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GLUCOSYLATED FLAVONOIDS AND OTHER PHENOLIC COMPOUNDS FROM SORGHUM

RENÉ GUJER, DANIÈLE MAGNOLATO* and RON SELF†

Nestlé Research Department, Nestec Ltd., Av. Nestlé 55, CH-1800 Vevey, Switzerland; †Agricultural and Food Research Council, Food Research Institute, Colney Lane, Norwich NR4 7UA, U.K.

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Key Word Index—Sorghum; Gramineae; flavanoids; glycosides; new oligomer glucosides; procyanidins; chalcone; identification.

Abstract—The principal tannin constituents of sorghum are proanthocyanidins or condensed tannins. Analysis of the methanolic extract of a Hungarian sorghum (szegedi toerpe) containing 6% catechin equivalents of tannins resulted in the separation and purification of 4 procyanidins having the basic formula epicatechin–(epicatechin)$_n$–catechin and one procyanidin trimer corresponding to epicatechin–catechin–epicatechin. Apart from these procyanidins, the monomeric flavonoids eriodictyol 5-glucoside and (+)-taxifolin 7-glucoside together with their aglycones eriodictyol and taxifolin were found. Glucosylated dimeric and trimeric flavanoids with eriodictyol or eriodictyol 5-glucoside as the lower unit were also identified with the help of negative ion FABMS. Polymeric flavonoids formed between a chalcone and a flavonoid, as yet not identified, are also present in the grain.

INTRODUCTION

During the past 15 years, the chemistry of proanthocyanidin dimers has been completely established by the use of ^{1}H and ^{13}C NMR spectroscopy [1–6]. Although the structure of higher oligomeric procyanidins has never been established in sorghum, trimeric and tetrameric forms of procyanidins from *Areca catechu* L. were described by Nonaka *et al.* [7]. In sorghum grain, Gupta and Haslam [8] identified (+)-catechin, procyanidin dimer B-1 and a procyanidin polymer corresponding to 5–6 (−)-epicatechin units linked to a (+)-catechin as chain termination unit. Flavan-4-ols such as luteoforol [9] and apiforol [10] have been reported and their possible role as bird repellents discussed by Butler [11]. Pigments were also studied, suggesting the presence of eriodictyol [12–14], pelargonidin [14] and glycosylated forms of luteolinidin and apigeninidin [15, 16]. As well as procyanidins having 2,3-*cis* stereochemistry, Brandon *et al.* [17] found 20% of the units with 2,3-*trans* stereochemistry and 8% with a prodelphinidin oxidation pattern. Neither glycosylated forms of proanthocyanidins nor glycosylated forms of condensed flavonoids have been reported in sorghum. Porter *et al.* [18] recently reported the presence of procyanidin polymers containing *O*-β-glucopyranoside functions from ripe fruits of *Cydonia oblonga* and from barks of *Pinus brutia* and *Picea abies*. The fact that unripe quince fruits contained procyanidins which became *O*-glycosylated in ripe fruit was also mentioned by Porter [19]. In this paper we describe the isolation, purification and identification of monomeric compounds, procyanidin trimers, a tetramer and a pentamer together with new glucosylated forms of polymeric flavanoids linked by a 4,8-interflavan bond. It also shows the contribution of FABMS in the identification of condensed flavonoids. A preliminary report of this work was presented at a symposium earlier this year [20].

*To whom correspondence should be addressed.

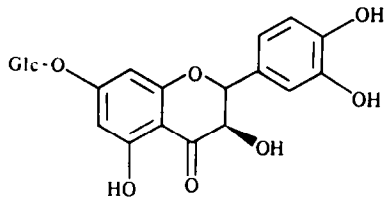
RESULTS

(a) *Monomers*

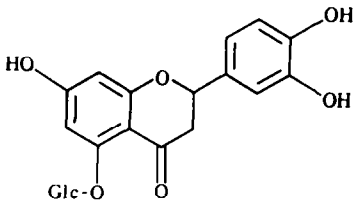
Methanolic extracts of sorghum grain eluted from Sephadex LH-20 with ethanol and further fractionated on silica gel contained seven low molecular weight phenolics. These were identified as (+)-catechin, naringenin, (+)-taxifolin and eriodictyol by HPLC co-chromatography with commercial products, mmp and ^{1}H NMR, along with three monomeric glycosides. The sugar in all three glycosides was shown to be glucose after analysis by the Hakomori method [21], acid and enzymatic hydrolysis. $[M-H]^-$ and diagnostic fragments were obtained by negative ion FABMS analysis. The position of the glucose was deduced from UV shift measurements with $AlCl_3$, $AlCl_3$–HCl, NaOAc and NaOMe.

Compound **1a** was identified as taxifolin 7-*O*-β-glucoside by $[M-H]^- = 465$ (M = 466). The fragment corresponding to the loss of glucose at *m/z* 303 $[(M-H)-162]^-$ gave the molecular weight of the aglycone. Acid and enzymatic hydrolysis gave taxifolin and glucose. The UV spectrum gave no shift with NaOAc and a bathochromic shift of 23 nm with $AlCl_3$ confirmed that the glucose was linked at position 7. This compound was found by Markham *et al.* [21] in *Podocarpus nivalis*.

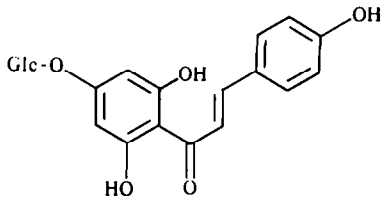
Compound **1b** was identified as eriodictyol 5-*O*-β-glucoside by $[M-H]^- = 449$ (M = 450) and the fragment corresponding to the loss of glucose at *m/z* 287 $[(M-H)-162]^-$, gave the molecular weight of the aglycone. Acid and enzymatic hydrolysis gave glucose and eriodictyol. The UV spectrum showed a bathochromic shift of 39 nm with NaOAc and no shift with $AlCl_3$ which is consistent with the glucose located at position 5. This compound is reported for the first time.



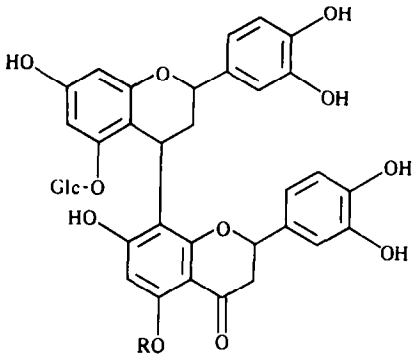
1a



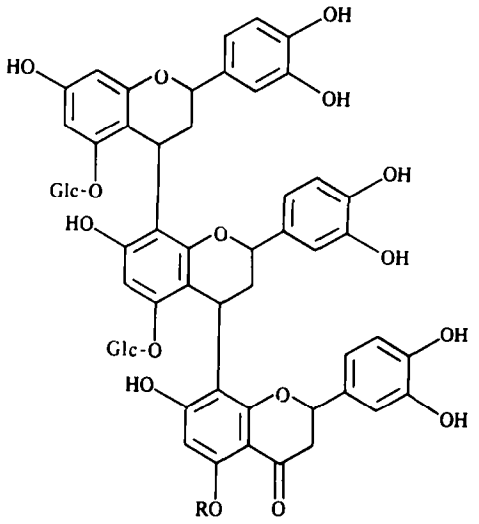
1b



1c



2a R = H
2b R = Glucose



2c R = H
2d R = Glucose

Compound **1c** was identified as 4,2',4',6'-tetrahydroxychalcone 4'-*O*-*β*-glucoside by $[M-H]^- = 433$ and a fragment corresponding to the loss of glucose at *m/z* 271 $[(M-H)-162]^-$ gave the mass of the aglycone. Acid and enzymatic hydrolysis gave glucose and naringenin. Isomerization of a chalcone into a flavanone was reported by Imperato [23]. The UV spectrum showed a bathochromic shift of 42 nm with $AlCl_3$ which is consistent with a chalcone skeleton with a hydroxyl group at position 2'. The 78 nm bathochromic shift with an increase in peak intensity in the presence of NaOMe indicated a free hydroxyl group at position 4. The absence of a shift with NaOAc confirmed the presence of glucose at the 4' position. These values are in agreement with those reported by Gilbert [24] for the 4'-glucoside of chalconaringenin extracted from the flowers of *Antirrhinum majus*.

(b) *Dimeric compounds*

Methanolic extracts of sorghum grain eluted from Sephadex LH-20 with EtOH–MeOH (1:1) and further chromatographed on silica gel contained five phenolic compounds as shown by TLC and HPLC.

Compound **3a** was identified as the procyanidin dimer B-1. The molecular weight was determined from $[M-H]^- = 577$ and the structure established by thiodegradation with toluene-α-thiol which gave (+)-catechin as the lower unit and 4-benzylthioepicatechin corresponding to (−)-epicatechin as the upper unit. ^1H NMR data of the peracetate derivatives of B-1 and of the 4-benzylthioepicatechin fit with the values reported respectively by Weinges [25] and Thompson *et al.* [1].

Compound **2a** was identified as the 5,7,3',4'-tetrahydroxyflavan-5-*O*-*β*-glucosyl-4,8-eriodictyol. The molecular weight was determined from $[M-H]^- = 721$. The ion at *m/z* 559 $[(M-H)-162]^-$ indicated the loss of hexose which was linked on the upper unit as shown by the fragment ion at *m/z* 433 $[(M-H)-288]^-$ corresponding to the loss of the eriodictyol lower unit. Acid hydrolysis gave eriodictyol, glucose and an anthocyanidin identified as luteolinidin by comparison with a synthetic compound obtained by reduction of eriodictyol with $NaBH_4$ followed by an oxidative dehydration as reported by Bate-Smith and Rasper [9] and Stafford [26]. The UV spectrum showed a bathochromic shift of 23 nm with

$AlCl_3$ which is consistent with a free hydroxyl at position 5 of the eriodictyol lower unit. The location of the glucose at position 5 of the upper flavan unit was confirmed by Hakomori permethylation followed by a mild hydrolysis. The permethylated aglycone gave a positive Gibbs test significant for a free *para* position of the hydroxyl group located at C-5.

The interflavan linkage was studied by bromination and ^1H NMR analysis of the brominated product obtained from the lower unit, adapting the methods reported by McGraw and Hemingway [27] for (+)-catechin and by Nonaka *et al.* [28] for A-1 type trimeric proanthocyanidin. Comparison with ^1H NMR spectra of synthesized 6- and 8-bromoeriodictyol indicated the formation of 6-bromoeriodictyol from **2a** significant for a 4,8-interflavan linkage.

Compound **2b** was tentatively identified as the 5,7,3'4'-tetrahydroxyflavan-5-*O*-β-glucosyl-4,8-eriodictyol 5-*O*-β-glucoside. The molecular weight determined from $[M-H]^-$ = 883 indicated that the molecule has one hexose more than **2a** as confirmed by the ion at *m/z* 721 $[(M-H)-162]^-$. Acid hydrolysis gave the same products as **2a**, which are eriodictyol, glucose and luteolinidin. The UV spectrum showed no shift with $AlCl_3$ which is consistent with the presence of glucose at position 5 of the lower unit. Location of the glucose on the upper flavan unit and interflavan linkage were analysed as for **2a**.

(c) *Trimers*

Compound **2c** was identified as the 5,7,3',4'-tetrahydroxyflavan-5-*O*-β-glucosyl-4,8-5,7,3',4'-tetrahydroxyflavan-5-*O*-β-glucosyl-4,8-eriodictyol. The molecular weight was determined from $[M-H]^-$ = 1155. Fragments $[(M-H)-162]^-$ at *m/z* 993 represented the loss of one hexose, and *m/z* 433 was interpreted to be the same as the fragment found with **2a** and corresponding to the upper unit. The fragment $[(M-H)-288]^-$ at *m/z* 867 is consistent with an unglucosylated eriodictyol as the lower unit. The bathochromic shift of 25 nm with $AlCl_3$ confirmed the free hydroxyl group at position 5 of the lower eriodictyol unit. Total hydrolysis gave eriodictyol, glucose and double the amount of luteolinidin formed from **2a**. The position of the glucose on C-5 of the upper flavan unit was confirmed by a positive Gibbs test [29] indicating a free *para* position of a hydroxyl group located on the C-5 of the permethylated and hydrolysed product. The interflavan linkage between C-4 of the middle flavan unit and C-8 of the lower eriodictyol unit was shown by bromination followed by hydrolysis and analysis of the ^1H NMR of the 6-bromoeriodictyol formed from the lower unit. The interflavan linkage between the two upper flavan units could not be established by the same method.

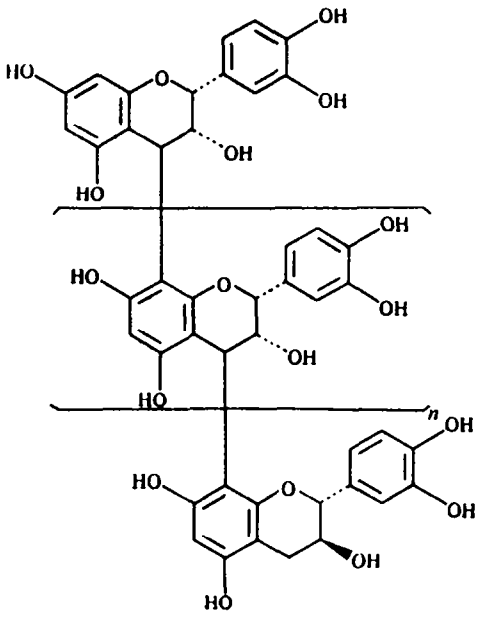
By analogy with the observation that 4 → 8 linked dimeric procyanidins predominate over their 4 → 6 linked isomers by as much as 10:1 [1, 3, 30], the polymeric proluteolinidin 5-*O*-glucosides would be expected to behave similarly.

Small amounts of 4,6-isomers might be found among the compounds not yet identified, which have similar UV spectra and very close retention times in HPLC. Similarly, as 5-*O*-glucosylation is found in all the other similar compounds, it would be expected that the middle flavan unit would be glucosylated at this position, and therefore this trimer is assigned structure **2b**.

Compound **2d** was tentatively identified as the 5,7,3',4'-tetrahydroxyflavan-5-*O*-β-glucosyl-4, 8-5, 7, 3', 4'-tetrahydroxyflavan-5-*O*-β-glucosyl-4, 8-eriodictyol-5-*O*-β-glucoside by analogy with **2b**. The molecular weight was obtained from $[M-H]^-$ = 1317 corresponding to **2b** with one hexose more. The fragment $[(M-H)-162]^-$ at *m/z* 1155 represents the loss of one hexose. The total acid hydrolysis yielded eriodictyol, glucose and luteolinidin. The absence of shift with $AlCl_3$ is consistent with the presence of glucose in position 5 of the lower eriodictyol unit. The glucosylation of the position 5 of the upper flavan unit was established by a positive Gibbs test. The FABMS data of **2a** and **2c** (dimeric monoglucoside and trimeric diglucoside) gave the same fragments with $[M-H]^-$, $[(M-H)-162]^-$ and $[(M-H)-288]^-$ corresponding to the loss of glucose and eriodictyol respectively. Unfortunately, FAB mass spectra of **2b** and **2d** (dimeric diglucoside and trimeric triglucoside) contained relatively fewer fragments.

The yellow products **2e** and **2f** had UV spectra characteristic of those for chalcone containing products. FABMS fragmentation suggested the presence of dimeric diglycosides and trimeric triglycosides, the structure of which have not yet been identified.

Proanthocyanidin dimer **3a**, trimer **3b**, tetramer **3c** and pentamer **3d** having the formula epicatechin-(epicatechin)$_n$-catechin with n = 0, 1, 2, 3 is consistent with the procyanidins found in sorghum by Gupta and Haslam [8] and in *Areca catechu* by Nonaka *et al.* [7]. The structure of the trimer **3e** corresponding to epicatechin-catechin-epicatechin was unequivocally proved by acid-catalysed thiodegradation and analysis with ^1H NMR spectroscopy of the intermediate products. Total hydrolysis yielded equal amount of epicatechin, 4-



n = 0, 1, 2, 3
n = 0; 3a
n = 1; 3b
n = 2; 3c
n = 3; 3d

benzylthio-(−)-epicatechin and 4-benzylthio-(+)-catechin, thus establishing epicatechin as the terminal unit. Epicatechin–catechin as chain extender was deduced from the presence of the dimeric procyanidin B-4 isolated as an intermediate product of the thiodegradation. Although the biosynthetic pathway leading to the monomeric and oligomeric forms of proanthocyanidins is not well established, it is difficult to explain the presence of trimeric procyanidin **3e** when (+)-catechin was the only monomeric flavan-3-ol found in sorghum.

EXPERIMENTAL

Plant material. Sorghum 'szegedi toerpe' was supplied by Kräuchi AG, Switzerland.

Standards and materials. Naringenin, naringenin 7-*O*-β-glucoside, eriodictyol, (+)-taxifolin, (+)-catechin, (−)-epicatechin from Roth, Germany. Sephadex LH-20 (Pharmacia) and silica gel 60 70–230 mesh ASTM (Merck) were used for LC. TLC was carried out on HPTLC plates silica gel 60 F_{254} from Merck. TLC spray: 1% $FeCl_3$ soln (H_2O). UV Bausch & Lomb Spectronic 2000, NMR Varian FT 80 MHz and Brucker 400 MHz, EI MS Kratos MS 3074, FABMS Kratos MS 9/50 TC. HPLC: Kontron Uvikon 720 LC detector, Kontron Programmer Mod 200, Shimadzu C R1A printer. Columns Lichrosorb RP-8 25–40 μm (25 × 10) for semi-prep. and Lichrosorb RP-8 10 μm (25 × 4).

Solvent systems. (A) Toluene–Me_2CO (2:1); (B) toluene–Me_2CO–H_2O (20:40:1); (C) toluene–Me_2CO–H_2O (10:20:1); (D) EtOAc–*iso*-PrOH–H_2O (10:7:1); (E) toluene–Me_2CO–H_2O (5:20:3). HPLC gradients: (A) 0–50% H_2O in MeCN in 50 min, flow rate 3.0 ml/min. (B) 0–50% 0.005% H_3PO_4 in MeCN in 50 min, flow rate 1.0 ml/min, UV detection at 280 nm.

Isolation of proanthocyanidins and oligomeric flavonoid glucosides. Sorghum grains (200 g) were powdered using a 0.5 mm sieve and extracted with MeOH (1000 ml × 4) containing $K_2S_2O_8$ (50 mg). The insoluble parts were filtered off and the red–brown soln evaporated under red. pres. to dryness (40°). The residue (8.4 g) was dissolved in 80% aq. MeOH (600 ml) and washed with petrol (500 ml × 3). The lower phase was evaporated, yielding a red–brown amorphous product (6.8 g) which was dissolved in hot MeOH (100 ml) and chromatographed on a Sephadex LH-20 column (3 × 30 cm) eluted successively with EtOH (fractions 1–50), EtOH–MeOH (1:1) (fractions 51–65) and MeOH (fractions 66–200) of 20 ml fractions. Fractions 21–25 contained 20 mg of a pale yellow powder, analysed as naringenin by MS, ^{1}H NMR and comparison with commercial products. Fractions 26–35 gave 59 mg of an orange–brown product identified as apigenin by MS, ^{1}H NMR and mmp with authentic samples. Fractions 36–45 represented a mixture (234 mg) of three monomers and three glucosides which were rechromatographed on a silica gel column (2 × 25 cm). Elution with solvent system (A) gave eriodictyol (18 mg; R_f 0.48), taxifolin (56 mg; R_f 0.46) and catechin (42 mg; R_f 0.39). These products were analysed by ^{1}H NMR, MS and comparison with commercial standards. Further elution with (B) gave chalcone glucoside **1c** (14 mg; R_f 0.29) and a mixture of taxifolin 7-glucoside **1a** (R_f 0.19) and eriodictyol 5-glucoside, **1b** (R_f 0.2). The two glucosides were separated by semi-prep. HPLC eluted with gradient system (A) and yielded **1a** (61 mg; R_t 16.5 min) and **1b** (20 mg; R_t 21.5 min).

Mass spectrometry. FABMS were obtained on a MS 9/50 TC mass spectrometer (Kratos Analytical) using a 5–7 xenon beam (source pressure 10^{-5} Torr). Samples were dissolved in glycerol on the copper tip of the FAB insertion probe. Spectra were recorded at 8 kV accelerating potential and at 1000 resolution.

(+)-*Taxifolin* 7-O-β-*glucoside* (**1a**) [22]. FABMS: $[M-H]^-$ = 465, *m/z* 303 (aglycone); ^{13}C NMR C sugars: c_1 = 104.2 ppm, c_2 = 73.2 ppm, c_3 = 76.2 ppm, c_4 = 70 ppm, c_5 = 77.2 ppm, c_6 = 60.8 ppm [31]. UV λ_{max}^{MeOH} nm: 286, 340 sh; $AlCl_3$: 309, 380; $AlCl_3$/HCl: 285, 340 sh; NaOMe: 287, 360 decomp.; NaOAc: 285, 340 sh. Acid and enzymatic hydrolysis with β-glucosidase gave taxifolin (HPLC, TLC, mmp) and glucose, respectively identical with authentic samples (TLC solvent system D).

Eriodictyol 5-O-β-*glucoside* (**1b**). FABMS: $[M-H]^-$ = 449, *m/z* 287 (aglycone). UV λ_{max}^{MeOH} nm: 283, 330 sh; $AlCl_3$: 283. $AlCl_3$–HCl: 283. NaOMe: 322 increased intensity. NaOAc: 322. Acid and enzymatic hydrolysis with β-glucosidase gave eriodictyol (HPLC, TLC, MS, mmp) and glucose.

4,2',4',6'-*Tetrahydroxychalcone* 4'-*glucoside* (**1c**) [24]. FABMS: $[M-H]^-$ = 433, *m/z* 271 (aglycone). UV λ_{max}^{MeOH} nm: 366; $AlCl_3$: 408; NaOMe: 444 incr. intensity; NaOAc: 366. Acid and enzymatic hydrolysis with β-glucosidase gave naringenin (HPLC, TLC, mmp) [23] and glucose. Isomerization with NaOAc [24, 32] yielded naringenin 7-*O*-β-glucoside identical with commercial product.

Fractions 56–65 afforded a mixture (206 mg) of proanthocyanidin dimer B-1 and 6 flavonoid glucosides. Separation on silica gel (20 g), column (2 × 25 cm), using solvent system (C) yielded **3a** (29.2 mg; R_f 0.52), **2a** (26.5 mg; R_f 0.49), **2c** (16.3 mg; R_f 0.30), **2b** (33.9 mg; R_f 0.26), **2d** (14.4 mg; R_f 0.14) and two yellow products **2e** (10.2 mg; R_f 0.36) and **2f** (11.1 mg; R_f 0.16).

Analysis. Compound **3a** was purified by semi-prep. HPLC using gradient system (A) as a white amorphous product (R_t 18.9 min) $[\alpha]_D^{20}$ + 38° (*c* 1; MeOH). ^{1}H NMR was identical with decaacetate derivative of epicatechin–catechin dimer B-1 [25]. FABMS: $[M-H]^-$ = 577. Thiodegradation [1] with toluene-α-thiol gave (+)-catechin and 4-benzylthio-(−)-epicatechin. ^{1}H NMR: see ref. [1].

5,7,3',4'-Tetrahydroxyflavan-5-*O*-β-glucosyl-(4,8)-eriodictyol (**2a**) was obtained as a white amorphous product, $[\alpha]_D^{20}$ −99° (*c* 1; MeOH), HPLC (R_t: 30.9 min), gradient system (B). FABMS: $[M-H]^-$ = 721, $[(M-H)-162]^-$ (glucose) = 559 (aglycone), $[(M-H)-288]^-$ (eriodictyol) = 433. Hydrolysis with CF_3COOH (90°, 30 min) gave eriodictyol (HPLC, TLC, mmp) and luteolinidin [UV λ_{max}^{MeOH} nm: 500; $AlCl_3$: 550] and glucose [TLC, solvent system (D); UV λ_{max}^{MeOH} nm: 287, 322 sh; $AlCl_3$: 310]. Hakomori permethylation [21, 33] and mild acid hydrolysis gave the permethylated aglycone; EIMS: $[M]^+$ = 657 which gave a positive Gibbs test [29]. Bromination with pyridine HBr–Br_2 and further hydrolysis [28] gave 6-bromoeriodictyol together with deeply coloured bromoanthocyanidins.

5,7,3',4'-*Tetrahydroxyflavan*-5-O-β-*glucosyl*-4,8-*eriodictyol* 5-O-β-*glucoside dimer* (**2b**). $[\alpha]_D^{20}$ −124° (*c* 1 MeOH), HPLC: R_t 25.5 min; FABMS: $[M-H]^-$ = 883, $[(M-H)-162]^-$ (glucose) = 721 (**2a**); UV λ_{max}^{MeOH} nm: 284, 324 sh; $AlCl_3$: 284. Acid hydrolysis gave eriodictyol, luteolinidin and glucose. Hakomori permethylation [21, 33] and mild acid hydrolysis gave the permethylated aglycone; EIMS: $[M]^+$ = 643 which gave a positive Gibbs test [29]. Bromination and hydrolysis furnished 6-bromoeriodictyol.

5,7,3',4'-*Tetrahydroxyflavan*-5-O-β-*glucosyl*-4,8-5,7,3',4'-*tetrahydroxyflavan*-5-O-β-*glucosyl*-4,8-*eriodictyol trimer* (**2c**). $[\alpha]_D^{20}$ −110° (*c* 1; MeOH), HPLC: R_t 30.5 min. White amorphous powder. FABMS: $[M-H]^-$ = 1155, $[(M-H)-162]^-$ (glucose) = 993 (monoglucoside), $[(M-H)-288]^-$ (eriodictyol) = 867, *m/z* 433 glucosylated form of 3,4-flavan. UV λ_{max}^{MeOH} nm: 284, 324 sh; $AlCl_3$: 284. Acid hydrolysis gave eriodictyol, luteolinidin and glucose. Bromination and further hydrolysis gave 6-bromoeriodictyol and brominated anthocyanidins.

5,7,3',4'-*Tetrahydroxyflavan*-5-O-β-*glucosyl*-4,8-5,7,3',4'-*tetra*-

hydroxyflavan-5-O-*β*-*glucosyl*-4,8-*eriodictyol* 5-O-*β*-*glucoside* trimer (**2d**). $[\alpha]_D^{20}$ −134° (c 1 MeOH); HPLC: R_t: 26.4 min; FABMS: $[M-H]^-$ = 1317, $[(M-H)-162]^-$ (glucose) = 1155 (**2e**); UV λ_{max}^{MeOH} nm: 284, 322 sh; $AlCl_3$: 284. Acid hydrolysis gave eriodictyol, luteolinidin and glucose. Bromination and further hydrolysis furnished 6-bromoeriodictyol.

Eriodictyol. ^{1}H NMR (400 MHz, Me_2CO-d_6): δ7.032–7.052 (1H, *d*, *J* = 2 Hz, H-2'), 6.878 (1H, *s*, H-6'), 6.872 (1H, *s*, H-5'), 5.954–5.965 (1H, *d*, *J* = 2.5 Hz, H-6), 5.940–5.948 (1H, *d*, *J* = 2 Hz, H-8), 5.378–5.422 (1H, *dd*, J_A = 25 Hz, J_B = 6 Hz, H-2), 3.108–3.182 (1H, *dd*, J_A = 17 Hz, J_B = 12 Hz, H-3), 2.702–2.755 (1H, *dd*, J_A = 17 Hz, J_B = 3.5 Hz, H-3).

8-*Bromoeriodictyol*. ^{1}H NMR (400 MHz, Me_2CO-d_6): δ7.028–7.038 (1H, *d*, *J* = 2 Hz, H-2'), 6.875 (1H, *s*, H-6'), 6.869 (1H, *s*, H-5'), 6.180 (1H, *s*, H-6), 5.410–5.457 (1H, *dd*, J_A = 12 Hz, J_B = 3 Hz, H-2), 3.159–3.237 (1H, *dd*, J_A = 17 Hz, J_B = 13 Hz, H-3), 2.749–2.802 (1H, *dd*, J_A = 17 Hz, J_B = 3.5 Hz, H-3).

6-*Bromoeriodictyol*. ^{1}H NMR (400 MHz, Me_2CO-d_6): δ7.085–7.095 (1H, *d*, *J* = 2 Hz, H-2'), 6.90–6.91 (1H, *dd*, J_A = 2.0 Hz, J_B = 0.7 Hz, H-6'), 6.863–6.887 (1H, *d*, *J* = 8 Hz, H-5'), 6.156 (1H, *s*, H-8), 5.534–5.578 (1H, *dd*, J_A = 12 Hz, J_B = 3 Hz, H-2), 3.161–3.295 (1H, *dd*, J_A = 17 Hz, J_B = 12 Hz, H-3), 2.842–2.896 (1H, *dd*, J_A = 7 Hz, J_B = 3.5 Hz, H-3).

Sugar analysis. The glucosides were permethylated following the Hakomori method [21] as described in ref. [33]. The methylated glucosides were hydrolysed with 0.5 N HCl or by the HCO_2H–H_2SO_4 method [34]. The sugars were analysed as their permethylalditol acetates [35].

Compound **2e**, FABMS: $[M-H]^-$ = 867 indicated a dimer diglycoside; **2f**, FABMS: $[M-H]^-$ = 1301 indicated a trimer triglycoside. UV data of **2e** and **2f** were characteristic for chalcones, however the exact structures have not yet been determined.

Fractions 66–75 afforded a slightly brown mixture (178 mg) which was separated on silica gel (column 2 × 25 cm), using solvent system (E) as eluent. Two trimeric proanthocyanidins **3a** (50.3 mg; R_f 0.28) and **3b** (8.2 mg; R_f 0.26) were found.

Epicatechin-4,8-*epicatechin*-4,8-*catechin trimer* (**3b**). After purification on semi-prep. HPLC (R_t 19.1 min) using the gradient system (A), a sl. brown amorphous product was obtained, $[\alpha]_D^{20}$ + 76° (*c* 1; MeOH), FABMS: $[M-H]^-$ = 865, $[(M-H)-288]^-$ = 577 (dimer), $[(M-H)-152]^-$ = 713. Thiodegradation furnished after 4 hr four products: epicatechin-4,8-catechin dimer (B-1) (R_t 18.9 min), (+)-catechin (R_t 20.5 min), 4-benzylthio-(−)-epicatechin (R_t 40.1 min) and 4-benzylthio-(−)-epicatechin-epicatechin (R_t 36.8 min). After 29 hr reaction duration, only (+)-catechin and 4-benzylthio-(−)-epicatechin (^{1}H NMR) [1] were present, ratio 1:2.

Epicatechin-4,8-*catechin*-4,8-*epicatechin trimer* (**3c**). HPLC: (R_t 21.2 min), sl. brown amorphous products, $[\alpha]_D^{20}$ + 51° (*c* 1; MeOH); FABMS: identical with **3b**, thiodegradation gave after 4 hr, four products: (−)-epicatechin (R_t 22.1 min), 4-benzylthio-(−)-epicatechin (R_t 40.1 min), 4-benzylthio-epicatechin–catechin (R_t 44.1 min) and the catechin–epicatechin dimer B-4 (R_t 17.1 min) which was isolated and analysed as its peracetate (^{1}H NMR) identical with B-4 [25]. After 20 hr reaction duration, only epicatechin, 4-benzylthio-(−)-epicatechin and 4-benzylthio-(+)-catechin were found in the ratio 1:1:1.

Fractions 76–100 were a mixture of oligomeric polyphenols (216 mg), from which two proanthocyanidins were separated by TLC on silica gel (column 2 × 25 cm) using solvent system (E).

Epicatechin–epicatechin–epicatechin–catechin tetramer (**3c**). Obtained as a sl. brown powder (8.9 mg), $[\alpha]_D^{20}$ + 95° (*c* 1; MeOH); TLC (R_f 0.28); FABMS: $[M-H]^-$ = 1153, $[(M-H)-288]^-$ = 865 (trimer), HPLC: R_t 23.5 min.

Epicatechin–epicatechin–epicatechin–epicatechin–catechin pentamer. Obtained as a sl. brown powder (12.1 mg), $[\alpha]_D^{20}$ + 104°(*c* 1; MeOH); TLC: R_f 0.26; FABMS: $[M-H]^-$ = 1441, $[(M-H)-288]^-$ = 1153 (tetramer); HPLC: R_t 24.7 min). Thiodegradation of **3c** and **3d** gave only 4-benzylthio-(−)-epicatechin and (+)-catechin in ratios of 3:1 and 4:1, respectively.

Fractions 101–200 contained a mixture of dark brown products (4.15 g) corresponding to higher M_r phenolics not yet identified.

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