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FLAVONOL GLYCOSIDE GALLATES FROM *TELLIMA GRANDIFLORA**

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Key Word Index—*Tellima grandiflora*; Saxifragaceae; flavonol glycoside gallates; quercetin 3-*O*-(6″-*O*-galloyl)-β-D-glucoside; kaempferol; myricetin; NMR spectroscopy.

Abstract—Quercetin 3-*O*-(6″-*O*-galloyl)-β-D-glucoside has been identified as a constituent of *Tellima grandiflora* (Saxifragaceae). In all, twelve gallates were encountered: two isomeric gallates of quercetin 3-*O*-glucoside and two of quercetin 3-*O*-galactoside, a similar set involving kaempferol, and a similar set involving myricetin.

INTRODUCTION

During a previous investigation [1] of the flavonoids of *Tellima grandiflora* (Pursh) Dougl. a complex series of acylated flavonol monoglycosides was encountered. In the present communication the structure of the major compound, referred to as tellimoside, is described. In addition, evidence is presented concerning the structures of eleven other closely related acylated flavonol glycosides from this plant.

* Part II in the series "Chemotaxonomic Studies in the Saxifragaceae". For Part I see Ref. 1.

RESULTS AND DISCUSSION

TLC of the methanolic leaf extract of *Tellima grandiflora* showed the presence of a mixture of neutral flavonol glycosides which were extremely alkali labile and which exhibited a stronger affinity for polyamide than do simple flavonol monosides. Considerable difficulty was experienced in the separation of these compounds; however, 12 related compounds were obtained.

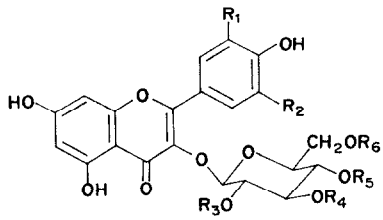
The UV spectral properties [2] of each of these compounds was typical of a 3-*O*-substituted flavonol but an absorption in the range 265–275 nm

Table 1. TLC data for flavonol acylglycosides from *Tellima grandiflora*

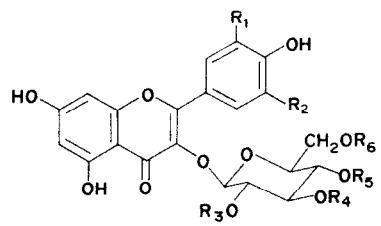
Compound	Relative amount*	$R_f \times 100$† (1)	(2)	(3)	(4)	(5)
(1) Km 3-(6″-galloylglucoside)	+++	13	22	23	31	50
(2) Km 3-galloylglucoside	+	13	23	25	27	39
(3) Km 3-(6″-galloylgalactoside)	++	13	23	25	35	46
(4) Km 3-galloylgalactoside	trace	13	22	23	31	36
(5) Qu 3-(6″-galloylglucoside)	+++++	12	13	15	25	41
(6) Qu 3-galloylglucoside	+	12	17	17	22	32
(7) Qu 3-(6″-galloylgalactoside)	++	12	14	15	27	37
(8) Qu 3-galloylgalactoside	+	12	18	16	25	28
(9) My 3-(6″-galloylglucoside)	+++	13	8	9	19	30
(10) My 3-galloylglucoside	trace	13	13	13	17	24
(11) My 3-(6″-galloylgalactoside)	+	13	9	9	21	28
(12) My 3-galloylgalactoside	trace	13	13	13	18	21

* Estimated by visual examination of TLC plates after $AlCl_3$ spray. Km = kaempferol, Qu = quercetin, My = myricetin.

† Chromatographic systems: (1) polyamide DC 6·6, H_2O–*n*-BuOH–Me_2CO (14:3:3); (2) polyamide DC 6·6, C_6H_6–MeOH–MeCOEt–H_2O (55:20:22:3) developed 2×; (3) polyamide DC 6·6, $CHCl_3$–MeOH–MeCOEt 50% HOAc (65:20:11:4) dev. 2×; (4) Avicel, 10% HOAc; (5) Si gel S, *n*-BuOAc–MeCOEt–HOAc–H_2O (60:25:12:3).



(1) $R_1R_2R_3R_4R_5$ = H R_6 = galloyl
(2) $R_1R_2R_6$ = H $R_3R_4R_5$ = (H,H,galloyl)
(5) $R_2R_3R_4R_5$ = H R_1 = OH R_6 = galloyl
(6) R_2R_6 = H R_1 = OH $R_3R_4R_5$ = (H,H,galloyl)
(9) $R_3R_4R_5$ = H R_1R_2 = OH R_6 = galloyl
(10) R_6 = H R_1R_2 = OH $R_3R_4R_5$ = (H,H,galloyl)
(1a) $R_1R_2R_3R_4R_5R_6$ = H
(5a) $R_2R_3R_4R_5R_6$ = H R_1 = OH
(9a) $R_3R_4R_5R_6$ = H R_1R_2 = OH



(3) $R_1R_2R_3R_4R_5$ = H R_6 = galloyl
(4) $R_1R_2R_6$ = H $R_3R_4R_5$ = (H,H,galloyl)
(7) $R_2R_3R_4R_5$ = H R_1 = OH R_6 = galloyl
(8) R_2R_6 = H R_1 = OH $R_3R_4R_5$ = (H,H,galloyl)
(11) $R_3R_4R_5$ = H R_1R_2 = OH R_6 = galloyl
(12) R_6 = H R_1R_2 = OH $R_3R_4R_5$ = (H,H,galloyl)
(3a) $R_1R_2R_3R_4R_5R_6$ = H
(7a) $R_2R_3R_4R_5R_6$ = H R_1 = OH
(12a) $R_3R_4R_5R_6$ = H R_1R_2 = OH

suggested the presence of an additional aromatic moiety, possibly a hydroxybenzoic acid [3,4]. Based upon spectral response to shift reagents the components of the mixture could be divided into three groups of four spectroscopically identical compounds: **1**–**4**, **5**–**8**, and **9**–**12**.

Total acid hydrolysis of compounds **1**–**4** produced kaempferol, a sugar, and gallic acid in the approx ratio of 1:1:1. Compounds **1** and **2** yielded glucose; compounds **3** and **4** yielded galactose. Thus, the first group consisted of two isomeric kaempferol 3-*O*-glucoside monogallates and two isomeric kaempferol 3-*O*-galactoside monogallates. Comparable results were obtained with the remaining two groups leading to the assignment of compounds **5** and **6** as isomeric quercetin 3-*O*-glucoside monogallates, **7** and **8** as quercetin 3-*O*-galactoside monogallates, **9** and **10** as myricetin 3-*O*-glucoside monogallates, and **11** and **12** as myricetin 3-*O*-galactoside monogallates.

Attempted enzymic hydrolysis using β-glucosidase failed to produce an aglycone with any of the twelve acylated compounds. From the mild alkaline hydrolysate of compounds **1** or **2** kaempferol 3-*O*-β-D-glucoside (**1a**) was identified by co-chromatography with an authentic standard (TLC, five solvents) and by further degradation to kaempferol and glucose by the action of β-glucosidase. Under identical conditions compounds **3** and **4** yielded kaempferol 3-β-D-galactoside (**3a**). Quercetin 3-*O*-β-D-glucoside (**5a**), quercetin 3-*O*-β-D-galactoside (**7a**), myricetin 3-*O*-β-D-glucoside (**9a**), and myricetin 3-*O*-β-D-galactoside (**11a**) were likewise identified after mild alkaline hydrolysis of the isomeric pairs **5**,**6**; **7**,**8**; **9**,**10**; and **11**,**12**, respectively. Evidence that in each compound the gallic acid residue was attached to the sugar moiety was obtained by mild acid hydrolysis. In addition to the aglycone, sugar, gallic acid and the corresponding flavonol 3-*O*-monoside, small amounts of other products were observed. These compounds reacted as reducing sugars and gave gallic acid and either glucose or galactose upon further acidic hydrolysis. These compounds were not further characterized due to the small amounts available.

The major flavonol glycoside gallate, tellimoside (**5**), was recrystallized from water, mp 199–200°. The IR spectrum showed the presence of absorptions at 1700, 1450 and 1270 cm^{-1} typical of sugar gallates [5]. These bands were absent from the spectrum of unacylated glycoside **5a**. NMR spectra of underivatized tellimoside were determined in acetone-d_6 at 100 MHz. Spectra of the pertrimethylsilyl derivative were determined in CCl_4 at 100 and 220 MHz. The presence of the galloyl function was clearly seen in all spectra. Integration showed (ignoring hydroxyl groups) five protons attributable to quercetin, seven protons attributable to glucose, and two protons attributable to the gallate residue. These data establish a 1:1:1 ratio of aglycone to sugar to gallic acid.

The NMR spectrum of perTMStellimoside determined in deuteriobenzene at 100 MHz provided the most useful data concerning the position of the gallic acid. The two proton singlet at δ 7·52, (δ 7·18 in CCl_4; δ 7·05 in acetone-d_6) was assigned to the two equivalent protons of the galloyl residue [6, 7, 8] while the remaining low field resonances

were characteristic of quercetin [2]. The appearance of an anomeric proton signal at δ 6·21 confirmed the β-linkage of the glucosyl portion of the molecule and suggested the $^4C_1$ conformation of a pyranose ring [9, 10]. The remaining glucose protons consisted of four groups of resonances centered at δ 4·60, δ 4·27, δ 3·82 and δ 3·52 which integrated for 1, 1, 3 and 1 protons, respectively. The highest field multiplet was assigned to the H-2″ proton by comparison with pentaTMS-β-D-glucopyranose [10] and verified by double resonance techniques.

The two lower field quartets at δ 4·60 and δ 4·27 appeared as the AB-portion of an ABX system (J_{AB} 12 Hz, J_{AX} 2·5 Hz, J_{BX} 4·5 Hz). Based upon the following evidence, these two resonances were assigned to the geminal glucosyl protons of the C-6″ to which the galloyl function is attached: (1) the apparent coupling constants $J_{6''6''}$ 12 Hz; $J_{5''6''}$ 4·5 Hz; $J_{5''6'}$ 2·5 Hz are similar to those of the geminally coupled C-6 protons (J_{gem} 11·0 Hz) and the vicinal C-5 proton $J_{5,6}$ 4·9 Hz; $J_{5,6'}$ 2·8 Hz) for pentaTMS-β-D-glucopyranose [10] and acylated glycopyranoside derivatives [11, 12]; (2) the downfield shifts of about 0·5 ppm relative to penta TMS-β-D-glucopyranose [10] and about 0·75 ppm relative to perTMS flavonol glucosides [2] are consistent with an expected deshielding effect of the galloyl function on the two adjacent C-6 protons; (3) double resonance experiments eliminated the 2″ and 3″ OH groups as likely sites of acylation while suggesting that the two lower field resonances (δ 4·60 and δ 4·27) are probably coupled.

Thus, in tellimoside, the galloyl function appears to be attached to the glucosyl C-6″ alcoholic group. The isomeric glucoside monogallate **6** must therefore be acylated at either 4″, 3″ or 2″ position. Careful comparison of the properties of **5** and **6** with those of the deacyl compound **5a**, and the other acyl-deacyl groups, suggested that an exactly analogous relationship exists between the isomeric pairs of glucoside monogallates and galactoside monogallates of kaempferol, quercetin and myricetin. Accordingly, one isomer of each pair contains the galloyl function at the 6″ position of either glucose (**1**,**5** and **9**) or galactose (**3**, **7** and **11**) while the other consists of the 2″, 3″ or 4″-galloyl analogue (**2**, **6**, **10** and **4**, **8**, **12**).

Since completion of this work, the NMR spectra of phenyl galactoside gallates and two flavonol galactoside gallates have appeared in the literature. The NMR spectrum of tellimoside closely resembles that published [13] for quercetin 3-*O*-(6″-galloyl)-β-D-galactoside. The reported deshielding effect of the galloyl function on the C-6″ geminal sugar protons resulted in a downfield shift of *ca* 0·75 ppm in support of the present assignment for tellimoside. Comparison of chromatographic mobilities and resistance to β-glucosidase for quercetin 3-*O*-(6″-galloyl)- and -(2″-galloyl-β-D-galactoside [13] are in complete agreement with formulations **7** and **8** for the isomeric 3-*O*-(galloyl) galactosides in this study.

The occurrence of flavonoid glycoside gallates in *Tellima grandiflora* (tribe Saxifragoideae–Saxifragaceae) increases to three the number of families in which they are known. Quercetin 3-*O*-(6″-galloyl)-β-D-galactoside (**7**) and the 2″-galloyl isomer (possibly **8**) have recently been found [13] to occur in *Euphorbia platiphyllos* and *E. verrucosa* (Euphorbiaceae), respectively. A gallate ester of naringenin 7-*O*-glucoside (prunin) has been isolated from *Acacia farnesiana* (Leguminosae) [14] and shown to be narignenin 7-*O*-β-D-[6″-*O*-galloyl]-glucopyranoside [15]. In the Saxifragoideae, 2′- and 6′-galloyl arbutin occur in the leaves of *Bergenia* species along with gallic acid esters of flavanols [16]. Bergenin, thought to be biosynthetically derived from 2′-galloyl glucose, is not uncommon in the tribe [17]. In addition, hydrolysable tannins composed of glucose, gallic and hexahydroxybiphenic acid have been detected in *Tellima grandiflora* in these laboratories (unpublished data). A survey of the tribe for bound forms of gallic acid is currently underway.

EXPERIMENTAL

NMR spectra were recorded on Varian HA-100, XL-100 and HR-220 instruments using TMS as an internal standard. UV spectra were determined according to Mabry *et al.* [2]. IR spectra were obtained using KBr disks. A Kofler hot stage apparatus was used to determine mp (uncorr).

Plant material. Flowering plants of *Tellima grandiflora* (Pursh) Dougl. were collected near the campus (UBC) in early June and partially air-dried. Voucher specimens have been deposited in the University of British Columbia Herbarium.

Extraction and chromatography. Acylated flavonoids were obtained by 2 procedures. The first is described in Ref. [1]. The second procedure involved TLC of the initial EtOAc fraction directly without preliminary column fractionation. TLC purification involved repeated bandings using the solvent systems described [1]. Solvent 5, the butyl acetate based system, was used only for analytical purposes after difficulty was encountered in

elution of the compounds from the gel. The relative amounts and chromatographic properties of the acylated flavonol glycosides are summarized in Table 1.

UV spectra. The 3 groups of spectroscopically identical compounds exhibited the following spectral properties: Group I (ie. compounds **1–4**) λ_{max} nm (MeOH) 267, 290*s*, 352; (+ $AlCl_3$) 275, 300, 402; (+ $AlCl_3$/HCl) 275, 300, 350, 400; (+ NaOAc) 275, 315, 384; (+ NaOAc/H_3BO_3) 268, 298, 355; (+ NaOMe) 266, 326, 405. Group II (i.e. **5–8**) λ_{max} nm (MeOH) 259*s*, 266, 291*s*, 360; (+ $AlCl_3$) 278, 308*s*, 439; (+ $AlCl_3$/HCl) 272, 304*s*, 367, 404; (+ NaOAc) 274, 318, 402; (+ NaOAc/H_3BO_3) 264, 296, 382; (+ NaOMe) 270, 327, 409 decomp. Group III (i.e. **9–12**) λ_{max} nm (MeOH) 267, 293*s*, 264; (+ $AlCl_3$) 276, 302*s*, 442; (+ $AlCl_3$/HCl) 274, 302, 374, 410; (+ NaOAc) 272, 320, 425; (+ NaOAc/H_3BO_3) 266, 290*s*, 390; (+ NaOMe) 270, 330 decomp.

Acid hydrolysis. Total acid hydrolysis was performed using 0·1 N HCl at 100° for 90 min. Partial acid hydrolysis was carried out with 0·1 N HCl for various time periods at 100°. Hydrolyzates were cooled then passed through a short column of polyamide SC-6 and eluted with H_2O to remove the sugars and the HCl. The flavonol aglycone, or glycoside in the case of partial hydrolyses, and gallic acid were recovered from the column by elution with 80% MeOH containing several drops of conc HOAc.

Identification of hydrolysis products. The sugar–HCl eluate was neutralized using *N,N*-dioctylmethylamine [18]. Identification of the sugars was based on co-chromatography (TLC, Avicel, EtOAc–Pyr–H_2O 67:20:13 and EtOAc–HOAc–HCO_2H–H_2O 68:12:4:16), and on colour reactions with spray reagents *p*-anisidine phthalate and diphenylamine-phosphoric acid. Flavonol aglycones were identified by co-chromatography (TLC, polyamide DC-6·6, C_6H_6–MeOH–MeCOEt–H_2O 55:20:23:2 and toluene–EtOAc–EtOH, 2:1:1) and by spectroscopic procedures [2]. Gallic acid was identified by co-chromatography (TLC, Avicel, H_2O–HOAc 94:6 and *i*-BuOH–HOAc–H_2O 14:1:5), and colour reaction with diazotized *p*-nitroaniline. Galloyl sugar esters were located by short wave UV, diazotized *p*-nitroaniline and/or *p*-anisidine phthalate. Further hydrolysis of the esters was carried out using 0·1 N HCl at 100° for 90 min followed by identification of gallic acid and the sugar components as described above.

Alkaline hydrolysis. Alkaline hydrolysis was carried out with 0·05 N NH_4OH in 50% MeOH at room temp for various time periods from 10 to 60 min. Neutralization was effected by removal of NH_4OH *in vacuo* at 20° on a rotary evaporator.

Enzymatic hydrolysis. β-Glucosidase in 0·05 N acetate buffer, pH 5·1 was incubated with an aq soln of the flavonol glycoside at room temp for 24 hr.

Tellimoside. [Quercetin 3-*O*-(6″-galloyl)-β-D-glucopyranoside]: pale yellow needles from cold H_2O, mp 199–200°, IR ν_{max}^{KBr} 3350, 1700, 1658, 1610, 1567, 1506, 1449, 1360, 1302, 1272, 1238, 1203, 1169, 1084, 1038, 1010, 930, 876, 815, 767 cm^{-1}, NMR δ_{ppm} 100 MHz: acetone-d_6 7·73, 7·66*m* (H-2′, H-6′) 7·05*s* (galloyl H-2″/6‴) 6·85*d*, *J* 8 Hz (H-5′) 6·48*d*, *J* 2 Hz (H-8) 6·27*d*, *J* 2 Hz (H-6) 5·34*d*, *J* 7·5 Hz (H-1″) 4·4–3·2 (glucosyl protons + H_2O).

Pertrimethylsilyltellimoside. NMR δ_{ppm} 100 MHz: CCl_4 7·40*q*, *J* 2 Hz, 8 Hz (H-6′) 7·32*d* *J* 2 Hz (H-2′) 7·18*s*, (galloyl H-2‴/6‴) 6·70*d*, *J* 8 Hz (H-5′) 6·40*d*, *J* 2·5 Hz (H-8) 6·14*d*, *J* 2·5 Hz (H-6) 5·79*d*, *J* 7·5 Hz (H-1″) 4·41*d*, *J* 12 Hz (H-6″) 4·08*d*, *J* 12 Hz (H-6″) 3·72–3·32 (4 glucosyl protons). 220 MHz: CCl_4 7·36*q*, *J* 2·0 Hz, 8·0 Hz (H-6′) 7·29*d*, *J* 2·0 Hz (H-2′) 7·14*s* (galloyl H-2‴/6‴) 6·65*d*, *J* 8·0 Hz (H-5′) 6·36*d*, *J* 2·5 Hz (H-8) 6·12*d*, *J* 2·5 Hz (H-6) 5·71*d*, *J* 7·5 Hz (H-1″) 4·38*d*, *J* 12 Hz (H-6″) 4·03*d*, *J* 12 Hz (H-6″) ≈3·5 (3 glucosyl protons) 3·41*m* *J* ≈ 7 Hz, 8 Hz (H-2″). 100 MHz: C_6H_6-d_6 7·85*q*, *J* 8·5 Hz, 2·0 Hz (H-6′) 7·52*s* (galloyl H-2‴/6‴) 6·72*d*, *J* 8·5 Hz (H-5′) 6·53*d*, *J* 2·5 Hz (H-8) 6·43*d*, *J* 2·5 Hz (H-6) 6·21*d*, *J* 7·0 Hz (H-1″) 4·60*q*, *J* 12 Hz, 2·5 Hz (H-6″) 4·27*q*, *J* 12 Hz, 4·5 Hz (H-6″) ≈3·8 (3 glucosyl protons) 3·52*m* *J* ≈ 7 Hz, 8 Hz (H-2″).

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