anal. Calc. for $C_{21}H_{26}O_{11}$: C, 55.51; H, 5.73. Found: C, 55.77; H, 5.72.

4-β-L-Fucopyranosyloxy-3,5-dimethoxybenzaldehyde (**7**). — This compound was obtained from **6** by the general procedure for deacetylation. The progress of the reaction was monitored by t.l.c. on silica gel in 4:1 chloroform–methanol [R_F 0.95 (**6**), 0.60 (**7**)]. Compound **7** crystallized from ethanol as needles, m.p. 148–149°, $[\alpha]_D^{25}$ + 17° (*c* 1, *N,N*-dimethylformamide), $[\alpha]_{546}^{25}$ + 20° (*c* 1, *N,N*-dimethylformamide); ν_{max}^{KBr} 3500–3400 (OH) and 1690 cm^{-1} (CO formyl); m.s.: *m/z* (%) 346 (1, MNH_4^+), 200 (2, **29**·NH_4^+), 183 (100, **29**·H^+), 164 (8, MNH_4^+ – **29**), 146 (2, 164 – H_2O), and 129 (3, MH^+ – **29** – H_2O).

Anal. Calc. for $C_{15}H_{20}O_8$: C, 54.88; H, 6.10. Found: C, 54.51; H, 6.16.

3,5-Dimethoxy-4-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyloxy)benzaldehyde (**9**). — This compound was obtained by the general procedure for glycosylation from syringaldehyde (**29**; 373 mg, 2.05 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**8**; 800 mg, 1.95 mmol). After acetylation and concentration to dryness, t.l.c. on silica gel in 1:1 chloroform–ethyl acetate showed **9** (R_F 0.52) as the major component, some 4-acetoxy-3,5-dimethoxybenzaldehyde (R_F 0.77), and some 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose (R_F 0.70). Compound **9** was purified by chromatography on a silica gel column (2.2 × 75 cm) with 1:1 chloroform–ethyl acetate as solvent and was isolated as a colorless foam (564 mg, 57%), $[\alpha]_D^{25}$ + 0.5° (*c* 1, chloroform), $[\alpha]_{546}^{25}$ + 1° (*c* 1, chloroform); ν_{max}^{KBr} 1745 (OAc) 1685 (CO formyl), and 1225 cm^{-1} (CO methoxy and sugar); m.s.: *m/z* (%) 530 (100, MNH_4^+), 331 (30, MH^+ – **29**), and 183 (5, **29**·H^+).

Anal. Calc. for $C_{23}H_{28}O_{13}$: C, 53.91; H, 5.47. Found: C, 53.74; H, 5.49.

4-β-D-Galactopyranosyloxy-3,5-dimethoxybenzaldehyde (**10**). — This compound was obtained from **9** by the general procedure for deacetylation; t.l.c. (4:1 chloroform–methanol) R_F 0.35. It crystallized from ethanol as needles, m.p. 214.5–215.5°, $[\alpha]_D^{25}$ – 11° (*c* 1, *N,N*-dimethylformamide), $[\alpha]_{546}^{25}$ – 13° (*c* 1, *N,N*-dimethylformamide); λ_{max} 284 nm (log ε 4.02); ν_{max}^{KBr} 3380 (OH), 1690 (CO formyl), 830 and 780 cm^{-1} (CH arom.); m.s.: *m/z* (%) 362 (2, MNH_4^+), 200 (9, **29**·NH_4^+), 183 (100, **29**·H^+), and 180 (12, MNH_4^+ – **29**).

Anal. Calc. for $C_{15}H_{20}O_9$: C, 52.33; H, 5.81. Found: C, 52.51; H, 6.02.

3,5-Dimethoxy-4-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyloxy)benzoic acid (**11**). — 3,5-Dimethoxy-4-hydroxy-benzoic acid (**30**, syringic acid; 400 mg, 2.02 mmol) was dissolved in 0.571M NaOH (7 mL, 4 mmol) and subjected to glycosylation. After acetylation and concentration to dryness, the solid residue was triturated in 10:10:1 chloroform–ethyl acetate–acetic acid (9 mL) to give a suspension. Salts (sodium bromide and sodium syringate) were removed by centrifugation (10 min at 5000 r.p.m.) and the supernatant was chromatographied on a silica gel column (2.2 × 75 cm) with 10:10:1 chloroform–ethyl acetate–acetic acid as solvent to give 4-acetoxy-3,5-dimethoxybenzoic acid (R_F 0.76), 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose (R_F 0.67), and **11** (R_F 0.58) as the major component. Compound **11** was isolated as a yellowish foam (460 mg, 45%), $[\alpha]_D^{25}$ + 4° (*c* 1, chloroform), $[\alpha]_{546}^{25}$ + 5° (*c* 1, chloroform); ν_{max}^{KBr} 1730 (OAc), 1695 (CO acid), and 1220 cm^{-1} (CO); m.s.: *m/z* (%) 546 (100, MNH_4^+), 366 (12, MNH_4^+ – 3 CH_3CO_2H), 348 (8, MNH_4^+ – **30**), 331 (65, MH^+ – **30**), 306 (15, MNH_4^+ – 4 CH_3CO_2H), and 289 (12, MH^+ – 4 CH_3CO_2H).

Anal. Calc. for $C_{23}H_{28}O_{14}$: C, 52.27; H, 5.30. Found: C, 52.47; H, 5.59.

4-β-D-Galactopyranosyloxy-3,5-dimethoxybenzoic acid (**12**). — To a solution of **11** (127 mg, 0.24 mmol) in anhydrous methanol (1.5 mL) was added dropwise a M solution of sodium methoxide in methanol (252 μL, 252 μmol). The progress of the reaction was monitored by t.l.c. on silica gel in 20:5:1 chloroform–methanol–acetic acid [R_F 0.82 (**11**), 0.23 (**12**)]. The solution was stirred for 5 h at room temperature and concentrated to dryness. The residue was dissolved in water (1 mL) and to this solution was added a solution of $KHSO_4$ (35 mg, 257 μmol) in water (1 mL). The gel formed was solidified by trituration and isolated by filtration (61 mg, 71%). The filtrate afforded an additional crop (26%). Compound **12** crystallized from water as needles, m.p. 216–218° (dec.), $[\alpha]_D^{25}$ −12° (*c* 1, *N,N*-dimethylformamide), $[\alpha]_{546}^{25}$ −17° (*c* 1, *N,N*-dimethylformamide); λ_{max} 251 nm (log ε 3.92); ν_{max}^{KBr} 3540 (OH acid), 3300 (OH), 1665 (CO acid), 1255 (OH acid), 1220 (CO), 1050 (CO sugar), and 865 cm^{-1} (CH arom.); m.s.: *m/z* (%) 378 (33, MNH_4^+), 361 (5, MH^+), 343 (3, $MH^+ - H_2O$), 216 (100, **30**·NH_4^+), 198 (57, $\mathbf{30}^+$), 180 (25, $MNH_4^+ - \mathbf{30}$), and 145 (5, 343 − **30**).

Anal. Calc. for $C_{15}H_{20}O_{10}\cdot 1.5\,H_2O$: C, 46.51; H, 5.94. Found: C, 46.85; H, 5.92.

3,5-Dimethoxy-4-[O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyloxy]acetophenone (**21**). — This compound was obtained from acetosyringone (**28**; 402 mg, 2.05 mmol) and 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-maltosyl bromide (**20**; 1.365 g, 1.95 mmol) by the general procedure for glycosylation. After acetylation and concentration to dryness, chromatography on a silica gel column (2.2 × 75 cm) using 1:1 chloroform–ethyl acetate as solvent gave **21** as a colorless foam (510 mg, 32%; R_F 0.40), $[\alpha]_D^{25}$ +49° (*c* 1, chloroform), $[\alpha]_{546}^{25}$ +56° (*c* 1, chloroform); ν_{max}^{KBr} 1735 (OAc), 1670 (CO ketone), and 1225 cm^{-1} (CO methoxy and sugar); ^{1}H-n.m.r. ($CDCl_3$): δ 7.16 (s, 2 H, H-2,6), 5.41 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1″α), 5.33 (dd, 1 H, $J_{2'',3''}$ 10.5 $J_{3'',4''}$ 9.5 Hz, H-3″), 5.23 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 8.5 Hz, H-3′), 5.21 (d, 1 H, $J_{1',2'}$ 7 Hz, H-1′β), 5.14 (dd, 1 H, $J_{1',2'}$ 7, $J_{2',3'}$ 8 Hz, H-2′), 5.03 (t, 1 H, $J_{3'',4''} = J_{4'',5''}$ 10 Hz, H-4″), 4.83 (dd, 1 H, $J_{1'',2''}$ 4, $J_{2'',3''}$ 10.5 Hz, H-2″), 4.42 (dd, 1 H, $J_{5',6'a}$ 3, $J_{6'a,6'b}$ 12 Hz, H-6′a), 4.22 (dd, 1 H, $J_{5'',6''a}$ 4, $J_{6''a,6''b}$ 12.5 Hz, H-6″a), 4.21 (dd, 1 H, $J_{5',6'b}$ 5, $J_{6'a,6'b}$ 11.5 Hz, H-6′b), 4.20 (t, 1 H, $J_{3',4'} = J_{4',5'}$ 9 Hz, H-4′), 4.04 (dd, 1 H, $J_{5'',6''b}$ 2.5, $J_{6''a,6''b}$ 12.5 Hz, H-6″b), 3.95 (m, 1 H, $J_{4'',5''}$ 10, $J_{5'',6''a}$ 4, $J_{5'',6''b}$ 2.5 Hz, H-5″), 3.85 (s, 6 H, 2 OCH_3), 3.70 (m, 1 H, $J_{4',5'}$ 9.5, $J_{5',6'a}$ 3, $J_{5',6'b}$ 4.5 Hz, H-5′), 2.55 (s, 3 H, CH_3-8), 2.07 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), 1.99 (s, 3 H, OAc), 1.98 (s, 3 H, OAc), and 1.97 (s, 3 H, OAc); m.s.: *m/z* (%) 832 (100, MNH_4^+), 654 (10, **26**·NH_4^+), 636 (5, $MNH_4^+ - \mathbf{28}$), 619 (25, $MH^+ - \mathbf{28}$), 594 (7, 654 − CH_3CO_2H), 559 (15, 619 − CH_3CO_2H), 366 (6, **19**·NH_4^+), 331 (7, **19**·$H^+ - H_2O$), 197 (40, **28**·H^+), and 181 (6, $\mathbf{28}^+ - CH_3$).

Anal. Calc. for $C_{36}H_{46}O_{21}\cdot H_2O$: C, 51.92; H, 5.77. Found: C, 52.04; H, 5.67.

4-[O-α-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]-3,5-dimethoxyacetophenone (**22**). — This compound was obtained from **21** by the usual procedure of deacetylation. The progress of the reaction was monitored by t.l.c. on silica gel in 4:1 chloroform–methanol [R_F 0.95 (**21**), 0.18 (**22**)]. The deacetylation was quantitative and **22** crystallized from ethanol, m.p. 185.5–187°, $[\alpha]_D^{25}$ +53° (*c* 1, *N,N*-dimethylformamide), $[\alpha]_{546}^{25}$ +60° (*c* 1, *N,N*-dimethylformamide); λ_{max} 276 nm (log ε 4.00); ν_{max}^{KBr} 3380

(OH), 1670 (CO ketone), 1210 (CO methoxy), 1060 (CO sugar), 855 and 810 cm^{-1} (CH arom.); ^{1}H-n.m.r. (D_2O): δ 7.34 (s, 2 H, H-2,6), 5.43 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1''α), 5.19 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1'β), 3.92 (s, 6 H, 2 OCH_3), 3.87–3.55 (m, 10 H, H-2',3',4',6'a,6'b,2'',3'', 4'',6''a,6''b), 3.50 (m, 1 H, $J_{4'',5''}$ 9.5, $J_{5'',6''a}$ 2, $J_{5'',6''b}$ 4.5 Hz, H-5''), 3.41 (t, 1 H, $J_{4',5'}$ 9.5 Hz, H-5'), and 2.65 (s, 3 H, CH_3-8); m.s.: *m/z* (%) 538 (12, MNH_4^+), 361 (8, MNH_4^+ − D-Glc + H_2O − CH_3), 342 (65, MNH_4^+ − **28**), 325 (5, MH^+ − **28**), 197 (100, **28**·H^+), 181 (40, **28**$^+$ − CH_3), and 43 (20, CH_3CO^+).

Anal. Calc. for $C_{22}H_{32}O_{14}\cdot H_2O$: C, 49.07; H, 6.32. Found: C, 49.25; H, 6.20.

*3,5-Dimethoxy-4-[*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyloxy]benzaldehyde* (**23**). — This compound was obtained by the general procedure for glycosylation from syringaldehyde (**29**; 373 mg, 2.05 mmol) and 2,3,6,2',3',4',6'-hepta-*O*-acetyl-α-maltosyl bromide (**20**; 1.365 g, 1.95 mmol). After acetylation and concentration to dryness, t.l.c. in 1:1 chloroform–ethyl acetate showed **23** (R_F 0.42), some 4-acetoxy-3,5-dimethoxybenzaldehyde (R_F 0.77), and 1,2,3,6, 2',3',4',6'-octa-*O*-acetylmaltose (R_F 0.56). Compound **23** was isolated by column chromatography on silica gel as a yellowish foam (370 mg, 24%), $[\alpha]_D^{25}$ + 54° (*c* 1, chloroform), $[\alpha]_{546}^{25}$ + 64° (*c* 1, chloroform); ν_{max}^{KBr} 1740 (OAc), 1685 (CO formyl), 1220 (CO methoxy and sugar), and 1035 cm^{-1} (CO sugar); ^{1}H-n.m.r. ($CDCl_3$): δ 9.86 (s, 1 H, H-7), 7.10 (s, 2 H, H-2,6), 5.42 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1''α), 5.34 (t, 1 H, $J_{2'',3''} = J_{3'',4''}$ 10 Hz, H-3''), 5.26 (m, 1 H, H-3'), 5.23 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'β), 5.15 (t, 1 H, $J_{1',2'} = J_{2',3'}$ 7.5 Hz, H-2'), 5.04 (t, 1 H, $J_{3'',4''} = J_{4'',5''}$ 10 Hz, H-4''), 4.84 (dd, 1 H, $J_{1'',2''}$ 4, $J_{2'',3''}$ 10.5 Hz, H-2''), 4.43 (dd, 1 H, $J_{5',6'a}$ 3, $J_{6'a,6'b}$ 12 Hz, H-6'a), 4.26–4.18 (m, 3 H, H-4',6'b,6''a), 4.05 (dd, 1 H, $J_{5'',6''b}$ 2, $J_{6''a,6''b}$ 12.5 Hz, H-6''b), 3.97 (m, 1 H, $J_{4'',5''}$ 10, $J_{5'',6''a}$ 3.5, $J_{5'',6''b}$ 2 Hz, H-5''), 3.88 (s, 6 H, 2 OCH_3), 3.72 (m, 1 H, $J_{4',5'}$ 10, $J_{5',6'a}$ 3, $J_{5',6'b}$ 4 Hz, H-5'), 2.08 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), 2.02 (s, 6 H, 2 OAc), 2.01 (s, 3 H, OAc), 2.00 (s, 3 H, OAc), and 1.99 (s, 3 H, OAc); m.s.: *m/z* (%) 818 (100, MNH_4^+), 654 (15, **26**·NH_4^+), 636 (5, MNH_4^+ − **29**), 619 (15, MH^+ − **29**), 594 (57, 654 − CH_3CO_2H), 559 (10, 619 − CH_3CO_2H), 366 (8, **19**·NH_4^+), 331 (7, **19**·H^+ − H_2O), and 183 (20, **29**·H^+).

Anal. Calc. for $C_{35}H_{44}O_{21}\cdot H_2O$: C, 51.34; H, 5.62. Found: C, 51.60; H, 5.69.

*4-[*O*-α-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]-3,5-dimethoxybenzaldehyde* (**24**). — This compound was obtained from **23** by the general procedure for deacetylation. The progress of the reaction was monitored by t.l.c. on silica gel in 4:1 chloroform–methanol [R_F 0.92 (**23**), 0.17 (**24**)]. Compound **24** crystallized from 2-propanol, m.p. 137–139°, $[\alpha]_D^{25}$ + 56° (*c* 1, *N,N*-dimethylformamide), $[\alpha]_{546}^{25}$ + 63° (*c* 1, *N,N*-dimethylformamide); λ_{max} 284 nm (log ε 3.99); ν_{max}^{KBr} 3380 (OH), 1680 (CO formyl), 1070 (CO sugar), and 830 cm^{-1} (CH arom.); ^{1}H-n.m.r. (D_2O) : δ 9.87 (s, 1 H, H-7), 7.40 (s, 2 H, H-2,6), 5.47 (d, 1 H, $J_{1'',2''}$ 4 Hz, H-1''α), 5.27 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'β), 3.99 (s, 6 H, 2 OCH_3), 3.92–3.57 (m, 11 H, H-2',3',4',6'a,6'b, 2'',3'',4'',5'', 6''a,6''b), and 3.45 (t, 1 H, $J_{4',5'}$ 9 Hz, H-5'); m.s.: *m/z* (%) 524 (3, MNH_4^+), 342 (100, MNH_4^+ − **29**), 325 (4, MH^+ − **29**), 198 (12, D-Glc·NH_4^+), 180 (35, D-Glc·NH_4^+ − H_2O), and 163 (9, D-Glc·H^+ − H_2O).

Anal. Calc. for $C_{21}H_{30}O_{14}\cdot H_2O$: C, 48.09; H, 6.11. Found: C, 48.03; H, 6.16.

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